DISS. ETH No. 29528

Advancing Drug Utilisation and Drug Safety Research Through Interdisciplinary Methods

A thesis submitted to attain the degree of
DOCTOR OF SCIENCES of ETH ZURICH
(Dr. sc. ETH Zurich)

presented by

MARIA LUISA MARQUES DE SÁ FAQUETTI

M.Sc. in Medicinal and Industrial Pharmaceutical Sciences,
Eidgenössische Technische Hochschule Zürich (ETH Zürich)

born on 22.12.1986

accepted on the recommendation of
Prof. Dr. Andrea M. Burden, doctoral thesis supervisor
Prof. Dr. Gisbert Schneider, co-examiner
Prof. Dr. Milo A. Puhan, co-examiner
Prof. Dr. Francesca Grisoni, co-examiner

2023

Diss No 29528

Cover image: Created based on picture by PHC-Vector on iStock (illustration ID:1162417560). Modifications were kindly executed by Enriqueta Vallejo-Yagüe.

Language translation: The German translation of the Summary (Zusammenfassung) was conducted by Lukas Leu and revised by Dominik Stämpfli, Franziska Ulrich, and Lucia Gasparovic.

Pictures: In Chapter 1, Figure 1.1.4 was created in BioRender (https://www.biorender.com). The other figures throughout the chapter were created using free icons available on Flaticon (a Freepik Website – https://www.flaticon.com).

To the women who raised me –

My grandmother, Dona Neli, who's been a rock of stability and endless source of love and support throughout my life,

and my mother, Heloisa, whose encouragement made me the girl I wanted to be.

To my beloved husband –

Lukas, who has been cherishing and supporting me in whatever hobbies and business I put my hand in.

“The pursuit of science is a never-ending journey of curiosity, perseverance, and wonder, and its rewards are immeasurable”.

Jennifer Doudna

Contents

Acknowledgements

My PhD journey would not have been possible without the help, support, and mentoring of many people. It is, therefore, my great pleasure to express my gratitude to all those who made this thesis possible through their encouragement, partnership, support, and mentorship.

First and foremost, I would like to express my profound gratitude to my supervisor Prof Dr Andrea M. Burden, for introducing me to the field of pharmacoepidemiology, for assisting me in all regards along the way, and for giving me many degrees of freedom to grow as a researcher. Thanks to her, I could independently delve into an exciting topic during my PhD. She has been an inspiring mentor and group leader, not only from scientific and technical perspectives but from her values of respect, trust, and plurality in the work environment.

I am also grateful to my research committee members, Prof Dr Gisbert Schneider, Prof Dr Milo A. Puhan and Prof Dr Francesca Grisoni. Their mentoring has been instrumental not only in the successful completion of my dissertation work but also in my growth as a researcher. A special thank you to Prof Dr Andrew deMello for chairing my dissertation committee.

I truly appreciate the contribution of the other co-authors in the projects of this thesis, including Laura Slappendel, Hélène Bigonne, Dr Georg Aichinger, Prof Dr Shana Sturla, Dr Petra Schneider, Dr Theresa Burkard, Dr Lene Dreyer, Dr René Cordtz, Prof Dr Guillaume Obozinski, and Prof Dr Fernando Perez-Cruz. Without your valuable work, our significant publications were not possible. A special thanks to my master's students Valentina Bicvic, Divyia Koshi, and Géraldine Frey, for their rich contribution to this thesis, and my colleagues from the Swiss Data Science Centre (SDSC) for the fruitful discussions and scientific exchange.

My sincerest thanks to the whole Pharmacoepidemiology Group at ETH Zurich. I am very thankful to Dr Stefan Weiler for his willingness to help and discuss any topic (particularly the Beers list!). Dr Dominik Stämpfli for his thoughtful discussions (scientific-related or not) and for being a reference in supervising students inside our group. Anke and Tibor for ensuring everything else was right. This journey would not have been the same without my PhD colleagues (and friends) Dr Adrian Martinez De la Torre and Dr Enriqueta Vallejo-Yagüe who took me by the hand and kindly taught me how to navigate the new seas. Annika Vestergaard Kvist, Franziska Ulrich, Marcel Rainer, Lucia Gasparovic, Emanuele Palumbo, Pritibha Singh, and Lukas Higi, you all have been an inexhaustible source of encouragement, companionship, friendship, and joy inside and outside the office. Also, thank you for your advice, revisions,

scientific inputs, and artwork, throughout my work in the PhD, particularly in the thesis. It has been a pleasure working with you.

I also extend my deepest gratitude to my colleagues from DCHAB. I would be remiss in not mentioning Lukas Lätsch. Our discussions and scientific jokes kept my motivation high. Also, I would like to sincerely thank the Computer-Assisted Drug Design (CADD) group for your hospitality and warmth throughout my days in your lab. Special thanks to Dr Jan Hiss and Sarah Haller for their technical support, patience, coffee, and encouragement. Just as importantly, a big thank you to Kurt Hauenstein for your kindness, help, and generosity throughout the time I have been at the ETHZ.

I am also very grateful for the EBPhD programme at UZH and the fantastic graduate students I met over the last four years. My experience during the PhD was enriched by this supportive and intelligent group of students, who became so much more than colleagues. A special thanks to Dr Sarah Ziegler and Dr Marco Kauffmann for taking care of all bureaucratic details, and my PhD buddy Dr Dominik Menges, who I will always be indebted to you for your support and the vital role you played in the course of this journey.

To my previous supervisors, Prof Karl-Heinz Altmann, Prof Jeffrey Bode, and Prof Dr Marcus Vinicius Craveiro, I am grateful for your mentorship and encouragement during my undergraduate research projects, which inspired me to pursue a career in Natural Sciences.

My heartfelt thanks go to my friends Vivian, Camila, Talita, Clau, Milene for her unvaluable help on my development during my PhD time, Biro, Stephanie, Sofia, Matthias, Fabian, Andrea, and Raffael for their unwavering support and encouragement.

Finally, my most sincere and deepest gratitude to my family. To my grandparents, Neli and José Roberto (Chuba), your devotion to our family and unconditional love is precious. I would not be where I am today without your unrestricted support and encouragement. I am incredibly grateful for the best education you could provide me and the great values you raised me. You have always stressed the importance of education, making me the person I am today. Words cannot express my gratitude for all that you have done for me. To my mother, Heloisa, who gave me a strong foundation and, most importantly, the freedom to be who I am and choose my path. Thank you for instilling in me the values of freedom, respect, curiosity, and self-belief. Your unwavering support and encouragement have shaped who I am today.

Finally, words cannot express my love and gratitude to my husband, Lukas. Without his sunny morning optimism, I would be a much grumpier person. He has shared this fantastic journey with me and has seen me through the ups and downs along the way. I want to thank

him for showing me the beauty of life, for his love and support, and for making my mind relax during the hard times of my PhD. Finally, to my parents-in-law, Vreni and Seppinho, thank you for embracing me into your family with open arms and providing me with a loving and nurturing environment in Switzerland. Your constant support and words of wisdom have been a source of inspiration and strength. I am truly grateful for the love and care you have shown me.

List of Figures

List of Tables

List of Abbreviations, Acronyms and Symbols

15-ALOX: Arachidonate 15-Lipoxygenase

AAK1: AP2 Associate Protein Kinase 1

AA2AR: Adenosine Receptor A2A

AAB: Alpha-Adrenoceptor Blocker

Aβ: Amyloid Beta

ACE: Angiotensin Converting Enzyme

AChEi: Acetyl-Cholinesterase Inhibitor

AD: Alzheimer's Disease

ADH: Anti-Diuretic Hormone

ADME: Absorption, Distribution, Metabolism, and Excretion

ADORA3: Adenosine Receptor A3

ADR: Adverse Drug Reaction

AF: Atrial Fibrillation

AGS: American Geriatrics Society

AH: Anti-Histamine

AMP: Adenosine Monophosphate

APP: Amyloid Precursor Protein

ARB: Angiotensin Receptor Blocker

ATC: Anatomical Therapeutic Chemical

ATP: Adenosine 5'-Triphosphate

AUC: Area Under the Curve

BACE1: β-Secretase

BB: Beta Blocker

bDMARD: Biologic Disease-Modifying Antirheumatic Drug

BMI: Body Mass Index

BNF: British National Formulary

BPH: Benign Prostatic Hyperplasia

CATS2: Chemically Advanced Template Search Version 2

CCB: Calcium Channel Blocker

CCP: Cyclic Citrullinated Peptides

CI: Confidence Interval

CKD: Chronic Kidney Disease

C_{max}: Maximum Concentration Plasma

CMV: Cytomegalovirus

CNS: Central Nervous System

COPD: Chronic Obstructive Pulmonary Disease

CPR: Civil Personal Registration

csDMARD: Conventional Synthetic Disease-Modifying Antirheumatic Drug

CV: Cardiovascular

CYP: Cytochrome P450

DAS28: 28-joint Disease Activity Score

DANBIO: Danish Rheumatologic Database

DCK: Deoxycytidine Kinase

DDD: Defined Daily Dose

DDIs: Drug-Drug Interactions

DPP-4: Dipeptidyl Peptidase 4

EC_{50}: Half Maximal Effective Concentration

EC: Enzyme Commission

e.g.: For example, '*exempli gratia*'

eGFR: Estimated Glomerular Filtration Rate

EGFR: Epidermal Growth Factor Receptor

EHRs: Electronical Health Records

EMA: European Medicines Agency

EULAR: European Alliance of Associations for Rheumatology

FAERS: Food and Drug Administration Adverse Event Reporting System

FDA: Food and Drug Administration

GDPR: General Data Protection

GH: Growth Hormone

GI: Gastrointestinal

GLP-1: Glucagon-Like Peptide 1

GP: General Practitioner

HAQ: Health Assessment Questionnaire

HAQ-DI: Health Assessment Questionnaire-Disability Index

HbA1c: Hemoglobin A1c

HCV: Hepatitis C

HF: Heart Failure

HFrEF: Heart Failure with Reduced Ejection Fraction

HR: Hazard Ratio

HSV: Thymidine Kinase

HTRF: Homogeneous Time-Resolved Fluorescence

HZ: Herpes Zoster

IC_{50}: Half Maximal Inhibitory Concentration

ICD: International Classification of Diseases

i.e.: That is, '*id est*'

IFN: Interferon

IL: Interleukin

IMRD-UK: IQVIA Medical Research Database in the UK

iNOS: Inducible Nitric Oxide Synthase

INR: International Normalised Ratio

IR: Incidence Rates

IQR: Interquartile Range

IV: Intravenous

JAK: Janus Kinase

JAKis: Janus Kinase inhibitors

K_d: Binding Constant

K_i: Inhibitor Constant

M: Molar

MACE: Major Adverse Cardiovascular Events

MAP3K12: Dual Leucine Ziper Kinase

mM: Milimolar

□M: Micromolar

RMS: Root Mean Square

RMSD: Root Mean Square Deviation

NA: Not Accessed

NIAD: Non-Insulin Antidiabetic Drug

NICE: National Institute for Health and Care Excellence

nM: Nanomolar

NSAID: Nonsteroidal Anti-Inflammatory Drug

OTC: Over the Counter

PAH: Pulmonary Arterial Hypertension

PBPK: Physiologically Based Pharmacokinetic

PDB: Protein Data Bank

PDE10A: Phosphodiesterase 10A

PDE10A2: Phosphodiesterase 10A2

pH: Potential of Hydrogen

PIPs: Potentially Inappropriate Prescriptions

PKN2: Serine/Threonine-Protein Kinase N2

PLP: Proximal Ligand Potential

PPIs: Proton Pump Inhibitors

PROMPT: Prescribing Optimally in Middle-Aged People's Treatments

qPCR: Quantitative Polymerase Chain Reaction

QSAR: Quantitative Structure-Activity Relationship

QIVIVE: Quantitative *In vitro* to *In vivo* Extrapolation

RA: Rheumatoid Arthritis

RCT: Randomized Clinical Trial

RF: Rheumatoid Factor

RTK: Receptor Tyrosine Kinase

RWD: Real-World Data

RWE: Real-World Evidence

SARS-CoV-2: Acute Respiratory Syndrome Coronavirus 2

SD: Standard Deviation

SEA: Similarity Ensemble Approach

SEM: Standard Error of the Mean

SGLT-2: Sodium-Glucose Co-Transporter 2

SMILES: Simplified Molecular-Input Line-Entry System

SOM: Self-Organizing Map

SPiDER: Self-Organizing Map-Based Prediction of Drug Equivalence Relationships

SSRI: Selective Serotonin Reuptake Inhibitor

START: Screening Tool to Alert to Right Treatment

STOPP: Screening Tool of Older Persons' Prescriptions

SU: Sulfonylurea

T2DM: Type 2 Diabetes Mellitus

TCA: Tricyclic Antidepressant

TCPS: Time Conditional Propensity Score

TIGER: Target Inference Generator

THIN: The Health Improvement Network

TNF: Tumour Necrosis Factor

TNF-αis: Tumour Necrosis Factor Alpha Inhibitors

TRPC3: Short Transient Receptor Potential Channel 3

TRPC6: Short Transient Receptor Potential Channel 6

TRPM6: Transient Receptor Potential Cation Channel Subfamily M member 6

tsDMARD: Targeted Synthetic Disease-Modifying Antirheumatic Drug

TYK2: Non-Receptor Tyrosine-Protein Kinase

Ube2N: Ubiquitin-Conjugating Enzyme E2 N

UGIB: Upper Gastrointestinal Bleeding

UK: United Kingdom

US: United States

UTI: Urinary Tract Infection

VAS: Visual Analogue Scale

VTE: Venous Thromboembolism

WHO: World Health Organization

XPO1: Exportin-1

Summary

Randomized clinical trials (RCTs) are fundamental for evaluating the safety and efficacy during the development of new drugs. However, RCTs are often limited in their generalisability and validity due to low diversity and number of participants, and duration of exposure. Pharmacoepidemiologic research allows the evaluation of the use and effects of drugs in a more extensive and diverse patient population, reflecting real-world conditions. Moreover, the increasing ability to electronically capture real-world data (RWD) related to patient health status and transform it into new knowledge, so-called real-world evidence (RWE), has opened new opportunities for pharmacoepidemiologists to close gaps between research and clinical care. These electronic healthcare databases can be used to study the utilisation, safety, and effectiveness of drugs at population level.

The use of RWD in pharmacoepidemiologic research also poses some challenges. For example, while studies on drug utilisation provide an overview of patterns in use and adverse events, capturing complex drug utilisation patterns in large databases may be a difficult task when thousands of drugs are approved and used in routine practice. Thus, there is a need to combine multidisciplinary approaches to uncover valuable information on prescription patterns using large patient datasets. Another central challenge in pharmacoepidemiology is the investigation of adverse events with unknown mechanisms of action. Pharmacoepidemiologic studies alone are limited in the ability to study only known drug effects based on the known pharmacokinetic and pharmacodynamics principles, and thus, are limited to infer conclusions when adverse events may be due to unknown mechanisms of action. Thus, in this dissertation, we focus on leveraging methodologies from pharmacoepidemiology, data science, and medicinal chemistry to address unanswered questions in the study of the utilisation and safety of drugs used to treat chronic conditions.

In the first part of this dissertation (**Chapter 3**), we proposed a novel application of the Apriori algorithm, an established data mining algorithm, to overcome the inherent computational challenges of assessing real-world prescription patterns at the compound level using RWD (**Chapter 3.1**). The analysis revealed a high prevalence of polypharmacy (i.e., use of ≥5 concomitant medications) in patients with diabetes. Additionally, we evaluated an extensive array of individual drugs and drug combinations that are frequently prescribed, surpassing the existing knowledge on polypharmacy patterns based on drug classes. Subsequently, we shifted the focus from identifying harmful combinations of drugs to identifying opportunities for

optimising pharmacotherapy in patients starting their first oral antidiabetic medication by estimating the prevalence of potentially inappropriate prescriptions (PIPs; [Chapter 3.2]). The analysis indicated that the prevalence of PIPs was higher in patients receiving polypharmacy and in older patients. Thus, starting treatment with oral antidiabetic drugs, particularly in those patients receiving polypharmacy, should involve a comprehensive review of the medications to optimise prescribing decisions.

Moving forward, in the second part of this dissertation (Chapter 4), we combined interdisciplinary approaches to investigate the safety profile of Janus Kinase inhibitors (JAKis) following rising safety concerns leading to black-box warnings, despite unknown mechanisms. First, we leveraged computational and experimental approaches to investigate whether the safety concerns on thrombosis and viral infection associated with tofacitinib and baricitinib was potentially due to off-target effects (Chapter 4.1). Although our findings did not confirm the hypothesis of elevated risk of thrombosis and viral infection explained by drug-target interactions, previously unknown off-targets of baricitinib and tofacitinib were identified, suggesting them as potential candidates for drug repurposing. Subsequently, we proposed the novel prevalent new-user cohort study to investigate the incidence and risk of major adverse cardiovascular events (MACE), venous thromboembolism (VTE), and viral infection/reactivation associated with JAKi use in the Danish rheumatologic database (DANBIO) (Chapter 4.2). The use of an advanced study design and the DANBIO aims to overcome challenges posed by confounding biases in observational studies, and to provide valuable safety knowledge on the treatment of patients with rheumatoid arthritis (RA) using JAKis.

Finally, we extended our investigation on the off-target profile of tofacitinib and baricitinib with a focus on repurposing within Alzheimer's disease (AD; [Chapter 5]). The combined approach of a machine learning-based tool and experimental validation allowed the identification and characterisation of previously unknown baricitinib off-targets potentially relevant for AD progression. Although baricitinib is unlikely to be successfully repurposed for AD, the findings contribute to our understanding of JAKis off-target effects and their potential implications.

Overall, this dissertation demonstrated the ability of pharmacoepidemiology to collaborate with other disciplines and leverage different methodologies. By integrating diverse approaches, it becomes possible to identify new risks and benefits, explore off-target effects, and potentially uncover new indications for approved drugs. This interdisciplinary collaboration

has the potential to impact early stages of drug development and improve prescribing decisions, highlighting the benefits of fusing alternative approaches to tackle common goals.

Zusammenfassung

Randomisierte klinische Studien (RCTs) sind essenziell, um die Sicherheit and Wirksamkeit von Arzneimitteln während ihrer Entwicklung zu untersuchen. Die Erkenntnisse sind jedoch oft nur begrenzt verallgemeinbar und valide, da die Studienpopulation meist klein und homogen und die Behandlungsdauer kurz ist.

Die pharmakoepidemiologische Forschung ermöglicht die Evaluierung der Verwendung und der Wirkungen von Arzneimitteln in grösseren und vielfältigeren Patientenpopulationen, die die klinische Realität abbilden. Die zunehmende Verfügbarkeit von elektronischen Gesundheitsdaten der Patienten (real-world data, RWD) erleichtert Pharmakoepidemiologen den Gewinn neuer Erkentnisse, so genannte Real-World Evidence (RWE), mit denen Lücken zwischen Forschung und klinischer Versorgung geschlossen werden können. Diese elektronischen Gesundheitsdatenbanken können genutzt werden, um die Verwendung, Sicherheit und Wirksamkeit von Arzneimitteln auf der Bevölkerungsebene zu untersuchen.

Die Verwendung von RWD in der pharmakoepidemiologischen Forschung bringt auch einige Herausforderungen mit sich. Die Erkennung komplexer Muster im Arzneimittelgebrauch wird beispielsweise dadurch erschwert, dass Tausende von Arzneimitteln und Formulierungen zugelassen sind, die in der Routinepraxis verschrieben und kombiniert werden. Multidisziplinäre Ansätze können diese Hürde überwinden und aus grossen Patientendatensätzen wertvolle Informationen über Verschreibungsmuster gewinnen. Eine weitere zentrale Herausforderung in der Pharmakoepidemiologie ist die Untersuchung unerwünschter Wirkungen mit unbekannten Wirkmechanismen. Pharmakoepidemiologische Studien allein sind begrenzt auf die Untersuchung von bekannten Arzneimittelwirkungen basierend auf bekannten pharmakokinetischen und pharmakodynamischen Prinzipien und können daher nur bedingt Schlussfolgerungen ziehen, wenn unerwünschte Wirkungen auf unbekannte Wirkmechanismen zurückzuführen sind. In dieser Dissertation nutzen wir Methoden aus der Pharmackepidemiologie, den Datenwissenschaften und der medizinischen Chemie, um unbeantwortete Fragen über den Gebrauch und die Sicherheit von Arzneimitteln zur Behandlung chronischer Erkrankungen zu klären.

Im ersten Teil dieser Dissertation (**Kapitel 3**) haben wir eine neuartige Anwendung des Apriori-Algorithmus auf RWD vorgeschlagen, um die rechnerischen Herausforderungen bei der Bewertung von realen Verschreibungsmustern auf der Ebene der Präparate zu bewältigen (**Kapitel 3.1**). Die Analyse zeigte eine hohe Prävalenz von Polypharmazie (gleichzeitige Einnahme

von ≥5 Medikamenten) bei Patienten mit Diabetes auf. Zusätzlich werteten wir ein umfangreiches Spektrum an einzelnen Arzneimitteln und Arzneimittelkombinationen aus, die häufig verschrieben werden, und ergänzten damit das vorhandene Wissen über Polypharmazie basierend auf Arzneimittelklassen. Anschliessend verlagerten wir den Schwerpunkt auf die Erkennung von Möglichkeiten zur Optimierung der Pharmakotherapie bei Patienten, die ihre erste orale antidiabetische Medikation beginnen, indem wir die Prävalenz potenziell unangemessener Verschreibungen (PIPs; [**Kapitel 3.2**]) bestimmten. Die Analyse ergab, dass die Prävalenz von PIPs insbesondere bei Patienten mit Polypharmazie und bei älteren Patienten hoch ist. Daher sollte zu Beginn der Behandlung mit oralen Antidiabetika, insbesondere bei Patienten mit Polypharmazie, eine umfassende Überprüfung der Medikamente erfolgen, um die Verschreibungsentscheidungen zu optimieren.

Im zweiten Teil dieser Dissertation (**Kapitel 4**) haben wir interdisziplinäre Ansätze kombiniert, um das Sicherheitsprofil von Janus-Kinase-Inhibitoren (JAKis) zu untersuchen, nachdem es vermehrt Sicherheitsbedenken gab, die zu Black-Box-Warnungen führten, deren Mechanismus aber unbekannt ist. Zunächst untersuchten wir mit computerbasierten und experimentellen Ansätzen, ob das erhöhte Risiko für Thrombosen und Virusinfektionen mit Tofacitinib und Baricitinib möglicherweise auf Off-Target-Effekte zurückzuführen ist (**Kapitel 4.1**). Obwohl unsere Ergebnisse die Hypothese eines erhöhten Thrombose- und Virusinfektionsrisikos aufgrund von Wechselwirkungen zwischen Wirkstoff und Zielmolekül nicht bestätigten, wurden bisher unbekannte Off-Targets von Baricitinib und Tofacitinib identifiziert, was darauf hindeutet, dass sie sich als potenzielle Kandidaten für ein Repurposing eignen. Anschließend schlugen wir eine neuartige Kohortenstudie für Patienten mit einer Erstverschreibung vor, um die Häufigkeit und das Risiko von schwerwiegenden unerwünschten kardiovaskulären Ereignissen (major adverse cardiac events, MACE), venösen Thromboembolien (VTE) und viralen Infektionen/Reaktivierungen im Zusammenhang mit der Anwendung von JAKi in der dänischen Rheumadatenbank (DANBIO) zu untersuchen (**Kapitel 4.2**). Die Verwendung eines fortschrittlichen Studiendesigns und der DANBIO-Datenbank zielt darauf ab, die Herausforderungen von störenden Verzerrungen (confounding biases) in Beobachtungsstudien zu überwinden und liefert wertvolle Erkenntnisse zur Sicherheit bei der Behandlung von Patienten mit rheumatoider Arthritis (RA) mit JAKis.

Schliesslich haben wir unsere Untersuchung des Off-Target-Profils von Tofacitinib und Baricitinib im Sinne eines Repurposing bei der Alzheimer-Demenz (AD; [**Kapitel 5**]) erweitert. Der kombinierte Einsatz eines auf Machine Learning basierenden Ansatzes und einer

experimentellen Validierung ermöglichte die Identifizierung und Charakterisierung von bisher unbekannten Off-Targets von Baricitinib, die für das Fortschreiten der Alzheimer-Demenz von Bedeutung sein könnten. Obwohl es unwahrscheinlich ist, dass Baricitinib erfolgreich bei einer Alzheimer-Demenz eingesetzt werden kann, tragen die Ergebnisse zu unserem Verständnis der Off-Target-Effekte von JAKis und deren möglichen Auswirkungen bei.

Insgesamt zeigte die Dissertation die Fähigkeit der Pharmakoepidemiologie, mit anderen Disziplinen zusammenzuarbeiten und verschiedene Methoden zu nutzen. Durch die Integration verschiedener Ansätze ist es möglich, neue Risiken und Vorteile zu identifizieren, Off-Target-Effekte zu erforschen und potenziell neue Indikationen für zugelassene Arzneimittel zu entdecken. Diese interdisziplinäre Zusammenarbeit hat das Potenzial, sich auf frühe Phasen der Arzneimittelentwicklung auszuwirken und Verschreibungsentscheidungen zu verbessern. Sie unterstreicht den Wert der Verbindung verschiedener wissenschaftlicher Disziplinen zur Erreichung gemeinsamer Ziele.

Chapter 1

Introduction

1.1 Pharmacoepidemiology

1.1.1 Placing pharmacoepidemiology in the drug development process

Pharmacoepidemiology is the scientific discipline that studies the use, safety, and effectiveness of medications at a population level. It is a bridge science encompassing epidemiology, the study of health-related questions in populations, and clinical pharmacology, the study of the effects of drugs on humans.[1] Although pharmacoepidemiology is built on top of these two core sciences, it also draws upon the insights of other disciplines like medicinal chemistry and data science (**Figure 1.1.1**). By harnessing the synergies among various domains, pharmacoepidemiology has evolved into a comprehensive discipline that offers valuable insights into medication usage, its effects, and the resulting impact on public health.

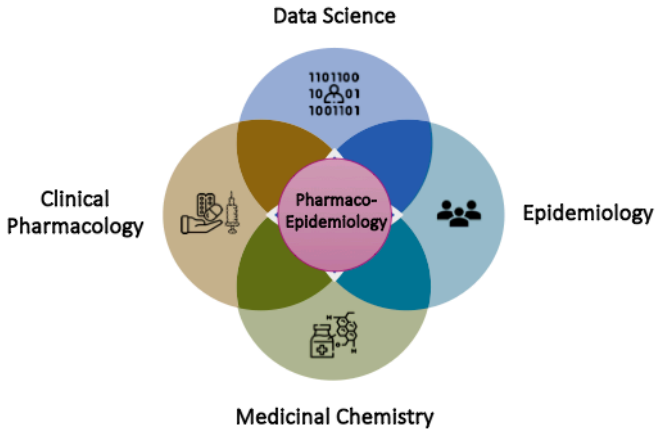


Figure 1.1.1 Schematic illustration of pharmacoepidemiology core sciences (clinical pharmacology and epidemiology) and synergy with medicinal chemistry and data science.

While randomized clinical trials (RCTs) are designed to assess safety and efficacy of a medication under ideal and controlled settings, pharmacoepidemiology evaluates safety and effectiveness of medications under the reality of the daily clinical practice where there is no longer strict exclusion criteria or randomization of treatment allocation. Pharmacoepidemiologic studies complement the knowledge gained from the pre-clinical and clinical phases of drug development. First, while pre-clinical studies provide information on a

drug's pharmacological properties, potential toxicities, pharmacokinetics and pharmacodynamics, RCTs evaluate the efficacy and safety of drugs in carefully selected populations. Nonetheless, these studies may not capture the full range of drug effects, particularly in broader patient populations and more diverse settings, such as older adults, individuals with multiple concomitant conditions, and pregnant women. Moreover, as RCTs are conducted under controlled conditions, they are limited, by design, in the number of patients, exposure time, setting, and patient profile.[2] Thus, the ability to detect rare adverse events and long-term effects of drugs in RCTs is also limited.

Second, pharmacoepidemiology may provide insight into patterns of drug use, prescribing practices, and treatment outcomes in real-world clinical practice.[1] This information can be used to optimize pharmacotherapy, improve patient outcomes, and inform health policy decisions, such as prescription recommendations. Therefore, even after successful drug development and approval, investigating the utilisation, safety, and effectiveness of drugs in real-world clinical practice remains of interest. Third, pharmacoepidemiology can play a role throughout drug discovery and development. With the rapid growth of real-world data (RWD), pharmacoepidemiology offers an alternative approach to exploring the use and effects of drugs that were initially approved for a different indication, also called drug repurposing.[3–6] Large healthcare datasets offer a perspective rooted in actual clinical care, providing extensive longitudinal diagnostic and pathophysiological patient data. These datasets play a crucial role in generating and validating of drug repurposing hypotheses.[6–8]

1.1.2 Pharmacoepidemiology methods

The increasing ability to electronically capture RWD related to patient health status and transform it into new knowledge, so-called real-world evidence (RWE), has opened new opportunities for pharmacoepidemiologists to close gaps between research and clinical care.[9] Pharmacoepidemiology uses a wide range of RWD sources (e.g., electronical health records [EHRs], medical claims data, pharmacovigilance data, disease registries, and data gathered from other sources such as digital health technologies) that can inform of the health status of patients.[2] These large databases often comprise millions of patients and multiple years of observation, spanning information on patient characteristics, medical diagnoses, medications prescription, and sometimes lifestyle variables such as body mass index (BMI), smoking status, and alcohol consumption. While these databases are comprehensive, the data are collected during routine clinical practice, and thus, the methodology of pharmacoepidemiology is

observational in nature as there is no intervention regarding treatment assignment. As such, there are a variety of methodological challenges, such as bias, confounding variables, and data quality. In the following sections, the different types of RWD and pharmacoepidemiology studies are described in more detail, along with their potential challenges and limitations.

1.1.3 Real-world data sources for pharmacoepidemiologic research

In pharmacoepidemiology, the source of data significantly influences research outcomes. There are two main approaches for data collection: data specifically tailored to answer a research question or for use in a specific study ('primary data collection') or data derived from healthcare services collected for non-research purposes ('secondary use of data').[10] Although secondary data do not include systematic data collection tailored to the research question, it has several advantages. First, it is more representative of routine clinical practice in the general population due to its large size, permitting the analysis of specific groups.[11] This is particularly important to study drug use, safety and effectiveness in populations excluded from RCTs, such as older adults and patients with multiple concomitant drugs and conditions. Second, secondary data can frequently be linked with data from other sources (e.g., primary care, vital statistics, and cancer registries) to generate a more complete view of patients' healthcare trajectories and facilitate causal assessments.

As different research studies demand different types of data, a primary concern in pharmacoepidemiology research is correctly choosing the appropriate database to address the research question. Although various sources of RWD for pharmacoepidemiologic research exist, no single data source captures all the desired information. Notably, healthcare databases vary in quality and completeness, population representativeness, breadth and detail of information they contain, and liability with other data sources.[10] Thus, it is crucial to understand their strengths and limitations to produce appropriate RWE. For the purpose of this dissertation, in the subsequent pages, we elaborate on two secondary data sources – The IQVIA Medical Research Database in the United Kingdom (IMRD-UK), a Cegedim database incorporating data from The Health Improvement Network (THIN) database, and the Danish rheumatologic database (DANBIO).

1.1.3.1 The IQVIA Medical Research Database UK (IMRD-UK)

Electronic Health Records (EHRs) occupy a pivotal position within the landscape of RWD. They encompass the systematic collection of medical data in digital format during routine clinical

care.[11] A main advantage of EHRs is their longitudinal and interconnected nature, allowing for a comprehensive panorama of patients' journey within the healthcare system across time. By weaving together various pieces of the healthcare puzzle, EHRs provide a holistic lens to understand patients' trajectories and the evolving tapestry of their medical experiences.

The most common sources of EHRs data are derived from primary care, or general practitioners (GPs).[1] While GPs act as gatekeepers of the health care system in countries such as the UK and the Netherlands, EHRs not only provide information on primary care attention but include information on all medical events that GPs are involved in or informed of, such as admission to emergency rooms or medications initiated by specialists. Thus, EHRs typically contain patient demographics, medical history, medication prescriptions, immunization status, laboratory results, information on some lifestyle factors, and feedback from secondary care or hospitalizations.[11]

This dissertation uses data from the IMRD-UK, a Cegedim database incorporating data from the THIN database, which is a database of anonymised EHRs generated from the daily record of GPs.[12] The IMRD-UK is an extensive database containing longitudinal primary care information, including detailed information about patient characteristics (e.g., year of birth, sex, practice registration date, practice de-registration date, ethnicity), laboratory results (e.g., estimated glomerular filtration rate [eGFR] and haemoglobin A1c [HbA1c]), medical conditions (i.e., diagnoses with dates, referrals to hospitals, symptoms), medications (i.e., drug name, formulation, prescription date, strength, quantity, dosing instructions), and other patient-level data (e.g., smoking status, height, weight, alcohol use, pregnancy, birth, death dates). A schematic representation of information collected in the primary care IMRD-UK is illustrated in **Figure 1.1.2**.

IMRD-UK includes data from more than 18 million patients from over 800 general medical practices in the UK, covering approximately 6% of the UK population.[13] The database is representative of the UK and valid regarding age and sex comparisons and a wide range of diseases.[12] By using IMRD-UK, researchers can gain insights into a patient's complete healthcare journey, as GPs are the gatekeepers to all other medical services in the UK. Moreover, the database represents an important data source for drug utilisation studies, particularly in the study of prescription patterns.[1] IMRD-UK includes every drug prescribed to patients within the GP practice, and thus the data reflects the behaviour of the prescriber.[14,15]

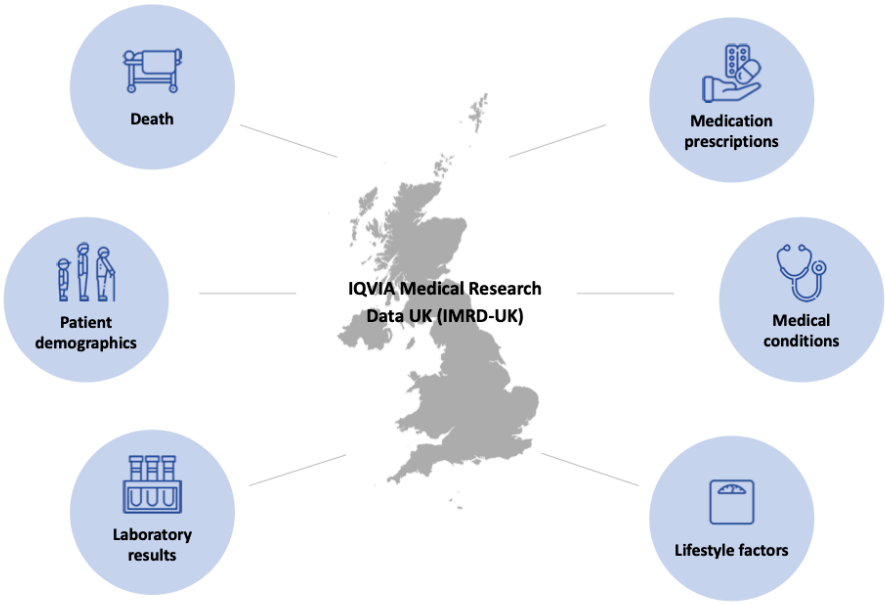


Figure 1.1.2 Information in the IQVIA Medical Research Data in the UK (IMRD-UK), a Cegedim database incorporating data from The Health Improvement Network (THIN) database. The IMRD-UK encompasses longitudinal primary care information, including medication prescriptions, patient demographics, medical conditions, laboratory results, patient demographics, and other patient-level data such as lifestyle factors and death.

Despite the many advantages of EHRs, some limitations pose several methodological challenges. For example, these databases typically lack information on prescriptions from specialists, hospitalizations, or nursing homes. Additionally, while GPs receive feedback letters regarding diagnoses made during hospitalizations or from specialists, details of the care from external providers are not captured completely. While certain types of information can be found in EHRs compared to claims data or pharmacy dispensing databases (e.g., BMI status and occupation), they might not be routinely recorded.

1.1.3.2 The Danish Nationwide Clinical Registries

While EHRs provide important clinical information, one of the main limitations comes to the inability to evaluate costly medications that are most frequently prescribed by specialists or in hospitals. Thus, to evaluate such medications, the use of drug or disease registries is required. Registry data are typically designed to monitor the use, safety, and effectiveness of specific drug classes (i.e., biologics) or diseases (i.e., cancer).[16] The primary advantage of registries is the

detailed nature of data collection for the specific drug or disease of interest, thereby providing a more comprehensive and pragmatic picture of a drug's safety profile. This level of granularity allows researchers to control for potential confounders and conduct more precise analyses, such as adjusting for baseline characteristics or exploring subgroup effects.

In Denmark, for example, all patients when first diagnosed with rheumatoid arthritis (RA) or starting more advanced therapies such as biological disease-modifying antirheumatic drugs (bDMARDs) for the treatment of inflammatory rheumatic conditions must be registered into the Danish rheumatologic database (DANBIO) registry.[17] The Danish rheumatological registry is a comprehensive database that provides longitudinal data on specific and detailed clinical information, including measures of disease activity, treatment effect, adverse events, and change of treatment.[18] Since 2005, DANBIO's primary objective has been first, to serve as a robust research database for investigating multiple aspects, such as treatment effectiveness, adverse events, and quality of life, and second, to facilitate clinical decision-making by providing a disease chronicle to rheumatologists, thereby enhancing the quality of care delivered to patients.[19]

While registry data serve as a valuable resource in pharmacoepidemiology, offering extensive and representative information on real-world patient populations, they are limited to the variables captured within the registry, which might not cover all potential confounders or outcomes of interest. As registry data alone might not provide a complete picture of the patient's healthcare trajectory, additional data can be further acquired by linking registries to other sources of medical information (i.e., medical records, drug prescriptions, hospital data) enabling a more comprehensive view of patients' disease trajectories to evaluate the safety profile of drugs in pharmacoepidemiologic studies properly. Countries with linked population-wide healthcare, such as Denmark and Canada, provide rich longitudinal data that permit an assessment of patient care and trajectory over time. This is ideal for studying adverse drug reactions (ADRs), where knowing the temporal relationship between exposure and outcome is essential to determine a causal relationship.[20]

In this context, Denmark provides an optimal environment for conducting pharmacoepidemiologic research as individuals are assigned a unique civil personal registration (CPR) number that follows them throughout life.[21] The CPR number serves as the key identifier bridging the DANBIO registry with various medical and administrative national databases (**Figure 1.1.3**), enabling seamless data linkage across diverse Danish data sources.[21–26] Hence, this data

linkage facilitates the investigation of research questions that cannot be addressed by individual registries alone as it depends on comprehensive information about patients.[19]

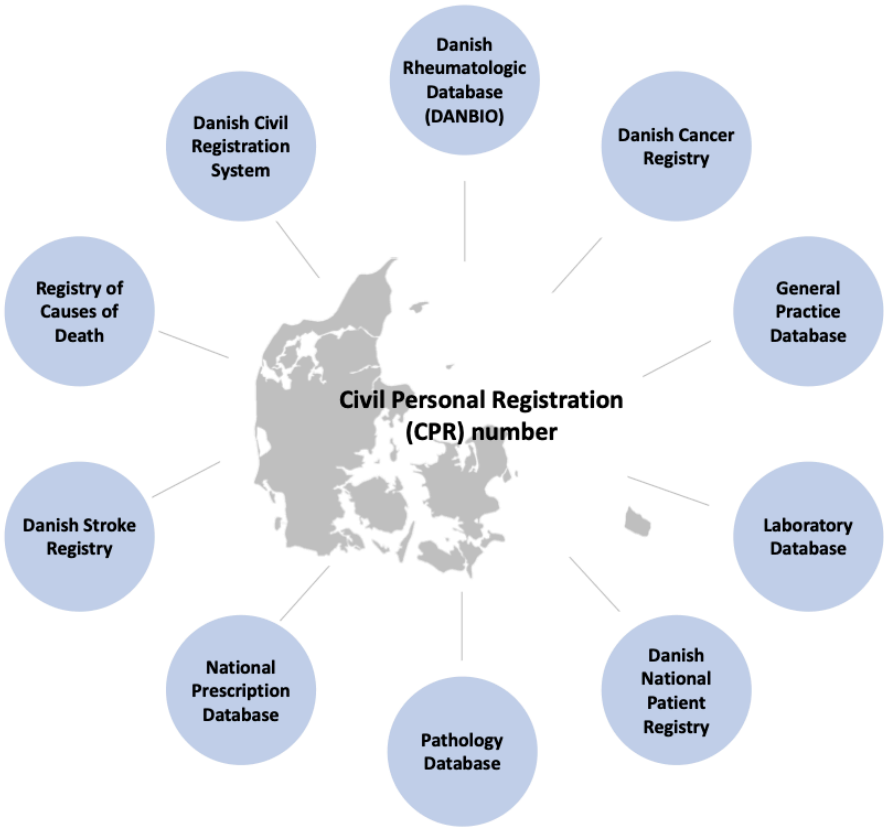


Figure 1.1.3 Individual-level record linkage between Danish databases using the civil personal register (CPR) number.

1.1.4 Challenges and opportunities in pharmacoepidemiology to be addressed using interdisciplinary methods

Pharmacoepidemiology encompasses studies on drug utilisation, safety, and effectiveness of medications in large observational healthcare databases, as described above. Typically, observational designs used in pharmacoepidemiology can be grouped into descriptive and analytic analyses. The primary distinction between the two is the presence of hypothesis testing. In the following section, the different types of studies and the challenges and limitations are described.

1.1.4.1 Methodological challenges and current limitations in descriptive drug utilisation analysis

Descriptive analyses of drug utilisation aim to describe the extent and pattern of drug exposure (i.e., prescription, dispense and consumption of medicines) at the population level or within a group of patients. While these studies are descriptive in nature, they are important facets of pharmacoepidemiology as they provide information on the prevalence of prescriptions use and can facilitate the safe and effective use of drugs in large populations.[1] Moreover, drug utilisation research can be particularly useful to identify potential over- and underuse of drugs, identify gaps between current practices and guidelines and potential areas for improvement,[27] explore patterns of drug prescribing within a population, dispensing and consuming, acquire knowledge on the perceptions of clinicians, pharmacists, and patients, and assess the impact of interventions to improve drug use.[27] Additionally, drug utilisation in pharmacovigilance studies can further include the prevalence of ADRs to evaluate if there are correlations between drug use and safety outcomes. Ultimately, it may help authorities and regulators drive public health policies and interventions to optimize healthcare delivery and improve patient outcomes.[27]

While drug utilisation studies provide an overview of patterns in use and ADRs, there are significant methodological challenges and limitations. For example, capturing complex drug utilisation patterns in large databases may be challenging when thousands of drugs are approved and used in daily practice. Thus, the investigation of complex prescription patterns among patients receiving multiple concomitant medications in large population-based datasets is poorly explored. Polypharmacy is commonly defined as the concomitant use of five or more medications, and it is becoming increasingly prevalent worldwide as populations age and the number of people with multiple long-term conditions rises.[28] Although multiple medications may be needed to address the numerous medical needs of a patient, the risk of experiencing an ADR due to a drug-drug interaction (DDI) increases substantially with the number of medications.[29] A DDI arises based on a pharmacologic interaction between two drugs in a manner that the effectiveness or toxicity of one or more drugs is altered.[30]

Despite the availability of extensive data sources containing drug prescription information, studies investigating prescription patterns among patients with polypharmacy frequently focus on examining combinations of drug classes co-prescribed in specific groups of patients. Nevertheless, the occurrence of DDIs in patients receiving polypharmacy is not generally a problem related to the class of the medication but rather the individual drugs (i.e., single drug compounds). For example, when using cyclosporine, a cytochrome P450 (CYP) 3A4

inhibitor, it is recommended to avoid statins that undergo metabolism via this enzyme, such as simvastatin, lovastatin, and pitavastatin. Instead, non-CYP3A4 substrate statins like pravastatin, fluvastatin, and rosuvastatin represent a more suitable choice to avoid a potential DDI.[31] Thus, identifying combinations of drugs at the drug compound level is of interest to minimize ADRs and improve safety in patients receiving polypharmacy.

The challenge arises with the need to compute a higher order of drug combinations in patients receiving polypharmacy. For example, patients receiving polypharmacy may receive up to 40 different drugs concomitantly,[32] that is, up to 10^{12} possible combinations of drugs. However, assessing all possible drug combinations in such patients is not computationally feasible. Therefore, novel interdisciplinary approaches such as machine learning-based models[33] and data mining techniques[34,35] are needed to uncover valuable information on prescription patterns using large population-based datasets.

1.1.4.2 Methodological challenges, current limitations, and opportunities in analytic drug safety analysis

Drug safety encompasses the assessment, management, and prevention of adverse effects associated with medication use in populations.[1] It involves the evaluation of the risks and benefits of drugs, both before and after they are approved for use, as well as the identification and management of any safety concerns, such as new ADRs. During the post-marketing phase, also known as phase IV of drug development, drug safety monitoring becomes paramount. While RCTs conducted in earlier phases provide crucial safety information, they are often limited in terms of sample size and duration of exposure. Phase IV allows for evaluating drug safety in a more extensive and diverse patient population, reflecting real-world conditions and longer-term use. Thus, in phase IV, the focus shifts from controlled clinical settings to broader patient populations and routine clinical practice. This phase provides an opportunity to gather additional data on the drug's safety profile, effectiveness in different patient subgroups, and the occurrence of rare or long-term side effects that may not have been evident during earlier phases.

Despite the crucial role of pharmacovigilance in identifying safety signals in post-marketing approval, it has some limitations including under-reporting and uncertainty regarding the causal relationship between the drug and the reported event.[1] Therefore, observational studies are often necessary to further evaluate apparent associations between drugs and adverse events. Although descriptive studies provide insight into new safety concerns, further

causality assessment with longitudinal clinical data is needed. These studies typically include the cohort, case-control, or case-only designs and are the hallmark of pharmacoepidemiology.[1]

While analytical pharmacoepidemiologic studies provide a natural solution to study drug effects, there are significant challenges to consider. Assessing causality with these studies represents a main challenge due to the non-random treatment allocation, which leads to confounding (i.e., a difference between patients exposed to a treatment of interest and a comparator group).[36] Moreover, due to the complexities of disease mechanisms and treatment responses, not all findings from safety and effectiveness studies make natural sense. Nevertheless, uncovering findings that challenge initial expectations can lead to a deeper understanding of the complex dynamics of medications at play.

As routine clinical care does not follow strict protocol and randomization of treatment, there are a number of biases and confounding factors that need to be addressed when conducting drug safety or effectiveness studies. Moreover, a big challenge in pharmacoepidemiology is the investigation of ADRs with unclear pathways. Pharmacoepidemiology is limited in the ability to study only known drug effects based on the known pharmacokinetic and pharmacodynamics principles, and thus it is limited to infer conclusions when adverse events with unknown mechanisms of action. The emergence of safety signals with unknown mechanisms in pharmacoepidemiologic RWD-based studies may be due to the presence of confounding factors (e.g., age, underlying diseases, concomitant medications, or lifestyle factors) and biases,[37–40] which is depicted in **Figure 1.1.4** (**a**), or the binding of a drug molecule to a protein target in the human body other than the intended target, also called off-target interactions, depicted in **Figure 1.1.4** (**b**).[41]

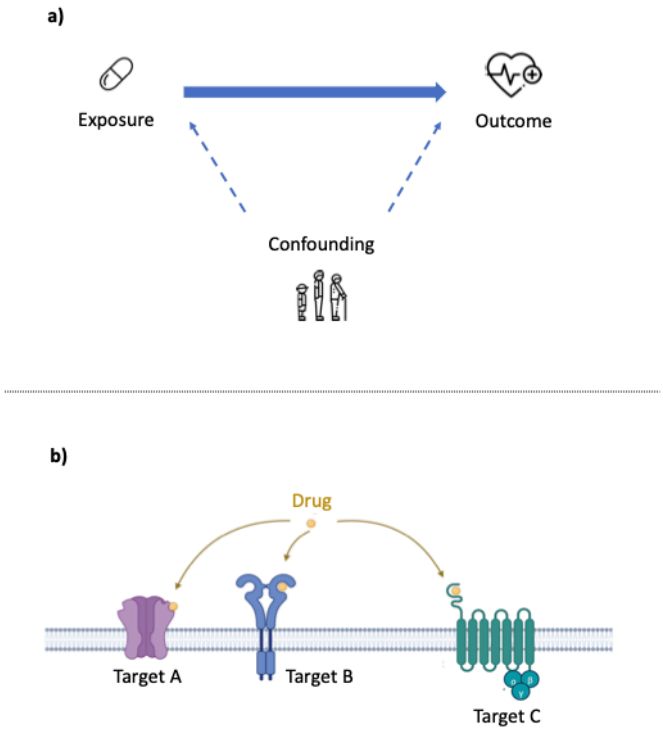


Figure 1.1.4 The emergence of safety signals with unknown mechanisms of action may be due to (a) confounding (e.g., age) and biases or (b) off-target interactions. Although most drugs are designed to interact with particular targets (represented by the interaction between the drug and Target A in panel b) to trigger a biochemical response, some drugs may additionally interact with other targets in the human body (represented by the drug interaction with Targets B and C in panel b), leading to unintended and often unknown effects).

Bias is characterized as a systematic flaw embedded in the study's design or the process of data collection, analysis, interpretation, and reporting. This flaw consistently leads to results that deviate from the truth.[42] For example, consider a typical cohort study where patients are followed from treatment through to the occurrence of an outcome of interest. If the study design includes patients who had previously received one of the drugs being investigated, the resulting estimates could be biased.[43] This bias arise because patients already exposed to the drug before the study's start do not have the same risk as those exposed at the study start, as the former have already survived the initial exposure. Consequently, if the treatment effect on the outcome varies over time, any early effect shortly after starting the drug would be missed, skewing the overall treatment effect (referred to as prevalent user bias).[44] To address that,

observational studies often involve an incident new-user cohort design for head-to-head comparisons between two medications, leaving out prevalent users.

Confounding is the distortion of the association between an exposure and a health outcome by an extraneous variable (or confounder) and can seriously distort the effect estimates.[45] It refers to systematic differences between a group exposed to an intervention and a selected comparator group. In pharmacoepidemiology, confounding often arises when the factors influencing physician treatment decisions and patient medication use also independently affect health outcomes.[36] From this process, several sources of bias can result. Accounting for confounding in pharmacoepidemiologic research poses significant challenges as exposure is determined by a complex interaction of factors (e.g., patient, clinician, and healthcare system) and RWD often lack detailed clinical information on many confounders.[11] Conversely, RCTs are less prone to confounding, as patients are randomly assigned to one of the investigation groups before intervention.[2]

In clinical practice, treatment choice is often linked to a patient's health status, disease severity, and prognosis, which can result in significant and challenging confounding by indication.[37] This type of confounding commonly occurs due to the principles of good medical practice, whereby physicians tend to prescribe medications and perform procedures on patients who are likely to benefit the most. For example, patients with a more severe illness are potentially subjected to more intensive treatment than less severe patients. Consequently, the more intensive approach may seemly yield unfavourable outcomes when assessing and comparing different treatments. This type of confounding is one of the most challenging to address in pharmacoepidemiology as it frequently stems from difficulties in accurately assessing medical indications, underlying disease severity, and prognosis.[46,47] Different techniques at different research stages can be used to deal with confounding and biases in pharmacoepidemiology (e.g., careful selection of study design, propensity score methods, matching and stratification, and multivariable regression adjustment).[38,40,43,47–50]

Besides the potential for safety signals with unknown mechanisms of action occurring due to potential confounding and biases, the emergence of safety signals with unknown mechanisms of action can also be due to off-target interactions. While most drugs are designed to bind specifically to a particular target protein to produce a therapeutic effect, they can additionally interact with other proteins in the body, also known as off-targets, leading to unintended effects.[41,51] Off-target interactions can occur due to similarity in the structure of the drug molecule to other proteins, structural similarities among targets, or due to the promiscuity of

the drug molecule (i.e., the ability to interact with multiple targets or biological pathways in the body).[41,52,53] It is known that individual drugs may act on multiple macromolecular targets and/or disease pathways – termed the polypharmacology of a drug.[41,54,55] For example, imatinib, a kinase inhibitors used in cancer therapy, was primarily designed to inhibit specific kinases during drug development. However, additional studies have shown that imatinib also interacts with other targets, leading to dysregulation of bone remodeling.[56] While previous studies have identified that the target profile of approved drugs is far more comprehensive than initially known, it is not feasible for researchers or clinicians to fully elucidate all possible targets and outcomes.[41,51,57]

In observational safety studies, researchers aim to identify potential associations between drugs and ADRs by carefully accounting for confounding factors and bias. In this context, if an ADR is observed even after sufficiently adjusting for confounding factors and bias, it is commonly interpreted as suggestive evidence of an association between the drug and the specific outcome of interest. However, it becomes challenging to rule out the possibility of unmeasured confounding when the underlying mechanism for the ADR is unknown. Thus, in order to overcome the challenges arising from the presence of confounding in pharmacoepidemiologic research, there is a need to carefully select the study design, analytical methods, and data source appropriately to reduce bias and safeguard the validity and accuracy of the results of observational studies. Additionally, there is a need to combine multidisciplinary approaches (e.g., identification of additional drug-target interactions using machine learning-based approaches and experimental testing) to explore unintended drug-target interactions and, ultimately, to improve knowledge on unexplained safety signals.

Not only can we combine observational pharmacoepidemiologic studies with computational and experimental methods to assess new unexplained safety signals, but there is a great opportunity for finding new therapeutic uses for existing drugs, also known as drug repurposing. For example, suppose computation and experimental models identify additional drug-target activity for a drug already approved. In that case, pharmacoepidemiology studies can be conducted to identify if the new benefit of a drug is already being observed in clinical practice (**Figure 1.1.5**, arrow I). Or vice versa, if new benefits of a drug are observed in clinical practice and confirmed in RWD studies, this information can feed back to earlier stages of drug development to better understand the target profile of a drug (**Figure 1.1.5**, arrow II).

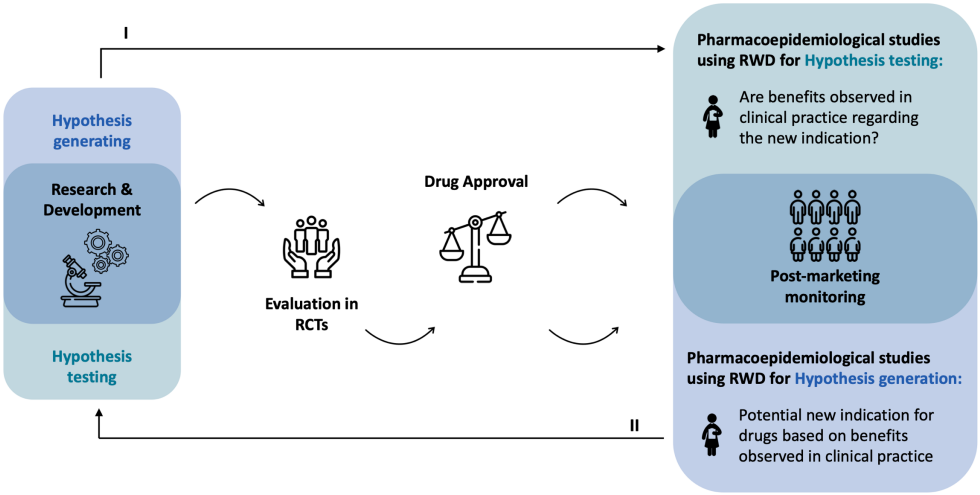


Figure 1.1.5 Combining pharmacoepidemiologic studies with computational and experimental methods may provide an opportunity for drug repurposing. If computation and experimental models identify additional drug-target activity for a specific drug that already has approval (hypothesis generation), pharmacoepidemiology studies can be conducted to identify if the new benefit of a drug is already being observed in clinical practice (hypothesis testing [arrow I]). If new benefits of a drug are observed in pharmacoepidemiologic RWD-based studies (hypothesis generation), this information can feed back to earlier stages of drug development to better understand the target profile of a drug, and, ultimately, to leading to a new indication (hypothesis testing [arrow II]). RCTs Randomized clinical trials; RWD Real-world data.

1.2 Case examples used in this dissertation

In this dissertation, we selected three case examples to examine the application of interdisciplinary approaches to overcoming the abovementioned limitations when assessing drug safety and utilisation using RWD-based pharmacoepidemiologic studies.

1.2.1 Polypharmacy in patients with type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM) is a complex chronic metabolic disorder characterized by increased blood glucose levels due to insulin resistance or impaired secretion.[58] Almost 90% of the total cases of diabetes worldwide, which is estimated to be around 537 million, are attributed to T2DM.[59] Pharmacological management of patients with T2DM is often complex due to the heterogeneity of the disease. This complexity is further increased with additional chronic conditions and a range of complications associated with T2DM, frequently leading to polypharmacy.[60] Furthermore, the lack of clinical guidelines with guidance on how to treat

patients with more than two additional conditions may further increase the problem of polypharmacy.

Although the correct combination of drugs in patients with T2DM can improve their health status and quality of life, polypharmacy increases the likelihood of potentially inappropriate prescriptions (PIPs), which can thereby increase the risk of experiencing ADRs, DDIs, or drug-disease interactions.[29,61,62] A critical aspect of understanding the intricacies of polypharmacy in patients with T2DM lies in identifying the patterns of medication use at a drug compound level, which is crucial to identify potential risks associated with specific drugs and drug combinations prescribed within these patient populations. Thus, to address the challenges of descriptive drug utilisation studies described above, there is a need to combine interdisciplinary approaches integrating pharmacoepidemiology and data mining techniques. Additionally, as patients with polypharmacy are more likely to have potentially inappropriate medication use, assessing the appropriateness of medication use in this population may help unveil areas for potential improvement in treatment strategies.

1.2.2 Investigation of safety concerns associated with Janus Kinase inhibitors in rheumatoid arthritis

Janus Kinase inhibitors (JAKis) are a novel class of targeted synthetic disease-modifying antirheumatic drugs (tsDMARD) and represent an important alternative to treat patients with moderate to high rheumatoid arthritis (RA) disease activity.[63] JAKis target the JAK family of kinases (JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase [TYK2]), disrupting intracellular activation of proinflammatory cytokine cascades in RA by interfering with adenosine 5'-triphosphate (ATP)-binding sites.[64] As a consequence, the production of multiple pro-inflammatory cytokines, such as interleukin (IL)-6, IL-10, and interferon (IFN)-γ is inhibited.[65]

Different JAKis with varying affinities for one or more JAK enzymes have been approved worldwide to treat RA, including tofacitinib, baricitinib, and updacitinib.[65–67] Nevertheless, safety concerns have emerged towards the increased risk of serious viral infections, venous thromboembolism (VTE), and major adverse cardiovascular events (MACE) associated with JAKis, leading to warnings and precautions on the drug labels regardless of their selectivity.[63,68–73] While safety assessment of JAKis in RCTs is limited by the study conditions (e.g., time restrictions, population inclusion and exclusion criteria), it may not represent a real-world clinical setting. Moreover, as the exact mechanisms to explain these safety concerns remain unclear, the increased risk of VTE, MACE, and serious viral infection in JAKis users is heavily

debated. Finally, there is a lack of RWE on the safety concerns associated with the JAKis use, and thus, safety concerns remain.

Observational studies comparing the effects of drugs typically utilize a new-user cohort design to capture potential early effects and address prevalent user bias. Additionally, an appropriate comparison group typically receiving an active comparator drug with a similar indication is defined.[11] Nevertheless, even if similar indications, comparisons of treatments given at different stages of the disease may introduce bias related to disease duration and progression (e.g., time-lag bias).[74,75] While finding a contemporaneous comparator with a similar indication is ideal to avoid bias in observational studies, this can be highly challenging.

JAKis are commonly prescribed after the prior failure of bDMARDs (e.g., tumour necrosis factor α inhibitors [TNF-$\alpha$is]).[76,77] In this scenario, a new user design would lead to the exclusion of the majority of JAKis users, resulting in a substantial reduction in the sample size and thus decreased generalizability of study results. Despite available RWE on the safety concerns associated with the JAKis use,[78–81] previous studies focused on treatment-naïve users. Thus, there is a need to (i) design cohort studies to address the limitation of comparing JAKis with an "older drug" from which many JAKis users may have switched without reducing the generalizability of study results, and (ii) have appropriate analytical methods to control for confounding, particularly due to inclusion of prevalent users, and providing balanced treatment groups at cohort entry necessary to estimate comparative effects with minimal bias.

Another main challenge in RWD-based observational studies to assess safety concerns associated with JAKis use arises from the fact that several risk factors associated with the outcomes are often not available, or only partially complete, due to RWD source limitations. To address this challenge, medical data linkage is extremely important to generate a more complete picture of a patient's healthcare trajectory, permit an assessment of causality, and minimize the risk of biases. Additionally, the lack of precise mechanisms to explain the observed safety concerns on MACE, VTE and viral infection, further complicates the safety assessment of JAKis. Given that kinase inhibitors have the potential to interact with multiple targets in the body, as described above, it is likely that the occurrence of those safety concerns could be attributed to off-target interactions, supporting the hypothesis of ADRs resulting from off-target effects.[82] Thus, interdisciplinary approaches providing a more comprehensive and holistic view of the safety profile of JAKis are needed to improve knowledge on whether the safety signals associated with JAKis use are a product of confounding in observational studies or if it is due to off-target effects.

1.2.3 Investigation of Janus Kinase inhibitors off-targets for potential drug repurposing in Alzheimer's disease

Alzheimer's disease (AD) is the primary cause of cognitive decline in older adults, and it poses a significant challenge to healthcare systems worldwide.[83] The hallmark features of AD are the accumulation of amyloid-β peptide (Aβ)-enriched neuritic plates and the formation of neurofibrillary tangles composed of microtubule-associated protein tau.[84] Additionally, the importance of inflammatory processes, mitochondrial function, and the protection and regeneration of neurons in the pathophysiology of AD has become increasingly apparent.[85] Despite efforts, the underlying mechanisms of AD and optimal treatment targets have not been fully elucidated.

Without effective prevention and treatment options, AD affects countless individuals globally, with projections indicating a significant surge due to the growing life expectancy rates.[86] This trend has significant implications for public health and economic welfare, as the long-term care of affected individuals places a substantial burden on healthcare systems and caregivers. As such, there is a pressing need for new approaches to preventing and treating AD that can address the underlying disease mechanisms and improve patient outcomes.

Drug repurposing approaches could be especially valuable in the case of AD, where multiple distinct disease drivers exist and the underlying mechanisms are poorly understood.[83] One strategy to identify potential drug candidates to repurpose in AD involves generating hypotheses by identifying approved drugs used to treat other conditions that may target genetic or environmental regulators linked to the initial molecular factors of AD pathology.[4] This approach focuses on pathways that can initiate or amplify early AD neuropathology, hastening the manifestation of clinical symptoms associated with the disease.[87]

Previous studies suggested that inhibition of the JAK-STAT and IFN signalling pathways by JAKis might be beneficial in the context of AD as they are likely to modulate immune-driven neuroinflammation.[88,89] Nevertheless, the potential influence of off-target effects in AD progression remains unclear. Thus, the use of an interdisciplinary approach combining computational and experimental models to identify additional drug-target activity may improve the current knowledge of the JAKis off-target profile, opening a door for potential repurposing.

Chapter 2

Thesis Goal

This dissertation aims to address unanswered questions in the study of the utilisation and safety of drugs used to treat chronic conditions. In particular, this dissertation focuses on leveraging methodologies from other disciplines, such as data science and medicinal chemistry, to advance our understanding of drug utilisation and drug safety.

Chapter 3 delves into two critical issues surrounding drug utilisation in patients with type 2 diabetes mellitus (T2DM) receiving multiple concomitant medications, also known as polypharmacy. Firstly, we address the computational challenge of assessing real-world polypharmacy patterns at a drug compound level. To accomplish this, we applied the Apriori algorithm, a data mining technique which allows us to assess an extremely high number of drug combinations surpassing the existing knowledge of polypharmacy based solely on drug classes.[32] This approach is key as drug-drug interactions (DDIs) are not restricted only to drug classes, but they can occur at the level of individual drug compounds. Thus, given the increasing prevalence of T2DM and the complexity of prescription patterns in patients receiving polypharmacy, it is essential to accurately identify and quantify polypharmacy patterns at the drug compound level.

Additionally, in **Chapter 3**, we explore opportunities for optimising pharmacotherapy in patients with T2DM by estimating the prevalence of potentially inappropriate prescriptions (PIPs) using explicit process measures of the appropriateness of prescribing derived from real-world data (RWD).[90] As antidiabetic therapy carries its challenges and risks, it becomes paramount to minimise the risks associated with the use of additional medications. Furthermore, given the challenges of avoiding polypharmacy in patients with T2DM, identifying potential opportunities for optimise medication use becomes crucial to implement measures to review indications, address DDI, and consider comorbidities, reducing the likelihood of inappropriate prescribing in patients with T2DM.

In **Chapter 4**, we present a novel multidisciplinary approach for investigating safety concerns associated with the use of Janus Kinase inhibitors (JAKis) in a real-world clinical setting, particularly with regard to adverse events with unknown mechanisms of action. Firstly, we determine the drug-target binding profile of JAKis to identify potential drug-target interactions that may explain a thrombotic profile or the ability of JAKis to modulate viral assembly and viral endocytosis.[91] This analysis combines computational and experimental methods. Subsequently, we propose a prevalent new-user cohort study to investigate the incidence and risk of major adverse cardiovascular events (MACE), venous thromboembolism (VTE) and viral infections associated with JAKis use at a population level.[92] This study design aims to overcome challenges posed by confounding factors in drug safety studies using RWD.

In **Chapter 5**, our investigation extends to previously unknown drug-target interactions of JAKis that may be a factor in the use of these drugs in the context of Alzheimer's disease (AD). This investigation combines computational and experimental approaches to shed light on the off-target effects of the JAKis in conditions other than its primary indication.[93]

Throughout this dissertation, we strive to address research gaps, incorporate interdisciplinary methodologies, and employ RWD to enhance our understanding of drug utilisation and safety in treating chronic conditions.

Chapter 3

Descriptive Drug Utilisation Analyses

Chapter 3.1

Identification of polypharmacy patterns in new-users of metformin using the Apriori algorithm: a novel framework for investigating concomitant drug utilisation through association rule mining

Authors

Maria L. Faquetti, Adrian Martinez-De la Torre, Theresa Burkard, Guillaume Obozinski, Andrea M. Burden

Remarks

Maria L. Faquetti contributed to the study protocol preparation, statistical analysis, data interpretation, manuscript preparation, and critical revisions.

Publication

Faquetti ML *et al.*, Identification of polypharmacy patterns in new-users of metformin using the Apriori algorithm: A novel framework for investigating concomitant drug utilisation through association rule mining. *Pharmacoepidemiology & Drug Safety.*32(3):366-381 (2023). https://doi.org/10.1002/pds.5583

3.1.1 Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by high blood glucose levels, resulting from the body's inefficient use or production of insulin.[94–96] Management of individuals with T2DM is often complex due to the heterogeneity of the disease, and the coexistence of additional chronic conditions and complications of T2DM, which may result in complex prescription patterns.[60,97] Furthermore, to treat concomitant conditions, multiple drugs may be used at the same time, a problem that is also referred to as polypharmacy.

Polypharmacy is commonly defined as the simultaneous use of five or more drugs, and is common among patients with T2DM.[98] Polypharmacy is driven by the increasing prevalence of comorbidities (40% of adults with diabetes have at least three additional chronic conditions) and aging.[99,100] Moreover, it can be further increased by current T2DM treatment guidelines, which rarely offer guidance on how to treat patients with more than two additional chronic conditions.[101–103]

Medication errors, poor treatment adherence, and adverse drug reactions (ADRs) are consequences of polypharmacy.[104] Importantly, polypharmacy increases the risk of several unintended adverse health outcomes due to harmful drug-drug interactions (DDIs).[105–108] The prevalence of potential DDIs in primary care is estimated to be higher than 60%,[109] with up to 12% being clinically significant[109,110] with elevated morbidity and mortality and high healthcare costs.[111,112]

Despite increasing awareness of polypharmacy, specific drug combinations in diabetic patients receiving polypharmacy remain poorly characterized with the focus on the number of drugs rather than the individual drug combinations.[113,114] For example, a recent study revealed that 77.9% of patients with T2DM had polypharmacy, with an average of 7.7 drugs per patient.[115] Similarly, it was previously found that 60% of patients with T2DM aged ≥75 years receive ≥5 drugs.[113] Only a handful of studies have identified medication patterns at the drug compound level, but these studies only included a limited number of drug compounds in the analyses.[116–123]

Understanding polypharmacy patterns at a drug compound level is important as the occurrence of DDIs is not a problem related only to classes of drugs, but to single drug compounds. Therefore, this study aimed to use a novel approach in order to identify

prescription patterns at the compound level among patients with T2DM receiving their first non-insulin antidiabetic drug (NIAD).

3.1.2 Materials and methods

3.1.2.1 Data source

We conducted a descriptive cohort study using the IQVIA Medical Research Data (IMRD; incorporating data from THIN, A Cegedim Database of anonymized electronic health records in the UK), between 2016 and 2019. IMRD is an extensive database containing longitudinal non-identified primary care medical records from over 18 million patients, of which approximately 2.9 million are currently active. IMRD has been shown to be generally representative of the UK and valid in terms of age and sex comparisons and a wide range of diseases.[12] The database comprises a range of information, including demographics (e.g., year of birth and sex), diagnosis, lab tests (e.g., estimated glomerular filtration rate [eGFR] and hemoglobin A1c [HbA1c]), drug prescriptions recorded by general practitioners (GPs), and other information (e.g., smoking status, pregnancy, and death). Medications were recorded in the database using the British National Formulary (BNF) classification, and then were mapped according to the international anatomical therapeutic codes (ATC) classification system. All diagnoses were recorded using Read codes.[124]

3.1.2.2 Study population

Patients who received a first-ever metformin prescription and aged ≥18 years between January 2016 and December 2019 were included. Cohort entry (index date) was defined as the date of the first-ever metformin prescription. Patients with a previous NIAD treatment were excluded. Patients were allowed to have additional co-prescribed NIADs at the index date. All patients were required to have a minimum period of 1-year of database history and at least one GP visit prior to the index date. We only consider patients registered with a practice with a valid up-to-standard date and who had an acceptable patient flag. Patients diagnosed with gestational diabetes, polycystic ovary syndrome, or insulin therapy previous or at index date were excluded.

3.1.2.3 Polypharmacy definition

We identified patients with polypharmacy at the start of metformin therapy, which was defined as the use of ≥5 different prescribed drug compounds (i.e., metformin + ≥4 additional unique ATC codes on the 5th level) on, or within 90-days before the index date. The average prescription length of drugs to treat chronic illnesses in the UK is three months, and therefore, we assumed that drugs prescribed in a 90-days period are deemed to be co-prescribed. Although treatments for acute illnesses (e.g., antibiotics and pain-killers) usually have shorter duration, we used sensitivity analyses with shorter prescription time frames to explore polypharmacy patterns with different exposure window lengths. Thus, polypharmacy was further defined as the prescription of ≥5 different drug compounds at: (i) the index date or within 30-days previous to index date; and (ii) index date or within 14-days around index date (i.e., 7 days □ index date). We only considered drugs and diagnosis (i.e., history of comorbidities) that had a valid flag in the database. Additional details on the selection of drugs for the polypharmacy analysis are available in the Appendix.

3.1.2.4 Statistical analysis

We describe patient characteristics at index date as a mean and standard deviation (SD) or median and interquartile range (IQR) or counts and proportions, as appropriate. Alcohol use and smoking were identified based on the most recent value recorded in the database (i.e., at or at any time before index date). Body mass index (BMI) was calculated using the value closest to the index date for the weight (i.e., at or before index date), nonetheless, as the height of an individual is not likely to vary significantly during adulthood, the closest value to the index date was considered regardless if recorded before, at, or after the index date. The mean HbA1c and eGFR were summarized using the most recently recorded values up to previous six months prior to the index date, and a history of comorbidities was assessed if ever registered in the database previous to index date. All characteristics are reported among those with and without polypharmacy, and stratified by sex.

To determine co-prescribed drugs, we performed association rule and frequent-set analysis using the Apriori algorithm.[125] This methodology represents a novel approach to identify prescription patterns in large databases when it is not computationally feasible to assess extremely high number of drug combinations (e.g., patients with up to 40 drugs, resulting in $1.1x10^{12}$ possible combinations). Additional details on the polypharmacy analysis using the Apriori and its metrics are included in the Appendix and **Table 8.1.1**, respectively. In this study,

the frequency and proportion of co-prescribed drugs in patients receiving polypharmacy were ranked by frequency of prescribing stratified by sex.

After identifying and quantifying the different sets of drugs, we ranked the combinations of metformin with one to up to six additional drugs based on the frequency of prescription. In this way, we captured unique prescription patterns prescribed in patients with T2DM. Moreover, quantifying the 'at least' particular combinations of drugs is important for knowing all patients exposed to any given combination of drugs.

Within each stratum (i.e., sex), we assessed the top five most frequently occurring drug combinations in patients receiving polypharmacy. If >1 drug combination shared the same position in the rank, we presented all combinations with the same frequency. In the secondary analysis, we repeated the analysis stratified by age groups. Numbers fewer than seven are suppressed according to data use agreement with IMRD. All analyses were performed using the R version (4.1.2).

3.1.3 Results

3.1.3.1 Patients' characteristics at metformin first-ever prescription

We identified 34,169 metformin initiators between 2016 and 2019 (**Figure 3.1.1**). The demographic characteristics at index are provided in **Table 3.1.1**, stratified by sex and polypharmacy status. Overall, 20,854 (61.0%) received ≥4 additional drugs in addition to metformin at index date or within 90-days before index date, and were categorized as having polypharmacy. The mean age of individuals with polypharmacy was 63.1, while for individuals with no polypharmacy was 53.7. Patients with polypharmacy had a higher prevalence of comorbidities than those without. When stratified by sex, there was a higher proportion of women receiving polypharmacy (65.6%) compared to men (57.4%), and women were more frequently receiving ≥20 drugs concomitantly (women=2.0%; men=0.9%). Moreover, women with polypharmacy were more often obese and had a lower eGFR, while men had higher levels of HbA1c and received more frequently combined antidiabetic pharmacotherapy (i.e., additional NIAD at metformin first prescription).

The demographics characteristics for the secondary analysis stratified by age groups is provided in **Table 8.1.2**. The proportion of patients receiving polypharmacy increased with age (18 – 39 years=30.4%, 40 – 59 years=50.5%, 60 – 74 years=70.9%, and ≥75 years=84.3%). The most prevalent concomitant condition among patients with polypharmacy aged ≥40 years was

hypertension (40 – 59 years=43.9%, 60 – 74 years=65.3%, and ≥75 years=71.8%), while depression was most the prevalent concomitant condition in 18- to 39-year-olds (38.3%) with polypharmacy. Additionally, patients younger than 60 years had more often a diagnosis for asthma, depression, and were more likely to be obese. On the other hand, patients aged ≥60 years had more often a diagnosis of cardiovascular diseases, osteoarthritis, hypothyroidism, and cancer.

The sensitivity analyses using shorter exposure windows to identify concomitant drugs are available in **Table 8.1.3** and **Table 8.1.4** for exposure window at index date and within 30-days previous to index date, and **Table 8.1.5** and **Table 8.1.6** for 14-days exposure window around the index date. Overall, the proportion of patients receiving polypharmacy declined from 61.0% in the primary analysis to 43.8% with a 30-days window and 20.9% with a 14-days window. Similarly, the mean number of drugs prescribed declined from 9.0 in the primary analysis to 8.1 and 7.7 with a 30- and 14-days window, respectively.

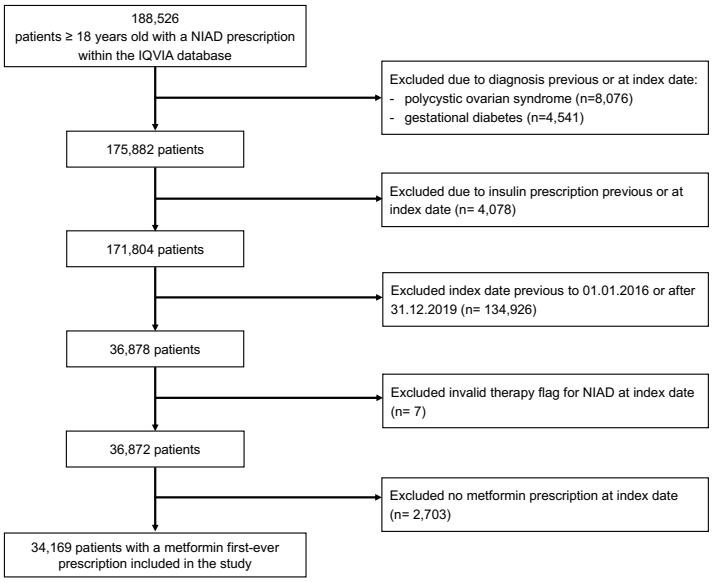


Figure 3.1.1 Flow diagram of patient selection. NIAD=non-insulin antidiabetic drug.

Table 3.1.1 Baseline characteristics of metformin new-users, stratified by sex.

	Overall study patients (N=34,169)		Women (N=14,964)		Men (N=19,205)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	13,315 (39.0%)	20,854 (61.0%)	5,141 (34.4%)	9,823 (65.6%)	8,174 (42.6%)	11,031 (57.4%)
Mean age (SD)	53.7 (13.3)	63.1 (12.7)	52.1 (15.2)	62.8 (13.6)	54.7 (11.9)	63.4 (12.0)
Median number of drugs (IQR)	3 (2 - 4)	8 (6 - 11)	3 (2 - 4)	8 (6 - 11)	3 (2 - 4)	8 (6 - 10)
Mean number of drugs (SD)	2.6 (1.1)	9.0 (3.9)	2.7 (1.1)	9.4 (4.2)	2.6 (1.1)	8.6 (3.6)
Distribution of drugs n (%)						
1 (only metformin)	2,532 (7.4%)	-	953 (6.4%)	-	1,579 (8.2%)	-
2 - 4	10,783 (31.6%)	-	4,188 (28.0%)	-	6,595 (34.3%)	-
5 - 9	-	13,635 (39.9%)	-	6,002 (40.1%)	-	7,633 (39.7%)
10 - 19	-	6,742 (19.7%)	-	3,517 (23.5%)	-	3,225 (16.8%)
≥20	-	477 (1.4%)	-	304 (2.0%)	-	173 (0.9%)
Max nº of drugs	-	40	-	40	-	38
Number of NIADs prescribed at index date n (%)						
≥2	410 (3.1%)	696 (3.3%)	99 (1.9%)	284 (2.9%)	311 (3.8%)	412 (3.7%)
Smoking status[1], n (%)						
Current	2,282 (17.1%)	3,757 (18.0%)	792 (15.4%)	1,820 (18.5%)	1,490 (18.2%)	1,937 (17.6%)
Never	7,348 (55.2%)	9,175 (44.0%)	3,206(62.4%)	4,938 (50.3%)	4,142 (50.7%)	4,237 (38.4%)
Former	3,576 (26.9%)	7,832 (37.6%)	1,092 (21.2%)	3,017 (30.7%)	2,484 (30.4%)	4,815 (43.6%)
Unknown / Missing value	109 (0.8%)	90 (0.4%)	51 (1.0%)	48 (0.5%)	58 (0.7%)	42 (0.4%)
Alcohol use[1], n (%)						
Current	9,147 (68.7%)	13,463 (64.5%)	3,086 (60.0%)	5,637 (57.4%)	6,061 (74.1%)	7,826 (71.0%)
Never (Lifelong teetotaler)	2,616 (19.6%)	5,155 (24.7%)	1,381 (26.9%)	3,168 (32.3%)	1,235 (15.1%)	1,987 (18.0%)
Former	409 (3.1%)	1,303 (6.2%)	154 (3.0%)	542 (5.5%)	255 (3.1%)	761 (6.9%)
Unknown / Missing value	1,143 (8.6%)	933 (4.5%)	520 (10.1%)	476 (4.8%)	623 (7.6%)	457 (4.1%)
BMI[2], n (%)						
Obese (BMI ≥30.0 kg/m^2)	7,969 (59.8%)	13,884 (66.6%)	3,169 (61.6%)	6,849 (69.7%)	4,800 (58.7%)	7,035 (63.8%)
Overweight (BMI 25.0 – 29.9 kg/m^2)	3,627 (27.2%)	5,036 (24.1%)	1,182 (23.0%)	2,034 (20.7%)	2,445 (29.9%)	3,002 (27.2%)
Normal/ Underweight (BMI <25.0 kg/m^2)	1,167 (8.8%)	1,456 (7.0%)	576 (11.2%)	715 (7.3%)	591 (7.2%)	741 (6.7%)
Missing value	552 (4.2%)	478 (2.3%)	214 (4.2%)	225 (2.3%)	338 (4.1%)	253 (2.3%)
History of disease, n (%)						
Hypertension	3,674 (27.6%)	12,282 (58.9%)	1,273 (24.7%)	5,653 (57.6%)	2,401 (29.4%)	6,629 (60.1%)
Coronary heart disease	174 (1.3%)	3,936 (18.9%)	32 (0.6%)	1,138 (11.6%)	142 (1.7%)	2,798 (25.4%)
Heart failure	17 (0.2%)	1,007 (4.8%)	<7	325 (3.3%)	18 (0.2%)	682 (6.2%)
Cerebrovascular disease	208 (1.6%)	1,822 (8.7%)	51 (1.0%)	743 (7.6%)	157 (1.9%)	1,079 (9.8%)
Chronic kidney disease	25 (0.2%)	244 (1.2%)	14 (0.3%)	111 (1.1%)	11 (0.1%)	133 (1.2%)
Chronic liver disease	294 (2.2%)	883 (4.2%)	102 (2.0%)	455 (4.6%)	192 (2.4%)	428 (3.9%)

Table 3.1.1 (cont.) Baseline characteristics of metformin new-users, stratified by sex.

	Overall study patients (N=34,169)		Women (N=14,964)		Men (N=19,205)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
History of disease, n (%)						
COPD	140 (1.1%)	2,151 (10.3%)	49 (1.0%)	972 (9.9%)	91 (1.1%)	1,179 (10.7%)
Asthma	1,214 (9.1%)	4,088 (19.6%)	538 (10.5%)	2,367 (24.1%)	676 (8.3%)	1,721 (15.6%)
Diabetic retinopathy	292 (2.2%)	736 (3.5%)	95 (1.9%)	328 (3.3%)	197 (2.4%)	408 (3.7%)
Sleep disorders	629 (4.7%)	1,959 (9.4%)	196 (3.8%)	870 (8.9%)	433 (5.3%)	1,089 (9.9%)
Depression	2088 (15.7%)	6,024 (28.9%)	1,103 (21.5%)	3,603 (36.7%)	985 (12.1%)	2,421 (22.0%)
Alzheimer/Dementia	23 (0.2%)	344 (1.7%)	12 (0.2%)	195 (2.0%)	11 (0.1%)	149 (1.4%)
Hypothyroidism	564 (4.2%)	2,449 (11.7%)	387 (7.5%)	1,856 (18.9%)	177 (2.2%)	593 (5.4%)
Osteoarthritis	1,258 (9.5%)	5,365 (25.7%)	561 (10.9%)	2,967 (30.2%)	697 (8.5%)	2,398 (21.7%)
Osteoporosis	71 (0.5%)	702 (3.4%)	56 (1.1%)	559 (5.7%)	15 (0.2%)	143 (1.3%)
Cancer	545 (4.1%)	1,985 (9.5%)	275 (5.4%)	1,014 (10.3%)	270 (3.3%)	971 (8.8%)
Lab values						
Most recent HbA1c measurement six months prior to index date n (%)[3]						
<6.5% (48 mmol/mol)	545 (4.1%)	754 (3.6%)	320 (6.2%)	439 (4.5%)	225 (2.8%)	315 (2.9%)
6.5 – 7.4% (48 – 57 mmol/mol)	2,956 (22.2%)	6,410 (30.7%)	1,263 (24.6%)	3,137 (31.9%)	1,963 (20.7%)	3,273 (29.7%)
7.5 – 8.5% (58 – 69 mmol/mol)	2,486 (18.7%)	5,142 (24.7%)	878 (17.1%)	2,383 (24.3%)	1,608 (19.7%)	2,759 (25.0%)
>8.5% (69 mmol/mol)	5,418 (40.7%)	6,334 (30.4%)	1,559(30.3%)	2,722 (27.7%)	3,859 (47.2%)	3,612 (32.7%)
Most recent eGFR measurement (mL/min/1.73 m^2) six months prior to index date n (%)[3]						
<30	<7	19 (0.1%)	<7	12 (0.1%)	<7	7 (0.1%)
30 – 59	487 (3.7%)	2,400 (11.5%)	227 (4.4%)	1,314 (13.4%)	260 (3.2%)	1,086 (9.8%)
≥60	8,652 (65.0%)	12,874 (61.7%)	3,009 (58.5%)	5,812 (59.2%)	5,643 (69.0%)	7,062 (64.0%)
Missing value	4,173 (31.3%)	561 (26.7%)	1,904 (37.0%)	2685 (27.3%)	2,269 (27.8%)	2,876 (26.1%)

Acronyms: NIAD: non-insulin antidiabetic drug; SD: standard deviation; IQR: interquartile range; BMI: body mass index; COPD: chronic obstructive pulmonary disease; HbA1c: hemoglobin A1c; eGFR: Estimated glomerular filtration rate.

[1] Identified values closest to the index date (i.e., at or before index date).

[2] BMI was assessed using the most recently recorded value for weight (at or at any time before index date) and height (at or at any time before or after index date).

[3] The most recent value (at index date and previous six-months) registered in the database with a valid unit was considered.

Note: A history of comorbidities was assessed if ever registered in the database previous to or at index date. Numbers fewer than seven are suppressed according to data use agreement with IQVIA Medical Research Data (IMRD).Drug prescription patterns in patients receiving polypharmacy.

3.1.3.2 Drug prescription patterns in patients receiving polypharmacy

Table 3.1.2 shows the proportions of patients prescribed each of the top five most co-prescribed drugs and combinations of up to six drugs additional to metformin overall patients with polypharmacy and stratified by sex. Atorvastatin was the most frequently co-prescribed drug with metformin overall (38.7%), in women (34.3%) and men (42.6%), with a higher proportion of men receiving the medication. Overall, frequently prescribed drugs and drug combinations mainly treat manifestations of metabolic syndrome.

We repeated the analysis of drug prescription patterns stratifying by age groups (**Table 3.1.3**). Atorvastatin was the most co-prescribed drug in addition to metformin in adults ≥40 years old with polypharmacy (40-59 years [37.6%]; 60-74 years [42.6%]; ≥75 years [37.2%]). A second statin (simvastatin) was among the most prescribed drugs in patients aged ≥60 years (60-74 years [26.5%]; ≥75 years [28.9%]). On the other hand, salbutamol was often prescribed in 40- to 59-year-olds (20.6%), and sertraline (13.5%) among adults <40 years.

In the sensitivity analyses, similar results were obtained for overall patients with polypharmacy and stratified by sex and age using a 30-days window (**Table 8.1.7** and **Table 8.1.8**, respectively) and 14-days window (**Table 8.1.9** and **Table 8.1.10**, respectively), as compared to our primary and secondary analyses.

Table 3.1.2 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy at index date and within 90-days before index date, and stratified by sex.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Overall (20,854)	1	1	8,064 (38.7%)	atorvastatin
		2	5,798 (27.8%)	omeprazole
		3	4,808 (23.1%)	amlodipine
		4	4,713 (22.6%)	simvastatin
		5	4,650 (22.3%)	ramipril
	2	1	2,437 (11.7%)	aspirin, atorvastatin
		2	2,388 (11.5%)	amlodipine, atorvastatin
		3	2,263 (10.9%)	ramipril, atorvastatin
		4	2,185 (10.5%)	omeprazole, atorvastatin
		5	2,139 (10.3%)	bisoprolol, atorvastatin
	3	1	1,220 (5.9%)	aspirin, bisoprolol, atorvastatin
		2	924 (4.4%)	aspirin, ramipril, atorvastatin
		3	857 (4.1%)	bisoprolol, ramipril, atorvastatin
		4	845 (4.1%)	aspirin, bisoprolol, ramipril
		5	708 (3.4%)	amlodipine, ramipril, atorvastatin
	4	1	577 (2.8%)	aspirin, bisoprolol, atorvastatin, ramipril
		2	334 (1.6%)	omeprazole, aspirin, bisoprolol, atorvastatin
		3	325 (1.6%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		4	300 (1.4%)	aspirin, glyceryl trinitrate, bisoprolol, atorvastatin
		5	260 (1.2%)	aspirin, amlodipine, bisoprolol, atorvastatin
	5	1	159 (0.8%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin
		2	152 (0.7%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	144 (0.7%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	95 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, amlodipine
		5	89 (0.4%)	aspirin, isosorbide mononitrate, bisoprolol, atorvastatin, ramipril
	6	1	41 (0.2%)	lansoprazole, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril
		2	39 (0.2%)	ticagrelor, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril
		3	38 (0.2%)	aspirin, omeprazole, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril
		4	37 (0.2%)	lansoprazole, aspirin, clopidogrel, bisoprolol, atorvastatin, ramipril
		5	34 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, atorvastatin, ramipril
Women (9,823)	1	1	3,365 (34.3%)	atorvastatin
		2	2,955 (30.1%)	omeprazole
		3	2,176 (22.2%)	salbutamol
		4	2,014 (20.5%)	co-codamol

Table 3.1.2 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy at index date and within 90-days before index date, and stratified by sex.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Women (9,823)	1	5	2,009 (20.5%)	simvastatin
	2	1	992 (10.1%)	omeprazole, atorvastatin
		2	881 (9.0%)	amlodipine, atorvastatin
		3	751 (7.6%)	ramipril, atorvastatin
		4	750 (7.6%)	aspirin, atorvastatin
		5	723 (7.4%)	salbutamol, omeprazole
	3	1	314 (3.2%)	prednisolone, salbutamol, amoxicillin
		2	313 (3.2%)	aspirin, bisoprolol, atorvastatin
		3	253 (2.6%)	prednisolone, salbutamol, omeprazole
		4	251 (2.6%)	aspirin, omeprazole, atorvastatin
		5	236 (2.4%)	aspirin, ramipril, atorvastatin
	4	1	124 (1.3%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	115 (1.2%)	prednisolone, salbutamol, amoxicillin, omeprazole
		3	104 (1.1%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	96 (1.0%)	prednisolone, salbutamol, amoxicillin, atorvastatin
		5	84 (0.9%)	prednisolone, salbutamol, doxycycline, omeprazole
	5	1	41 (0.4%)	omeprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	36 (0.4%)	prednisolone, salbutamol, amoxicillin, co-codamol, omeprazole
		3	34 (0.3%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		4	32 (0.3%)	prednisolone, salbutamol, amoxicillin, omeprazole, paracetamol
		5	31 (0.3%)	prednisolone, salbutamol, amoxicillin, tiotropium bromide, omeprazole
	6	1	14 (0.1%)	prednisolone, doxycycline, salbutamol, amoxicillin, montelukast, atorvastatin
		2	13 (0.1%)	furosemide, prednisolone, doxycycline, salbutamol, paracetamol, tiotropium bromide
		2	13 (0.1%)	prednisolone, salbutamol, atorvastatin, lansoprazole, amoxicillin, tiotropium bromide
		3	12 (0.1%)	prednisolone, amoxicillin, salbutamol, paracetamol, tiotropium bromide, omeprazole
		3	12 (0.1%)	prednisolone, amoxicillin, salbutamol, tiotropium bromide, omeprazole, carbocisteine
		3	12 (0.1%)	omeprazole, aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		4	11 (0.1%)	prednisolone, doxycycline, salbutamol, tiotropium bromide, carbocisteine, atorvastatin
		4	11 (0.1%)	prednisolone, amoxicillin, salbutamol, clarithromycin, co-codamol, omeprazole
		4	11 (0.1%)	prednisolone, amoxicillin, salbutamol, atorvastatin, formoterol and budesonide, tiotropium bromide
		4	11 (0.1%)	prednisolone, vitamins with minerals, alendronic acid, salbutamol, tiotropium bromide, omeprazole
		4	11 (0.1%)	prednisolone, doxycycline, salbutamol, atorvastatin, salmeterol and fluticasone, tiotropium bromide
		4	11 (0.1%)	furosemide, prednisolone, amoxicillin, salbutamol, omeprazole, tiotropium bromide

Table 3.1.2 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy at index date and within 90-days before index date, and stratified by sex.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Women (9,823)	6	4	11 (0.1%)	prednisolone, amoxicillin, co-codamol, salbutamol, atorvastatin, tiotropium bromide
		4	11 (0.1%)	omeprazole, prednisolone, paracetamol, salbutamol, tiotropium bromide, atorvastatin
		4	11 (0.1%)	furosemide, prednisolone, amoxicillin, paracetamol, salbutamol, omeprazole
		4	11 (0.1%)	prednisolone, amoxicillin, co-codamol, salbutamol, omeprazole, amitriptyline
		4	11 (0.1%)	lansoprazole, prednisolone, amoxicillin, co-codamol, salbutamol, atorvastatin
		5	10 (0.1%)	furosemide, prednisolone, doxycycline, tiotropium bromide, salbutamol, carbocisteine
		5	10 (0.1%)	prednisolone, paracetamol, salbutamol, tiotropium bromide, carbocisteine, omeprazole
		5	10 (0.1%)	prednisolone, paracetamol, salbutamol, tiotropium bromide, carbocisteine, atorvastatin
		5	10 (0.1%)	omeprazole, prednisolone, salbutamol, tiotropium bromide, carbocisteine, atorvastatin
		5	10 (0.1%)	amlodipine, prednisolone, doxycycline, salbutamol, atorvastatin, montelukast
		5	10 (0.1%)	lansoprazole, prednisolone, amoxicillin, salbutamol, atorvastatin, montelukast
		5	10 (0.1%)	lansoprazole, prednisolone, amoxicillin, salbutamol, atorvastatin, formoterol and budesonide
		5	10 (0.1%)	prednisolone, doxycycline, salbutamol, atorvastatin, formoterol and beclometasone, tiotropium bromide
		5	10 (0.1%)	prednisolone, amoxicillin, sabutamol, tiotropium bromide, omeprazole, formoterol and beclometasone
		5	10 (0.1%)	prednisolone, omeprazole, sabutamol, tiotropium bromide, formoterol and beclometasone, atorvastatin
		5	10 (0.1%)	prednisolone, alendronic acid, vitamins with minerals, paracetamol, salbutamol, omeprazole
		5	10 (0.1%)	lansoprazole, prednisolone, doxycycline, salbutamol, tiotropium bromide, atorvastatin
		5	10 (0.1%)	prednisolone, levothyroxine sodium, amoxicillin, salbutamol, tiotropium bromide, omeprazole
		5	10 (0.1%)	lansoprazole, prednisolone, doxycycline, salbutamol, tiotropium bromide, atorvastatin
		5	10 (0.1%)	predisolone, amoxicillin, paracetamol, salbutamol, tiotropium bromide, atorvastatin
		5	10 (0.1%)	omeprazole, prednisolone, amoxicillin, co-codamol, tiotropium bromide, salbutamol
		5	10 (0.1%)	furosemide, bisoprolol, prednisolone, paracetamol, tiotropium bromide, salbutamol
		5	10 (0.1%)	furosemide, omeprazole, prednisolone, paracetamol, tiotropium bromide, salbutamol
		5	10 (0.1%)	prednisolone, paracetamol, tiotropium bromide, salbutamol, atorvastatin, amitriptyline
		5	10 (0.1%)	prednisolone, omeprazole, tiotropium bromide, salbutamol, atorvastatin, amitriptyline
		5	10 (0.1%)	aspirin, bisoprolol, ramipril, atorvastatin, co-codamol, omeprazole
Men (11,031)	1	1	4,699 (42.6%)	atorvastatin
		2	3,053 (27.7%)	aspirin
		3	2,940 (26.7%)	amlodipine
		4	2,929 (26.6%)	ramipril
		5	2,843 (25.8%)	omeprazole
	2	1	1,687 (15.3%)	aspirin, atorvastatin
		2	1,512 (13.7%)	ramipril, atorvastatin
		3	1,507 (13.7%)	amlodipine, atorvastatin

Table 3.1.2 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy at index date and within 90-days before index date, and stratified by sex.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Men (11,031)	2	4	1,450 (13.1%)	bisoprolol, atorvastatin
		5	1,398 (12.7%)	bisoprolol, aspirin
	3	1	907 (8.2%)	aspirin, bisoprolol, atorvastatin
		2	688 (6.2%)	aspirin, ramipril, atorvastatin
		3	661 (6.0%)	aspirin, bisoprolol, ramipril
		4	648 (5.9%)	atorvastatin, bisoprolol, ramipril
		5	491 (4.5%)	amlodipine, ramipril, atorvastatin
	4	1	453 (4.1%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	248 (2.2%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	230 (2.1%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	222 (2.0%)	aspirin, bisoprolol, atorvastatin, glyceryl trinitrate
		5	198 (1.8%)	aspirin, bisoprolol, atorvastatin, amlodipine
	5	1	131 (1.2%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	118 (1.1%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	103 (0.9%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	75 (0.7%)	aspirin, amlodipine, bisoprolol, atorvastatin, ramipril
		5	72 (0.7%)	aspirin, isosorbide mononitrate, bisoprolol, atorvastatin, ramipril
	6	1	37 (0.3%)	lansoprazole, aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		2	31 (0.3%)	lansoprazole, aspirin, clopidrogrel, bisoprolol, ramipril, atorvastatin
		3	30 (0.3%)	glyceryl trinitrate, aspirin, ticagrelor, bisoprolol, atorvastatin, ramipril
		4	27 (0.2%)	isosorbide mononitrate, aspirin, lansoprazole, bisoprolol, atorvastatin, ramipril
		4	27 (0.2%)	clopidogrel, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril
		5	26 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin
		5	26 (0.2%)	omeprazole, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril

Table 3.1.3 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing stratified by age in new metformin users receiving polypharmacy at index date and within 90-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
18 - 39 years (862)	1	1	213 (24.7%)	omeprazole
		2	210 (24.4%)	salbutamol
		3	153 (17.7%)	co-codamol
		4	123 (14.3%)	amoxicillin
		5	116 (13.5%)	sertraline
	2	1	59 (6.8%)	amoxicillin, salbutamol
		1	59 (6.8%)	beclomethasone, salbutamol
		2	48 (5.6%)	co-codamol, omeprazole
		3	47 (5.5%)	prednisolone, salbutamol
		4	44 (5.1%)	omeprazole, salbutamol
		5	42 (4.9%)	omeprazole, naproxen
	3	1	28 (3.2%)	prednisolone, amoxicillin, salbutamol
		2	16 (1.9%)	amoxicillin, beclometasone, salbutamol
		3	14 (1.6%)	prednisolone, salbutamol, omeprazole
		3	14 (1.6%)	aspirin, bisoprolol, atorvastatin
		4	13 (1.5%)	doxyclycline, prednisolone, salbutamol
		4	13 (1.5%)	prednisolone, salbutamol, beclometasone
		4	13 (1.5%)	amoxicillin, salbutamol, co-codamol
		4	13 (1.5%)	omeprazole, amoxicillin, salbutamol
		5	12 (1.4%)	amlodipine, ramipril, atorvastatin
		5	12 (1.4%)	omeprazole, diazepam, amitriptyline
		5	12 (1.4%)	omeprazole, co-codamol, salbutamol
	4	1	10 (1.2%)	omeprazole, prednisolone, amoxicillin, salbutamol
		2	9 (1.0%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	9 (1.0%)	indapamide, amlodipine, atorvastatin, ramipril
		3	8 (0.9%)	prednisolone, amoxicillin, beclometasone, salbutamol
		3	8 (0.9%)	prednisolone, doxycycline, amoxicillin, salbutamol
		3	8 (0.9%)	prednisolone, amoxicillin, salbutamol, co-codamol
		4	7 (0.8%)	aspirin, glyceryl trinitrate, bisoprolol, atorvastatin
		4	7 (0.8%)	aspirin, glyceryl trinitrate, ramipril, atorvastatin
		4	7 (0.8%)	amoxicillin, salbutamol, predinisolone, clarithromycin

Table 3.1.3 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing stratified by age in new metformin users receiving polypharmacy at index date and within 90-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
40 - 59 years (7,007)	1	1	2,635 (37.6%)	atorvastatin
		2	2,003 (28.6%)	omeprazole
		3	1,635 (23.3%)	ramipril
		4	1,451 (20.7%)	amlodipine
		5	1,441 (20.6%)	salbutamol
	2	1	813 (11.6%)	ramipril, atorvastatin
		2	700 (10.0%)	omeprazole, atorvastatin
		3	692 (9.9%)	amlodipine, atorvastatin
		4	664 (9.5%)	aspirin, atorvastatin
		5	618 (8.8%)	bisoprolol, atorvastatin
	3	1	408 (5.8%)	aspirin, bisoprolol, atorvastatin
		2	318 (4.5%)	aspirin, ramipril, atorvastatin
		3	305 (4.4%)	bisoprolol, ramipril, atorvastatin
		4	301 (4.3%)	aspirin, bisoprolol, ramipril
		5	240 (3.4%)	amlodipine, ramipril, atorvastatin
	4	1	227 (3.2%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	121 (1.7%)	aspirin, glyceryl trinitrate, bisoprolol, atorvastatin
		3	111 (1.6%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		4	105(1.5%)	omeprazole, aspirin, bisoprolol, atorvastatin
		5	90 (1.3%)	lansoprazole, aspirin, ramipril, atorvastatin
	5	1	66 (0.9%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	61 (0.9%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	58 (0.8%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	47 (0.7%)	aspirin, ticagrelor, bisoprolol, atorvastatin, ramipril
		5	39 (0.6%)	lansoprazole, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin
	6	1	19 (0.3%)	aspirin, ticagrelor, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		2	18 (0.3%)	lansoprazole, aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		2	18 (0.3%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin, clopidogrel
		3	16 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, atorvastatin, ramipril
		4	15 (0.2%)	clopidogrel, aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril
		5	13 (0.2%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin, isosorbide mononitrate

Table 3.1.3 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing stratified by age in new metformin users receiving polypharmacy at index date and within 90-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
60 - 74 years (9,073)	1	1	3,861 (42.6%)	atorvastatin
		2	2,516 (27.8%)	omeprazole
		3	2,407 (26.5%)	amlodipine
		4	2,401 (26.5%)	simvastatin
		5	2,375 (26.2%)	aspirin
	2	1	1,264 (13.9%)	aspirin, atorvastatin
		2	1,258 (13.9%)	amlodipine, atorvastatin
		3	1,062 (11.7%)	ramipril, atorvastatin
		4	1,058 (11.7%)	omeprazole, atorvastatin
		5	1,044 (11.5%)	bisoprolol, atorvastatin
	3	1	604 (6.7%)	aspirin, bisoprolol, atorvastatin
		2	440 (4.8%)	aspirin, ramipril, atorvastatin
		3	408 (4.5%)	aspirin, bisoprolol, ramipril
		4	402 (4.4%)	bisoprolol, ramipril, atorvastatin
		5	400 (4.4%)	amlodipine, aspirin, atorvastatin
	4	1	261 (2.9%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	167 (1.8%)	omeprazole, aspirin, bisoprolol, atorvastatin
		3	159 (1.8%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		4	146 (1.6%)	aspirin, bisoprolol, atorvastatin, glyceryl trinitrate
		4	146 (1.6%)	aspirin, bisoprolol, atorvastatin, amlodipine
		5	120 (1.3%)	aspirin, amlodipine, ramipril, atorvastatin
	5	1	73 (0.8%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	71 (0.8%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	58 (0.6%)	omeprazole, aspirin, bisoprolol, ramipril, atorvastatin
		4	54 (0.6%)	aspirin, bisoprolol, amlodipine, ramipril, atorvastatin
		5	47 (0.5%)	aspirin, bisoprolol, atorvastatin, glyceryl trinitrate, isosorbide mononitrate
	6	1	20 (0.2%)	lansoprazole, aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		2	17 (0.2%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin, isosorbide mononitrate
		2	17 (0.2%)	amlodipine, aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		2	17 (0.2%)	omeprazole, aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	16 (0.2%)	glyceryl trinitrate, aspirin, bisoprolol, ramipril, atorvastatin, furosemide
		3	16 (0.2%)	lansoprazole, clopidogrel, aspirin, bisoprolol, ramipril, atorvastatin
		4	15 (0.2%)	furosemide, prednisolone, doxycycline, tiotropium bromide, salbutamol, carbocisteine
		4	15 (0.2%)	aspirin, prednisolone, salbutamol, tiotropium bromide, atorvastatin, carbocisteine

Table 3.1.3 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing stratified by age in new metformin users receiving polypharmacy at index date and within 90-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
60 - 74 years (9,073)	6	4	15 (0.2%)	aspirin, omeprazole, glyceryl trinitrate, isosorbide mononitrate, bisoprolol, atorvastatin
		4	15 (0.2%)	lansoprazole, aspirin, furosemide, bisoprolol, ramipril, atorvastatin
		5	14 (0.2%)	aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		5	14 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, atorvastatin, ramipril
		5	14 (0.2%)	prednisolone, amoxicillin, salbutamol, omeprazole, tiotropium bromide, salmeterol and fluticasone
		5	14 (0.2%)	aspirin, glyceryl trinitrate, isosorbide mononitrate, bisoprolol, atorvastatin, omeprazole
		5	14 (0.2%)	glyceryl trinitrate, aspirin, bisoprolol, ramipril, atorvastatin, salbutamol
		5	14 (0.2%)	aspirin, prednisolone, doxycycline, salbutamol, atorvastatin, amoxicillin
		5	14 (0.2%)	omeprazole, atorvastatin, prednisolone, doxycyline, amoxicillin, salbutamol
		5	14 (0.2%)	aspirin, prednisolone, salbutamol, atorvastatin, amoxicillin, bisoprolol
		5	14 (0.2%)	omeprazole, amlodipine, ramipril, atorvastatin, aspirin, bisoprolol
≥75 years (3,912)	1	1	1,455 (37.2%)	atorvastatin
		2	1,130 (28.9%)	simvastatin
		3	1,114 (28.5%)	aspirin
		4	1,066 (27.2%)	omeprazole
		5	1,009 (25.8%)	bisoprolol
	2	1	493 (12.6%)	aspirin, atorvastatin
		2	454 (11.6%)	bisoprolol, atorvastatin
		3	409 (10.5%)	amlodipine, atorvastatin
		4	399 (10.2%)	omeprazole, atorvastatin
		5	381 (9.7%)	aspirin, simvastatin
	3	1	194 (5.0%)	aspirin, bisoprolol, atorvastatin
		2	155 (4.0%)	aspirin, ramipril, atorvastatin
		3	150 (3.8%)	omeprazole, aspirin, atorvastatin
		4	140 (3.6%)	furosemide, bisoprolol, atorvastatin
		4	140 (3.6%)	bisoprolol, ramipril, atorvastatin
		5	134 (3.4%)	aspirin, amlodipine, atorvastatin
	4	1	80 (2.0%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	57 (1.5%)	omeprazole, aspirin, bisoprolol, atorvastatin
		3	52 (1.3%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		4	51 (1.3%)	omeprazole, aspirin, ramipril, atorvastatin
		5	45 (1.2%)	aspirin, bisoprolol, atorvastatin, furosemide

Table 3.1.3 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing stratified by age in new metformin users receiving polypharmacy at index date and within 90-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
≥75 years (3,912)	5	1	24 (0.6%)	omeprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	21 (0.5%)	warfarin, digoxin, furosemide, bisoprolol, atorvastatin
		3	18 (0.5%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin
		3	18 (0.5%)	prednisolone, amoxicillin, salbutamol, furosemide, atorvastatin
		4	16 (0.4%)	furosemide, aspirin, ramipril, bisoprolol, atorvastatin
		5	15 (0.4%)	aspirin, bisoprolol, ramipril, atorvastatin, isosorbide mononitrate
		5	15 (0.4%)	warfarin, digoxin, furosemide, paracetamol, bisoprolol
		5	15 (0.4%)	warfarin, furosemide, bisoprolol, paracetamol, atorvastatin
	6	1	8 (0.2%)	omeprazole, aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin
		2	7 (0.2%)	lansoprazole, warfarin, digoxin, furosemide, bisoprolol, atorvastatin
		2	7 (0.2%)	warfarin, digoxin, furosemide, bisoprolol, atorvastatin, paracetamol

3.1.4 Discussion

This population-based study explored prescription patterns in patients with new-onset T2DM receiving multiple medications. This is the first study to identify polypharmacy patterns at the drug compound level, which is essential when investigating the potential for DDIs. Additionally, this is the first study to use the Apriori algorithm in a large prescription database to overcome the computational challenges of assessing an extremely high number of drug combination. The Apriori algorithm computes all possible combinations of drugs per patient, including the combinations prescribed to no patient in the data. This methodology allowed us to precisely identify and quantify polypharmacy patterns at a compound level, particularly challenging in patients receiving extremely high number of medications, moving away from the existing knowledge of polypharmacy based on drug classes.[115,122,126]

We identified that 61.0% of patients initiating metformin received ≥4 additional drugs at index date or within 90-days before index date, and polypharmacy was more prevalent among women and patients aged ≥75 years. For example, it was previously found that 77.9% of patients with T2DM in an Asian population received polypharmacy (i.e., the use of ≥5 drugs in a day), of which 60.4% are women.[115] Moreover, this study identified an average of 7.7 different drugs taken per patient, with a mean age of 62.8 years. Similarly, it was estimated that adults with T2DM receive an average of 8.4 different drugs within the same day in Germany.[126]

We pinpointed several examples of potential DDIs in our study. For instance, a pharmacokinetic interaction between atorvastatin (metabolized by the cytochrome P450 [CYP] 3A4) and CYP3A4 substrates, inhibitors, and inducers (i.e., bisoprolol, ticagrelor, and lansoprazole). This drug combination is highly prevalent in overall and adults aged 40 to 59 years. While the individual use of weak CYP3A4 inhibitors has minimal effect on statin plasma levels,[127] it is unclear whether the use of multiple drugs affecting CYP3A4 in many ways and, thus, atorvastatin metabolism may increase the risk of DDIs and ADRs in those patients. An additional example of drug combination that may result in hypoglycaemia is discussed in the Appendix.

Although most drugs identified in our study prevent diabetes complications and are likely important drugs that cannot be de-prescribed, it is essential to consider individual characteristics of drugs (e.g., CYP enzyme interactions) in patients receiving multiple drug treatments. For instance, statins are commonly prescribed in diabetic patients with cardiovascular risk,[98] and some members of this class are CYP3A4-metabolized (e.g., simvastatin

and atorvastatin), while others are non-CYP3A4 metabolized (e.g., rosuvastatin). When CYP3A4-metabolized statins are used concomitantly with the antidiabetic drug glyburide, the maximum plasma concentration (C_{max}) and the area under the curve (AUC) of the antidiabetic drug may increase by up to 20%, increasing the risk for ADE. Moreover, additional drugs affecting CYP3A4 activity may be co-prescribed, adding to the risk of a multi-drug interaction with clinical significance. Therefore, the particular combination of glyburide and a CYP3A4-metabolized statin may be inappropriate in diabetic patients with T2DM receiving polypharmacy, and replacement by a non-CYP3A4 metabolized statin should be considered.

3.1.4.1 Strengths and limitations

A key strength of this study is the first-time use of the Apriori algorithm in a large prescription database, which allowed to assess an extremely high number of drug combinations at a compound level. Apriori and other algorithms used to find frequent item-sets (e.g., Toivonen's and PCY algorithms) are efficient approaches for finding frequent patterns in large databases. Thus, we moved beyond polypharmacy quantification at a drug class level as seen in previous studies.[114,118,128] These analyses failed to consider the omission of potentially harmful medications at a pharmaceutical ingredient level, as DDIs is not a problem related only to classes of drugs but to single drug compounds. IMRD allowed studying a large number of patients starting a metformin treatment who are representative of the UK population, where GPs usually manage T2DM.[13] Moreover, most of the results of our primary analysis remained consistent in the sensitivity analyses.

Our study also has some limitations. First, the IMRD only captures drug prescriptions, and therefore, we would not identify over-the-counter drugs.[129] Second, the use of primary care data means that prescriptions made in secondary care are not available, as well as medications required in hospital administration, special safety monitoring, or expensive drugs are often not fully captured (e.g., biologic disease-modifying antirheumatic drugs, methotrexate, atypical antipsychotics, and cancer treatments). Therefore, we might have underestimated actual drug utilisation and interactions. However, as the GP acts as the gatekeeper, chronic diseases and prescriptions are often monitored by the physician. Thus, we do not expect this limitation to greatly influence the interpretation of the results. Similarly, this study did not consider variations in dosages, formulation types, or between drugs within the same therapeutic class. Nevertheless, we believe this constraint has not interfered with the study's primary objective.

Third, we acknowledge that the concomitant use of drugs may have been overestimated as, in some cases, the prescription length may be shorter than 90-days, and, thus, patients will have stopped one drug before starting another. Moreover, drug exposure was defined based on unique ATC codes on the 5th level, and therefore, the concomitant use of drugs may have been underestimated when one ATC code combined >1 active pharmaceutical ingredient. Since all prescriptions are considered 'exposures' through the entire window length (i.e., 90-, 30-, or 14-days), longer windows increased the mean drug exposure counts, and thus the frequency (i.e., ranking) of prescriptions with short durations (e.g., antibiotics or pain-killers) was increased. Thus, the extent of polypharmacy decreased when we shortened the exposure window in which we may have missed many of the repeated prescriptions for drugs treating chronic diseases, where the mode of prescriptions is a 90-days' supply in the UK. Nevertheless, the prescription patterns are consistent over different exposure windows. Thus, we believe that it plausibly reflects prescribed polypharmacy. As for most data sources on drug utilisation, whether patients actually consumed the medications remains a limitation. Finally, although the potential DDIs identified in this study were conceivable, no adverse health events were assessed.

3.1.5 Conclusion

This study is the first to precisely identify and quantify polypharmacy patterns at a drug compound level by assessing the frequency of item-sets. The use of Apriori is efficient for finding frequent patterns in large databases and allowed us to assess an extremely high number of drug combinations at a compound level, moving away from the existing knowledge of polypharmacy based on drug classes. Polypharmacy was identified in 61% of metformin new-users, with a higher proportion of women and adults (≥75 years). The high prevalence of polypharmacy at all age strata indicates the need to optimize drug therapies to minimize DDI and ADR. Most drugs identified in our study were not relevant for drug deprescribing, however, they highlight the importance of considering the characteristics of individual drug compounds (e.g., CYP enzyme interactions) in patients receiving polypharmacy. While potential clinically relevant DDIs and drug combinations were identified, results should be interpreted cautiously, and longitudinal studies will be needed to investigate causal associations.

Chapter 3.2

Examining inappropriate medication in UK primary care for type 2 diabetes patients with polypharmacy

Authors

Maria L. Faquetti, Géraldine Frey, Dominik Stämpfli, Stefan Weiler, Andrea M. Burden

Remarks

Maria L. Faquetti contributed to the conceptualization of the study, methodology, formal analysis, investigation, data interpretation, manuscript preparation, and critical revisions.

Publication

Faquetti ML *et al*. Examining inappropriate medication in UK primary care for type 2 diabetes patients with polypharmacy. medRxiv. (2023). https://doi.org/10.1101/2023.05.24.23290466

3.2.1 Introduction

Type 2 diabetes mellitus (T2DM) is a complex chronic metabolic disorder affecting approximately 537 million adults worldwide, with nearly 90% having T2DM.[130] T2DM is characterised by insulin resistance and progressive decline of insulin production by pancreatic β-cell dysfunction.[131] While T2DM incidence rises with age, rates of T2DM onset in younger patients are increasing, leading to new public health challenges.[132] Pharmacological management of individuals with T2DM is often complex, especially when concomitant chronic conditions and diabetes complications are present, which may result in the use of multiple medications, also known as polypharmacy.[97] Moreover, patients with multimorbidity may attend multiple physicians, leading to fragmented, uncoordinated medication prescriptions.

Polypharmacy, generally defined as the concomitant use of five or more drugs, may be required to manage multiple coexisting conditions, presenting a risk of both undertreatment and overtreatment.[133] Although the correct combination of drugs in patients with T2DM can improve their health status and quality of life, polypharmacy increases the likelihood of potentially inappropriate prescriptions (PIPs), which can thereby increase the risk of experiencing an adverse drug event (ADE), drug-drug interaction (DDI), or drug-disease interaction.[29,61]

Furthermore, patients and society expect the benefits of medicines to outweigh their risks. However, patients are not equally receptive to beneficial effects and not equally susceptible to ADE. Factors such as comorbidities, concurrent prescribing of drugs with potential for interaction, and inappropriate prescribing can all contribute to this variability.[134] Therefore, it is crucial to ensure that medications are prescribed appropriately, considering individual patients' situations, to minimise the risks and maximise the benefits.

The appropriateness of prescribing medication can be assessed by using implicit process measures (i.e., judgement based, focused on individual patients) or by using explicit process measures (i.e., criterion-based). Among the most commonly used explicit process measures are the American Geriatrics Society (AGS) Beers criteria[135] and the Prescribing Optimally in Middle-age People's Treatments (PROMPT),[136] which were tailored to identify drugs to be avoided in older and middle-aged adults, respectively, independent of a diagnosis or in the context of a disease. While previous studies, predominantly among the elderly, have identified that the prevalence of PIPs is between 20% and 79%, depending on the population and setting,[104,128,137–140] less is known among patients with T2DM, particularly non-elderly.

The study by Oktora and colleagues revealing that the prevalence of ≥1 PIP among patients aged ≥45 years with T2DM and polypharmacy using Beers and PROMPT ranged from 24.9% to 39.0% was limited to information on drug prescriptions, which hindered implementation of most of the criteria.[128] Thus, no large population-based study encompassing clinical information has applied these measures to identify drugs to be avoided in the context of diseases or under specific situations in patients with T2DM.

The overall aim of this study was to estimate the prevalence of PIPs in middle-aged and older adults starting their first non-insulin antidiabetic treatment (NIAD) using two explicit process measures of appropriateness of prescribing medication in a primary care database from the UK, stratified by age and polypharmacy status. This study aimed to identify if pharmacotherapy could be optimised and, ultimately, to provide clinical insights into the management of patients with T2DM receiving multiple medications in primary care.

3.2.2 Materials and methods

3.2.2.1 Study design and data source

We conducted a descriptive cohort study using data from primary care practices in the UK between 2016 and 2019 to assess PIPs in patients at the start of their first NIAD, stratified by age and polypharmacy status. The data were obtained from IQVIA Medical Research Data (IMRD; incorporating data from THIN, A Cegedim Database of anonymised electronic health records in the UK). IMRD is an extensive database containing longitudinal non-identified electronic medical records collected from general practices in the UK from over 18 million patients, of which approximately 2.9 million are currently active. IMRD has been shown to be representative of the UK and valid in terms of age and sex comparisons and a wide range of diseases.[12]

Demographic information, clinical outcomes and diagnosis, lab tests (e.g., estimated glomerular filtration rate [eGFR] and haemoglobin A1c [HbA1c]), drug prescriptions, specialist referrals, and other information (e.g., smoking status, pregnancy, and death) are recorded in the database by general practitioners (GPs). Medications are recorded using the British National Formulary (BNF) classification and were manually mapped to their corresponding International Anatomical Therapeutic Classification (ATC) code. As the IMRD consists of primary care records, medication dispensed during hospitalisation, nor those prescribed by specialists, are included in the database. All diagnoses are recorded using Read codes.[12]

3.2.2.2 Study population

The study population consisted of all adults aged ≥45 years who received a first-ever NIAD prescription between January 2016 and December 2019. Cohort entry (index date) was defined as the date of the first-ever NIAD prescription. Patients included were classified by age at index date as older adults (≥65 years) or middle-aged adults (45-64 years). Patients were allowed to have multiple co-prescribed NIADs at the index date. All patients were required to have ≥1 year of database history and at least one GP visit before the index date. We only considered patients registered with a practice with a valid up-to-standard date, who had valid entries (i.e., a valid patient flag), and with a first NIAD prescription with a valid flag in the database. Patients diagnosed with gestational diabetes, polycystic ovary syndrome, cancer (except by non-melanoma skin cancers), or insulin therapy previous to, or at, the index date were excluded. Additionally, patients in palliative care or at end-of-life were excluded. All comorbidity or palliative/end-of-life care patients were identified using Read codes.

3.2.2.3 Polypharmacy definition

We leveraged an existing cohort of patients with T2DM and polypharmacy, which is described in detail elsewhere.[32] In brief, polypharmacy was defined as the prescription of ≥5 different drug compounds on or within 90 days before the index date. We identified patients with polypharmacy at the start of NIAD therapy and only considered drugs and diagnoses (i.e., history of comorbidities and medical conditions in the Beers or PROMPT criteria for assessing PIP) with a valid flag in the database. Additional details on the selection of drugs for the polypharmacy analysis are available in the Appendix.

3.2.2.4 Potentially inappropriate prescriptions (PIPs)

We used the AGS Beers criteria for assessment of PIPs in patients aged ≥65 years,[135] and the PROMPT criteria for patients aged 45 to 64 years, at the start of the first NIAD.[136] The Beers criteria includes lists of potentially inappropriate drugs that should be avoided in patients aged ≥65 years as a whole or those with certain medication conditions or at an increased risk of an ADE or DDI.[135] The 2015 version was the latest published version for the years 2016-2019. The PROMPT criteria are used to optimise medication prescribing practices for middle-aged adults (i.e., 45-64 years old)[136] It includes recommendations relevant to this age group considering age-related factors, such as differences in the prevalence of diseases and polypharmacy.

While Beers list encompasses 92 criteria, PROMPT has 22 criteria. For the assessment of PIPs, we included only those criteria that can be fully or partially applied in a primary care database, without information on drugs prescribed during hospital stay or in secondary care. We excluded 17 criteria from Beers (i) because of unavailability of data (e.g., severity of disease, medication prescribed during hospital stay, or dose reduction information in patients with reduced kidney function), or (ii) they were ruled out by study design (i.e., due to exclusion criteria), resulting in 75 indicators from Beers and 22 from PROMPT included in the analysis. Nevertheless, the availability of clinical and prescription information allowed a larger number of Beers and PROMPT criteria to be applied than in previous studies.[12,32,104,128,140,141] Moreover, a multidisciplinary team collaborated to align the applicability of each criterion using primary care data, aiming to find consensus through the lists and achieve optimal results in a consistently manner. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including drugs from the BNF previously published.[142] As PROMPT was tailored to the UK and the Republic of Ireland, no adaptation regarding the drug list was needed.

The list of criteria used, their recommendation, rationale, applicability using primary care data (i.e., fully applied or partially applied), limitation, and an extensive description used in each criterium to assess PIPs according to Beers and PROMPT are listed in **Table 8.2.1** and **Table 8.2.2**, respectively. Additionally, information on ATC codes considered for the identification of the corresponding drugs and Read codes considered for identification of the corresponding diagnosis in each criterion are listed in **Table 8.2.3** for Beers and **Table 8.2.4** for PROMPT criteria. All codes were manually reviewed and confirmed by a medical doctor and clinical pharmacist.

Patients were categorised into those who received a PIP criteria drug, drug combination, or drug-disease combination. Criteria which specified a particular dosage not to exceed, such as prescription of proton pump inhibitors (PPIs) above recommended maintenance dosages for >8 weeks (PROMPT criteria 22), were assessed by calculating the daily dose using the strength, quantity prescribed, and the dose per day or duration for each prescription in the period of assessment.

Whenever dosage information was not available in the data set for drugs of which judgment of appropriate use was dependant on prescribed dose or duration, the cumulative dose (CD) was calculated per drug compound in the database using the defined daily dose (DDD) by the ATC index whenever provided (available at https://www.whocc.no/atc_ddd_index/). This index represents an average maintenance dose per day for a drug used for its main indication

in adults, and thus allows for comparisons of drug consumption at an international level. Moreover, the ATC index is freely available worldwide. Whenever the ATC index was not available, we adopted the (minimal) daily dose recommended by the BNF formulary, as specified in the 'PIPs definition' in **Table 8.2.1** and **Table 8.2.2**. Finally, for multiple prescriptions with only one record having information on dosage/frequency, we assumed the same prescription mode for all prescriptions of the same drug compound for the same patient.

3.2.2.5 Statistical analysis

We reported patient characteristics at index date, stratified by age groups (40-64 years and ≥65 years) and polypharmacy status (<5 drugs and ≥5 drugs), as a mean and standard deviation or median and interquartile range (IQR) or counts and proportions, as applicable. We identified alcohol use and smoking based upon the most recent value recorded in the database (i.e., either at or before index date). Body mass index was calculated using the value closest to the index date. However, as the height of an individual is not likely to vary significantly during adulthood, the closest value to the index date (before or after) was considered. We summarised the mean HbA1c and eGFR using the most recently recorded values up to the previous six months prior to the index date, and a history of comorbidities was assessed if ever registered in the database before the index date. We reported all patient characteristics among older and middle-aged adults, stratified by polypharmacy status.

We estimated the number of PIPs per patient at the start of the first NIAD treatment in each of the strata, which was identified as the number of patients with at least one day with any PIPs. Additionally, for the individual PIPs criteria, prevalence was reported in patients receiving at least one day of the drug(s) under evaluation of PIPs at or within 90 days to index date. As a sensitivity analysis, for drugs of which judgment of PIPs was dependent on prescribed dose or duration, the prevalence of PIPs was reported separately for patients having complete information on dose or duration by dividing the patient number by the total number of patients with complete information on dose or duration. Numbers fewer than seven are suppressed according to data use agreement with IMRD. All analyses were performed using the R software version (4.1.2). The study protocol was approved by the IQVIA Scientific Research Committee (study reference number: 22SRC047).

3.2.3 Results

A total of 28,604 patients initiating a NIAD between 2016 and 2019 (**Figure 3.2.1**) were eligible for inclusion. The demographic characteristics at the index are provided in **Table 3.2.1**, stratified by age and polypharmacy status. Overall, 18,494 (64.7%) patients had polypharmacy at the index date or within 90 days before the index date and were categorised as having polypharmacy. The prevalence of polypharmacy increased with age (older adults=77.8%; middle-aged adults=55.2%), and sulfonylureas (SU), dipeptidyl peptidase 4 (DPP-4) inhibitors, and sodium-glucose co-transporter (SGLT-2) inhibitors were prescribed more frequently (alone or in combination with metformin) in patients receiving polypharmacy compared to no polypharmacy regardless of age.

Among older patients without polypharmacy, 6.5% received ≥1 PIP with a maximum of four PIPs per patient, while 39.6% of those with polypharmacy had ≥1 PIP with a maximum of 10 PIPs per patient (**Table 3.2.2**). Similarly, the prevalence of PIPs was higher among middle-aged adults with polypharmacy than in those without. Among middle-aged adults without polypharmacy, few PIPs were identified, with 98.5% of patients having no PIPs, while 22.7% of those with polypharmacy had ≥1 PIP with a maximum of four PIPs per patient (**Table 3.2.2**).

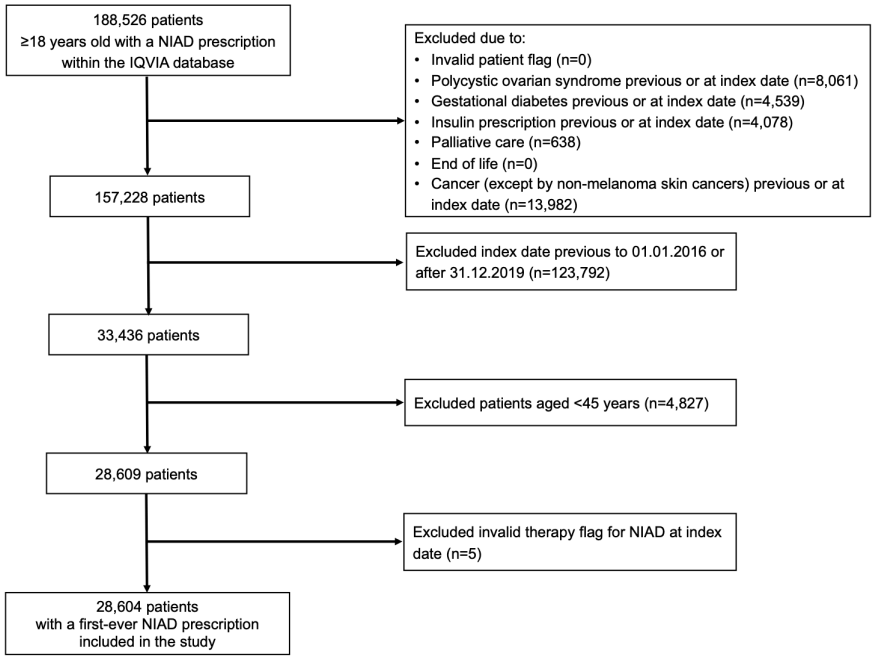


Figure 3.2.1 Flow diagram of patient selection. NIAD, non-insulin antidiabetic drug.

Table 3.2.1 Baseline characteristics of non-insulin antidiabetic drug (NIAD) new-users, stratified by age and polypharmacy status.

	Overall study cohort (N=28,604)		**Middle-aged (≥45 and <65 years) (N=16,654)**		**Older adults (≥65 years) (N=11,950)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	10,110 (35.3%)	18,494 (64.7%)	7,457 (44.8%)	9,197 (55.2%)	2,653 (22.2%)	9,297 (77.8%)
Females	3,462 (34.2%)	8,429 (45.6%)	2,446 (32.8%)	4,163 (45.3%)	1,016 (38.3%)	4,266 (45.9%)
Mean age (SD)	58.8 (9.3)	64.9 (10.8)	54.3 (5.4)	55.9 (5.4)	71.4 (5.5)	73.7 (6.6)
Median number of drugs (IQR)	3 (2 – 4)	8 (6 – 11)	3 (2 – 4)	8 (6 – 11)	3 (2 – 4)	8 (6 – 11)
Mean number of drugs (SD)	2.7 (1.1)	9.1 (4.0)	2.6 (1.1)	8.8 (4.0)	3.0 (1.0)	9.5 (4.0)
Distribution of drugs n (%)						
1 (NIAD)	1,653 (5.8%)	-	1,376 (8.2%)	-	277 (2.3%)	-
2 - 4	8,457 (29.6%)	-	6,081 (36.5%)	-	2,376 (19.9%)	-
5 - 9	-	11,848 (41.4%)	-	6,259 (37.6%)	-	5,589 (46.8%)
10 - 19	-	6,180 (21.6%)	-	2,714 (16.3%)	-	3,466 (29.0%)
≥20	-	466 (1.6%)	-	224 (1.3%)	-	242 (2.0%)
Max nº of drugs	4	41	4	41	4	40
Number of NIADs prescribed at index date n (%)						
≥2	293 (2.9%)	557 (3.0%)	230 (3.1%)	348 (3.8%)	63 (2.4%)	209 (2.2%)
NIAD class[1]						
Biguanides (Metformin)	9,611 (96.8%)	17,082 (94.5%)	7,231 (97.0%)	8,890 (96.7%)	2,551 (96.2%)	8,592 (92.4%)
Sulfonylureas	615 (6.2%)	1,319 (7.4%)	472 (6.3%)	659 (7.2%)	157 (5.9%)	712 (7.7%)
DPP-4 inhibitors	112 (1.1%)	503 (2.8%)	61 (0.8%)	125 (1.4%)	53 (2.0%)	393 (4.2%)
SGLT-2 inhibitors	47 (0.5%)	115 (0.6%)	45 (0.6%)	77 (0.8%)	<7	40 (0.4%)
GLP-1 analogues	10 (0.1%)	22 (0.1%)	12 (0.2%)	18 (0.2%)	<7	<7
Thiazolidinediones	8 (0.08%)	12 (0.06%)	<7	<7	<7	7 (0.8%)
Others	7 (0.07%)	18 (0.1%)	<7	14 (0.2%)	<7	<7
Smoking status[2], n (%)						
Current	1,629 (16.1%)	3,243 (17.5%)	1,336 (17.9%)	2,242 (24.4%)	293 (11.0%)	1,001 (10.8%)
Never	5,513 (54.5%)	8,096 (43.8%)	4,080 (54.7%)	3,897 (42.4%)	1,433 (54.0%)	4,199 (45.2%)
Former	2,910 (28.8%)	7,079 (38.3%)	2,003 (26.9%)	3,012 (32.7%)	907 (34.2%)	4,067 (43.7%)
Unknown / Missing value	58 (0.6%)	76 (0.4%)	38 (0.5%)	46 (0.5%)	20 (0.8%)	30 (0.3%)

Table 3.2.1 (cont.) Baseline characteristics of non-insulin antidiabetic drug (NIAD) new-users, stratified by age and polypharmacy status.

	Overall study cohort (N=28,604)		Middle-aged (≥45 and <65 years) (N=16,654)		Older adults (≥65 years) (N=11,950)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
Alcohol use[2], n (%)						
Current	7,313 (72.3%)	11,950 (64.6%)	5,412 (72.6%)	6,030 (65.6%)	1,901 (71.7%)	5,920 (63.7%)
Never (Lifelong teetotaler)	1,826 (18.1%)	4,590 (24.8%)	1,332 (17.9%)	2,146 (23.3%)	494 (18.6%)	2,444 (26.3%)
Former	322 (3.2%)	1,187 (6.4%)	219 (2.9%)	591 (6.4%)	103 (3.9%)	596 (6.4%)
Unknown / Missing value	649 (6.4%)	767 (4.1%)	494 (6.6%)	430 (4.7%)	155 (5.8%)	337 (3.6%)
BMI[3], n (%)						
Obese (BMI ≥30.0 kg/m^2)	5,921 (58.6%)	12,083 (65.3%)	4,675 (62.7%)	6,862 (74.6%)	1,246 (47.0%)	5,221 (56.1%)
Overweight (BMI 25.0 – 29.9 kg/m^2)	2,944 (29.1%)	4,616 (25.0%)	1,973 (26.5%)	1,708 (18.6%)	971 (36.6%)	2,908 (31.3%)
Normal/ Underweight (BMI <25.0 kg/m^2)	878 (8.7%)	1,380 (7.5%)	540 (7.2%)	404 (4.4%)	338 (12.7%)	976 (10.5%)
Missing value	367 (3.6%)	415 (2.2%)	269 (3.6%)	223 (2.4%)	98 (3.7%)	192 (2.1%)
History of disease, n (%)						
Hypertension	3,246 (32.1%)	11,409 (61.7%)	2,321 (28.5%)	4,939 (53.7%)	1,123 (42.3%)	6,470 (69.6%)
Coronary heart disease	172 (1.7%)	3,780 (20.4%)	90 (1.2%)	1,373 (14.9%)	82 (3.1%)	2,407 (25.9%)
Heart failure	10 (0.1%)	782 (4.2%)	<7	234 (2.5%)	<7	548 (5.9%)
Cerebrovascular disease	185 (1.8%)	1,773 (9.6%)	101 (1.4%)	561 (6.1%)	84 (3.2%)	1,212 (13.0%)
Chronic kidney disease	376 (3.7%)	2,558 (13.8%)	156 (2.1%)	504 (5.5%)	220 (8.3%)	2,054 (22.1%)
Chronic liver disease	257 (2.5%)	823 (4.5%)	192 (2.6%)	496 (5.4%)	65 (2.5%)	327 (3.5%)
COPD	208 (2.1%)	2,408 (13.0%)	114 (1.5%)	922 (10.0%)	94 (3.5%)	1,486 (16.0%)
Asthma	810 (8.0%)	3,703 (20.0%)	653 (8.8%)	2,056 (22.4%)	57 (5.9%)	1,617 (17.7%)
Sleep disorders	707 (7.0%)	2,847 (15.4%)	566 (7.6%)	1,604 (17.4%)	141 (5.3%)	1,243 (13.4%)
Depression	1,993 (19.7 %)	6,655 (36.0%)	1,600 (21.5%)	4,013 (43.6%)	393 (14.8%)	2,642 (28.4%)
Alzheimer/Dementia	39 (0.4%)	406 (2.2%)	12 (0.2%)	32 (0.3%)	27 (1.0 %)	374 (4.0 %)
Hypothyroidism	426 (4.2%)	2,224 (12.0%)	281 (3.8%)	978 (10.6%)	145 (5.5%)	1,246 (13.4%)
Osteoarthritis	1,169 (11.6%)	5,003 (27.1%)	592 (7.9%)	1,621 (17.6%)	577 (21.7%)	3,382 (36.4%)
Osteoporosis	94 (0.9%)	829 (4.5%)	32 (0.4%)	169 (1.8%)	62 (2.3%)	660 (7.1%)
Lab values						
Most recent HbA_{1c} measurement six months prior to index date n (%)[4]						
<6.5% (48 mmol/mol)	304 (3.0%)	647 (3.5%)	235 (3.2%)	325 (3.5%)	69 (2.6%)	322 (3.5%)
6.5 – 7.4% (48 – 57 mmol/mol)	2,410 (23.8%)	5,594 (30.2%)	1,672 (22.4%)	2,618 (28.5%)	738 (27.8%)	2,976 (32.0%)

Table 3.2.1 (cont.) Baseline characteristics of non-insulin antidiabetic drug (NIAD) new-users, stratified by age and polypharmacy status.

	Overall study cohort (N=28,604)		Middle-aged (≥45 and <65 years) (N=16,654)		Older adults (≥65 years) (N=11,950)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
7.5 – 8.5% (58 – 69 mmol/mol)	2,010 (19.9%)	4,614 (24.9%)	1,389 (18.6%)	2,087 (22.7%)	621 (23.4%)	2,527 (27.2%)
>8.5% (69 mmol/mol)	4,419 (43.7%)	5,781 (31.3%)	3,429 (46.0%)	3,211 (34.9%)	990 (37.3%)	2,570 (27.6%)
Missing value	967 (9.6%)	1,858 (10.0%)	732 (9.8%)	956 (10.4%)	235 (8.9%)	902 (9.7%)
Most recent eGFR measurement (mL/min/1.73 m²) six months prior to index date n (%)[4]						
<30	8 (0.1%)	73 (0.4%)	<7	13 (0.1%)	<7	60 (0.6%)
30 – 59	452 (4.5%)	2,406 (13.0%)	184 (2.5%)	499 (5.4%)	268 (10.1%)	1,907 (20.5%)
≥60	6,886 (68.1%)	11,188 (60.5%)	5,190 (69.6%)	6,067 (66.0%)	1,696 (63.9%)	5,121 (55.1%)
Missing value	2,764 (27.3%)	4,827 (27.6%)	2,083 (27.9%)	2,618 (28.5%)	683 (25.7%)	2,209 (23.8%)

Abbreviations: NIAD: non-insulin antidiabetic drug; SD: standard deviation; IQR: interquartile range; DPP-4: Dipeptidyl peptidase 4; SGLT-2: Sodium-glucose co-transporter 2; GLP-1: Glucagon-like peptide-1; BMI: body mass index; COPD: chronic obstructive pulmonary disease; HbA1c: hemoglobin A1c; eGFR: Estimated glomerular filtration rate;

[1] The summed proportion value is above 100%, as a patient may receive more than one NIAD compound at the index date.

[2] Identified values closest to the index date (i.e., at or before index date).

[3] BMI was assessed using the most recently recorded value for weight (at or at any time before index date) and height (at or at any time before or after index date).

[4] The most recent value (at index date and previous six-months) registered in the database with a valid unit was considered.

Note: A history of comorbidities was assessed if ever registered in the database previous to or at index date. Numbers fewer than seven are suppressed according to data use agreement with IMRD.

Table 3.2.2 Prevalence of potentially inappropriate prescriptions (PIPs) in older and middle-aged adults according to the American Geriatrics Society (AGS) Beers criteria 2015 and the Prescribing Optimally in Middle-aged People's Treatments (PROMPT) criteria, respectively, stratified by polypharmacy status.

Age group	Older adults (≥65 years)		Middle-aged (≥45 and <65 years)	
Number of PIPs	no polypharmacy (N=2,653)	polypharmacy (N=9,297)	no polypharmacy (N=7,457)	polypharmacy (N=9,297)
0	2,480 (93.5%)	5,615 (60.4%)	7,342 (98.5%)	7,113 (77.3%)
1	142 (5.4%)	2,488 (26,8%)	*	1,535 (16.7%)
2	25 (0.9%)	701 (7.5%)	*	425 (4.6%)
3	<7	179 (1.9%)	0 (0.0%)	108 (1.2%)
4	<7	103 (1.1%)	0 (0.0%)	16 (0.2%)
≥5	0 (0.0%)	211 (2.3%)	0 (0.0%)	0 (0.0%)
max	4	10	2	4
≥1	173 (6.5%)	3,682 (39.6%)	114 (1.5%)	2,084 (22.7%)

* These numbers were suppressed due to patient privacy.
Abbreviations: PIPs: Potentially inappropriate prescriptions.

Table 3.2.3 provides the prevalence of PIPs among older adults according to the Beers criteria. The most frequently occurring PIP was the use of PPIs for >8 weeks unless for high-risk patients (criteria 33 [n=1,034, 11.1%]), followed by the use of high anticholinergic antidepressants monotherapy or in combination to other drugs for management of depression (criteria 16 [n=951, 10.2%]), the use of antipsychotics, benzodiazepines (BZD), non-BZD, BDZ receptor agonist hypnotics, tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI) in patients with a history of falls or fractures (criteria 3 [n=911, 9.8%]), and the use of peripheral alpha-1 blockers in patients with hypertension (criteria 2 [n=495, 5.3%]). Among patients having no polypharmacy, the most frequent PIPs were PPI use >8 weeks unless for high-risk patients, erosive esophagitis, Barrett's esophagitis, pathological hypersecretory condition, or demonstrated need for maintenance treatment (criteria 33 [n=46, 1.7%]) and the use of antidepressants monotherapy or in combination to other drugs for management of depression (criteria 16 [n=31, 1.2%]). The sensitivity analysis identifying the prevalence of individual PIPs among patients having complete information on dose or duration is shown in Table 3.2.3.

The prevalence of individual PIPs in middle-aged adults at the start of the first NIAD treatment is provided in Table 3.2.4. The highest prevalence in middle-aged patients with polypharmacy was observed for strong opioids without co-prescription of laxatives (criteria 17 [n=381, 4.1%]); followed by the combination of non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin (acetylsalicylic acid) or SSRI without adequate gastrointestinal protection

(criteria 21 [n=361, 3.9%]), long-term use of BZD (criteria 14 [n=293, 3.2%]), and PPI use above maintenance dosages for >8 weeks (criteria 2 [n=269, 2.9%]). In contrast, in patients having no polypharmacy, the highest prevalence was observed for first-generation antihistamines as first-line treatment >7 days (criteria 8 [n=14, 0.2%]) and for PPI above maintenance dose for >8 weeks (criteria 2 [n=13, 0.2%]). The sensitivity analysis identifying the prevalence of individual PIPs among patients having complete information on dose or duration is shown in **Table 3.2.4**.

Table 3.2.3 Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
2	1	Avoid first generation AH	<7	170 (1.8%)		
2	2	Avoid antiparkinsonian agents in combination with antipsychotics	0 (0.0%)	0 (0.0%)		
2	3	Avoid antispasmodics	<7	90 (1.0%)		
2	4	Avoid dipyridamole oral short action	0 (0.0%)	<7		
2	5	Avoid ticlopidine	0 (0.0%)	0 (0.0%)		
2	6	Avoid nitrofurantoin if (i) creatinine clearance <30ml/min or (ii) long-term suppression of bacteria	0 (0.0%) [i] 0 (0.0%) [ii]	20 (0.2%) [i] 37 (0.4%) [ii]	0 (0.0%) [ii]	23 (0.2%) [ii]
2	7	Avoid peripheral alpha-1 blockers in hypertension	13 (0.5%)	495 (5.3%)		
2	8	Avoid clonidine (as first-line therapy), guanabenz, guanfacine, methyldopa and reserpine (>0.1 mg/day) in hypertension	0 (0.0%)	<7		
2	9	Avoid disopyramide	0 (0.0%)	<7		
2	10	Avoid dronedarone in permanent AF or severe/recently decompensated HF	0 (0.0%)	0 (0.0%)		
2	11	Avoid digoxin as first-line therapy for AF	0 (0.0%)	36 (0.4%)		
2	12	Avoid digoxin as first-line therapy for HF	0 (0.0%)	<7		
2	13	If digoxin is used for AF or HF, avoid dosages >125 mcg/day	<7	72 (0.8%)	<7	72 (0.8%)
2	14	Avoid nifedipine immediate release	0 (0.0%)	13 (0.1%)		
2	15	Avoid amiodarone as first-line therapy for AF unless patient has HF or substantial left ventricular hypertrophy	0 (0.0%)	<7		
2	16	Avoid antidepressants[3] alone or in combination	31 (1.2%)	951 (10.2%)		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
2	17	Avoid first- and second- generation antipsychotics (except for schizophrenia, bipolar disorder, or short-term use as antiemetic during chemotherapy)	24 (0.9%)	333 (3.6%)		
2	18	Avoid barbiturates	<7	<7		
2	19	Avoid short- and intermediate-acting BZD	<7	139 (1.5%)		
2	20	Avoid long-acting BZD	10 (0.4%)	333 (3.6%)		
2	21	Avoid meprobamate	0 (0.0%)	0 (0.0%)		
2	22	Avoid BZD-receptor agonist hypnotics	<7	330 (3.5%)		
2	23	Avoid Ergoloid mesylates and isoxsuprine	0 (0.0%)	0 (0.0%)		
2	24	Avoid androgens unless hypogonadism	0 (0.0%)	23 (0.2%)		
2	25	Avoid desiccated thyroid	0 (0.0%)	0 (0.0%)		
2	26	Avoid oral and topical patch estrogens	<7	34 (0.4%)		
2	27	Avoid GH, except as hormone replacement after pituitary gland removal	0 (0.0%)	<7		
2	28	Avoid megestrol	0 (0.0%)	0 (0.0%)		
2	29	Avoid long duration SU (chlorpropamide)	0 (0.0%)	0 (0.0%)		
2	30	Avoid long duration SU (glyburide)	0 (0.0%)	<7		
2	31	Avoid metoclopramide unless gastroparesis	<7	65 (0.7%)		
2	32	Avoid mineral oil given orally	0 (0.0%)	0 (0.0%)		
2	33	Avoid PPI use for >8weeks	46 (1.7%)	1,034 (11.1%)	30 (1.1%)	772 (8.3%)
2	34	Avoid meperidine, especially in individuals with CKD	0 (0.0%)	0 (0.0%)		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
2	35	Avoid chronic use of oral non-cyclooxygenase-NSAID	<7	29 (0.3%)	0 (0.0%)	12 (0.1%)
2	36	Avoid indomethacin, ketorolac (including parenteral)	<7	16 (0.2%)		
2	37	Avoid pentazocine	0 (0.0%)	0 (0.0%)		
2	38	Avoid skeletal muscle relaxants[4]	0 (0.0%)	15 (0.2%)		
2	39	Avoid desmopressin for treatment of nocturia or nocturnal polyuria	0 (0.0%)	<7		
3	40	In patients with HF, avoid (i) NSAID, COX-2 inhibitors, thiazolidinediones (pioglitazone, rosiglitazone), and cilostazol, (ii) dronedarone in severe or recently decompensated HF, and (iii) nondihydropyridine CCB (diltiazem, verapamil) only in patients with HFrEF.	0 (0.0%) (i) 0 (0.0%) (ii) NA (iii)	18 (0.2%) [(i)] 0 (0.0%) [(ii)] NA [(iii)]		
3	41	Avoid AchEi, peripheral alpha-1 blockers, tertiary TCA, chlorpromazine, thioridazine, olanzapine in patients with syncope	0 (0.0%)	<7		
3	42	Avoid bupropion, chlorpromazine, clozapine, maprotiline, olanzapine, thioridazine, thiothixene, and tramadol if chronic seizures or epilepsy	<7	<7		
3	43	Avoid (i) anticholinergics, BZD chlorpromazine, corticosteroids, H2-receptor blockers, meperidine, sedative hypnotics in patients with or at risk of delirium. Avoid (ii) antipsychotics for behavioral problems of dementia or delirium.	0 (0.0%) [(i)] <7 [(ii)]	14 (0.2%) [(i)] 46 (0.5%) [(ii)]		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
3	44	Avoid anticholinergics, antipsychotics, H2-receptor antagonist, BZD, non-BZD, BZD receptor agonist hypnotics in patients with dementia or cognitive impairment	<7	232 (2.5%)		
3	45	In patients with history of falls or fractures, avoid (i) anticonvulsants (except for seizure and mood disorders), (ii) antipsychotics, BZD, non- BZD, BZD receptor agonist hypnotics, TCA, SSRI (unless safer alternatives are not available)[5], and (iii) opioids (except for pain management due to recent fractures or joint replacement).	<7 (i) 35 (1.3%) (ii) 8 (0.3%) (iii)	144 (1.5%) (i) 911 (9.8%) (ii) 379 (4.1%) (iii)		
3	46	Avoid CNS stimulants (oral decongestants, stimulants, and theobromines) in patients with insomnia	0 (0.0%)	65 (0.7%)		
3	47	Antipsychotics (except aripiprazole, quetiapine, and clozapine) and antiemetics in patients with Parkinson's disease avoid	0 (0.0%)	7 (0.1%)		
3	48	Avoid aspirin (>325 mg/day) and non-COX2 NSAID in patients with history of gastric ulcers unless other alternatives are not effective and patient can take gastroprotective agent	0 (0.0%)	7 (0.1%)		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
3	49	Avoid NSAID (non-COX and COX-selective, oral and parenteral) in CKD stages IV or less	0 (0.0%)	0 (0.0%)		
3	50	Avoid estrogen (oral and transdermal) and peripheral alpha-1 blockers in women with urinary incontinency	0 (0.0%)	0 (0.0%)		
3	51	Avoid strong anticholinergics drugs (except antimuscarinics for urinary incontinence) in men with lower urinary tract symptoms or BPH	0 (0.0%)	17 (0.2%)		
5	52	Avoid use of ACE inhibitors and amiloride or triamterene;	0 (0.0%)	<7		
5	53	Avoid using ≥2 anticholinergic drugs	0 (0.0%)	0 (0.0%)		
5	54	Avoid SSRI [(i)] or TCA [(ii)] and ≥2 other CNS-active drugs	<7 0 (0.0%)	163 (1.8%) [(i)] 142 (1.5%) [(ii)]		
5	55	Avoid antipsychotics and ≥2 other CNS-active drugs	<7	86 (0.9%)		
5	56	Avoid BZD and non-BZD, BZD receptor agonist hypnotics and ≥2 additional CNS-active drugs	0 (0.0%)	197 (2.1%)		
5	57	Avoid corticosteroids + NSAID, if not possible, provide GI protection	0 (0.0%)	22 (0.2%)		
5	58	Avoid lithium together with ACE inhibitors, monitor lithium concentration	0 (0.0%)	7 (0.1%)		
5	59	Avoid lithium together with loop diuretics, monitor lithium concentration	0 (0.0%)	<7		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
5	60	Avoid opioids and ≥2 other CNS-active drugs	<7	214 (2.3%)		
5	61	Avoid peripheral alpha-1 blockers together with loop diuretics in older women unless conditions warrant both drugs	0 (0.0%)	19 (0.2%)		
5	62	Avoid theophylline in combination with cimetidine	0 (0.0%)	0 (0.0%)		
5	63	Avoid warfarin in combination with amiodarone when possible	0 (0.0%)	11 (0.1%)		
5	64	Avoid warfarin in combination with NSAID when possible	0 (0.0%)	<7		
5	65	Avoid amiodarone if creatinine clearance <30 mL/min	0 (0.0%)	<7		
6	66	Avoid apixaban if creatinine clearance <25 mL/min	0 (0.0%)	<7		
6	67	Avoid dabigatran if creatinine clearance <30 mL/min	0 (0.0%)	<7		
6	68	Avoid edoxaban if creatinine clearance <30 or >95 mL/min	0 (0.0%)	<7		
6	69	Avoid fondaparinux if creatinine clearance <30 mL/min	0 (0.0%)	0 (0.0%)		
6	70	Avoid rivaroxaban if creatinine clearance <30 mL/min	0 (0.0%)	<7		
6	71	Avoid spironolactone if creatinine clearance <30 mL/min	0 (0.0%)	11 (0.1%)		
6	72	Avoid triamterene if creatinine clearance <30 mL/min	0 (0.0%)	0 (0.0%)		

Table 3.2.3 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in older adults with new-onset type 2 diabetes (T2DM) according to the American Geriatrics Society (AGS) Beers criteria 2015, stratified by polypharmacy status.

					Sensitivity Analysis (Patients with dosage information)[2]	
Table Number[1]	**Criteria Number**	**Recommendation**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**	**Patients receiving PIP without polypharmacy (N=2,653)**	**Patients receiving PIP with polypharmacy (N=9,297)**
6	73	Avoid duloxetine if creatinine clearance <30 mL/min	0 (0.0%)	<7		
6	74	Avoid tramadol extended release if creatinine clearance <30 mL/min	0 (0.0%)	0 (0.0%)		
6	75	Avoid probenecid if creatinine clearance <30 mL/min	0 (0.0%)	0 (0.0%)		

[1] Original table number from the 2015 Beers criteria (doi: 10.1111/jgs.13702).

[2] Drugs of which judgment of PIP was dependant on prescribed dose or duration, prevalence of PIPs was reported separately for patients having complete information on dose or duration.

[3] Amitriptyline, amoxapine, clomipramine, desipramine, doxepin (>6mg/day), imipramine, nortriptyline, paroxetine, protriptyline, trimipramine.

[4] Carisoprodol, chlorzoxazone, cyclobenzaprine, metaxalone, methocarbamol, orphenadrine.

[5] Criteria partially applied (detailed information at the Table 8.2.1).

Abbreviations: ACE: Angiotensin-converting enzyme ; AchEi: acetylcholinesterase inhibitors; AH: Anti-histamine; AF: Atrial fibrillation; ARB: Angiotensin receptor blocker; BB: Beta blockers; BPH: Benign prostatic hyperplasia; BZD: Benzodiazepines; CCB: calcium-channel blockers; CKD: Chronic kidney disease; CNS: Central nervous system; GH: Growth hormone; GI: Gastrointestinal; HF: Heart failure; HFrEF: Heart failure with reduced ejection fraction; NA: Not assessed; NSAID: non-steroidal anti-inflammatory drugs; PIP: Potentially inappropriate prescription; PPI: Proton pump inhibitor; SSRI: selective serotonin reuptake inhibitor; SU: sulfonylureas; TCA: Tricyclic antidepressants.

Table 3.2.4 Prevalence of individual potentially inappropriate prescriptions (PIPs) in middle-aged adults with new-onset type 2 diabetes (T2DM) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria, stratified by polypharmacy status.

				Sensitivity Analysis (Patients with dosage information[1])	
Criteria Number	**Recommendation**	**Patients receiving PIP without polypharmacy (N=7,457)**	**Patients receiving PIP with polypharmacy (N=9,197)**	**Patients receiving PIP without polypharmacy (N=7,457)**	**Patients receiving PIP with polypharmacy (N=9,197)**
1	Avoid stimulant laxatives as first-line treatment in constipation for >4 weeks other than for opioid-induced constipation	<7	25 (0.3%)	<7	10 (0.1%)
2	Avoid PPI above recommended maintenance dose for >8 weeks	13 (0.2%)	269 (2.9%)	13 (0.2%)	269 (2.9%)
3	Avoid omeprazole and esomeprazole in combination with clopidogrel	0 (0.0%)	107 (1.2%)		
4	Avoid use of alpha-adrenoceptor blocking drugs as monotherapy for hypertension.	8 (0.1%)	<7		
5	Aspirin dose should not exceed 150 mg/ day for anti-platelet therapy	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
6	Avoid cardio selective CCB in combination with BB	0 (0.0%)	27 (0.3%)		
7	Avoid oral short-acting dipyridamole as monotherapy in antiplatelet treatment	0 (0.0%)	0 (0.0%)		
8	Avoid first-generation antihistamines as first-line treatment for >7 days.	14 (0.2%)	185 (2.0%)	9 (0.1%)	108 (1.2%)
9	Avoid theophylline monotherapy for asthma or COPD	0 (0.0%)	<7		
10	Concomitant bisphosphonate if oral corticosteroids >3 months	11 (0.1%)	113 (1.2%)	<7	30 (0.3%)
11	Avoid mucolytic agents in stable COPD	<7	65 (0.7%)		
12	Avoid SSRI + venlafaxine	0 (0.0%)	8 (0.08%)		
13	Avoid TCA as first-line treatment of depression	7 (0.09%)	101 (1.1%)		
14	Avoid BZD long term use	<7	293 (3.2%)	<7	131 (1.4%)
15	Avoid non-BZD hypnotics long term use	7 (0.09%)	245 (2.7%)	<7	127 (1.4%)

Table 3.2.4 (cont.) Prevalence of individual potentially inappropriate prescriptions (PIPs) in middle-aged adults with new-onset type 2 diabetes (T2DM) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria, stratified by polypharmacy status.

				Sensitivity Analysis (Patients with dosage information[1])	
Criteria Number	**Recommendation**	**Patients receiving PIP without polypharmacy (N=7,457)**	**Patients receiving PIP with polypharmacy (N=9,197)**	**Patients receiving PIP without polypharmacy (N=7,457)**	**Patients receiving PIP with polypharmacy (N=9,197)**
16	Avoid carbamazepine with clarithromycin or erythromycin	0 (0.0%)	<7		
17	Strong opioids should be prescribed together with laxatives	11 (0.1%)	381 (4.1%)		
18	Avoid nitrofurantoin >7 days in the treatment of uncomplicated lower UTI	10 (0.1%)	32 (0.3%)	<7	19 (0.2%)
19	Avoid use of long acting SU	0 (0.0%)	0 (0.0%)		
20	Avoid long term NSAID	11 (0.1%)	283 (3.1%)	<7	119 (1.3%)
21	Avoid combination of NSAID and 1) low dose aspirin or 2) SSRI, without adequate gastrointestinal protection	8 (0.1%)	361 (3.9%)		
22	Avoid ≥2 drugs from the same pharmacological class – Example 1: Opioids (excl. morphine and methadone)	0 (0.0%)	204 (2.2%)		
22	Avoid ≥2 drugs from the same pharmacological class – Example 2: SSRI	<7	24 (0.3%)		
22	Avoid ≥2 drugs from the same pharmacological class – Example 3: PPI	<7	40 (0.4%)		

[1] Drugs of which judgment of PIP was dependant on prescribed dose or duration, prevalence of PIPs was reported separately for patients having complete information on dose or duration.

Abbreviations: COPD: Chronic obstructive pulmonary disease; SSRI: Selective serotonin reuptake inhibitors; eGFR: Estimated glomerular filtration rate; ADR: Adverse drug reaction; ADH: antidiuretic hormone; NSAID: Non-steroidal anti-inflammatory drugs; PIP: Potentially inappropriate prescription; PPI: Proton pump inhibitors; CCB: calcium-channel blockers; BB: Beta blocker; BZD: Benzodiazepines; UTI: Urinary tract infections; SU: Sulfonylureas.

3.2.4 Discussion

This is the first study to apply the Beers and the PROMPT and list to a large population-based primary care database to evaluate the prevalence of PIPs among both older and middle-aged adults initiating therapy for T2DM with and without polypharmacy. The prevalence of PIPs was overall higher in patients receiving polypharmacy compared to no polypharmacy. While the prevalence of PIPs was higher among older patients with polypharmacy (39.6%), we found that 22.7% of middle-aged patients with polypharmacy received ≥1 PIPs. At the individual PIP level, long-term use of PPIs and strong opioids without laxatives were the most frequently identified PIPs among older patients (criteria 33) and middle-aged patients (criteria 17), respectively, with polypharmacy (11.1% and 4.1%, respectively). The results of this study highlight the frequency of PIPs among patients with T2DM and the subsequent importance of medication reviews to avoid harmful adverse events due to PIPs.

While this is the first study to apply the Beers and PROMPT criteria to patients initiating their first NIAD treatment, our results align with the existing literature.[104,128,139,143–146] The only study to date, that has applied both Beers and PROMPT in patients with T2DM receiving polypharmacy to assess PIPs, observed higher PIPs prevalence in middle-aged patients (36.9% to 39.0%) than in older patients (24.9% to 28.0%).[128] Limitations included the absence of clinical information, and, therefore, fewer criteria were assessed. The latter is particularly important for the criteria related to medication with multiple indications. While our study was also limited by missing information, we could address more criteria due to the availability of clinical parameters and synchronization of data and criteria list through a multidisciplinary team, including a clinical pharmacologist and a clinical pharmacist. For example, in PROMPT criteria number 13, the lack of information on depression diagnosis implicates the overestimation of PIPs, particularly because TCA is used within different indications. While we acknowledge the diagnosis codes for depression are subject to misclassification and are an underestimation of true prevalence, we were able to combine our prescribing data with previous diagnosis and clinical patient records to overcome this limitation.

Another study using Beers criteria to assess PIPs in elderly with T2DM and polypharmacy identified that the prevalence of PIPs was 54.5%.[139] While this is substantially higher than the 39.6% found in our study, we expect the difference is largely due to differences in the study population. The study by Formiga *et al*. included patients age ≥75 years who were admitted to the hospital, while we included all older adults initiating a NIAD in general practice with a mean

age of 73.7 years.[139] Thus, we expect that our results more likely reflect the prevalence of PIPs in the general population of older adults with diabetes.

As the number of studies assessing the Beers and PROMPT criteria in patients with T2DM is limited, we also note that our results are comparable to the reported prevalence of PIPs in the general population. Studies applying Beers and PROMPT showed PIPs prevalence ranging from 34.2% to 64.8% in older adults,[104,141,145,146] and 21.1% to 46.2% in middle-aged adults.[141,143,144] Nonetheless, it is challenging to draw an accurate one-to-one comparison with most studies, as very often, only part of the criteria was applied due to limitations on the dataset. Among the studies applying the Beers criteria, most observational studies implemented a subset of the criteria list.[147] Nevertheless, one study that implemented the entire list of criteria used administrative claims data from the USA and included patients ≥65 years, with no stratification between polypharmacy status. The study found the prevalence of PIPs ranged between 34.2% to 37.6%. As the study did not stratify by polypharmacy status, it is difficult to directly compare our results. Overall, 3,855 patients (173 without polypharmacy and 3,682 with polypharmacy) had at least one PIP, which corresponds to a PIP prevalence of 32.3% among our older adults. While this is marginally lower, than the study by Jirón *et al*., the marginal disparities between these estimates may be due to differences between US and UK clinical settings and data sources used in each study.

At the individual PIP level in older adults, the long-term use of PPIs is of particular concern as it may increase the risk of adverse drug reactions, such as chronic kidney disease, particularly in high doses,[148] and B12 deficiency.[149] Studies in primary care and emergency settings indicate that PPIs are frequently prescribed for inappropriate indications or for indications where their use provides little to no benefit.[150] Moreover, patients may stay long-term on PPIs, often indefinitely, even without a clear clinical indication.[151,152] Therefore, our findings indicate the need for carefully reviewing indications of PPIs to minimise the risk of long-term PPI-related adverse outcomes in older patients with diabetes, particularly in those receiving polypharmacy.

The use of high anticholinergic antidepressants (criteria 16 [10.2%]), and SSRIs, BZD, non-BZD hypnotics, and TCA (criteria 45 [9.8%]) in patients with a history of falls and fractures were among the most frequent PIPs in older adults found this study as well as in previous work.[128,139] Anticholinergic antidepressants may cause ADRs such as dry mouth, blurred vision, constipation, urinary dysfunction, sedation, orthostatic hypotension, and cognitive impairment, which all reduce the patient's quality of life, may lead to increased risk of falls, substantial morbidity, and even mortality.[153] Older adults have a higher sensitivity to anticholinergic effects, with might

worsen pre-existing symptoms.[154,155] Patients with dementia and depression may become more confused due to the anticholinergic side effects of some antidepressants as well as patients with orthostatic hypotension may collapse and fall. These concerns become particularly important in patients with diabetes complications, such as neuropathy, retinopathy, and diabetic cardiomyopathy.[156,157] Although anticholinergic antidepressants are not the first-line pharmacological therapy for treating depression according to the clinical guidelines in the UK,[158] we found high prevalence of drug prescription in older patients. While further research on the appropriateness is needed, our results indicate that clinicians do not withhold from prescribing highly anticholinergic antidepressants to older patients with diabetes and polypharmacy, despite their potential to cause ADRs and the availability of safer alternatives.

The use of SSRIs, BZD, non-BZD, and TCA is associated with an increased risk of falls and fractures,[159–161] a primary concern for elderly adults with T2DM. These drugs are often prescribed to the elderly for prolonged periods, without the repeat prescription being reviewed.[161,162] BZD have long been recognised as a contributory factor for falls. Non-BZD hypnotics are considered safer than BZD, however, they have an increased risk for falls when used in long-term.[160] Notably, in the UK, TCA drugs such as amitriptyline, prescribed in low doses for longer term, are considered appropriate to treat neuropathic pain.[163] Thus, although indications cannot be determined from our dataset, the high prevalence of TCA prescription in patients with T2DM may be, at least partially, for its use in pain management. Additionally, despite warnings on the clinical guideline for treating depression in the UK regarding the use of antidepressants in older people with increased risk of falls and fractures,[158] our study indicates that clinicians do not refrain from prescribing these drugs to older patients with a history of falls and fractures which are risk factors for new events. This is of particular concern in older patients with T2DM: besides the risk of falls and fractures increase with age,[164] complications of diabetes (e.g., neuropathy and vision loss) and NIAD treatment adverse effects (e.g., hypoglycaemia in patients receiving SU), can further increase this risk.[165]

At the individual PIP level in middle-aged adults, this study indicates that opioid-related constipation may not be adequately prevented by clinicians, despite being a significant concern and burden to patients.[166] Nonetheless, a possible reason for not adding a laxative drug to opioid treatment could be that patients already benefit from laxatives as over-the-counter (OTC) drugs, and thus, we may have missed those patients in the primary care dataset.

The combination of NSAIDs and SSRIs or low-dose aspirin without gastroprotection (3.9%) was also identified to be broadly used in middle-aged adults receiving polypharmacy. While drug

combinations with NSAIDs, such as SSRIs and aspirin,[167–169] are risk factors for upper gastrointestinal bleeding (UGIB), the risk of outcomes associated with these two combinations is attenuated by concomitant use of a PPI.[169] Thus, this study indicates the need for clinical improvement and may help clinicians in tailoring therapy to minimise the risk of UGIB in middle-aged adults with T2DM starting their antidiabetic treatment.

Many of the most frequent PIP criteria in this study relate to the inappropriate duration of medication use. Although short-term use of medications may confide a different risk-benefit ratio than long-term use and may not be considered inappropriate in specific conditions, the high prevalence of the most frequent PIPs found in our study is not compatible with only appropriate drug prescriptions. To approach that, documenting and communicating the intended treatment duration or planned review dates in the medical records can provide valuable information to clinicians, facilitating regular review and discontinuation of inappropriate prescriptions.[170] Moreover, open communication with patients about prescription duration can also help manage expectations and reduce resistance to changing medication regimens.

While GPs may be less likely to discontinue drug prescriptions from secondary care, they play a critical role in conciliating prescriptions from different settings as well as evaluating the benefit of continuing drugs based on a risk-benefit assessment and their patient needs. The lack of evidence and the challenges in assessing treatment benefits and harms were previously identified as barriers to appropriate prescribing, particularly in older patients with multiple comorbidities.[170] To address that, evidence-based guidelines have been developed to support deprescribing of specific drugs, such as PPIs and BZD, which were among the most prevalent PIPs in this study.[171,172] These guidelines may offer valuable guidance to optimize medication regimens, enhance patient safety, and improve outcomes.

A key strength of this study is the detailed insights into the prevalence of PIPs in patients with new-onset T2DM, applying the 2015 AGS Beers criteria adapted to the UK to 65-year-olds and older and the PROMPT criteria to 45- to 64-year-old patients. The availability of primary care clinical data allowed for a more thorough assessment of PIPs specifying conditions in patients with diabetes compared to previous studies.[128,139] The IMRD allowed studying a large number of patients starting a NIAD treatment who are representative of the UK population. In the UK, GPs are central players managing chronic diseases, coordinating drug prescriptions that may be initially prescribed in secondary care, and thus have a favourable position to assess their patients' medications across diseases. The sensitivity analysis confirmed the robustness of the

results, where assumptions were necessary. Another strength is the availability of a clear and detailed definition of each criterion used in this study, which can be applied in future studies to assess PIPs using large population-based databases, facilitating interpretation and comparability between studies.

Our study also has some limitations. First, the Beers criteria is an extensively used and validated explicit process measure developed in the US with the intention of providing a valuable tool for assessing the quality of prescribing in older adults, regardless of their place of residence and their level of frailty. Thus, to accommodate the differences between the US and UK drug availability, we adapted the Beers criteria by including drugs from the British National Formulary, as previously published by Ble *et al*. We note that while several drugs in the Beers criteria are listed as PIPs, they are not inappropriate in older adults according to the National Institute for Health Care and Excellence (NICE) clinical guidelines in the UK (e.g., amitriptyline, clomipramine, desipramine, and nortriptyline are indicated for management of depression,[158] and nitrofurantoin long-term low dose prophylaxis of recurrent urinary tract infection[173]), whereas other non-recommended drugs (e.g., theophylline as monotherapy for asthma and COPD) are omitted. Similarly, the Beers criteria do not consider varying indications for certain drugs (e.g. amitriptyline for the management of neuropathic pain in the UK).[174] Thus, while Beers identifies those as PIPs, we acknowledge that this may overestimate inappropriate use in the UK. On the other hand, our estimation of PIP prevalence using Beers is likely to be more conservative, as Beers seems to underestimate PIP compared to explicit methods tailored to the context of Europe, such as Screening Tool of Older Persons' Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) criteria.[139,175] Moreover, although Beers was not tailored to the context of Europe prescribing, clinically relevant issues associated with the inappropriate use of drugs identified using Beers remain relevant in Europe. Finally, Beers was readily adapted to our dataset compared to the STOPP/START criteria.

In addition to the above, we expect that we have likely underestimated medication use, and subsequently PIPs due to limitations with the IMRD. The IMRD only captures drug prescriptions made by GPs. Thus, we were not able to identify OTC drugs, such as NSAIDs and laxatives. Additionally, drugs given during hospitalisation and specialist prescriptions are not captured within the database.

Another limitation is that the Beers list was not created for use in observational data, and therefore, not all of the criteria could be applied due to limitations in our observational data. Failure to apply the full criteria list may have resulted in lower estimates of the overall PIPs

prevalence compared to other studies, as well as overestimation of individual PIPs whenever it was partially applied. A complete overview of the limitations associated with each specific criterion are available in **Table 8.2.1** and **Table 8.2.2**. Similarly, due to missing data on some clinical parameters, not all patients had complete information. To overcome this limitation, we conducted a sensitivity analysis among only those patients with complete information, and results remained largely similar.

Finally, while the DDD by the ATC index does not necessarily correspond to the recommended daily dose, we had no access to the indication of drugs due to limitations on our dataset, and thus the exclusive use of BNF formulary as a source of standard daily dose to estimate CD was not feasible. Nevertheless, as the ATC index is freely available worldwide and allows for comparisons of drug consumption at an international level, the use of the DDD ATC index represents a valuable alternative to estimate CD.

3.2.5 Conclusion

This analysis using primary care data revealed opportunities for optimizing pharmacotherapy in patients newly treated with oral antidiabetics. While the burden of polypharmacy, and thus PIPs, increases with age, this problem is not exclusive to the elderly. Among patients with polypharmacy, 39.6% of older adults had at ≥1 PIP, compared to 22.7% of middle-aged adults with ≥1 PIP. Antidiabetic therapy carries its own challenges and risks, emphasizing the importance of minimizing risks associated with existing medications. Given the challenges of avoiding polypharmacy in adults aged ≥45 years with diabetes, it is crucial to implement measures that review indications, address drug interactions, and consider comorbidities, reducing the likelihood of inappropriate prescribing. Initiating treatment with NIADs in patients with polypharmacy should trigger a comprehensive medication review to optimize prescribing decisions.

Chapter 4

Analytical Drug Safety Analyses

Chapter 4.1

Identification of novel off-targets of baricitinib and tofacitinib by machine learning with a focus on thrombosis and viral infection

Authors

Maria L. Faquetti, Francesca Grisoni, Petra Schneider, Gisbert Schneider, Andrea M. Burden

Remarks

Maria L. Faquetti contributed to the design of the computational workflow, performance of experiments, formal analysis, results interpretation, drafting of the manuscript, and critical revisions.

Publication

Faquetti ML *et al*. Identification of novel off targets of baricitinib and tofacitinib by machine learning with a focus on thrombosis and viral infection. *Scientific Reports.* 12, 7843 (2022). https://doi.org/10.1038/s41598-022-1187

4.1.1 Introduction

Janus Kinase (JAK) (EC number 2.7.10.2) inhibitors are novel targeted synthetic disease-modifying antirheumatic drugs. The new class of small molecule drugs represents an important alternative to treat moderate-to-severe rheumatoid arthritis (RA) patients with non- or inadequate response to conventional synthetic disease-modifying antirheumatic drugs and biological disease-modifying antirheumatic drugs.[77] The JAK inhibitors target one or more kinases of the JAK family (JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase TYK2) and inhibit multiple pro-inflammatory cytokines critical to the pathogenesis of autoimmunity, such as interleukin (IL)-6, IL-10, and interferon (IFN)-γ.[176–178] Baricitinib (JAK1/JAK2 inhibitor) and tofacitinib (JAK1/JAK3 inhibitor) are the first members of this class approved in the United States (US) and Europe to treat RA (**Figure 4.1.1**).

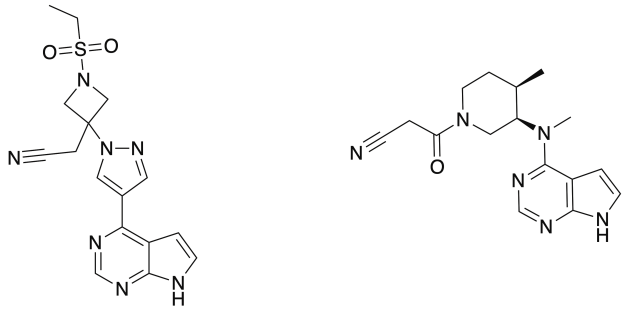


Figure 4.1.1 Chemical structure of baricitinib and tofacitinib. The two drugs were the first Janus Kinase (JAK) inhibitors to receive approval in the United States and Europe to treat rheumatoid arthritis.

Safety concerns associated with JAK inhibitors, such as the increased risk for thrombosis and viral infection or reactivation, have emerged worldwide, and boxed warnings are included on all approved JAK inhibitors used to treat inflammatory conditions.[72,81,179–183] While a dose-response effect was observed in the risk of thrombosis in clinical trials of both baricitinib and tofacitinib, there is no known mechanism associated with the pharmacological on-target effect that could explain the risk of thrombosis associated with baricitinib and tofacitinib. Although the use of baricitinib and tofacitinib is expected to increase infections due to modulation of IFNs,[64] the incidence of Herpes Zoster (HZ), particularly associated with JAK inhibitors drugs, remains unclear.[184,185] Thus, the increased risk of these safety concerns is heavily debated.

It is well established that unintended off-target activity may interfere with multiple biological processes, inducing undesired side effects.[186] Nevertheless, given the complexity of the human proteome, complete elucidation of all biological targets of a drug before its entrance into the market is often unfeasible. In this context, machine learning can be used to predict the potential for an approved drug to interact with off-targets and identify potential safety-related concerns.[187] The identification of additional drug-target interactions using chemo-centric and machine learning approaches and experimental confirmation may help to determine mechanisms of adverse drug events.[188,189] For example, previously unknown drug-target interactions for the approved compound Celecoxib were identified using a ligand-based method, which is based on the principle that structural similarity reflects functional similarity, supporting the biological plausibility of reported cardiovascular adverse drug events.[51,190] Moreover, off-target profiling is frequently used to identify candidate drugs for repurposing. For example, computational studies using machine learning identified baricitinib as a promising JAK inhibitor for repurposing in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or COVID-19).[191,192] Baricitinib was considered a potential candidate for repurposing in COVID-19 based on the high affinity for AP-2-associated protein kinase 1 (AAK1) (EC number 2.7.11.1), which is critical in regulating viral endocytosis, and its inhibition may reduce the ability of the virus to infect lung cells.[193]

Improving our understanding of the target space of JAK inhibitors drugs is essential in order to explain the mechanisms of unexpected side effects associated with these drugs and to identify opportunities for repurposing. Although several binding screens of tofacitinib and baricitinib have been published, they are mostly limited to a few protein families, such as protein kinases and lipid kinases.[82,194,195] Thus, the community would benefit from a more extensive characterization of the target profile of tofacitinib and baricitinib.

In light of the currently unexplained thrombotic and viral infection risk and the previously observed off-target binding potential of baricitinib, here we investigate if the thrombosis and viral infection/reactivation risk may be a result of an off-target effect. We, therefore, aimed to leverage well-established machine learning methods to identify off-target drug-protein interactions for baricitinib and tofacitinib and validate such predictions *in vitro* assays. Previously unknown off-targets of baricitinib and tofacitinib were predicted and confirmed drug-target interactions suggest an attenuation of pulmonary vascular remodelling, modulation of Hepatitis C (HCV) viral response, and hypomagnesemia. Nevertheless, the identified off-target

interactions could not explain the elevated thrombosis or viral infections/reactivation risk. These results suggest both JAK inhibitors as potential candidates for drug repurposing.

4.1.2 Materials and methods

4.1.2.1 Data preparation and molecular representation for target prediction

Baricitinib and tofacitinib were provided as Simplified Molecular Input Line Entry Specification (SMILES) and processed in KNIME v3.7.2[196] with the MOE v.2019.0102[197] "wash" function employing the following options: "disconnect salts", "remove lone pairs", "deprotonate strong acids", "remove minor component", "protonate strong bases," and "add hydrogen". Chemically advanced template search version 2 (CATS2)[198] descriptors and two-dimensional MOE descriptors ('QSAR descriptors' node of KNIME; 'Forcefield'=MMFF94*) were calculated for all generated molecules and used as input for the target prediction tools.

4.1.2.2 Macromolecular target prediction and selection

Target Inference Generator ([TIGER v. 19.07], inSili.com. LLC, Zurich)[51] and Self-organizing map (SOM)-based prediction of drug equivalence relationships (SPiDER)[199] softwares were used for target activity prediction. Targets with statistically meaningful predictions from SPiDER ($p<0.05$) and/or TIGER (score>1) were selected for *in vitro* characterization if they were considered to have a potential influence on thrombosis or viral infection/reactivation.

4.1.2.3 *In vitro* characterization

Baricitinib (99.97% purity) and tofacitinib (99.96% purity) compounds were purchased from MedChem Express LLC (New Jersey, www.medchemexpress.com). *In vitro* characterization was performed on a fee-for-service basis at Eurofins (www.eurofins.com) if the assay was commercially available. For the biochemical assays, compound targets showing an experimental readout greater than 25% (inhibition or stimulation) at 30 µM were selected for follow-up, and dose-response curve characterization and determination IC_{50}/EC_{50} (two or three replicates, multiple concentrations, maximum 100 µM concentration). Additional details on the conduct of *in vitro* assays are included in the Appendix.

4.1.2.4 Computational ligand docking

Protein crystal structures of Serine/threonine-protein kinase N2 (PKN2) (PDB ID: 4CRS)[200] and Phosphodiesterase 10A (PDE10A) (PDB ID: 5C28),[201] were retrieved from the worldwide Protein Data Bank (wwPDB, https://www.rcsb.org/) and prepared for docking using MOE software (v.2019.0102),[197] applying MOE QuickPrep ('Delete Water Molecules Farther than 4.5 Å from Ligand or Receptor'=True; 'Retain QuickPrep Minimization Restraints'=True;), and MOE minimize for energy minimization with Amber10:EHT. After the model quality inspection by Ramachandran plots, all compounds were standardized at a pH of 7 prior to docking using MOE. Crystalized ligands and JAK inhibitors were docked using the software GOLD (v. 5.5)[202] within MOE software (v.2016.08)[197] (Efficiency='default', Score Efficiency=100; Early Termination=[number:3, RMS=1.5]), using either GoldScore (PDB ID: 4CRS) or Piecewise linear potential (PLP; PDB IDs: 5C28) as scoring functions (Rigid Receptor). The poses were refined with MOE GBVI/WSA dG. For each ligand, 90 poses were generated, and 15 were refined and scored using the assigned scoring function.

The scoring function for each macromolecular target considered (GoldScore or PLP) was chosen based on a re-docking analysis (i.e., the scoring function minimizing the root mean square deviation [RMSD] of the crystallized ligand was selected). Re-docking of the crystallized ligand phosphothiophosphoric acid-adenylate ester in the binding site of PKN2 led to led to a RMSD value of 0.80 Å for 4CRS while re-docking of 6-chloro-2-cyclopropyl-5-methylpyrimidin-4-amine in the binding site of PDE10A led to a value of RMSD=0.23 Å for 5C28. JAK inhibitors were docked into the crystalized structure (baricitinib and tofacitinib using GoldScore scoring function on PKN2 [PDB ID: 4CRS], and baricitinib using PLP scoring function on PDE10A [PDB ID: 5C28]) and the minimum energy pose was chosen for the analysis.

4.1.2.5 Statistical Analysis

4.1.2.5.1 Transient Receptor Potential Cation Channel Subfamily M member 6 (TRPM6)

The binding constant (K_d) was calculated with a standard dose-response curve using the Hill equation (**Equation 4.1.1**):

$$Response = Background + \frac{Signal - Background}{1 + (K_d^{Hill\ Slope} / Dose^{Hill\ Slope})} \qquad \textbf{(Equation 4.1.1)}$$

The curve was fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm using RStudio Team (2020) v. 1.3.1073 (RStudio, PBC, Boston, MA, http://www.rstudio.com/).

4.1.2.5.2 Protein Kinase N2 (PKN2)

The IC_{50} values for baricitinib and tofacitinib were determined by a non-linear, least squares regression analysis using RStudio Team (2020) v. 1.3.1073 (RStudio, PBC, Boston, MA, http://www.rstudio.com/). Inhibitory constant (*Ki*) values were estimated from experimental IC_{50} values using a web-based tool, based on the equation of Cheng and Prusoff[203] and the observed IC_{50} of the tested compound.

4.1.2.5.3 Phosphodiesterase 10A2 (PDE10A2)

The IC_{50} value and the standard error of the mean (SEM) were determined by a non-linear, least squares regression analysis using GraphPad Prism Version 9.0.2 for Macintosh (GraphPad Software, San Diego, California USA, https://www.graphpad.com). The dose-response curve was plotted using RStudio Team (2020), v. 1.3.1073 (RStudio, PBC, Boston, MA, http://www.rstudio.com/). Inhibitory constant (*Ki*) values were estimated from experimental IC_{50} values using a web-based tool, based on the equation of Cheng and Prusoff[203] and the observed IC_{50} of the tested compound.

4.1.3 Results

4.1.3.1 Off-target profiling of baricitinib and tofacitinib by machine learning revealed additional drug-target interactions

Macromolecular targets of baricitinib and tofacitinib were predicted using two previously published machine learning approaches: Target Inference Generator (TIGER)[51] and SOM-based Prediction of Drug Equivalence Relationships (SPiDER).[199] Both approaches follow the chemical similarity principle, in which molecules sharing similar structures are likely to have similar bioactivity.[204]

SPiDER uses a neural network (SOM), and drug-target relationships are inferred based on the descriptor similarity of a query compound to reference ligands without directly considering the target similarity. The method uses topological and physicochemical information about molecules to suggest a functional similarity between compounds. TIGER extends SPiDER using a

more extensive set of targets, as well as a different prediction algorithm and scoring function. Both approaches have been extensively applied to de novo-designed compounds, natural products with biological activity, and approved drugs.[205–207]

Overall, 40 potential targets for baricitinib and 58 for tofacitinib (SPiDER [p<0.05] and/or TIGER [score>1]) were predicted by TIGER and SPiDER. The list of predicted targets accompanied by the score for TIGER, and p-values for SPiDER, are shown in **Table 8.3.1** and **Table 8.3.2**, respectively. The cut-off values were chosen based on recent prospective studies where the target prediction tools led to bioactivity confirmation *in vitro*.[51,208,209] The resulting predictions reflect both known and unknown potential drug-target interactions. From all predicted targets, nine targets for baricitinib and eight for tofacitinib were identified as being relevant for thrombosis and viral infection/reactivation (**Table 4.1.1**).

4.1.3.2 *In vitro* characterization confirmed previously unknown baricitinib and tofacitinib drug-target interactions

Of the 98 predicted, a total of 11 drug-target interactions were experimentally validated using biochemical or cell-based assays, based on the availability of fee-based *in vitro* testing services (**Table 4.1.2**). Among the predicted targets, two members of the Transient Receptor Potential superfamily of calcium channels were suggested, namely short transient receptor potential channels 6 (TRPC6) and 3 (TRPC3). Commercial assays were unavailable for TRPC6 or TRPC3, and therefore, these targets could not be tested. Instead, transient receptor potential cation channel subfamily M member 6 (TRPM6) (EC number 2.7.11.1) was employed for the respective binding assays. Additionally, while serine/threonine-protein kinase N2 (PKN2) (EC number 2.7.11.13) was among the predicted targets for tofacitinib but not for baricitinib, PKN2-baricitinib binding affinity was previously determined in baricitinib (apparent dissociation constant [$K_{d\ app}$]=269 nM and IC_{50}=284 nM).[82] Thus, PKN2 was included in the list of targets tested for baricitinib, allowing a direct comparison between tofacitinib and baricitinib inhibitory activity on this target in the same experimental conditions.

From the 11 drug-target interactions tested, five showed an experimental readout greater than 25% drug-target interaction at 30 □M and were selected for further *in vitro* characterization (**Table 4.1.2**). Four out of five drug-target interactions were confirmed by further *in vitro* evaluation, with IC_{50} and K_i or K_d values in the nanomolar range (baricitinib and tofacitinib on PKN2) and in the micromolar range (baricitinib on Phosphodiesterase 10A2 (PDE10A2) EC number 3.1.4.17; tofacitinib on TRPM6). The investigated drugs were considered

as active if the determined IC_{50} was lower than 30 □M. The raw *in vitro* data for drug-binding activity using biochemical assays is available in **Table 8.3.3**. Dose-response curves for targets showing activity are available in **Figures 8.3.1 – 8.3.3**.

Table 4.1.1 Suggested targets with impact on thrombosis and viral infection per Janus Kinase (JAK) inhibitor drug and target prediction approach.

Drug	Predicted target	Approach		Thrombosis	Viral infection/ reactivation
		TIGER	SPiDER		
Baricitinib	Protein Kinase C Beta (PKC-β)	x		x	
	Adenosine Receptor A2A (AA2AR)	x		x	
	Inducible Nitric Oxide Synthase (iNOS)	x		x	
	Phosphodiesterase 10A (PDE10A)	x	x	x	
	Ras Related Protein Rab-7a	x		x	
	Epidermal growth factor receptor (EGFR) kinase	x			x
	Deoxycytidine kinase (DCK)	x			x
	Serine/threonine-protein kinase N2 (PKN2) [a]		x		x
	Thymidine kinase (HSV) [b]	x			x
Tofacitinib	Arachidonate 15-Lipoxygenase (15-ALOX)	x		x	
	Adenosine Receptor A2A (AA2AR)	x		x	
	Short transient receptor potential channel 6 (TRPC6)	x		x	
	Short transient receptor potential channel 3 (TRPC3)	x		x	
	Adenosine Receptor A3 (ADORA3)	x		x	
	Exportin-1 (XPO1)	x			x
	Serine/threonine-protein kinase N2 (PKN2)	x	x		x
	Ubiquitin-conjugating enzyme E2 N (Ubc13)	x			x

[a] PKN2 was included in the list of targets tested for baricitinib, which allowed us to make a direct comparison between tofacitinib and baricitinib inhibitory activity on this target.

[b] Human herpesvirus 1 (strain SC16).

Note: Commercial assays were unavailable for TRPC6 or TRPC3, and therefore, these targets could not be validated. Instead, transient receptor potential cation channel subfamily M member 6 (TRPM6) was employed for the respective binding assays.

Table 4.1.2 *In vitro* findings for baricitinib and tofacitinib off-target activity.

Drug	Safety issue	Target	IC_{50} (□M) [a]	K_i or K_d (□M)
Baricitinib	Thrombosis	Adenosine Receptor A2A (AA2AR) [b]	Inactive	n.d.
		Inducible NOS (iNOS)	Inactive	n.d.
		PI3 Kinase (p110b/p85a)	Inactive	n.d.
		Phosphodiesterase 10A2 (PDE10A2)	28 □ 2 [d][e]	K_i=6.1
	Viral infection	Serine/threonine-protein kinase N2 (PKN2)	0.24, 0.21 [e]	K_i=0.082, 0.069 [e]
		Epidermal growth factor receptor (EGFR)	Inactive	n.d.
Tofacitinib	Thrombosis	Adenosine Receptor A3 (ADORA3) [b,c]	Inactive	n.d.
		Arachidonate 15-lipoxygenase (15-ALOX)	Inactive	n.d.
		Transient receptor potential cation channel subfamily M member 6 (TRPM6)*	n.d.	K_d=6.1, 7.7 [e]
		Adenosine Receptor A2A (AA2AR) [c]	Inactive	n.d.
	Viral infection	Serine/threonine-protein kinase N2 (PKN2)	0.71, 0.74 [e]	K_i=0.24, 0.25 [e]

[a] JAK inhibitors were tested at a concentration of 30 □M. During follow-up experiments, JAK inhibitors were tested in multiple concentrations (top concentration of 100 □M) for dose-response curve characterization and determination of IC_{50}/EC_{50} (two or three replicates).

[b] Antagonistic effect.

[c] Agonistic effect.

[d] Values are the mean □ standard error of the mean (SEM) for the number of replicates (n) >2.

[e] For n=2, no averaging was made, and both values are presented.

n.d.: not determined.

*Commercial assays were unavailable for TRPC6 or TRPC3, and therefore, these targets could not be validated. Instead, transient receptor potential cation channel subfamily M member 6 (TRPM6) was employed for the respective binding assays.

Note: All *in vitro* testing was performed on a fee-for-service basis at Eurofins Cerep (www.eurofins.com).

4.1.3.3 Computational ligand docking predicted potential modes of baricitinib and tofacitinib-target interaction

Computational ligand docking (**Figure 4.1.2**) predicted potential modes of interaction (i.e., three-dimensional orientations of the drug molecule and a target) for baricitinib and tofacitinib in the binding pocket of the identified macromolecular targets (PKN2 [PDB-ID: 4CRS34]; PDE10A [PDB-ID: 5C2835]) using docking algorithms, and it provided the respective score for each orientation predicted. TRPM6 was not considered due to the unavailability of an experimentally determined structure.

Molecular docking of baricitinib to PKN2, shown in **Figure 4.1.2** (**a**), suggests an interaction between the nitrile nitrogen on the drug structure and Mg^{2+}. Similar to the crystallized ligand, the two residues, Phee668 and Gly666, are hydrogen donors and interact with one oxygen from the sulfonyl group. Additionally, two arene-H interactions between pyrrole on the tofacitinib structure and the hydrogen on the amino groups of PKN2 residue Gly666 were suggested in **Figure 4.1.2** (**b**). A third arene-H interaction between pyrimidine on the tofacitinib molecule and the hydrogen on the amino groups of Lys686 was indicated. Like the crystallized ligand, an interaction with Phe668 is predicted. Additionally, interactions between Mg^{2+} and two nitrogen atoms are suggested.

In **Figure 4.1.2** (**c**), the Gln716 is making a hydrogen bond, donating a bond to the core pyrimidine nitrogen. Another interaction is suggested between the pyrimidine core of baricitinib and the protein - a π-stacking interaction with the key residue Phe719. Some structural equivalence between baricitinib and the co-crystallized ligand is observed, such as the pyrimidine core in the earliest and the aromatic ring in the latest. The interactions with Gln716 and Phe716 are key for recognition of PDE10A inhibitors by the enzyme.[210]

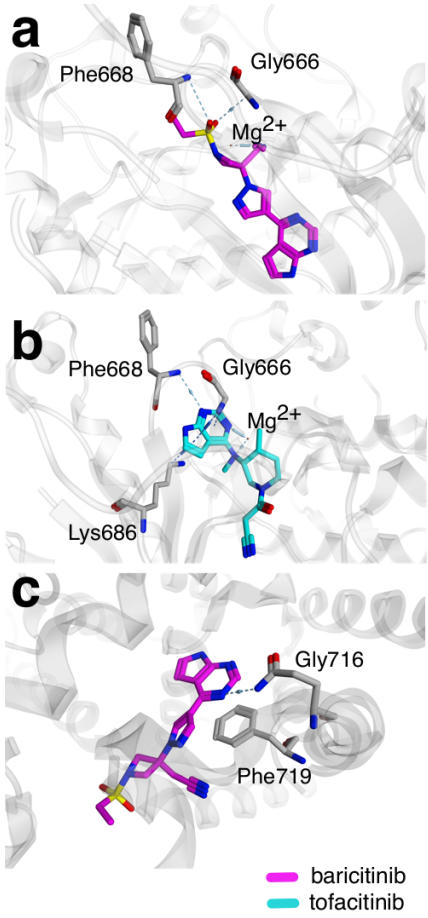


Figure 4.1.2 Predicted docking pose of baricitinib and tofacitinib on the identified targets. Predicted docking pose of baricitinib (**a**) and tofacitinib (**b**) in the binding site of PKN2 (PDB-ID: 4CRS[200]). Predicted binding pose of baricitinib (**c**) in the binding site of PDE10A (PDB-ID: 5C28[201]).

4.1.4 Discussion

Both baricitinib and tofacitinib were confirmed as "promiscuous drugs" since they bind to proteins from families other than the primary therapeutic target.[51] Thus, both drugs may be potential candidates for adverse drug effects and further repurposing. The confirmed drug-target interactions suggest an attenuation of pulmonary vascular remodelling (inhibition of PDE10A), modulation of Hepatitis C (HCV) viral response (inhibition of PKN2), and hypomagnesemia (inhibition of TRPM6). Therefore, we did not identify off-target interactions that could explain the elevated thrombosis or viral infections/reactivation risk observed in the clinical setting.[72,211,212]

The thrombotic and cardiovascular risk associated with JAK inhibitors remains debated, which is largely due to a lack of a clear mechanism associated with the therapeutic target that could explain the increased risk. In our analysis, we aimed to investigate if there may be a plausible off-target interaction that could explain the observed effects. While the computational approaches identified several targets relevant for blood coagulation and platelet aggregation (e.g., Adenosine receptor A2A [AA2AR] and Arachidonate 15-lipoxygenase [15-ALOX] EC number 1.13.11.33), neither baricitinib nor tofacitinib was found to interact with those receptors *in vitro*, ruling them out as potential off-targets.

Nonetheless, the drugs were shown to inhibit two targets related to thrombosis – PDE10A and TRPM6. PDE10A, which was recently validated as a novel target to treat pulmonary arterial hypertension (PAH) due to its central role in progressive pulmonary vascular remodelling,[210,213] was identified as a target of baricitinib. The preliminary *in vitro* results of this study showed moderate inhibition of baricitinib for PDE10A2. Molecular docking in the active site of PDE10A (**Figure 4.1.2** [**c**]) suggested a similar binding pose of baricitinib to the crystallized inhibitor (PDB ID: 5C28), with a predicted π-stacking interaction with the Phe719 residue, crucial for biological activity.[214] Additionally, among important regions for ligand binding is the occupation of a hydrophobic clamp formed by two phenylalanine residues, Phe719 and Phe686. The arene-H-type interaction between the pyrazole structure in baricitinib and Phe686 residue while occupying the hydrophobic clamp suggests that baricitinib has a similar binding mode to PQ-10, a papaverine analogue having IC_{50} equal to 6 □M.

Clinically, PDE10A inhibition is expected to decrease the risk of thrombosis, particularly in patients with PAH. Thus, the expected positive clinical impact of PDE10A inhibition on the risk of thrombosis is not in line with a potential link to an elevated thrombosis risk. Rather, baricitinib might improve progressive pulmonary vascular remodelling.

This study further identified previously unknown off-target interactions of tofacitinib with TRPM6, with moderate binding affinity. While our computational approach identified TRPC6 and TRPC3 as potential targets, we were unable to experimentally validate these targets due to a lack of commercially available *in vitro* assays. Thus, we can only speculate that the binding affinity observed with TRPM6 may translate to binding in TRPC6 and TRPC3. Additional experiments are needed to confirm if the C subfamily is also a potential target of tofacitinib. This aspect is important as TRPC6 is known to regulate human clot retraction, physiological hemostasis, and thrombus formation, and its inhibition is thought to have a positive effect on thrombotic outcomes.[215] Thus, further research is needed to confirm if tofacitinib binds to TRPC6.

Cumulatively, the active targets in this study suggest that JAK inhibitors may have a beneficial effect on cardiovascular risk, and therefore do not support a hypothesis that the risk of thrombosis is related to an off-target drug effect (in the framework of the macromolecular targets investigated in this study). Nevertheless, we note that recent US-based cohort studies have identified no difference in thrombosis risk between tofacitinib and tumour necrosis factor (TNF)-α inhibitors,[216–218] thereby suggesting that much of the observed risk seen in pharmacovigilance studies may be due to underlying risk factors rather than a drug effect.[71,179] For example, standardized incidence rates (IR) of venous thromboembolism or pulmonary embolism were comparable among patients with RA using tofacitinib (IR=1.05 [0.78-1.39]) and biological disease-modifying antirheumatic drugs (bDMARDs; IR=0.94 [0.85-1.03]) within MarketScan database cohorts.[218] Conversely, recent analysis using pharmacovigilance data of the US FDA Adverse Event Report System (FAERS) did not identify a signal of disproportionate reporting for venous thromboembolism and/or pulmonary embolism events with tofacitinib.[218] Therefore, an improved understanding of the underlying risk factors for thrombosis in patients with JAK inhibitors is urgently needed.

The risk of thrombosis can be further increased in RA patients with high disease activity, cardiovascular risk factors (e.g., obesity), immobility, and hormonal replacement therapy.[219,220] Patients using JAK inhibitors frequently have high disease activity with non- or inadequate response to conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and bDMARDs and multiple chronic conditions (e.g., cardiovascular disorders and depression), which can make the attribution of thrombotic events in patients treated with JAK inhibitors even more intricate.

In addition to thrombosis, targets related to viral infection and viral reactivation were investigated. Therapies targeting the JAK family of enzymes may interfere with a normal antiviral response, including inhibition of IFN-γ activity, and may potentially increase the risk of infection/ reactivation of several viral infectious diseases, particularly HZ in patients with RA[184,212] The computational approaches identified several targets expected to play a role in viral endocytosis and viral response, including epidermal growth factor receptor (EGFR) kinase (EC number 2.7.10.1) and PKN2. Although baricitinib and tofacitinib were not found to interact with EGFR in our experimental setting used, both showed PKN2 inhibitory activity.

Clinically, PRK2 is of great importance as a target for antiviral therapy, particularly anti-HCV, as its suppression leads to viral replication blockage in humans. PKN2 inhibitors, in combination with other antiviral therapies, have demonstrated synergistic antiviral activity for chronic HCV treatment.[221,222] While, to date, three studies have evaluated tofacitinib binding activity on PKN2, the results are contradictory.[82,194,223]

The preliminary *in vitro* results of the current study suggest PKN2 inhibition with both baricitinib and tofacitinib, as the IC_{50} and K_i values are in the nanomolar range. Moreover, the molecular docking in the PKN2 crystalized structure suggested a similar binding mode, shape, and certain molecular features (i.e., pharmacophore) of baricitinib and tofacitinib (**Figure 4.1. 1**) as to the co-crystalized ligand at the protein binding site. The model indicates that the two drugs interact with the Mg^{2+} similarly to the crystallized ligand – a PKN2 inhibitor – on the kinase functional pocket.[224] Similar to the crystallized ligand, each Phe668 and Gly666 as hydrogen donors and interact with one oxygen from the sulfonyl group in the baricitinib molecule, suggesting the role of this group for drug anchoring in the binding pocket. Additionally, the Phe668 residue backbone interacts with one nitrogen from the pyrimidine in tofacitinib, suggesting a similar binding mode to the crystallized ligand on the active pocket of PKN2. However, the impact of PKN2 inhibition is proposed to have a positive effect on viral suppression,[222] and therefore does not explain the elevated risk of HZ in RA patients. The exact mechanism of HZ viral reactivation remains unclear.

Outside of its role in viral suppression, PKN2 may play an essential role in various cellular processes, such as cellular proliferation, migration, and signalling pathways.[225–227] Moreover, PKN2 is involved in autoinflammatory disorders,[228] heart failure,[229] and it is a target of interest in cancer.[227,230,231] As concerns regarding the risk of malignancy and major adverse cardiovascular events (MACE) in patients treated with tofacitinib have been raised by the European Medicines Agency, it is important to consider the potential role of PKN2 inhibition.[232]

However, in mice models, PKN2 activation was the cause of cardiac dysfunctions,[229] and therefore, the clinical impact of PKN2 inhibition is contradictory to the risk of cancer and MACE in RA patients.

Off-target profiling using computational approaches has been widely used to identify candidates for drug repurposing.[233,234] Indeed, JAK inhibitors were recently established as potential candidate therapies for SARS-CoV-2 based on in silico methods.[235–237] Our computational methods identified 98 drug-target predictions, and the preliminary *in vitro* results found inhibitory activity on several proteins other than the primary therapeutic target, thereby confirming baricitinib and tofacitinib as promiscuous drugs and candidates for drug repurposing studies. For example, PDE10A inhibition has been primarily studied in psychiatric and neurological conditions, such as schizophrenia[238] and Huntington's disease,[239] and, to a lesser extent, in multiple peripheral pathological conditions (e.g., osteogenic differentiation).[240,241] Additionally, PDE10A inhibition by baricitinib is hypothesized to have a synergistic pharmacological effect in combination with other COVID-19 treatments (e.g., antiviral and corticosteroids drugs) due to the anti-fibrotic and anti-inflammatory effects of phosphodiesterase's inhibitors on the treatment of COVID-19 and its associated conditions (e.g., thrombosis, inflammation, and fibrosis).[242,243] Therefore, the confirmed PDE10A inhibition identified in this study supports the potential for baricitinib as a potential candidate outside of rheumatology.

Moreover, while TRPM6 was not initially predicted, the moderate inhibitory activity is worth investigating. TRPM6 inhibition is not fully elucidated. However, it is mainly involved in magnesium homeostasis in the intestine and kidney,[244,245] and it has been demonstrated to have expression levels modulated by hormones such as estrogen[246] and angiotensin II,[247] immunosuppressant,[248] and diuretics drugs,[249] and epidermal growth factor (EGF).[250] Moreover, the decreased expression of TRPM6 in cancer patients treated with EGFR-targeted therapies (e.g., cetuximab) seems to positively contribute to the oncologic efficacy of these therapies, as decreased magnesium availability inhibits cell proliferation and slows down tumour growth.[250,251]Thus, we encourage further investigation into the clinical relevance of TRPM6 inhibition by tofacitinib in oncology.

The results also highlight the complementarity of the two approaches, TIGER and SPiDER. JAK3 (TIGER score=6.9) and JAK1 (TIGER score=1.9) ranked fourth and twenty-sixty, respectively, on the list of predicted target proteins for tofacitinib, while JAK3 (TIGER score=8.4) ranked second for baricitinib using the TIGER approach. Tyrosine Kinase (EC number 2.7.10.1)

(tofacitinib [p-value=0.01]; baricitinib [p-value=0.02]) ranked second on the list for the two JAK inhibitors using SPiDER. Although lacking the subfamily specificity, SPiDER correctly identified the target family, which encompasses the JAK kinases.

The top predictions for tofacitinib suggested D-Amino-Acid Oxidase (EC number 1.4.3.3; TIGER score=11.3), and Phosphodiesterase (3',5'-Cyclic-Nucleotide Phosphodiesterase; EC number 3.1.4.17; SPiDER p-value=0.009), while the top predictions for baricitinib pointed to Deoxycytidine Kinase (EC number 2.7.1.74; TIGER score=8.8), and Monoamine Oxidase (EC number 1.4.3.4; SPiDER p-value=0.016). Experimental validation of the remaining top-ranking predicted targets, including Deoxycytidine Kinase suggested as a new target of baricitinib, will be considered for future study.

Only a small fraction (~10%) of the 98 predicted off-targets were experimental tested in this study. However, as we did observe active binding on three distinct targets, this study suggests that there might be other interactions among the list of predicted targets. Thus, further testing might help to elucidate the molecular mechanisms of these JAK inhibitors and open the door for an improved understanding of the safety concerns and repurposing in other conditions (e.g., in neurodegenerative diseases, diabetes, and viral infections).

The use of computational and experimental approaches in this study allowed for the identification and characterization of previously unknown off-target interactions for the two JAK inhibitors (e.g., baricitinib-PDE10A and tofacitinib-TRPM6), which adds to the target space of tofacitinib and baricitinib. TIGER and SPiDER identified additional targets of tofacitinib and baricitinib that other approaches, such as the Similarity Ensemble Approach (SEA)[53] and the SwissTargetPrediction,[252] were unable to capture (**Table 8.3.4**). For example, both SEA and SwissTargetPrediction failed to assign TRPM6 (tofacitinib) and PKN2 (baricitinib) identified by TIGER and SPiDER, respectively. Nevertheless, comparisons between different target prediction approaches should be performed with caution, as extensive experimental studies are essential for validating the hypotheses and demonstrating the potential impact of each approach.

Moreover, TIGER and SPiDER use a large set of targets, encompassing a broad scope of protein families that allows for identifying drug promiscuity. Additionally, the inclusion of multiple protein families helps to predict a broad off-target drug profile and point out potential targets for repurposing studies. This is particularly important for understudied druggable proteins and targets with no approved drugs. Ultimately, it increases knowledge on the potential drug effects of tofacitinib and baricitinib.

Despite the encouraging results of our study, we are mindful of some limitations. As identified, we could not experimentally validate all predicted targets related to thrombosis (e.g., TRPC6) or viral infection/reactivation (e.g., deoxycytidine kinase [DCK; EC number 2.7.1.74], Thymidine kinase [HSV; EC number 2.7.1.21], Exportin-1 [XPO1], or Ubiquitin-conjugating enzyme E2 N [Ube2N; EC number 2.3.2.23]). As such, we cannot conclude if these targets may play an important role in thrombosis or viral infection/reactivation risk and are limited in the conclusions we can draw. Thus, we encourage researchers with access to the appropriate assays to validate these targets. Moreover, there might be additional targets of relevance that were not predicted by our computational tools.

The provided docking poses constitute an additional support to the experimentally determined values and that shall not be considered as a binding hypothesis. Thus, future computational studies including X-ray crystallography analysis are needed, and they may provide insights on the binding mode of the JAK inhibitors on the new targets. We also acknowledge that the activity of small molecule drugs using *in vitro* assays does not always translate into activity in the cellular environment. Thus, the results should still be interpreted with caution and treated as preliminary evidence for the off-target binding of baricitinib and tofacitinib.

4.1.5 Conclusion

In summary, previously unknown off-targets of baricitinib and tofacitinib were identified and characterized using a combination of machine learning and experimental methods. The confirmed target interactions suggest an attenuation of pulmonary vascular remodelling, modulation of HCV viral response, and hypomagnesemia. Thus, it does not endorse the hypothesis of elevated thrombosis or viral infections/reactivation risk explained by one (or more) drug-target interactions. Consequently, the current safety concerns may be due to underlying patient-specific factors (confounders) or to targets not detected by our computational pipeline. Additionally, as not all of the predicted targets were experimentally validated, further research is warranted. Finally, baricitinib and tofacitinib may be potential candidates for repurposing, as they were identified as drugs with promiscuous binding activity.

Chapter 4.2

JAK-inhibitors and risk on serious viral infection, venous thromboembolism and cardiac events in patients with rheumatoid arthritis: A protocol for a prevalent new-user cohort study using the Danish nationwide DANBIO register

Authors

Maria Luisa Faquetti[¶], Enriqueta Vallejo-Yagüe[¶], René Cordtz, Lene Dreyer, Andrea M. Burden

Remarks

Maria L. Faquetti contributed to the design of the study, drafting of the manuscript, and critical revisions.

[¶]These authors contributed equally to this work and shared the first authorship.

Publication

This study is published in its current form as an open-access article distributed under the terms of the CC-BY 4.0 license:

Faquetti ML *et al*. JAK-inhibitors and risk on serious viral infection, venous thromboembolism and cardiac events in patients with rheumatoid arthritis: A protocol for a prevalent new-user cohort study using the Danish nationwide DANBIO register. *PLOS ONE*. 18(7):e0288757 (2023). https://doi:10.1371/journal.pone.0288757

4.2.1 Introduction

Janus Kinase inhibitor (JAKi) drugs are novel targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) and represent an important alternative to treat patients with moderate-to-serious rheumatoid arthritis (RA) disease.[77] The JAKis target kinases of the JAK family (JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase TYK2), inhibiting the production of multiple pro-inflammatory cytokines, such as interleukin (IL)-6, IL-10, and interferon IFN-γ.[65,253] For the treatment of RA, JAKi drugs with different affinities for one or more JAK enzymes have been approved worldwide, such as tofacitinib, baricitinib, and updacitinib. While tofacitinib, for example, preferentially inhibits JAK1 and JAK3, baricitinib is designed to target JAK1 and JAK2, and upadacitinib is selective for JAK1.[65,183,254] However, safety concerns on the increased risk of serious viral infections, venous thromboembolism (VTE), and major adverse cardiovascular events (MACE) associated with JAKis have emerged worldwide, regardless of their selectivity.[63,68–72,232]

Patients with rheumatoid arthritis (RA) have increased risk of serious infection, thrombosis and cardiovascular risk factors.[255–257] An increasing amount of literature suggests that the use of JAKi drug may further increase the risk of cardiovascular diseases (e.g., increased serum lipid levels) and, thus, potentially increased the risk of MACE.[69,211,258,259] Nevertheless, the underlying mechanism of cardiovascular outcomes due to JAKis use is not fully understood. Recent analyses of thromboembolic events as suspected adverse drug reactions for JAKis using real-world data support the need to investigate further this potential safety issue on different drugs of this class.[71,179] While, conversely, integrated analyses of randomized clinical trials (RCTs) of JAKis did not reveal elevated risk of VTE,[68,70,260,261] safety assessment in RCTs is limited by the study conditions (e.g., time restrictions, population inclusion and exclusion criteria) and, therefore, it may not represent a real-world clinical setting. Thus, due to the paucity of evidence on the safety of JAKis at a population level, safety concerns regarding VTE and MACE associated with JAKis remain.

Therapies targeting the JAK family of enzymes may interfere with normal antiviral response, including inhibition of IFN-γ activity and may potentially further increase the risk of infection and reactivation of viral infectious diseases in RA patients. Pooling data from tofacitinib RCTs revealed an increased incidence rate of herpes zoster (HZ) than the observed rate in patients on biological disease-modifying antirheumatic drugs (bDMARDs).[68] Similarly, Curtis *et al*. reported a 2-fold increased risk for HZ in RA patients treated with tofacitinib compared to RA

patients using biologics (including, but not exclusively, tumour necrosis factor α inhibitor [TNF-αi] drugs) in a real-world study.[81] Nevertheless, there is a lack of real-world evidence on the risk of other serious infections (e.g., cytomegalovirus and Epstein-Barr virus) in patients with RA using JAKi drugs.

In light of the need to enrich the safety profile of JAKis in real-world data, we aim to quantify the incidence and risk of MACE, VTE and serious viral infections in RA patients registered in the Danish rheumatologic database (DANBIO).

4.2.2 Materials and methods

4.2.2.1 Study objectives

The study objectives are to investigate the incidence (frequency) and risk of (1) major adverse cardiovascular events (MACE), (2) venous thromboembolism (VTE), and (3) serious viral infection (or reactivation) associated to the use of JAKis compared to TNF-αis among RA patients.

4.2.2.2 Study design and setting

We will conduct a population-based cohort study using a prevalent new-user design.[262] Clinical data, medications, and outcomes will be obtained for patients enrolled in the Danish rheumatologic database (DANBIO) between January 1st, 2017, and December 31st, 2022 (or the latest available in the database). Since 2006, DANBIO registry collects data prospectively using a web-based system used in routine care at Danish hospital Departments of Rheumatology or private rheumatologic clinics.

4.2.2.3 Data source

The study will use data from the DANBIO with additional information obtained from the Danish National Patient Registry, the Danish Civil Registration System in Denmark, Danish National Database of Reimbursed Prescriptions, and the Danish National Prescription Registry. The unique civil registration (CPR) number from the Danish Civil Registration System [263] enables linkage between these national data sources on an individual-based level.

The DANBIO registry provides information on RA diagnosis, patient demographics (sex and age), patient characteristics (e.g., smoking status), RA disease duration, RA disease activity score

[e.g., 28-joint disease activity score (DAS28)], health assessment questionnaire (HAQ), biologic markers [e.g., rheumatoid factor (RF) and cyclic citrullinated peptides] and inflammatory markers [e.g., C-reactive protein (CRP)], patient-reported outcomes [e.g., visual analogue scale (VAS) on pain], other clinical endpoints, and anti-rheumatic medication (with start and stop dates) including conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), corticosteroids, tsDMARD, and biologics (e.g., TNF-αi and non- TNF-αi biologics). No distinction between biologics and biosimilars will be done in this study.

The Danish National Patient Registry provides information on disease diagnoses [classified by the International Classification of Diseases, 10th revision (ICD-10) codes].[22] Moreover, it has complete data regarding hospitalizations and outpatient care. Thus, information on comorbidities and outcomes (e.g., cardiovascular diseases, VTE, and serious viral infections) will be collected from the Danish National Patient Registry. Additionally, the Danish National Prescription Registry provides data on non-rheumatic medication [identified by Anatomical Therapeutic Chemical Classification (ATC) codes].[264] Finally, the Danish Civil Registration System provides information on vital status, sex, date of birth, and migration date and status.

The Danish healthcare model is similar to other countries such as Canada, Italy, or France, where there is a single-payer Universal healthcare system. Thus, patients in Denmark receive bDMARDs or JAKi at no cost as they are provided free of charge within the hospital setting. Regarding access to medication, JAKis are eligible as a third-line therapy option, after csDMARDs and bDMARDs. Thus, patients receiving JAKis within Denmark will have failed or be contraindicated for bDMARDs prior to receiving a JAKi. Finally, clinical practice in Denmark traditionally follows the European Alliance of Associations for Rheumatology (EULAR) guidelines,[265] and therefore, we expect our results to be representative to other countries following EULAR guidance.

4.2.2.4 Ethics statement

The study complies with the Declaration of Helsinki. Registry-based research does not require approval from the Danish National Research Ethics Committee. The study was approved by the DANBIO (ref. DANBIO-2018-11-30) steering committee and registered at the North Region's inventory (ref. P-2018–182).

4.2.2.5 Patient consent for publication

DANBIO is approved as a national quality registry and by the Danish Data Protection Agency, which is based on the Act on Processing of Personal Data and ensures that data security, protection and individual rights, are dealt with correctly. This constitutes the necessary legal requirements, and informed consent is not required per Danish legislation.[266] Therefore, informed patient consent is not needed to register or participate in the DANBIO registry.[18]

4.2.2.6 Data management plan

To protect the participants' privacy and to maintain confidentiality, only completely anonymized data from patients registered in DANBIO will be included in the study. Thus, the authors will have no access to information that could identify participants during data analysis. The data will be analyzed securely with remote access to Statistics Denmark. Also, only aggregated data with sufficient masking (i.e., ≥3 events per cell) will be made available on reasonable request.

4.2.2.7 Study Population

4.2.2.7.1 Base cohort

We will identify all RA patients in DANBIO database, ≥18 years old, receiving a JAKi or a TNF-αi (i.e., incident and prevalent users) between January 1st, 2017 and December 31st, 2022 (or latest available in the database). TNF-αi users with previous use of a JAKi, patients missing age or sex, and patients with a diagnosis of cancer (except non-melanoma skin cancer) ever before T_0 (i.e., study cohort entry date for JAKi users and the corresponding matching date for TNF-αi users) will be excluded. Two cohorts will be created to evaluate the outcomes of interest ([1] MACE or VTE, and [2] serious viral infections) independently. Thus, additionally, for the population that will lead to MACE or VTE base cohort, patients with a diagnosis of mitral stenosis, valvular heart diseases, valve replacement, heart transplantation, MACE- or VTE-events (i.e., defined as per the outcomes) ever before T_0 will not be eligible for matching in the exposure set. For the serious viral infection base cohort, a diagnosis for serious viral infection (i.e., defined as per the outcomes), or antiviral therapy (acyclovir, valaciclovir and famciclovir) within the 365 days prior T_0 (i.e., wash-out period) will not be eligible for matching in the exposure set. Patients with a diagnosis of human immunodeficiency virus (HIV) ever before T_0 in the serious viral infection cohort will be excluded.

A graphical representation of base cohorts for MACE/VTE outcomes and serious viral infection, depicting inclusion and exclusion criteria, is shown in **Figure 4.2.1** and **Figure 4.2.2**, respectively. Starting from patients in the DANBIO registry database, we may define subsequent key population steps in this study: base cohorts, exposure sets, and study cohorts. These are explained in detail later in the protocol, here in brief: As illustrated in **Figure 4.2.1** and **Figure 4.2.2**, a base cohort will be obtained after applying the inclusion and exclusion criteria. This will include JAKis and TNF-αi initiators (i.e., potential controls) starting the drug treatment within the study period. From the base cohort, JAKis users will be identified as members of the JAKi group and subsequently matched with selected TNF-αi users within their exposure set. For that, the exposure set for each JAKi user will include a pool of TNF-αi users from the base cohort who are eligible to be their controls (i.e., due to similar exposure until the time of JAKi start). Notably, the exposure sets are the distinction between prevalent and incident users of JAKs. Lastly, the matched JAKi users and TNF-αi users with similar treatment history at the JAKi start constitute the study cohorts.

A complete list for RA diagnoses (i.e., inclusion criteria) using the International Classification of Diseases 10th revision (ICD-10) codes is available in **Table 8.4.1**. A Complete list of ICD-10 codes for diagnosis and the Anatomical Therapeutic Chemical (ATC) classification codes for antiviral therapy used as exclusion criteria is available in **Table 8.4.2**.

The base cohorts to evaluate the outcomes of interest will be created including patients with at least one day of treatment with JAKi or TNF-αi during the study period. The base cohort entry date for the two cohorts will be defined as the date of treatment initiation (i.e., 'treatment start date') for a JAKi or a TNF-αi. Patients will be allowed to enter the base cohort a maximum of two times, first with a TNF-αi and second with a JAKi (but not vice versa given the use of the prevalent new-user design). Use of JAKis prior to the study period is not expected due to later approval of these drugs in Europe. Use of TNF-αi or other bDMARD prior to the study period will not be penalized.

The base cohort will therefore include:

1. Incident new-users of JAKis (i.e., new user of JAKi without prior use of TNF-αi);
2. Prevalent new-users of JAKis, also called switchers (i.e., new user of JAKis with previous use of TNF-αi drug);
3. Incident new-users of TNF-αi (i.e., new user of TNF-αi without prior use of TNF-αi);
4. Prevalent new-users of TNF-αi (i.e., new user of TNF-αi with prior use of TNF-αi).

Subsequently, exposure sets will be created for each JAKi user as described in the below subsections, and as depicted in **Figure 4.2.3**.

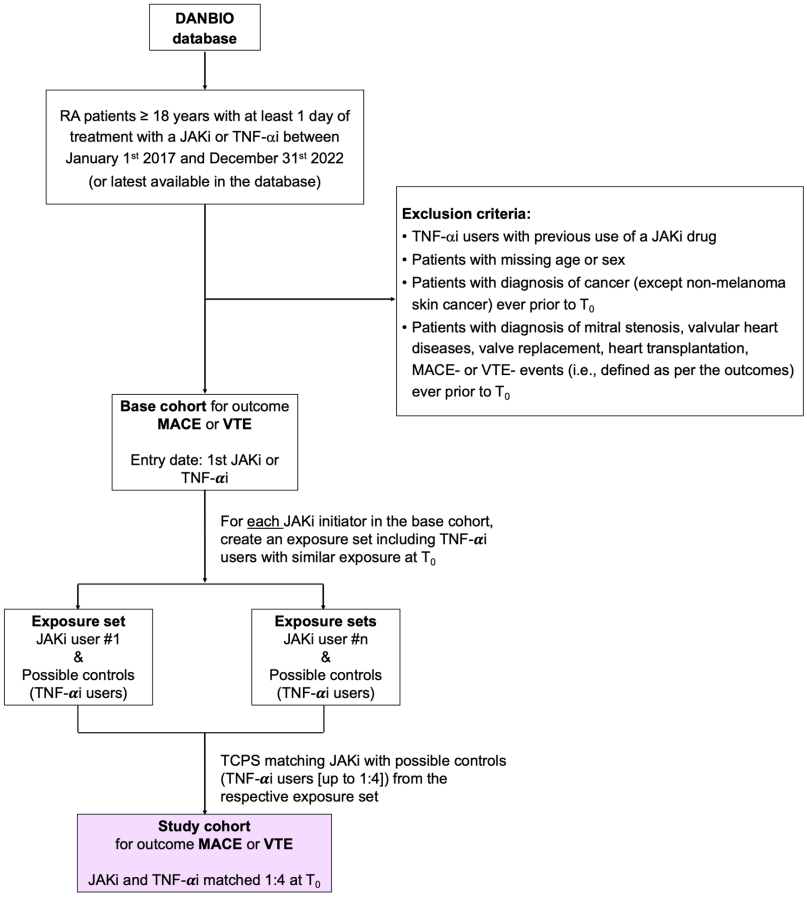


Figure 4.2.1 Base cohorts for major adverse cardiovascular events (MACE) and venous thromboembolism (VTE) outcomes, inclusion and exclusion criteria, exposure sets, and study cohorts. Abbreviations: RA Rheumatoid Arthritis; JAKi Janus Kinase inhibitor; TNF-αi Tumour Necrosis Factor α inhibitor; DANBIO Danish rheumatologic database. TCPS time-conditional propensity score; T_0 will be defined as the study cohort entry date for JAKi users (i.e., first start of JAKi treatment) and the corresponding matching date for TNF-αi users.

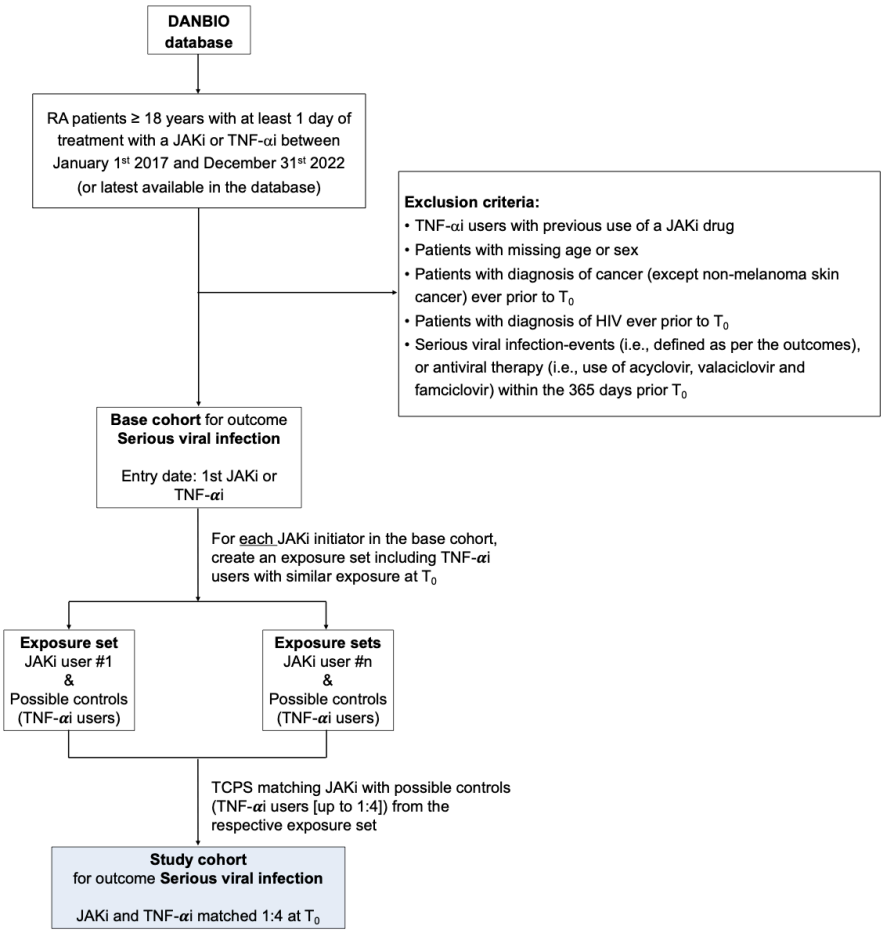
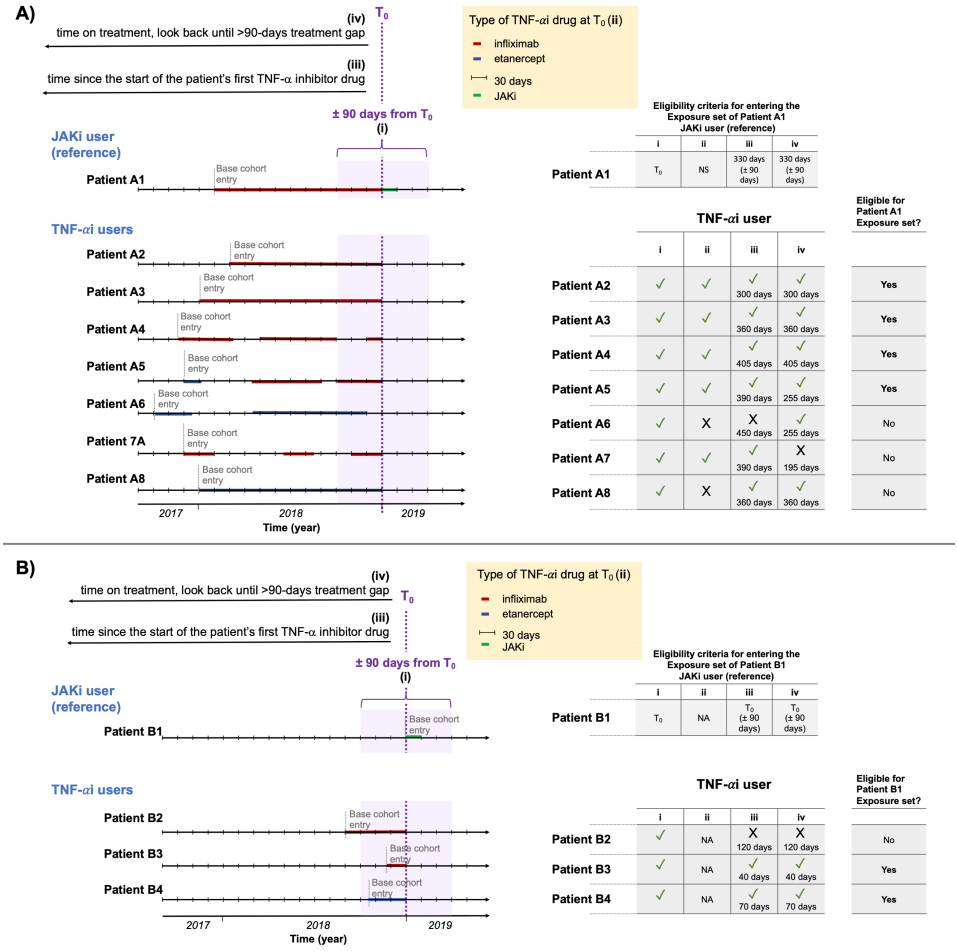


Figure 4.2.2 Base cohorts for the outcome of serious viral infection, inclusion and exclusion criteria, exposure sets, and study cohorts. Abbreviations: RA Rheumatoid Arthritis; JAKi Janus Kinase inhibitor; TNF-αi Tumour Necrosis Factor α inhibitor; HIV Human Immunodeficiency Virus; DANBIO Danish rheumatologic database. TCPS time-conditional propensity score; T_0 will be defined as the study cohort entry date for JAKi users (i.e., first start of JAKi treatment) and the corresponding matching date for TNF-αi users.

4.2.2.7.2 Exposure sets

Among the patients included in the base cohorts, a set of potential controls (i.e., exposure set) will be defined for each JAKi user at the time of the JAKi treatment initiation (T_0). The exposure set (i.e., potential controls) for each JAKi user will include TNF-αi users with similar exposure history at T_0, who will be chosen using time-based exposure sets.

Exposure sets will be defined by i) calendar time (TNF-αi drug use within □ 90 days from T_0), (ii) type of TNF-αi drug , (iii) time since the start of the first TNF-αi drug within a difference of □ 90 calendar days (i.e., look back period from study cohort entry date up to base cohort entry), and (iv) duration (days) of the most recent TNF-αi treatment course, starting looking from T_0 and continue backwards in time until a period free of treatment >90 days to account for direct and delayed switchers. A graphical description of the time-dependent exposure set for an example of JAKi prevalent new-user (patient A1) is depicted in **Figure 4.2.3** (panel A) The figure shows several TNF-αi users from the base cohort as potential controls (patients A2 to A8). Additionally, in **Figure 4.2.3** (panel B), potential controls to be included in the exposure set for a JAKi user with no previous TNF-αi use (patient B1). TNF-αi users may enter more than one exposure set, at different times, eventually matching the respective JAKi users T_0. Note that the base cohort entry may be previous to the study period for TNF-αi users.



	Eligibility criteria for entering the Exposure set of Patient A1 JAKi user (reference)				
	i	ii	iii	iv	
Patient A1	T_0	NS	330 days (± 90 days)	330 days (± 90 days)	
	TNF-αi user				
	i	ii	iii	iv	Eligible for Patient A1 Exposure set?
Patient A2	✓	✓	✓ 300 days	✓ 300 days	Yes
Patient A3	✓	✓	✓ 360 days	✓ 360 days	Yes
Patient A4	✓	✓	✓ 405 days	✓ 405 days	Yes
Patient A5	✓	✓	✓ 390 days	✓ 255 days	Yes
Patient A6	✓	X	X 450 days	✓ 255 days	No
Patient A7	✓	✓	✓ 390 days	X 195 days	No
Patient A8	✓	X	✓ 360 days	✓ 360 days	No

	Eligibility criteria for entering the Exposure set of Patient B1 JAKi user (reference)			
	i	ii	iii	iv
Patient B1	T_0	NA	T_0 (± 90 days)	T_0 (± 90 days)
	TNF-αi user				
	i	ii	iii	iv	Eligible for Patient B1 Exposure set?
Patient B2	✓	NA	X 120 days	X 120 days	No
Patient B3	✓	NA	✓ 40 days	✓ 40 days	Yes
Patient B4	✓	NA	✓ 70 days	✓ 70 days	Yes

Figure 4.2.3 Graphical description of the time-dependent exposure set for Janus Kinase inhibitors (JAKis) users. (A) We depict an example of potential controls (patients A2, A3, A4, A5) to be included in the exposure set for a JAKi user with previous TNF-αi use (patient A1), and three examples (patients A6, A7, and A8) who are not eligible to be controls for that JAKi user based on: (i) calendar time (TNF-αi use within □ 90 days from T_0), (ii) type of TNF-αi at T_0 (not applicable for JAKis users with no previous use of TNF-αi), (iii) time since the start of the patient's first TNF-αi drug within a difference of □ 90 calendar days (i.e., look back period from study cohort entry date up to base cohort entry), and (iv) duration (days) of the most recent TNF-αi treatment course, starting looking from T_0 and continue backwards in time until a period free of treatment >90 days. (B) Potential controls (patients B3 and B4) to be included in the exposure set for a JAKi user with no previous TNF-αi use (patient B1), and one example (patient B2) who is not eligible to be a control for that JAKi user based on the requirements above (i), (iii), and (iv). While infliximab and etanercept were used to exemplify patients using different TNF-α drugs, the list of TNF-αis used in the study is not restricted to these two drugs. Abbreviations: NA not applicable; JAKi Janus Kinase inhibitor; TNF-αi tumour necrosis factor α inhibitor; T_0 will be defined as the study cohort entry date for JAK inhibitor users and the corresponding matching date for TNF-αi users.

4.2.2.7.3 Study cohort

Within the corresponding exposure set, each JAKi user will be matched to one TNF-αi user at T_0 using time-conditional propensity scores (TCPS). Therefore, the study cohorts will include the matched JAKis - TNF-αi pairs, as depicted in **Figure 4.2.1** and **Figure 4.2.2**. T_0 will be defined as the study cohort entry date for JAKi users, and the corresponding matching date for TNF-αi users. Note that for JAKi incident new-users, the base cohort entry date and T_0 day will be identical. Patients will be allowed to enter the study cohort a maximum of two times, first with a TNF-αi and second with a JAKi (but not vice versa given the use of the prevalent new-user design).

4.2.2.8 Sample size considerations

The number of outcome events required in each group (JAKi and TNF-αi) was estimated by outcome type using current literature. All calculations assume 80% power and alpha 0.05.

For estimating the risk of infection, Curtis and collaborators previously found an adjusted HR for HZ with tofacitinib compared to biologic agents (including TNF-αi and non- TNF-αi) of 2.01 (95% CI 1.40 to 2.88).[81] Assuming (i) an equivalent risk of HZ for the composite outcome of serious viral infections used in this study, and (ii) that the incidence risk is similar for baricitinib and upadacitnib to tofacitinib, 64 events are required.[267]

Molander and collaborators previously assessed incident VTE events with JAKis compared TNF-αis in patients with RA.[78] In their study, the adjusted HR for VTE was 1.73 (95% CI 1.24 to 2.42) for JAKi users compared to TNF-αi users. Therefore, 104 events are required.[267] The adjusted HR for MACE in tofacitinib users compared to TNF-αi users found by Ytterberg and collaborators in RA patients aged >50 years and having at least one cardiovascular risk factor was 1.33 (95% CI 0.91 to 1.94).[69] Thus, assuming a similar risk of MACE for baricitinib and upadacitnib to tofacitinib, 386 events are required given the suggested relative hazard.[267]

In the event that study is not adequately powered, we will also report the unadjusted incident rates and incidence rate ratio's as the topic is of high clinical and regulatory importance. Additionally, since this is a real-world study in registry data, every patient in DANBIO database fulfilling the eligibility criteria will be included in the study.

4.2.2.9 Study exposure

Patients will be classified into one of the two mutually exclusive groups at entry into the study cohort: current use of JAKis or current use of TNF-αis. Exposure will be defined using the as-treated approach (i.e., defined at study cohort entry and considered time-fixed). A complete list of ATC codes for JAK and TNF-αi is available in the **Table 8.4.3**.

4.2.2.10 Follow-up and censoring

We will follow all patients in the study cohort from the study cohort entry date until the earliest of the following: occurrence of first ICD-10 code for an outcome event, end of study period, or censoring as a result of change in the exposure status. Change in exposure status will be defined as (i) treatment discontinuation (defined by the earliest of the following: a recorded treatment discontinuation date, or at the beginning of a treatment gap of >90 days) or (ii) switching from a JAKi to a non-JAKi drug, or (iii) switching from a TNF-αi to a non-TNF-αi drug, or (iv) death. We will accept a permissible gap of up to 90 days between courses of the same exposure treatment. During follow-up, the TNF-αis will be treated as a class.

4.2.2.11 Study outcome(s)

MACE, VTE and serious viral infections will be identified using the ICD-10 codes in the Danish National Patient Registry. MACE will be defined as a composite of myocardial infarction, stroke, and cardiovascular (CV) death. VTE will be defined as a composite of pulmonary embolism, deep vein thrombosis, and other embolisms. Serious viral infection (or viral reactivation) will be defined as a composite of herpes zoster (HZ), cytomegalovirus (CMV), and Epstein-Barr. A complete list of outcome codes is available in the **Table 8.4.4**.

4.2.2.12 Covariates

A list of covariates to be assessed at T_0 is provided in **Table 8.4.5**. Comorbidities will be identified in the Danish National Patient Registry as diagnosed in the previous 10 years from the study cohort entry date, or otherwise specified in **Table 8.4.5**, in the 'comments' column. Indicators of disease severity (e.g., disease activity score-28 [DAS28] and the Stanford Health Assessment Questionnaire-Disability Index [HAQ-DI]) and disease duration (i.e., time since RA diagnosis) will be collected at T_0, or will be defined as the closest value looking back up to 90 days prior to T_0. Comedication will be defined as drug use at T_0, looking back up to 180 days prior to T_0 or

otherwise specified in **Table 8.4.6**. Previous immunization for HZ will be assessed at T_0, looking back up to 5 years. Ascertainment periods for covariates in **Table 8.4.5** and **Table 8.4.6** were chosen based on clinical and methodological rationale.

Note that TNF-αi users may be included in none, one, or more exposure sets. In the case of a TNF-αi user included in several exposure sets, covariates for these patients will be assessed in each exposure set, and therefore a TNF-αi user may have different covariate values in different exposure sets. The complete lists with ATC codes for comedication and HZ immunization are available in the **Table 8.4.6** and **Table 8.4.7**, respectively.

4.2.2.13 Handling missing data

The number or frequency of missing data in relevant variables will be indicated for transparency purposes. For matching purposes, missing values on disease duration, HAQ-DI, DAS28, RF factor, anti-cyclic citrullinated peptides (CCP) status, smoking status, and alcohol consumption, will be considered as a separate category. Nevertheless, we do not expect an elevated frequency of missing data for key variables due to the high completeness of data in DANBIO database.[18,268] For comorbidities (e.g., depression, diabetes, and psoriasis) we will assume that the absence of an ICD-10 code means that the patient was not diagnosed with that condition. For comedication we will assume that if the ATC code is not recorded, the patient does not use the drug.

4.2.2.14 Time-conditional propensity score (TCPS) matching

We will construct TCPS separately for incident and prevalent new-users by using conditional logistic regression with time-varying covariates, including the covariates provided in **Table 8.4.5**, and stratifying by exposure set (therefore "conditional" on exposure set). Hence, an individual may have different scores for different exposure sets they enter, depending on the time of entry.

We will match JAKi users to TNF-αi users within exposure sets on nearest TCPS and on a variable ratio (i.e., one JAKi user will be matched with up to four TNF-αi controls), without replacement and in chronological order. However, if more than 10% exposure sets have no suitable match available after trimming the distribution of TCPS, we will perform matching with replacement. Note that a patient may be matched as TNF-αi user to a JAKi user and later during the follow-up initiate with a JAKi. In this case, the subject will be included as a new user of a JAKi from the point of switch onwards and a matched comparator will be identified at the switching

point. While the TCPS for incident new users will be the probability of initiating a JAKi compared to a TNF-αi, for prevalent new-users it will be the probability of a patient switching treatment from TNF-αi to JAKi.

A Caliper of 0.06 standard deviation (SD) of logit TCPS within each exposure set will be used to define the range of estimated TCPS within which to select the TNF-αi patient. Unbalanced variables will be adjusted in the statistical analysis (Cox proportional hazard analysis). To satisfy the positivity assumption, we will exclude exposure sets where the TCPS of patients treated with TNF-αi are not within the range of the TCPS distribution of the corresponding JAKi exposure set.

4.2.2.15 Statistical analysis

4.2.2.15.1 Primary analysis

We will assess patients' characteristics and covariate distribution among the JAKi users and TNF-αi users included in exposure sets at T_0. This will be performed before and after TCPS-matching in the two exposure groups in each cohort. Similarly, the mean follow-up time in person-years before, to evaluate the quality of the TCPS matching and potential imbalanced censoring. The accuracy of matching among covariates will be assessed using the absolute value of the standardized differences (with a value of 0.1 or more to be considered important). Categorical variables will be presented as counts and percentage, and continuous variables as mean and SD or median and interquartile range (IQR).

The study analyses will be performed separately for each cohort ([1] MACE or VTE, and [2] serious viral infection) on an outcome basis. We will describe the cumulative incidence of outcome new events in each exposure group, stratified by outcome type (MACE, VTE or serious viral infection). Aalen-Johansen method will be applied to estimate the survival function of time-to-event from study cohort entry, stratified by outcome type (MACE, VTE or serious viral infection).

Subsequently, the Cox proportional hazard model will be used to conduct the statistical analysis between matched pairs of patients exposed to JAKis and patients exposed to TNF-αis in the two cohorts. Thus, we will estimate the hazard ratios (HR) with 95% confidence interval (CI) of each outcome (MACE, VTE and serious viral infection) associated to JAKis use compared to TNF-αis use. Crude incidence rates and crude and adjusted HR estimates and the corresponding 95% confidence intervals (95% CIs) will be presented for each outcome MACE,

VTE and serious viral infection. The Cox proportional hazard analysis will be performed using the maximum partial likelihood method for estimation with a Newey-West covariance matrix to account for the same patient contributing TNF-αi-exposed person time and JAKi-exposed person time and if matching with replacement is used.

4.2.2.15.2 Subgroup Analysis

Secondarily, we will perform a subgroup analysis by sex, similarly to above described for the overall study cohorts. However, if the reduced sample size does no longer enable to performed these subgroup analyses as planned, we will alternatively only report the incidence risk ratio (crude and adjusted). Other sub-group analyses would only be performed if sample size allows it (e.g., JAKi drug, dose, age).

4.2.2.15.3 Sensitivity analyses

We will repeat our primary analysis (i) stratified by follow-up period (≤1 year and >1 year) to examine the impact of follow-up duration in our results, (ii) varying the treatment gap to define continuous drug use to 0 and 180 days, (iii) varying the period free of treatment in the previous 365 days varying from 0 and ≤180 days to estimate the cumulative time on TNF-α treatment (in days) at T_0.

4.2.2.15.4 Other analysis for evaluating the impact on unmeasured confounding

We will apply the E-value as initial method to assess severity of unmeasured confounding (no unmeasured confounding assumption by the TCPS).[269] A strength of the E-value approach is that it makes minimal assumptions regarding the structure of unmeasured confounding (e.g., it does not assume the unmeasured confounder is binary), nor does it assume that there is a single unmeasured confounder). It also does not require assumptions regarding the prevalence or distribution of the unmeasured confounder(s).

A large E-value in context of study design and existing TCPS method for confounding control, indicates that, besides reporting E-value, further sensitivity analysis may not be needed. Conversely, small E-value can be due to an unmeasured confounder that is (i) a single binary variable which the prevalence is quite high or quite low, or (ii) not a single binary variable or prevalence of a single binary confounder is unknown. If the first occurs, we will perform the rule-out method [270] as second sensitivity analysis, and if the latter occurs (or moderate or large

impact is observed from the rule-out analysis), we will further evaluate the impact of unmeasured confounders using, for example, propensity score calibration.[271]

4.2.2.16 Dissemination plan

Studies from this project will be submitted for publication in high-impact international journals and be presented at international rheumatology and pharmacoepidemiology conferences.

4.2.2.17 Status and timeline of the study

Data analysis will start when DANIO data is available. The data is expected to be available at any time from January 31st, 2023. The timeline of the study is depicted in **Table 4.2.1**.

Table 4.2.1 Timeline of the study.

Year	Activity
2023	Acquiring the DANBIO data
2023	Data Analysis
2024	Publication of results
2024	Conference Presentations

4.2.3 Discussion

This population-based cohort study aims to investigate the incidence and risk of MACE, VTE, and serious viral infection associated with the use of JAKis compared to TNF-αis among RA patients in real-world clinical practice. Moreover, to our knowledge, this will be the first study in DANBIO database to investigate the risk of MACE, VTE and serious viral infection (or reactivation) associated with JAKis in RA patients.

When it comes to JAKis approved to treat RA, information from safety trials have let to warnings and precautions on the labels of JAKi.[272,273] For example, the post-marketing ORAL Surveillance study, which compared tofacitinib versus TNF-αis in RA patients aged 50 years and older with cardiovascular risk factors, have led the recommendation for using JAKis with caution.[69,273] Although RCTs are the gold standard to determine drug safety and provide initial data on safety risks, it is limited by the study conditions (e.g., time restrictions, population

inclusion and exclusion criteria). Therefore, RCTs may miss the various nuances of different patients in a real-world clinical setting. For example, patients included in clinical trials often have less comorbidities and receive less polypharmacy compared to the overall population, due, potentially presenting lower risk of infection and fewer cardiovascular risk factors, resulting in lower adverse event rates. Thus, real-world evidence on the matter is of interest as it provides additional insights into the real-world incidence and patterns of adverse events.

Nevertheless, pharmacoepidemiologic studies on real-world data to assess safety of JAKis are challenging, due to the common prescription of JAKi after previous failure of bDMARD (e.g., TNF-αi). While a traditional new-user design if often preferable to investigate safety concerns (because it mimics RCT design in real-world setting), this applied to our research question would lead to the exclusion of the majority of JAKi users (i.e., exclusion of JAKi users with previous use of TNF-αi). This would result in strong reduction of sample size and, importantly, reduced generalizability of study results. Thus, a key strength of this study is the methodological approach to overcome this challenge, by using the prevalent new-user design.[262]

At the same time the prevalent new-user design enables the inclusion of most JAKi users, it reduces potential confounding by indication and the potential consequences of depletion of susceptible patients (i.e., prevalent-user bias) by time-conditional propensity score matching JAKi users with TNF-αi controls on previous history.

Another strength of this study is the use of DANBIO database. The register has high Danish nationwide coverage of patients with rheumatic diseases and high completeness on key data variables, such as RA diagnosis, disease scores, biomarkers, and history of cs/bDMARD therapy. Moreover, DANBIO database can be linked to other data sources in Denmark (e.g., the Danish National Patient Registry, a nationwide administrative registry that covers all hospitalizations and outpatient visits). Therefore, by using DANBIO database, this study will integrate the entire patient history to obtain a complete view of the patient history. This protocol has been designed for the DANBIO registry, and thus, a Danish patient population. Denmark has a universal healthcare system and follows the EULAR guidelines for treatment recommendations, and as such, it may have some inherent differences from other countries. For example, we may expect fewer socio-economic differences between our patient groups and will provide comparisons between our cohort and other published studies. However, one of the strengths of the study design is that the prevalent-new user design with propensity score matching addresses circumstances in which the study and reference drugs are not prescribed under the same criteria. While we would not expect substantially different results in different patient settings,

it is possible that finding optimal bDMARD matches for JAK inhibitor patients may be more challenging in some populations.

Finally, despite the overall completeness and validity of outcome diagnoses in the Danish National Patient Registry,[22] the potential for bias due to misclassification of outcomes should not be completely ruled out. This is an intrinsic limitation of RWD-based studies, as data is often collected for non-research purposes.

Chapter 5

Off-target profile with a focus on drug repurposing

Chapter 5.1

Baricitinib and tofacitinib off-target profile with a focus on Alzheimer's disease

Authors

Maria L. Faquetti[¶], Laura Slappendel[¶], Hélène Bigonne[¶], Francesca Grisoni, Petra Schneider, Georg Aichinger, Gisbert Schneider, Shana J. Sturla, Andrea M. Burden

Remarks

Maria L. Faquetti contributed to the conceptualisation of the study, design of the computational workflow for target prediction, performance of experiments and analysis, drafting of the manuscript, and critical revisions.

[¶]These authors contributed equally to this work and shared the first authorship.

Publication

This study is published in its current form as an open-access article distributed under the terms of the CC-BY-NC 4.0 license:

Faquetti ML *et al*. Baricitinib and Tofacitinib Off-Target Profile, with a Focus on Alzheimer's Disease. ChemRxiv. (2023). https://doi.org/10.26434/chemrxiv-2023-b108s.

5.1.1 Introduction

Repurposing Janus Kinase (JAK) inhibitor drugs for Alzheimer's disease (AD) has received increasing attention.[5,89] Members of this class were designed to target the JAK family (JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase TYK2) inhibiting the production of multiple pro-inflammatory cytokines, such as interleukin (IL)-6, IL-10, and interferon (IFN)-γ.[65,253] As AD progression and severity are associated with immune-driven neuroinflammation,[274,275] the potential role of some JAK inhibitors in treating AD is supported by their capacity to reduce neuroinflammation and modulate immunoregulatory processes.[276,277]

Two JAK inhibitors, baricitinib and tofacitinib, currently approved to treat rheumatoid arthritis, were recently identified among potential drug candidates for repurposing in AD.[5,89] Besides the potential effects of baricitinib and tofacitinib in AD due to pharmacological inhibition of one or more targets of the JAK family, these drugs are known to have additional off-targets (i.e., protein targets different than the primary therapeutic target).[89,91,191] The unintended off-target activity of JAK inhibitor drugs adds to the evidence that they may modulate additional targets on specific pathways and cellular processes in other diseases.

Previous work has explored the association between JAK inhibitors' target profile and AD in the context of potential repurposing using a multi-step machine learning (ML) framework.[89] The framework first identified potential associations between a list of genes and AD severity and then produced a ranked list of possible repurposing drugs. Following, the highly rated drugs were evaluated for common trends among their targets (i.e., primary targets and additional drug-target interactions previously identified). Therefore, although baricitinib and tofacitinib were previously identified as promising candidates for repurposing in AD, the potential influence of previously unknown targets in AD progression remains unclear.

In this work, we aimed to identify previously unknown drug-target interactions of baricitinib and tofacitinib that may be a factor in the use of these drugs in the context of AD. We used a multidisciplinary approach that combined experimental ligand-based ML for target prediction (Target Inference Generator – TIGER),[51] *in vitro* testing of predicted targets, and physiologically based pharmacokinetic (PBPK) modelling. A similar approach recently allowed us to profile tofacitinib and baricitinib, focusing on targets related to thrombosis and viral infections, and led to the identification of two previously unknown off-targets.[91]

5.1.2 Materials and methods

5.1.2.1 Macromolecular target prediction and selection

Target Inference Generator ([TIGER v. 19.07], inSili.com. LLC, Zurich)[51] software was used for target activity prediction. This approach leverages the chemical similarity principle – stating that molecules sharing similar structural features (potential pharmacophore points) are likely to have similar bioactivity [204] – to perform target prediction.

Baricitinib and tofacitinib were provided as Simplified Molecular Input Line Entry System (SMILES)[278] strings and pre-processed in KNIME v3.7.2[196], using the MOE v.2019.0102[197] "wash" function for structure standardisation (employing the following options: "disconnect salts", "remove lone pairs", "deprotonate strong acids", "remove minor component", "protonate strong bases," and "add hydrogen"). Chemically advanced template search version 2 (CATS2)[198] descriptors and two-dimensional MOE descriptors ('QSAR descriptors' node of KNIME; 'Forcefield'=MMFF94*) were calculated for all generated molecules and used as input for target prediction. Targets with TIGER score >1 were retained for follow-up analysis. The cut-off value on the TIGER score was chosen based on recent prospective studies where predicted targets were successfully confirmed experimentally.[51,206,208] Targets were further filtered based on their relevance on AD after screening scientific literature. Finally, targets that were not previously identified as off-targets of baricitinib or tofacitinib were selected for *in vitro* testing.

5.1.2.2 *In vitro* characterisation

Baricitinib (99.97% purity) and tofacitinib (99.96% purity) were purchased from MedChem Express LLC (New Jersey, www.medchemexpress.com) and Biosynth Carbosynth (Berkshire, www.carbosynth.com). Both drugs were tested *in vitro* on a selection of protein targets associated with AD. The protein targets (**Table 5.1.1**) were chosen using the TIGER target prediction. Glutaminyl cyclase and Ras-related protein RAB-7 experiments were conducted by directly visualising protein inhibition. The concentrations of baricitinib and tofacitinib were increased incrementally, up to a maximum concentration of 200 µM (see **Table 8.5.1**). Other targets were performed on a fee-for-service basis at Eurofins (www.eurofins.com). In these *in vitro* biochemical assays, if the drug showed inhibition or stimulation exceeding 25% at a concentration of 30 µM in two separate measurements, binding constants (K_d) were determined. Assay details are included in **Chapter 8.5**.

5.1.2.3 Brain concentration prediction

Due to the lack of existing information on baricitinib's potential to cross the blood-brain barrier in humans, a permeation assay was performed by Eurofins in MDCKII cell line. The mean permeability of baricitinib from the apical to the basolateral side (A to B) was $4.5x10^{-6}$ cm/s, and the B to A was $5.5x10^{-6}$ cm/s. According to Palmer and Alavijeh,[279] this moderate permeability value falls within the acceptable range for a desired target profile of a central nervous system (CNS) drug candidate.

A male human PBPK model for baricitinib was developed in Berkeley Madonna 10 (www.berkeleymadonna.com). Parameters were extracted from physiological values,[280–283] from a previous model,[284] or calculated from logP and pK_a values.[285] All used parameters are included in **Chapter 8.5**.

Oral 4mg (maximum recommended daily dose) baricitinib administration was modelled. The conceptual representation of the model is available in **Figure 8.5.1**. From the gut, uptake to the liver was modelled with the first order rate constant determined in a previous study,[281] then distributed to the systemic circulation. The model estimation of the concentration of baricitinib in the blood plasma over time was validated with a previous model.[284] Other organs were categorised as slowly perfused (i.e., muscles, adipose, bone, skin) or rapidly perfused (i.e., heart, lung, spleen, kidneys) tissue. Urinary excretion was modelled based on previously established clearance values.[284]

Concentrations of baricitinib in the brain were computed by different approaches (**Figure 8.5.1**). Firstly, it was estimated to be 0.91% of blood concentration, as suggested by the Quantitative Structure-Activity Relationship (QSAR) tool of the PreADMET webserver (https://preadmet.bmdrc.kr).[286] This estimation is further referred to as "Prediction 1 [QSAR]". The second approach, "Prediction 2 [Mouse exp.]", relied on a brain-to-plasma concentration ratio of 20%, which was experimentally observed in mice.[287] Finally, the third approach involved modelling of the blood-brain barrier permeation using the quantitative *in vitro – in vivo* scaling methodology developed by Ball *et al*.[283] This last estimation of baricitinib concentration in the brain tissue is herein mentioned as "Prediction 3 [QIVIVE BBB]".

Then, to address the impact of parameter uncertainty on these predicted concentrations of baricitinib in the brain, their calculation has been iteratively repeated 1000 times, with the most sensitive parameters (having the greatest influence on the results) being re-sampled in each iteration. The sensitivity analysis developed for this purpose by Evans and Andersen[288] was used (see method and results in **Table 8.5.2**) and model parameters found with normalised

sensitivity coefficients >0.1 were considered the most sensitive. These parameters values were randomly sampled in Monte-Carlo simulations,[289,290] according to their assumed distribution (**Table 8.5.3**).

5.1.3 Results

The list of 78 potential drug-target interactions predicted with TIGER score >1 (baricitinib [n=31]; tofacitinib [n=47]) was published elsewhere.[91] Of those 78, we selected nine potential drug-target interactions which are known to be relevant for AD (**Table 5.1.1**, [baricitinib [n=6]; tofacitinib [n=3]) [291–296] and experimentally validated these nine predictions using biochemical or cell-based assays.

Table 5.1.1 Target predictions for potential baricitinib and tofacitinib drug-target interactions suggested by Target Inference Generator (TIGER) relevant for Alzheimer's disease (AD).

Drug	Predicted targets associated with AD
baricitinib	Metabotropic glutamate receptor 1 (MGlu1)
	Dual leucine zipper kinase (MAP3K12)
	Casein kinase II subunit β (CK2-β)
	Carbonic anhydrase II (CA2)
	PI3-kinase P110 subunit α (PIK3CA)
	Ras-related protein Rab-7a (RAB7a)
tofacitinib	Phosphodiesterase 8B (PDE8A)
	Glutaminyl cyclase (GC)
	Inducible nitric oxide synthase (iNOS)

Abbreviations: AD: Alzheimer's Disease.

Of the nine drug-target interactions tested, CK2-α2 and dual leucine zipper kinase [MAP3K12] were inhibited by more than 25% and were characterised further by quantification of binding constants (**Table 5.1.2**). The K_d values were in the micromolar range (two-point measurements, CK2-α2-baricitinib K_d=5.8 □M [5.4 □M; 6.1 □M]; MAP3K12-baricitinib K_d=5.8 □M [5.5 □M; 6.1 □M], **Table 8.5.4** and **8.5.5**, respectively). Concentration-dependent inhibition profiles for CK2-α2-baricitinib and MAP3K12-baricitinib are shown in **Figure 8.5.2** and **Figure 8.5.3**, respectively.

Table 5.1.2 *In vitro* characterisation of baricitinib and tofacitinib for inhibiting the selected macromolecular targets.

Drug	Predicted target	Assay type	K_d (μM)
baricitinib	Casein Kinase II subunit α2 (CK2-α2) *,†	binding	5.4, 6.1‡
	Carbonic Anhydrase II (CA2)§	binding	inactive
	PI3-Kinase P110-α Subunit (PIK3CA)§	binding	inactive
	Metabotropic Glutamate receptor 1 (MGlu1) *,†	cell-based	inactive
	Dual leucine zipper kinase (MAP3K12)†	binding	5.5, 6.1‡
	Ras-Related Protein Rab-7a (RAB7a)	binding	inactive
tofacitinib	Glutaminyl Cyclase (GC)	binding	inactive
	Inducible Nitric Oxide Synthase (iNOS)§	binding	inactive
	Phosphodiesterase 8A (PDE8A)§	binding	inactive

Abbreviation: K_d: binding constant.
*Antagonistic effect.
† The drug was tested in multiple concentrations (top concentration of 100 μM).
‡ K_d determination with N=2. No averaging was made, and both values were presented.
§ The drug was tested at a concentration of 30 μM.

Among estimations of the concentration of baricitinib available in the brain, Predictions 2 [Mouse exp.] and 3 [QIVIVE BBB] were similar (see concentration versus time profiles in **Figure 8.5.4**). The distribution of maximal brain concentrations predicted by Monte Carlo simulations of the PBPK model is presented in **Table 5.1.3**. Median Cmax values range from 1.3 nM (for Prediction 1 [QSAR]) to 23 nM (for Prediction 2 [Mouse exp.] and Prediction 3 [QIVIVE BBB]), which is respectively over 4,000 times and 200 times lower than the minimum K_d value from *in vitro* experiments (**Table 5.1.2**).

Table 5.1.3 Predictions of maximal baricitinib concentrations in the brain by 1,000 Monte Carlo simulations of the physiologically-based pharmacokinetic (PBPK) model after 4 mg oral intake.

		Model brain tissue concentrations		
		Prediction 1 [QSAR]	Prediction 2 [Mouse exp.]	Prediction 3 [QIVIVE BBB]
Cmax (nM)	1st Quartile	1.1	19	18
	Median	1.3	23	23
	3rd Quartile	1.6	27	29

Abbreviation: Cmax: Maximum concentration of baricitinib in the brain, Mouse exp.: experimentally observed in mouse, QIVIVE BBB: quantitative *in vitro-in vivo* extrapolation of blood-brain-barrier permeation, QSAR: quantitative structure-activity relationship.

5.1.4 Discussion

In this work, we employed a multidisciplinary approach to explore the off-target effects of JAK inhibitors in the context of AD. We combined ligand- and machine learning-based target prediction to identify previously unknown drug-target interactions of baricitinib and tofacitinib. We subsequently conducted *in vitro* experiments to confirm the predicted drug-target interactions. This led to the identification of two enzymes previously unknown to be inhibited by baricitinib (CK2-α2 [K_d=5.8 □M]; MAP3K12 [K_d=5.8 □M]). Additionally, we predicted concentrations of baricitinib in brain tissue using PBPK modelling. The predicted maximum concentrations were found to be between 1.3 and 23 nM, which is two to three orders of magnitude below the corresponding binding constant. The putative off-target effect of baricitinib adds to the evidence that the drug potentially modulates the activity of additional proteins on pathways and cellular processes involved in the pathogenesis of AD. Nevertheless, target affinities are too low compared to anticipated drug bioavailability at the target.

CK2 is an active serine-threonine protein kinase that modulates multiple signalling pathways. [297,298] Abnormal CK2 signalling is associated with several diseases, including numerous neurological conditions.[299] The high activation of CK2 in AD is associated with abnormal phosphorylation of tau protein.[300] This abnormal phosphorylation contributes to the formation of neurofibrillary tangles, which are linked to the progression of the disease. In addition, elevated CK2 activity may also enhance β-secretase (BACE1) transcription, which is the first and rate-limiting step in the production of Aβ, the main constituent of amyloid plaques.[294,295]

While inhibition of BACE1 cleavage of amyloid precursor protein (APP) seemed to be an attractive approach to treat AD, potent BACE1 inhibitors, such as atabecestat, verubecestat, and

lanabecestat, were developed and tested regarding efficacy and safety during clinical studies in patients with AD.[301–304] However, these compounds failed later phases of randomised clinical trials due to the lack of efficacy or safety reasons. Although the complete pharmacological inhibition of BACE1 activity leads to detrimental adverse events in the RCTs, it remains to be established if only a low degree of BACE1 inhibition levels (as a result of the off-target inhibition of CK2 activity by baricitinib, for example) may be needed to decrease Aβ production.

Another critical signalling pathway in neurological disorders is the c-Jun N-terminal kinase (JNK). This family of protein kinases plays a crucial role in neuronal plasticity, regeneration, cell death, and regulation of cellular senescence.[305] MAP3K12 works as an injury sensor that initiates the JNK-dependent stress response in neurons to mediate context-dependent axon re- and degeneration.[306] Notably, inhibition of MAP3K12 is suggested to selectively regulate a JNK pathway that mediates neuronal degeneration and apoptosis. Therefore, there is considerable interest in identifying MAP3K12 inhibitors for use in chronic neurodegenerative indications.[293,307–309]

Moving forward, to further investigate baricitinib potential treatment in AD, we predicted its brain concentration using a simple PBPK model. Implementing PBPK models is a key aspect of drug development to predict *in vivo* absorption, distribution, metabolism, and excretion (ADME) processes, for a large variety of applications while reducing costs, time and ethical issues associated with animal experimentation.[310] Due to the difficulty in measuring *in vivo* human blood-brain barrier permeability, this effort is especially relevant for CNS drug candidates, which have a higher failure rate.[311,312]

The effect of baricitinib treatment in AD was evaluated using the established PBPK model to perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE). Predicted concentrations in the organ of interest (i.e., brain), ranging from 1.3 to 23 nM, were compared with *in vitro* binding constants to off-target proteins, for which minimum obtained value was 5.4 µM. Considering the orders of magnitude differences between these values (more than 200 times lower for Predictions 2 and 3 and over 4,000 times lower for Prediction1), potential off-target effects should be expected to be negligible for the oral administration of 4 mg baricitinib. Additionally, although both CK2 and MAP3K12 are expressed in different tissues,[298,313] effects due to a drug-target binding outside the brain are not likely, given the low plasma concentration 97 nM measured in clinical study.[284]

While the evidence is limited, our findings align with the existing real-world evidence, which has failed to identify a significant association between the use of JAKis and AD. An

observational study using electronic health records (EHRs) was recently conducted to test whether the use of JAK inhibitors was associated with the risk of AD in a large population.[6] The study found no differences in the risk of AD in patients treated with tofacitinib compared with those treated with abatacept, a tumour necrosis factor (TNF)-α inhibitor drug. However, it is worth noting that the mean follow-up duration was relatively short, only six months. As AD is a slow progressive disease, it is likely that the short follow-up period resulted in limited statistical power to detect smaller differences in magnitude and was not able to detect delayed treatment outcomes. While the authors of the study suggest the null finding could be due to the short follow-up duration in their study, thereby limiting the statistical power and ability to detect delayed treatment outcomes, the findings are in line with our experimental evidence.

The combined use of computational and experimental approaches allowed us to identify and characterise previously unknown off-target interactions for baricitinib (CK2-α2 and MAP3K12), which adds to the known target space of baricitinib. We further assessed the brain distribution of baricitinib and found that its low permeability considerably reduces its suitability for repurposing in AD. While additional research is needed to evaluate the implications of potential baricitinib off-target in the context of AD, this comprehensive approach can help optimise drug repurposing efforts by increasing the chances of successful potential candidates for repurposing in AD.

There are a few limitations in our approach. First, there might be additional targets of relevance that were not predicted by the TIGER computational tool. Moreover, although TIGER encompasses multiple protein families, it is limited by the manual annotation of the molecules' target information in the collection of biologically active reference molecules.[314] Second, we acknowledge that the activity of small molecule drugs using *in vitro* assays does not always translate into activity in the cellular environment. Thus, the results should still be interpreted cautiously and treated as preliminary evidence for the off-target binding of baricitinib and tofacitinib. Third, as the predictive accuracy of the PBPK model it is highly dependent on available pharmacokinetic data for baricitinib, the computation of drug concentration in the brain could be improved if information related to the unbound fraction in the brain or intrinsic transcellular permeability is available.[312] Moreover, the PBPK model prediction only addresses the concentration of baricitinib in a compartment of interest, not its pharmacological effect. A pharmacodynamic model capable of integrating ligand-binding interactions would provide additional justification for the prediction of the drug effect.[315]

Finding potential drug candidates among already approved medication for other indications to address the urgent need for disease-modifying pharmacological treatments for AD remains a goal worthy of pursuing. By synergistically integrating multiple approaches used in drug development, we have successfully identified previously unknown drug-target interactions for baricitinib. Although our results suggest a low likelihood of successfully repurposing baricitinib and tofacitinib in AD, our approach harnesses the power of multidisciplinary methods in drug discovery to identify promising candidates for repurposing efficiently.

5.1.5 Conclusion

Due to recent interest in JAK inhibitors as promising drug candidates for treatment of AD, we designed a multidisciplinary approach to investigate this potential effect for baricitinib and tofacitinib. Using machine learning, we predicted new off-targets of baricitinib related to AD. Previously unknown inhibition of two enzymes (CK2-α2 and MAP3K12) by baricitinib were confirmed using *in vitro* experiments. While our PBPK model suggested a low likelihood of successfully repurposing this drug in AD due to low brain permeability even at the maximum recommended daily dose, we have demonstrated the added benefit of a multidisciplinary approach that combines ML target prediction, *in vitro* confirmation, and PBPK modelling may optimise efforts in drug repurposing in AD.

Chapter 6

General Discussion

6.1 Summary of findings

In this dissertation, we addressed methodological challenges and limitations in the descriptive analysis of drug utilisation (**Chapter 3**) and analytical studies on drug safety (**Chapters 4** and **5**) by using an interdisciplinary approach and leveraging methodologies from pharmacoepidemiology, data science, and medicinal chemistry. In **Chapter 3.1**, we proposed a novel application of the Apriori algorithm in order to overcome the inherent computational challenges of assessing real-world polypharmacy patterns at the compound level.[32] The study revealed that 61% of new metformin users received polypharmacy. Furthermore, the analysis stratified by sex and age showed higher polypharmacy prevalence among women and older adults. Additionally, we evaluated an extensive array of drugs and drug combinations frequently prescribed, surpassing the existing knowledge on polypharmacy patterns based on drug classes. Although the most frequently prescribed drugs and drug combinations identified in our study are used to prevent diabetes complications and thus are likely important drugs that cannot be deprescribed, our findings highlight the importance of considering the characteristics of individual drug compounds to minimise the risk of drug interactions.

In **Chapter 3.2**, we shifted the focus from identifying harmful combinations to identifying opportunities for optimising pharmacotherapy in patients starting their first oral antidiabetic medication by estimating the prevalence of potentially inappropriate prescriptions (PIPs).[90] The analysis stratified by polypharmacy status and age indicated that the prevalence of PIPs was higher in patients receiving polypharmacy and in older patients. At the individual level, long-term proton pump inhibitors (PPIs) and strong opioids without laxatives were the most frequently identified PIPs in older and middle-aged patients with polypharmacy, respectively. Starting treatment with oral antidiabetic drugs, particularly in those patients receiving polypharmacy, should trigger a comprehensive review of the medications to optimise prescribing decisions.

Moving on from descriptive analyses of drug utilisation to analytical drug safety studies, in **Chapter 4**, we combined interdisciplinary approaches to investigate the safety profile of Janus Kinase inhibitors (JAKis) from different perspectives. In **Chapter 4.1**, we leveraged computational and experimental approaches to investigate whether the risk of thrombosis and viral infection/reactivation associated with tofacitinib and baricitinib was potentially due to off-target effects.[91] Although our findings did not confirm the hypothesis of elevated risk of thrombosis or viral infection/reactivation explained by drug-target interactions, previously

unknown off-targets of baricitinib and tofacitinib were identified, suggesting the two JAKis suitability as potential candidates for drug repurposing.

Subsequently, in **Chapter 4.2**, we proposed the novel prevalent new-user cohort study to investigate the incidence and risk of major adverse cardiovascular events (MACE), venous thromboembolism (VTE), and viral infection/reactivation associated with JAKi use at a population level.[92] The use of an advanced study design and the Danish rheumatologic database (DANBIO) aimed to overcome challenges posed by confounding biases in observational studies, providing valuable safety knowledge on the treatment of patients with rheumatoid arthritis (RA) using JAKis.

Finally, in **Chapter 5**, we extended our investigation on the off-target profile of tofacitinib and baricitinib with a focus on repurposing within Alzheimer's disease (AD).[93] The combined use of a machine learning-based approach and experimental validation allowed the identification and characterisation of previously unknown baricitinib off-targets potentially relevant for AD progression. Nevertheless, a potential positive effect of baricitinib in AD progression is unlikely. When baricitinib is administered in doses approved to treat RA, our physiologically-based pharmacokinetic (PBPK) model indicates a low likelihood of successfully repurposing the JAKi in AD due to low brain permeability and the correspondingly insignificant concentrations of the drug predicted to reach the brain. Finally, while additional research is needed to evaluate the potential impact of off-target interactions on AD, our approach may help to prioritize the drugs with a higher likelihood of being effective in repurposing in AD.

6.2 Impact and relevance

The studies in this dissertation are connected by the common thread of applying interdisciplinary methods to study the use and safety of drugs in real-world settings. In addition to the individual relevance discussed in each respective chapter, the collection of studies in this dissertation allowed us to address several challenges on descriptive and analytical observational studies and improve the knowledge on the utilisation and safety of drugs used in the management of chronic conditions.

The range of health conditions experienced by patients with type 2 diabetes mellitus (T2DM), often resulting in polypharmacy, highlights the complexity of managing this population and the importance of maintaining a comprehensive and collaborative approach to clinical care. However, a significant limitation in managing this group of patients is the lack of current evidence-based guidelines tailored to the management of patients with T2DM receiving

polypharmacy.[107] The evidence base for the management of chronic diseases, such as those issued by the National Institute for Health and Care Excellence (NICE) in the UK used throughout **Chapter 3**, is drawn primarily from trials of interventions for single conditions, from which multimorbid patients receiving polypharmacy are often excluded.[28] Therefore, in clinical practice, patients might receive treatment regimens which are tailored to several single conditions and do not account for the co-existence of further morbidities and their respective treatments. These patients are thus more likely to experience adverse drug reactions (ADRs), drug-drug interactions (DDIs), and PIPs.

There is a need to improve the knowledge of challenges and complexities associated with prescribing multiple medications if we are to tailor medication treatment to individual patients. Furthermore, a comprehensive view of pharmacotherapy is essential to better evaluate the potential for multi-drug interactions (i.e., interactions of at least three drugs) and avoid prescribing cascade (i.e., the process of prescribing more medications to address the unintended consequences of previous medications, leading to an unnecessary and potentially problematic medication regimen).[316–319] In the absence of specific guidelines tailored to patients with polypharmacy, this dissertation contributes to improving the assessment of benefits and risks of complex pharmacotherapy regimens. Moreover, the use of data mining in this dissertation (**Chapter 3.1**) addresses the question of the most frequently prescribed combinations of drugs at the drug compound level in these patients. Ultimately, our findings contribute to improving patient care in patients with T2DM in general, and particularly in those receiving polypharmacy.

Although polypharmacy is not always inappropriate, it is essential to continuously assess whether each individual drug has been appropriately prescribed, both individually and in the context of the medication regimen as a whole.[320] Our findings in **Chapter 3.2** underscore the high frequency of PIP in patients with T2DM in the UK and highlight the importance of performing medication reviews to mitigate the risk of harmful ADRs resulting from PIP, particularly in patients with polypharmacy. Moreover, while polypharmacy is associated with increased potentially inappropriate medication use, ensuring multiple drug prescriptions are limited to what is needed for patients' best care is a significant challenge. By identifying areas of potential optimisation in pharmacotherapy, this study contributes to enhancing patient safety and improving the appropriateness of prescribing practices by general practitioners (GPs) in the management of T2DM. Identifying PIP in large population-based databases may represent a first

step towards a more patient-centred approach to risk assessment and more appropriate and tailored therapeutic interventions.

The assessment of PIPs identifies specific medications that may warrant further evaluation and facilitates personalized medication reviews, where healthcare providers can assess the appropriateness of each medication in light of comorbidities, comedication, and other factors that are important in patients with diabetes. This personalized approach can, ultimately, help tailoring the medication regimen to better suit the patient's needs and ensure that patients receive appropriate medications based on their individual conditions and regardless their polypharmacy status. Within the UK system, where the GP is the gatekeeper to other healthcare providers, they can serve as an ideal setting for assessing PIP.

Moving on from descriptive drug utilisation to analytical safety studies, a central point of this thesis is to combine observational studies as well as computational and experimental investigations to complement each other. While the underlying mechanisms of VTE and viral infection/reactivation associated with JAKis use are poorly understood, we hypothesised that the increased risk of these safety concerns could be due to an off-target effect. By combining computational and experimental approaches in **Chapter 4.1**, we identified and characterised off-targets for tofacitinib and baricitinib. Although we have not identified an off-target interaction that could explain the safety concerns, our results revealed the potential of JAKis for drug repurposing. Moreover, our results led to the subsequent investigation of these safety concerns from a pharmacoepidemiologic perspective (**Chapter 4.2**).

Complementarily, in **Chapter 4.2**, we developed a protocol to study the safety concerns on MACE, VTE and viral infection/reactivation associated with JAKis in high-quality data from Denmark and implementing an advanced study design with particular attention to minimising confounding. Without a known mechanism, it is possible that the elevated risk of MACE, VTE, and viral infection are primarily due to underlying confounding factors – namely confounding by indication – rather than a direct drug effect. Thus, addressing this in a large observational study is essential to understand the safety profile of JAKis. However, addressing this research question in the real-world setting is challenging, due to potentially intrinsic differences because persons prescribed JAKi or TNF-αi. Therefore, traditional pharmacoepidemiology studies investigating these concerns may have been subject of confounding, one of the most challenging concepts in pharmacoepidemiology. First, the absence or insufficient capture of potential confounding factors (e.g., lifestyle factors, over-the-counter drugs, frailty, and disease severity) is a common limitation of data sources such as claims data and electronic health records (EHRs),[11,321–323]

which renders it difficult or even impossible to adjust for such factors, in order to control for confounding.[36] Therefore, the linkage of the DANBIO registry to other Danish medical databases is a key strength of the study, since provides a broad overview of patients' health status minimizing the occurrence of unmeasured confounding.

A second challenge pertains to the issue of confounding by indication. A conservative method to address this would have been the typical new-user design. However, the new-user design would exclude a large number of JAKi users who had previously received a TNF-αi, reducing the generalisability of study results. Conversely, our approach, the prevalent new-user design, allows the use of most patients exposed to JAKis, providing a more complete assessment of drug safety.[262] The use of time-based exposure sets provides equivalent points in the disease course at which patient confounders can be measured. Moreover, matching on previous patient history helps to reduce potential confounding by indication, time-lag bias, and the potential consequences of susceptible patients (i.e., prevalent-user bias).[75,324,325] Therefore, we optimised the study design and conditions aiming for robust answers to ongoing safety concerns.

One of the main areas of impact of **Chapter 4.2** is the relevance to the research community. In this chapter we highlight the importance of data linkage to create comprehensive and detailed patient profiles, facilitate longitudinal analysis, control for confounding variables, improve generalizability, and enhance the ability to detect rare events. Thus, in this chapter, we encourage other countries and data holders to build bridges between their databases. By building bridges between databases in different countries, researchers can access a wider range of data sources and gain insights into the use of medications, health outcomes, and other relevant factors across varied populations. This can lead to a more comprehensive understanding of the effects of medications in different healthcare settings, considering diverse patient demographics, cultural contexts, and healthcare practices.

In addition to investigating the mechanisms behind new unexplained safety signals, as we did in **Chapter 4.1**, the combination of computational and experimental methods also provides a great opportunity to identify new indications for approved drugs. The search for potential candidates for repurposing among approved drugs remains a worthwhile goal, particularly for diseases such as AD, where there is a significant demand for disease-modifying treatments and traditional drug development has not been successful so far. In **Chapter 5**, we identified previously unknown drug-target interactions of baricitinib involved in AD pathomechanism. However, the JAKi has a low likelihood of being successfully repurposed in AD due to the small concentrations of the drug reaching the human brain.

Overall, the individual projects in this dissertation demonstrate the ability of pharmacoepidemiology to overlap with different disciplines. While there is a seemingly natural overlap with machine learning (**Chapter 3.1**), the work in **Chapters 4.1** and **5** highlight the potential to use pharmacoepidemiology to identify new risks and benefits in synergy with molecular chemistry to explore off-target effects. While our analyses did not identify biologically relevant off-target interactions, this interdisciplinary collaboration can help contribute to finding new indications for approved drugs. Such insights may feedback early drug development stages, aiding in the assessment of potential risks and benefits when prescribing repurposed medications. Ultimately, a strong impact of this dissertation lies on its relevance on the research community. These findings contributed to scientific and health gaps in drug utilisation, safety, and development, and provided advanced methodologies and strategies to address challenging research questions. Therefore, this dissertation is an example of the benefits of bridging different scientific disciplines and fusing alternative approaches to tackle common goals.

6.3 Global limitations

In addition to those already presented in the individual chapters, we acknowledge that the studies conducted within the scope of this dissertation do contain limitations and need to be viewed in light of some methodological considerations. Issues around real-world data (RWD)-based studies include a lack of detailed clinical information and a limited ability to control for confounding, although they are not unique to them. Moreover, while computational models such as target prediction, docking, and PBPK models offer valuable tools for studying drug safety and evaluating the potential of drugs for repurposing, they are also limited and may not always align with the complexity of the actual biological systems. Thus, it is important to recognize these limitations and use computational models as one component of a comprehensive research approach integrating multiple sources of evidence.

6.3.1 Database limitations

The studies conducted within the scope of this dissertation use real-world data, which is collected during routine clinical practice. Missing data is a common issue in RWD-based studies, referring to the absence of information on the phenomena of interest.[326] Since clinical information may not be measured or recorded consistently over time and for all individuals, this can result in missing data. There are various reasons for data to be missing, including errors during data entry or transfer, patient non-adherence, and loss of follow up.[327] In EHRs or claims data, data elements needed for answering specific research questions may not be measured in

the database. In large data registries, data incompleteness can occur due to missed appointment and incomplete capture of individuals in the database. This limitation, inherent to observational studies compared to randomized controlled trials (RCTs), implies that residual confounding bias cannot be completely eliminated due to unmeasured factors in the data.[328]

Missing data can also lead to some measurement error (e.g., misclassification of exposure or outcomes),[329,330] whereby we may not capture the complete information on each patient, as well as non-random missingness can introduce selection bias, distorting estimates of medication effectiveness and safety.[331] This bias arises when certain patient subgroups or specific outcome data are more likely to be missing, compromising the reliability of study findings. Additionally, the decrease in sample size due to missing data reduces statistical power, limiting the ability to detect associations.[327] It also handicaps sensitivity analyses and the exploration of different assumptions, limiting researchers' ability to fully address uncertainty and evaluate the medication's effects. To mitigate the consequences of missing data in pharmacoepidemiology, strategies such as sensitivity analyses, multiple imputation techniques, and careful consideration of missing data mechanisms are often employed.[327,330,332,333]

While EHRs are an important data source for research on drug utilisation, information on risk factors, such as BMI, alcohol intake, and smoking, is often recorded incompletely or missing for large proportions of the population. Moreover, data on prescribed medication does not necessarily reflect whether patients actually filled their prescriptions and, ultimately, if they took the drug or not. Therefore, this may result in misclassification bias as patients are classified as exposed while they are actually not exposed, or vice versa.[334] Moreover, as EHRs used in this dissertation do not contain information on drug use during hospital stays, every hospitalisation represents a period with missing drug exposure information by design, which may become relevant for longer stays. Due to the above limitations of EHRs, the descriptive studies in Chapter 3 might have underestimated polypharmacy in patients starting their antidiabetic treatment, for example.

Another concern is that EHRs do not always contain all the information about drug dosage and the duration of drug treatments prescribed by clinicians. This can lead to misclassification bias in drug exposure assessment. Moreover, in actual practice, the time between consecutive prescriptions may be longer than the prescription duration. Patients rarely collect a subsequent prescription on the day that the last dose has been used from the previous prescription. Thus, we have to account for irregular treatment patterns with both overlapping and late refill patterns.

To address these challenges in **Chapter 3.2**, we used three different approaches in the estimation of PIP: (i) the use of defined daily dose (DDD) to estimate treatment duration of treatment,[335] (ii) the use of a permissible gap to define treatment episodes (i.e., continuation or discontinuation of medication) when information on treatment duration was required,[336] and (iii) complete-case analysis including only patients with complete information on dosage and treatment duration. While (i) and (ii) were used in the primary analysis, (iii) was conducted as sensitivity analyses.[332] The advantage of using the DDD approach lies in the dose being the average use of the drug in an adult patient for its main indication, facilitating comparisons across studies worldwide.[337] However, the actual dosage prescribed is typically a function of other characteristics such as age, weight, height, and other health status-related factors.[338] Thus, the assumption of DDD used to estimate treatment duration may have resulted in over- or under-estimation of treatment exposure, with the magnitude and direction depending on the degree of missingness, drug indication, and length of treatment.

Additionally, in a second approach, we extended the calculated days' supply by using a permissible gap when information on the duration of treatment was necessary.[336] However, by using this approach, if a patient discontinues drug use without finishing the supply, the prevalence of PIP could be overestimated. As in the descriptive analysis our main goal was identifying patients exposed to PIP for hypothesis generation, having higher sensitivity (i.e., the ability to identify true positives correctly) was preferred over specificity (i.e., the ability to identify true negatives correctly).

While the above limitations related to exposure misclassification are unavoidable and intrinsic to most studies using secondary data, a complete case analysis conducted as a sensitivity analysis confirmed the robustness of the results in our PIP study. Thus, since we assumed that the missingness on dose and duration to be unrelated to the outcome (i.e., PIP), we expect unbiased estimates.

In addition to information on exposure status, we also note that health outcomes may also be misclassified in RWD-based studies due to a lack of detailed clinical information, completeness and validity of outcome codes, data entry errors or delay, and provisional diagnosis.[340] Thus, absence of information about the outcome in the database does not always reflect the absence of a particular condition. For example, in **Chapter 4.2**, a patient with herpes zoster (HZ) infection who did not go to see a physician is misclassified as not having the outcome when she or he actually has. In this case, the point estimate for the cumulative incidence of incidence ratio of HZ would be biased towards the null. In our case, however, the rate of false

negatives is estimated to be small as patients are unlikely to not visit a physician for diagnosis, due to the rapid and intense onset of the outcome.

6.3.2 Limitations on the computational approaches

In this dissertation, we used two previously published ligand-based target prediction approaches for off-target profiling of JAKis (**Chapters 4.1** and **5**). Both approaches consist of comparing the chemical signature of two drugs to identify whether there are chemical similarities, thus suggesting shared biological activity. Nevertheless, these computational methods have their limitations. Prediction models such as TIGER and SPiDER draw all their power from data and are thus limited by their reference library. In this case, the library of biologically active reference molecules is limited by the manual annotation of the molecules' target information and by the need to meet exceptionally high standards in terms of data cleanness and structure for the models to be applied.[314] Lastly, both models are trained only with true positives, which can lead to the identification of some false positives.

We use computational ligand docking to predict potential modes of drug-target in **Chapter 4.1**, which brings several challenges and limitations. For example, although substantial progress has recently been made in x-ray crystallography, 3D structures were not always available for some protein targets of interest, such as the TRPC6.[341] Moreover, besides improvements in the last years, differences among different software packages and some limitations in predictability (e.g., mode of binding and entropic effects) depending on the docking algorithm remain.[342]

6.4 Outlook

6.4.1 Drug utilisation studies

The results of **Chapter 3.1** and **3.2** highlighted the frequency of polypharmacy and potentially harmful drug combinations among patients with T2DM. The next steps will include investigating the potential multi-drug interactions and adverse health outcomes. Due to ethical aspects, studies designed to examine adverse health effects of potential drug interactions in humans are almost always non-interventional. As observational research on this matter is primarily limited to studies involving two drug compounds (i.e., DDI), there is limited evidence on the health outcomes of multi-drug interaction (i.e., at least three drug interactions). In general, pharmacoepidemiologic studies of drug triads have been limited to examinations of the

associations between the use of drug classes (e.g., ≥3 central nervous system drugs) without identifying specific drug combinations of concern.[343–345]

Significant limitations hinder the progress in conducting pharmacoepidemiologic research on multi-drug interaction, particularly concerning RWD sources. For example, studies investigating the health effects of three or more drugs face challenges due to the scarcity of patients simultaneously receiving specific combinations of three or more drugs and because the study outcomes are often rare. Additionally, there are also challenges related to addressing limitations in study design to study drug interactions and confounding by indication[346–348] Although an active comparator (i.e., another drug with the same indication) can help addressing bias when confounding by indication is present and when appropriate for the research question of interest, it can be difficult to identify suitable comparators when evaluating a combination of three or four drugs.

In light of the current limitations of RWD sources and traditional pharmacoepidemiologic studies to explore the association between multi-drug interactions and health outcomes, different systems across the translational spectrum may be used in follow up studies. Among those are in vitro studies of drug-enzyme interactions, experimental studies on DDIs' effects on serum drug concentrations using animal models, and integration of genetic information in pharmacoepidemiologic studies.[349–351] Additionally, artificial intelligence presents vast potential for advancing the understanding of the cumulative impact of mild adverse events in these patients.[29,352,353] Complex combinations of drugs can shift the beneficial effects of individual drugs to detrimental outcomes when used in a complex drug regimen.[354] These complex combinations can lead to cumulative interactions seen in polypharmacy patients. For example, exploring the target profile of “drug cocktails” using machine-learning-based approaches may offer a valuable opportunity to explore the potential for an additive pharmacodynamic effect when multiple medications acting on the same target are used by a single patient. This perspective has yet to be fully explored. Thus, our findings can feed back into earlier stages of drug development to better understand the effects of multi-drug interaction and the target profile drug cocktails.

In **Chapter 3.2**, we highlighted the high prevalence of PIP in patients starting their first antidiabetic medication in the UK, particularly in older adults and those receiving polypharmacy. Following our findings, longitudinal observational studies are needed to determine whether hospital admission is associated with PIP in patients with T2DM and to investigate the incidence and risk of adverse health outcomes associated with the most frequent individual PIP criteria.

In particular, it would be important to identify inappropriate medication use and the risk of falls, a primary concern in patients with T2DM, elderly, and those receiving polypharmacy. Ultimately, while there are no criteria explicitly tailored to evaluate medication appropriateness in patients with T2DM, it would be of interest to compile an explicit list of combinations that are best avoided by patients with T2DM, considering risk-benefit assessment in this population.

6.4.2 Drug Safety analyses

Following our research on the investigation of safety concerns in JAKi users that did not identify off-target interactions that could explain the increased risk of thrombosis and viral infection/reactivation, the next step is to conduct a large observational study to tackle confounding. Thus, in **Chapter 4.2** we propose a prevalent new-user cohort study protocol to be implemented once Danish data is available. This study will investigate the incidence and risk of MACE, VTE, and viral infection/reactivation associated with JAKi use at a population level. The study aims to overcome challenges posed by confounding biases in observational studies, providing valuable safety knowledge on the treatment of RA patients with JAKis. If RWE no longer show an increased risk of MACE, thrombosis, and viral infection/reactivation in JAKi users compared to a TNF-αi, the increased risk previously observed is likely to be due to confounding factors, which were appropriately addressed by our study. Conversely, if RWE still shows an increased risk of MACE, thrombosis, and viral infection/reactivation in JAKi users, the explanation for the increased risk remains unknown and requires additional investigation.

Naturally, any remaining risk could be due to residual confounding (mostly by indication) as previously discussed above, leading to a biased estimate. Second, safety concerns in JAKi users could be due to an unknown DDI. Finally, as inflammation is a common characteristic of MACE, thrombosis, and viral infection/reactivation, the on-target modulation of inflammatory pathways could lead to an imbalance in homeostasis and, thus, increase the risk of MACE, thrombosis, and viral infection/reactivation in JAKi users.

While our findings in **Chapter 5** do not support the use of tofacitinib and baricitinib in AD, the collaborative approach leveraging ligand based-target prediction, experimental validation, and PBPK modelling used in this dissertation holds great potential for identification of new therapeutic uses for existing drugs. Such investigations shed light on novel therapeutic approaches and may contribute to the development of effective treatments for diseases with a high need for pharmacologic treatment such as AD. In addition to the integration of computational and experimental methods, the combination of pharmacoepidemiologic

methods using RWD further enhances the prospect of drug repurposing efforts.[4] Incorporating pharmacoepidemiology in future initiatives on drug repurposing allows for the assessment of the potential benefits and risks of repurposed drugs in real-world clinical practice.[52,53] By analysing data from EHRs, claims databases, and registries, researchers can identify signals of effectiveness or ADRs associated with the repurposed drug candidate. These findings not only provide valuable insights into the drug's performance in a broader population but also help guide the design of further RCTs. By combining computational methods, experimental validation, and pharmacoepidemiologic studies, the drug repurposing process becomes more comprehensive, robust, translatable, and resource-efficient, fostering the identification of novel therapeutic uses for existing drugs with a higher likelihood of success in clinical practice.

6.5 Conclusions

In this dissertation, we addressed unanswered questions in the study of the utilisation and safety of drugs used to treat chronic conditions by leveraging methodologies from other disciplines, such as data science and medicinal chemistry. First, we assessed real-world polypharmacy patterns at the drug compound level in patients with T2DM using the Apriori algorithm, highlighting the importance of considering the characteristics of individual drug compounds to minimise the risk of drug interactions (**Chapter 3.1**).[32] Next, we estimated the prevalence of PIPs in patients starting their first oral antidiabetic treatment, which indicated higher prevalence of PIPs in patients receiving polypharmacy and in older patients (**Chapter 3.2**).[90] Our findings have shown the need for a comprehensive review of the medications to optimise prescribing decisions in those patients.

Subsequently, we conducted two studies to investigate safety concerns on MACE, thrombosis, and viral infection/reactivation associated with JAKis providing a more comprehensive assessment of JAKis' drug safety. First, combined computational and experimental approaches to investigate whether the safety concerns were due to an off-target effect (**Chapter 4.1**), which was not confirmed in our study.[91] Next, as a coherent continuation of our investigation, we proposed a prevalent new-user cohort study using the DANBIO registry to investigate whether the increased risk of safety concerns at a population level was due to confounding factors (**Chapter 4.2**).[92] Lastly, the combination of computational and experimental approaches to investigate the off-target profile of JAKis with a focus on AD (**Chapter 5**) suggested that a positive effect of tofacitinib and baricitinib in AD progression is unlikely, and thus, our findings may help to prevent waste of resources on future interventional studies.[93]

Moving forward, we expect that deeper understanding on the interaction of several frequently co-prescribed drugs in patients with T2DM having polypharmacy can impact T2DM treatment guidelines towards more comprehensive approaches for treating patients with diabetes. Moreover, understanding the incidence and risk of adverse health outcomes associated with inappropriate medication bring forward interventions to improve prescribing in patients with T2DM. We expect that properly addressing confounding factors in RWD will deepen the understanding of safety concerns associated with JAKi use, thus also improving patient care. We expect that the use of interdisciplinary and collaborative approaches will allow to exploit the full potential of drug repurposing.

Ultimately, the studies in this dissertation show the benefit of increasing the synergy between pharmacoepidemiology and various scientific disciplines to improve medication use and effects, and their impact on public health. This dissertation highlights how RWD, pharmacoepidemiology, and other scientific disciplines can be used in synergy to advance treatment safety and effectiveness, with the overarching aim to meet the complex pharmacological needs of patients.

Chapter 7

References

1. Strom BL, Kimmel SE, Hennessy S. *Pharmacoepidemiology*. Sixth edition. John Wiley & Sons Ltd; 2020.

2. Sherman RE, Anderson SA, Dal Pan GJ, et al. Real-World Evidence — What Is It and What Can It Tell Us? *N Engl J Med*. 2016;375(23):2293-2297.

3. Czaja AS, Ross ME, Liu W, et al. Electronic health record (EHR) based postmarketing surveillance of adverse events associated with pediatric off-label medication use: A case study of short-acting beta-2 agonists and arrhythmias. *Pharmacoepidemiol Drug Saf*. 2018;27(7):815-822.

4. Pushpakom S, Iorio F, Eyers PA, et al. Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov*. 2019;18(1):41-58.

5. Desai RJ, Varma VR, Gerhard T, et al. Targeting abnormal metabolism in Alzheimer's disease: The Drug Repurposing for Effective Alzheimer's Medicines (DREAM) study. *Alzheimer's Dement: Transl Res Clin Interv*. 2020;6(1):e12095.

6. Desai RJ, Varma VR, Gerhard T, et al. Comparative Risk of Alzheimer Disease and Related Dementia Among Medicare Beneficiaries With Rheumatoid Arthritis Treated With Targeted Disease-Modifying Antirheumatic Agents. *JAMA Netw Open*. 2022;5(4):e226567.

7. Zhang Z, Zhou L, Xie N, et al. Overcoming cancer therapeutic bottleneck by drug repurposing. *Sig Transduct Target Ther*. 2020;5(1):1-25.

8. Zong N, Wen A, Moon S, et al. Computational drug repurposing based on electronic health records: a scoping review. *npj Digit Med*. 2022;5(1):1-8.

9. Bérard A. Pharmacoepidemiology Research-Real-World Evidence for Decision Making. *Front Pharmacol*. 2021;12.

10. Sørensen HT, Sabroe S, Olsen J. A Framework for Evaluation of Secondary Data Sources for Epidemiological Research. *Int J Epidemiol*. 1996;25(2):435-442.

11. Schneeweiss S, Avorn J. A review of uses of health care utilization databases for epidemiologic research on therapeutics. *J Clin Epidemiol*. 2005;58(4):323-337.

12. Lewis JD, Schinnar R, Bilker WB, Wang X, Strom BL. Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. *Pharmacoepidemiol Drug Saf*. 2007;16(4):393-401.

13. Blak BT, Thompson M, Dattani H, Bourke A. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. *Inform Prim Care*. 2011;19(4):251-255.

14. Galozy A, Nowaczyk S, Sant'Anna A, Ohlsson M, Lingman M. Pitfalls of medication adherence approximation through EHR and pharmacy records: Definitions, data and computation. *Int J Med Inform*. 2020;136:104092.

15. Bosworth HB, Granger BB, Mendys P, et al. Medication adherence: A call for action. *Am Heart J*. 2011;162(3):412-424.

16. McGettigan P, Alonso Olmo C, Plueschke K, et al. Patient Registries: An Underused Resource for Medicines Evaluation: Operational proposals for increasing the use of patient registries in regulatory assessments. *Drug Saf*. 2019;42(11):1343-1351.

17. Danish Rhreumatologic Database (DANBIO). What is DANBIO? Danish Rhreumatologic Database (DANBIO). Published 2015. Accessed June 1, 2023. https://danbio-online.dk/om-danbio/information-in-english

18. Ibfelt EH, Jensen D, Hetland ML. The Danish nationwide clinical register for patients with rheumatoid arthritis: DANBIO. *Clin Epidemiol*. 2016;8:737-742.

19. Hetland ML. DANBIO-powerful research database and electronic patient record. *Rheumatol*. 2011;50(1):69-77.

20. Schneeweiss S. Improving therapeutic effectiveness and safety through big healthcare data. *Clin Pharmacol Ther*. 2016;99(3):262-265.

21. Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol*. 2014;29(8):541-549.

22. Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol*. 2015;2015:449.

23. Gjerstorff ML. The Danish Cancer Registry. *Scand J Public Health*. 2011;39(7):42-45.

24. Baadsgaard M, Quitzau J. Danish registers on personal income and transfer payments. *Scand J Public Health*. 2011;39(7):103-105.

25. Jensen VM, Rasmussen AW. Danish Education Registers. *Scand J Public Health*. 2011;39(7):91-94.

26. Helweg-Larsen K. The Danish Register of Causes of Death. *Scand J Public Health*. 2011;39(7):26-29.

27. Elseviers M, Wettermark B, Almarsdóttir AB, et al. *Drug Utilization Research: Methods and Applications*. John Wiley & Sons Inc; 2016.

28. Guthrie B, Makubate B, Hernandez-Santiago V, Dreischulte T. The rising tide of polypharmacy and drug-drug interactions: population database analysis 1995–2010. *BMC Med*. 2015;13(74).

29. Molokhia M, Majeed A. Current and future perspectives on the management of polypharmacy. *BMC Fam Pract*. 2017;18(1).

30. Jankel CA, Speedie SM. Detecting Drug Interactions: A Review of the Literature. Hartshorn EA, ed. *DICP*. 1990;24(10):982-989.

31. Kellick KA, Bottorff M, Toth PP, The National Lipid Association's Safety Task Force null. A clinician's guide to statin drug-drug interactions. *J Clin Lipidol*. 2014;8(3):S30-46.

32. Faquetti ML, la Torre AM, Burkard T, Obozinski G, Burden AM. Identification of polypharmacy patterns in new-users of metformin using the Apriori algorithm: A novel framework for investigating concomitant drug utilization through association rule mining. *Pharmacoepidemiol Drug Saf*. 2023;32(3):279-396.

33. Lavecchia A. Machine-learning approaches in drug discovery: methods and applications. *Drug Discov Today*. 2015;20(3):318-331.

34. Wu X, Kumar V, Ross Quinlan J, et al. Top 10 algorithms in data mining. *Knowl Inf Syst*. 2008;14(1):1-37.

35. Iavindrasana J, Cohen G, Depeursinge A, Müller H, Meyer R, Geissbuhler A. Clinical data mining: a review. *Yearb Med Inform*. 2009;18(1):121-133.

36. Brookhart MA, Stürmer T, Glynn RJ, Rassen J, Schneeweiss S. Confouding Control in Healthcare Database Research: Challenges and Potential Approaches. *Med Care*. 2010;48(6):S114-S120.

37. Walker AM. Confounding by Indication. *Epidemiology*. 1996;7(4):335.

38. Schneeweiss S, Suissa S. Advanced Approaches to Controlling Confounding in Pharmacoepidemiologic Studies. In: Strom BL, Kimmel SE, Hennessy S, eds. *Pharmacoepidemiology*. 1st ed. Wiley; 2019:1078-1107.

39. Mansournia MA, Etminan M, Danaei G, Kaufman JS, Collins G. Handling time varying confounding in observational research. *BMJ*. 2017;359:j4587.

40. Uddin MdJ, Groenwold RHH, Ali MS, et al. Methods to control for unmeasured confounding in pharmacoepidemiology: an overview. *Int J Clin Pharm*. 2016;38:714-723.

41. Reddy AS, Zhang S. Polypharmacology: drug discovery for the future. *Expert Rev Clin Pharmacol*. 2013;6(1).

42. Porta MS. *A Dictionary of Epidemiology*. 5th ed. Oxford University Press; 2008.

43. Ray WA. Evaluating Medication Effects Outside of Clinical Trials: New-User Designs. *Am J Epidemiol*. 2003;158(9):915-920.

44. Applebaum KM, Malloy EJ, Eisen EA. Left Truncation, Susceptibility, and Bias in Occupational Cohort Studies. *Epidemiology*. 2011;22(4):599-606.

45. Rothman KJ, Greenland S, Lash TL. *Modern Epidemiology*. 3rd ed. Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008.

46. Bosco JLF, Silliman RA, Thwin SS, et al. A most stubborn bias: no adjustment method fully resolves confounding by indication in observational studies. *J Clin Epidemiol*. 2010;63(1):64-74.

47. McMahon AD. Approaches to combat with confounding by indication in observational studies of intended drug effects. *Pharmacoepidemiol Drug Saf*. 2003;12(7):551-558.

48. Hernán MA, Hernández-Díaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. *Am J Epidemiol*. 2002;155(2):176-184.

49. Groenwold RHH, Klungel OH, Altman DG, van der Graaf Y, Hoes AW, Moons KGM. Adjustment for continuous confounders: an example of how to prevent residual confounding. *Can Med Assoc J*. 2013;185(5):401-406.

50. Austin PC. An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. *Multivariate Behav Res*. 2011;46(3):399-424.

51. Schneider P, Schneider G. A computational method for unveiling the target promiscuity of pharmacologically active compounds. *Angew Chem Int Ed*. 2017;56(38):11520-11524.

52. Zhang M, Liu Y, Jang H, Nussinov R. Strategy toward Kinase-Selective Drug Discovery. *J Chem Theory Comput*. 2023;19(5):1615-1628.

53. Keiser MJ, Roth BL, Armbruster BN, Ernsberger P, Irwin JJ, Shoichet BK. Relating protein pharmacology by ligand chemistry. *Nat Biotechnol*. 2007;25(2):197-206.

54. Peters JU. Polypharmacology – Foe or Friend? *J Med Chem*. 2013;56(22):8955-8971.

55. Zhang W, Bai Y, Wang Y, Xiao W. Polypharmacology in Drug Discovery: A Review from Systems Pharmacology Perspective. *Curr Pharm Des*. 2016;22(21):3171-3181.

56. Vandyke K, Fitter S, Dewar AL, Hughes TP, Zannettino ACW. Dysregulation of bone remodeling by imatinib mesylate. *Blood*. 2010;115(4):766-774.

57. Schneider G, Schneider P. Macromolecular target prediction by self-organizing feature maps. *Expert Opinion on Drug Discovery*. 2017;12(3):271-277.

58. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature*. 2019;576(7785):51-60.

59. Ahmad E, Lim S, Lamptey R, Webb DR, Davies MJ. Type 2 diabetes. *Lancet*. 2022;400(10365):1803-1820.

60. Iglay K, Hannachi H, Joseph Howie P, et al. Prevalence and co-prevalence of comorbidities among patients with type 2 diabetes mellitus. *Curr Med Res Opin*. 2016;32(7):1243-1252.

61. Li SJ, Hwang HF, Yu WY, Lin MR. Potentially inappropriate medication use, polypharmacy, and falls among hospitalized patients. *Geriatr Gerontol Int*. 2022;22(10):857-864.

62. Maher RL, Hanlon J, Hajjar ER. Clinical consequences of polypharmacy in elderly. *Expert Opin Drug Saf*. 2014;13(1):57-65.

63. Winthrop KL. The emerging safety profile of JAK inhibitors in rheumatic disease. *Nat Rev Rheumatol*. 2017;13(4):234-243.

64. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discov*. 2017;16(12):843-862.

65. McInnes IB, Byers NL, Higgs RE, et al. Comparison of baricitinib, upadacitinib, and tofacitinib mediated regulation of cytokine signaling in human leukocyte subpopulations. *Arthritis Res Ther*. 2019;21(1):183.

66. Xeljanz® (tofacitinib) Product Information. European Medicines Agency (EMA). 2017. Accessed June 16, 2020. https://www.ema.europa.eu/en/documents/product-information/xeljanz-epar-product-information_en.pdf

67. Olumiant® (baricitinib) Product Information. European Medicines Agency (EMA). 2017. Accessed June 15, 2020. https://www.ema.europa.eu/en/documents/product-information/olumiant-epar-product-information_en.pdf

68. Cohen SB, Tanaka Y, Mariette X, et al. Long-term safety of tofacitinib up to 9.5 years: a comprehensive integrated analysis of the rheumatoid arthritis clinical development programme. *RMD Open*. 2020;6(3):e001395.

69. Ytterberg SR, Bhatt DL, Mikuls TR, et al. Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis. *N Engl J Med*. 2022;386(4):316-326.

70. Cohen SB, van Vollenhoven RF, Winthrop KL, et al. Safety profile of upadacitinib in rheumatoid arthritis: integrated analysis from the SELECT phase III clinical programme. *Ann Rheum Dis*. 2021;80(3):304-311.

71. Vallejo-Yagüe E, Weiler S, Micheroli R, Burden AM. Thromboembolic safety reporting of tofacitinib and baricitinib: An analysis of the WHO VigiBase. *Drug Saf*. 2020;43(9):881-891.

72. Scott IC, Hider SL, Scott DL. Thromboembolism with Janus Kinase (JAK) inhibitors for rheumatoid arthritis: How real is the risk? *Drug Saf*. 2018;41(7):645-653.

73. Pfizer. Pfizer shares co-primary endpoint results from post-marketing required safety study of XELJANZ® (tofacitinib) in subjects with rheumatoid arthritis (RA). Published January 27, 2021. Accessed April 22, 2021. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-shares-co-primary-endpoint-results-post-marketing

74. Iudici M, Porcher R, Riveros C, Ravaud P. Time-dependent biases in observational studies of comparative effectiveness research in rheumatology. A methodological review. *Ann Rheum Dis*. 2019;78(4):562-569.

75. Suissa S, Azoulay L. Metformin and the Risk of Cancer. *Diabetes Care*. 2012;35(12):2665-2673.

76. Claxton L, Taylor M, Soonasra A, Bourret JA, Gerber RA. An Economic Evaluation of Tofacitinib Treatment in Rheumatoid Arthritis After Methotrexate or After 1 or 2 TNF Inhibitors from a U.S. Payer Perspective. *J Manag Care Spec Pharm*. 2018;24(10).

77. Smolen JS, Landewé RBM, Bijlsma JWJ, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2019 update. *Ann Rheum Dis*. 2020;70:685-699.

78. Molander V, Bower H, Frisell T, Delcoigne B, Di Giuseppe D, Askling J. Venous thromboembolism with JAK inhibitors and other immune-modulatory drugs: a Swedish comparative safety study among patients with rheumatoid arthritis. *Ann Rheum Dis*. 2022;82(2).

79. Machado MA de Á, Moura CS de, Guerra SF, Curtis JR, Abrahamowicz M, Bernatsky S. Effectiveness and safety of tofacitinib in rheumatoid arthritis: a cohort study. *Arthritis Res Ther*. 2018;20(1):60.

80. Pawar A, Desai RJ, Gautam N, Kim SC. Risk of admission to hospital for serious infection after initiating tofacitinib versus biologic DMARDs in patients with rheumatoid arthritis: a multidatabase cohort study. *Lancet Rheumatol*. 2020;2(2):e84-e98.

81. Curtis JR, Xie F, Yun H, Bernatsky S, Winthrop KL. Real-world comparative risks of herpes virus infections in tofacitinib and biologic-treated patients with rheumatoid arthritis. *Ann Rheum Dis*. 2016;75(10):1843-1847.

82. Klaeger S, Heinzlmeir S, Wilhelm M, et al. The target landscape of clinical kinase drugs. *Science*. 2017;358(6367).

83. Alzheimer's Disease International (ADI). *World Alzheimer Report 2015, The Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends*. 2015. Accessed August 1, 2023. https://www.alzint.org/u/WorldAlzheimerReport2015.pdf

84. Jack CR, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol*. 2013;12(2):207-216.

85. Khan A, Corbett A, Ballard C. Emerging treatments for Alzheimer's disease for non-amyloid and non-tau targets. *Expert Rev Neurother*. 2017;17(7):683-695.

86. Alzheimer's Association. *2021 Alzheimer's Disease Facts and Figures*. 2021. Accessed May 16, 2023. https://onlinelibrary.wiley.com/doi/10.1002/alz.12328

87. Thambisetty M. Understanding mechanisms and seeking cures for Alzheimer's disease: why we must be "extraordinarily diverse." *Am J Physiol Cell Physiol*. 2017;313(4):C353-C361.

88. Taylor JM, Moore Z, Minter MR, Crack PJ. Type-I interferon pathway in neuroinflammation and neurodegeneration: focus on Alzheimer's disease. *J Neural Transm*. 2018;125(5):797-807.

89. Rodriguez S, Hug C, Todorov P, et al. Machine learning identifies candidates for drug repurposing in Alzheimer's disease. *Nat Commun*. 2021;12(1):1033.

90. Faquetti ML, Frey G, Stämpfli D, Weiler S, Burden AM. Examining inappropriate medication in UK primary care for type 2 diabetes patients with polypharmacy. *medRxiv*. Preprint published online May 28, 2023.

91. Faquetti ML, Grisoni F, Schneider P, Schneider G, Burden AM. Identification of novel off targets of baricitinib and tofacitinib by machine learning with a focus on thrombosis and viral infection. *Sci Rep*. 2022;12(1):7843.

92. Faquetti ML, Vallejo-Yagüe E, Cordtz R, Dreyer L, Burden AM. JAK-inhibitors and risk on serious viral infection, venous thromboembolism and cardiac events in patients with rheumatoid arthritis: A protocol for a prevalent new-user cohort study using the Danish nationwide DANBIO register. *PLOS ONE*. 2023;18(7):e0288757.

93. Faquetti ML, Slappendel L, Bigonne H, et al. Baricitinib and tofacitinib off-target profile, with a focus on Alzheimer's disease. Preprint published online August 3, 2023.

94. World Health Organization. *Global Report on Diabetes*. 2016.

95. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nat Rev Endocrinol*. 2018;14(2):88-98.

96. DeFronzo RA. From the Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. *Diabetes*. 2009;58(4):773-795.

97. Teljeur C, Smith SM, Paul G, Kelly A, O'Dowd T. Multimorbidity in a cohort of patients with type 2 diabetes. *Eur J Gen Pract*. 2013;19(1):17-22.

98. American Diabetes Association. Standards of Medical Care in Diabetes—2014. *Diabetes Care*. 2014;37(1):S14-S80.

99. Wolff JL, Starfield B, Anderson G. Prevalence, expenditures, and complications of multiple chronic conditions in the elderly. *Arch Intern Med*. 2002;162(20):2269-2276.

100. Maddigan SL, Feeny DH, Johnson JA. Health-related quality of life deficits associated with diabetes and comorbidities in a Canadian National Population Health Survey. *Qual Life Res*. 2005;14(5):1311-1320.

101. Kessler C, Ward MJ, McNaughton CD. Reducing Adverse Drug Events: The Need to Rethink Outpatient Prescribing. *JAMA*. 2016;316(20):2092.

102. Shehab N, Lovegrove MC, Geller AI, Rose KO, Weidle NJ, Budnitz DS. US Emergency Department Visits for Outpatient Adverse Drug Events, 2013-2014. *JAMA*. 2016;316(20):2115-2125.

103. Tinetti ME, Bogardus ST, Agostini JV. Potential Pitfalls of Disease-Specific Guidelines for Patients with Multiple Conditions. *N Engl J Med*. 2004;351(27):2870-2874.

104. Jirón M, Pate V, Hanson LC, Lund JL, Jonsson Funk M, Stürmer T. Trends in Prevalence and Determinants of Potentially Inappropriate Prescribing in the United States: 2007 to 2012. *J Am Geriatr Soc*. 2016;64(4):788-797.

105. Beyth RJ, Shorr RI. Epidemiology of Adverse Drug Reactions in the Elderly by Drug Class: *Drugs Aging*. 1999;14(3):231-239.

106. Wallace E, Salisbury C, Guthrie B, Lewis C, Fahey T, Smith SM. Managing patients with multimorbidity in primary care. *BMJ*. 2015;350.

107. Duerden M, Avery T, Payne R. *Polypharmacy and Medicines Optimisation: Making It Safe and Sound*. The King's Fund; 2013.

108. Austin RP. Polypharmacy as a Risk Factor in the Treatment of Type 2 Diabetes. *Diabetes Spectr*. 2006;19(1):13-16.

109. Teixeira JJV, Crozatti MTL, dos Santos CA, Romano-Lieber NS. Potential Drug-Drug Interactions in Prescriptions to Patients over 45 Years of Age in Primary Care, Southern Brazil. Laks J, ed. *PLOS ONE*. 2012;7(10):e47062.

110. Doubova SV, Reyes-Morales H, Torres-Arreola L del P, Suárez-Ortega M. Potential drug-drug and drug-disease interactions in prescriptions for ambulatory patients over 50 years of age in family medicine clinics in Mexico City. *BMC Health Serv Res*. 2007;7(1):147.

111. Johnson JA, Bootman JL. Drug-Related Morbidity and Mortality: A Cost-of-Illness Model. *Arch Intern Med*. 1995;155(18):1949-1956.

112. Classen DC, Pestotnik SL, Evans RS, Lloyd JF, Burke JP. Adverse Drug Events in Hospitalized Patients: Excess Length of Stay, Extra Costs, and Attributable Mortality. *JAMA*. 1997;277(4):301-306.

113. Franchini M, Pieroni S, Fortunato L, Molinaro S, Liebman M. Poly-Pharmacy Among the Elderly: Analyzing the Co-Morbidity of Hypertension and Diabetes. *Curr Pharm Des*. 2014;21.

114. Horii T, Iwasawa M, Kabeya Y, Atuda K. Polypharmacy and oral antidiabetic treatment for type 2 diabetes characterised by drug class and patient characteristics: A Japanese database analysis. *Sci Rep*. 2019;9(1):12992.

115. İnci H. Evaluation of multiple drug use in patients with type 2 diabetes mellitus. *Diabetol Int*. 2021;12(4):399-404.

116. Guisado-Clavero M, Violán C, López-Jimenez T, et al. Medication patterns in older adults with multimorbidity: a cluster analysis of primary care patients. *BMC Fam Pract*. 2019;20.

117. Menditto E, Miguel AG, Juste AM, et al. Patterns of multimorbidity and polypharmacy in young and adult population: Systematic associations among chronic diseases and drugs using factor analysis. *PLOS ONE*. 2019;14(2):e0210701.

118. Calderón-Larrañaga A, Gimeno-Feliu LA, González-Rubio F, et al. Polypharmacy Patterns: Unravelling Systematic Associations between Prescribed Medications. Carvajal A, ed. *PLOS ONE*. 2013;8(12):e84967.

119. Quinn KJ, Shah NH. A dataset quantifying polypharmacy in the United States. *Sci Data*. 2017;4.

120. Khan A, Srinivasan U, Uddin S. Development and exploration of polymedication network from Pharmaceutical and Medicare Benefits Scheme data. In: *Proceedings of the Australasian Computer Science Week Multiconference*. ACM; 2019.

121. Oort S, Rutters F, Warlé-van Herwaarden MF, et al. Characteristics associated with polypharmacy in people with type 2 diabetes: the Dutch Diabetes Pearl cohort. *Diabet Med*. 2021;38(4).

122. Noale M, Veronese N, Cavallo Perin P, et al. Polypharmacy in elderly patients with type 2 diabetes receiving oral antidiabetic treatment. *Acta Diabetol*. 2016;53(2):323-330.

123. Ishizaki T, Mitsutake S, Hamada S, et al. Drug prescription patterns and factors associated with polypharmacy in >1 million older adults in Tokyo. *Geriatr Gerontol Int*. 2020;20(4):304-311.

124. Chisholm J. The Read clinical classification. *BMJ*. 1990;300(6732):1092-1092.

125. Agrawal R, Srikant R. Fast Algorithms for Mining Association Rules. In: *Proceedings of the 20th International Conference on Very Large Data Bases*.2004:487-499.

126. Bauer S, Nauck MA. Polypharmacy in people with Type 1 and Type 2 diabetes is justified by current guidelines—a comprehensive assessment of drug prescriptions in patients needing inpatient treatment for diabetes-associated problems. *Diabet Med*. 2014;31(9):1078-1085.

127. Jacobson TA. Comparative pharmacokinetic interaction profiles of pravastatin, simvastatin, and atorvastatin when coadministered with cytochrome P450 inhibitors. *Am J Cardiol*. 2004;94(9):1140-1146.

128. Oktora MP, Alfian SD, Bos HJ, et al. Trends in polypharmacy and potentially inappropriate medication (PIM) in older and middle-aged people treated for diabetes. *Br J Clin Pharmacol*. 2021;87(7):2807-2817.

129. Qato DM, Wilder J, Schumm LP, Gillet V, Alexander GC. Changes in Prescription and Over-the-Counter Medication and Dietary Supplement Use Among Older Adults in the United States, 2005 vs 2011. *JAMA Intern Med*. 2016;176(4):473-482.

130. Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119.

131. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. *Lancet*. 2017;389(10085):2239-2251.

132. Lascar N, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S. Type 2 diabetes in adolescents and young adults. *Lancet Diabetes Endocrinol*. 2018;6(1):69-80.

133. Sinnige J, Braspenning JC, Schellevis FG, et al. Inter-practice variation in polypharmacy prevalence amongst older patients in primary care. *Pharmacoepidemiol Drug Saf*. 2016;25(9):1033-1041.

134. Eichler HG, Abadie E, Breckenridge A, et al. Bridging the efficacy–effectiveness gap: a regulator's perspective on addressing variability of drug response. *Nat Rev Drug Discov*. 2011;10(7):495-506.

135. By the American Geriatrics Society 2015 Beers Criteria Update Expert Panel. American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults. *J Am Geriatr Soc*. 2015;63(11):2227-2246.

136. Cooper JA, Ryan C, Smith SM, et al. The development of the PROMPT (PRescribing Optimally in Middle-aged People's Treatments) criteria. *BMC Health Serv Res*. 2014;14(1):484.

137. Gallagher P, Lang PO, Cherubini A, et al. Prevalence of potentially inappropriate prescribing in an acutely ill population of older patients admitted to six European hospitals. *Eur J Clin Pharmacol*. 2011;67(11):1175.

138. Gallagher P, O'Mahony D. STOPP (Screening Tool of Older Persons' potentially inappropriate Prescriptions): application to acutely ill elderly patients and comparison with Beers' criteria. *Age Ageing*. 2008;37(6):673-679.

139. Formiga F, Vidal X, Agustí A, et al. Inappropriate prescribing in elderly people with diabetes admitted to hospital. *Diabet Med*. 2016;33(5):655-662.

140. Fialová D, Topinková E, Gambassi G, et al. Potentially Inappropriate Medication Use Among Elderly Home Care Patients in Europe. *JAMA*. 2005;293(11):1348-1358.

141. Guillot J, Maumus-Robert S, Marceron A, Noize P, Pariente A, Bezin J. The Burden of Potentially Inappropriate Medications in Chronic Polypharmacy. *J Clin Med*. 2020;9(11):3728.

142. Ble A, Masoli JAH, Barry HE, et al. Any versus long-term prescribing of high risk medications in older people using 2012 Beers Criteria: results from three cross-sectional samples of primary care records for 2003/4, 2007/8 and 2011/12. *BMC Geriatr*. 2015;15(1):146.

143. Cooper JA, Moriarty F, Ryan C, et al. Potentially inappropriate prescribing in two populations with differing socio-economic profiles: a cross-sectional database study using the PROMPT criteria. *Eur J Clin Pharmacol*. 2016;72(5):583-591.

144. Moriarty F, Cahir C, Bennett K, Hughes CM, Kenny RA, Fahey T. Potentially inappropriate prescribing and its association with health outcomes in middle-aged people: a prospective cohort study in Ireland. *BMJ Open*. 2017;7(10):e016562.

145. Davidoff AJ, Miller GE, Sarpong EM, Yang E, Brandt N, Fick DM. Prevalence of Potentially Inappropriate Medication Use in Older Adults Using the 2012 Beers Criteria. *J Am Geriatr Soc*. 2015;63(3):486-500.

146. Alhawassi TM, Alatawi W, Alwhaibi M. Prevalence of potentially inappropriate medications use among older adults and risk factors using the 2015 American Geriatrics Society Beers criteria. *BMC Geriatr*. 2019;19(1):154.

147. Thomas RE, Thomas BC. A Systematic Review of Studies of the STOPP/START 2015 and American Geriatric Society Beers 2015 Criteria in Patients ≥65 Years. *Curr Aging Sci*. 2019;12(2):121-154.

148. Yang H, Juang SY, Liao KF. Proton pump inhibitors use and risk of chronic kidney disease in diabetic patients. *Diabetes Res Clin Pract*. 2019;147:67-75.

149. Scarpignato C, Gatta L, Zullo A, et al. Effective and safe proton pump inhibitor therapy in acid-related diseases – A position paper addressing benefits and potential harms of acid suppression. *BMC Med*. 2016;14(1):179.

150. Heidelbaugh JJ, Kim AH, Chang R, Walker PC. Overutilization of proton-pump inhibitors: what the clinician needs to know. *Therap Adv Gastroenterol*. 2012;5(4):219-232.

151. Boath EH, Blenkinsopp A. The rise and rise of proton pump inhibitor drugs: patients' perspectives. *Soc Sci Med*. 1997;45(10):1571-1579.

152. Pottegård A, Broe A, Hallas J, de Muckadell OBS, Lassen AT, Lødrup AB. Use of proton-pump inhibitors among adults: a Danish nationwide drug utilization study. *Therap Adv Gastroenterol*. 2016;9(5):671-678.

153. van Eijk MEC, Bahri P, Dekker G, et al. Use of prevalence and incidence measures to describe age-related prescribing of antidepressants with and without anticholinergic effects. *J Clin Epidemiol*. 2000;53(6):645-651.

154. Beers MH, Ouslander JG. Risk Factors in Geriatric Drug Prescribing: A Practical Guide to Avoiding Problems. *Drugs*. 1989;37(1):105-112.

155. Nolan L, O'Malley K. Adverse Effects of Antidepressants in the Elderly. *Drugs Aging*. 1992;2(5):450-458.

156. Krolewski AS, Warram JH, Cupples A, Gorman CK, Szabo AJ, Christlieb AR. Hypertension, orthostatic hypotension and the microvascular complications of diabetes. *J Chronic Dis*. 1985;38(4):319-326.

157. De Boer IH, Bangalore S, Benetos A, et al. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. *Diabetes Care*. 2017;40(9):1273-1284.

158. National Institute for Health and Care Excellence (NICE). Depression in adults: treatment and management. 2022. Accessed January 14, 2023. www.nice.org.uk/guidance/ng222

159. Airagnes G, Pelissolo A, Lavallée M, Flament M, Limosin F. Benzodiazepine Misuse in the Elderly: Risk Factors, Consequences, and Management. *Curr Psychiatry Rep*. 2016;18(10):89.

160. Scharner V, Hasieber L, Sönnichsen A, Mann E. Efficacy and safety of Z-substances in the management of insomnia in older adults: a systematic review for the development of recommendations to reduce potentially inappropriate prescribing. *BMC Geriatr*. 2022;22(1):87.

161. Darowski A, Chambers SACF, Chambers DJ. Antidepressants and Falls in the Elderly: *Drugs Aging*. 2009;26(5):381-394.

162. Dell'osso B, Lader M. Do Benzodiazepines Still Deserve a Major Role in The Treatment of Psychiatric Disorders? A Critical Reappraisal. *Eur Psychiatry*. 2013;28(1):7-20.

163. National Institute for Health and Care Excellence (NICE). Chronic pain (primary and secondary) in over 16s: assessment of all chronic pain and management of chronic primary pain. 2021. Accessed December 22, 2022. www.nice.org.uk/guidance/ng193

164. Ensrud KE. Epidemiology of Fracture Risk With Advancing Age. *J Gerontol A Biol Sci Med Sci*. 2013;68(10):1236-1242.

165. Yang Y, Hu X, Zhang Q, Zou R. Diabetes mellitus and risk of falls in older adults: a systematic review and meta-analysis. *Age Ageing*. 2016;45(6):761-767.

166. Wertli MM, Held U, Signorell A, Steurer J, Blozik E, Burgstaller JM. Opioid Prescription in Switzerland: Appropriate Comedication use in Cancer and Noncancer Pain. *Pain Physician*. 2019;22(6):537-548.

167. Masclee GMC, Valkhoff VE, Coloma PM, et al. Risk of Upper Gastrointestinal Bleeding From Different Drug Combinations. *Gastroenterology*. 2014;147(4):784-792.e9.

168. Lanas A, Scheiman J. Low-dose aspirin and upper gastrointestinal damage: epidemiology, prevention and treatment. *Curr Med Res Opin*. 2007;23(1):163-173.

169. Targownik LE, Bolton JM, Metge CJ, Leung S, Sareen J. Selective Serotonin Reuptake Inhibitors Are Associated With a Modest Increase in the Risk of Upper Gastrointestinal Bleeding. *Am J Gastroenterol*. 2009;104(6):1475-1482.

170. Anderson K, Stowasser D, Freeman C, Scott I. Prescriber barriers and enablers to minimising potentially inappropriate medications in adults: a systematic review and thematic synthesis. *BMJ Open*. 2014;4(12):e006544.

171. Pottie K, Thompson W, Davies S, et al. Deprescribing benzodiazepine receptor agonists. *Can Fam Physician*. 2018;64(5):339-351.

172. Farrell B, Pottie K, Thompson W, et al. Deprescribing proton pump inhibitors: Evidence-based clinical practice guideline. *Can Fam Physician*. 2017;63(5):354-364.

173. East Kent Prescribing Group (Representing Ashford CCG, Canterbury and Coastal CCG, South Kent Coast CCG and Thanet CCG), National Health Service (NHS). Long-term nitrofurantoin safety monitoring guidance for prescribers. 2020. Accessed January 10, 2023. https://www.eastkentformulary.nhs.uk/media/1539/ekpg_nitrofurantoin-safety-monitoring-guidance-patients-final-jan2020.pdf

174. National Institute for Health and Care Excellence (NICE). Neuropathic pain in adults: pharmacological management in non-specialist settings. 2013. Accessed April 14, 2023. https://www.nice.org.uk/guidance/cg173

175. O'Mahony D, O'Sullivan D, Byrne S, O'Connor MN, Ryan C, Gallagher P. STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. *Age Ageing*. 2014;44(2):213-218.

176. Leonard WJ, O'Shea JJ. JAKS and STATS: Biological implications. *Annu Rev Immunol*. 1998;16:293-322.

177. Shao F, Pang X, Baeg GH. Targeting the JAK/STAT Signaling Pathway for Breast Cancer. *Curr Med Chem*. 2021;28(25):5137-5151.

178. Chong ZZ, Souayah N. SARS-CoV-2 Induced Neurological Manifestations Entangles Cytokine Storm That Implicates For Therapeutic Strategies. *Curr Med Chem*. 2021;28.

179. Verden A, Dimbil M, Kyle R, Overstreet B, Hoffman KB. Analysis of Spontaneous Postmarket Case Reports Submitted to the FDA Regarding Thromboembolic Adverse Events and JAK Inhibitors. *Drug Saf*. 2018;41(4):357-361.

180. Food and Drug Administration. FDA approves Boxed Warning about increased risk of blood clots and death with higher dose of arthritis and ulcerative colitis medicine tofacitinib (Xeljanz®, Xeljanz XR®). FDA Drug Safety Communication. Accessed February 5, 2021. https://www.fda.gov/drugs/drug-safety-and-availability/fda-approves-boxed-warning-about-increased-risk-blood-clots-and-death-higher-dose-arthritis-and

181. European Medicines Agency. Increased risk of blood clots in lungs and death with higher dose Xeljanz® (tofacitinib) for rheumatoid arthritis. European Medicines Agency. Published March 20, 2019. Accessed June 16, 2020. https://www.ema.europa.eu/en/news/increased-risk-blood-clots-lungs-death-higher-dose-xeljanz-tofacitinib-rheumatoid-arthritis

182. Olumiant® (baricitinib) FDA Approval History. Drugs.com. 2018. Accessed February 5, 2021. https://www.drugs.com/history/olumiant.html

183. European Medicines Agency. Olumiant® (baricitinib) Product Information. 2018. Accessed June 15, 2020. https://www.ema.europa.eu/en/medicines/human/EPAR/olumiant

184. Winthrop KL, Curtis JR, Lindsey S, et al. Herpes Zoster and Tofacitinib: Clinical Outcomes and the Risk of Concomitant Therapy. *Arthritis Rheumatol*. 2017;69(10):1960-1968.

185. Winthrop KL, Yamanaka H, Valdez H, et al. Herpes zoster and tofacitinib therapy in patients with rheumatoid arthritis. *Arthritis Rheumatol*. 2014;66(10):2675-2684.

186. Peters JU. *Polypharmacology in Drug Discovery*. Wiley; 2012.

187. Keiser MJ, Setola V, Irwin JJ, et al. Predicting new molecular targets for known drugs. *Nature*. 2009;462(7270):175-181.

188. de Azevedo WF. Application of Machine Learning Techniques for Drug Discovery. *Curr Med Chem*. 2021;28(38):7805-7807.

189. Wójcikowski M, Siedlecki P, Ballester PJ. *Docking Screens for Drug Discovery*. Springer New York; 2019.

190. Bajorath J. Computational approaches in chemogenomics and chemical biology: current and future impact on drug discovery. *Expert Opin Drug Discov*. 2008;3(12):1371-1376.

191. Richardson P, Griffin I, Tucker C, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet*. 2020;395(10223):e30-e31.

192. Segler MHS, Preuss M, Waller MP. Planning chemical syntheses with deep neural networks and symbolic AI. *Nature*. 2018;555(7698):604-610.

193. Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395(10224):565-574.

194. Karaman MW, Herrgard S, Treiber DK, et al. A quantitative analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2008;26(1):127-132.

195. Eberl HC, Werner T, Reinhard FB, et al. Chemical proteomics reveals target selectivity of clinical Jak inhibitors in human primary cells. *Sci Rep*. 2019;9(1):14159.

196. Berthold MR, Cebron N, Dill F, et al. KNIME: The Konstanz Information Miner. In: Preisach C, Burkhardt H, Schmidt-Thieme L, Decker R, eds. *Data Analysis, Machine Learning and Applications*. Studies in Classification, Data Analysis, and Knowledge Organization. Springer; 2008:319-326.

197. Chemical Computing Group ULC. Molecular Operating Environment (MOE). Published online 2020.

198. Reutlinger M, Koch CP, Reker D, et al. Chemically Advanced Template Search (CATS) for Scaffold-Hopping and Prospective Target Prediction for 'Orphan' Molecules. *Mol Inform*. 2013;32(2):133-138.

199. Reker D, Rodrigues T, Schneider P, Schneider G. Identifying the macromolecular targets of de novo-designed chemical entities through self-organizing map consensus. *PNAS*. 2014;111(11):4067-4072.

200. Mathea A, Elkins JM, Shrestha L, et al. Human protein kinase N2 (PKN2, PRKCL2) in complex with ATPgammaS. Worldwide Protein Data Bank. Published March 12, 2014. Accessed November 9, 2020. https://www.rcsb.org/structure/4CRS

201. Shipe WD, Sharik SS, Barrow JC, et al. Discovery and optimization of a series of pyrimidine-based phosphodiesterase 10A (PDE10A) inhibitors through fragment screening, structure-based design, and parallel synthesis. *J Med Chem*. 2015;58(19):7888-7894.

202. Jones G, Willett P, Glen RC, Leach AR, Taylor R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol*. 1997;267(3):727-748.

203. Yung-Chi C, Prusoff WH. Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. *Biochem Pharmacol*. 1973;22(23):3099-3108.

204. Maggiora G, Vogt M, Stumpfe D, Bajorath J. Molecular Similarity in Medicinal Chemistry. *J Med Chem*. 2014;57(8):3186-3204.

205. Schneider P, Röthlisberger M, Reker D, Schneider G. Spotting and designing promiscuous ligands for drug discovery. *Chem Commun*. 2016;52(6):1135-1138.

206. Grisoni F, Merk D, Friedrich L, Schneider G. Design of Natural-Product-Inspired Multitarget Ligands by Machine Learning. *ChemMedChem*. 2019;14(12):1129-1134.

207. Schneider P, Schneider G. Polypharmacological Drug–target Inference for Chemogenomics. *Mol Inf*. 2018;37(9-10):1800050.

208. Bruns D, Merk D, Santhana Kumar K, Baumgartner M, Schneider G. Synthetic Activators of Cell Migration Designed by Constructive Machine Learning. *ChemistryOpen*. 2019;8(10):1303-1308.

209. Merk D, Grisoni F, Friedrich L, Gelzinyte E, Schneider G. Computer-Assisted Discovery of Retinoid X Receptor Modulating Natural Products and Isofunctional Mimetics. *J Med Chem*. 2018;61(12):5442-5447.

210. Huang YY, Yu YF, Zhang C, et al. Validation of phosphodiesterase-10 as a novel target for pulmonary arterial hypertension via highly selective and subnanomolar inhibitors. *J Med Chem*. 2019;62(7):3707-3721.

211. Xie W, Huang Y, Xiao S, Sun X, Fan Y, Zhang Z. Impact of Janus kinase inhibitors on risk of cardiovascular events in patients with rheumatoid arthritis: systematic review and meta-analysis of randomised controlled trials. *Ann Rheum Dis*. 2019;78(8):1048-1054.

212. Bechman K, Subesinghe S, Norton S, et al. A systematic review and meta-analysis of infection risk with small molecule JAK inhibitors in rheumatoid arthritis. *Rheumatology*. 2019;58(10):1755-1766.

213. Tian X, Vroom C, Ghofrani HA, et al. Phosphodiesterase 10A upregulation contributes to pulmonary vascular remodeling. *PLOS ONE*. 2011;6(4).

214. Liras S, Bell AS. *Phosphodiesterases and Their Inhibitors*. Wiley-VCH; 2014.

215. Vemana HP, Karim ZA, Conlon C, Khasawneh FT. A critical role for the transient receptor potential channel type 6 in human platelet activation. *PLOS ONE*. 2015;10(4).

216. Desai RJ, Pawar A, Weinblatt ME, Kim SC. Comparative Risk of Venous Thromboembolism in Rheumatoid Arthritis Patients Receiving Tofacitinib Versus Those Receiving Tumor Necrosis Factor Inhibitors: An Observational Cohort Study. *Arthritis Rheumatol*. 2019;71(6):892-900.

217. Desai RJ, Pawar A, Khosrow-Khavar F, Weinblatt ME, Kim SC. Risk of venous thromboembolism associated with tofacitinib in patients with rheumatoid arthritis: a population-based cohort study. *Rheumatology*. 2021; 61(1):121-130.

218. Mease P, Charles-Schoeman C, Cohen S, et al. Incidence of venous and arterial thromboembolic events reported in the tofacitinib rheumatoid arthritis, psoriasis and psoriatic arthritis development programmes and from real-world data. *Ann Rheum Dis*. 2020;79(11):1400-1413.

219. Molander V, Bower H, Frisell T, Askling J. Risk of venous thromboembolism in rheumatoid arthritis, and its association with disease activity: a nationwide cohort study from Sweden. *Ann Rheum Dis*. 2021;80(2):169-175.

220. Cushman M. Epidemiology and Risk Factors for Venous Thrombosis. *Semin Hematol*. 2007;44(2):62-69.

221. Lee SH, Moon JS, Pak BY, et al. HA1077 displays synergistic activity with daclatasvir against hepatitis C virus and suppresses the emergence of NS5A resistance-associated substitutions in mice. *Sci Rep*. 2018;8(1):12469.

222. Kim SJ, Kim JH, Sun JM, Kim MG, Oh JW. Suppression of hepatitis C virus replication by protein kinase C-related kinase 2 inhibitors that block phosphorylation of viral RNA polymerase. *J Viral Hepat*. 2009;16(10):697-704.

223. Davis MI, Hunt JP, Herrgard S, et al. Comprehensive analysis of kinase inhibitor selectivity. *Nat Biotechnol*. 2011;29(11):1046-1051.

224. Mukai H, Ono Y. Purification and kinase assay of PKN. In: *Methods in Enzymology*. Vol 406. Elsevier; 2006:234-250.

225. Schmidt A, Durgan J, Magalhaes A, Hall A. Rho GTPases regulate PRK2/PKN2 to control entry into mitosis and exit from cytokinesis. *EMBO J*. 2007;26(6):1624-1636.

226. Lachmann S, Jevons A, De Rycker M, et al. Regulatory domain selectivity in the cell-type specific PKN-dependence of cell migration. *PLOS ONE*. 2011;6(7):e21732.

227. Lin W, Huang J, Yuan Z, Feng S, Xie Y, Ma W. Protein kinase C inhibitor chelerythrine selectively inhibits proliferation of triple-negative breast cancer cells. *Sci Rep*. 2017;7(1):2022.

228. Park YH, Wood G, Kastner DL, Chae JJ. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat Immunol*. 2016;17(8):914-921.

229. Sakaguchi T, Takefuji M, Wettschureck N, et al. Protein kinase N promotes stress-induced cardiac dysfunction through phosphorylation of myocardin-related transcription factor A and disruption of Its interaction with actin. *Circulation*. 2019;140(21):1737-1752.

230. O'Sullivan AG, Mulvaney EP, Hyland PB, Kinsella BT. Protein kinase C-related kinase 1 and 2 play an essential role in thromboxane-mediated neoplastic responses in prostate cancer. *Oncotarget*. 2015;6(28):26437-26456.

231. Rajagopalan P, Nanjappa V, Patel K, et al. Role of protein kinase N2 (PKN2) in cigarette smoke-mediated oncogenic transformation of oral cells. *J Cell Commun Signal*. 2018;12(4):709-721.

232. Pfizer. Pfizer shares co-primary endpoint results from post-marketing required safety study of XELJANZ® (tofacitinib) in subjects with rheumatoid arthritis (RA). 2021. Accessed March 26, 2021. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-shares-co-primary-endpoint-results-post-marketing

233. Chong CR, Sullivan DJ. New uses for old drugs. *Nature*. 2007;448(7154):645-646.

234. Oprea TI, Nielsen SK, Ursu O, et al. Associating drugs, targets and clinical outcomes into an integrated network affords a new platform for computer-aided drug repurposing. *Mol Inform*. 2011;30(2-3):100-111.

235. Napolitano M, Fabbrocini G, Patruno C. Potential role of Janus kinase inhibitors in COVID-19. *J Am Acad Dermatol*. 2020.

236. Spinelli FR, Conti F, Gadina M. HiJAKing SARS-CoV-2? The potential role of JAK inhibitors in the management of COVID-19. *Sci Immunol*. 2020;5(47).

237. Stebbing J, Krishnan V, de Bono S, et al. Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients. *EMBO Mol Med*. 2020;12.

238. Helal CJ, Kang Z, Hou X, et al. Use of structure-based design to discover a potent, selective, in vivo active phosphodiesterase 10A inhibitor lead series for the treatment of schizophrenia. *J Med Chem*. 2011;54(13):4536-4547.

239. Kleiman RJ, Kimmel LH, Bove SE, et al. Chronic suppression of phosphodiesterase 10A alters striatal expression of genes responsible for neurotransmitter synthesis, neurotransmission, and signaling pathways implicated in Huntington's disease. *J Pharmacol Exp Ther*. 2011;336(1):64-76.

240. Chen S, Zhang Y, Lighthouse JK, et al. A novel role of cyclic nucleotide phosphodiesterase 10A in pathological cardiac remodeling and dysfunction. *Circulation*. 2020;141(3):217-233.

241. Müller-Deubert S, Ege C, Krug M, et al. Phosphodiesterase 10A is a mediator of osteogenic differentiation and mechanotransduction in bone marrow-derived mesenchymal stromal cells. *Stem Cells Int*. 2020.

242. Giorgi M, Cardarelli S, Ragusa F, et al. Phosphodiesterase inhibitors: could they be beneficial for the treatment of COVID-19? *Int J Mol Sci*. 2020;21(15).

243. Dalamaga M, Karampela I, Mantzoros CS. Commentary: Phosphodiesterase 4 inhibitors as potential adjunct treatment targeting the cytokine storm in COVID-19. *Metabolism*. 2020;109:154282.

244. Chubanov V, Mittermeier L, Gudermann T. Role of kinase-coupled TRP channels in mineral homeostasis. *Pharmacol Ther*. 2018;184:159-176.

245. Schlingmann KP, Waldegger S, Konrad M, Chubanov V, Gudermann T. TRPM6 and TRPM7—Gatekeepers of human magnesium metabolism. *Biochim Biophys Acta Mol Basis Dis*. 2007;1772(8):813-821.

246. Touyz RM, He Y, Montezano ACI, et al. Differential regulation of transient receptor potential melastatin 6 and 7 cation channels by ANG II in vascular smooth muscle cells from spontaneously hypertensive rats. *Am J Physiol Regul Integr Comp Physiol*. 2006;290(1):R73-78.

247. Nijenhuis T, Hoenderop JGJ, Bindels RJM. Downregulation of Ca2+ and Mg2+ transport proteins in the kidney explains tacrolimus (FK506)-induced hypercalciuria and hypomagnesemia. *J Am Soc Nephrol*. 2004;15(3):549-557.

248. Nijenhuis T, Vallon V, van der Kemp AWCM, Loffing J, Hoenderop JGJ, Bindels RJM. Enhanced passive Ca2+ reabsorption and reduced Mg2+ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. *J Clin Invest*. 2005;115(6):1651-1658.

249. Groenestege WMT, Thébault S, van der Wijst J, et al. Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest*. 2007;117(8):2260-2267.

250. Vincenzi B, Santini D, Galluzzo S, et al. Early magnesium reduction in advanced colorectal cancer patients treated with cetuximab plus irinotecan as predictive factor of efficacy and outcome. *Clin Cancer Res*. 2008;14(13):4219-4224.

251. Nasulewicz A, Wietrzyk J, Wolf FI, et al. Magnesium deficiency inhibits primary tumor growth but favors metastasis in mice. *Biochim Biophys Acta Mol Basis Dis*. 2004;1739(1):26-32.

252. Daina A, Michielin O, Zoete V. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res*. 2019;47(W1):W357-W364.

253. Genovese MC, Fleischmann R, Combe B, et al. Safety and efficacy of upadacitinib in patients with active rheumatoid arthritis refractory to biologic disease-modifying anti-rheumatic drugs (SELECT-BEYOND): a double-blind, randomised controlled phase 3 trial. *Lancet*. 2018;391(10139):2513-2524.

254. European Medicines Agency. Xeljanz® to be used with caution for all patients at high risk of blood clots. 2019. Accessed May 2, 2021. https://www.ema.europa.eu/en/documents/referral/xeljanz-article-20-procedure-xeljanz-be-used-caution-all-patients-high-risk-blood-clots_en.pdf

255. Zöller B, Li X, Sundquist J, Sundquist K. Risk of subsequent ischemic and hemorrhagic stroke in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. *BMC Neurol*. 2012;12:41.

256. Holmqvist ME, Neovius M, Eriksson J, et al. Risk of venous thromboembolism in patients with rheumatoid arthritis and association with disease duration and hospitalization. *JAMA*. 2012;308(13):1350-1356.

257. Listing J, Gerhold K, Zink A. The risk of infections associated with rheumatoid arthritis, with its comorbidity and treatment. *Rheumatology*. 2013;52(1):53-61.

258. Serhal L, Edwards CJ. Upadacitinib for the treatment of rheumatoid arthritis. *Expert Rev Clin Immunol*. 2019;15(1):13-25.

259. Kume K, Amano K, Yamada S, et al. Tofacitinib improves atherosclerosis despite up-regulating serum cholesterol in patients with active rheumatoid arthritis: a cohort study. *Rheumatol Int*. 2017;37(12):2079-2085.

260. Liu L, Yan YD, Shi FH, Lin HW, Gu ZC, Li J. Comparative efficacy and safety of JAK inhibitors as monotherapy and in combination with methotrexate in patients with active rheumatoid arthritis: A systematic review and meta-analysis. *Front Immunol*. 2022;13.

261. Smolen JS, Genovese MC, Takeuchi T, et al. Safety Profile of Baricitinib in Patients with Active Rheumatoid Arthritis with over 2 Years Median Time in Treatment. *J Rheumatol*. 2019;46(1):7-18.

262. Suissa S, Moodie EEM, Dell'Aniello S. Prevalent new-user cohort designs for comparative drug effect studies by time-conditional propensity scores. *Pharmacoepidemiol Drug Saf*. 2017;26(4):459-468.

263. Pedersen CB. The Danish Civil Registration System. *Scand J Public Health*. 2011;39(7):22-25.

264. Pottegård A, Schmidt SAJ, Wallach-Kildemoes H, Sørensen HT, Hallas J, Schmidt M. Data Resource Profile: The Danish National Prescription Registry. *Int J Epidemiol*. 2017; 46(3):798-798f

265. Smolen JS, Landewé RBM, Bergstra SA, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2022 update. *Ann Rheum Dis*. 2023;82(1):3-18.

266. Thygesen LC, Daasnes C, Thaulow I, Brønnum-Hansen H. Introduction to Danish (nationwide) registers on health and social issues: structure, access, legislation, and archiving. *Scand J Public Health*. 2011;39(7):12-16.

267. Kohn JS. Sample size – Survival analysis | Sample Size Calculators. Accessed January 20, 2023. https://sample-size.net/sample-size-survival-analysis/

268. Ibfelt EH, Sørensen J, Jensen DV, et al. Validity and completeness of rheumatoid arthritis diagnoses in the nationwide DANBIO clinical register and the Danish National Patient Registry. *Clin Epidemiol*. 2017;9:627-632.

269. VanderWeele TJ. Sufficient Cause Interactions and Statistical Interactions. *Epidemiology*. 2009;20(1):6-13.

270. Schneeweiss S. Sensitivity analysis and external adjustment for unmeasured confounders in epidemiologic database studies of therapeutics. *Pharmacoepidemiol Drug Saf*. 2006;15(5):291-303.

271. Stürmer T, Schneeweiss S, Rothman KJ, Avorn J, Glynn RJ. Performance of propensity score calibration -a simulation study. *Am J Epidemiol*. 2007;165(10):1110-1118.

272. Food and Drug Administration. Safety trial finds risk of blood clots in the lungs and death with higher dose of tofacitinib (Xeljanz®, Xeljanz XR®) in rheumatoid arthritis patients; Food and Drug Administration. 2019. Accessed June 16, 2020. https://www.fda.gov/drugs/drug-safety-and-availability/safety-trial-finds-risk-blood-clots-lungs-and-death-higher-dose-tofacitinib-xeljanz-xeljanz-xr

273. Food and Drug Administration. FDA requires warnings about increased risk of serious heart-related events, cancer, blood clots, and death for JAK inhibitors that treat certain chronic inflammatory conditions. Food and Drug Administration. 2021. Accessed November 20, 2022. https://www.fda.gov/drugs/drug-safety-and-availability/fda-requires-warnings-about-increased-risk-serious-heart-related-events-cancer-blood-clots-and-death

274. Heneka MT, Carson MJ, Khoury JE, et al. Neuroinflammation in Alzheimer's disease. *Lancet Neurol*. 2015;14(4):388-405.

275. Querfurth HW, LaFerla FM. Alzheimer's Disease. *N Engl J Med*. 2010;362(4):329-344.

276. Gavegnano C, Haile WB, Hurwitz S, et al. Baricitinib reverses HIV-associated neurocognitive disorders in a SCID mouse model and reservoir seeding in vitro. *J Neuroinflammation*. 2019;16(1):182.

277. Clark JD, Flanagan ME, Telliez JB. Discovery and Development of Janus Kinase (JAK) Inhibitors for Inflammatory Diseases. *J Med Chem*. 2014;57(12):5023-5038.

278. Weininger D. SMILES, a chemical language and information system. 1. Introduction to methodology and encoding rules. *J Chem Inf Comput Sci*. 1988;28(1):31-36.

279. Palmer AM, Alavijeh MS. Translational CNS medicines research. *Drug Discov Today*. 2012;17(19):1068-1078.

280. Willmann S, Höhn K, Edginton A, et al. Development of a Physiology-Based Whole-Body Population Model for Assessing the Influence of Individual Variability on the Pharmacokinetics of Drugs. *J Pharmacokinet Pharmacodyn*. 2007;34(3):401-431.

281. Leibman KC. Editorial: Drug Metabolism and Disposition: the biological fate of chemicals. *Drug Metab Dispos*. 1973;1(2):487-488.

282. Wang X, He B, Shi J, Li Q, Zhu HJ. Comparative Proteomics Analysis of Human Liver Microsomes and S9 Fractions. *Drug Metab Dispos*. 2020;48(1):31-40.

283. Ball K, Bouzom F, Scherrmann JM, Walther B, Declèves X. Development of a Physiologically Based Pharmacokinetic Model for the Rat Central Nervous System and Determination of an In Vitro–In Vivo Scaling Methodology for the Blood–Brain Barrier Permeability of Two Transporter Substrates, Morphine and Oxycodone. *J Pharm Sci*. 2012;101(11):4277-4292.

284. Posada MM, Cannady EA, Payne CD, et al. Prediction of Transporter-Mediated Drug-Drug Interactions for Baricitinib. *Clin Transl Sci*. 2017;10(6):509-519.

285. Punt A, Pinckaers N, Peijnenburg A, Louisse J. Development of a Web-Based Toolbox to Support Quantitative In-Vitro-to-In-Vivo Extrapolations (QIVIVE) within Nonanimal Testing Strategies. *Chem Res Toxicol*. 2021;34(2):460-472.

286. Saber-Ayad M, Hammoudeh S, Abu-Gharbieh E, et al. Current Status of Baricitinib as a Repurposed Therapy for COVID-19. *Pharmaceuticals*. 2021;14(7):680.

287. Richardson PJ, Ottaviani S, Prelle A, Stebbing J, Casalini G, Corbellino M. CNS penetration of potential anti-COVID-19 drugs. *J Neurol*. 2020;267(7):1880-1882.

288. Evans MV. Sensitivity Analysis of a Physiological Model for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Assessing the Impact of Specific Model Parameters on Sequestration in Liver and Fat in the Rat. *Toxicol Sci*. 2000;54(1):71-80.

289. Clewell HJ, Jarnot BM. Incorporation of Pharmacokinetics in Noncancer Risk Assessment: Example with Chloropentafluorobenzene. *Risk Anal*. 1994;14(3):265-276.

290. Clewell HJ, Gearhart JM, Gentry PR, et al. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal*. 1999;19(4):547-558.

291. Coimbra JR, Sobral PJ, Santos AE, Moreira PI, Salvador JA. An overview of glutaminyl cyclase inhibitors for Alzheimer's disease. *Future Med Chem*. 2019;11(24):3179-3194.

292. Pérez-Torres S, Cortés R, Tolnay M, Probst A, Palacios JM, Mengod G. Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. *Exp Neurol*. 2003;182(2):322-334.

293. Rodriguez L, Mohamed NV, Desjardins A, Lippé R, Fon EA, Leclerc N. Rab7A regulates tau secretion. *J Neurochem*. 2017;141(4):592-605.

294. Masliah E, Iimoto DS, Mallory M, Albright T, Hansen L, Saitoh T. Casein kinase II alteration precedes tau accumulation in tangle formation. *Am J Pathol*. 1992;140(2):263-268.

295. Patel S, Cohen F, Dean BJ, et al. Discovery of Dual Leucine Zipper Kinase (DLK, MAP3K12) Inhibitors with Activity in Neurodegeneration Models. *J Med Chem*. 2015;58(1):401-418.

296. Patel S, Meilandt WJ, Erickson RI, et al. Selective Inhibitors of Dual Leucine Zipper Kinase (DLK, MAP3K12) with Activity in a Model of Alzheimer's Disease. *J Med Chem*. 2017;60(19):8083-8102.

297. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem*. 2003;369(1):1-15.

298. Cozza G, Bortolato A, Moro S. How druggable is protein kinase CK2? *Med Res Rev*. 2010;30(3):419-462.

299. Castello J, Ragnauth A, Friedman E, Rebholz H. CK2—An Emerging Target for Neurological and Psychiatric Disorders. *Pharmaceuticals*. 2017;10(4):7.

300. Greenwood JA, Scott CW, Spreen RC, Caputo CB, Johnson GV. Casein kinase II preferentially phosphorylates human tau isoforms containing an amino-terminal insert. Identification of threonine 39 as the primary phosphate acceptor. *J Biol Chem*. 1994;269(6):4373-4380.

301. Novak G, Streffer JR, Timmers M, et al. Long-term safety and tolerability of atabecestat (JNJ-54861911), an oral BACE1 inhibitor, in early Alzheimer's disease spectrum patients: a randomized, double-blind, placebo-controlled study and a two-period extension study. *Alzheimers Res Ther*. 2020;12(1):58.

302. Egan MF, Kost J, Tariot PN, et al. Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *N Engl J Med*. 2018;378(18):1691-1703.

303. Egan MF, Kost J, Voss T, et al. Randomized Trial of Verubecestat for Prodromal Alzheimer's Disease. *N Engl J Med*. 2019;380(15):1408-1420.

304. Wessels AM, Tariot PN, Zimmer JA, et al. Efficacy and Safety of Lanabecestat for Treatment of Early and Mild Alzheimer Disease: The AMARANTH and DAYBREAK-ALZ Randomized Clinical Trials. *JAMA Neurol*. 2020;77(2):199-209.

305. Yarza R, Vela S, Solas M, Ramirez MJ. c-Jun N-terminal Kinase (JNK) Signaling as a Therapeutic Target for Alzheimer's Disease. *Front Pharmacol*. 2016;6.

306. Gallo KA, Johnson GL. Mixed-lineage kinase control of JNK and p38 MAPK pathways. *Nat Rev Mol Cell Biol*. 2002;3(9):663-672.

307. Sengupta Ghosh A, Wang B, Pozniak CD, Chen M, Watts RJ, Lewcock JW. DLK induces developmental neuronal degeneration via selective regulation of proapoptotic JNK activity. *J Cell Biol*. 2011;194(5):751-764.

308. Tiziana Borsello, Gianluigi Forloni. JNK Signalling: A Possible Target to Prevent Neurodegeneration. *Curr Pharm Des*. 2007;13(18):1875-1886.

309. Manning AM, Davis RJ. Targeting JNK for therapeutic benefit: from junk to gold? *Nat Rev Drug Discov*. 2003;2(7):554-565.

310. Jones H, Chen Y, Gibson C, et al. Physiologically based pharmacokinetic modeling in drug discovery and development: A pharmaceutical industry perspective. *Clin Pharmacol Ther*. 2015;97(3):247-262.

311. Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *Neurotherapeutics*. 2005;2(4):554-571.

312. Ball K, Bouzom F, Scherrmann JM, Walther B, Declèves X. Physiologically Based Pharmacokinetic Modelling of Drug Penetration Across the Blood–Brain Barrier—Towards a Mechanistic IVIVE-Based Approach. *AAPS J*. 2013;15(4):913-932.

313. Reddy UR, Pleasure D. Cloning of a novel putative protein kinase having a leucine zipper domain from human brain. *Biochem Biophys Res Commun*. 1994;202(1):613-620.

314. Schneider P, Schneider G. Collection of bioactive reference compounds for focused library design. *QSAR Comb Sci*. 2003;22(7):713-718.

315. Salahudeen MS, Nishtala PS. An overview of pharmacodynamic modelling, ligand-binding approach and its application in clinical practice. *Saudi Pharm J*. 2017;25(2):165-175.

316. on behalf of the Scottish Diabetes Research Network (SDRN) Epidemiology Group, Höhn A, Jeyam A, et al. The association of polypharmacy and high-risk drug classes with adverse health outcomes in the Scottish population with type 1 diabetes. *Diabetologia*. 2021;64(6):1309-1319.

317. Azoulay L, Filion KB, Platt RW, et al. Association Between Incretin-Based Drugs and the Risk of Acute Pancreatitis. *JAMA Intern Med*. 2016;176(10):1464-1473.

318. Duerden M, Avery T, Payne R. *Polypharmacy and Medicines Optimisation: Making It Safe and Sound*. The King's Fund; 2013.

319. Rochon PA, Gurwitz JH. The prescribing cascade revisited. *Lancet*. 2017;389(10081):1778-1780.

320. Aronson JK. A prescription for better prescribing. *Br J Clin Pharmacol*. 2006;61(5):487-491.

321. Kim DH, Schneeweiss S. Measuring frailty using claims data for pharmacoepidemiologic studies of mortality in older adults: evidence and recommendations. *Pharmacoepidemiol Drug Saf*. 2014;23(9):891-901.

322. Schneeweiss S, Setoguchi S, Brookhart MA, Kaci L, Wang PS. Assessing residual confounding of the association between antipsychotic medications and risk of death using survey data. *CNS Drugs*. 2009;23(2):171-180.

323. Strom BL. Methodologic challenges to studying patient safety and comparative effectiveness. *Med Care*. 2007;45(10):S13-15.

324. Lu B. Propensity Score Matching with Time-Dependent Covariates. *Biometrics*. 2005;61(3):721-728.

325. Danaei G, Tavakkoli M, Hernán MA. Bias in Observational Studies of Prevalent Users: Lessons for Comparative Effectiveness Research From a Meta-Analysis of Statins. *Am J Epidemiol*. 2012;175(4):250-262.

326. McKnight PE, McKnight KM, Sidani S, Figueredo AJ. *Missing Data: A Gentle Introduction*. Guilford Press; 2007.

327. Thabane L, Mbuagbaw L, Zhang S, et al. A tutorial on sensitivity analyses in clinical trials: the what, why, when and how. *BMC Med Res Methodol*. 2013;13:92.

328. Rubin DB. Estimating Causal Effects from Large Data Sets Using Propensity Scores. *Ann Intern Med*. 1997;127(8):757.

329. Van Buuren S. *Flexible Imputation of Missing Data*. Chapman and Hall/CRC; 2012.

330. Sterne JAC, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ*. 2009;338:b2393.

331. Graham JW. Missing Data Analysis: Making It Work in the Real World. *Annu Rev Psychol*. 2009;60(1):549-576.

332. van Smeden M, Penning de Vries BBL, Nab L, Groenwold RHH. Approaches to addressing missing values, measurement error, and confounding in epidemiologic studies. *J Clin Epidemiol*. 2021;131:89-100.

333. Eekhout I, de Boer RM, Twisk JWR, de Vet HCW, Heymans MW. Missing Data: A Systematic Review of How They Are Reported and Handled. *Epidemiology*. 2012;23(5):729.

334. European Medicines Agency. The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP). Guide on Methodological Standards in Pharmacoepidemiology (Revision 10). EMA/95098/2010. 2022. Accessed May 22, 2023. http://www.encepp.eu/standards_and_guidance

335. Pazzagli L, Brandt L, Linder M, et al. Methods for constructing treatment episodes and impact on exposure-outcome associations. *Eur J Clin Pharmacol*. 2020;76(2):267-275.

336. Pazzagli L, Andersen M, Sessa M. Pharmacological and epidemiological considerations while constructing treatment episodes using observational data: A simulation study. *Pharmacoepidemiol Drug Saf*. 2022;31(1):55-60.

337. Merlo J, Wessling A, Melander A. Comparison of dose standard units for drug utilisation studies. *E J Clin Pharmacol*. 1996;50(1):27-30.

338. National Institute for Health and Care Excellence. NICE British National Formulary (BNF). NICE. Accessed February 24, 2023. https://bnf.nice.org.uk

339. Swift A, Heale R, Twycross A. What are sensitivity and specificity? *Evid Based Nurs*. 2020;23(1):2-4.

340. Hall GC, Lanes S, Bollaerts K, Zhou X, Ferreira G, Gini R. Outcome misclassification: Impact, usual practice in pharmacoepidemiology database studies and an online aid to correct biased estimates of risk ratio or cumulative incidence. *Pharmacoepidemiol Drug Saf*. 2020;29(11):1450-1455.

341. Cooke RM, Brown AJH, Marshall FH, Mason JS. Structures of G protein-coupled receptors reveal new opportunities for drug discovery. *Drug Discov Today*. 2015;20(11):1355-1364.

342. Pagadala NS, Syed K, Tuszynski J. Software for molecular docking: a review. *Biophys Rev*. 2017;9(2):91-102.

343. Aspinall SL, Springer SP, Zhao X, et al. Central Nervous System Medication Burden and Risk of Recurrent Serious Falls and Hip Fractures in Veterans Affairs Nursing Home Residents. *J Am Geriatr Soc*. 2019;67(1):74-80.

344. Hanlon JT, Zhao X, Naples JG, et al. Central Nervous System Medication Burden and Serious Falls in Older Nursing Home Residents. *J Am Geriatr Soc*. 2017;65(6):1183-1189.

345. Nace D, Aspinall S, Castle N, et al. Central Nervous System (CNS) Medication Burden & Risk of Serious Falls in Older Nursing Home Residents. *JAMDA*. 2017;18(3):B21.

346. Delaney JAC, Suissa S. The case-crossover study design in pharmacoepidemiology. *Stat Methods Med Res*. 2009;18(1):53-65.

347. Maclure M, Fireman B, Nelson JC, et al. When should case-only designs be used for safety monitoring of medical products? *Pharmacoepidemiol Drug Saf*. 2012;21(S1):50-61.

348. Mittleman MA, Mostofsky E. Exchangeability in the case-crossover design. *Int J Epidemiol*. 2014;43(5):1645-1655.

349. Peng Y, Cheng Z, Xie F. Evaluation of Pharmacokinetic Drug–Drug Interactions: A Review of the Mechanisms, In Vitro and In Silico Approaches. *Metabolites*. 2021;11(2):75.

350. Grimsley A, Gallagher R, Hutchison M, Pickup K, Wilson ID, Samuelsson K. Drug–drug interactions and metabolism in cytochrome P450 2C knockout mice: Application to troleandomycin and midazolam. *Biochem Pharmacol*. 2013;86(4):529-538.

351. Zhang D, Luo G, Ding X, Lu C. Preclinical experimental models of drug metabolism and disposition in drug discovery and development. *Acta Pharm Sin B*. 2012;2(6):549-561.

352. Tsigelny IF. Artificial intelligence in drug combination therapy. *Brief Bioinform*. 2019;20(4):1434-1448.

353. Romm EL, Tsigelny IF. Artificial intelligence in drug treatment. *Annu Rev Pharmacol Toxicol*. 2020;60:353-369.

354. Finkelstein J, Friedman C, Hripcsak G, Cabrera M. Potential utility of precision medicine for older adults with polypharmacy: a case series study. *Pharmgenomics Pers Med*. 2016;9:31-45.

355. Bolen S, Feldman L, Vassy J, et al. Systematic Review: Comparative Effectiveness and Safety of Oral Medications for Type 2 Diabetes Mellitus. *Ann Intern Med*. 2007;147(6):386-399.

356. Herings RMC, de Boer A, Leufkens HGM, Porsius A, Stricker BHCh. Hypoglycaemia associated with use of inhibitors of angiotensin converting enzyme. *Lancet*. 1995;345(8959):1195-1198.

357. Morris AD, Boyle DI, McMahon AD, et al. ACE Inhibitor Use Is Associated With Hospitalization for Severe Hypoglycemia in Patients With Diabetes. *Diabetes Care*. 1997;20(9):1363-1367.

358. Pfizer. Highlights of Prescribing Information. ALTACE® (ramipril) capsules, for oral use. Pfizer. 2017. Accessed May 30, 2022. https://labeling.pfizer.com/ShowLabeling.aspx?id=716

359. Dimakos J, Cui Y, Platt RW, Renoux C, Filion KB, Douros A. Concomitant Use of Sulfonylureas and Warfarin and the Risk of Severe Hypoglycemia: Population-Based Cohort Study. *Diabetes Care*. 2022;45(10):e131-e133.

Chapter 8

Appendices

Chapter 8.1

Identification of polypharmacy patterns in new-users of metformin using the Apriori algorithm: a novel framework for investigating concomitant drug utilisation through association rule mining

Selection of drugs included in the polypharmacy analysis

The database contained 2,256 anatomical therapeutic chemical (ATC) codes. Of these, vaccines, insulin products, other therapeutic products (e.g., cosmetics and disinfectants) were excluded. Non-insulin antidiabetic drugs (NIADs) containing two active ingredients in the formulation were split and counted as exposure to two drug compounds (e.g., sitagliptin and metformin [A10B D07] was split in metformin [A10B A02] and sitagliptin [A10B H01]). We restricted our analysis to include drugs with a prescription frequency of ≥5 in the database (624 unique ATC codes) during the study period.

Apriori method

The Apriori algorithm is one of the most popular algorithms for generating frequent item-sets and it was first proposed by Agrawal *et al*.[125] Apriori addresses the computational limitation derived from examining extremely high number of individual combinations as they appear when trying to assess drug combinations in large databases. The frequency of each potential combination of k drugs in patients taking combinations of n drugs, is given by **Equation 8.1.1**.

$$C_{n,k} = \frac{n!}{k!(n-k)!} \qquad \textbf{(Equation 8.1.1)}$$

According to the **Equation 8.1.1**, patients receiving 15 drugs, for example, have 32,752 possible combinations of two up to 15 drugs, while patients receiving 40 drugs have 1.1×10^{12} possibilities of drug combinations. Therefore, assessing the most frequent drug combinations, particularly in patients receiving extremely high polypharmacy is very challenging. The Apriori overcomes this challenge by excluding most large sets as candidates by looking first at smaller sets and recognizing that a large set cannot be frequent unless all its subsets are.

In this study, Apriori was applied to identify and quantify combinations of drugs that are commonly prescribed (i.e., frequent item-sets). The algorithm is based on a concept called 'support', defined by the number of patients in which a specific drug compound (or combination of drugs) occurs. The first step was to compute the support of each individual drug in the database (i.e., to compute to how many patients each drug was prescribed). After that, a support threshold of 5 was used to filter out some of the drugs that were not frequently prescribed (i.e., drugs prescribed to ≤5 patients in the database during the study period). In the following step, the algorithm repeated the analysis using combinations of two drugs. At this

step, without the use of Apriori, the number of possible combinations quickly increased due to the large number of drugs available in the database. Thus, an advantage of using Apriori compared to the aforementioned approach is that the algorithm will not count pairs of drugs (or combinations of drugs) that contain any of the non-frequent items, and therefore, the number of drug combinations computed is significantly lower than when computing all possible drug combinations. In a next step, Apriori repeats iteratively the same process for sets of three up to 40 drugs, not accounting for the sets that were eliminated in the previous round. Thus, the Apriori algorithm, as well as other algorithms used to find frequent item-sets (e.g., Toivonen's and PCY algorithms), are efficient approaches for finding frequent patterns in large databases.

Polypharmacy analysis using Apriori algorithm

The parameters "support" set for the analysis of each stratum are shown in the **Table 8.1.1**. Support refers to the frequency of a drug and can be calculated by finding number of drug combinations including particular drug (e.g., drug A) divided by total number of drug combinations in the stratum. The support requirements vary with different number of drugs in each stratum. Confidence is a conditional probability that refers to the likelihood that a drug B is also taken if drug A is taken, and this parameter was set in 0.1 in all strata in our analysis. We restricted our analysis to drugs with a frequency of prescription >5 in the respective strata.

Drug combination and potential hypoglycaemia

Metformin as monotherapy has little or no risk of hypoglycaemia based on its mechanism of action.[355] However, when combined with additional drugs (polypharmacy), hypoglycaemic effects can occur either due to the inherent effect of an additional drug itself, to a drug-drug interaction (DDI), or to additive drug effects. For example, although a pairwise drug interaction with metformin may not be clinically significant, the DDI still affects drug metabolism. In a context of polypharmacy, the altered metabolism due to multiple drug intake may significantly increase the risk of hypoglycaemia in patients with type 2 diabetes mellitus (T2DM).

For example, ramipril, an angiotensin-converting enzyme (ACE) inhibitor, is highly prevalent among metformin initiators and evidence suggests it may increase insulin sensitivity, leading to an elevated risk of hypoglycaemia.[356,357] Thus, the combination of metformin and ramipril may potentiate metformin's hypoglycaemic effects, particularly in older patients and/or with renal impairment.[358] Additionally, the risk of hypoglycaemic effects can be further exacerbated by adding a sulfonylurea (dual therapy) to the drug cocktail for improving insulin

secretion and a beta-blocker agent (i.e., propranolol), for example.[359] However, further evidence on the clinical significance of this drug combination is needed.

Table 8.1.1 The Apriori algorithm parameters for polypharmacy analysis.

Stratum	Total number of ATC codes in the analysis	Max. number of additional dugs in the polypharmacy analysis	Support
Overall population	621	39	0.001
Male	502	36	0.001
Female	510	38	0.001
18- 39 years	196	25	0.005
40-59 years	446	36	0.001
60-74 years	477	38	0.001
≥75 years	385	31	0.001

Abbreviations: ATC: Anatomical therapeutic chemical.

Table 8.1.2 Baseline characteristics of metformin new-users, stratified by age.

	18 – 39 years N=2,838 (8.3%)		40 – 59 years N=13,885 (40.6%)		60 – 74 years N=12,804 (37.5%)		≥75 years N=4,642 (13.6%)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	1,976 (69.6%)	862 (30.4%)	6,878 (49.5%)	7,007 (50.5%)	3,731 (29.1%)	9,073 (70.9%)	730 (15.7%)	3,912 (84.3%)
Mean age (SD)	31.8 (5.5)	33.2 (5.1)	50.8 (5.3)	52.0 (5.1)	65.8 (4.2)	67.0 (4.2)	79.4 (4.1)	80.5 (4.6)
Median number of drugs (IQR)	2 (1 - 3)	7 (5 - 9)	3 (2 - 3)	7 (6 - 10)	3 (2 - 4)	8 (6 - 11)	3 (3 - 4)	9 (7 - 12)
Mean number of drugs (SD)	2.3 (1.1)	7.9 (3.6)	2.6 (1.1)	8.6 (3.9)	2.9 (1.0)	9.1 (4.0)	3.1 (0.9)	9.7 (3.9)
Distribution of drugs								
1 (only metformin)	624 (22.0%)	-	1,379 (9.9%)	-	481 (3.8%)	-	84 (1.0%)	-
2 - 4	1,352 (47.6%)	-	5,499 (39.6%)	-	3,250 (25.4%)	-	682 (14.7%)	-
5 - 9	-	669 (23.6%)	-	4,882 (35.2%)	-	5,884 (45.9%)	-	2,200 (47.4%)
10 - 19	-	181 (6.4%)	-	1,975 (14.2%)	-	2,972 (23.2%)	-	1,614 (34.8%)
≥20	-	12 (0.4%)	-	150 (1.1%)	-	217 (1.7%)	-	98 (2.1%)
Max nº of drugs	-	30	-	38	-	40	-	33
Number of NIADs prescribed at index date								
≥2	69 (3.5%)	45 (5.2 %)	241 (3.5%)	304 (4.3%)	89 (2.4%)	240 (2.6%)	11 (1.5%)	107 (2.7%)
Smoking status[1]								
Current	441 (22.3%)	271 (31.4%)	1,293 (18.8%)	1,862 (26.6%)	492 (13.2%)	1,384 (15.2%)	56 (7.7%)	240 (6.1%)
Never	1,168 (58.8%)	404 (46.9%)	3,755 (54.6%)	2,979 (42.5%)	2,020 (54.1%)	3,772 (41.6%)	412 (56.4%)	2,020 (51.7%)
Former	328 (16.6%)	179 (20.8%)	1,787 (26.0%)	2,133 (30.4%)	1,206 (32.3%)	3,885 (42.8%)	255 (34.9%)	1,635 (41.8%)
Unknown / Missing value	46 (2.3%)	8 (0.9%)	43 (0.6%)	33 (0.5%)	13 (0.4%)	32 (0.4%)	7 (1.0%)	17 (0.4%)
Alcohol use[1]								
Current	1,034 (52.3%)	463 (53.7%)	4,824 (70.1%)	4,542 (64.8%)	2,803 (75.1%)	6,040 (66.6%)	486 (66.6%)	2418 (61.8%)
Never (Lifelong teetotaler)	488 (24.7%)	236 (27.4%)	1,357 (19.7%)	1,672 (23.9%)	599 (16.1%)	2,122 (23.4%)	172 (23.6%)	1,15 (28.8%)
Former	47 (2.4%)	42 (4.9%)	207 (3.0%)	448 (6.4%)	125 (3.3%)	584 (6.4%)	30 (4.1%)	229 (5.8%)
Unknown / Missing value	407 (20.6%)	121 (14.0%)	490 (7.1%)	345 (4.9%)	204 (5.5%)	37 (3.6%)	42 (5.7%)	140 (3.6%)

Table 8.1.2 (cont.) Baseline characteristics of metformin new-users, stratified by age.

	18 – 39 years N=2,838 (8.3%)		**40 – 59 years N=13,885 (40.6%)**		**60 – 74 years N=12,804 (37.5%)**		**≥75 years N=4,642 (13.6%)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	
BMI[2]								
Obese (BMI ≥30.0 kg/m^2)	1,207 (61.1%)	660 (76.6%)	4,477 (65.1%)	5,404 (77.1%)	2,043 (54.8%)	6,038 (66.5%)	242 (33.2%)	1,782 (45.5%)
Overweight (BMI 25.0 – 29.9 kg/m^2)	371 (18.8%)	105 (12.2%)	1,700 (24.7%)	1,155 (16.5%)	1,253 (33.6%)	2,320(25.6%)	303 (41.5%)	
Normal/Underweight (BMI <25.0 kg/ m^2)	259 (13.1%)	58 (6.7%)	440 (6.4%)	276 (3.9%)	322 (8.6%)	560 (6.2%)	146 (20.0%)	
Missing value	139 (7.0%)	39 (4.5%)	21 (3.8%)	172 (2.5%)	113 (3,0%)	155 (1.7%)	39 (5.3%)	
History of disease								
Hypertension	127 (6.4%)	147 (17.1%)	1,636 (23.8%)	3,079 (43.9%)	1,423 (38.1%)	5,923 (65.3%)	355 (48.6%)	
Coronary heart disease	<7	16 (1.9%)	48 (0.7%)	741 (10.6%)	81 (2.2%)	1,952 (21.5%)	35 (4.8%)	
Heart failure	<7	12 (1.4%)	6 (0.1%)	170 (2.4%)	10 (0.3%)	436 (4.8%)	<7	
Cerebrovascular disease	7 (0.4%)	9 (1.0%)	71 (1.0%)	303 (4.3%)	82 (2.2%)	817 (9.0%)	42 (5.8%)	656 (16.8%)
Chronic kidney disease	<7	<7	<7	25 (0.4%)	14 (0.4%)	102 (1.1%)	<7	111 (2.8%)
Chronic liver disease	27 (1.4%)	36 (4.2%)	154 (2.2%)	331 (4.7%)	97 (2.6%)	397 (4.4%)	<7	88 (2.3%)
COPD	<7	<7	41 (0.6%)	362 (5.2%)	77 (2.1%)	1,208 (13.3%)	19 (2.6%)	520 (13.3%)
Asthma	324 (16.4%)	286 (33.2%)	621 (9.0%)	1,516 (21.6%)	212 (5.7%)	1,569 (17.3%)	30 (4.1%)	598 (15.3%)
Diabetic retinopathy	7 (0.4%)	<7	115 (1.7%)	140 (2.0%)	125 (3.4%)	335 (3.7%)	39 (5.3%)	235 (6.0%)
Sleep disorders	79 (4.0%)	81 (9.4%)	357 (5.2%)	663 (9.5%)	145 (3.9%)	894 (9.9%)	28 (3.8%)	256 (6.5%)
Depression	354 (17.9%)	330 (38.3%)	1,113 (16.2%)	2,415 (34.5%)	503 (13.5%)	2,455 (27.1%)	55 (7.5%)	665 (17.0%)
Alzheimer/Dementia	<7	<7	<7	<7	9 (0.2%)	79 (0.9%)	13 (1.9%)	257 (6.6%)
Hypothyroidism	78 (4.0%)	64 (7.4%)	265 (3.9%)	649 (9.3%)	160 (4.3%)	1,097 (12.1%)	50 (6.9%)	581 (14.9%)
Osteoarthritis	9 (0.5%)	14 (1.6%)	367 (5.3%)	808 (11.5%)	628 (16.8%)	2,743 (30.2%)	215 (29.5%)	1,679 (42.9%)
Osteoporosis	<7	<7	11 (0.2%)	78 (1.1%)	34 (0.9%)	285 (3.1%)	23 (3.2%)	332 (8.5%)
Cancer	10 (0.5%)	11 (1.3%)	135 (2.0%)	268 (3.8%)	265 (7.1%)	1,004 (11.1%)	112 (15.3%)	667 (17.1%)

Table 8.1.2 (cont.) Baseline characteristics of metformin new-users, stratified by age.

	18 – 39 years N=2,838 (8.3%)		40 – 59 years N=13,885 (40.6%)		60 – 74 years N=12,804 (37.5%)		≥75 years N=4,642 (13.6%)	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
Lab values								
HbA1c[3]								
<6.5% (48 mmol/mol)	188 (9.5%)	69 (8.0%)	224 (3.3%)	251 (3.6%)	107 (2.9%)	311 (3.4%)	26 (3.6%)	123 (3.1%)
6.5 – 7.4% (48 – 57 mmol/mol)	203 (10.3%)	133 (15.4%)	1,527 (22.2%)	1,923 (27.4%)	1,016 (27.2%)	3,057 (33.7%)	210 (28.8%)	1,297 (33.2%)
7.5 – 8.5% (58 – 69 mmol/mol)	200 (10.1%)	127 (14.7%)	1,228 (17.8%)	1,614 (23.0%)	861 (23.1%)	2,323 (25.6%)	198 (27.1%)	1,078 (27.6%)
>8.5% (69 mmol/mol)	588 (29.8%)	268 (31.1%)	3,163 (46.0%)	2,494 (35.6%)	1,429 (38.3%)	2,565 (28.3%)	238 (32.6%)	1,007 (25.7%)
Missing value	797 (40.3%)	265 (30.7%)	737 (10.7%)	725 (10.3%)	318 (8.5%)	817 (9.0%)	58 (7.9%)	407 (10.4%)
eGFR (mL/min/1.73 m²)[3]								
<30	<7	<7	<7	<7	<7	<7	<7	11 (0.3%)
30 – 59	12 (0.6%)	8 (0.9%)	140 (2.0%)	306 (4.4%)	192 (5.1%)	1,012 (11.2%)	143 (19.6%)	1,074 (27.4%)
≥60	943 (47.7%)	486 (56.4%)	4,744 (69.0%)	4,666 (66.6%)	2,550 (68.3%)	5,731 (63.2%)	415 (56.8%)	1,991 (50.9%)
Missing value	1,021 (51.7%)	368 (42.7%)	1,994 (29.0%)	2,030 (29.0%)	989 (26.5%)	2,327 (25.6%)	169 (23.2%)	836 (21.4%)

Acronyms: NIAD: non-insulin antidiabetic drug; SD: standard deviation; IQR: interquartile range; BMI: body mass index; COPD: chronic obstructive pulmonary disease; HbA1c: hemoglobin A1c; eGFR: Estimated glomerular filtration rate.

[1] Identified values closest to the index date (i.e., at or before index date).

[2]BMI was assessed using the most recently recorded value for weight (at or at any time before index date) and height (at or at any time before or after index date).

[3]The most recent value (at index date and previous six-months) registered in the database with a valid unit was considered.

Note: A history of comorbidities was assessed if ever registered in the database previous to or at index date. Numbers fewer than seven are suppressed according to data use agreement with IMRD.

Table 8.1.3 Baseline characteristics of metformin new-users receiving polypharmacy at index date, with exposure window at index date and within 30-days before index date, stratified by sex.

	Overall study patients (N=34,169)		**Women (N=14,964)**		**Men (N=19,205)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	19,187 (56.2%)	14,982 (43.8%)	7,884 (52.7%)	7,080 (47.3%)	11,303 (58.9%)	7,902 (41.1%)
Mean age (SD)	56.1 (13.7)	63.8 (12.6)	54.9 (15.4)	60.8 (13.1)	56.9 (12.4)	63.7 (12.1)
Median number of drugs (IQR)	2 (1 - 3)	7 (6 - 10)	2 (1 - 3)	7 (6 - 10)	2 (1 - 3)	7 (6 - 9)
Mean number of drugs (SD)	2.3 (1.1)	8.1 (3.2)	2.4 (1.1)	8.4 (3.4)	2.3 (1.1)	7.9 (3.0)
Distribution of drugs						
1 (only metformin)	5,762 (16.9%)	-	2,309 (15.4%)	-	3,453 (18.0%)	-
2 - 4	13,425 (39.3%)	-	5,575 (37.2%)	-	7,850 (40.9%)	-
5 - 9	-	11,094 (32.5%)	-	5,007 (33.5%)	-	6,087 (31.7%)
10 - 19	-	3,767 (11.0%)	-	2,000 (13.4%)	-	1,767 (9.2%)
≥20	-	121 (0.3%)	-	73 (0.5%)	-	48 (0.2%)
Max nº of drugs	-	37	-	37	-	30

Abbreviations: SD: standard deviation; IQR: interquartile range.

Table 8.1.4 Baseline characteristics of metformin new-users receiving polypharmacy at index date, with exposure window at the index date and within 30-days before index date, stratified by age.

	18 – 39 years N=2,838 (8.3%)		**40 – 59 years N=13,885 (40.6%)**		**60 – 74 years N=12,804 (37.5%)**		**≥75 years N=4,642 (13.6%)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	2,364 (83.3%)	474 (16.7%)	8,945 (64.4%)	4,940 (35.6%)	6,219 (48.6%)	6,585 (51.4%)	1,659 (35.7%)	2,983 (64.3%)
Mean age (SD)	32.0 (5.4)	33.4 (5.2)	51.0 (5.3)	52.1 (5.1)	66.2 (4.2)	67.0 (4.2)	79.7 (4.3)	80.7 (4.7)
Median number of drugs (IQR)	2 (1 - 3)	7 (5 - 9)	2 (1 - 3)	7 (6 - 9)	2 (1 - 3)	7 (6 - 10)	3 (1 - 4)	8 (6 - 10)
Mean number of drugs (SD)	2.0 (1.0)	7.5 (3.1)	2.3 (1.1)	7.9 (3.2)	2.3 (1.1)	8.2 (3.2)	2.5 (1.1)	8.5 (3.1)
Distribution of drugs								
1 (only metformin)	963 (33.9%)		2,685 (19.3%)		1,676 (13.1%)		438 (9.4%)	
2 - 4	1,401 (49.4%)		6,260 (45.1%)		4,543 (35.5%)		1,221 (26.3%)	
5 - 9		391 (13.8%)		3,775 (27.2%)		4,861 (38.0%)		2,067 (44.5%)
10 - 19		78 (2.7%)		1,128 (8.1%)		1,673 (13.1%)		888 (19.1%)
≥20		5 (0.2%)		37 (0.3%)		51 (0.4%)		28 (0.6%)
Max nº of drugs	-	37	-	37	-	37	-	27

Abbreviations: SD: standard deviation; IQR: interquartile range

Table 8.1.5 Baseline characteristics of metformin new-users receiving polypharmacy at index date, with exposure window within 14-days around the index date (i.e., from index date to ± 7 days), stratified by sex.

	Overall study patients (N=34,169)		**Women (N=14,964)**		**Men (N=19,205)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	27,020 (79.1%)	7,149 (20.9%)	11,539 (77.1%)	3,425 (22.9%)	15,481(80.6%)	3,724 (19.4%)
Mean age (SD)	58.3 (13.8)	63.9 (12.9)	57.7 (15.2)	63.8 (13.4)	58.7 (12.6)	63.9 (12.3)
Median number of drugs (IQR)	2 (1 - 2)	7 (6 - 9)	2 (1 - 3)	7 (6 - 9)	2 (1 - 2)	7 (5 - 9)
Mean number of drugs (SD)	1.8 (1.0)	7.7 (2.9)	1.9 (1.0)	7.9 (3.1)	1.8 (1.0)	7.6 (2.8)
Distribution of drugs						
1 (only metformin)	13,289 (38.9%)	-	5,577 (37.3%)	-	7,712 (40.1%)	-
2 - 4	13,731 (40.2%)	-	5,692 (39.8%)	-	7,769 (40.5%)	-
5 - 9	-	5,577 (16.3%)	-	2,593 (17.3%)	-	2,984 (15.5%)
10 - 19	-	1,545 (4.5%)	-	816 (5.5%)	-	729 (3.8%)
≥20		27 (0.1%)		16 (0.1%)		11 (0.1%)
Max nº of drugs	-	26	-	26	-	26

Abbreviations: SD: standard deviation; IQR: interquartile range

Table 8.1.6 Baseline characteristics of metformin new-users receiving polypharmacy at index date, with exposure window within 14-days around the index date (i.e., from index date to ± 7 days), stratified by age.

	18 – 39 years N=2,838 (8.3%)		**40 – 59 years N=13,885 (40.6%)**		**60 – 74 years N=12,804 (37.5%)**		**≥75 years N=4,642 (13.6%)**	
Characteristic	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**	**no polypharmacy**	**polypharmacy**
N (%)	2,573 (90.7%)	265 (9.3%)	11,612 (83.6%)	2,273 (16.4%)	9,680 (75.6%)	3,124 (24.4%)	3,155 (68.0%)	1,487 (32.0%)
Mean age (SD)	32.1 (5.4)	33.2 (5.1)	51.3 (5.3)	52.1 (5.1)	66.5 (4.2)	66.9 (4.3)	80.1 (4.4)	80.9 (4.8)
Median number of drugs (IQR)	1 (1 – 2)	6 (5 – 8)	2 (1 – 2)	7 (5 – 9)	1 (1 – 2)	7 (6 – 9)	2 (1 – 3)	7 (6 – 9)
Mean number of drugs (SD)	1.7 (0.9)	6.9 (2.7)	1.9 (1.0)	7.6 (3.0)	1.8 (1.0)	7.8 (3.0)	1.9 (1.0)	8.0 (2.8)
Distribution of drugs								
1 (only metformin)	1,141 (50.8%)	-	5,418 (39.0%)	-	4,868 (38.0%)	-	1,562 (33.6%)	-
2 - 4	1,132 (39.9%)	-	6,194 (44.6%)	-	4,812 (37.6%)	-	1,593 (34.3%)	-
5 - 9	-	231 (8.1%)	-	1,799 (13.0%)	-	2,425 (18.9%)	-	1,122 (24.2%)
10 - 19	-	32 (1.1%)	-	465 (3.3%)	-	686 (5.4%)	-	362 (7.8%)
≥20		<7		9 (0.1%)		13 (0.1%)		<7
Max nº of drugs	-	26	-	26	-	25	-	25

Abbreviations: SD: standard deviation; IQR: interquartile range

Table 8.1.7 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Overall (14,982)	1	1	5,704 (38.1%)	atorvastatin
		2	3,883 (25.9%)	omeprazole
		3	3,330 (22.2%)	ramipril
		4	3,293 (22.0%)	aspirin
		5	3,282 (21.9%)	amlodipine
	2	1	1,746 (11.7%)	aspirin, atorvastatin
		2	1,629 (10.9%)	amlodipine, atorvastatin
		3	1,622 (10.8%)	ramipril, atorvastatin
		4	1,592 (10.6%)	bisoprolol, atorvastatin
		5	1,452 (9.7%)	omeprazole, atorvastatin
	3	1	910 (6.1%)	aspirin, bisoprolol, atorvastatin
		2	668 (4.5%)	aspirin, ramipril, atorvastatin
		3	632 (4.2%)	bisoprolol, ramipril, atorvastatin
		4	627 (4.2%)	aspirin, bisoprolol, ramipril
		5	491 (3.3%)	amlodipine, ramipril, atorvastatin
	4	1	430 (2.9%)	aspirin, bisoprolol, atorvastatin, ramipril
		2	241 (1.6%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	217 (1.4%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	186 (1.2%)	aspirin, amlodipine, bisoprolol, atorvastatin
		5	175 (1.2%)	lansoprazole, bisoprolol, ramipril, atorvastatin
	5	1	119 (0.8%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin
		2	91 (0.6%)	aspirin, omeprazole, bisoprolol, ramipril, atorvastatin
		2	91 (0.6%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	64 (0.4%)	aspirin, bisoprolol, ramipril, atorvastatin, amlodipine
		4	63 (0.4%)	aspirin, bisoprolol, ticagrelor, ramipril, atorvastatin,
		5	62 (0.4%)	aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin

Table 8.1.7 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Overall (14,982)	6	1	27 (0.2%)	lansoprazole, ticagrelor, aspirin, bisoprolol, atorvastatin, ramipril
		2	24 (0.2%)	aspirin, glyceryl trinitrate, bisoprolol, atorvastatin, ramipril, lansoprazole
		2	24 (0.2%)	aspirin, clopidogrel, lansoprazole, bisoprolol, atorvastatin, ramipril
		3	23 (0.2%)	lansoprazole, aspirin, furosemide, bisoprolol, atorvastatin, ramipril
		4	21 (0.1%)	aspirin, ticagrelor, atorvastatin, glyceryl trinitrate, bisoprolol, ramipril
		5	19 (0.1%)	aspirin, clopidogrel, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		5	19 (0.1%)	omeprazole, aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin
Women (7,080)	1	1	2,411 (30.1%)	atorvastatin
		2	1,993 (28.1%)	omeprazole
		3	1,390 (19.6%)	simvastatin
		4	1,355 (19.1%)	levothyroxine sodium
		5	1,303 (18.4%)	salbutamol
	2	1	680 (9.6%)	omeprazole, atorvastatin
		2	624 (8.8%)	amlodipine, atorvastatin
		3	552 (7.8%)	ramipril, atorvastatin
		4	546 (7.7%)	aspirin, atorvastatin
		5	526 (7.4%)	bisoprolol, atorvastatin
	3	1	235 (3.3%)	aspirin, bisoprolol, atorvastatin
		2	179 (2.5%)	aspirin, ramipril, atorvastatin
		3	175 (2.5%)	aspirin, omeprazole, atorvastatin
		4	161 (2.3%)	amlodipine, ramipril, atorvastatin
		5	159 (2.2%)	bisoprolol, ramipril, atorvastatin
	4	1	93 (1.3%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	74 (1.0)	aspirin, bisoprolol, omeprazole, atorvastatin
		3	57 (0.8%)	aspirin, ramipril, omeprazole, atorvastatin
		4	52 (0.7%)	aspirin, bisoprolol, lansoprazole, atorvastatin
		5	49 (0.7%)	aspirin, bisoprolol, amlodipine, atorvastatin

Table 8.1.7 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Women (7,080)	5	1	26 (0.4%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		2	20 (0.3%)	aspirin, bisoprolol, paracetamol, atorvastatin, omeprazole
		3	19 (0.3%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole
		3	19 (0.3%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		4	18 (0.3%)	aspirin, bisoprolol, co-codamol, atorvastatin, omeprazole
		5	17 (0.2%)	lansoprazole, furosemide, bisoprolol, ramipril, atorvastatin
		5	17 (0.2%)	aspirin, furosemide, bisoprolol, ramipril, atorvastatin
	6	1	7 (0.1%)	aspirin, ticagrelor, furosemide, bisoprolol, atorvastatin, ramipril
		1	7 (0.1%)	prednisolone, vitamins with minerals, alendronic acid, salbutamol, tiotropium bromide, omeprazole
		1	7 (0.1%)	omeprazole, aspirin, bisoprolol, ramipril, co-codamol, atorvastatin
Men (7,902)	1	1	3,293 (41.7%)	atorvastatin
		2	2,197 (27.8%)	aspirin
		3	2.080 (26.3%)	ramipril
		4	1,992 (25.2%)	amlodipine
		5	1,917 (24.3%)	bisoprolol
	2	1	1,200 (15.2%)	aspirin, atorvastatin
		2	1,070 (13.5%)	ramipril, atorvastatin
		3	1,066 (13.5%)	bisoprolol, atorvastatin
		4	1,037 (13.1%)	bisoprolol, aspirin
		5	1,005 (12.7%)	amlodipine, atorvastatin
	3	1	675 (8.5%)	aspirin, bisoprolol, atorvastatin
		2	498 (6.3%)	aspirin, ramipril, bisoprolol
		3	489 (6.2%)	atorvastatin, aspirin, ramipril
		4	473 (6.0%)	atorvastatin, bisoprolol, ramipril
		5	330 (4.2%)	ramipril, amlodipine, atorvastatin

Table 8.1.7 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Men (7,902)	4	1	337 (4.3%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	189 (2.4%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	143 (1.8%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	138 (1.7%)	ramipril, bisoprolol, atorvastatin, lansoprazole
		5	137 (1.7%)	amlodipine, aspirin, bisoprolol, atorvastatin
	5	1	100 (1.3%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	72 (0.9%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		3	65 (0.8%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	51 (0.6%)	aspirin, isosorbide mononitrate, bisoprolol, atorvastatin, ramipril
		5	50 (0.6%)	aspirin, amlodipine, bisoprolol, atorvastatin, ramipril
	6	1	23 (0.3%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, glyceryl trinitrate
		2	22 (0.3%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, clopidogrel
		3	21 (0.3%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, ticagrelor
		4	19 (0.2%)	aspirin, ramipril, bisoprolol, atorvastatin, glyceryl trinitrate, clopidogrel
		5	17 (0.2%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, furosemide

Table 8.1.8 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window at index date or within 30-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
18 – 39 years (474)	1	1	114 (24.1%)	omeprazole
		2	106 (22.4%)	salbutamol
		3	84 (17.7%)	co-codamol
		4	77 (16.2%)	atorvastatin
		5	62 (13.1%)	sertraline
	2	1	31 (6.5%)	beclomethasone, salbutamol
		2	30 (6.3%)	ramipril, atorvastatin
		3	24 (5.1%)	omeprazole, naproxen
		4	23 (4.9%)	omeprazole, co-codamol
		5	21 (4.4%)	omeprazole, sertraline
	3	1	12 (2.5%)	aspirin, bisoprolol, atorvastatin
		2	9 (1.9%)	indapamide, amlodipine, ramipril
		2	9 (1.9%)	indapamide, amlodipine, atorvastatin
		2	9 (1.9%)	indapamide, ramipril, atorvastatin
		2	9 (1.9%)	aspirin, ramipril, atorvastatin
		2	9 (1.9%)	amlodipine, ramipril, atorvastatin
		2	9 (1.9%)	bisoprolol, ramipril, atorvastatin
		3	8 (1.7%)	aspirin, bisoprolol, ramipril
		4	7 (1.5%)	aspirin, glyceryl trinitrate, bisoprolol
		4	7 (1.5%)	aspirin, glyceryl trinitrate, atorvastatin
		4	7 (1.5%)	prednisolone, amoxicillin, salbutamol
		4	7 (1.5%)	tramadol, omeprazole, amitriptyline
		4	7 (1.5%)	lansoprazole, amitriptyline, salbutamol
	4	1	8 (1.7%)	indapamide, amlodipine, atorvastatin, ramipril
		2	8 (1.7%)	aspirin, ramipril, atorvastatin, bisoprolol

Table 8.1.8 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window at index date or within 30-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
40 – 59 years (4,940)	1	1	1,847 (37.4%)	atorvastatin
		2	1,336 (27.0%)	omeprazole
		3	1,153 (23.3%)	ramipril
		4	977 (19.8%)	Amlodipine
		5	869 (17.6%)	salbutamol
	2	1	568 (11.5%)	ramipril, atorvastatin
		2	493 (10.0%)	aspirin, atorvastatin
		3	468 (9.5%)	amlodipine, atorvastatin
		4	467 (9.5%)	bisoprolol, atorvastatin
		5	428 (8.7%)	omeprazole, atorvastatin
	3	1	317 (6.4%)	aspirin, bisoprolol, atorvastatin
		2	234 (4.7%)	aspirin, ramipril, atorvastatin
		3	232 (4.7%)	aspirin, bisoprolol, ramipril
		4	227 (4.6%)	bisoprolol, ramipril, atorvastatin
		5	162 (3.3%)	amlodipine, atorvastatin, ramipril
	4	1	173 (3.5%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	89 (1.8%)	aspirin, bisoprolol, lansoprazole, atorvastatin
		3	81 (1.6%)	glyceryl trinitrate, aspirin, bisoprolol, atorvastatin
		4	69 (1.4%)	omeprazole, bisoprolol, aspirin, atorvastatin
		5	66 (1.3%)	lansoprazole, bisoprolol, ramipril, atorvastatin
	5	1	48 (1.0%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole
		2	42 (0.9%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	40 (0.8%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	36 (0.7%)	aspirin, bisoprolol, ramipril, atorvastatin, ticagrelor
		5	29 (0.6%)	aspirin, bisoprolol, glyceryl trinitrate, atorvastatin, lansoprazole
	6	1	13 (0.3%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole, ticagrelor

Table 8.1.8 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window at index date or within 30-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
40 – 59 years (4,940)	6	1	13 (0.3%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate, lansoprazole
		2	12 (0.2%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate, ticagrelor
		3	10 (0.3%)	clopidogrel, aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	10 (0.3%)	clopidogrel, aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole
		4	8 (0.2%)	lansoprazole, aspirin, ticagrelor, glyceryl trinitrate, atorvastatin, bisoprolol
		4	8 (0.2%)	omeprazole, aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin
60 – 74 years (6,585)	1	1	2,723 (41.4%)	atorvastatin
		2	1,692 (25.7%)	aspirin
		3	1,679 (25.5%)	omeprazole
		4	1,647 (25.0%)	amlodipine
		5	1,601 (24.3%)	simvastatin
	2	1	901 (13.7%)	aspirin, atorvastatin
		2	854 (13.0%)	amlodipine, atorvastatin
		3	775 (11.8%)	ramipril, atorvastatin
		4	767 (11.6%)	bisoprolol, atorvastatin
		5	699 (10.6%)	bisoprolol, aspirin
	3	1	444 (6.7%)	aspirin, bisoprolol, atorvastatin
		2	320 (4.9%)	aspirin, ramipril, atorvastatin
		3	299 (4.5%)	aspirin, bisoprolol, ramipril
		4	295 (4.5%)	bisoprolol, ramipril, atorvastatin
		5	273 (4.1%)	aspirin, amlodipine, atorvastatin
	4	1	194 (2.9%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	115 (1.7%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	109 (1.7%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	102 (1.5%)	amlodipine, bisoprolol, atorvastatin, aspirin
		5	82 (1.2%)	ramipril, amlodipine, atorvastatin, aspirin

Table 8.1.8 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window at index date or within 30-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
60 – 74 years (6,585)	5	1	58 (0.9%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	38 (0.6%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	37 (0.6%)	aspirin, bisoprolol, ramipril, atorvastatin, amlodipine
		4	33 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		5	30 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, isosorbide mononitrate
	6	1	14 (0.2%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, furosemide
		2	12 (0.2%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, clopidogrel
		3	11 (0.2%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin, ticagrelor
		4	9 (0.1%)	aspirin, bisoprolol, ramipril, atorvastatin, isosorbide mononitrate, glyceryl trinitrate
		4	9 (0.1%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole, glyceryl trinitrate
		4	9 (0.1%)	omeprazole, aspirin, bisoprolol, amlodipine, ramipril, atorvastatin
		5	8 (0.1%)	omeprazole, aspirin, bisoprolol, atorvastatin, glyceryl trinitrate, nicorandil
		5	8 (0.1%)	furosemide, aspirin, bisoprolol, atorvastatin, isosorbide mononitrate, nicorandil
		5	8 (0.1%)	warfarin, digoxin, furosemide, bisoprolol, ramipril, atorvastatin
		5	8 (0.1%)	furosemide, aspirin, bisoprolol, atorvastatin, glyceryl trinitrate, ramipril
		5	8 (0.1%)	omeprazole, aspirin, bisoprolol, atorvastatin, glyceryl trinitrate, ramipril
		5	8 (0.1%)	lansoprazole, aspirin, bisoprolol, atorvastatin, isosorbide mononitrate, ramipril
		5	8 (0.1%)	amlodipine, aspirin, bisoprolol, atorvastatin, isosorbide mononitrate, ramipril
		5	8 (0.1%)	furosemide, aspirin, bisoprolol, atorvastatin, co-codamol, ramipril
		5	8 (0.1%)	lansoprazole, aspirin, bisoprolol, atorvastatin, co-codamol, ramipril
		5	8 (0.1%)	lansoprazole, aspirin, bisoprolol, atorvastatin, amlodipine, ramipril
≥75 years (2,983)	1	1	1,057 (35.4%)	atorvastatin
		2	803 (26.9%)	simvastatin
		3	797 (26.7%)	aspirin

Table 8.1.8 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window at index date or within 30-days before index date.

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
≥75 years (2,983)	1	4	779 (26.15)	bisoprolol
		5	754 (25.3%)	omeprazole
	2	1	342 (11.5%)	bisoprolol, atorvastatin
		2	339 (11.4%)	aspirin, atorvastatin
		3	287 (9.6%)	amlodipine, atorvastatin
		4	272 (9.1%)	omeprazole, atorvastatin
		5	261 (8.7%)	aspirin, simvastatin
	3	1	137 (4.6%)	aspirin, bisoprolol, atorvastatin
		2	105 (3.5%)	ramipril, aspirin, atorvastatin
		3	102 (3.4%)	bisoprolol, atorvastatin, furosemide
		4	101 (3.4%)	bisoprolol, atorvastatin, ramipril
		5	99 (3.3%)	omeprazole, aspirin, atorvastatin
	4	1	55 (1.8%)	aspirin, bisoprolol, atorvastatin, ramipril
		2	36 (1.2%)	omeprazole, aspirin, atorvastatin, ramipril
		3	35 (1.2%)	omeprazole, aspirin, atorvastatin, bisoprolol
		4	34 (1.1%)	lansoprazole, aspirin, atorvastatin, bisoprolol
		5	33 (1.1%)	lansoprazole, furosemide, atorvastatin, bisoprolol
	5	1	15 (0.5%)	omeprazole, aspirin, atorvastatin, bisoprolol, ramipril
		2	11 (0.4%)	lansoprazole, aspirin, atorvastatin, bisoprolol, ramipril
		3	10 (0.3%)	warfarin, digoxin, furosemide, atorvastatin, bisoprolol
		3	10 (0.3%)	lansoprazole, furosemide, atorvastatin, bisoprolol, paracetamol
		4	10 (0.3%)	aspirin, furosemide, bisoprolol, ramipril, atorvastatin
		5	9 (0.3%)	warfarin, digoxin, furosemide, lansoprazole, bisoprolol
		5	9 (0.3%)	atorvastatin, digoxin, furosemide, lansoprazole, bisoprolol
		5	9 (0.3%)	atorvastatin, ramipril, furosemide, lansoprazole, bisoprolol
		5	9 (0.3%)	aspirin, bisoprolol, amlodipine, ramipril, atorvastatin

Table 8.1.9 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Overall (7,149)	1	1	2,759 (38.6%)	atorvastatin
		2	1,785 (25.0%)	omeprazole
		3	1,606 (22.5%)	aspirin
		4	1,572 (22.0%)	ramipril
		5	1,518 (21.2%)	amlodipine
	2	1	854 (11.9%)	aspirin, atorvastatin
		2	825 (11.5%)	ramipril, atorvastatin
		3	801 (11.2%)	bisoprolol, atorvastatin
		4	771 (10.8%)	amlodipine, atorvastatin
		5	712 (10.0%)	bisoprolol, aspirin
	3	1	466 (6.5%)	aspirin, bisoprolol, atorvastatin
		2	344 (4.8%)	aspirin, ramipril, atorvastatin
		3	339 (4.7%)	bisoprolol, ramipril, atorvastatin
		4	323 (4.5%)	aspirin, bisoprolol, ramipril
		5	246 (3.4%)	amlodipine, ramipril, atorvastatin
	4	1	232 (3.2%)	aspirin, bisoprolol, atorvastatin, ramipril
		2	136 (1.9%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	110 (1.5%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	96 (1.3%)	lansoprazole, bisoprolol, ramipril, atorvastatin
		5	91 (1.3%)	lansoprazole, aspirin, ramipril, atorvastatin
	5	1	71 (1.00%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin
		2	48 (0.7%)	aspirin, omeprazole, bisoprolol, ramipril, atorvastatin
		2	48 (0.7%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	38 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, amlodipine
		3	38 (0.5%)	aspirin, ticagrelor, ramipril, bisoprolol, atorvastatin,
		4	37 (0.5%)	clopidogrel, aspirin, bisoprolol, ramipril, atorvastatin

Table 8.1.9 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Overall (7,149)	5	5	34 (0.5%)	aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin
		5	34 (0.5%)	furosemide, bisoprolol, aspirin, ramipril, atorvastatin
	6	1	19 (0.3%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin, clopidogrel
		2	17 (0.2%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin, ticagrelor
		3	15 (0.2%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin, furosemide
		4	14 (0.2%)	aspirin, clopidogrel, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		5	13 (0.2%)	aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
Women (3,425)	1	1	1,152 (33.6%)	atorvastatin
		2	912 (26.6%)	omeprazole
		3	650 (19.0%)	levothyroxine sodium
		4	629 (18.4%)	simvastatin
		5	609 (17.8%)	salbutamol
	2	1	317 (9.3%)	omeprazole, atorvastatin
		2	283 (8.3%)	amlodipine, atorvastatin
		3	281 (8.2%)	ramipril, atorvastatin
		4	266 (7.8%)	bisoprolol, atorvastatin
		5	255 (7.4%)	aspirin, atorvastatin
	3	1	125 (3.6%)	aspirin, bisoprolol, atorvastatin
		2	88 (2.6%)	bisoprolol, ramipril, atorvastatin
		3	85 (2.5%)	aspirin, ramipril, atorvastatin
		4	82 (2.4%)	omeprazole, aspirin, atorvastatin
		5	76 (2.2%)	ramipril, amlodipine, atorvastatin
	4	1	51 (1.5%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	40 (1.2%)	aspirin, bisoprolol, omeprazole, atorvastatin
		3	31 (0.9%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		4	25 (0.7%)	omeprazole, aspirin, ramipril, atorvastatin
		5	22 (0.6%)	furosemide, ramipril, bisoprolol, atorvastatin

Table 8.1.9 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Women (3,425)	5	1	15 (0.4%)	omeprazole, aspirin, bisoprolol, ramipril, atorvastatin
		2	13 (0.4%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin
		3	9 (0.3%)	furosemide, aspirin, bisoprolol, atorvastatin, ramipril
		3	9 (0.3%)	aspirin, ramipril, atorvastatin, bisoprolol, levothyroxine sodium
		4	8 (0.2%)	ticagrelor, aspirin, ramipril, atorvastatin, bisoprolol
		4	8 (0.2%)	aspirin, isosorbide mononitrate, bisoprolol, ramipril, omeprazole
		4	8 (0.2%)	omeprazole, aspirin, isosorbide mononitrate, bisoprolol, atorvastatin
		4	8 (0.2%)	lansoprazole, aspirin, ramipril, salbutamol, atorvastatin
		5	7 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, atorvastatin
		5	7 (0.2%)	aspirin, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		5	7 (0.2%)	omeprazole, spironolactone, bisoprolol, atorvastatin, aspirin
		5	7 (0.2%)	warfarin, digoxin, furosemide, bisoprolol, ramipril
		5	7 (0.2%)	aspirin, isosorbide mononitrate, bisoprolol, atorvastatin, levothyroxine sodium
		5	7 (0.2%)	digoxin, furosemide, bisoprolol, ramipril, atorvastatin
		5	7 (0.2%)	lansoprazole, furosemide, bisoprolol, ramipril, atorvastatin
		5	7 (0.2%)	omeprazole, furosemide, bisoprolol, aspirin, atorvastatin
		5	7 (0.2%)	aspirin, bisoprolol, amlodipine, ramipril, atorvastatin
		5	7 (0.2%)	aspirin, bisoprolol, atorvastatin, omeprazole, levothyroxine sodium
Men (3,725)	1	1	1,607 (43.1%)	atorvastatin
		2	1,077 (28.9%)	aspirin
		3	971 (26.1%)	ramipril
		4	955 (25.6%)	bisoprolol
		5	932 (25.05)	amlodipine
	2	1	599 (16.1%)	aspirin, atorvastatin
		2	544 (14.6%)	ramipril, atorvastatin
		3	535 (14.4%)	bisoprolol, atorvastatin
		4	522 (14.0%)	bisoprolol, aspirin

Table 8.1.9 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by sex, with exposure window at index date or within 30-days before index date.

Sex (N)	Nº of drugs	Rank	N (%)	Drug compound
Men (3,725)	2	5	488 (13.1%)	amlodipine, atorvastatin
	3	1	341 (9.2%)	aspirin, bisoprolol, atorvastatin
		2	259 (7.0%)	aspirin, atorvastatin, ramipril
		3	255 (6.8%)	aspirin, bisoprolol, ramipril
		4	251 (6.7%)	atorvastatin, bisoprolol, ramipril
		5	170 (4.6%)	atorvastatin, ramipril, amlodipine
	4	1	181 (4.9%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	105 (2.8%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	74 (2.0%)	lansoprazole, ramipril, bisoprolol, atorvastatin
		4	72 (1.9%)	ramipril, bisoprolol, aspirin, lansoprazole
		4	72 (1.9%)	lansoprazole, aspirin, ramipril, atorvastatin
		5	70 (1.9%)	aspirin, bisoprolol, amlodipine, atorvastatin
		5	70 (1.9%)	omeprazole, bisoprolol, atorvastatin, aspirin
	5	1	58 (1.6%)	lansoprazole, aspirin, bisoprolol, ramipril, atorvastatin
		2	41 (1.1%)	glyceryl trinitrate, aspirin, bisoprolol, ramipril, atorvastatin
		3	33 (0.9%)	clopidogrel, aspirin, bisoprolol, atorvastatin, ramipril
		3	33 (0.9%)	omeprazole, aspirin, ramipril, atorvastatin, bisoprolol
		4	31 (0.8%)	amlodipine, aspirin, ramipril, atorvastatin, bisoprolol
		5	30 (0.8%)	aspirin, bisoprolol, amlodipine, ramipril, atorvastatin
	6	1	17 (0.5%)	lansoprazole, aspirin, clopidogrel, bisoprolol, ramipril, atorvastatin
		2	14 (0.4%)	lansoprazole, aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin
		2	14 (0.4%)	glyceryl trinitrate, aspirin, clopidogrel, bisoprolol, ramipril, atorvastatin
		3	12 (0.3%)	glyceryl trinitrate, aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin
		3	12 (0.3%)	isosorbide mononitrate, aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin
		3	12 (0.3%)	aspirin, lansoprazole, bisoprolol, ramipril, atorvastatin, furosemide
		4	11 (0.3%)	aspirin, ticagrelor, glyceryl trinitrate, bisoprolol, ramipril, atorvastatin
		5	10 (0.3%)	lansoprazole, aspirin, bisoprolol, amlodipine, ramipril, atorvastatin

Table 8.1.10 Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window within 14 days around the index date (i.e., from index date to ± 7 days)

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
18 - 39 years (265)	1	1	66 (24.9%)	salbutamol
		2	54 (20.4%)	omeprazole
		3	41 (15.5%)	atorvastatin
		4	40 (15.1%)	co-codamol
		5	38 (14.3%)	ramipril
	2	1	22 (8.3%)	beclomethasone, salbutamol
		2	14 (5.3%)	ramipril, atorvastatin
		3	11 (4.2%)	amlodipine, ramipril
		3	11 (4.2%)	omeprazole, naproxen
		3	11 (4.2%)	omeprazole, co-codamol
		3	11 (4.2%)	amlodipine, atorvastatin
		4	10 (3.8%)	bisoprolol, ramipril
		4	10 (3.8%)	ramipril, omeprazole
		4	10 (3.8%)	salbutamol, omeprazole
		5	9 (3.4%)	formoterol and budesonide, salbutamol
		5	9 (3.4%)	omeprazole, citalopram
		5	9 (3.4%)	bisoprolol, atorvastatin
		5	9 (3.4%)	salbutamol, amitriptyline
		5	9 (3.4%)	atorvastatin, omeprazole
40 – 59 years (2,273)	1	1	884 (38.9%)	atorvastatin
		2	590 (26.0%)	omeprazole
		3	560 (24.6%)	ramipril
		4	429 (18.9%)	amlodipine
		5	398 (17.5%)	aspirin
	2	1	302 (13.3%)	ramipril, atorvastatin
		2	252 (11.1%)	aspirin, atorvastatin

Table 8.1.10 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window within 14 days around the index date (i.e., from index date to ± 7 days).

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
40 – 59 years (2,273)	2	3	241 (10.6%)	bisoprolol, atorvastatin
		4	220 (9.7%)	bisoprolol, aspirin
		5	211 (9.3%)	omeprazole, atorvastatin
	3	1	172 (7.6%)	aspirin, bisoprolol, atorvastatin
		2	130 (5.7%)	bisoprolol, ramipril, atorvastatin
		3	128 (5.6%)	aspirin, atorvastatin, ramipril
		4	121 (5.3%)	bisoprolol, ramipril, aspirin
		5	82 (3.6%)	amlodipine, ramipril, atorvastatin
	4	1	97 (4.3%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	52 (2.3%)	aspirin, bisoprolol, lansoprazole, atorvastatin
		3	41 (1.8%)	glyceryl trinitrate, aspirin, bisoprolol, atorvastatin
		4	39 (1.7%)	lansoprazole, aspirin, atorvastatin, ramipril
		5	38 (1.7%)	lansoprazole, bisoprolol, ramipril, atorvastatin
	5	1	30 (1.3%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole
		2	25 (1.1%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		3	23 (1.0%)	aspirin, bisoprolol, ticagrelor, atorvastatin, ramipril
		4	19 (0.8%)	aspirin, bisoprolol, clopidogrel, atorvastatin, ramipril
		5	18 (0.8%)	aspirin, bisoprolol, glyceryl trinitrate, atorvastatin, lansoprazole
	6	1	12 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole, clopidogrel
		2	10 (0.4%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate, lansoprazole
		3	9 (0.4%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate, ticagrelor
		3	9 (0.4%)	clopidogrel, aspirin, bisoprolol, ramipril, atorvastatin, lansoprazole
		4	8 (0.4%)	clopidogrel, aspirin, bisoprolol, lansoprazole, atorvastatin, glyceryl trinitrate
		4	8 (0.4%)	clopidogrel, aspirin, ramipril, atorvastatin, glyceryl trinitrate, bisoprolol
		5	7 (0.3%)	lansoprazole, aspirin, ticagrelor, glyceryl trinitrate, bisoprolol, atorvastatin
		5	7 (0.3%)	aspirin, isosorbide mononitrate, bisoprolol, ramipril, atorvastatin, lansoprazole

Table 8.1.10 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window within 14 days around the index date (i.e., from index date to ± 7 days).

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
60 – 74 years (3,124)	1	1	1,303 (41.7%)	atorvastatin
		2	832 (26.6%)	aspirin
		3	788 (25.2%)	omeprazole
		4	774 (24.8%)	amlodipine
		5	710 (22.7%)	simvastatin
	2	1	440 (14.1%)	aspirin, atorvastatin
		2	419 (13.4%)	amlodipine, atorvastatin
		3	381 (12.2%)	bisoprolol, atorvastatin
		4	373 (11.9%)	ramipril, atorvastatin
		5	354 (11.3%)	bisoprolol, aspirin
	3	1	217 (6.9%)	aspirin, bisoprolol, atorvastatin
		2	159 (5.1%)	aspirin, ramipril, atorvastatin
		3	150 (4.8%)	bisoprolol, ramipril, atorvastatin
		3	150 (4.8%)	bisoprolol, ramipril, aspirin
		4	129 (4.1%)	ramipril, amlodipine, atorvastatin
		5	127 (4.1%)	aspirin, amlodipine, atorvastatin
	4	1	100 (3.2%)	aspirin, bisoprolol, ramipril, atorvastatin
		2	64 (2.1%)	lansoprazole, aspirin, bisoprolol, atorvastatin
		3	57 (1.8%)	omeprazole, aspirin, bisoprolol, atorvastatin
		4	48 (1.5%)	aspirin, amlodipine, bisoprolol, atorvastatin
		5	47 (1.5%)	lansoprazole, aspirin, ramipril, bisoprolol
	5	1	34 (1.1%)	lansoprazole, aspirin, ramipril, bisoprolol, atorvastatin
		2	22 (0.7%)	aspirin, amlodipine, bisoprolol, ramipril, atorvastatin
		3	21 (0.7%)	aspirin, bisoprolol, ramipril, atorvastatin, omeprazole
		4	16 (0.5%)	aspirin, bisoprolol, ramipril, atorvastatin, glyceryl trinitrate
		5	15 (0.5%)	aspirin, ramipril, bisoprolol, atorvastatin, isosorbide mononitrate

Table 8.1.10 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window within 14 days around the index date (i.e., from index date to ± 7 days).

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
60 – 74 years (3,124)	6	1	10 (0.3%)	lansoprazole, aspirin, furosemide, bisoprolol, ramipril, atorvastatin
		2	7 (0.2%)	lansoprazole, aspirin, ticagrelor, bisoprolol, ramipril, atorvastatin
≥75 years (1,487)	1	1	531 (35.7%)	atorvastatin
		2	385 (25.9%)	bisoprolol
	2	3	365 (24.5%)	simvastatin
		4	361 (24.3%)	aspirin
		5	353 (23.7%)	omeprazole
		1	170 (11.4%)	bisoprolol, atorvastatin
		2	155 (10.4%)	aspirin, atorvastatin
		3	139 (9.3%)	amlodipine, atorvastatin
		4	136 (9.1%)	ramipril, atorvastatin
		5	131 (8.8%)	aspirin, bisoprolol
	3	1	71 (4.8%)	aspirin, bisoprolol, atorvastatin
		2	54 (3.6%)	ramipril, bisoprolol, atorvastatin
		3	52 (3.5%)	aspirin, ramipril, atorvastatin
		4	49 (3.3%)	bisoprolol, atorvastatin, lansoprazole
		4	49 (3.3%)	furosemide, bisoprolol, atorvastatin
		5	48 (3.2%)	aspirin, ramipril, bisoprolol
	4	1	31 (2.1%)	aspirin, bisoprolol, atorvastatin, ramipril
		2	19 (1.3%)	aspirin, atorvastatin, lansoprazole, bisoprolol
		2	19 (1.3%)	bisoprolol, atorvastatin, omeprazole, aspirin
		3	17 (1.1%)	ramipril, atorvastatin, furosemide, bisoprolol
		3	17 (1.1%)	aspirin, atorvastatin, furosemide, bisoprolol
		4	16 (1.1%)	lansoprazole, bisoprolol, ramipril, atorvastatin

Table 8.1.10 (cont.) Rank of co-prescribed drug compounds and combinations of up to 6 drugs (excl. metformin) by frequency of prescribing in overall new metformin users receiving polypharmacy and stratified by age, with exposure window within 14 days around the index date (i.e., from index date to ± 7 days).

Age (N)	Nº of drugs	Rank	N (%)	Drug compound
≥75 years (1,487)	4	5	15 (1.0%)	aspirin, bisoprolol, amlodipine, atorvastatin
		5	15 (1.0%)	omeprazole, aspirin, ramipril, bisoprolol
		5	15 (1.0%)	lansoprazole, furosemide, atorvastatin, bisoprolol
	5	1	8 (0.5%)	aspirin, atorvastatin, omeprazole, bisoprolol, ramipril
		2	7 (0.5%)	bisoprolol, atorvastatin, furosemide, aspirin, ramipril

Chapter 8.2

Examining inappropriate medication in UK primary care for type 2 diabetes patients with polypharmacy

Selection of drugs included in the polypharmacy analysis

The database contained 2,256 ATC codes. Of these, vaccines, insulin products, surgical dressings, other non-therapeutic products (e.g., cosmetics and disinfectants), general nutrients as well as records with invalid ATC codes were excluded from the calculation of polypharmacy. NIADs containing two active ingredients in the formulation were split and counted as exposure to two drug compounds (e.g., sitagliptin and metformin [A10BD07] was split in metformin [A10BA02] and sitagliptin [A10BH01]). We restricted our analysis to include drugs with a prescription frequency of ≥5 in the database (624 unique ATC codes) during the study period.

Table 8.2.1 List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	1	Avoid first-generation AH Brompheniramine Carbinoxamine Chlorpheniramine Clemastine Cyproheptadine Dexbrompheniramine Dexchlorpheniramine Dimenhydrinate Diphenhydramine (except if oral for severe allergic reactions) Doxylamine Hydroxyzine Meclizine Promethazine Triprolidine	Highly anticholinergic; clearance reduced with advanced age, confusion, dry mouth, constipation, and other anticholinergic effects or toxicity Use of diphenhydramine in situations such as acute treatment of severe allergic reaction may be appropriate	PIP was defined as ≥1 prescription of first-generation AH at the index date or previous 90 days (except if diphenhydramine with a diagnosis of severe allergic reactions in the 14-days previous to drug prescription).		Fully applied
2	2	Avoid antiparkinsonian agents (benztropine oral and trihexyphenidyl) in combination with antipsychotics	Not recommended for extrapyramidal symptoms with antipsychotics	PIP was defined as ≥1 prescription of antiparkinsonian agent at the index date or previous 90 days and ≥1 prescription of antipsychotic within the 90-days period. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including orphenadrine and and methocarbamol.[2]	Potential underestimation of PIP as some antipsychotics in the UK (e.g., clozapine) are only prescribed in secondary care and, therefore, were not included in the assessment of PIP. Potential overestimation of PIP, as we assumed that patients having a prescription for antiparkinsonian agent and antipsychotic medication within the 90 days interval were used concomitantly.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	3	Avoid antispasmodics Atropine Belladona alkaloids Clinidium-Chlordiazepoxide Dicyclomine Hyoscyamine Propantheline Scopolamine	Highly anticholinergic, uncertain effectiveness	PIP was defined as ≥1 prescription of antispasmodic agents at the index date or previous 90 days. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including dicycloverine and hyoscine.[2]		Fully applied
2	4	Avoid dipyridamole oral short-action (does not apply to extended release combination with aspirin)	May cause orthostatic hypotension; IV formulation is acceptable for use in cardiac stress testing	PIP was defined as ≥1 prescription of dipyridamole (oral short-action formulation) at the index date or previous 90 days.	Potential underestimation of PIP, as we have not included IV formulations due to limitation of our data (i.e., patients receiving IV formulation other than for cardiac stress may have been misclassified as not receiving PIP).	Fully applied
2	5	Avoid ticlopidine	Safer, effective alternatives available	PIP was defined as ≥1 prescription of triclopidine at the index date or previous 90 days.		Fully applied
2	6	Avoid nitrofurantoin if creatinine clearance <30ml/min or for long-term suppression of bacteria. To accommodate differences between the US and UK, we set creatinine clearance <45 mL/min as per the recommendation of NHS upon toxicity assessment.[2]	Potential pulmonary toxicity, hepatoxicity, peripheral neuropathy, especially with long-term use	PIP was defined as ≥1 prescription of nitrofurantoin at the index date or previous 90 days in patients with (i) ≥2 measurements of eGFR <45 mL/min in the year previous to index date, (ii) a diagnosis code for CKD stages 3B, 4, or 5 ever) prior index, or (iii) if long-term suppression of bacteria (i.e., ≥1 prescription of nitrofurantoin with total length >180 days, allowing for gap of maximum 14 days between (cont.)	Potential overestimation of PIP as we used as a proxy for low creatinine clearance ≥2 eGFR measurements with value <45 mL/min in the year prior to index date or a diagnosis code for CKD stages 3B, 4, or 5 ever before the index. By using the minimal cumulative dose prescription approach, we may have misclassified patients receiving high doses of nitrofurantoin <6 months as PIP. (cont.)	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	6			(cont.) prescriptions whenever patients had information on dosage or minimal cumulative dose prescription (prescription of >9,000 mg) of nitrofurantoin within the aforementioned period whenever dosage information was missing. The minimal threshold of minimal cumulative dose prescription within 180 days was calculated based on the standard dosage recommended by NHS for long term nitrofurantoin use (i.e., 50 to 100 mg/day for long-term low dose prophylaxis of recurrent UTI).[3]	(cont.) Although NHS recommends assessing toxicity every 3-6 months in patients receiving long-term nitrofurantoin, we have not assessed whether patients were checked on nitrofurantoin toxicity in the assessment of PIP due to the limitations of our dataset.	
2	7	Avoid peripheral alpha-1 blockers (doxazosin, prazosin, and terazosin) as an antihypertensive	High risk of orthostatic hypotension;	PIP was defined as ≥1 prescription of alpha-1 blockers at the index date or previous 90 days in, in patients with prior diagnosis of hypertension.	Potential overestimation of PIP. We have not assessed the indication of alpha-1 blockers (i.e., we assumed it was used in the treatment of hypertension whenever prescribed to patients with hypertension)	Fully applied
2	8	Avoid central alpha blockers (clonidine as first-line anti-hypertensive. Guanfacine, methyldopa and reserpine [>0.1 mg/ day])	High risk of adverse CNS effects, bradycardia, orthostatic hypotension; Not recommended as routine treatment for hypertension	PIP was defined as ≥1 prescription of (i) guanabenz, guanfacine, methyldopa, or reserpine at index or prior 90 days in patients with hypertension, or (ii) clonidine at index or prior 90 days in patients with hypertension and no prior use of antihypertensive drug.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	9	Avoid disopyramide	Potent negative inotrope and therefore may induce HF in older adults; strongly anticholinergic	PIP was defined as ≥1 prescription of disopyramide at the index date or previous 90 days.		Fully applied
2	10	Avoid dronedarone in individuals with permanent AF or severe or recently decompensated HF	Worse outcomes have been reported in patients taking dronedarone having permanent AF or severe/decompensated HF	PIP was defined as ≥1 prescription of dronedarone at the index date or previous 90 days with no prior diagnosis of permanent AF or severe/decompensated HF.		Fully applied
2	11	Avoid digoxin as first-line therapy for AF	It may be associated with increased mortality	PIP was defined as ≥1 prescription of digoxin at the index date or previous 90 days in patients with prior diagnosis of AF and with no previous use of a first-line agent for rate control in AF as recommended by the NICE guidelines on management of AF[4] (i.e., standard BB [excl. sotalol] or CCB [verapamil and diltiazem]).	We may have misclassified patients receiving digoxin as first-line treatment for AF who had used a BB or CCB prior to AF event for other indications as not receiving PIP. Moreover, in patients receiving PIP, we have not assessed the reason for prescribing digoxin as first-line therapy due to limitations of our dataset (i.e., according to the NICE guidelines, digoxin monotherapy may be appropriate in people with no or very little physical exercise or when other rate-limiting drug options are ruled out because of comorbidities or the person's preferences).	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	12	Avoid digoxin as first-line therapy for HF	Questionable effects on risk of hospitalization and may be associated with increased mortality in older adults with HF	PIP was defined as ≥1 prescription of digoxin at the index date or previous 90 days in patients with prior diagnosis of HF (except if severe HF with reduced ejection fraction) and no previous use of other antiarrhythmics as recommended by the NICE guidelines on management of chronic HF[5] (i.e., BB [bisoprolol, carvedilol, and nebivolol], ARB, mineralocorticoid receptor antagonists, or ACE inhibitor).	We may have misclassified patients receiving digoxin as first-line treatment for HF who had used other antiarrhythmics medication prior to AF event for other indications as not receiving PIP. Moreover, we have not assessed the reason why patient received digoxin as first-line treatment due to limitation of our data. Nevertheless, digoxin is recommended by the NICE guidelines for worsening or severe HF with reduced ejection fraction despite first-line treatment for HF.	Fully applied
2	13	If digoxin is used for AF or HF, avoid dosages >125 mcg/day	Decreased real clearance of digoxin may lead to increased risk of toxic effects;	PIP was defined as ≥1 prescription of digoxin with daily dose >125 mcg at the index date or previous 90 days in patients with a diagnosis of HF or AF.	Potential underestimation of PIP as only patients having complete dosage information were included in the analysis.	Fully applied
2	14	Avoid nifedipine immediate release	Potential for hypotension; risk of precipitating myocardial ischemia	PIP was defined as ≥1 prescription of nifedipine immediate release at the index date or previous 90 days.		Fully applied
2	15	Avoid amiodarone as first-line therapy AF unless patient has HF or substantial left ventricular hypertrophy	Amiodarone is effective for maintaining sinus rhythm but has greater toxicities than other antiarrhythmics used in AF; it may be reasonable first-line therapy in patients with concomitant (cont.)	PIP was defined as ≥1 prescription of amiodarone at the index date or previous 90 days in patients with a previous diagnosis of AF and no prior use of beta blockers (except sotalol) or CCB (verapamil and diltiazem) - except in patients with additional diagnosis of HF or left ventricular hypertrophy previous to index date.	We may have misclassified patients receiving amiodarone as first-line treatment for AF who had used a BB or CCB prior to AF event for other indications as not receiving PIP.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	15		(cont.) HF or substantial left ventricular hypertrophy if rhythm control is preferred over rate control.			Fully applied
2	16	Avoid the following antidepressants, alone or in combination Amitriptyline Amoxapine Clomipramine Desipramine Doxepin (>6mg/day) Imipramine Nortriptyline Paroxetine Protriptyline Trimipramine	Highly anticholinergic, sedating, and cause orthostatic hypotension;	PIP was defined as ≥1 prescription of the antidepressants at the index date or previous 90 days.	Potential overestimation of PIP. First, the use of tricyclic antidepressants is recommended in the management of depression by the NICE guidelines[6], and thus the prescription of these drugs it might not be considered inappropriate. Second, we have not assessed the indication of the antidepressant (i.e., the NICE guidelines for management of neuropathic pain in adults in non-specialists' settings recommends amitriptyline as initial treatment for neuropathic pain [except trigeminal neuralgia], and thus it would be appropriate).[7]	Fully applied
2	17	Avoid first-(conventional) and second- (atypical) generation antipsychotics, unless non-pharmacological options have failed and patient is threatening substantial harm to self or others	Increased risk of stroke and greater rate of cognitive decline and mortality in persons with dementia. Avoid antipsychotics for behavioural problems of dementia or delirium unless nonpharmacological options have failed or are not possible and the older adult is threatening substantial harm to self and others	PIP was defined as ≥1 prescription of antipsychotics at the index date or previous 90 days (except in patients with diagnosis of schizophrenia or bipolar disorder ever prior to index date).	Patients with a cancer diagnosis were excluded due to the study design (i.e., exclusion criteria); thus, the short-term use of antipsychotics during chemotherapy described in the criteria was not assessed in our analysis. Potential overestimation of PIP as we have not assessed if patients had previously failed nonpharmacological options and if an older adult is threatening substantial harm to themselves and others due to limitations on the dataset. Moreover, some antipsychotics in the UK (e.g., clozapine) are exclusively prescribed in secondary care; therefore, we have no access to their prescription data.	Partially applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	18	Avoid barbiturates Amobarbital Butabarbital Butalbital Mephobarbital Pentobarbital Phenobarbital Secobarbital	High rate of physical dependence, tolerance to sleep benefits great risk of overdose at low dosages.	PIP was defined as ≥1 prescription of barbiturates at the index date or previous 90 days.		Fully applied
2	19	Avoid short- and intermediate-acting BZD Alprazolam Estazolam Lorazepam Oxazepam Temazepam Triazolam	Older adults have increased sensitivity to BZD and decreased metabolism of long-acting agents; In general, all BZD increase risk of cognitive impairment, delirium, falls, fractures, and motor vehicle crashes in older adults.	PIP was defined as ≥1 prescription of short- and intermediate-acting BZD at the index date or previous 90 days. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including clobazam, ketazolam, and medazepam.[2]		Fully applied
2	20	Avoid long-acting BZD Clorazepate Chlordiazepoxide Clonazepam Diazepam Flurazepam Quazepam	Elderly have decreased metabolism of long-acting BZDs. Increased risk of cognitive impairment, delirium, falls, fractures, motor vehicle crashes. May be appropriate for seizure disorders, (cont.)	PIP was defined as ≥1 prescription of long-acting BZD at the index date or previous 90 days. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including bromazepam, lormetazepam, and nitrazepam.[2]	Potential overestimation of PIP. We have not assessed the indication of the BZD (i.e., it may be appropriate in seizure disorders, rapid eye movement sleep disorders, BZD withdrawal, ethanol withdrawal, severe generalized anxiety disorder, and periprocedural anaesthesia) due to limitations in our dataset.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	20		(cont.), rapid eye movement sleep disorders, BZD withdrawal, ethanol withdrawal, generalized anxiety disorder, and periprocedural anaesthesia.			Fully applied
2	21	Avoid meprobamate	High rate of physical dependence; very sedating	PIP was defined as ≥1 prescription of meprobamate at the index date or previous 90 days.		Fully applied
2	22	Avoid nonBZD, BZD receptor agonist hypnotics Eszopiclone Zolpidem Zaleplon	ADR similar to BZDs in elderly, delirium, falls, fractures, increased emergency department visits, hospitalizations, motor vehicle crashes	PIP was defined as ≥1 prescription of BZD-receptor agonists hypnotics at the index date or previous 90 days. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including zopiclone.[2]		Fully applied
2	23	Avoid Ergoloid mesylates (dehydrogenated ergot alkaloids) Isoxsuprine	Lack of efficacy	PIP was defined as ≥1 prescription of Ergoloid mesylates or isoxsuprine at the index date or previous 90 days.		Fully applied
2	24	Avoid androgens unless confirmed hypogonadism with clinical symptoms Methyltestosterone Testosterone	Potential for cardiac problems; contraindicated in men with prostate cancer	PIP was defined as ≥1 prescription of androgen at the index date or previous 90 days in male except if previous diagnosis of hypogonadism.	Potential overestimation of PIP. We have not considered hypogonadism symptoms (only the previous diagnosis) to assess the PIP. However, the hypogonadism diagnosis is made upon low serum testosterone levels and the presence of clinical symptoms. Thus, we considered that if previous a diagnosis recorded in the database, then androgen is appropriate.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	25	Avoid desiccated thyroid	Concerns about cardiac affects	PIP was defined as ≥1 prescription of desiccated thyroid at the index date or previous 90 days.		Fully applied
2	26	Avoid oral and topical patch estrogens with or without progestins (vaginal cream or tablets are acceptable to use low-dose intravaginal estrogen for management of dyspareunia, lower UTI and other vaginal symptoms).	Carcinogenic potential (breast and endometrium); lack of cardioprotective effect and cognitive protection in older women; Evidence indicates that vaginal estrogens for the treatment of vaginal dryness are safe and effective.	PIP was defined as ≥1 prescription of estrogen at the index date or previous 90 days.	Potential underestimation of PIP as we excluded all vaginal cream tablets formulations, regardless the dose or indication.	Fully applied
2	27	Avoid GH, except as hormone replacement after pituitary gland removal	Impact on body composition is small and associated with edema, arthralgia, carpal tunnel syndrome, gynecomastia, impaired fasting glucose.	PIP was defined as ≥1 prescription of GH at the index date or previous 90 days (except if pituitary gland removal diagnosis recorded ever before).		Fully applied
2	28	Avoid megestrol	Minimal effect on weight; increases risk of thrombotic events and possibly death in older adults	PIP was defined as ≥1 prescription of megestrol at the index date or previous 90 days		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	29	Avoid sulfonylureas long duration - chlorpropamide	Prolonged half-life in older adults; can cause prolonged hypoglycaemia; causes syndrome of inappropriate antidiuretic hormone secretion	PIP was defined as ≥1 prescription of chlorpropamide at the index date or previous 90 days.		Fully applied
2	30	Avoid sulfonylureas long duration - glyburide	Higher risk of severe prolonged hypoglycaemia in older adults	PIP was defined as ≥1 prescription of glyburide at the index date or previous 90 days. To accommodate differences between the US and UK drug availability, we adapted the Beers criteria by including glibenclamide.[2]		Fully applied
2	31	Avoid metoclopramide unless gastroparesis	Can cause extrapyramidal symptoms, including tardive dyskinesia; risk may be greater in frail older adults	PIP was defined as ≥1 prescription of metoclopramide at the index date or previous 90 days (except in patients with prior diagnosis of gastroparesis)		Fully applied
2	32	Avoid mineral oil given orally	Potential for aspiration and adverse events	PIP was defined as ≥1 prescription of mineral oil (oral) at the index date or previous 90 days.		Fully applied
2	33	Avoid PPI use for >8weeks, unless for high risk patients (e.g., oral corticosteroids or chronic NSAID use) (cont.)	Risk of *Clostridium difficile* infection and bone loss and fractures	PIP was defined as ≥1 prescription of PPI with a total length of >56 days at the index date or previous 90 days, allowing for a gap of maximum 7 days between prescriptions when dosage information is available, (cont.)	We assumed that patients with ≥1 prescription of corticosteroids within 90 days had concomitant use with PPI. Also, we have only included corticosteroids and oral formulations of corticosteroids. Due to data limitations, we cannot access the reason for drug discontinuation (cont.)	Partially applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	33	(cont.) erosive esophagitis, Barrett's esophagitis, pathological hypersecretory condition, or demonstrated need for maintenance treatment (e.g., due to failure of drug discontinuation trial or H2-receptor blockers)		(cont.) or (ii) minimal cumulative dose prescription (see below) of a PPI within the period above whenever dosage information was missing: Omeprazole: 56 days*20 mg (>1,120 mg prescribed) Lansoprazole: 56 days*30 mg (>1,680 mg prescribed) Esomeprazole: 56 days*30 mg (>1,680 mg prescribed) Pantoprazole: 56 days*40 mg (>2,240 mg prescribed) Rabeprazole: 56 days*20 mg (>1,120 mg prescribed Except if patients fulfil ≥1 following condition(s): (iii) concomitant use of oral corticosteroids (i.e., ≥1 prescription at index or previous 90 days, regardless of prescription length), (iv) prior use of H2-receptor blocker, (v) prior diagnosis of erosive esophagitis or Barrett's esophagitis prior to/at PPI prescription, (vii) pathological hypersecretory condition [i.e., Zollinger-Ellison syndrome], or (viii) concomitant use of NSAID (i.e., ≥1 prescription with total length >90 days allowing for a gap of maximum 7 days between prescriptions) (cont.)	(cont.) (e.g., treatment failure). Thus, we assumed that if a patient had previously used an H2-receptor blocker, it was discontinued due to treatment failure. Similarly, we have not assessed if failure in discontinuing PPIs previously due to limitations in our dataset. Potential PIP overestimation. By using the minimal cumulative dose prescription approach (as a proxy for use of PPI >56 days) we may have misclassified patients receiving high doses of PPI for <8 weeks as receiving PIP. Moreover, we defined drug use >8 weeks as the prescription of >56 days of the drug, considering patients received a pack size for 56 days of treatment. Thus, patients receiving a prescription for 8 weeks with a pack size of 60 days may be misclassified as PIP (applicable for NSAID use >90 days).	Partially applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	33			(cont.) when dosage information is available, or minimal cumulative dose prescription (see below) of a NSAID within the period above whenever dosage information was missing: Ibuprofen: 90 days*1,2 g (>108 g prescribed) Naproxen: 90 days*500 mg (>45,000 mg prescribed) Etodolac 600mg: 90 days*400 mg (>36,000 mg prescribed) Meloxican: 90 days*15 mg (>1,350 mg prescribed) Diclofenac: 90days*100 mg (>9,000 mg prescribed) Etoricoxib: 90 days*60 mg (>5,400 mg prescribed) Mefenamic acid: 90 days*1 g (>90 g prescribed) Nabumetone: 90 days*1 g (>90g prescribed) Indometacin: 90 days*100 mg (>9,000 mg prescribed) Tolfenamic acid: 90 days*300 mg (>27,000 mg prescribed)		Partially applied
2	34	Avoid meperidine, especially in individuals with CKD	Not effective in dosages commonly used; may have higher risk of neurotoxicity than other opioids	PIP was defined as ≥1 prescription of meperidine at the index date or previous 90 days.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	35	Avoid NSAID chronic use unless other alternatives are not effective, and patient can take gastroprotective agent. Aspirin >325 mg/day Diiclofenac Diflunisal Fenoprofen Etodolac Ibuprofen Meclofenamate Mefenamic acid Meloxicam Nabumetone Naproxen Oxaprozin Piroxicam Sulindac Tolmetin	increased risk of GI bleeding or peptic ulcer disease in high-risk groups, including those aged >75 or taking oral or parenteral corticosteroids anticoagulants, or antiplatelets; Use of PPI or misoprostol reduces but do not eliminate risk. Upper GI ulcers, gross bleeding perforation caused by NSAID occur in ~ 1% of patients treated for 3-6 months and in 2-4% of patients treated for 1 year; these trends continue with longer duration of use.	PIP was defined as ≥1 prescription of NSAID with total length >90 days at the index date or previous 90 days allowing for a gap between prescription of up to 7 days when information on dosage is available, or minimal cumulative dose prescription (see below) of a NSAID within the period above whenever dosage information was missing, except if ≥1 prescription of PPI or misoprostol within 90-days interval. Ibuprofen: 90 days*1,2 g (>108g prescribed) Naproxen: 90 days*500 mg (>45,000 mg prescribed) Etodolac: 90 days*400 mg (>36,000 mg prescribed) Meloxican: 90 days*15 mg (>1,350 mg prescribed) Diclofenac: 90 days*100 mg (>9,000 mg prescribed) Mefenamic acid: 90 days*1 g (>90 g prescribed) Nabumetone: 90 days*1 g (>90 g prescribed)	Chronic use of NSAID was defined as use >90 days. Potential overestimation of PIP, as we have not assessed whether other alternatives were effective due to limitations of the dataset.	Partially applied
2	36	Avoid indomethacin, ketorolac (including parenteral)	Indomethacin is more likely than other NSAID to have adverse CNS effects. Increased risk of GI bleeding, (cont.)	PIP was defined as ≥1 prescription of indomethacin or ketorolac at the index date or previous 90 days.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
2	36		(cont.) peptic ulcer disease, and acute kidney			
2	37	Avoid pentazocine	Opioid analgesic that causes CNS adverse effects more commonly than other opioids analgesic drugs; is also a mixed agonist and antagonist	PIP was defined as ≥1 prescription of pentazocine at the index date or previous 90 days.		Fully applied
2	38	Avoid skeletal muscle relaxants Carisoprodol Chlorzoxazone Cyclobenzaprine Metaxalone Methocarbamol Orphenadrine	Most muscle relaxants poorly tolerated by older adults because some have anticholinergic adverse effects, sedation, increased risk of fractures; effectiveness at dosages tolerated by older adults questionable	PIP was defined as ≥1 prescription of skeletal muscle relaxants at the index date or previous 90 days.		Fully applied
2	39	Avoid desmopressin for treatment of nocturia or nocturnal polyuria	High risk of hyponatremia	PIP was defined as ≥1 prescription of desmopressin at the index date or previous 90 days in patients with a previous diagnosis of nocturia or nocturnal polyuria.		Fully applied
3	40	In patients with HF, avoid (i) NSAID, COX-2 inhibitors, (cont.)	Potential to promote fluid retention and exacerbate HF	PIP was defined as ≥1 prescription of the listed drugs at the index date or previous 90 days in patients with previous diagnosis of HF. (cont.)	Potential underestimation of PIP as we have not assessed (iii) due to limitations on the database (i.e., no diagnosis code for HFrEF in the dataset, as patients with HFrEF are managed by secondary care in the UK).	Partially applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	40	(cont.) thiazolidinediones (pioglitazone, rosiglitazone), and cilostazol, (ii) dronedarone in severe or recently decompensated HF, and (iii) nondihydropyridine CCB (diltiazem, verapamil) in patients with HFrEF.		(cont.) Additionally, in (ii) patients should have a diagnosis of severe/ recently decompensated HF recorded at dronedarone prescription or prior 90 days (i.e., proxy for severe or recently decompensated HF).		Partially applied
3	41	In patients with syncope, avoid AchEi Peripheral α-1 blockers Doxazosin Prazosin Terazosin Tertiary TCA Chlorpromazine Thioridazine Olanzapine	Increases risk of orthostatic hypotension or bradycardia	PIP was defined as ≥1 prescription of AChEi, peripheral alpha-1 blockers (doxazosin, prazosin, terazosin), tertiary TCAs, chlorpromazine, thioridazine or olanzapine at the index date or previous 90 days in patients with a diagnosis for syncope ever prior to drug prescription.		Fully applied
3	42	Avoid Bupropion Chlorpromazine Clozapine Maprotiline Olanzapine Thioridazine Thiothixene Tramadol (cont.)	Lowers seizure threshold; may be acceptable in patients with will controlled seizures in whom alternative agents have not been effective.	PIP was defined as ≥1 prescription of at least one of the drugs listed at the index date or previous 90 days in patients with chronic seizures and epilepsy. Patients with well controlled seizures (i.e., no diagnosis of seizures in the previous 6 months to index as a proxy for no epileptic event and ≥1 prescription of anti-seizure medication within 90-days previous to index) were excluded.	Potential overestimation of PIP. We have not assessed if alternative agents failed previously due to limitation of the database and different indications (e.g., antidepressants, antipsychotics, and opioids). Moreover, we used a combination of absence of diagnosis record of seizure or epileptic event within 6 months prior to index and ≥1 prescription of anti-seizure medication at index or prior 90 days as a proxy for well controlled epilepsy. (cont.)	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	42	(cont.) if chronic seizures or epilepsy			(cont.) Thus, we assumed that if a patient had no diagnosis of epilepsy within 6 months prior to index and had co-medication for epilepsy, then drug prescription was not inappropriate. Potential underestimation of PIP as some antipsychotics in the UK (e.g., clozapine) are exclusively prescribed in secondary care; therefore, we have no access to their prescription data.	Fully applied
3	43	In patients with or at risk of delirium avoid (i) Anticholinergics BZD Chlorpromazine Corticosteroids H2-receptor blockers Cimetidine Famotidine Nizatidine Ranitidine Meperidine Sedative hypnotics avoid (ii) antipsychotics for behavioural problems of dementia or delirium.	Potential of these drugs of inducing or worsening delirium. Avoid antipsychotics for behavioural problems of dementia or delirium unless non-pharmacological options have failed or are not possible and the older adult is threatening substantial harm to self and others. Antipsychotics are associated with greater risk of stroke and mortality in persons with dementia.	PIP was defined as ≥1 prescription of ≥1 listed drug at the index date or previous 90 days in patients with diagnosis of delirium in the previous year. Additionally, patients receiving antipsychotics were defined as PIP if they had a previous diagnosis of dementia.	Potential underestimation of PIP as only patients having a diagnosis of delirium prior to index were included in the analysis due to limitations of our data on capturing the risk of delirium. Moreover, some antipsychotics in the UK (e.g., clozapine) are exclusively prescribed in secondary care; therefore, we have no access to their prescription data. Potential overestimation of PIP as we have not assessed if other non-pharmacological options have failed previously or were not possible, as well as if older adults represented substantial harm to self or others (not well captured in the database).	Partially applied
3	44	In patients with dementia or cognitive impairment (cont.)	Avoid because of CNS effects; Avoid antipsychotics (cont.)	PIP was defined as ≥1 prescription of anticholinergics, BZD, H2-receptor antagonists, (cont.)	Potential overestimation of PIP as we have not assessed if other non-pharmacological options have failed previously (cont.)	

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	44	(cont.) avoid Anticholinergics Antipsychotics H2-receptor antagonist BZD Non-BZD, BZD receptor agonist hypnotics	(cont.) for behavioural problems of dementia unless nonpharmacological options have failed or are not possible and the older adult is threatening substantial harm to self and others. Antipsychotics are associated with greater risk of stroke and mortality in persons with dementia.	(cont.) non-BZDs BZD receptor agonist hypnotics or antipsychotics at the index date or previous 90 days in patients with diagnosis of dementia or cognitive impairment at or prior to index date.	(cont.) or were not possible, as well as if older adults represented substantial harm to self or others (not well captured in the database). Potential underestimation of PIP as some antipsychotics in the UK (e.g., clozapine) are exclusively prescribed in secondary care; therefore, we have no access to their prescription data.	Fully applied
3	45	In patients with history of falls or fractures, avoid (i) Anticonvulsants (except for seizure and mood disorders), (ii) Antipsychotics, BZD, non- BZD, BZD receptor agonist hypnotics, TCA, SSRI unless safer alternatives are not available, and (iii) Opioids (except for pain management due to recent fractures or joint replacement).	May cause ataxia, impaired psychomotor function, syncope, additional falls; shorter-acting BZDs are not safer than long acting ones. If one of the drugs must be used consider reducing use of other CSN-active medications that increase risk of falls and fractures (i.e., anticonvulsants, opioid-receptor agonists, anti-psychotics, antidepressants, [cont.]	PIP was defined as ≥1 prescription of ≥1 drug listed in patients with fracture and/or falls history at the index date or previous 90 days (except if patients with anticonvulsants had an ever-prior diagnosis of seizures or mood disorders, or patients with opioids had a diagnosis of fracture or joint replacement in the previous 30 days).	In patients receiving opioids or anticonvulsants, we could not assess if GPs considered reducing the use of other CSN-active medications that increase the risk of falls and fractures (i.e., antipsychotics, antidepressants, BZDs-receptor agonists, other sedatives, and hypnotics) as well as if they implemented other strategies to reduce the risk of fall. Moreover, due to limitations on the database we could not assess if safer alternatives to antipsychotics, BZDs, non-BZDs/BZDs receptor agonist hypnotics, TCAs, or SSRI. Potential underestimation of PIP as some antipsychotics in the UK (e.g., clozapine) are exclusively prescribed in secondary care; therefore, we have no access to their prescription data.	Partially applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	45		[cont.] BZDs-receptor agonists, other sedatives and implement other strategies to reduce fall risk)			Partially applied
3	46	In patients with insomnia avoid: Oral decongestants Pseudoephedrine Phenylephrine Stimulants Amphetamine Armodafinil Methylphenidate Modafinil Theobromines Theophylline Caffeine	CNS stimulants effect	PIP was defined as ≥1 prescription of oral decongestants, stimulants, or theobromines at the index date or previous 90 days in patients with a diagnosis of insomnia in the previous year.		Fully applied
3	47	In patients with Parkinson's disease avoid Antipsychotics (except aripiprazole, quetiapine, and clozapine) Antiemetics Metoclopramide Prochlopramide Promethazine	Dopamine-receptor antagonists with potential to worsen parkinsonian symptoms; Quetiapine, aripiprazole, clozapine, appear to be less likely to precipitate worsening of Parkinson disease	PIP was defined as ≥1 prescription of an antipsychotic or antiemetics listed at the index date or previous 90 days in patients with a previous diagnosis of Parkinson's disease.	Potential underestimation of PIP as some antipsychotics in the UK are exclusively prescribed in secondary care; therefore, we have no access to their prescription data.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	48	Avoid aspirin (>325 mg/day) and non-COX2 NSAID in patients with history of gastric ulcers unless other alternatives are not effective and patient can take gastroprotective agent (PPI or misoprostol).	May exacerbate existing ulcers or cause new or additional ulcers	PIP was defined as ≥1 prescription of aspirin (>325 mg/day) and non-COX2 selective NSAID at the index date or previous 90 days in patients with a diagnosis for gastric ulcer ever prior to drug prescription without gastric protection (i.e., >1 prescription of PPI or misoprostol at index date or previous 90 days)	We assumed that if a patient could take a gastroprotective agent, it was co-prescribed together aspirin or non-COX2 selective NSAIDs. Moreover, we assumed that a prescription of PPI or misoprostol within the 90-days interval meant that patients were using concomitantly to aspirin or non-COX2 selective NSAIDs. Potential overestimation of PIP as we could not assess the indication or weather previous alternatives were not effective;	Partially applied
3	49	Avoid NSAID (non-COX and COX-selective, oral and parenteral) in CKD stages IV or V (creatinine clearance <30 mL/min)	May increase risk of acute kidney injury and further decline of renal function	PIP was defined as ≥1 prescription of NSAID at the index date or previous 90 days in patients with a previous diagnosis of CKD stages IV or V (ever prior to to index date) and/or patients with ≤2 eGFR measurements ≤30mL/min in the previous year.		Fully applied
3	50	In women with urinary incontinency avoid Estrogen oral and transdermal (exclude intravaginal) Peripheral α-1 blockers Doxazosin Prazosin Terazosin	Aggravation of incontinence	PIP was defined as ≥1 prescription of estrogen or peripheral alpha-1 blockers at the index date or previous 90 days in women with a diagnosis of urinary incontinency in the prior 5 years.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
3	51	Avoid strong anticholinergics drugs (except antimuscarinics for urinary incontinence) in men with lower urinary tract symptoms or BPH	May decrease urinary flow and cause urinary retention	PIP was defined as ≥1 prescription of strong anticholinergics drugs at the index date or previous 90 days in men with ≥1 lower UT symptom diagnosis recorded in the last year, or diagnosis of BPH in the previous 5 years.		Fully applied
5	52	Avoid use of ACE inhibitors and amiloride or triamterene;	Increased risk of hypercalcemia	PIP was defined as ≥1 prescription of ACE inhibitors and ≥1 prescription of amiloride or triamterene at the index date or previous 90 days in patients without a diagnosis code of hypocalcaemia (or potassium measurement <3.5 mmol/L) at index or prior 6 months to index date.	Potential overestimation of PIP as we assumed that ACE inhibitors and amiloride or triamterene were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied
5	53	Avoid using two or more anticholinergic drugs	Increased risk of cognitive decline	PIP was defined as concomitant use of ≥2 anticholinergic drugs at the index date or previous 90 days. Concomitant use was defined as ≥1-day prescription overlaps of >2 drugs.	Potential overestimation of PIP as we assumed that two or more anticholinergic drugs were concomitantly used as long as the they were prescribed within the 90-days period.	Fully applied
5	54	Avoid antidepressants (i.e., SSRI or TCA) and at least two other CNS-active drugs	Increased risk of falls	PIP was defined as ≥1 prescription of SSRI or TCA at the index date or previous 90 days and ≥2 other CNS-active drugs (i.e., distinct ATC codes at 5th level) prescribed within the 90-days period.	Potential overestimation of PIP as we assumed that SSRI or TCA and the other CNS active drugs were concomitantly used as long as the drugs were prescribed within the 90-days period.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
5	55	Avoid antipsychotics and two or more CNS-active drugs	Increased risk of falls	PIP was defined as ≥1 prescription of antipsychotics at the index date or previous 90 days with ≥2 other CNS-active drugs (i.e., distinct ATC codes at 5th level) prescribed within the 90-days period.	Potential overestimation of PIP as we assumed that antipsychotics and the other CNS active drugs were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied
5	56	Avoid prescribing BZD and non-BZD, BZD receptor agonist hypnotics and at least two additional CNS-active drugs	Increased risk of falls and fractures	PIP was defined as ≥1 prescription of BZD and/or non-BZD, BZD hypnotics at the index date or previous 90 days with ≥2 other CNS-active drugs (i.e., distinct ATC codes at 5th level) prescribed within the 90-days period.	Potential overestimation of PIP as we assumed that two or more drugs were concomitantly used as long as the they were prescribed within the 90-days period.	Fully applied
5	57	Avoid corticosteroids + NSAID, if not possible, provide GI protection (PPI or misoprostol)	Increased risk of peptic ulcer disease or GI bleeding	PIP was defined as ≥1 prescription of corticosteroids at the index date or previous 90 days with additional ≥1 prescription of NSAID within the 90-days period and without use of PPI, or misoprostol within the 90-days period.	Potential overestimation of PIP as we assumed that NSAIDs and corticosteroids were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied
5	58	Avoid lithium + ACE inhibitors, monitor lithium concentration	Increased risk of lithium toxicity	PIP was defined as ≥1 prescription of lithium and ≥1 prescription of ACE inhibitor at index date or 90 days previous to index in patients without lithium monitoring. Lithium monitoring was defined by ≥1 record of a diagnosis code for lithium monitoring or a lab measurement (regardless result value) within 6-months previous to index date.	Potential overestimation of PIP as we assumed that lithium and ACE inhibitors were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
5	59	Avoid lithium + loop diuretics, monitor lithium concentration	Increased risk of lithium toxicity	PIP was defined as ≥1 prescription of lithium and ≥1 prescription of loop diuretic at index date or 90 days before index in patients without lithium monitoring. Lithium monitoring was defined by ≥1 record of a diagnosis code for lithium monitoring or a lab measurement (regardless result value) within 6-months before index date.	Potential overestimation of PIP as we assumed that lithium and loop diuretics were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied
5	60	Avoid opioid receptor agonist analgesics + ≥2 other CNS-active drugs	Increased risk of falls	PIP was defined as ≥1 prescription of opioids at the index date or previous 90 days with ≥2 other CNS-active drugs (i.e., distinct ATC codes at 5th level) prescribed within the 90-days period.	Potential overestimation of PIP as we assumed that opioids and the additional CNS active drugs were concomitantly used as long as the drugs were prescribed within the 90-days period.	Fully applied
5	61	Avoid peripheral α-1 blockers in combination with loop diuretics in older women unless conditions warrant both drugs	Increased risk of urinary incontinence in older women	PIP was defined as ≥1 prescription of loop diuretics and ≥1 prescription alpha-1 blockers at index date or previous 90 days, except in patients with previous use of CCB + thiazide like diuretic + ARB + ACE inhibitors (as per recommendation of NICE guidelines to manage adults with hypertension)[8].	Potential overestimation of PIP as we assumed that alpha-1 blockers and loop diuretics were concomitantly used as long as the two drugs were prescribed within 90 days. Moreover, we assumed that the previous use of CCB + thiazide-like diuretic + ARB or ACE inhibitors (ever prior index) warranty a condition for appropriate prescription of loop diuretic and alpha-1-blocker.	Fully applied
5	62	Avoid theophylline in combination with cimetidine	Increased risk of theophylline toxicity	PIP was defined as ≥1 prescription of theophylline and ≥1 prescription of cimetidine at index date or 90 days before index.	Potential overestimation of PIP as we assumed that theophylline and cimetidine were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
5	63	Avoid warfarin in combination with amiodarone when possible; monitor international normalized ratio closely	Increased risk of bleeding	PIP was defined as ≥1 prescription of warfarin and ≥1 prescription amiodarone at index date or previous 90 days without diagnosis code for warfarin monitoring or INR monitoring, or ≥1 INR measurement (regardless the value measured) recorded within the 90-days period.	Potential overestimation of PIP as we assumed that warfarin and amiodarone were concomitantly used as long as the two drugs were prescribed within the 90-days period. Moreover, we assumed that PIP occurs if no INR monitoring during a 90-days period when using the combination of drugs.	Fully applied
5	64	Avoid warfarin in combination with non-steroidal anti-inflammatory drugs when possible; if used together, monitor bleeding closely	Increased risk of bleeding	PIP was defined as ≥1 prescription of warfarin and ≥1 prescription NSAID at index date or previous 90 days without diagnosis code for warfarin monitoring or INR monitoring, or ≥1 INR measurement (regardless the value measured) recorded within the 90-days period.	Potential overestimation of PIP as we assumed that warfarin NSAID were concomitantly used as long as the two drugs were prescribed within the 90-days period.	Fully applied
6	65	Avoid amiloride if creatinine clearance <30 mL/min	Increased potassium and decreased sodium	PIP was defined as ≥1 prescription of amiloride at the index date or previous 90 days in patients with ≥2 measurements of eGFR <30 mL/min in the year previous to index date or a diagnosis code for CKD stages 4 or 5 ever prior index.		Fully applied
6	66	Avoid apixaban if creatinine clearance <25 mL/min	Increased risk of bleeding	PIP was defined as ≥1 prescription of apixaban at the index date or previous 90 days in patients with ≥2 eGFR measurement <25 mL/min within 1-year period prior to the index date.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
6	67	Avoid dabigatran if creatinine clearance <30 mL/min	Increased risk of bleeding	PIP was defined as ≥1 prescription of dabigatran at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	68	Avoid edoxaban if creatinine clearance <30 or >95 mL/min	Increased risk of bleeding	PIP was defined as ≥1 prescription of edoxaban at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 or >95 mL/min within 1-year period prior to the index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	69	Avoid fondaparinux if creatinine clearance <30 mL/min	Increased risk of bleeding	PIP was defined as ≥1 prescription of fondaparinux at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	70	Avoid rivaroxaban if creatinine clearance <30 mL/min	Increased risk of bleeding	PIP was defined as ≥1 prescription of rivaroxaban at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
6	71	Avoid spironolactone if creatinine clearance <30 mL/min	increased potassium	PIP was defined as ≥1 prescription of spironolactone at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	72	Avoid triamterene if creatinine clearance <30 mL/min	increased potassium and decreased sodium	PIP was defined as ≥1 prescription of triamterene at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	73	Avoid duloxetine if creatinine clearance <30 mL/min	increased GI adverse effects (nausea, diarrhoea)	PIP was defined as ≥1 prescription of duloxetine at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied
6	74	Avoid tramadol extended release if creatinine clearance <30 mL/min	CNS adverse effects	PIP was defined as ≥1 prescription of tramadol modified-release formulations at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		Fully applied

Table 8.2.1 (cont.) List of 2015 American Geriatrics Society Beers criteria used for the assessment of potential inappropriate prescription (PIP) in older adults.

Table Number[1]	Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Applicability
6	75	Avoid probenecid if creatinine clearance <30 mL/min	Loss of effectiveness	PIP was defined as ≥1 prescription of probenecid at the index date or previous 90 days in patients with ≥2 eGFR measurement <30 mL/min in the year previous to index date or a diagnosis code for CKD stage 5 ever prior index.		

[1]Adapted from the " American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults" , by the American Geriatrics Society 2015 Beers Criteria Update Expert Panel, 2015*, J Am Geriatr Soc*, 63:2227–46. https://doi.org/10.1111/jgs.13702.

[2]Adapted from the " Any versus long-term prescribing of high-risk medications in older people using 2012 Beers Criteria: results from three cross-sectional samples of primary care records for 2003/4, 2007/8 and 2011/12" , by Ble A. *et al.*, 2015, *BMC Geriatr*, 15:146. https://doi.org/10.1186/s12877-015-0143-8.

[3]Adapted from the " Long-term nitrofurantoin safety monitoring guidance for prescribers" , by East Kent Prescribing Group (Representing Ashford CCG, Canterbury and Coastal CCG, South Kent Coast CCG and Thanet CCG), 2020, *National Health Service (NHS).*

Available at: https://www.eastkentformulary.nhs.uk/media/1539/ekpg_nitrofurantoin-safety-monitoring-guidance-patients-final-jan2020.pdf; 2020 [accessed 11 April 2023].

[4]Adapted from the " Atrial fibrillation: diagnosis and management" , by National Institute for Health and Care Excellence (NICE), 2021.

Available at: www.nice.org.uk/guidance/ng196; 2021 [accessed 20 April 2023].

[5]Adapted from the " Chronic heart failure in adults: diagnosis and management" , by National Institute for Health and Care Excellence (NICE), 2018.

Available at: www.nice.org.uk/guidance/ng106; 2018 [accessed 3 April 2023].

[6]Adapted from the " Depression in adults: treatment and management" , by National Institute for Health and Care Excellence (NICE), 2018.

Available at: https://www.nice.org.uk/guidance/ng222; 2022 [accessed 20 April 2023].

[7]Adapted from the " Neuropathic pain in adults: pharmacological management in non-specialist settings" , by National Institute for Health and Care Excellence (NICE), 2013. Available at: https://www.nice.org.uk/guidance/cg173; 2013 [accessed 14 April 2023].

[8]Adapted from the " Hypertension in adults" , by National Institute for Health and Care Excellence (NICE), 2013. Available at: www.nice.org.uk/guidance/ng136; 2013 [accessed 3 April 2019]

Abbreviations: ACE: Angiotensin converting enzyme; AchEi: acetylcholinesterase inhibitors; AF: Atrial fibrillation; AH: Anti-histamine; ARB: Angiotensin II receptor blockers; BB: Beta blockers; BPH: Benign prostatic hyperplasia; BZD: Benzodiazepines; CCB: Calcium-channel blockers; CKD: Chronic kidney disease; CNS: Central nervous system; HFrEF: Heart failure with reduced ejection fraction; eGFR: Estimated glomerular filtration rate; GH: Growth hormone; GI: Gastrointestinal; GP: General practitioner; HF: Heart failure; INR: International normalized ratio; IV: Intravenous; NHS: National Health Service; NICE: National Institute for the Heath and Care Excellence; NSAID: Non-steroidal anti-inflammatory drugs; OTC: Over the counter; PIP: Potential inappropriate prescription; PPI: Proton pump inhibitors; SSRI: Selective serotonin reuptake inhibitor; TCA: Tricyclic antidepressants; UK: United Kingdom; UTI: Urinary tract infections;

Table 8.2.2 List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
1	Other than for opioid-induced constipation, stimulant laxatives should not be prescribed as first-line treatment in constipation for >4 weeks.	Stimulant laxatives are not suitable for continuous long-term use, other than for opioid induced constipation.	≥1 prescription of stimulant laxatives at or within 90-days to index date, without a prescription of opioid drug and no previous use of other laxative drug prior 5 years to the index date (proxy for first line treatment). Use of stimulant laxatives ≥4 weeks was defined as (i) ≥1 prescription with cumulative length >28 days, allowing for a gap of up to 7 days between prescriptions, whenever information on dosage for all prescriptions was available, or (ii) minimal cumulative dose prescription (see below) of a stimulant laxative drug for constipation within the aforementioned period when dosage information was missing: Senna all formulations: 28 days*7.5 mg (prescription of >210 mg) Senna fruit 12.4%: 28 days*4 g sachet (prescription of >112 g) Bisacodyl oral formulations: 28 days*10 mg (prescription of >280 mg) Bisacodyl suppositories: 28 days*10 mg (prescription of >280 mg) Sodium picosulfate all formulations: 28 days*5 mg (prescription of >140 mg)	Potential underestimation of PIP, as we look back 5 years prior to the index date for assessing previous use of other laxative drugs. Potential overestimation of PIP by using a minimal cumulative dose prescription approach in patients with missing information on dosage (e.g., patients using high dose of laxatives for short time may be misclassified as having PIP). Moreover, PIP may be overestimated as we defined the drug use >4 weeks equal to prescription of >28 days of drug assuming that pack size is equal to 28 days of treatment (i.e., patients receiving a prescription for 4 weeks with a pack size of 30 days may be misclassified as PIP).	Fully applied
2	PPI should not be prescribed above recommended maintenance dose for >8 weeks, unless treatment (cont.)	PPI can lead to adverse reactions such as headaches, dizziness, skin rash, abdominal pain, diarrhoea, back pain, (cont.)	PIP was defined as use of PPI in high doses (see below) for >8 weeks in absence of a rare condition (i.e., ≥1 prescription of a PPI with cumulative length of >56 days allowing for a gap of maximum 7 days between prescriptions). Only included patients having complete information on dosage. (cont.)	Potential underestimation of PIP due to inclusion of only complete cases in the analysis. Moreover, patients starting at normal dose and increasing the value above threshold over the 8 weeks period were not classified as PIP (i.e., only classified as PIP patients at high dose for at least >56 days). PIP may be overestimated as we defined (cont.)	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
2	(cont.) is indicated for rare conditions (e.g., Zollinger-Ellison syndrome)	(cont.) and upper respiratory infections	(cont.) High dose per PPI drug: omeprazole >20 mg/day lansoprazole >30 mg/day esomeprazole >20 mg/day pantoprazole >40 mg/day rabeprazole >20 mg/day	(cont.) the drug use >8 weeks equal to prescription of >56 days of drug assuming (pack size for 56 days of treatment). Thus, patients receiving a prescription for 8 weeks with a pack size of 60 days may be misclassified as PIP.	Fully applied
3	Avoid omeprazole and esomeprazole in combination with clopidogrel	Reduction of antiplatelet effect that may lead to thrombosis	PIP was defined as the concomitant use of clopidogrel and esomeprazole or omeprazole. Concomitant use was defined as ≥1-day overlap prescription between the two drugs.	Potential underestimation of PIP as only patients with complete dosage information for at least one of the drugs were included. Potential overestimation of PIP as whenever the two drugs were not prescribed at the same day but had ≥1-day overlap, we assumed the first drug was not advised to be stopped at the prescription of the second drug.	Fully applied
4	Avoid use of AAB drugs as monotherapy for hypertension.	Increased risk of orthostatic hypotension	PIP was defined as AAB monotherapy (i.e., ≥1 prescription of AAB at or within 90-days to index date and no prescription of additional hypertensive drug in the 90-days period) in patients previously diagnosed with hypertension.	Potential overestimation of PIP as we assumed that a prescription of AAB and another anti-hypertensive drug within the 90-days period meant that patients were using the drugs concomitantly.	Fully applied
5	Aspirin doses should not exceed 150 mg/day for anti-platelet therapy	Risk of bleeding	PIP was defined as ≥1 prescription of aspirin with antiplatelet indication (ATC B01AC06) with dose >150 mg/ day.	Potential underestimation of PIP as only patients having complete dosage information were included in the analysis. Moreover, we used the ATC code as a proxy to ascertain the indication (we have not included aspirin formulations with other indication), and we assumed that all patients receiving aspirin ≤150 mg was for antiplatelet therapy.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
6	Avoid cardio-selective CCB in combination with BB	Risk of atrioventricular block and risk of myocardial depression	PIP was defined as the concomitant use of CCB and BB. Concomitant use of drugs was defined as ≥1 day of use of both drugs.	Potential underestimation of PIP as only patients with complete dosage information on the two drugs prescribed on the same date were included. Potential overestimation of PIP as we assumed that patients having a prescription for CCB and BB with ≥1-day overlap received concomitantly the two drugs (we have not considered that physicians could have advised patients to stop one drug before initiating the other).	Fully applied
7	Avoid oral short-acting dipyridamole as monotherapy in antiplatelet treatment	Risk of orthostatic hypotension	PIP was defined as use of short-acting dipyridamole with no additional antiplatelet medication use.	Potential underestimation of PIP as we assumed that whenever patients received a prescription of dipyridamole with another antiplatelet drug within 90-days period the two drugs were used concomitantly.	Fully applied
8	Avoid 1st generation AH drug as first-line treatment for >7 days.	May cause addiction and/or exert anticholinergic Effects causing unwanted side effects (e.g., constipation, drowsiness, psychomotor impairment)	PIP was defined as ≥1 prescription of first-generation oral AH for >7 days, with no use of other AH drug within 1-year before index date. Only oral formulations were included in the analysis. We defined >7 days drug use as (i) ≥1 prescription with cumulative length >7 days, allowing for a gap of ≤1 day between prescriptions (when information on dosage is available), or (ii) minimal cumulative dose prescription (see below) of a first-generation AH drug within the aforementioned period whenever dosage information was missing. Minimal threshold of cumulative drug prescribed (in mg per drug) within 90-day period previous to index. (cont.)	Potential overestimation of PIP as we assumed that first-generation AH was only used for short-term periods. Moreover, PIP may be overestimated as we defined the drug use >7 days (i.e., prescription of >7 days of drug) assuming that pack size is equal to a number that is multiple of 7.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
8			(cont.) Promethazine hydrochloride all formulations: 7 days*25 mg (prescription of >175 mg) Diphenhydramine 25 mg tablets: 7days*200 mg (prescription of >1400 mg) Chlorphenamine all formulations: 7 days*12 mg (prescription of >84 mg) Alimemazine 10mg tablets: 7 days*30 mg (prescription of ≥210 mg) Hydroxyzine all formulations: 7 days*75 mg (prescription of ≥525 mg)		Fully applied
9	Avoid theophylline as monotherapy for asthma or COPD	Increased risk of arrhythmias	PIP was defined as ≥1 prescription of theophylline at index date or previous 90-days with no additional medication for asthma/COPD was prescribed in the 90-days period.	PIP potential overestimation as we assumed patients having a prescription for theophylline and other medication for asthma or COPD within the 90 days interval were used concomitantly.	Fully applied
10	Concomitant bisphosphonate should be prescribed if oral corticosteroids are used >3 months	Long-term use of an oral corticosteroids increases the risk of osteoporosis and subsequent bone fracture	The use of corticosteroids for >90 days was defined as (i) ≥1 prescription of corticosteroids with a total length of >90 days in patients with dosage information, or (ii) minimal cumulative dose prescription in mg (see below) of an oral corticosteroid drug within the period above whenever dosage information was missing. The cumulative dose of oral corticosteroids was calculated among current users and expressed as prednisone equivalents using an online tool.[1] The minimal threshold in prednisone equivalent was 900 mg (10 mg*90 days) in patients with no dosage information. We have not included budesonide as it is a glucocorticoid with limited systemic bioavailability due to extensive (90%) first-pass hepatic metabolism by the cytochrome p-450 enzyme system.[2,3] These properties limit systemic adverse effects.	As we used the minimal cumulative dose prescription of prednisone equivalents in patients with missingness of dosage information, patients receiving high dosages for shorter time may be misclassified as having PIP. Potential overestimation of PIP, as we have not accounted for bisphosphonates administered in the hospital (e.g., zoledronic acid) due to limitations of the database.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
11	Avoid mucolytic agents in stable COPD	There is little benefit from the use of mucolytic agents in stable COPD	PIP was defined as ≥1 prescription for mucolytic at index or previous 90 days in patients with stable COPD. Stable COPD was defined as no diagnosis record for exacerbations within 1-year previous to the mucolytic agent prescription.	Potential overestimation of PIP as we assumed that patients with no diagnosis code for exacerbation registered by the GP in the previous year had no exacerbation event, and therefore, were considered stable.	Fully applied
12	Avoid SSRI in combination with venlafaxine	Serotonin syndrome	PIP was defined as ≥1 prescription of SSRI and ≥1 prescription of venlafaxine with ≥1 day overlap treatment.	Potential overestimation of PIP as we assume patients with prescriptions overlapping at least one day were using the two drugs concomitantly (we have not considered that physicians could have advised patients to stop one drug before initiating another). Also, potential underestimation of PIP, as only patients with complete dosage information for at least one of the two drugs were included in the analysis.	Fully applied
13	Avoid TCA as first-line treatment of depression	Anticholinergic effects; constipation; dry mouth; drowsiness	PIP was defined as ≥1 TCA prescription at index date or previous 90 days in patients with a previous diagnosis of depression who had not used other AD after diagnosis of depression (i.e., proxy for TCA as first line therapy for depression).	Potential underestimation of PIP. In the UK, antidepressants such as TCA are also used as treatment for chronic primary pain in patients over 16 years old.[4] Therefore, we have only included in the analysis patients with a previous diagnosis of depression in the database. If multiple diagnosis were recorded in the database, we used the first one. Moreover, we assumed that patients receiving TCA or other antidepressant before the diagnosis had a different indication than treating depression (e.g., pain management), and thus, should not rule out the potential for misclassification of patients that received other antidepressants with indication to treat this condition before the registry of depression diagnosis in the database.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
14	Avoid BZD drugs long term use (>4 weeks)	Risk of dependency; daytime sedation; cognitive impairment; agitation; irritability	PIP was defined as (i) ≥1 prescription of BZD with total length >30 days, allowing for a gap between prescriptions of ≤7 days in patients with complete dosage information, or (ii) minimal cumulative dose prescription (see below) of a BZD drug within the aforementioned period whenever dosage information was missing: Diazepam all formulations: 28 days*10 mg (prescription of >280 mg) Clonazepam all formulations: 28 days*8 mg (prescription of >224 mg) Temazepam all formulations: 28 days*20 mg (prescription of >560 mg) Chlordiazepoxide all formulations: 28 days*30 mg (prescription of >840 mg) Lorazepam 1 mg tablets: 28 days*2.5 mg (prescription of >70 mg) Loprazolam 1 mg tablets: 28 days*1 mg (prescription of >28 mg) Oxazepam all formulations: 28 days*50 mg (prescription of >1,400 mg) Nitrazepam 5 mg tablets: 28 days*5 mg (prescription of >140 mg) Clobazam 10 mg tablets: 28 days*20 mg (prescription of >560 mg) Alprazolam 250 mcg tablets: 28 days*1 mg (prescription of >28 mg)	Potential PIP overestimation. By using the minimal cumulative dose prescription approach, we may have misclassified patients receiving high doses of BZD for <4 weeks as receiving PIP. Moreover, we defined drug use >4 weeks as the prescription of >28 days of the drug, considering patients received a pack size for 28 days of treatment. Thus, patients receiving a prescription for 4 weeks with a pack size of 30 days may be misclassified as PIP.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
15	Avoid non-BZD hypnotics (zolpidem, zaleplon and zopiclone) use >4 weeks	Non-BZD hypnotics have ADR similar to those of BZD (e.g., daytime sedation, cognitive impairment, agitation, and irritability)	PIP was defined as (i) ≥1 prescription of non-BZD drugs with total length >30 days, allowing for a gap between prescriptions of ≤7 days in patients with complete dosage information, or (ii) minimal cumulative dose prescription (see below) of a non-BZD drug within the aforementioned period whenever dosage information was missing: Zopiclone all formulations: 28 days*7.5 mg (prescription of >210 mg) Zolpidem all formulations: 28 days*10 mg (prescription of >280 mg)	Potential PIP underestimation. By using the minimal cumulative dose prescription approach, we may have misclassified patients receiving high doses of non-BZD hypnotics for <4 weeks as receiving PIP. Moreover, we defined drug use >4 weeks as the prescription of >28 days of the drug, considering patients received a pack size for 28 days of treatment. Thus, patients receiving a prescription for 4 weeks with a pack size of 30 days may be misclassified as PIP.	Fully applied
16	Avoid carbamazepine in combination with clarithromycin or erythromycin	Inhibition of carbamazepine metabolism; headache; drowsiness; nausea;	PIP was defined as the concomitant use of carbamazepine and clarithromycin or erythromycin. Concomitant use was defined as ≥1 day of prescription overlap between the two drugs.	PIP potential overestimation as we assumed that patients with prescriptions overlapping ≥1 day received the two drugs concomitantly (we have not considered that physicians could have advised patients to stop one drug before initiating another).	Fully applied
17	Strong opioids should not be prescribed without laxatives	Constipation	PIP was defined as ≥1 prescription of strong opioids at index date or previous 90 days, with no prescription for laxatives within the 90 days period.	Potential overestimation of PIP. Laxatives are OTC drugs; thus, patients may have used laxatives without a prescription.	Fully applied
18	Nitrofurantoin should not be prescribed >7 days for uncomplicated UTI	Potential for pulmonary toxicity	PIP was defined ≥1 prescription of nitrofurantoin with total length >7 days, allowing for gap of ≤1 day between prescriptions for patients with information on dosage, or (ii) minimal cumulative dose prescription of >1,400 mg of nitrofurantoin (7 days*200 mg) within the aforementioned period whenever dosage information was missing	Potential PIP overestimation as we consider that nitrofurantoin was only indicated for the management of UTI.	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
19	Avoid use of long acting SU for management of diabetes	Oral long-acting SU can cause prolonged hypoglycaemia (or syndrome of inappropriate ADH secretion;	PIP was defined as ≥1 prescription of long acting SU at index date or previous 90 days.		Fully applied
20	Avoid long-term NSAID treatment	Long-term NSAID treatment should be reviewed periodically due to increased risk of thrombotic effects, and the lowest effective dose should be prescribed for the shortest period.	PIP was defined as (i) ≥1 prescription NSAID with total length >90 days at index date or previous 90 days, allowing a gap of ≤7 days between prescriptions for patients with information on dosage, or (ii) minimal cumulative dose prescription (see below) of a NSAID within the aforementioned period whenever dosage information was missing: Ibuprofen all formulations: 90 days*1,2 g (prescription of >108 g) Naproxen all formulations: 90 days*500 mg (prescription of >45,000 mg) Etodolac 600 mg: 90 days*400 mg (prescription of >36,000 mg) Meloxican all formulations: 90 days*15 mg (prescription of >1,350 mg) Diclofenac all formulations: 90 days*100 mg (prescription of >9,000 mg) Etoricoxib all formulations: 90 days*60 mg (prescription of >5,400 mg) Mefenamic acid all formulations: 90 days*1 g (prescription of >90 g) Celecoxib all formulations: 90 days*200 mg (prescription of >18,000 mg) Aceclofenac 100mg tablets: 90 days*200 mg (prescription of >18,000 mg) (cont.)	Potential PIP underestimation. By using the minimal cumulative dose prescription approach, we may have misclassified patients receiving high doses of non-BZD hypnotics for <4 weeks as receiving PIP. Moreover, we may have underestimated the use of NSAID (over-the counter drugs).	Fully applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
20			(cont.) Nabumetone 500mg tablets: 90 days*1 g (prescription of >90 g) Indometacin 50mg capsules: 90 days*100 mg (prescription of >9,000 mg) Piroxicam all formulations 90 days*20 mg (prescription of >1,800 mg)		Fully applied
21	Avoid combination of NSAID with low dose aspirin or SSRI, without adequate GI protection	Risk of gastro-intestinal bleeding	PIP was defined as ≥1 prescription of NSAID and ≥1 prescription of SSRI or aspirin, with ≥1-day prescription overlap, at or in the prior to 90-days, with no additional prescription of PPI or H2-receptor antagonist within the 90-days period.	Potential underestimation of PIP. We assumed patients with a prescription for PPI or H2-receptors within 90 days had the gastroprotective medication while using the combination of NSAID + SSRI or aspirin.	Fully applied
22	Avoid using ≥2 drugs from the same pharmacological class, unless used for additive effects in line with current clinical guidelines – Example 1: duplication of opioids (excluding methadone and morphine)	Possible unwanted duplication of effect, increasing risk of side effects and adverse events	PIP was defined as ≥1 prescription of two different opioids (i.e., different ATC codes at 5th level) at index date or within 90 days previous to index date, with ≥1 day overlap between prescriptions.	We assumed that patients with prescriptions overlapping at least one day were using the two drugs concomitantly. We have not considered that physicians could have advised patients to stop one drug before initiating another). Also, potential underestimation of PIP, as we considered only patients with information on dosage for at least one drug in the analysis.	Partially applied

Table 8.2.2 (cont.) List of Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria used for the assessment of potential inappropriate prescription (PIP) in middle-aged adults.

Criteria Number	Recommendation	Rationale	PIP definition	Limitations	Criteria Applicability
22	Avoid using ≥2 drugs from the same pharmacological class, unless used for additive effects in line with current clinical guidelines – Example 2: duplication of SSRI	Possible unwanted duplication of effect, increasing risk of side effects and adverse events	PIP was defined by ≥1 prescription of two different SSRI (i.e., different ATC codes at 5th level) at index date or within 90 days previous to index date, with ≥1 day overlap between prescriptions.	Potential overestimation of PIP as the co-prescription of two SSRI may be due to medication transition. We have not considered that physicians could have advised patients to stop one drug before initiating another. Also, potential underestimation of PIP as we considered only patients with information on dosage for at least one drug in the analysis.	Partially applied
22	Avoid using ≥2 drugs from the same pharmacological class, unless used for additive effects in line with current clinical guidelines – Example 3: duplication of PPI		PIP was defined by ≥1 prescription of two different PPI (i.e., different ATC codes at the 5th level) at the index date or within 90 days previous to the index date, with ≥1-day overlap between prescriptions.	We have not considered that physicians could have advised patients to stop one drug before initiating another). Also, potential underestimation of PIP, as we considered only patients with information on dosage for at least one drug in the analysis.	Partially applied

[1] Calculated by the Corticosteroid Conversion Calculator - ClinCalc.com., https://clincalc.com/corticosteroids/; [accessed 11 April 2023].

[2] Adapted from the " Budesonide for the Induction and Maintenance of Remission in Crohn' s Disease: Systematic Review and Meta-Analysis for the Cochrane Collaboration" , by Kuenzig ME *et al.*, 2018, *J Can Assoc Gastroenterol.* (4):159-173. doi:10.1093/jcag/gwy018

[3] Adapted from " Long-term oral budesonide treatment and risk of osteoporotic fractures in patients with microscopic colitis" , by Reilev M *et al.,* 2020, *Aliment Pharmacol Ther*. 51(6):644-651. doi:10.1111/apt.15648

[4] Adapted from the " Chronic pain (primary and secondary) in over 16s: assessment of all chronic pain and management of chronic primary pain" , by National Institute for Health and Care Excellence (NICE), 2021. Available at: https://www.nice.org.uk/guidance/ng193; 2021 [accessed 11 April 2023].

Abbreviations: AAB: alpha-adrenoceptor blocking drugs; ADH: antidiuretic hormone; ADR: adverse drug reaction; AH: antihistamine; ATC: Anatomical therapeutic chemical classification ; BB: beta blockers; BZD: benzodiazepine; CCB: calcium channel blockers; COPD: chronic obstructive pulmonary disease; GP: General practitioner; NSAID: non-steroidal anti-inflammatory drugs; PIP: Potentially inappropriate prescription; PPI: proton pump inhibitors; SSRI: selective serotonin reuptake inhibitor; SU: sulfonylureas; TCA: Tricyclic antidepressant; UTI: Urinary tract infections.

Table 8.2.3 List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	1	**First-generation antihistamines**: "R06AB01" "R05X" "R01BA52" "R01BA51" "R06AB51" "R06AB04" "R06AB54" "R06AA54" "R06AA04" "R06AX02" "N07CA52" "R06AA02" "N02BE71" "R06AA52" "N07XX59" "R01BA02" "R05DA20" "R05FB02" "R01BA52" "R06AA09" "R05CA03" "R01AA04" "N05B B01" "R06AE05" "R06AE55" "R06AD02" "N02AB52" "R06AX07"	**Severe allergy:** "2227.11" "3359.00" "3368.00" "66G..00" "66G3.00" "66GZ.00" "7Q08400" "8Hld.00" "8HVK000" "8T0C.00" "9b99.00" "9b9W.00" "9NlX.00" "AB63300" "D310.00" "D310011" "D310z00" "D403300" "F401400" "F4C0611" "F4D3100" "F4D3111" "M280.00" "SN50.11" "SN50000" "SN50100" "SN59000" "SN59100" "SN59200" "SN59300" "SN59400" "SN5A.00" "ZL18200" "ZL5A600" "ZL9A200" "ZLD3200" "ZLE6100"
2	2	**Antiparkinsonian agents:** "N04AC01" "N04AA01" "N04AB02" "M03BA03" **Antipsychotics:** "N05A" "N06C" "A03CA01" "N01AH51"	
2	3	**Antispasmodics (exclude ophthalmic):** "A03BA01" "A04A D04" "A03B A01" "G04BE02 in combination with atropine" "A03CA02" "A03AA07" "A02AA02" "A03BB01" "A04AD01" "A03AB05"	
2	4	**Dipyridamole (oral short-acting):** "B01AC07"	
2	5	**Triclopidine:** "B01AC05"	
2	6	**Nitrofurantoin:** "J01XE01"	**Chronic Kidney disease stages 3B, 4 and 5:** "1Z13.00" "1Z14.00" "1Z16.00" "1Z1F.00" "1Z1G.00" "1Z1H.00" "1Z1J.00" "1Z1K.00" "1Z1L.00" "K054.00" "K055.00"
2	7	**Alpha-1 blockers (doxazosin, prazosin, and terazosin):** "C02CA04" "C02CA01" "G04CA03"	**Hypertension:** "14A2.00" "661M600" "661N600" "662..12" "6627.00" "6628.00" "662b.00" "662c.00" "662d.00" "662F.00" "662G.00" "662O.00" "662P.00" "662P000" "662P100" "662q.00" "8B26.00" "8BL0.00" "8CR4.00" "8HT5.00" "8I3N.00" "8IA6.00" "9OI1.00" "9OI3.00" "9OI4.00" "9OI5.00" "9OI6.00" "9OI7.00" "9OI8.00" "9OIA.11" "9OIZ.00" "G2...00" "G2...11" "G20..00" "G20..12" "G200.00" "G201.00" "G202.00" "G203.00" "G20z.00" "G20z.11" "G21..00" "G210.00" "G210000" "G210100" "G210z00" "G211.00" "G211000" "G211100" "G211z00" "G21z.00" "G21z000" "G21z011" "G21z100" "G21zz00" "G22..00" "G220.00"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	7		(cont.) "G221.00" "G222.00" "G22z.00" "G22z.11" "G23..00" "G230.00" "G231.00" "G232.00" "G233.00" "G234.00" "G23z.00" "G24..00" "G240.00" "G240000" "G240z00" "G241.00" "G241000" "G241z00" "G244.00" "G24z.00" "G24z000" "G24z100" "G24zz00" "G25..00" "G25..11" "G250.00" "G251.00" "G26..00" "G26..11" "G27..00" "G28..00" "G2y..00" "G2z..00" "G672.11" "G8y3.00" "Gyu2.00" "Gyu2000" "Gyu2100" "L12..00" "L122.00" "L122000" "L122100" "L122300" "L122400" "L122z00" "L127.00" "L127000" "L127100" "L127200" "L127300" "L127400" "L127z00" "L128.00" "L128000" "L128100" "L128200" "L12B.00" "Lyu1.00"
2	8	**Clonidine, guanabenz, guanfacine, methyldopa, and reserpine:** "C02AC02" "C02AB01" "C02LB01" "C02AA02" "C03AA01 (whenever the formulation combines reserpine)" "C03AA03 (whenever the formulation combines reserpine)" "R03DA07 (whenever the formulation combines reserpine)" "N02CX02" "C02AC01" **Other antihypertensive drugs:** "C02 except C02CA" "C09AA" "C09C" "C09D" "C08C" "C08D" "C03A" "C03B" "C03C" "C03D" "C03E" "C03XA" "C07" "C09XA02" "C02CA"	**Hypertension**: see criteria number 7.
2	9	**Disopyramide:** "C01BA03"	
2	10	**Dronedarone:** "C01BD07"	**Persistent atrial fibrillation:** "G573500" "G573400" **Decompensated heart failure:** "G580200"
2	11	**Digoxin:** "C01AA05" **Beta blockers:** "C07" (except C07AA07) **Calcium-channel blockers:** "C08DB01" "C08DA01" "C08DA51" "C09BB10""C08DB01"	**Atrial fibrillation:** "14AD.00" "14AN.00" "2JS..00" "3272" "3283" "662S.00" "6A9..00" "9Os..00" "9Os0.00" "9Os1.00" "9Os2.00" "9Os3.00" "9Os4.00" "G573.00" "G573000" "G573200" "G573300" "G573400" "G573500" "G573700" "G573z00" "G574.00" "G574000" "G574011" "G574z00"
2	12	**Digoxin:** see criteria number 11 **Beta blockers:** "C07AB07" "C07AB12" "C07AG02" **Calcium-channel blockers:** see criteria 11 Angiotensin receptor blockers: "C09C" "C09D" Angiotensin-converting enzyme inhibitors: "C09AA" "C09B" **Mineralocorticoid receptor antagonists:** "H02AA02"	**Heart failure:** "14A6.00" "14AM.00" "1O1..00" "2JZ..00" "661M500" "661N500" "662p.00" "662T.00" "662W.00" "679W100" "679X.00" "67D4.00" "8B29.00" "8CL3.00" "8CMK.00" "8H2S.00" "8HBE.00" "8HHz.00" "8Hk0.00" "8HTL000" "8IE0.00" "8IE1.00" "9Or..00" "9Or0.00" "9Or1.00" "9Or2.00" "9Or3.00" "9Or4.00" "9Or5.00" "G232.00" "G58..00" "G58..11" "G580.00" "G580.11" "G580.12" "G580000" "G580100" "G580200" "G580300" "G580400" "G582.00" "G583.00" "G583.11" "G583.12" "G58z.00" "G58z.12" "G5y4z00" "L09y200" "Q48y100" "SP11100" "SP11111"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	13	**Digoxin:** see criteria number 11	**Atrial fibrillation:** see criteria number 11 **Heart failure:** see criteria number 12
2	14	**Nifedipine:** "C08CA05"	
2	15	**Amiodarone:** "C01BD01" **Other antiarrhythmic drugs:** "C07" (except C07AA07) "C08DB01" "C08DA01" "C08DA51" "C09BB10" "C08DB01"	**Atrial fibrillation:** see criteria number 11 **Heart failure:** see criteria number 12 **Left ventricular hypertrophy:** "G5y3411"
2	16	**Amitriptyline, amoxapine, clomipramine, desipramine, doxepin (>6mg/day), imipramine, nortriptyline, paroxetine, protriptyline, trimipramine:** "N06AA09" "N06CA01" "N06AA17" "N06AA04" "N06AA01" "N06AA12" "N06AA02" "N06AA10" "N06AB05" "N06AA11" "N06AA06"	
2	17	**Antipsychotics:** "N05AA" "N06CA01" "N05AB03" "N05AB04" "N05AB06" "N05AB02" "A03CA01" "N05AC02" "N05AC04" "N05AD01" "N05AD02" "N05AD07" "N05AD08" "N01AH51 whenever formulation combined with antipsychotics" "N05AE01" "N05AF01" "N05AF03" "N05AF05" "N05AG02" "N05AH" "N05AL01" "N05AL04" "N05AL05" "N05A X08" "N05AB02" "N05AE05" "N05AE03" "N05AG01" "N05AN01" "N05AX11" "N05AX13"	**Schizophrenia:** "13L3.12" "13Y2.00" "1464.00" "212T.00" "212W.00" "E10..00" "E100.00" "E100.11" "E100000" "E100100" "E100200" "E100300" "E100400" "E100500" "E100z00" "E101.00" "E101000" "E101100" "E101200" "E101300" "E101400" "E101500" "E101z00" "E102.00" "E102000" "E102100" "E102200" "E102300" "E102400" "E102500" "E102z00" "E103.00" "E103000" "E103100" "E103200" "E103300" "E103400" "E103500" "E103z00" "E104.00" "E105.00" "E105000" "E105100" "E105200" "E105300" "E105400" "E105500" "E105z00" "E106.00" "E106.11" "E107.00" "E107.11" "E107000" "E107100" "E107200" "E107300" "E107400" "E107500" "E107z00" "E10y.00" "E10y.11" "E10y000" "E10y100" "E10yz00" "E10z.00" "E122.00" "E14z.11" "E212.00" "E212000" "E212200" "E212z00" "Eu05200" "Eu05212" "Eu2..00" "Eu20.00" "Eu20000" "Eu20011" "Eu20100" "Eu20111" "Eu20200" "Eu20212" "Eu20213" "Eu20214" "Eu20300" "Eu20311" "Eu20400" "Eu20500" "Eu20511" "Eu20512" "Eu20600" "Eu20y00" "Eu20y11" "Eu20y12" "Eu20y13" "Eu20z00" "Eu21.00" "Eu21.11" "Eu21.12" "Eu21.13" "Eu21.14" "Eu21.15" "Eu21.16" "Eu21.17" "Eu21.18" "Eu22013" "Eu23000" "Eu23100" "Eu23111" "Eu23112" "Eu23200" "Eu23211" "Eu23212" "Eu23214" "Eu25.00" "Eu25000" "Eu25011" "Eu25012" "Eu25100" "Eu25111" "Eu25112" "Eu25200" "Eu25211" "Eu25212" "Eu25y00" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	17		(cont.) "Eu25z00" "Eu25z11" "Eu60100" "Eu84512" "ZRhi.00" "ZS7C611" "ZV11000" **Bipolar disorder:** "E11..11" "E114.00" "E114000" "E114100" "E114200" "E114300" "E114400" "E114500" "E114600" "E114z00" "E115.00" "E115000" "E115100" "E115200" "E115300" "E115400" "E115500" "E115600" "E115z00" "E116.00" "E116000" "E116100" "E116200" "E116300" "E116400" "E116500" "E116600" "E116z00" "E117.00" "E117000" "E117100" "E117200" "E117300" "E117400" "E117500" "E117600" "E117z00" "Eu30.11" "Eu31.00" "Eu31000" "Eu31100" "Eu31200" "Eu31300" "Eu31500" "Eu31600" "Eu31700" "Eu31800" "Eu31900" "Eu31911" "Eu31y00" "Eu31y11" "Eu31z00" "ZRby100"
2	18	**Barbiturates:** "N05CB01" "N05CA02" "N03AA01" "N03AA02" "R03DA74" "N05CA06"	
2	19	**Short- and intermediate-acting benzodiazepines:** "N05BA12" "N05BA06" "N05BA04" "N05CD07" "N05CD05" "N05BA09" "N05BA10" "N05BA03"	
2	20	**Long-acting benzodiazepines:** "N05BA05" "N05BA02" "N06CA01" "A03CA02" "N03AE01" "N05BA01" "N05CD01" "N05CD02" "N05CD06" "N05BA08"	
2	21	**Meprobamate:** "N05BC01" "N02BA71" "C03AA01 whenever formulation combined with meprobamate"	
2	22	**BZD-receptor agonist hypnotics**: "N05CF01" "N05CF02" "N05CF03"	
2	23	**Ergoloid mesylates (dehydrogenated ergot alkaloids), Isoxsuprine:** "C04AA01" "C04AE01"	
2	24	**Androgens:** "G03BA02" "G03BA03"	**Hypogonadism:** "C139.00" "C163.11" "C163200" "C163300" "C172.11" "C172.12" "F146.00"
2	25	**Desiccated thyroid:** "H03AA05"	
2	26	**Estrogens:** "G03FA14" "G03FA11" "G03CA03" "G03AA14" "G03FA17" "G03FA01" "G03FB06" "G03FB08" "G03FB05" "G03FB09" "G03FB01" "G03CA53" "G03AA11" "G03AA09" "G03AA10" "G03AA12" "G03AA07" "G03AB04" "G03AB06" "G03CA01" "G03AA05" "G03AA01" "G03DC02" "G03BA02" "G03CA04" "L02A A01" "G03C X01"	**Transphenoidal hypophysectomy**: 7100300

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	27	**Somatotropin:** "H01AC01	
2	28	**Megestrol:** "L02AB01"	
2	29	**Chlorpropamide:** "A10BB02"	
2	30	**Glyburide / Glibenclamide:** "A10BB01"	
2	31	**Metoclopramide:** "A03FA01" "N02BE51 whenever formulation combined with metoclopramide"	**Gastroparesis:** "C10FR00" "C10FR11" "J16y900"
2	32	**Mineral oil:** "A06AA01" "A06AA51"	
2	33	**Proton pump inhibitors:** "A02BC" "M01AE53" "A02BD" "M01AE52" **Oral corticosteroids:** "H02AB01" "H02AB02" "H02AB04" "H02AB06" "H02AB07" "H02AB08" "H02AB09" "A01AC03" "H02AB10" "H02AB13" "A07EA07" "A07EA06" **Non-steroidal anti-inflammatory drugs:** "M01A" (excluding topical formulations, suppositories, eye drops, and plasters) **H2-receptor antagonist:** "A02BA"	
2	34	**Meperidine:** "N02AB02"	
2	35	**Non-cyclooxygenase-selective non-steroidal anti-inflammatory drugs:** "N02BA01" "N02AJ07" "N02BA51" "N06BC01" "M01AB05" "M01AB55" "N02BA11" "M01AB08" "M01AE04" "M01AE51" "M01AE01" "N02BE51 whenever in combination with ibuprofen" "N02AJ08" "N02BE71 whenever in combination with ibuprofen" "M01AE03" "M01AE53" "M01AG01" "M01AC06" "M01AX01" "M01AE02" "M01AE52" "M01AC01" "M01AB02" "M01AB03" **Proton pump inhibitor:** "A02BC" "M01AE53" "A02BD" "M01AE52" **Misoprostol:** "A02BB01"	
2	36	**Indomethacin, ketorolac:** "M01AB01" "M01AB15"	
2	37	**Pentazocine:** "N02AD01 "	

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
2	38	**Skeletal muscle relaxants:** "M03BA02" "M03BA03" "M03BA53" "N04AB02" "M03BC01" " M03BC51"	
2	39	**Desmopressin:** "H01BA02"	**Nocturia or nocturnal polyuria:** "1A13.00" "1A22011" "R084200"
3	40	**Non-steroidal anti-inflammatory drugs:** M01A" (excluding M01AH, topical formulations, suppositories, eye drops, and plasters) **COX-2 inhibitors:** "L01XX33" "M01AH01" "M01AH02" "M01AH03" "M01AH04" "M01AH05" "M01AH06" **Thiazides**: "A10BG03" "A10BD06" "A10BD03" "A10BG02" **Cilostozol:** "B01AC23" **Dronedarone:** "C01BD07" **Nondihydropyridine CCBs:** "C08DB01" "C08DA01" "C09BB10"	**Heart failure:** see criteria 12 **Severe/ decompensated heart failure**: "G580200"
3	41	**Alpha-1 blockers (doxazosin, prazosin, and terazosin):** "C02CA04" "C02CA01" "G04CA03" **Acetylcholinesterase inhibitors:** "N06DA" "N07AA" **Tertiary tricyclic antidepressant drugs:** "N06AA09" "N06CA01" "N06AA02" "N06AA06" "N06AA12" "N06AA04" **Other antipsychotics (chlorpromazine, thioridazine, olanzapine):** "N05AA01" "N05AC02" "N05AH03"	**Syncope:** "147A.00" "147B.00" "1B6..11" "1B6..12" "1B62.00" "1B68.00" "2244.00" "Eu46y16" "G33z200" "R002.00" "R002.11" "R002100" "R002400" "R002500" "R002600" "R002z00" "R062000" "SN21.00" "SN21.12"
3	42	**Bupropion, chlorpromazine, clozapine, mapritiline, olanzapine, thioridazine, thiothixene, and tramadol:** "N06AX12" "N05AA01" "N05AH02" "N06AA21" "N05AH03" "N05AC02" "N02AX02" "N02AJ13" "N02BE71"	**Seizures:** "1473.00" "14On.00" "1B26.00" "1B27.00" "1O30.00" "282..13" "6110.00" "667..00" "6671.00" "6672.00" "6674.00" "6677.00" "6678.00" "6679.00" "667A.00" "667B.00" "667C.00" "667D.00" "667E.00" "667F.00" "667G.00" "667H.00" "667J.00" "667K.00" "667L.00" "667M.00" "667N.00" "667Q.00" "667R.00" "667S.00" "667T.00" "667V.00" "667W.00" "667Z.00" "67AF.00" "67IJ000" "8BIF.00" "8CE7.00" "8Hlp.00" "8IAg.00" "8IAh.00" "8IAi.00" "8IB2.00" "8IB3.00" "8IB4.00" "8T0L.00" "9N0r.00" "9N4V.00" "9Of..00" "9Of0.00" "9Of1.00" "9Of2.00" "9Of3.00" "9Of4.00" "9Of5.00" "9Of6.00" "9Of7.00" "E201500" "Eu05212" "Eu06013" "Eu10800" "Eu44511" "Eu80300" "F132100" "F132z12" "F25..00" "F250.00" "F250000" "F250100" "F250200" "F250300" "F250400" "F250y00" "F250z00" "F251.00" "F251000" "F251011" "F251100" "F251200" "F251300" "F251400" "F251500" "F251600" "F251y00" "F251z00" "F254.00" "F254000" "F254100" "F255100" "F255200" "F255300" "F255311" "F255400" "F255500" "F255600" "F255y00" "F255z00" "F257.00" "F25A.00" "F25B.00" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	42		(cont.) "F255500" "F255600" "F255y00" "F255z00" "F257.00" "F25A.00" "F25B.00" "F25C.00" "F25D.00" "F25E.00" "F25F.00" "F25G.00" "F25H.00" "F25y.00" "F25y000" "F25y100" "F25y400" "F25yz00" "F25z.00" "Fyu5000" "Fyu5100" "Q480.12" "R003300" "R003400" "R003z11" "SC20000" "F254200" "F254300" "F254500" "F254z00" "F255.00" "F255000" "F255011" "F255012"
3	43	**Anticholinergics:** "R06AB01" "R06AA52" "R06AD52" "R01BA52" "R01BA51" "R06AB51" "R06AB04" "R06AA54" "R06AA04" "R06AX02" "N07CA52" "R06AA02" "N02BE71" "N07XX59" "R01BA02" "R05FB02" "R06AA09" "R05CA03" "R01AA04" "N05BB01" "R06AX07" "N04AA01" "N04AB02" "M03B C01" "N02B E01" "N06A A09" "N06CA01" "N06AA17" "N06AA04" "N06AA01" "N06AA12" "N06AA02" "N06AA10" "N06AB05" "N06AA11" "N06AA06" "N05AA01" "N05AH02" "N05AH01" "N05AH03" "N05AB03" "N05AC02" "N05AB06" "A03CB04" "C01BA03" "G04BD10" "G04BD11" "G04BD02" "G04BD04" "G04BD08" "G04CA53" "G04BD07" "G04BD09" "A07DA52" "A03CB02" "A03CB" "A06AB04" "A03BA04" "C02AA03" "A03CA02" "A03BA01" "A07DA01" "A04AD04" "A03BA01" "N02AA51" "G04BE02" "A03AB05" "N05AB04" "R06AD02" "N02AB52" **Antipsychotics (incl. chlorpromazine):** "N05AA" "N06CA01" "N05AB03" "N05AB04" "N05AB06" "N05AB02" "A03CA01" "N05AC02" "N05AC04" "N05AD01" "N05AD02" "N05AD07" "N05AD08" "N01AH51 whenever formulation combined with antipsychotics" "N05AE01" cont.) "N05AF01" "N05AF03" "N05AF05" "N05AG02" "N05AH" "N05AL01" "N05AL04" "N05AL05" "N05A X08" "N05AB02" "N05AE05" "N05AE03" "N05AG01" "N05AN01" "N05AX11" "N05AX13" **Benzodiazepines:** "N05BA12" "N05CD11" "N05BA06" "N05CD06" "N05BA04" "N05CD07" "N05CD05" "N05BA08" "N05BA09" "N05BA05" "N05BA02" "N06CA01" "A03CA02" "N03AE01" "N05BA01" "N05CD03" "N05CD01" "N05CD02" "N05BA11" "N05CD08" "N05BA03" "N05BA10" (cont.)	**Delirium:** "E001100" "E003.00" "E004100" "E010.00" "E010.11" "E010.12" "E02y000" "E030.11" "E031.11" "E133.11" "Eu04.00" "Eu04000" "Eu04100" "Eu04y00" "Eu04y11" "Eu04z00" "Eu10411" "Eu23011" "Eu23111" **Dementia:** "1461" "66h..00" "6AB..00" "8BM0200" "8BPa.00" "8CET.00" "8CMe000" "8CMG200" "8CMZ.00" "8CMZ000" "8CMZ100" "8CMZ300" "8CSA.00" "8IAe000" "8IAe200" "9Ou..00" "9Ou1.00" "9Ou2.00" "9Ou3.00" "9Ou4.00" "9Ou5.00" "E00..11" "E00..12" "E000.00" "E001.00" "E001000" "E001100" "E001200" "E001300" "E001z00" "E002.00" "E002000" "E002100" "E002z00" "E003.00" "E004.00" "E004.11" "E004000" "E004100" "E004200" "E004300" "E004z00" "E012.00" "E012.11" "E02y100" "E041.00" "Eu00.00" "Eu00000" "Eu00011" "Eu00012" "Eu00013" "Eu00100" "Eu00111" "Eu00112" "Eu00113" "Eu00200" "Eu00z00" "Eu00z11" "Eu01.00" "Eu01.11" "Eu01000" "Eu01100" "Eu01111" "Eu01200" "Eu01300" "Eu01y00" "Eu01z00" "Eu02.00" "Eu02000" "Eu02100" "Eu02200" "Eu02300" "Eu02400" "Eu02500" "Eu02y00" "Eu02z00" "Eu02z11" "Eu02z13" "Eu02z14" "Eu02z16" "Eu04000" "Eu04100" "Eu10711" "Eu84311" "F110.00" "F110000" "F110100" "F118100" "Fyu3000" "ZR1T.00" "ZS7C500" "E00z.00" "Eu02z12" "Eu02z15" "F111.00" "F112.00"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	43	(cont) **Corticosteroids:** "H02AB01" "H02AB02" "H02AB04" "H02AB06" "H02AB07" "H02AB08" "H02AB09" "A01AC03" "H02AB10" "H02AB13" "A07EA07" "H02BX01" **H2-receptor blockers:** "A02BA01" "A02BA51" "A02BA02" "A02BA07" "A02BA53" "A02BA03" "A02BA04" **Meperidine:** "N02AB02" **Sedative hypnotics:** "N05CF01" "N05CF02" "N05CF03" "N05CB01" "N05CA02" "N05CA03" "N03AA01" "N03AA02" " C02LA71" "N05CA06" "N05CC01" "N05CE05" "N05CM02"	
3	44	**Anticholinergics:** see criteria 43 **Benzodiazepines:** see criteria 43 **H2-receptor blockers:** see criteria 43 **Non-benzodiazepine, benzodiazepine receptor agonist hypnotics:** "N05CF01" "N05CF02" "N05CF03"	**Dementia and cognitive impairment:** "1461" "66h..00" "6AB..00" "8BM0200" "8BPa.00" "8CET.00" "8CMe000" "8CMG200" "8CMZ.00" "8CMZ000" "8CMZ100" "8CMZ300" "8CSA.00" "8IAe000" "8IAe200" "9Ou..00" "9Ou1.00" "9Ou2.00" "9Ou3.00" "9Ou4.00" "9Ou5.00" "E00..11" "E00..12" "E000.00" "E001.00" "E001000" "E001100" "E001200" "E001300" "E001z00" "E002.00" "E002000" "E002100" "E002z00" "E003.00" "E004.00" "E004.11" "E004000" "E004100" "E004200" "E004300" "E004z00" "E012.00" "E012.11" "E02y100" "E041.00" "Eu00.00" "Eu00000" "Eu00011" "Eu00012" "Eu00013" "Eu00100" "Eu00111" "Eu00112" "Eu00113" "Eu00200" "Eu00z00" "Eu00z11" "Eu01.00" "Eu01.11" "Eu01000" "Eu01100" "Eu01111" "Eu01200" "Eu01300" "Eu01y00" "Eu02.00" "Eu02000" "Eu02100" "Eu02200" "Eu02300" "Eu02400" "Eu02500" "Eu02y00" "Eu02z00" "Eu02z11" "Eu02z13" "Eu02z14" "Eu02z16" "Eu04000" "Eu04100" "Eu10711" "Eu84311" "F110.00" "F110000" "F110100" "F118100" "Fyu3000" "ZR1T.00" "ZS7C500" "E00z.00" "Eu02z12" "Eu02z15" "F111.00" "F112.00" "28E..00" "28E0.00" "28E1.00" "28E2.00" "28E3.00" "311B.00" "38Dv.00" "38Dv000" "38Qr.00" "38Qv.00" "38Qv.11" "3AD3.00" "3AE1.00" "3AE2.00" "3AE3.00" "3AE4.00" "3AE5.00" "3AE6.00" "3AF..00" "6AQ..00" "9OqG.11" "Eu05700" "Eu05800" "Z7C..00" "Z7C1.00" "Z7C2.00" "ZR1H.00" "ZR1I.00" "ZR3b.00" "ZR3b.11" "ZRa2.00" "ZRd..00" "ZRh6.00" "ZRh6.11" "ZRkL.00" "ZRlfE00" "ZRLfF00" "ZRV9.11" "ZRVa.00" "ZRVa.11" "ZRVt.00" "ZRVt.11" "ZS3..00"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45	**Benzodiazepines:** see criteria 43 **Non-benzodiazepine, benzodiazepine receptor agonist hypnotics:** see criteria 44 **Antipsychotics:** see criteria 43 **Anticonvulsants:** "N05BA01" "N03A" "R03DA07 whenever combined with phenobarbital" **Tricyclic antidepressants:** "N06AA09" "N06CA01" "N06AA02" "N06AA06" "N06AA12" "N06AA07" "N06AA17" "N06AA01" "N06AA10" "N06C" "N06AA11" "N06AA04" "N06AA13" "N06AA15" "N06AA16" "N06AA21" **Selective serotonin reuptake inhibitors:** "N06AB" **Opioids:** "N02AA01" "N02AA51" "N01AB02" "N02AA02" "N02AA03" "N02AA05" "N02AA55" "N02AJ" "N02AA08" "N02AA10" "N02AB02" "N02AB52" "N01AH" "N02AB03" "N02AD01" "N02BE01" "N02AC54" "N02AC01" "N02AC04" "N02AD02" "N07BC01" "N02AE01" "N07BC51" "N02AF02" "N02AX02" "N02AX" "R05FA02" "A07DA52" "A07DA02" "R06AA09 whenever in combination with opioids" "N02AA59" "N02AB52" "N07BC02" "N02AC52"	**Falls:** "16D..00" "16D1.00" "16D5.00" "16D6.00" "1Bb0.00" "38A..00" "38A0.00" "38A1.00" "66aF.00" "67IC.00" "67ID.00" "67IE.00" "8BIG.00" "8CM7.00" "8CMW400" "8Hk1.00" "8HTl.00" "R01z600" "R200.12" "TCy..00" "U10..00" **Fractures:** "14G6.00" "14G7.00" "14G8.00" "14G9.00" "14GA.00" "7J41.00" "7J41000" "7J41100" "7J41200" "7J41300" "7J41400" "7J41500" "7J41y00" "7J41z00" "7J42300" "7J42400" "7J42500" "7J42600" "7J42700" "7J42800" "7J42900" "7J42A00" "7J42a00" "7J42b00" "7J42B00" "7J42c00" "7J42C00" "7J42d00" "7J42D00" "7J42e00" "7J42E00" "7J42F00" "7J42f00" "7J42G00" "7J42g00" "7J42H00" "7J42h00" "7J42J00" "7J42j00" "7J42K00" "7J42k00" "7J42L00" "7J42l00" "7J42M00" "7J42m00" "7J42N00" "7J42P00" "7J42Q00" "7J42R00" "7J42S00" "7J42T00" "7J42U00" "7J42V00" "7J42W00" "7J42X00" "7J42Y00" "7J42Z00" "7J43.00" "7J43.11" "7J43000" "7J43100" "7J43200" "7J43211" "7J43212" "7J43300" "7J43400" "7J43700" "7J43800" "7J43900" "7J43A00" "7J43C00" "7J43D00" "7J43y00" "7J43z00" "7K14.00" "7K14y00" "7K14z00" "7K15.00" "7K15y00" "7K15z00" "7K1D.00" "7K1D511" "7K1Dy00" "7K1Dz00" "7K1Ez00" "7K1F.00" "7K1F300" "7K1F400" "7K1F500" "7K1Fy00""7K1Fz00" "7K1G200" "7K1Gy11" "7K1H200" "7K1H300" "7K1H400" "7K1J300" "7K1JH00" "7K1Jy00" "7K1Jz00" "7K1K000" "7K1K200" "7K1K700" "7K1K800" "7K1K900""7K1KA00" "7K1KB00" "7K1KC00" "7K1Ky00" "7K1Kz00" "7K1L011" "7K1L100" "7K1L211" "7K1L300" "7K1La00" "7K1Lb00" "7K1Le00" "7K1Lg00" "7K1LZ00" "7K1N900" "7K1NA00" "7K1T100" "7K6GN00" "7K6H200" "7K6H411" "7K6H700" "7K6Hh00" "7K6HX00" "7P20100" "8HTo.00" "B585000" "N1y1.00" "N1y2.00" "N331.00" "N331.13" "N331000" "N331100" "N331200" "N331300" "N331400" "N331500" "N331600" "N331700" "N331800" "N331900" "N331A00" "N331B00" "N331C00" "N331M00" "N331M11" "N331N00" "N331y00" "N331z00" "N338.00" "N338000" "N338100" "N338111" "N338200" "N338300" "N338400" "N338500" "N338600" "N338z00" "NyuB000" "NyuB800" "NyuCE00" "S1...00" "S10..00" "S10..11" "S10..12" "S100.00" "S100.11" "S100.12" "S100000" "S100100" "S100111" "S100200" "S100211" "S100300" "S100311" "S100400" "S100411" "S100500" "S100511" "S100600" "S100611" "S100700" "S100711" "S100800" "S100900" "S100A00" "S100B00" "S100C00" "S100D00" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "S100E00" "S100F00" "S100G00" "S100H00" "S100J00" "S100K00" "S100L00" "S100M00" "S100N00" "S100x00" "S100z00" "S101.00" "S101.11" "S105400" "S101.12" "S101000" "S101100" "S101111" "S101200" "S101211" "S101300" "S101311" "S101400" "S101411" "S101500" "S101511" "S101600" "S101611" "S101700" "S101711" "S101800" "S101900" "S101A00" "S101B00" "S101C00" "S101D00" "S101E00" "S101F00" "S101G00" "S101H00" "S101J00" "S101K00" "S101L00" "S101M00" "S101N00" "S101x00" "S101z00" "S102.00" "S102000" "S102100" "S102200" "S102300" "S102400" "S102500" "S102600" "S102y00" "S102z00" "S103.00" "S103000" "S103100" "S103200" "S103300" "S103400" "S103500" "S103600" "S104.00" "S104000" "S104100" "S104200" "S104300" "S104400" "S104500" "S104600" "S105.00" "S105000" "S105100" "S105200" "S105300" "S105500" "S105600" "S106.00" "S106000" "S106100" "S107.00" "S107000" "S107100" "S108.00" "S109.00" "S10A.00" "S10A000" "S10A100" "S10A200" "S10B.00" "S10B000" "S10B100" "S10B200" "S10B300" "S10B400" "S10B500" "S10B600" "S10x.00" "S10y.00" "S10z.00" "S11..00" "S11..11" "S11..12" "S110.00" "S110000" "S110100" "S110200" "S110300" "S110400" "S110600" "S110700" "S110800" "S110900" "S110A00" "S110z00" "S111.00" "S111000" "S111100" "S111200" "S111300" "S111400" "S111600" "S111700" "S111800" "S111900" "S111A00" "S111z00" "S112.00" "S112000" "S112100" "S112200" "S112300" "S112400" "S112600" "S112700" "S112800" "S112900" "S112A00" "S112z00" "S113.00" "S113000" "S113100" "S113200" "S113300" "S113400" "S113600" "S113700" "S113800" "S113900" "S113A00" "S113z00" "S114.00" "S114000" "S114100" "S114200" "S114300" "S114400" "S114500" "S115.00" "S115000" "S115100" "S115200" "S115300" "S115400" "S115500" "S115z00" "S116.00" "S116000" "S116100" "S116200" "S116300" "S116z00" "S117.00" "S117000" "S117100" "S117200" "S117300" "S117z00" "S118.00" "S118000" "S118100" "S118200" "S118300" "S118z00" "S119.00" "S119000" "S119100" "S119200" "S119300" "S119z00" "S11x.00" "S11y.00" "S11z.00" "S120.00" "S120000" "S120100" "S120200" "S120300" "S120400" "S120500" "S120600" "S120700" "S120800" "S120900" "S120A00" "S120z00" "S121.00" "S121000" "S121100" "S121200" "S121300" "S121400" "S121500" "S121600" "S121700" "S121800" "S121900" "S121z00" "S122.00" "S123.00" "S125200" "S126200" "S127.00" "S127000" "S127100" "S128.00" "S12X.00" "S12X000" "S12X100" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "S12y.00" "S12y000" "S12y100" "S12z.11" "S12z.12" "S13..00" "S130.00" "S130000" "S130100" "S130200" "S130300" "S130400" "S130500" "S130600" "S130y00" "S130z00" "S131.00" "S131000" "S131100" "S131200" "S131300" "S131400" "S131500" "S131600" "S131y00" "S131z00" "S132.00" "S132000" "S132100" S132200" "S132y00" "S132z00" "S133.00" "S133000" "S133100" "S133200" "S133y00" "S133z00" "S134.00" "S134000" "S134100" "S134300" "S134400" "S134500" "S134600" "S134700" "S134800" "S134z00" "S135.00" "S135000" "S135100" "S135300" "S135400" "S135500" "S135600" "S135700" "S135800" "S135y00" "S135z00" "S13y.00" "S13z.00" "S14..00" "S140.00" "S141.00" "S14z.00" "S15..00" "S150.00" "S150000" "S150100" "S1z..00" "S2...00" "S2...11" "S20..00" "S20..11" "S200.00" "S200000" "S200100" "S200200" "S200300" "S200z00" "S201.00" "S201000" "S201100" "S201200" "S201300" "S201z00" "S20z.00" "S21..00" "S21..11" "S210.00" "S210000" "S210100" "S210200" "S210300" "S210400" "S210500" "S210600" "S210z00" "S211.00" "S211000" "S211100" "S211200" "S211300" "S211400" "S211500" "S211600" "S211z00" "S21z.00" "S22..00" "S220.00" "S220000" "S220100" "S220200" "S220300" "S220400" "S220500" "S220600" "S220700" "S220z00" "S221.00" "S221.11" "S221000 "S221100" "S221200" "S221300" "S221400" "S221500" "S221600" "S221700" "S221z00" "S222.00" "S222000" "S222100" "S222z00" "S223.00" "S223000" "S223100" "S223z00" "S224.00" "S224.11" "S224000" "S224100" "S224200" "S224300" "S224400" "S224500" "S224600" "S224700" "S224800" "S224900" "S224x00" "S224z00" "S225.00" "S225.11" "S225000" "S225100" "S225200" "S225300" "S225400" "S225500" "S225600" "S225700" "S225800" "S225900" "S225x00" "S225z00" "S226.00" "S227.00" "S228.00" "S22z.00" "S23..00" "S23..11" "S230.00" "S230000" "S230100" "S230200" "S230300" "S230400" "S230500" "S230600" "S230700" "S230800" "S230900" "S230A00" "S230B00" "S230z00" "S231.00" "S231000" "S231100" "S231200" "S231300" "S231400" "S231500" "S231600" "S231700" "S231800" "S231900" "S231A00" "S231B00" "S231z00" "S232.00" "S232000" "S232100" "S232200" "S232300" "S232z00" "S233.00" "S233000" "S233100" "S233200" "S233300" "S233z00" "S234.00" "S234.11" ("S234000" "S234100" "S234111" "S234200" "S234211" "S234300" "S234400" "S234500" "S234600" "S234700" "S234800" "S234900" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "S234911" "S234912" "S234A00" "S234A11" "S234A12" "S234B00" "S234C00" "S234D00" "S234E00" "S234F00" "S234G00" "S234z00" "S235.00" "S235.11" "S235000" "S235100" "S235111" "S235200" "S235211" "S235300" "S235400" "S235500" "S235600" "S235700" "S235800" "S235900" "S235911" "S235912" "S235A00" "S235A11" "S235A12" "S235B00" "S235C00" "S235D00" "S235E00" "S235F00" "S235z00" "S236.00" "S237.00" "S238.00" "S239.00" "S23A.00" "S23B.00" "S23C.00" "S23x.00" "S23x000" "S23x100" "S23x111" "S23x200" "S23x211" "S23x300" "S23xz00" "S23y.00" "S23y000" "S23y100" "S23y200" "S23y300" "S23yz00" "S23z.00" "S24..00" "S24..11" "S240.00" "S240000" "S240100" "S240200" "S240300" "S240400" "S240500" "S240600" "S240700" "S240800" "S240900" "S240A00" "S240B00" "S240C00" "S240D00" "S240E00" "S240F00" "S240y00" "S240z00" "S241.00" "S241000" "S241100" "S241200" "S241300" "S241400" "S241500" "S241600" "S241700" "S241800" "S241900" "S241A00" "S241B00" "S241C00" "S241D00" "S241E00" "S241F00" "S241y00" "S241z00" "S242.00" "S242000" "S242100" "S242200" "S242300" "S24z.00" "S25..00" "S25..11" "S250.00" "S250000" "S250200" "S250300" "S250400" "S250500" "S250600" "S250700" "S250800" "S250A00" "S250B00" "S250C00" "S250x00" "S250z00" "S251.00" "S251000" "S251200" "S251300" "S251400" "S251500" "S251600" "S251700" "S251800" "S251A00" "S251B00" "S251C00" "S251x00" "S251z00" "S252.00" "S253.00" "S26..00" "S26..11" "S26..12" "S260.00" "S260000" "S260300" "S260400" "S260500" "S260600" "S260700" "S260800" "S260900" "S260A00" "S260B00" "S260C00" "S260D00" "S260E00" "S260F00" "S260G00" "S260H00" "S260J00" "S260K00" "S260L00" "S260M00" "S260N00" "S260P00" "S260Q00" "S260R00" "S260S00" "S260T00" "S260U00" "S260V00" "S260W00" "S260x00" "S260z00" "S261.00" "S261000" "S261300" "S261400" "S261500" "S261600" "S261700" "S261800" "S261900" "S261A00" "S261B00" "S261C00" "S261D00" "S261E00" "S261F00" "S261G00" "S261H00" "S261J00" "S261K00" "S261L00" "S261M00" "S261N00" "S261P00" "S261Q00" "S261R00" "S261S00" "S261T00" "S261U00" "S261V00" "S261W00" "S261x00" "S261z00" "S262.00" "S263.00" "S264.00" "S26z.00" "S27..00" "S270.00" "S271.00" "S27z.00" "S28..00" "S28..11" "S280.00" "S281.00" "S28z.00" "S29..11" "S29..12" "S29..13" "S292.00" "S292000" "S292100" "S293.00" "S294.00" "S294000" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "S294100" "S2A..00" "S2B..00" "S2z..00" "S3...00" "S3...11" "S30..00" "S30..11" "S300.00" "S300100" "S300200" "S300300" "S300311" "S300400" "S300600" "S300700" "S300800" "S300900" "S300A00" "S300y00" "S300y11" "S300z00" "S301.00" "S301100" "S301200" "S301300" "S301311" "S301400" "S301500" "S301600" "S301700" "S301800" "S301900" "S301A00" "S301y00" "S301y11" "S301z00" "S302.00" "S302011" "S302012" "S302100" "S302200" "S302400" "S303.00" "S303011" "S303012" "S303100" "S303200" "S303300" "S303400" "S303z00" "S304.00" "S305.00" "S30w.00" "S30x.00" "S30y.00" "S30y.11" "S30z.00" "S31..00" "S310.00" "S310000" "S310011" "S310012" "S310100" "S310z00" "S311.00" "S311000" "S311100" "S311z00" "S312.00" "S312.11" "S312000" "S312100" "S312200" "S312300" "S312400" "S312500" "S312600" "S312x00" "S312z00" "S313.00" "S313.11" "S313000" "S313100" "S313200" "S313300" "S313400" "S313500" "S313600" "S313x00" "S313z00" "S314.00" "S315.00" "S31z.00" "S32..00" "S320.00" "S320000" "S320100" "S320200" "S320300" "S320400" "S321.00" "S321000" "S321100" "S321200" "S321300" "S321400" "S32z.00" "S33..00" "S330.00" "S330000" "S330011" "S330012" "S330100" "S330200" "S330300" "S330400" "S330500" "S330600" "S330700" "S330800" "S330900" "S330z00" "S331.00" "S331000" "S331011" "S331012" "S331100" "S331200" "S331300" "S331400" "S331500" "S331600" "S331700" "S331800" "S331900" "S331A00" "S331z00" "S332.00" "S332000" "S332100" "S332200" "S332z00" "S333.00" "S333000" "S333100" "S333200" "S333z00" "S334.00" "S334000" "S334100" "S335.00" "S335000" "S335100" "S336.00" "S336000" "S337.00" "S338.00" "S339.00" "S339000" "S339100" "S33A.00" "S33B.00" "S33C.00" "S33x.00" "S33x.11" "S33x000" "S33x100" "S33x200" "S33xz00" "S33y.00" "S33y000" "S33y100" "S33y200" "S33yz00" "S33z.00" "S34..00" "S340.00" "S341.00" "S342.00" "S342000" "S342100" "S343.00" "S343000" "S343100" "S344.00" "S344.11" "S344.12" "S344000" "S344100" "S345.00" "S345000" "S345100" "S346.00" "S346000" "S346100" "S347.00" "S347000" "S347100" "S348.00" "S349.00" "S34x.00" "S34y.00" "S34z.00" "S35..00" "S35..11" "S35..12" "S350.00" "S350.11" "S350.12" "S350000" "S350100" "S351.00" "S351000" "S351100" "S352.00" "S352.11" "S352000" "S352100" "S352111" "S352200" "S352300" "S352400" "S352500" "S352600" "S352700" "S352800" "S352900" "S352A00" "S352B00" "S352C00" "S352D00" "S352E00" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "S352F00" "S352G00" "S352H00" "S352J00" "S352z00" "S353.00" "S353000" "S353100" "S353111" "S353200" "S353300" "S353400" "S353500" "S353600" "S353700" "S353800" "S353900" "S353A00" "S353B00" "S353C00" "S353D00" "S353E00" "S353F00" "S353G00" "S353H00" "S353J00" "S353z00" "S354.00" "S355.00" "S356.00" "S35z.00" "S36..00" "S36..11" "S360.00" "S360000" "S360100" "S360200" "S360300" "S361.00" "S361000" "S361100" "S361200" "S361300" "S362.00" "S362000" "S362100" "S363.00" "S36z.00" "S37..00" "S370.00" "S371.00" "S3X..00" "S3x..00" "S3x0.00" "S3x1.00" "S3x2.00" "S3x3.00" "S3x4.00" "S3xz.00" "S3z..00" "S3z..11" "S3z0.00" "S3z0000" "S3z1.00" "S3z2.00" "S3zz.00" "S4...13" "S4A..00" "S4A0.00" "S4A0000" "S4A0100" "S4A1.00" "S4A1000" "S4A1100" "S4A2.00" "S4A2000" "S4A2100" "S4A3.00" "S4A3000" "S4A3100" "S4B..00" "S4B0.00" "S4B0000" "S4B0100" "S4B1.00" "S4B1000" "S4B1100" "S4B2.00" "S4B2000" "S4B2100" "S4B3.00" "S4B3000" "S4B3100" "S4C..00" "S4C0.00" "S4C0000" "S4C0100" "S4C0200" "S4C0300" "S4C0400" "S4C0500" "S4C0600" "S4C0y00" "S4C1.00" "S4C1000" "S4C1100" "S4C1200" "S4C1300" "S4C1400" "S4C1500" "S4C1600" "S4C1y00" "S4C2.00" "S4C2000" "S4C2100" "S4C2200" "S4C2300" "S4C2400" "S4C2500" "S4C2600" "S4C2y00" "S4C3.00" "S4C3000" "S4C3100" "S4C3200" "S4C3300" "S4C3400" "S4C3500" "S4C3600" "S4C3y00" "S4D..00" "S4D0.00" "S4D0000" "S4D0100" "S4D0200" "S4D0300" "S4D0400" "S4D0500" "S4D0600" "S4D1.00" "S4D1000" "S4D1100" "S4D1200" "S4D1300" "S4D1400" "S4D1500" "S4D1600" "S4D2.00" "S4D2000" "S4D2100" "S4D2200" "S4D2300" "S4D2400" "S4D2500" "S4D2600" "S4D3.00" "S4D3000" "S4D3100" "S4D3200" "S4D3300" "S4D3400" "S4D3500" "S4D3600" "S4E..00" "S4E0.00" "S4E1.00" "S4E2.00" "S4E3.00" "S4F..00" "S4F0.00" "S4F1.00" "S4F2.00" "S4F3.00" "S4F4.00" "S4F5.00" "S4F6.00" "S4F7.00" "S4G..00" "S4G0.00" "S4G1.00" "S4G2.00" "S4G3.00" "S4H..00" "S4H0.00" "S4H0000" "S4H0100" "S4H0200" "S4H0400" "S4H0600" "S4H1.00" "S4H1000" "S4H1100" "S4H1200" "S4H1300" "S4H1400" "S4H1600" "S4H2.00" "S4H2000" "S4H2100" "S4H2200" "S4H2400" "S4H2600" "S4H3.00" "S4H3000" "S4H3100" "S4H3200" "S4H3300" "S4H3400" "S4H3600" "S4J..00" "S4J0.00" "S4J0000" "S4J0100" "S4J1.00" "S4J1000" "S4J1100" "S4J1200" "S4J1300" "S4J2.00" "S4J2000" "S4J2100" "S4J3.00" "S4J3000" "S4J3100" "S4J3200" "S4J3300" "SC0X.00" "SC0z.11" "SC20.00" "SC3C000" "SC3D400" "SD92000" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "SP04A00" "SP04C00" "SP22400" "SR1..00" "SR10.00" "SR10000" "SR10100" "SR11.00" "SR12.00" "SR12000" "SR12100" "SR13.00" "SR14.00" "SR14000" "SR14100" "SR15000" "SR15100" "SR16000" "SR16100" "SR1z.00" "SR1z000" "SR1z100" "Syu1500" "Syu1600" "Syu2700" "Syu2800" "Syu4200" "Syu4300" "Syu4400" "Syu5300" "Syu5400" "Syu6300" "Syu6400" "Syu6500" "Syu7200" "Syu8300" "Syu8D00" "Syu9400" "SyuA200" "SyuBB00" "SyuL100" "SyuL400" "TC7..00" "Z6G1900" "Zw01.00" "Zw02.00" "Zw02400" "Zw02500" "Zw02D00" "Zw02E00" "Zw02F00" "Zw02G00" "Zw02H00" "Zw02J00" **Seizure:** "1473.00" "14On.00" "1B26.00" "1B27.00" "1O30.00" "282..13" "6110.00" "667..00" "6671.00" "6672.00" "6674.00" "6677.00" "6678.00" "6679.00" "667A.00" "667B.00" "667C.00" "667D.00" "667E.00" "667F.00" "667G.00" "667H.00" "667J.00" "667K.00" "667L.00" "667M.00" "667N.00" "667Q.00" "667R.00" "667S.00" "667T.00" "667V.00" "667W.00" "667Z.00" "67AF.00" "67IJ000" "8BIF.00" "8CE7.00" "8Hlp.00" "8IAg.00" "8IAh.00" "8IAi.00" "8IB2.00" "8IB3.00" "8IB4.00" "8T0L.00" "9N0r.00" "9N4V.00" "9Of..00" "9Of0.00" "9Of1.00" "9Of2.00" "9Of3.00" "9Of4.00" "9Of5.00" "9Of6.00" "9Of7.00" "E201500" "Eu05212" "Eu06013" "Eu10800" "Eu44511" "Eu80300" "F132100" "F132z12" "F25..00" "F250.00" "F250000" "F250100" "F250200" "F250300" "F250400" "F250y00" "F250z00" "F251.00" "F251000" "F251011" "F251100" "F251200" "F251300" "F251400" "F251500" "F251600" "F251y00" "F251z00" "F254.00" "F254000" "F254100" "F254200" "F254300" "F254500" "F254z00" "F255.00" "F255000" "F255011" "F255012" "F255100" "F255200" "F255300" "F255311" "F255400" "F255500" "F255600" "F255y00" "F255z00" "F257.00" "F25A.00" "F25B.00" "F25C.00" "F25D.00" "F25E.00" "F25F.00" "F25G.00" "F25H.00" "F25y.00" "F25y000" "F25y100" "F25y400" "F25yz00" "F25z.00" "Fyu5000" "Fyu5100" "Q480.12" "R003300" "R003400" "R003z11" "SC20000" **Bipolar disorder**: "E11..11" "E114.00" "E114000" "E114100" "E114200" "E114300" "E114400" "E114500" "E114600" "E114z00" "E115.00" "E115000" "E115100" "E115200" "E115300" "E115400" "E115500" "E115600" "E115z00" "E116.00" "E116000" "E116100" "E116200" "E116300" "E116400" "E116500" (cont.)

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	45		(cont.) "E116600" "E116z00" "E117.00" "E117000" "E117100" "E117200" "E117300" "E117400" "E117500" "E117600" "E117z00" "Eu30.11" "Eu31.00" "Eu31000" "Eu31100" "Eu31200" "Eu31300" "Eu31500" "Eu31600" "Eu31700" "Eu31800" "Eu31900" "Eu31911" "Eu31y00" "Eu31y11" "Eu31z00" "Eu34100" "ZRby100" **Depression:** "12K8.00" "1465.00" "1B17.00" "1B17.11" "1B1U.00" "1B1U.11" "1BT..00" "1JJ..00" "1TC..00" "1TC0.00" "1TC1.00" "1TC2.00" "1TC3.00" "2257.00" "62T1.00" "6658000" "6659000" "6G00.00" "8BK0.00" "8CAa.00" "8HHq.00" "8HHq000" "9H90.00" "9H91.00" "9H92.00" "9HA0.00" "9k4..00" "9k40.00" "9kQ..00" "9kQ..11" "9Ov..00" "9Ov0.00" "9Ov1.00" "9Ov2.00" "9Ov3.00" "9Ov4.00" "E001300" "E002.00" "E002100" "E002z00" "E004300" "E02y300" "E11..12" "E112.00" "E112.11" "E112.12" "E112.13" "E112.14" "E112000" "E112100" "E112200" "E112300" "E112400" "E112500" "E112600" "E112z00" "E113.00" "E113.11" "E113000" "E113100" "E113200" "E113300" "E113400" "E113500" "E113600" "E113700" "E113z00" "E11y200" "E11z200" "E130.00" "E130.11" "E135.00" "E200300" "E204.00" "E204.11" "E211200" "E291.00" "E2B..00" "E2B0.00" "E2B1.00" "Eu02z16" "Eu32.00" "Eu32.11" "Eu32.12" "Eu32.13" "Eu32000" "Eu32100" "Eu32200" "Eu32211" "Eu32212" "Eu32213" "Eu32300" "Eu32311" "Eu32312" "Eu32313" "Eu32314" "Eu32400" "Eu32500" "Eu32600" "Eu32700" "Eu32800" "Eu32B00" "Eu32y00" "Eu32y11" "Eu32y12" "Eu32z00" "Eu32z11" "Eu32z12" "Eu32z13" "Eu32z14" "Eu33.00" "Eu33.11" "Eu33.12" "Eu33.13" "Eu33.14" "Eu33000" "Eu33100" "Eu33200" "Eu33211" "Eu33212" "Eu33213" "Eu33214" "Eu33300" "Eu33311" "Eu33313" "Eu33314" "Eu33315" "Eu33316" "Eu33400" "Eu33y00" "Eu33z00" "Eu34111" "Eu34112" "Eu34113" "Eu34114" "Eu3y111" "Eu41200" "Eu41211" "Eu53011" "Eu53012" "Eu92000" "Eu33z11"
3	46	**CNS stimulants:** "R01BA02" "R01BA52" "R01BA51" "N02BE71" "R05DA20" "R06AB51" "N02BE01" "R01BA03" " "R01AA04" "C01CA06" "R01AB01" "N06BA04" "N06BA07" "R03DA04" "C01CA26" "R03DB04" "N06BC01" "N02BA01 (whenever in combination with caffeine)" "R06AA09" "N06BC01" "R06AA02" "R05DA04" "N02CA52 whenever in combination with caffeine" "N02AC54 whenever in combination with caffeine"	**Insomnia:** "1B1B.00" "1B1B.11" "1B1B000" "1B1B100" "1B1B200" "38D1.00" "8Q0..00" "9Ngt.00" "E274.00" "E274.12" "E274100" "E274111" "E274200" "Eu51000" "Fy00.00" "K5A2100" "R005.11" "R005100" "R005200"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	47	**Antipsychotics:** see criteria 43 (except "N05AH02" "N05AH04" "N05AX12") **Antiemetics:** "A03FA01" "N05AB04" "R06AD02" "R05DA20 whenever in combination with antiemetics" "R06AD52" "N02AB52 whenever in combination with promethazine" "N02BE71 whenever in combination with promethazine "	**Parkinson's disease:** "147F.00" "297A.00" "2987" "2987.11" "2994" "2994.11" "38GM.00" "8Hx0.00" "8T06.00" "8T06000" "9NIe.00" "A94y100" "Eu02300" "F11x900" "F12..00" "F121.00" "F121.11" "F123.00" "F124.00" "F12W.00" "F12X.00" "F12z.00" "F130300" "Fyu2000" "Fyu2100" "Fyu2200" "Fyu2900" "Fyu2B00"
3	48	**Aspirin:** "N02BA01" "N02AJ07" "N02BA51" "N06BC01 whenever in combination with aspirin" **Non-COX2 non-steroidal anti-inflammatory drugs:** "M01AA01" "M01AB01" "M01AB02" "M01AB03" "M01AB05" "M01AB55" "M01AB08" "M01AB11" "M01AB15" "M01AB16" "M01AC01" "M01AC02" "M01AC05" "M01AC06" "M01AE01" "M01AE14" "M01AE02" "M01AE52" "M01AE03" "M01AE53" "M01AE17" "M01AE04" "M01AE05" "M01AE09" "M01AE11" "M01AG01" "M01AG02" "M01AX01" "M01AX04" **Proton pump inhibitors:** "A02BC" "M01AE53" "A02BD" "M01AE52" **Misoprostol:** "A02BB01"	**Gastric ulcers:** "14C1.00" "14C1.11" "14C1.12" "1956.00" "7612111" "7612500" "761D500" "761D600" "761J.00" "761J.11" "761J000" "761J100" "761J111" "761Jy00" "761Jz00" "7627.00" "7627000" "7627100" "7627200" "7627y00" "7627z00" "J080000" "J102000" "J11..00" "J110.00" "J110000" "J110100" "J110111" "J110200" "J110300" "J110400" "J110y00" "J110z00" "J111.00" "J111000" "J111100" "J111111" "J111200" "J111211" "J111300" "J111400" "J111y00" "J111z00" "J112.00" "J112z00" "J113.00" "J11y.00" "J11y000" "J11y100" "J11y200" "J11y300" "J11y400" "J11yy00" "J11yz00" "J11z.00" "J11z.12" "J12..00" "J120.00" "J120000" "J120100" "J120200" "J120300" "J120400" "J120y00" "J120z00" "J121.00" "J121000" "J121100" "J121111" "J121200" "J121211" "J121300" "J121400" "J121y00" "J121z00" "J122.00" "J124.00" "J125.00" "J125z00" "J126.00" "J12y.00" "J12y000" "J12y100" "J12y200" "J12y300" "J12y400" "J12yy00" "J12yz00" "J12z.00" "J13..00" "J130.00" "J130000" "J130100" "J130200" "J130300" "J130400" "J130y00" "J130z00" "J131.00" "J131000" "J131100" "J131200" "J131300" "J131400" "J131y00" "J131z00" "J13y.00" "J13y000" "J13y100" "J13y200" "J13y300" "J13y400" "J13yy00" "J13yz00" "J13z.00" "J14..00" "J14..12" "J14..13" "J14..15" "J140.00" "J140000" "J140100" "J140200" "J140300" "J140400" "J140y00" "J140z00" "J141.00" "J141000" "J141100" "J141200" "J141300" "J141400" "J141y00" "J141z00" "J14y.00" "J14y000" "J14y100" "J14y200" "J14y300" "J14y400" "J14yy00" "J14yz00" "J14z.00" "J17y800" "ZV12711" "ZV12712" "ZV12C00"
3	49	**Non-steroidal anti-inflammatory drugs:** "N02BA01" "N02AJ07" "N02BA51" "N06BC01 whenever in combination with aspirin" M01A" (excluding topical formulations, suppositories, eye drops, and plasters)	**Chronic kidney disease stages 4 and 5:** "1Z13.00" "1Z14.00" "1Z1H.00" "1Z1J.00" "1Z1K.00" "1Z1L.00" "K054.00" "K055.00"

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
3	50	**Alpha-1 blockers:** see criteria 7 **Estrogens:** "G03AA" "G03AB" "G03C" "G03F" "L02AA01"	
3	51	**Anticholinergic drugs to be avoided in men:** "R06AB01" "R06AA52" "R06AD52" "R01BA52" "R01BA51" "R06AB51" "R06AB04" "R06AA54" "R06AA04" "R06AX02" "N07CA52" "R06AA02" "N02BE71" "N07XX59" "R01BA02" "R05FB02" "R06AA09" "R05CA03" "R01AA04" "N05BB01" "R06AX07" "N04AA01" "N04AB02" "M03B C01" "N02B E01" "N06A A09" "N06CA01" "N06AA17" "N06AA04" "N06AA01" "N06AA12" "N06AA02" "N06AA10" "N06AB05" "N06AA11" "N06AA06" "N05AA01" "N05AH02" "N05AH01" "N05AH03" "N05AB03" "N05AC02" "N05AB06" "A03CB04" "C01BA03" "A07DA52" "A03CB02" "A03CB" "A06AB04" "A03BA04" "C02AA03" "A03CA02" "A03BA01" "A07DA01" "A04AD04" "A03BA01" "N02AA51" "A03AB05" "N05AB04" "R06AD02" "N02AB52"	**Urinary tract symptoms:** "14D4.00" "A32y300" "A981100" "A983100" "K15..00" "K150.00" "K151.00" "K151200" "K151z00" "K152.00" "K152000" "K152y00" "K152z00" "K153.11" "K154.00" "K154000" "K154100" "K154200" "K154300" "K154400" "K154500" "K154600" "K154700" "K154800" "K154z00" "K155.00" "K15y.00" "K15y000" "K15y100" "K15yz00" "K15z.00" "K213.00" "Kyu5000" "Kyu5100" "R081000" "R086.00" "R086z00" "1AZ6.00" "1AZ6000" "1AZ6100" "1AZ6200" **Benign prostatic hyperplasia:** "25Q2.11" "K20..00" "K20..14" "K20..15" "K200.00"
5	52	**ACE inhibitors:** "C09AA01" "C09BA01" "C09AA02" "C09BA02" "C09AA03" "C09BA03" "C09AA04" "C09BA04" "C09AA05" "C09BB05" "C09AA06" "C09BA06" "C09AA08" "C09AA09" "C09AA10" "C09BB10" "C09AA13" "C09AA16" **Amiloride and triamterene:** "C03DB01" "C03EB02" Whenever in combination with amiloride or triamterene: "C03EA02" "C07DB01" "C03EA01" "C07DA06" "C03EB01" "C03DB02"	**Hypokalemia:** "44I4200" "C368.11"
5	53	**Anticholinergics:** see criteria 43	
5	54	**Tricyclic antidepressants:** "N06AA09" "N06CA01" "N06AA02" "N06AA06" "N06AA12" "N06AA07" "N06AA17" "N06AA01" "N06AA10" "N06C" "N06AA11" "N06AA04" "N06AA13" "N06AA15" "N06AA16" "N06AA21" **Selective serotonin reuptake inhibitors:** "N06AB" **Additional central nervous system active drugs:** Opioids (see criteria 45), benzodiazepines (see criteria 43), non-benzodiazepine / benzodiazepine receptor agonist hypnotics (see criteria 22), antipsychotics (see criteria 17).	

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
5	55	**Antipsychotics:** see criteria 17 **Additional central nervous system active drugs:** opioids (see criteria 45), benzodiazepines (see criteria 43), non-benzodiazepine / benzodiazepine receptor agonist hypnotics (see criteria 22), selective serotonin reuptake inhibitors (see criteria 54), tricyclic antidepressants (see criteria 55).	
5	56	**Benzodiazepines**: see criteria 43 **Non-benzodiazepine / benzodiazepine receptor agonist hypnotics:** see criteria 22 **Additional central nervous system active drugs:** opioids (see criteria 45), selective serotonin reuptake inhibitors (see criteria 54), tricyclic antidepressants (see criteria 55), antipsychotics (see criteria 17).	
5	57	**Corticosteroids:** see criteria 43 **Non-steroid anti-inflammatory drugs:** see criteria 49 **PPI:** see criteria 48 **Misoprostol:** see criteria 48	
5	58	**Lithium:** "N05AN01" **Angiotensin-converting enzyme:** see criteria 12	**Lithium monitoring:** "44vE.00" "44W8.00" "44W8.11" "44W8000" "44W8100" "44W8200" "6657.11" "665J.00" "R105300"
5	59	**Lithium:** see criteria 58 **Loop diuretics:** "C03CA" "C03EB02" "C03DB01 whenever in combination with loop diuretic" "C03CB02" "C03CC01" "C03EB01" "C07CA23 whenever in combination with loop diuretic " "C03CB01"	**Lithium monitoring:** see criteria 58
5	60	**Opioids:** see criteria 45 **Additional central nervous system active drugs:** selective serotonin reuptake inhibitors (see criteria 54), tricyclic antidepressants (see criteria 55), antipsychotics (see criteria 17), benzodiazepines (see criteria 43), non-benzodiazepine / benzodiazepine receptor agonist hypnotics (see criteria 22)	

Table 8.2.3 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the 2015 American Geriatrics Society Beers criteria.

Table Number[1]	Criteria Number	ATC codes used	Read codes used
5	61	**Loop diuretics:** see criteria 59 **Peripheral alpha-1 blockers:** see criteria 7 **Angiotensin-converting enzyme:** see criteria 12 **Angiotensin receptor blockers:** see criteria 12 **Calcium-channel blockers:** see criteria 11 **Thiazide-like diuretics:** "C03A"	
5	62	**Theophylline:** "R03DA04" "C01CA26 whenever in combination with theophylline" "R03DB04" **Cimetidine:** "A02BA01" "A02BA51"	
5	63	**Warfarin:** "B01AA03" **Amiodarone:** "C01BD01"	**Warfarin monitoring and International normalized ratio monitoring:** "4130.00" "41C5.00" "42jr.00" "42QE.00" "42QE000" "42QE100" "42QE200" "66Q..00" "66Q7.00" "66Q7000" "66Q8.00" "66Q8000" "66QE.00" "66QG.00" "8HHW.00" "9k21.00" "9k22.00" "9k22.11" "9k25.00" "9k25.11"
5	64	**Warfarin:** see criteria 63 **Non-steroidal anti-inflammatory drugs:** M01A" (excluding topical formulations, suppositories, eye drops, and plasters)	**Warfarin monitoring and International normalized ratio monitoring:** see criteria 63
6	65	**Amiodarone:** see criteria 63	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	66	**Apixaban:** "B01AF02"	**Chronic kidney disease stage 5**: "1Z14.00" "1Z1K.00" "1Z1L.00" "K055.00"
6	67	**Dabigatran:** "B01AE07"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	68	**Edoxaban:** "B01AF03"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	69	**Fondaparinux:** "B01AX05"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	70	**Rivaroxaban:** "B01AF01"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	71	**Spironolactone:** "C03DA01" "C03EB01"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	72	**Triamterene:** "C03DB02" "C03EA06 whenever in combination with triamterene" "C03EB01 whenever in combination with triamterene "	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	73	**Duloxetine:** "N06AX21"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	74	**Tramadol:** "N02AX20"	**Chronic kidney disease stages 4 and 5:** see criteria 49
6	75	**Probenecid:** "M04AB01"	**Chronic kidney disease stages 4 and 5:** see criteria 49

[1] Adapted from the " American Geriatrics Society 2015 Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults" , by the American Geriatrics Society 2015 Beers Criteria Update Expert Panel, 2015, *J Am Geriatr Soc*, 63:2227–46. https://doi.org/10.1111/jgs.13702.

Table 8.2.4 List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria.

Criteria Number	ATC codes used	Read codes used
1	**Opioids:** "N02AA01" "N02AA51" "N01AB02" "N02AA02" "N02AA03" "N02AA05" "N02AA55" "N02AJ" "N02AA08" "N02AA10" "N02AB02" "N02AB52" "N01AH01" "N02AB03" "N01AH51" "N02AD01" "N02BE01" "N02AC54" "N02AC01" "N02AC04" "N02AD02" "N07BC01" "N02AE01" "N07BC51" "N02AF02" "N02AX02" "N02AX" **Stimulant laxatives:** "A06AB02" "A06AA02 (whenever in combination with a stimulant laxative)" "A06 C01" "A06AB06" "P02CB01" "A06A B58" "A06AB08" "A06AB05" "A06AB07" "A06AB53" "A06AA51" "A06AB04" "A06AD04" **Other laxative drugs:** "A06AC01" "A03AA04" "A06AC03" "A06AC53" "A06AC06" "A06AD15" "A06AD" "A11G (whenever in combination with macrogol)" "A06A G06" "A06AA02" "A06AG10 " "A06AA51" "A06AA01" "A06AX"	**Zollinger Ellison Syndrome:** C115.11
2	**Proton pump inhibitors:** "A02BC" "M01AE53" "A02BD" "M01AE52"	
3	**Esomeprazole and omeprazole:** "A02BC01" "M01AE53" "A02BC05" "M01AE52" **Clopidogrel:** "B01AC04"	
4	**Alpha-adrenoreceptor antagonists:** "C02CA04" "C02CA01" "G04CA03" **Other antihypertensive drugs:** "C02 except C02CA" "C09AA" "C09C" "C09D" "C08C" "C08D" "C03A" "C03B" "C03C" "C03D" "C03E" "C03XA" "C07" "C09XA02"	**Hypertension:** "14A2.00" "661M600" "661N600" "662..12" "6627.00" "6628.00" "662b.00" "662c.00" "662d.00" "662F.00" "662G.00" "662O.00" "662P.00" "662P000" "662P100" "662q.00" "8B26.00" "8BL0.00" "8CR4.00" "8HT5.00" "8I3N.00" "8IA6.00" "9OI1.00" "9OI3.00" "9OI4.00" "9OI5.00" "9OI6.00" "9OI7.00" "9OI8.00" "9OIA.11" "9OIZ.00" "G2...00" "G2...11" "G20..00" "G20..12" "G200.00" "G201.00" "G202.00" "G203.00" "G20z.00" "G20z.11" "G21..00" "G210.00" "G210000" "G210100" "G210z00" "G211.00" "G211000" "G211100" "G211z00" "G21z.00" "G21z000" "G21z011" "G21z100" "G21zz00" "G22..00" "G220.00" "G221.00" "G222.00" "G22z.00" "G22z.11" "G23..00" "G230.00" "G231.00" "G232.00" "G233.00" "G234.00" "G23z.00" "G24..00" "G240.00" "G240000" "G240z00" "G241.00" "G241000" "G241z00" "G244.00" "G24z.00" "G24z000" "G24z100" "G24zz00" "G25..00" "G25..11" "G250.00" "G251.00" "G26..00" "G26..11" "G27..00" "G28..00" "G2y..00" "G2z..00" "G672.11" "G8y3.00" "Gyu2.00" "Gyu2000" "Gyu2100" "L12..00" "L122.00" "L122000" "L122100" "L122300" "L122400" "L122z00" "L127.00" "L127000" "L127100" "L127200" "L127300" "L127400" "L127z00" "L128.00" "L128000" "L128100" "L128200" "L12B.00" "Lyu1.00"

Table 8.2.4 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria.

Criteria Number	ATC codes used	Read codes used
5	**Aspirin:** "B06AC06"	
6	**Calcium-channel blockers:** "C08DB01" "C08DA01" "C09BB10" **Beta blockers:** "C07AA" "C07AB" "C07AG" "C07BA" "C07BB" "C07C" "C07D"	
7	**Dipyridamole:** "B01AC07" **Other antiplatelet medication:** "B01AC04" "B01AC05" "B01AC24" "B01AC22" "B01AC23" "B01AC06"	
8	**First generation antihistamines** (oral formulations): "R06AB01" "R06AB51" "R06AB04" "R06AA54" "R06AA52" "R06AA04" "R06AX02" "R01BA52" "R06A A02" "R06AA52" "R01BA02" "R05DA20" "R05FB02" "R06AA09" "R05CA03" "R01AA04" "N05BB01" "R06AE05" "R06AD02" "R06AD52" "R06AX07" "R06AD01" "R06AD07" "R01BA01" "R01BA51" "R06AB05" "R06AC01" **Other oral anti-histamine drugs:** "R06AX13" "R06AX27" "R06AX26" "R06AE07" "R06AE09" "R06AX18" "R06AX25"	
9	**Theophylline:** "R03DA04" "R03DA02" "R03DB04" "R03DA54" "R03DA74"	
10	**Oral corticosteroids:** "H02AB01" "H02AB02" "H02AB04" "H02AB06" "H02AB07" "H02AB08" "H02AB09" "A01AC03" "H02AB10" "H02AB13" "A07EA07" **Bisphosphonates:** "M05BA01" "M05BB01" "M05BA02" "M05BA03" "M05BB03" "M05BA04" "M05BA05" "M05BA06" "M05BA08" "M05BA07" "M05BB04" "M05BB02"	
11	**Mucolytic agents:** "R05CB"	**Chronic obstructive pulmonary disease:** "66YB.00" "66Yg.00" "66YL.00" "66YL.11" "679V.00" "8CR1.00" "9Oi3.00" "9Oi4.00" "H3...00" "H3...11" "H32..00" "H322.00" "H36..00" "H37..00" "H38..00" "H3y..00" "H3z..00" **Exacerbations:** "8BP8.00" "H312200" "H3y1.00"
12	**Venlafaxine:** "N06AX16" **Selective serotonin reuptake inhibitors:** "N06AB"	

Table 8.2.4 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria.

Criteria Number	ATC codes used	Read codes used
13	**Tricyclic antidepressants:** "N06AA09" "N06CA01" "N06AA02" "N06AA06" "N06AA12" "N06AA07" "N06AA17" "N06AA01" "N06AA10" "N06C" "N06AA11" "N06AA04" "N06AA13" "N06AA15" "N06AA16" "N06AA21" **Other antidepressants:** "N06AX" "N06AB" "N06AG" "N06AF"	**Depression:** "12K8.00" "1465.00" "1B17.00" "1B17.11" "1B1U.00" "1B1U.11" "1BT..00" "1JJ..00" "1TC..00" "1TC0.00" "1TC1.00" "1TC2.00" "1TC3.00" "2257.00" "62T1.00" "6658000" "6659000" "6G00.00" "8BK0.00" "8CAa.00" "8HHq.00" "8HHq000" "9H90.00" "9H91.00" "9H92.00" "9HA0.00" "9k4..00" "9k40.00" "9kQ..00" "9kQ..11" "9Ov..00" "9Ov0.00" "9Ov1.00" "9Ov2.00" "9Ov3.00" "9Ov4.00" "E001300" "E002.00" "E002100" "E002z00" "E004300" "E02y300" "E11..12" "E112.00" "E112.11" "E112.12" "E112.13" "E112.14" "E112000" "E112100" "E112200" "E112300" "E112400" "E112500" "E112600" "E112z00" "E113.00" "E113.11" "E113000" "E113100" "E113200" "E113300" "E113400" "E113500" "E113600" "E113700" "E113z00" "E11y200" "E11z200" "E130.00" "E130.11" "E135.00" "E200300" "E204.00" "E204.11" "E211200" "E291.00" "E2B..00" "E2B0.00" "E2B1.00" "Eu02z16" "Eu32.00" "Eu32.11" "Eu32.12" "Eu32.13" "Eu32000" "Eu32100" "Eu32200" Eu32211" "Eu32212" "Eu32213" "Eu32300" "Eu32311" "Eu32312" "Eu32313" "Eu32314" "Eu32400" "Eu32500" "Eu32600" "Eu32700" "Eu32800" "Eu32B00" "Eu32y00" "Eu32y11" "Eu32y12" "Eu32z00" "Eu32z11" "Eu32z12" "Eu32z13" "Eu32z14" "Eu33.00" "Eu33.11" "Eu33.12" "Eu33.13" "Eu33.14" "Eu33000" "Eu33100" "Eu33200" "Eu33211" "Eu33212" "Eu33213" "Eu33214" "Eu33300" "Eu33311" "Eu33313" "Eu33314" "Eu33315" "Eu33316" "Eu33400" "Eu33y00""Eu33z00" "Eu34111" "Eu34112" "Eu34113" "Eu34114" "Eu3y111" "Eu41200" "Eu41211" "Eu53011" "Eu53012" "Eu92000" "Eu33z11"
14	**Benzodiazepines:** "N05BA12" "N05CD11" "N05BA06" "N05CD06" "N05BA04" "N05CD07" "N05CD05" "N05BA08" "N05BA09" "N05BA05" "N05BA02" "N06CA01" "A03CA02" "N03AE01" "N05BA01" "N05CD03" "N05CD01" "N05CD02" "N05BA11" "N05CD08" "N05BA03" "N05BA10"	
15	**Non-benzodiazepine hypnotics:** "N05CF01" "N05CF02" "N05CF03"	
16	Carbamazepine: "N03AF01" Erythromycin: "J01FA01" Clarithromycin: "J01FA09" "A02BD14" "A02BD12" "A02BD06" "A02BD07" "A02BD05" "A02BD07" "A02BD09" "A02BD11"	

Table 8.2.4 (cont.) List of Anatomical therapeutic chemical (ATC) classification codes and Read codes used to assess potential inappropriate prescription (PIP) according to the Prescribing Optimally in Middle-aged People' s Treatments (PROMPT) criteria.

Criteria Number	ATC codes used	Read codes used
17	**Strong opioids:** "N02AA01" "R05FA02" "N02AA51" "N01AB02 (whenever formulation has a combination with morphine)" "N02AA03" "N02AA05" "N02AA55" "N02AB02" "N02AB52" "N01AH01" "N02AB03" "N01AH51" "N02AD01" "N02BE01" "N07BC01" "N02AE01" "N07BC51" "N02AF02" "N07BC06" **Laxatives:** "A06AB02" "A06AA02 (whenever in combination with a stimulant laxative)" "A06AC01" "A06AB06" "P02CB01" "A06AB58" "A06AB08" "A06AB05" "A06AB07" "A06AB53" "A06AA51" "A06AB04" "A06AD04" "A06AC01" "A03AA04" "A06AC03" "A06AC53" "A06AC06" "A06AD15" "A06AD" "A11G (whenever in combination with macrogol)" "A06A G06" "A06AA02" "A06AG10 " "A06AA51" "A06AA01" "A06AX"	
18	**Nitrofurantoin:** "J01XE01"	
19	**Long-acting Sulfonylureas:** "A10BB01" "A10BB02"	
20	**Non-steroidal anti-inflammatory drugs:** M01A" (excluding topical formulations, suppositories, eye drops, and plasters)	
21	**Non-steroidal anti-inflammatory drugs:** see criteria 20 **Low dose aspirin:** "B06AC06" **H2-receptor antagonist:** "A02BA" **Selective serotonin reuptake inhibitors:** "N06AB" **Proton pump inhibitors:** "A02BC" "M01AE53" "A02BD" "M01AE52"	
22	**Selective serotonin reuptake inhibitors:** "N06AB" **Proton pump inhibitors:** "A02BC" "M01AE53" "A02BD" "M01AE52" **Opioids:** "N02AA01" "N02AA51" "N01AB02" "N02AA02" "N02AA03" "N02AA05" "N02AA55" "N02AJ" "N02AA08" "N02AA10" "N02AB02" "N02AB52" "N01AH01" "N02AB03" "N01AH51" "N02AD01" "N02BE01" "N02AC54" "N02AC01" "N02AC04" "N02AD02" "N07BC01" "N02AE01" "N07BC51" "N02A F02" "N02AX02" "N02AX"	

Abbreviations: ATC: Anatomical therapeutic chemical.

Chapter 8.3

Identification of novel off-targets of baricitinib and tofacitinib by machine learning with a focus on thrombosis and viral infection

Serine/threonine-protein Kinase N2 (PKN2) Assay

PKN2 (human) was incubated with 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1% 5-mercaptoethanol, 30 □M AKRRRLSSLRA, 10 mM magnesium acetate and [γ-33P]-ATP (15 □M). The reaction was initiated by the addition of the Mg/ATP mixture. After incubation for 40 minutes at 25 °C, the reaction was terminated by the addition of phosphoric acid (conc. 0.5%). 10 □L of the reaction was then spotted onto a P30 filtermat and washed four times for 4 minutes in 0.425% phosphoric acid and one time in methanol prior to drying and scintillation counting. Stausporine was used as reference compound. A primary screening in duplicated was carried out (30 □M) to determine the target activity. The result was expressed as a percentage of the positive control (i.e., % kinase activity remaining), and the mean of the replicates was calculated. Later, tofacitinib and baricitinib were tested at 9 concentrations (100 □M as the highest concentration and dilution factor in a half-log-scale) in duplicate for dose-response curve and IC_{50} determination. (Eurofins Cerep assay ID: 14-549KP).

Epidermal Growth Factor Receptor (EGFR) Assay

Baricitinib was combined to EGFR and incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 10 mM $MnCl_2$, 0.1 mg/mL poly (Glu, Tyr) 4:1, 10 mM magnesium acetate and [γ-33P]-ATP (10 □M). The reaction was initiated by the addition of the Mg/ATP mix. After incubation for 40 minutes at 25 °C, the reaction was terminated by the addition of phosphoric acid to a concentration of 0.5%. 10 □L of the reaction was subsequently spotted onto a Filtermat A and washed four times for 4 minutes in 0.425% phosphoric acid and one time in methanol prior to drying and scintillation counting. Stausporine was used as reference compound. A primary screening in duplicated was carried out (30 μM) to determine the target activity. The result was expressed as a percentage of the positive control (i.e., % kinase activity remaining), and the mean of the replicates was calculated. (Eurofins Cerep assay ID: 14-531KP).

Transient receptor potential cation channel subfamily M member 6 (TRPM6) Assay

HEK-293 cells were tagged with DNA for quantitative polymerase chain reaction (qPCR) detection. streptavidin-coated magnetic beads were treated with biotinylated affinity ligands for 30 minutes at 25 °C to produce affinity resins. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock [Pierce], 1% BSA, 0.05% Tween 20, 1 mM DTT) to remove unbound ligand and to reduce nonspecific binding. Binding reactions were performed by combining the kinase, liganded affinity beads and tofacitinib in 1x binding buffer (20% SeaBlock, 0.17× PBS, 0.05% Tween 20, 6 mM DTT). Tofacitinib was prepared as 111x stocks in DMSO and diluted into the assay environment, such as the final concentration of DMSO was 0.9

%. K_d was determined using an 11-point 3-fold compound dilution series with three DMSO control points and DMSO was added to control assays lacking tofacitinib. Tofacitinib highest concentration was equal to 30 □M and the analysis was performed in duplicate. The analysis was carried on in polypropylene 384-well plates incubated at 25 °C with shaking for 1 h in a final volume of 20 □L. The affinity beads were washed extensively with wash buffer (1× PBS, 0.05% Tween 20) to remove unbound protein. The beads were resuspended in elution buffer (1× PBS, 0.05% Tween 20, 0.5 □M nonbiotinylated affinity ligand) and incubated at 25 °C with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR. (Eurofins Cerep assay ID: 87-0007-1084).

Inducible NOS (iNOS) Assay

Mouse recombinant iNOS expressed in *Escherichia coli* (*E.coli*) was used to access the enzyme functional activity. Baricitinib and 1.5 U/mL of enzyme in Tris buffer pH 8.0 were combined with 0.1 mM L-Arginine. The mixture was incubated for 90 minutes at 37 °C, followed by the addition of Griess reagent. The binding was determined by photometry at 545 nm. 1400W was used as reference compound. Baricitinib enzyme inhibition effect was calculated as a % inhibition of control enzyme activity. A primary screening in duplicated was carried out (30 □M) to determine the target activity. (Eurofins Cerep assay ID: 3185).

PI3 Kinase (p110b/p85a)

The enzyme was incubated in assay buffer containing 10 □M phosphatidylinositol-4, 5-bisphosphate and Mg/ATP (200 □M). The reaction was initiated by the addition of the Mg/ATP mixture. After incubation for 30 minutes at 25 °C, the reaction was terminated by the addition of a solution containing EDTA and biotinylated phosphatidylinositol- 3,4,5-trisphosphate. Detection buffer containing europium-labelled anti-GST monoclonal antibody, GST-tagged GRP1 PH domain and streptavidin-allophycocyanin was added. Following, the plate was read in time-resolved fluorescence mode and the homogeneous time-resolved fluorescence (HTRF) signal was determined according to the formula HTRF=10000 x (Em665 nm/Em620 nm). PI-103 was used as reference compound. A primary screening in duplicated was carried out (30 μM) to determine the target activity. The result was expressed as a percentage of the positive control (i.e., % kinase activity remaining), and the mean of the replicates was calculated. (Eurofins Cerep assay ID: 14-603KP).

Arachidonate 15-lipoxygenase (15-ALOX)

Human recombinant 15-ALOX expressed in E. coli cells was used. Tofacitinib and 1% DMSO were combined with 16 µg/mL of enzyme for 15 minutes at 37 °C in 50 µM Tris buffer pH 7.4. The reaction was initiated by addition of 25 µM arachidonic acid and dye DHR123 for 30 minutes at 37 °C. The binding was determined by Spectrofluorimetric quantitation of rhodamine 123 (Excitation: 485 nm, Emission: 535 nm). 2-TEDC was used as a reference compound. A primary screening in duplicated was carried out (30 □M) to determine the target activity. Later, the analysis was repeated using 10 µM arachidonic acid and tofacitinib was tested at 8 concentrations (100 □M as the highest concentration and dilution factor in a log-scale) in triplicate for dose-response curve and IC_{50} determination. (Eurofins Cerep assay ID: 199017)

Phosphodiesterase 10A2 (PDE10A2)

The effects of baricitinib on the activity of human PDE10A2 was accessed by measuring the formation of 5'AMP from cAMP using human recombinant enzyme expressed in Sf9 cells. The test compound was added to a buffer containing 40 mM Tris/HCl (pH 7.4), 8 mM $MgCl_2$, and 0.2 □M [3H]cAMP + cAMP. Thereafter, enzyme (about 0.8 U) was added, and the mixture incubated for 20 minutes at 25 °C. SPA beads were added and the mixture was maintained at 25 °C for 30 minutes under shaking. [3H]5'AMP was quantified via scintillation counting. The results are expressed as a percent inhibition of the control enzyme activity. Papaverine was used as standard inhibitory reference compound. A primary screening in duplicated was carried out (30 □M) to determine the target activity. Later, baricitinib was tested at 8 concentrations (100 □M highest concentration and dilution factor in a log-scale) in triplicate for dose-response curve and IC_{50} determination. (Eurofins Cerep assay ID: 4080)

Adenosine A3 receptor (ADORA3)

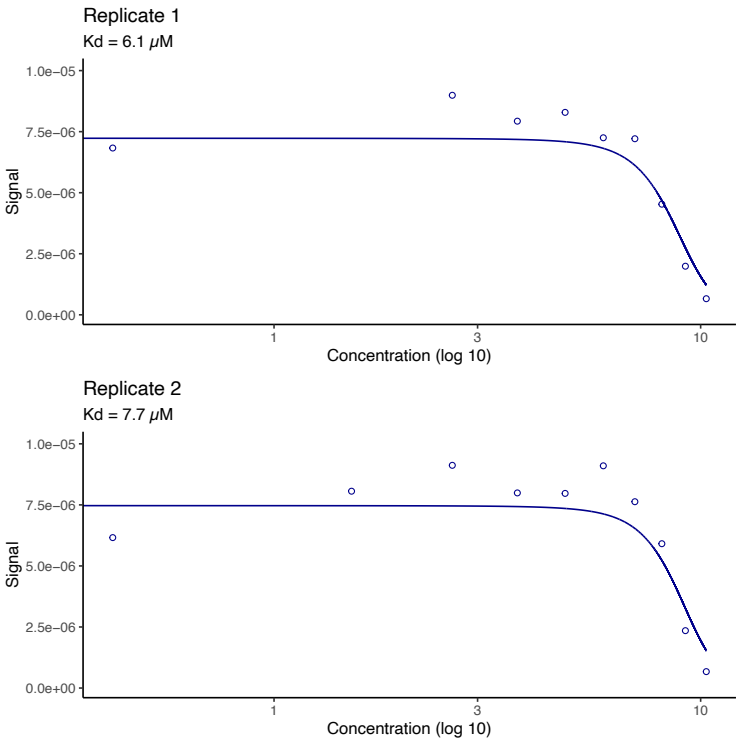
Functional activity of ADORA3 was evaluated in CHO cells transfected with human ADORA3. Cells were incubated with tofacitinib alone or in competition with control agonist (100 nM IB-MECA) in a first assay, and in a second one with antagonist (10 nM IB-MECA) for 20 minutes at 37 °C, and formation of cAMP was determined by homogeneous time resolved fluorescence (HTRF) at λex=337 nm and λem=620 and 665 nm using a microplate reader (Envison, Perkin Elmer). The cAMP concentration was determined by dividing the signal measured at 665 nm by that measured at 620 nm (ratio). Tofacitinib was tested at 8 concentrations (30 □M as the higher concentration and using log as dilution factor) for dose-response curve and IC_{50} determination and 8 concentrations (30 □M as the higher concentration and using log as dilution factor) for EC_{50} determination. For the agonist mode, results were expressed as a percent of the control

response to 100 nM IB-MECA, while for the antagonist mode, results were expressed as a percent inhibition of the control response to 10 nM IB-MECA. The standard reference agonist (IB-MECA) and antagonist (MRS 1220) were tested in the experiment at several concentrations to generate a concentration-response curve from which its EC_{50} and IC_{50} values, respectively, were calculated. (Eurofins Cerep assay ID: G107)

Adenosine A2A receptor (AA2AR)

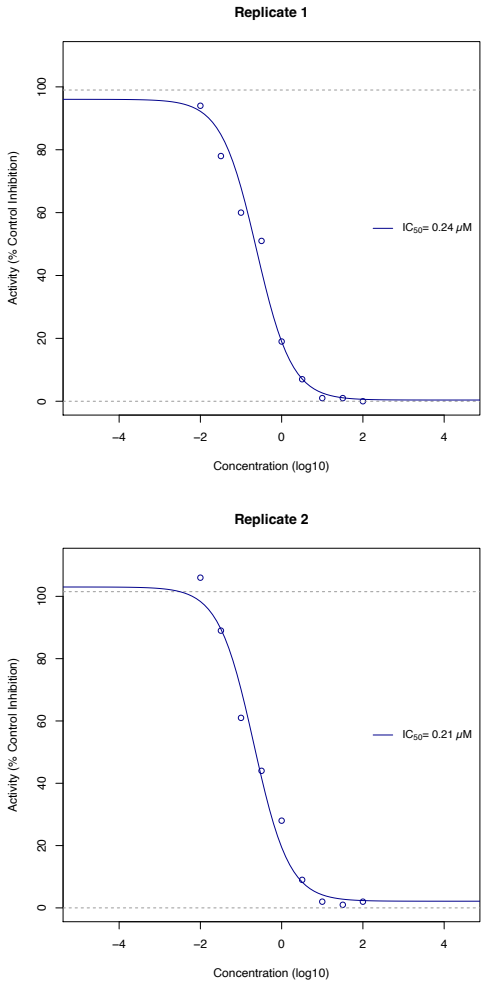
Functional activity on AA2AR was evaluated in a calcium flux assay in RBL-2H3 cells expressing human AA2AR. Cell lines were expanded from freezer stocks according to standard procedures. Cells were seeded in a total volume of 20 □L into black-walled, clear-bottom, Poly-D-lysine coated 384-well microplates and incubated at 37 °C for the appropriate time prior to testing. Assays were performed in 1 x Dye Loading Buffer consisting of 1x Dye, 1x Additive A and 2.5 mM Probenecid in HBSS / 20 mM Hepes. Cells were loaded with dye prior to testing. Media was aspirated from cells and replaced with 20 □L Dye Loading Buffer. Cells were incubated for 30-60 minutes at 37 °C. Cells were pre-incubated with test sample followed by addition of agonist control (0.045 □M NECA). Intermediate dilution of sample stocks was performed to generate 3x sample in assay buffer. After dye loading, cells were removed from the incubator and 10 □L 3x sample was added. Cells were incubated at 25 °C over 30 minutes in the dark to equilibrate plate temperature. Compound activity was measured on a FLIPR Tetra (MDS). Calcium mobilization was monitored for 2 minutes and 10 □M agonist control in HBSS / 20 mM Hepes was added to the cells 5 seconds into the assay. Baricitinib and Tofacitinib were tested at 10 concentrations (30 □M as the higher concentration and using log as dilution factor) for dose-response curve and IC_{50} determination. Cellular antagonist effect was calculated as a % inhibition of control reference agonist response. (Eurofins Cerep assay ID: 86-0011P-2409AN).

Figure 8.3.1 Dose-response curve of tofacitinib on transient receptor potential cation channel subfamily M member 6 (TRPM6). The amount of kinase measured qPCR (Signal; y-axis) is plotted against the corresponding compound concentration in nM in log10 scale (x-axis) per replicate (n=2).



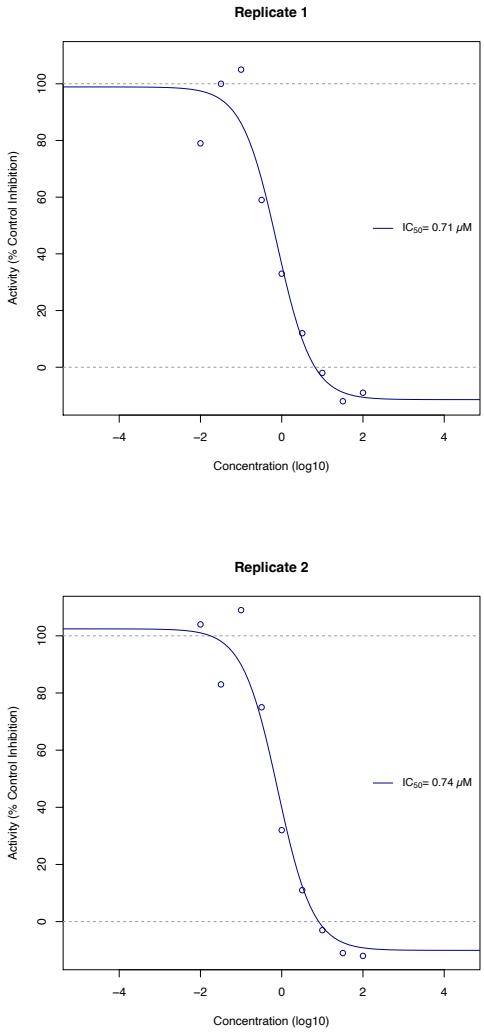
Abbreviations: K_d: Dissociation constant.

Figure 8.3.2 Dose-response curve of baricitinib on serine/threonine-protein Kinase N2 (PKN2). The activity measured (% of control; y-axis) is plotted against the corresponding compound concentration in Molar (M) in half-log10 scale (x-axis) per replicate (n=2).



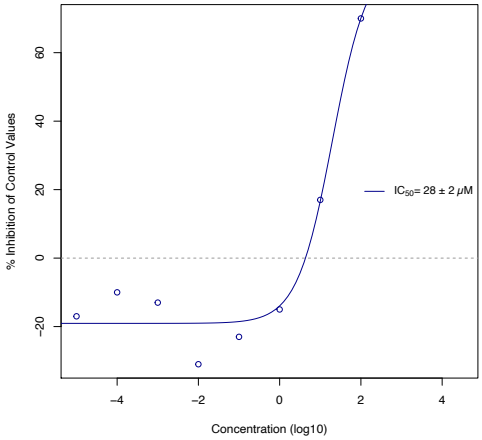
Abbreviations: IC_{50}: Half maximal inhibitory concentration.

Figure 8.3.3 Dose-response curve of tofacitinib on serine/threonine-protein Kinase N2 (PKN2). The activity measured (% of control; y-axis) is plotted against the corresponding compound concentration in Molar (M) in half-log10 scale (x-axis) per replicate (n=2).



Abbreviations: IC_{50}: Half maximal inhibitory concentration.

Figure 8.3.4 Dose-response curve of baricitinib on Phosphodiesterase 10A2 (PDE10A2). The activity measured (% of inhibition of control enzyme activity; y-axis) is plotted against the corresponding compound concentration in Molar (M) in log10 scale (x-axis).



Abbreviations: IC_{50}: Half maximal inhibitory concentration.

Table 8.3.1 Relevant drug-target interactions for baricitinib and tofacitinib predicted using the Target Inference Generator (TIGER). Targets with statistically meaningful predictions from TIGER (score >1) were identified.

Drug	Prediction	TIGER Score
Baricitinib	Deoxycytidine Kinase inhibitor	8.8
	Janus Kinase 3 Inhibitor	8.4
	Phosphodiesterase 6A Inhibitor	7.9
	Muscarinic Acetylcholine Receptor 4 Allosteric Modulator	6.8
	Protein Kinase C Beta Inhibitor	6.8
	DNA Gyrase subunit B (GyrB) Inhibitor (*Mycobacterium smegmatis*)	6
	Metabotropic Glutamate Receptor 1 Antagonist	6
	Leucine-Rich Repeat Serine-Threonine-Protein Kinase 2 Inhibitor	6
	Mitogen-Activated Protein Kinase Kinase Kinase 12 Inhibitor	5.3
	Adenosine Receptor A2A Inhibitor	4.5
	Epidermal Growth Factor Receptor Tyrosine Kinase 1 Inhibitor	4.2
	Leucine-Rich Repeat Serine-Threonine-Protein Kinase 2 Allosteric Modulator	4.1
	Calcium/Calmodulin Dependent Protein Kinase IV CaMK4 Inhibitor	3.8
	Inducible Nitric Oxide Synthase InOS Inhibitor	3.3
	Carbonic Anhydrase I Inhibitor	3
	Testis Specific Serine Kinase 2 Inhibitor	2.9
	Serine Threonine Kinase ILK1 (p59ILK, Integrin-Linked Kinase) Inhibitor	2.9
	MAP Kinase Interacting Serine Threonine Protein Kinase 1 Mnk Allosteric Modulator	2.8
	Methionine Aminopeptidase Inhibitor	2.8
	GPR119 (KIF19, AXOR103, G-Protein Coupled Receptor PGR2) Agonist	2.7
	Ribosomal Protein S6 Kinase A1 RSK1 (p90RSK) Inhibitor	2.4
	Phosphodiesterase 10A Inhibitor	2.1
	Methionine Aminopeptidase 1 Inhibitor	1.9
	Casein Kinase 2, Beta Polypeptide Subunit CK2-ß Inhibitor	1.8
	Polo-Like Kinase 4 (Serine Threonine Kinase Sak, STK18) Inhibitor	1.8
	Carbonic Anhydrase II Inhibitor	1.7
	PI3-Kinase P110-Alpha Subunit Inhibitor	1.7
	Ras-Related Protein Rab-7a Inhibitor	1.4
	Carbonic Anhydrase IX Inhibitor	1.3
	GPR39 Agonist	1.2
	Thymidine Kinase (HSV) Inhibitor	1.1
Tofacitinib	D-Amino-Acid Oxidase Inhibitor	11.3
	Phosphodiesterase 8B Inhibitor	10.8

Table 8.3.1 (cont.) Relevant drug-target interactions for baricitinib and tofacitinib predicted using the Target Inference Generator (TIGER). Targets with statistically meaningful predictions from TIGER (score >1) were identified.

Drug	Prediction	TIGER Score
	Metabotropic Glutamate Receptor 5 Inhibitor	7.3
	Janus Kinase 3 Inhibitor	6.9
	M18 Aspartyl Aminopeptidase Inhibitor	6.2
	Tyrosine-Protein Kinase TYK2 Inhibitor	5.3
	6-O-Methylguanine-DNA Methyltransferase Inhibitor	4.6
	Arachidonate 15-Lipoxygenase 15-ALOX Inhibitor	4.4
	Histone Lysine N-Methyltransferase EMTH1 (H3 Lysine-9 Specific 5) Inhibitor	4.3
	Histone Lysine N-Methyltransferase EHMT2 (Euchromatic Histone Methyltransferase 2, G9a) Inhibitor	3.8
	Ulp1 Endopeptidase, SuMO Specific Peptidase, Sentrin-Specific Protease 7 Inhibitor	3.8
	Glutaminyl Cyclase GC Inhibitor	3.1
	Ubiquitin-Conjugating Enzyme E2 N Inhibitor	3.1
	Transketolase Inhibitor	2.9
	Methionine Aminopeptidase 1 Inhibitor	2.8
Tofacitinib	Leucine-Rich Repeat Serine-Threonine-Protein Kinase 2 Allosteric Modulator	2.7
	Hypoxia-Inducible Factor Prolyl Hydroxylase 1 (egl-9 Family Hypoxia Inducible Factor 2) Inhibitor	2.6
	Adenosine Receptor A2A Antagonist	2.5
	TRP Canonical Subfamily M Member 6 Inhibitor	2.3
	Ulp1 Endopeptidase. SuMO Specific Peptidase. Sentrin-Specific Protease 6 Inhibitor	2.3
	TRP Canonical Subfamily M Member 3 Inhibitor	2.2
	Methionine Aminopeptidase Inhibitor	2.1
	Lysine-Specific Demethylase 4C (JHDM3C, Jumonj Domain Containing 2C) Inhibitor	2
	Doublecortin Like Kinase 3 Inhibitor	2
	Serine Threonine Kinase ILK1 (p59ILK, Integrin-Linked Kinase) Inhibitor	2
	Janus Kinase 1 Inhibitor	1.9
	Ulp1 Endopeptidase, SuMO Specific Peptidase, Sentrin-Specific Protease 8 Inhibitor	1.9
	Ribosomal Protein S6 Kinase A6 Inhibitor	1.8
	Serine Threonine Kinase 4 Inhibitor	1.8

Table 8.3.1 (cont.) Relevant drug-target interactions for baricitinib and tofacitinib predicted using the Target Inference Generator (TIGER). Targets with statistically meaningful predictions from TIGER (score >1) were identified.

Drug	Prediction	TIGER Score
Tofacitinib	Ectonucleotide Pyrophosphatase/phosphodiesterase Family Member 1 Inhibitor	1.7
	Non-Receptor Tyrosine-Protein Kinase TNK1 Inhibitor	1.7
	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 3 Inhibitor	1.7
	11Beta-Hydroxysteroid Dehydrogenase Inhibitor	1.6
	Protein Kinase N 1 Inhibitor	1.6
	Protein Kinase N 2 Inhibitor	1.6
	Microtubule Affinity Regulating Kinase 4 Inhibitor	1.6
	NUAK Family SNF1-Like Kinase 2 Inhibitor	1.6
	Glutathione S-Transferase GST P1-1 Inhibitor	1.4
	Glutathione S-Transferase Mu 2 Inhibitor	1.4
	Muscarinic Acetylcholine Receptor 5 Inhibitor	1.4
	Inducible Nitric Oxide Synthase Inhibitor	1.2
	NAD-Dependent Deacetylase Sirtuin 3 Inhibitor	1.2
	GPR6 Antagonist	1.2
	Dihydrofolate Reductase Inhibitor	1.1
	Adenosine Receptor A3 Antagonist	1.1
	CDGSH iron-Sulfur Domain-Containing Protein 1 Inhibitor	1.1

Abbreviations: TIGER: Target Inference Generator

Note: In the manuscript, we refer as the number of predicted targets as the number of different predictions. For TIGER, the same target can be predicted multiple times with different interaction mode (i.e., agonist, inhibitor, allosteric modulator and antagonist).

Table 8.3.2 Relevant drug-targets for baricitinib and tofacitinib predicted using SOM-based Prediction of Drug Equivalence Relationships (SPiDER). Targets with statistically meaningful predictions from SPiDER ($p < 0.05$) were identified.

Drug	Target	P-value
Baricitinib	Monoamine Oxidase	0.02
	Tyrosine Kinase	0.02
	Sodium Channel	0.02
	Transient Receptor Potential Ion Channel TRP	0.03
	Phosphodiesterase (3',5'-Cyclic-Nucleotide Phosphodiesterase)	0.03
	Metabotropic Glutamate Receptor	0.04
	Glucagon-Like Peptide Receptor	0.04
	Serine Threonine Kinase	0.04
	Potassium Channel	0.05
Tofacitinib	Phosphodiesterase (3',5'-Cyclic-Nucleotide Phosphodiesterase)	0.01
	Tyrosine Kinase	0.01
	Serine Threonine Kinase	0.02
	Metabotropic Glutamate Receptor	0.02
	Sodium Channel	0.02
	Endopeptidase (Cysteine Endopeptidase, Cysteine Protease)	0.02
	Potassium Channel	0.04
	Exopeptidase (Serine Exopeptidase, Serine Protease)	0.04
	Histamin Receptor	0.04
	Muscarinic Acetylcholine Receptor	0.05
	Androgen Receptor	0.05

Table 8.3.3 Raw *in vitro* results using biochemical screening assays. The experimental tests were performed at 30 µM and technical replicates (n=2).

Assay	Study ID	Eurofins Assay ID	Drug	% inhibition of control activity (1st)	% inhibition of control activity (2nd)	% inhibition (mean) activity	Follow-up experiment
PRK2 Human AGC Kinase Enzymatic Radiometric Assay	FR095-0022152	14-549KP	baricitinib	1[a]	2[a]	98%	yes
PI3Kbeta (PIK3CB) (p110b/p85a) Human PtdIns(4,5)P 3-Kinase Enzymatic HTRF Assay	FR095-0020731	14-603KP	baricitinib	92[a]	99[a]	4%	no
EGFR Human RTK Kinase Enzymatic Radiometric Assay	FR095-0020731	14-531KP	baricitinib	81[a]	77[a]	21%	no
iNOS Mouse Nitric Oxide Synthase Enzymatic Assay	FR095-0020731	3185	baricitinib	6.0	4.0	5%	no
PDE10A2 Human Phosphodiesterase Enzymatic Assay	FR095-0020731	4080	baricitinib	45.5	44.0	44.8%	yes
PRK2 Human AGC Kinase Enzymatic Radiometric Assay	FR095-0020731	14-549KP	tofacitinib	3[a]	0[a]	98%	yes
Transient Receptor Potential Cation Channel Subfamily M member 6 (TRPM6)	US073-0013667	87-0007-1084	tofacitinib	6.1[b]	5.7[b]	n.d.	no
15-LOX-1 Human Lipoxygenase Enzymatic Assay	FR095-0020731	199017	tofacitinib	19.3	34.0	27%	yes

[a] measured as % of positive control activity.

[b] activity measured as Kd (□M)

Abbreviations: EGFR: Epidermal Growth Factor Receptor; HTRF: Homogeneous Time Resolved Fluorescence; iNOS: Inducible Nitric Oxide Synthase; Kd binding constant; PDE10A2: Phosphodiesterase 10A2; PRK2: Serine/Threonine-Protein Kinase N2; RTK: Receptor Tyrosine Kinase.

Table 8.3.4 Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib	AP2-associated protein kinase 1	8.6174	8.9e-06	Tyrosine-protein kinase JAK3	1.0
	Ankyrin repeat and protein kinase domain-containing protein 1	13.7999	1.155e-05	Tyrosine-protein kinase JAK1	1.0
	BMP-2-inducible protein kinase	18.2298	3.938e-08	Tyrosine-protein kinase JAK2	1.0
	Bone morphogenetic protein receptor type-2	12.6351	5.146e-05	JAK3/JAK1	1.0
	Bone morphogenetic protein receptor type-1B	9.4168	3.192e-03	JAK1/JAK2/TYK2	1.0
	Peripheral plasma membrane protein CASK	20.8241	1.413e-09	JAK1/TYK2	1.0
	Death-associated protein kinase 1	10.4079	8.955e-04	JAK2/TYK2	1.0
	Death-associated protein kinase 2	10.8760	4.913e-04	Tyrosine-protein kinase TYK2	1.0
	Serine/threonine-protein kinase DCLK1	10.3159	1.008e-03	CDC7/DBF4 (Cell division cycle 7-related protein kinase/Activator of S phase kinase)	0.11
	Serine/threonine-protein kinase DCLK2	19.7327	5.729e-09	Leucine-rich repeat serine/threonine-protein kinase 2	0.11
	Serine/threonine-protein kinase DCLK3	20.0268	3.929e-09	Rho-associated protein kinase 1	0.11
	Myotonin-protein kinase	21.0990	9.933e-10	Neuropeptide Y receptor type 5	0.11
	Ephrin type-B receptor 6	8.6174	8.9e-06	ALK tyrosine kinase receptor	0.11
	Rhodopsin kinase GRK1	12.2963	7.948e-05	Beta-adrenergic receptor kinase 2	0.11
	G protein-coupled receptor kinase 4	12.2963	7.948e-05	G-protein coupled receptor kinase 2	0.11
	G protein-coupled receptor kinase 5	8.7348	7.656e-03	Orexin receptor 2	0.11
	Rhodopsin kinase GRK7	19.0775	1.328e-08	c-Jun N-terminal kinase 1	0.11
	Homeodomain-interacting protein kinase 1	9.0776	4.932e-03	Lysine-specific demethylase 4C	0.11
	Homeodomain-interacting protein kinase 3	10.1908	1.183e-03	Adenosine A1 receptor	0.11

Table 8.3.4 Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib	Tyrosine-protein kinase JAK2	95.2524	4.935e-51	Adenosine A2a receptor	0.11
	Tyrosine-protein kinase JAK1	119.2066	2.243e-64	Adenosine A2b receptor	0.11
	Tyrosine-protein kinase JAK2	21.2133	8.579e-10	Adenosine A3 receptor	0.11
	Tyrosine-protein kinase JAK3	17.4948	1.011e-07	Cyclin-dependent kinase 2	0.11
	Tyrosine-protein kinase JAK3	69.2252	1.551e-36	Glutaminyl-peptide cyclotransferase	0.11
	Tyrosine-protein kinase JAK3	111.8245	2.901e-60	Prokineticin receptor 1	0.11
	Calcium/calmodulin-dependent protein kinase type 1G	16.4586	3.818e-07	Nerve growth factor receptor Trk-A	0.11
	Calcium/calmodulin-dependent protein kinase type 1D	17.4635	1.052e-07	Neurotrophic tyrosine kinase receptor type 2	0.11
	Calcium/calmodulin-dependent protein kinase type II subunit α	12.8031	4.149e-05	NT-3 growth factor receptor	0.11
	Ribosomal protein S6 kinase α-6	18.2298	3.938e-08	Cytochrome P450 19A1	0.11
	Mitogen-activated protein kinase kinase kinase 15	25.2463	4.885e-12	Tyrosine-protein kinase ITK/TSK	0.11
	Mitogen-activated protein kinase kinase kinase 1	17.9162	5.887e-08	cAMP-dependent protein kinase alpha-catalytic subunit	0.11
	Mitogen-activated protein kinase kinase kinase 2	10.1908	1.183e-03	Phosphodiesterase 10A	0.11
	Mitogen-activated protein kinase kinase kinase 3	11.3960	2.522e-04	Corticotropin releasing factor receptor 1	0.11
	Microtubule-associated serine/threonine-protein kinase 1	20.8241	1.413e-09	Cyclin-dependent kinase 2/cyclin A	0.11
	Dual specificity mitogen-activated protein kinase kinase 2	9.2210	4.104e-03	Poly [ADP-ribose] polymerase-1	0.11
	Dual specificity mitogen-activated protein kinase kinase 3	11.6786	1.755e-04	Metabotropic glutamate receptor 5	0.11
	Dual specificity mitogen-activated protein kinase kinase 4	11.3960	2.522e-04	MAP kinase signal-integrating kinase 2	0.11

Table 8.3.4 Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib	Serine/threonine-protein kinase Nek3	29.8431	1.338e-14	Interleukin-8 receptor B	0.11
	Serine/threonine-protein kinase Nek7	18.7712	1.967e-08	Signal transducer and activator of transcription 3	0.11
	NUAK family SNF1-like kinase 2	17.4673	1.047e-07	CDC7/DBF4 (Cell division cycle 7-related protein kinase/Activator of S phase kinase)	0.11
	Serine/threonine-protein kinase OSR1	20.8241	1.413e-09	Glycogen synthase kinase-3 beta	0.11
	Serine/threonine-protein kinase PAK 5	13.7999	1.155e-05	Focal adhesion kinase 1	0.11
	Serine/threonine-protein kinase PAK 6	15.8308	8.54e-10	HERG	0.11
	Phosphorylase b kinase gamma catalytic chain, skeletal muscle/heart isoform	11.6786	1.755e-04	Tyrosine-protein kinase ABL	0.11
	Serine/threonine-protein kinase PknB	10.3159	1.008e-03	Anandamide amidohydrolase	0.11
	Serine/threonine-protein kinase RIO1	12.9969	3.236e-05	Carbonic anhydrase I	0.11
	Serine/threonine-protein kinase RIO2	12.9969	3.236e-05	Carbonic anhydrase IX	0.11
	Serine/threonine-protein kinase RIO3	12.9969	3.236e-05	Myosin light chain kinase, smooth muscle	0.11
	Receptor-interacting serine/threonine-protein kinase 4	15.8308	8.54e-10	Casein kinase II alpha	0.11
	Serine/threonine-protein kinase SBK1	16.4586	3.818e-07	Glycogen synthase kinase-3 alpha	0.11
	Uncharacterized serine/threonine-protein kinase SBK3	18.7712	1.967e-08	Histone acetyltransferase p300	0.11
	Serine/threonine-protein kinase SIK3	18.7712	1.967e-08	Orexin receptor 1	0.11
	Uncharacterized serine/threonine-protein kinase SBK3	18.7712	1.967e-08	Histone acetyltransferase p300	0.11
	Serine/threonine-protein kinase SIK3	18.7712	1.967e-08	Orexin receptor 1	0.11
	SRSF protein kinase 3	12.6351	5.146e-05	Dual-specificity tyrosine-phosphorylation regulated kinase 1A	0.11

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib	Serine/threonine-protein kinase 16	10.6360	6.684e-04	Dual specificity mitogen-activated protein kinase kinase 1	0.11
	Serine/threonine-protein kinase 25	13.3841	1.969e-05	Epidermal growth factor receptor erbB1	0.11
	Serine/threonine-protein kinase 26	10.1908	1.183e-03	Serine/threonine-protein kinase PIM1	0.11
	STE20/SPS1-related proline-alanine-rich protein kinase	20.8241	1.413e-09	Serine/threonine-protein kinase PIM2	0.11
	Threonine--tRNA ligase 1, cytoplasmic	21.3575	7.13e-13	Lysine-specific demethylase 4D-like	0.11
	Threonine--tRNA ligase	21.3575	7.13e-13	Cyclin-dependent kinase 5/CDK5 activator 1	0.11
	Serine/threonine-protein kinase TAO2	10.8760	4.913e-04	Cyclin-dependent kinase 4/cyclin D1	0.11
	Serine/threonine-protein kinase TNNI3K	30.4958	5.794e-15	Cyclin-dependent kinase 2/cyclin E	0.11
	Targeting protein for Xklp2	18.6374	2.335e-08	Cyclin-dependent kinase 1/cyclin B	0.11
	Non-receptor tyrosine-protein kinase TYK2	85.7151	1.013e-45	Lysine-specific demethylase 5C	0.11
	Serine/threonine-protein kinase ULK1	11.9781	1.195e-04	Casein kinase I delta	0.11
	Serine/threonine-protein kinase ULK2	11.6786	1.755e-04	Protein kinase C gamma (by homology)	0.11
	Serine/threonine-protein kinase ULK3	21.6896	4.657e-10	Cytochrome P450 17A1	0.11
	Serine/threonine-protein kinase VRK2	37.2957	9.45e-22	Lysine-specific demethylase 4A	0.11
				Lysine-specific demethylase 4D	0.11
				Neprilysin (by homology)	0.11
				Phosphodiesterase 5A	0.11
				Melatonin receptor 1A	0.11
				GABA-A receptor; alpha-1/beta-3/gamma-2	0.11
				Acyl coenzyme A:cholesterol acyltransferase	0.11
				G protein-coupled receptor kinase 7	0.11
				Serine/threonine-protein kinase TAO2	0.11

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib				Serine/threonine-protein kinase/endoribonuclease IRE1	0.11
				Serine/threonine-protein kinase OSR1	0.11
				STE20/SPS1-related proline-alanine-rich protein kinase	0.11
				Serine/threonine-protein kinase SBK1	0.11
				Serine/threonine-protein kinase VRK2	0.11
				Peripheral plasma membrane protein CASK	0.11
				Stem cell growth factor receptor	0.11
				Inhibitor of nuclear factor kappa B kinase beta subunit	0.11
				Kinesin-1 heavy chain/ Tyrosine-protein kinase receptor RET	0.11
				Dual specificity mitogen-activated protein kinase kinase 3	0.11
				Phosphorylase kinase gamma subunit 2	0.11
				Death-associated protein kinase 3	0.11
				CaM kinase I alpha	0.11
				Ribosomal protein S6 kinase alpha 1	0.11
				Death-associated protein kinase 1	0.11
				Casein kinase I alpha	0.11
				CaM kinase II	0.11
				cGMP-dependent protein kinase 2	0.11
				Dual specificity mitogen-activated protein kinase kinase 4	0.11
				Dual specificity mitogen-activated protein kinase kinase 2	0.11

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Baricitinib				Rho-associated protein kinase 2	0.11
				Serine/threonine-protein kinase PLK1	0.11
				JAK2/JAK1	0.11
Tofacitinib	Tyrosine-protein kinase ABL1	0.1191	0.3824	Tyrosine-protein kinase JAK3	0.78
	RAC-beta serine/threonine-protein kinase	43.2978	4.288e-22	Tyrosine-protein kinase JAK2	0.78
	RAC-gamma serine/threonine-protein kinase	42.6288	1.011e-21	Mitogen-activated protein kinase kinase kinase kinase 5	0.78
	RAC-alpha serine/threonine-protein kinase	16.2343	5.09e-10	Mitogen-activated protein kinase kinase kinase kinase 3	0.78
	Ankyrin repeat and protein kinase domain-containing protein 1	9.1999	4.216e-03	Tyrosine-protein kinase JAK1	0.67
	BMP-2-inducible protein kinase	30.6752	4.603e-15	Serine/threonine-protein kinase MST1	0.66
	Bromodomain-containing protein 9	15.7274	9.752e-07	Serine/threonine-protein kinase MST2	0.66
	C-C chemokine receptor type 10	25.6727	2.776e-12	Leucine-rich repeat serine/threonine-protein kinase 2	0.27
	Bromodomain-containing protein 9	15.7274	9.752e-07	Serine/threonine-protein kinase MST2	0.66
	C-C chemokine receptor type 10	25.6727	2.776e-12	Leucine-rich repeat serine/threonine-protein kinase 2	0.27
	C-C chemokine receptor type 10	36.1292	4.219e-18	G protein-coupled receptor kinase 7	0.27
	Cyclin-dependent kinase 8	4.6246	0.00149	Tyrosine-protein kinase FYN	0.27
	Cat eye syndrome critical region protein 2	43.2048	4.831e-22	Tyrosine-protein kinase ABL	0.27
	Peripheral plasma membrane protein CASK	13.9880	9.077e-06	Kinesin-1 heavy chain/ Tyrosine-protein kinase receptor RET	0.27
	Serine/threonine-protein kinase DCLK1	17.6241	8.563e-08	CaM kinase I alpha	0.27
	Serine/threonine-protein kinase DCLK2	13.2451	2.354e-05	Ribosomal protein S6 kinase alpha 1	0.27

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Tofacitinib	Serine/threonine-protein kinase DCLK3	33.6483	1.016e-16	Tyrosine-protein kinase LCK	0.27
	Myotonin-protein kinase	35.4233	1.043e-17	CaM kinase II	0.27
	Tyrosine-protein kinase Fyn	6.9580	7.476e-02	Rho-associated protein kinase 2	0.27
	Rhodopsin kinase GRK7	32.0774	7.622e-16	Protein kinase C delta	0.27
	Tyrosine-protein kinase JAK1	193.7198	7.023e-106	Protein kinase N2	0.27
	Tyrosine-protein kinase JAK2	159.1896	1.202e-86	JAK3/JAK1	0.27
	Tyrosine-protein kinase JAK2	68.3726	4.629e-36	JAK2/JAK1	0.27
	Tyrosine-protein kinase JAK3	56.5706	1.735e-29	Rho-associated protein kinase 1	0.27
	Tyrosine-protein kinase JAK3	221.3393	2.9e-124	JAK1/JAK2/TYK2	0.27
	Tyrosine-protein kinase JAK3	229.5137	8.113e-126	JAK1/TYK2	0.27
	Calcium/calmodulin-dependent protein kinase type 1	14.5576	4.372e-06	JAK2/TYK2	0.27
	Calcium/calmodulin-dependent protein kinase type 1D	21.3734	6.986e-10	Protein kinase N1	0.27
	Calcium/calmodulin-dependent protein kinase type 1G	11.0143	4.114e-04	Tyrosine-protein kinase TYK2	0.27
	Calcium/calmodulin-dependent protein kinase type II subunit alpha	22.3903	1.896e-10	Ribosomal protein S6 kinase alpha 2	0.27
	Calcium/calmodulin-dependent protein kinase type II subunit delta	6.8500	8.586e-02	CaM kinase II alpha	0.27
	Protein kinase C delta type	11.3562	2.654e-04	MAP kinase signal-integrating kinase 2	0.27
	Protein kinase C gamma type	6.8956	8.099e-02	BMP-2-inducible protein kinase	0.27
	Protein kinase C theta type	9.4697	2.983e-03	Ribosomal protein S6 kinase alpha 6	0.27
	Ribosomal protein S6 kinase alpha-1	13.8207	1.125e-05	Serine/threonine-protein kinase ULK3	0.27
	Ribosomal protein S6 kinase alpha-2	5.9388	0.0002763	CaM kinase I delta	0.27
	Ribosomal protein S6 kinase alpha-6	30.6752	4.603e-15	Myotonin-protein kinase	0.27

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Tofacitinib	Tyrosine-protein kinase Lck	2.0603	0.03918	Mitogen-activated protein kinase kinase kinase kinase 2	0.27
	Leucine-rich repeat serine/threonine-protein kinase 2	3.9951	0.003337	Non-receptor tyrosine-protein kinase TNK1	0.27
	Leukocyte tyrosine kinase receptor	9.5348	2.744e-03	Serine/threonine-protein kinase DCLK1	0.27
	Mitogen-activated protein kinase kinase kinase 15	16.9960	1.916e-07	NUAK family SNF1-like kinase 2	0.27
	Mitogen-activated protein kinase kinase kinase 1	12.0078	1.151e-04	Serine/threonine-protein kinase DCLK3	0.27
	Mitogen-activated protein kinase kinase kinase kinase 3	21.9976	3.137e-10	Serine/threonine-protein kinase AKT2	0.10
	Mitogen-activated protein kinase kinase kinase kinase 2	4.0298	0.003192	Poly [ADP-ribose] polymerase-1	0.10
	Mitogen-activated protein kinase kinase kinase kinase 5	4.6443	0.001452	Adrenergic receptor alpha-2	0.10
	Serine/threonine-protein kinase MARK2	8.4926	1.045e-02	Alpha-2b adrenergic receptor	0.10
	MAP/microtubule affinity-regulating kinase 3	3.9001	0.003768	Dual specificity mitogen-activated protein kinase kinase 7	0.10
	MAP/microtubule affinity-regulating kinase 4	21.1363	9.469e-10	Dopamine transporter (by homology)	0.10
	Microtubule-associated serine/threonine-protein kinase 1	13.9880	9.077e-06	Thrombin and coagulation factor X	0.10
	Mitogen-activated protein kinase 8	9.9263	1.661e-03	Dipeptidyl peptidase IV	0.10
	MAP kinase-interacting serine/threonine-protein kinase 2	2.7415	0.01655	Thrombin	0.10
	Serine/threonine-protein kinase Nek3	20.1200	3.487E-09	Vanilloid receptor	0.10
	Serine/threonine-protein kinase Nek7	12.5902	5.451E-05	Beta-secretase 1	0.10
	NUAK family SNF1-like kinase 2	29.4144	2.319E-14	Plasma kallikrein	0.10

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
	Serine/threonine-protein kinase OSR1	13.9880	9.077E-06	Serine/threonine-protein kinase AKT	0.10
	Serine/threonine-protein kinase PAK 5	9.1999	4.216E-03	Kinesin-like protein 1	0.10
	Serine/threonine-protein kinase PAK 6	10.5862	7.125E-04	Beta secretase 2	0.10
	Serine/threonine-protein kinase N1	20.3522	2.589E-09	Dopamine D1 receptor	0.10
	Serine/threonine-protein kinase N2	12.7536	4.421E-05	Glutaminyl-peptide cyclotransferase-like protein	0.10
	Serine/threonine-protein kinase PknB	17.6241	8.563E-08	Serine/threonine-protein kinase AKT	0.0
	Cyclin-dependent kinase 4	8.8598	6.522E-03	Protein kinase C (PKC)	0.0
	Proto-oncogene tyrosine-protein kinase receptor Ret	5.5367	0.0004626	MAP kinase ERK1	0.0
	Serine/threonine-protein kinase RIO1	8.6512	8.523E-03	Voltage-gated potassium channel subunit Kv1.5	0.0
Tofacitinib	Serine/threonine-protein kinase RIO2	8.6512	8.523E-03	Voltage-gated potassium channel subunit Kv1.3	0.0
	Serine/threonine-protein kinase RIO3	8.6512	8.523E-03	C-X-C chemokine receptor type 3	0.0
	Receptor-interacting serine/threonine-protein kinase 4	10.5862	7.125E-04	Histamine H4 receptor	0.0
	Rho-associated protein kinase 1	5.4818	0.0004964	Arachidonate 5-lipoxygenase	0.0
	Rho-associated protein kinase 2	17.4908	1.016E-07	Heat shock protein HSP 90-alpha	0.0
	Serine/threonine-protein kinase SBK1	11.0143	4.114E-04	Receptor protein-tyrosine kinase erbB-2	0.0
	Uncharacterized serine/threonine-protein kinase SBK3	12.5902	5.451E-05	Death-associated protein kinase 3	0.0
	Serine/threonine-protein kinase SIK2	17.8016	6.82e-11	Death-associated protein kinase 1	0.0
	Serine/threonine-protein kinase SIK3	12.5902	5.451E-05	Death-associated protein kinase 2	0.0
	STE20-like serine/threonine-protein kinase	5.5284	0.0004676	Serine/threonine-protein kinase 17B	0.0

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Tofacitinib	Serine/threonine-protein kinase 4	24.5822	1.144E-11	Melanocortin receptor 4	0.0
	Transcription initiation factor TFIID subunit 1	35.9930	5.024E-18	Glutaminyl-peptide cyclotransferase	0.0
	Serine/threonine-protein kinase TNNI3K	15.0347	2.371E-06	MAP kinase ERK2	0.0
	Non-receptor tyrosine-protein kinase TNK1	24.5822	1.144E-11	Trace amine-associated receptor 1 (by homology)	0.0
	Non-receptor tyrosine-protein kinase TYK2	152.3577	7.681E-83	Bile acid receptor FXR	0.0
	Serine/threonine-protein kinase ULK3	36.4012	2.976E-18	Interleukin-1 receptor-associated kinase 4	0.0
	Serine/threonine-protein kinase VRK2	25.1809	5.329E-12	Estradiol 17-beta-dehydrogenase 1	0.0
				Gamma-secretase	0.0
				Ephrin receptor	0.0
				Heat shock protein HSP 90-beta	0.0
				Serine/threonine-protein kinase RIPK2	0.0
				Nischarin	0.0
				P2X purinoceptor 7	0.0
				Cytochrome P450 19A1	0.0
				Serotonin 1d (5-HT1d) receptor	0.0
				Cyclooxygenase-1	0.0
				Sodium/hydrogen exchanger 1	0.0
				Serotonin 5a (5-HT5a) receptor	0.0
				Cyclin-dependent kinase 5/CDK5 activator 1	0.0
				Estrogen receptor alpha	0.0
				Trypsin I	0.0
				Dual-specificity tyrosine-phosphorylation regulated kinase 1A	0.0
				Estrogen receptor beta	0.0
				Serine/threonine-protein kinase Nek1	0.0

Table 8.3.4 (cont.) Drug-target interactions for baricitinib and tofacitinib predicted using the Similarity Ensemble Approach (SEA) and the Swiss Target Prediction.

	Prediction tool				
Drug	**SEA[a]**	**Z-score**	**P-value**	**Swiss Target Prediction (Probability)[b]**	**Probability**
Tofacitinib				Dopamine D2 receptor	0.0
				Voltage-gated L-type calcium channel alpha-1C subunit	0.0

[a] SEA, molecular descriptor: ECFP4 fingerprints; reference database: ChEMBL 16 binding data (activity <10 □M).
[b] Swiss Target Prediction, FP2 fingerprints; reference database: ChEMBL23 binding data (activity <10 □M).

Chapter 8.4

JAK-inhibitors and risk on serious viral infection, venous thromboembolism and cardiac events in patients with rheumatoid arthritis: A protocol for a prevalent new-user cohort study using the Danish nationwide DANBIO register

Table 8.4.1 List of diagnosis code using the International Classification of Diseases 10th revision (ICD-10) for inclusion criteria.

Condition	Diagnosis code (ICD-10)
Seropositive rheumatoid arthritis	M05.X
Seronegative rheumatoid arthritis	M06.0
Other specified rheumatoid arthritis	M06.8
Rheumatoid arthritis, unspecified	M06.9

Table 8.4.2 List of diagnosis code using the International Classification of Diseases 10th revision (ICD-10) and drug therapy using the Anatomical Therapeutic Chemical (ATC) classification codes for antiviral therapy used as exclusion criteria.

	Description	Diagnosis code (ICD-10)
Diagnosis	Human deficiency virus (HIV)	B20.X - B24.X
	Cancer	C00.X - C96.X (except C44.X)
	Heart transplant	Z94.1
	Heart valve transplant	Z95.2 - Z95.4
	Mitral stenosis	I05.X
	Heart valve disorders	I39.0 - I39.4
Antiviral therapy	aciclovir	J05AB01
	valaciclovir	J05AB11
	famciclovir	J05AB09

Table 8.4.3 List of drug therapy using the Anatomical Therapeutic Chemical (ATC) classification codes for exposure definition.

	Drug name	ATC code
TNF-α inhibitors	etanercept	L04AB01
	infliximab	L04AB02
	adalimumab	L04AB04
	certolizumab pegol	L04AB05
	golimumab	L04AB06
JAK inhibitors	tofacitinib	L04AA29
	baricitinib	L04AA37
	upadacitinib	L04AA44

Abbreviations: JAK=Janus Kinase, TNF=Tumour Necrosis Factor

Table 8.4.4 List of conditions using the International Classification of Diseases 10th revision (ICD-10) for outcome definition.

Outcome type	ICD-10	Condition
Major Adverse Cardiovascular Events (MACE)	I21.x - I23.x	Myocardial infarction
	I60.x, I61.x, I62.x, I63.8, I63.9, I64.x	Stroke
	I00 - I09, I11, I13, I20, I25.1-125.9, I25.0, I24, I21-I22, I33, I30 - I31, I40, I50, I26 - I28, I34 - I38, I42 - I49, I51, I10, I12, I15, I60 - I69, I71, I70, I72 - I78, I70,	Cardiovascular death
Venous Thromboembolism (VTE)	I80.2	Deep vein thrombosis
	I26.x	Pulmonary embolism
	I80.3, I80.8, I80.9, I81, I74.x, O22.3, O22.5, O22.9, H34.2, H34.8, O87.1, O87.3, O87.9, O88.2, I82.2, I82.3, I82.8, I82.9, M31.1, I51.3, N28.0, K55.0, I67.6, I67.6, I63.4, I63.1, I63.0, I24.0, G08, I66.x, G95.1, I21.9	Other embolisms
Serious Viral Infection	B25.x	Cytomegaloviral disease
	B27.1	Cytomegaloviral mononucleosis
	P35.1	Congenital cytomegalovirus infection
	B27.0	Mononucleosis due to Epstein-Barr virus
	B02.x	Herpes zoster

Table 8.4.5 List of covariates for Time-Conditional Propensity Score (TCPS).

Variable	Type of variable	Comments
Age	Continuous	
Sex	Binary: Female Male	
Socioeconomic status	Categorical	
Disease duration (time since rheumatoid arthritis diagnosis)	Categorical: <1 Year 1- 4.9 Years 5-10 Years >10 Years Missing	
Smoking status	Categorical: Past Current Never Missing	
Pregnancy	Binary	At T_0 looking back up to 365 days
Indicators of disease severity:		
Health Assessment Questionnaire (HAQ)	Categorical: 0 - 0.9 1 - 1.9 2 - 3 Missing	
28-joint disease activity score (DAS28)	Categorical: Remission (0 - 2.6) Low (2.7 - 3.2) Intermediate (3.3 - 5.0) High (>5.0) Missing	
Rheumatoid factor	Categorical: Positive Negative Missing	
Anti-citrullinated protein (anti-CCP) antibodies	Categorical: Positive Negative Missing	
Patient global disease activity	Continuous (Visual Analogue Scale)	
Physician global disease activity	Continuous (Visual Analogue Scale)	
Comorbidities:		
Viral infection (as per outcome definition)	Binary	Record between base cohort entry (incl. base cohort entry date) and study cohort entry date.
Thrombosis (as per outcome definition)	Binary	Record between base cohort entry (including base cohort entry date) and study cohort entry date.
Major Adverse Cardiovascular Events (as per outcome definition)	Binary	Record between base cohort entry (including base cohort entry date) and study cohort entry date.
Other cardiac disorders	Binary	
Cerebrovascular diseases	Binary	
Peripheral vascular diseases	Binary	
Hypertension	Binary	

Table 8.4.5 (cont.) List of covariates for Time-Conditional Propensity Score (TCPS).

Variable	Type of variable	Comments
Fractures	Binary	At T_0 looking back up to 180 days
Major surgery	Binary	At T_0 looking back up to 365 days
Psoriasis	Binary	
Other rheumatic conditions	Binary	
Transplanted organ	Binary	
Asthma	Binary	
Chronic obstructive pulmonary disease (COPD)	Binary	
Diabetes	Binary	
Alzheimer's disease	Binary	
Epilepsy	Binary	
Dementia	Binary	
Depression	Binary	
Parkinson's disease	Binary	
Osteoporosis	Binary	
Prolonged immobility	Binary	
Renal failure	Binary	
Chronic kidney disease	Binary	
Glomerular disorders	Binary	
Cystic kidney disease	Binary	
Liver diseases	Binary	
Ulcerative colitis	Binary	
Other noninfective gastroenteritis and colitis	Binary	
Chron disease	Binary	
Thrombophilia	Binary	
Medication:		
Previous immunization for Herpes Zoster	Binary	In the prior 5 years to T_0
Antiviral agents (except acyclovir, valaciclovir and famciclovir)	Binary	In the prior 365 days to T_0
Other anti-infective drugs		In the prior 365 days to T_0
Glucocorticoids use (cumulative dose in the past year)	Continuous	In the prior 365 days to T_0
csDMARD	Binary	At T_0
Number of previous biologics (non- TNF-α inhibitor)	1 >1	Ever prior to study cohort entry
Other TNF-α inhibitor previous to study cohort entry date	Binary	Ever prior to study cohort entry
NSAIDs	Binary	
Antidepressants	Binary	
Anti-epileptics	Binary	
Antipsychotics	Binary	
Anxiolytics	Binary	
Anti-hypertensives	Binary	
Diuretics	Binary	
Beta blockers	Binary	
Calcium channel blockers	Binary	
ACE inhibitors	Binary	
Angiotensin II blockers	Binary	
Statins	Binary	
Lipid lower agents	Binary	
Hormone replacement therapy	Binary	
Oral contraceptive	Binary	
Non-oral contraceptive	Binary	

Table 8.4.5 (cont.) List of covariates for Time-Conditional Propensity Score (TCPS).

	Variable	Type of variable	Comments
	Insulin	Binary	
	NIADs	Binary	
	anti-thrombotic agents (non-acetylsalicylic acid based)	Binary	
	Acetylsalicylic acid	Binary	
	Opioids	Binary	

Abbreviations: ACE: angiotensin-converting enzyme; csDMARD: Conventional synthetic disease-modifying antirheumatic drug; JAK: Janus Kinase; NIADs: Non-insulin oral anti-diabetic drugs; NSAIDs: non-steroidal anti-inflammatory drugs. TNF-α: Tumour necrosis factor alpha; T_0: study cohort entry date JAK inhibitors users and the corresponding matching date for TNF-α inhibitor users.

Table 8.4.6 List of Anatomical Therapeutic Chemical (ATC) classification codes for definition of comedication.

Drug	ATC code	Comments
Glucocorticoids for systemic use	H02AB (except H02AB06)	baseline use up to 90 days before T_0 (include dose at baseline)
Prednisolone	H02AB06	baseline use up to 90 days before T_0 (include dose at baseline)
Anti-inflammatory/antirheumatic agents in combination with corticosteroids	M01BA	baseline use up to 90 days before T_0 (include dose at baseline)
Methotrexate	L04AX03	ever before T_0 (include dose at baseline)
Etanercept	L04AB01	ever before T_0
Infliximab	L04AB02	ever before T_0
Adalimumab	L04AB04	ever before T_0
Certolizumab pegol	L04AB05	ever before T_0
Golimumab	L04AB06	ever before T_0
Rituximab	L01XC02	ever before T_0
Abatacept	L04AA24	ever before T_0
Tocilizumab	L04AC07	ever before T_0
Sarilumab	L04AC14	ever before T_0
Anakinra	L04AC03	ever before T_0
Hormonal contraceptives for systemic use	G03A (all codes)	
Androgens	G03B (all codes)	
Estrogens	G03C (all codes)	
Progestogens	G03D (all codes)	
Androgens and Female sex hormones in combination	G03E (all codes)	
Progestogens and estrogens in combination	G03F (all codes)	
Gonadotropins and other ovulation stimulants	G03G (all codes)	
Antiandrogens	G03H (all codes)	
Other sex hormones and modulators of the genital system	G03X (all codes)	
Butylpyrazolidines	M01AA (all codes)	
Acetic acid derivatives and related substances	M01AB (all codes)	
Oxicams	M01AC (all codes)	
Propionic acid derivatives	M01AE (all codes)	
Fenamates	M01AG (all codes)	
Coxibs	M01AH (all codes)	
Other anti-inflammatory and antirheumatic agents, non-steroids	M01AX (all codes)	
Antidepressants	N06A (all codes)	
Vitamin K antagonists	B01AA (all codes)	
Heparin group	B01AB (all codes)	
Platelet aggregation inhibitors excl. heparin	B01AC (all codes)	
Enzymes	B01AD (all codes)	
Direct thrombin inhibitors	B01AE (al codes)	
Direct factor Xa inhibitors	B01AF (all codes)	
Other antithrombotic agents	B01AX (all codes)	
Antiviral for systemic use	J05 (all codes)	baseline use up to 365 days before T_0 (include dose at baseline)

Table 8.4.6 (cont.) List of Anatomical Therapeutic Chemical (ATC) classification codes for definition of comedication.

Drug	ATC code	Comments
Antibacterials for systemic use	J01 (all codes)	baseline use up to 365 days before T_0 (include dose at baseline)
Antimycotics for systemic use	J02 (all codes)	baseline use up to 365 days before T_0 (include dose at baseline)
Antimycobacterials	J04 (all codes)	baseline use up to 365 days before T_0 (include dose at baseline)

Abbreviations: T_0: study cohort entry date JAK inhibitors users and the corresponding matching date for TNF-α inhibitor users; JAK: Janus Kinase; TNF-α: Tumour necrosis factor alpha;

Table 8.4.7 List of Anatomical Therapeutic Chemical (ATC) classification codes for definition of Herpes Zoster (HZ) immunization.

Vaccine name	ATC code
varicella/zoster immunoglobulin	J06BB03
zoster, live attenuated	J07BK02
zoster, purified antigen	J07BK03

Chapter 8.5

Baricitinib and tofacitinib off-target profile, with a focus on Alzheimer's disease

Glutaminyl cyclase inhibition

The inhibitory effect of tofacitinib on glutaminyl cyclase was tested with the Sensolyte® Green Glutaminyl Cyclase Activity Assay kit (Eurogentec, www.eurogentec.com) following the manufacturer's protocol (AS-72230). The assay involved a two-step procedure (Supplementary Fig. 1): First, the fluorogenic glutamine substrate was incubated with human glutaminyl cyclase and tofacitinib in a black 96-well plate for 30 min at 37 °C. Second, the pyroglutamate product was removed by incubating for 30 min at 37 °C with the developer to release a fluorescence signal. Fluorescence intensity was recorded with a Tecan Infinite 200 PRO series reader. The wavelengths used were excitation at 490 nm and emission at 520 nm. The increase in fluorescence was proportional to enzyme activity. For tofacitinib the following concentrations were tested: 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 and 100 µM. The assay was performed in triplicate. 1-Benzyl-imidazole was used as a negative control.

Ras related protein Rab7a inhibition

The capacity of tofacitinib and baricitinib to inhibit Rab7a was measured by the transcreener GDP FI assay kit for GTPase activity following the manufacturer's protocol (www.bellbrookslab.com) Recombinant Human Rab7 protein was purchased at Abcam (www.abcam.com, ab103507). GTP was converted to GDP by Rab7a, and the GDP released was bound by an antibody, which was coupled with a fluorescent signal. The assay was performed in buffer containing 50 mM HEPES pH 7.5, 4 mM $MgCl_2$, 1 mM EDTA, 0.01% Tween, 1% DMSO. GTP concentration was fixed at 200 nM. To measure inhibition, enzyme and inhibitor were added to assay buffer. Drug concentrations of 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 25, 50, 100 and 200 µM were tested. To start the reaction, GTP was added to the mixture, and it was allowed to incubate for 1 h at 37 °C. The GDP detection solution was added and the mixture was allowed to incubate for 1 h at room temperature. Fluorescence intensity was recorded with a Tecan Infinite 200 PRO series reader. The wavelengths used were excitation at 580 nm and emission at 620 nm. CID1067700 was used as negative control.

Inducible Nitric Oxide Synthase inhibition

Inducible nitric oxide synthase (iNOS) was purified from recombinant mouse Escherichia coli (E. Coli). Inhibition of the inducible NOS was measured by incubating the substrate L-Arginine (100 µM) and tofacitinib (30 µM) for 120 min at 37 °C in an enzyme buffer containing 40.5 mM Tris-HCl (pH 8.0), 0.5 mM NADPH, 4 µM FAD, 12 µM BH4, 3 mM DTT, 0.25% glycerol, 0.0025% BSA. Following incubation, Griess reagent containing 0.5 mg/ml naphtylene diamine and 5 mg/ mL sulphanilamide was added and the samples were incubated for 5 min at 22 °C. The amount of

nitrite produced then was quantified with a microplate reader (EnVision, Perkin Elmer) by measuring the absorbance at 550 nm. A mean value of -0.4% was found for tofacitinib. 1400W was used as negative control.

Phosphodiesterase 8A inhibition

Phosphodiesterase 8A (PDE8A1) was purified from human recombinant Sf9 cells. Inhibition of PDE8A1 was measured by incubating the substrate [3H] cAMP + cAMP (200 nM) and tofacitinib (30 µM) in a buffer containing 40 mM Tris/HCl (pH 7.4) and 8 mM $MgCl_2$, 180 nM cAMP and 0.1 µCi [3H]cAMP for 20 min at room temperature. Following incubation, SPA beads were added. After shaking for 30 min at 22 °C, the amount of [3H]5'AMP was quantified with a scintillation counter (MicroBeta, Perkin Elmer). Per cent inhibition was calculated relative to the negative control. A mean value of 3.2% inhibition was found for tofacitinib. Trequensin was used as negative control.

Carbonic anhydrase II inhibition

Carbonic anhydrase II was purified from human erythrocytes. Inhibition of carbonic anhydrase II was measured by incubating the substrate 4-nitrophenyl acetate (450 µM) and baricitinib (30 µM) in a buffer containing 50 mM MOPS (pH 7.5), 5.38 mM Na2SO4, 1 mM EDTA and 0.59 equivalents of carbonic anhydrase for 20 minutes at room temperature. The absorbance was then measured at 405 nm using a microplate reader (EnVision, Perkin Elmer). This measurement at t=0 was made to detect any compound interference with the spectrophotometric detection at this wavelength. Thereafter, the reaction was initiated by the addition of the substrate 4-nitrophenyl acetate at 450 µM, and the mixture was incubated for 20 min at room temperature. Following incubation, a second measurement of the absorbance at 405 nm was made (t=20). The enzyme activity was determined by subtracting the signal measured at t=0 from that measured at t=20. The inhibitory effects of the test compounds were calculated as % inhibition of negative control. A mean value of 1.9% inhibition was found for baricitinib. Acetazolamide was used as negative control.

Casein kinase subunit 2 α2 inhibition

Human Casein kinase subunit 2 alpha 2 (CK2-α2) was incubated with assay buffer containing 20 mM HEPES pH 7.6, 0.15 M NaCl, 0.1 mM EDTA, 5 mM DTT, 0.1% Triton X-100, 330 µM RRRDDDSDDD (Casein Kinase 2 substrate alpha-subunit), 10 mM Magnesium acetate and [γ-33P]-ATP (15 µM) and baricitinib (up to 30 µM). The reaction was initiated by the addition of the Mg/ATP mix. After incubation for 40 min at room temperature, the reaction was stopped by

adding phosphoric acid to reach a concentration of 0.5%. An aliquot of the reaction was then spotted onto a filter and washed four times for 4 min in 0.425% phosphoric acid and once in methanol prior to drying and scintillation counting. Baricitinib was tested in a concentration range between 3 nM and 30 µM.

PI3-Kinase P110-α Subunit inhibition

Human PI3-Kinase P110-α subunit (PI3K p110α(H1047R)/p85α) was incubated in an assay buffer containing 20 mM MOPS, 1 mM EDTA, 0.01% Brij-35, 5% Glycerol, 0.1% β-mercaptoethanol, 1 mg/mL BSA and phosphatidylinositol 4,5-bisphosphate (10 µM) and Mg/ATP, and baricitinib (30 µM). The reaction was initiated by the addition of the ATP solution (45 µM). After incubation for 30 min at room temperature, the reaction was quenched by adding a stop solution containing EDTA and biotinylated phosphatidylinositol-3,4,5-trisphosphate. Finally, detection buffer was added, which contains europium-labelled anti-GST monoclonal antibody, GST-tagged GRP1 PH domain and streptavidin allophycocyanin. The plate was read in time-resolved fluorescence mode and the homogeneous time-resolved fluorescence (HTRF) signal was determined according to the **Equation 8.5.1**.

$$\mathrm{HTRF} = 10000\left(\frac{\mathrm{Em\ 665\ nm}}{\mathrm{Em620nm}}\right) \qquad \textbf{(Equation 8.5.1)}$$

Dual leucine zipper kinase inhibition

Kinase-tagged T7 phage strains were prepared in an *E. coli* host derived from the BL21 strain. The lysates were centrifuged and filtered to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 min at room temperature to generate affinity resins for kinase assays. The ligand-bound beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1% BSA, 0.05% Tween 20, 1 mM DTT). Binding reactions were assembled by combining kinases, ligand-bound affinity beads, and baricitinib in binding buffer (20% SeaBlock, 0.17x PBS, 0.05% Tween 20, 6 mM DTT). Test compounds were prepared as 111X stocks in 100% DMSO. Baricitinib was then diluted directly into the assays such that the final concentration of DMSO was 0.9%. The assay plates were incubated at room temperature with shaking for 1 h and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 0.5 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 min. The kinase concentration in the eluates was measured by qPCR.

Metabotropic Glutamate Receptor inhibition

U2Os stably transfected cells were plated in a 384-well plate and incubated overnight at 37 ° C and 5% CO_2 to allow the cells to attach and grow. After overnight incubation, cell plating media was exchanged with 20 µL of Dye Loading buffer containing HBSS + 20 mM HEPES containing 1X Dye, 1X Additive A and 2.5mM Probenecid. Plates were incubated at 37 °C for 45 min. For inhibition testing, cells are pre-incubated with baricitinib (30 µM) followed by EC_{80} agonist challenge. Intermediate dilution of sample stocks was performed to generate 4X sample in assay buffer. 10 µL of 120 µM baricitinib was added to cells and incubated for 30 min at room temperature. Cell plates were loaded into FLIPR instrument. 10 µL of EC_{80} of control were added using FLIPR onboard robotics after 5 seconds of starting Calcium measurement. Final assay vehicle concentration was 1%. Compound enzyme inhibition effect was calculated as a % inhibition of control enzyme activity. A mean value of 0% was found for baricitinib.

Sensitivity Analysis

The impact of parameter deviation on the model's predictions was assessed by a sensitivity analysis. For this, each model parameter was individually increased by 5% and the associated impact on maximal brain tissue concentrations (Prediction 1 [QSAR], Prediction 2 [Mouse exp.] and Prediction 3 [QIVIVE BBB]) was computed. Oral administered dose was maintained at 4 mg. Normalized sensitivity coefficients (SC) were determined by using the **Equation 8.5.2**:

$$\mathrm{SC} = \frac{(C\prime - C)}{(P\prime - P)} \times \frac{P}{C} \qquad \textbf{(Equation 8.5.2)}$$

C and C' referred to the maximal concentration of baricitinib in brain tissue (Prediction 1 [QSAR], Prediction 2 [Mouse exp.] or Prediction 3 [QIVIIVE BBB]) with unchanged parameters or one elevated parameter, respectively, P and P' to the value of the unchanged or elevated parameter of interest.

Monte Carlo simulations

Monte Carlo simulations were performed with Berkeley Madonna 10 associated function for all the parameters found with an absolute value of normalized sensitivity coefficient >0.1 for at least one brain concentration (Prediction 1 [QSAR], Prediction 2 [Mouse exp.] or Prediction 3 [QIVIVE BBB]). Parameter simulated distributions were determined according to literature.[284,289,290] One thousand simulations were performed, and results were analysed by comparing first quartile, median and third quartile values for each time point for each of the

brain concentrations (Prediction 1 [QSAR], Prediction 2 [Mouse exp.] and Prediction 3 [QIVIVE BBB]).

Table 8.4.1 In vitro testing provider per target.

Drug	Target	Assay type	Provider
Baricitinib	Casein Kinase II subunit β (CK2-α2)	Biochemical	Eurofins
	Carbonic Anhydrase II (CA2)	Biochemical	Eurofins
	PI3-Kinase P110-α Subunit (PIK3CA)	Biochemical	Eurofins
	Metabotropic Glutamate receptor 1 (MGlu1)	Cell-based	Eurofins
	Dual leucine zipper kinase (MAP3K12)	Biochemical	Eurofins
	Ras-Related Protein Rab-7a (RAB7a)	Biochemical	in-house
Tofacitinib	Glutaminyl Cyclase (GC)	Biochemical	in-house
	Inducible Nitric Oxide Synthase (iNOS)	Biochemical	Eurofins
	Phosphodiesterase 8A (PDE8A)	Biochemical	Eurofins

Table 8.5.2 Normalized sensitivity coefficients (SC).

Parameter			SC (normalized)	
Category	**Name**	**Definition**	**Prediction 1 [QSAR] & Prediction 2 [Mouse exp.]**	**Prediction 3 [QIVIVE BBB]**
Physiology	BW	Bodyweight (kg)	-0.67	-0.67
	VSc	Relative slowly perfused tissues volume	-0.54	-0.54
Physico-chemical	PS	Partition coefficient slowly perfused tissues/blood	-0.54	-0.54
Metabolism	k_a	First-order absorption rate constant (h^{-1})	0.31	0.31
	CLr	Renal clearance (L/h)	-0.61	-0.24
	CLpgp	P-gp-mediated intrinsic clearance (µL/min/10^6 cells)	-	-0.59
	CLbcrp	BCRP-mediated intrinsic clearance (µL/min/10^6 cells)	-	-0.18
Other	Papp	Apparent passive permeability (cm/s)	-	0.79

Note: Due to similarity of calculations, results are identical for Prediction 1 [QSAR] and Prediction 2 [Mouse exp.]. Only absolute SC values >0.1 are shown.

Abbreviations: Mouse exp.: experimentally observed in mouse, QIVIVE BBB: quantitative *in vitro-in vivo* extrapolation of blood-brain-barrier permeation, QSAR: quantitative structure-activity relationship, SC: sensitivity coefficients.

Table 8.5.3 Parameters distributions used in the Monte Carlo analysis.

Parameter		Mean	CV (%)	Distribution
Tissue volume (fraction of body weight)				
BW	Bodyweight (kg)	73	26[a]	Lognormal [a]
VSc	Relative slowly perfused tissues volume	0.861	16[a]	Normal [a]
Partition coefficient for Baricitinib				
PS	Partition coefficient slowly perfused tissues/blood	0.548	30[a]	Lognormal [a]
Metabolic constant				
k_a	First-order absorption rate constant (h^{-1})	1.2	50[b]	Lognormal [b]
Others kinetic parameters				
CLr	Renal clearance (L/h)	11	29[c]	Lognormal
CLpgp	P-gp-mediated intrinsic clearance (µL/min/10^6 cells)	2.7	30	Lognormal
CLbcrp	BCRP-mediated intrinsic clearance (µL/min/10^6 cells)	0.8	30	Lognormal
Papp	Apparent passive permeability (cm/s)	$6.6e^{-6}$	15[c]	Lognormal

[a] Values obtained from Clewell *et al*. 1999 [reference 290].

[b] Values obtained from Clewell & Jarnot 1994 [reference 289].

[c] Values obtained from Posada *et al*. 2017 [reference 284].

Table 8.5.4 Raw data Casein kinase subunit 2 alpha 2 (CK2-α2) inhibition assay.

Concentration (nM)	Signal (1st replicate)	Signal (2nd replicate)
0	5.04E-07	6.80E-07
0	5.21E-07	5.02E-07
0	5.25E-07	5.60E-07
1.69	4.76E-07	4.49E-07
5.08	5.08E-07	5.64E-07
15.2	5.14E-07	5.48E-07
45.7	6.25E-07	5.62E-07
137	4.15E-07	4.96E-07
412	4.55E-07	5.28E-07
1230	4.48E-07	4.98E-07
3700	3.24E-07	3.26E-07
11100	1.49E-07	2.08E-07
33300	5.65E-08	5.78E-08
100000	1.29E-08	1.73E-08

Table 8.5.5 Raw data of inhibition with Baricitinib Dual leucine zipper kinase (MAP3K12).

Concentration (nM)	Signal (1st replicate)	Signal (2nd replicate)
0	5.21E-07	4.14E-07
0	5.51E-07	4.79E-07
0	4.47E-07	5.45E-07
3.05	4.86E-07	5.12E-07
9.14	4.87E-07	5.23E-07
27.4	5.32E-07	5.27E-07
82.3	5.12E-07	4.95E-07
247	5.00E-07	4.87E-07
741	4.12E-07	3.85E-07
2220	3.55E-07	3.27E-07
6670	2.47E-07	2.47E-07
20000	1.35E-07	1.29E-07
60000	5.27E-08	4.14E-08
180000	1.99E-08	2.00E-08

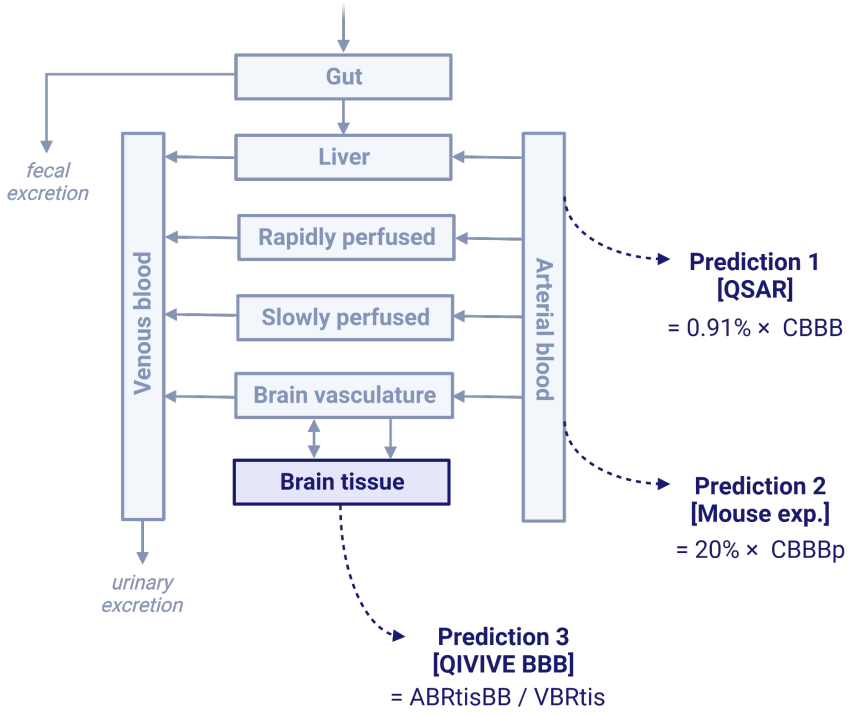


Figure 8.5.1 Conceptual physiologically-based pharmacokinetic (PBPK) model for predicting brain concentrations of baricitinib. Abbreviations: ABRtisBB: amount of baricitinib in the brain tissue, CBBB: concentration of baricitinib in the blood, CBBB: concentration of baricitinib in the plasma, Mouse exp.: experimentally observed in mouse, QIVIVE BBB: quantitative *in vitro-in vivo* extrapolation of blood-brain-barrier permeation, QSAR: quantitative structure-activity relationship, VBRtis: volume of brain tissue.

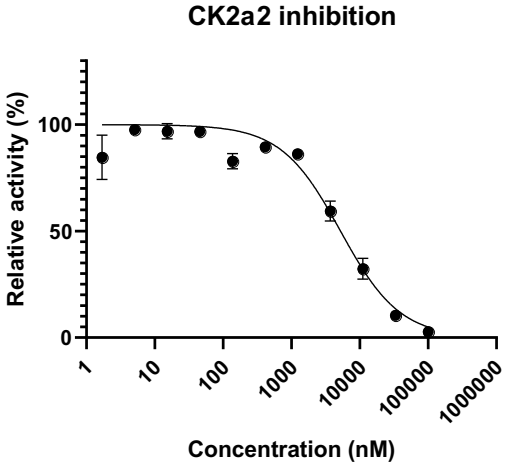


Figure 8.5.2 Baricitinib-induced inhibition of casein kinase subunit 2 alpha 2 (CK2-α2).

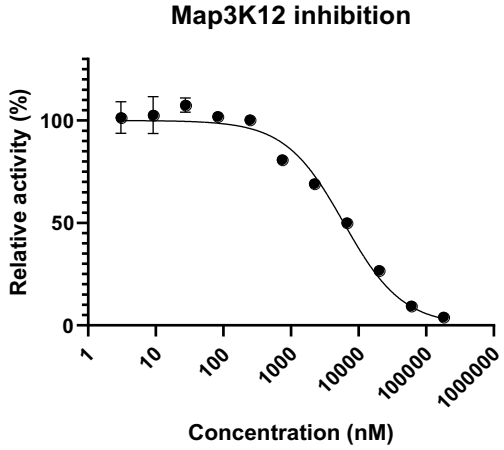


Figure 8.5.3 Baricitinib-induced inhibition of dual leucine zipper kinase (MAP3K12).

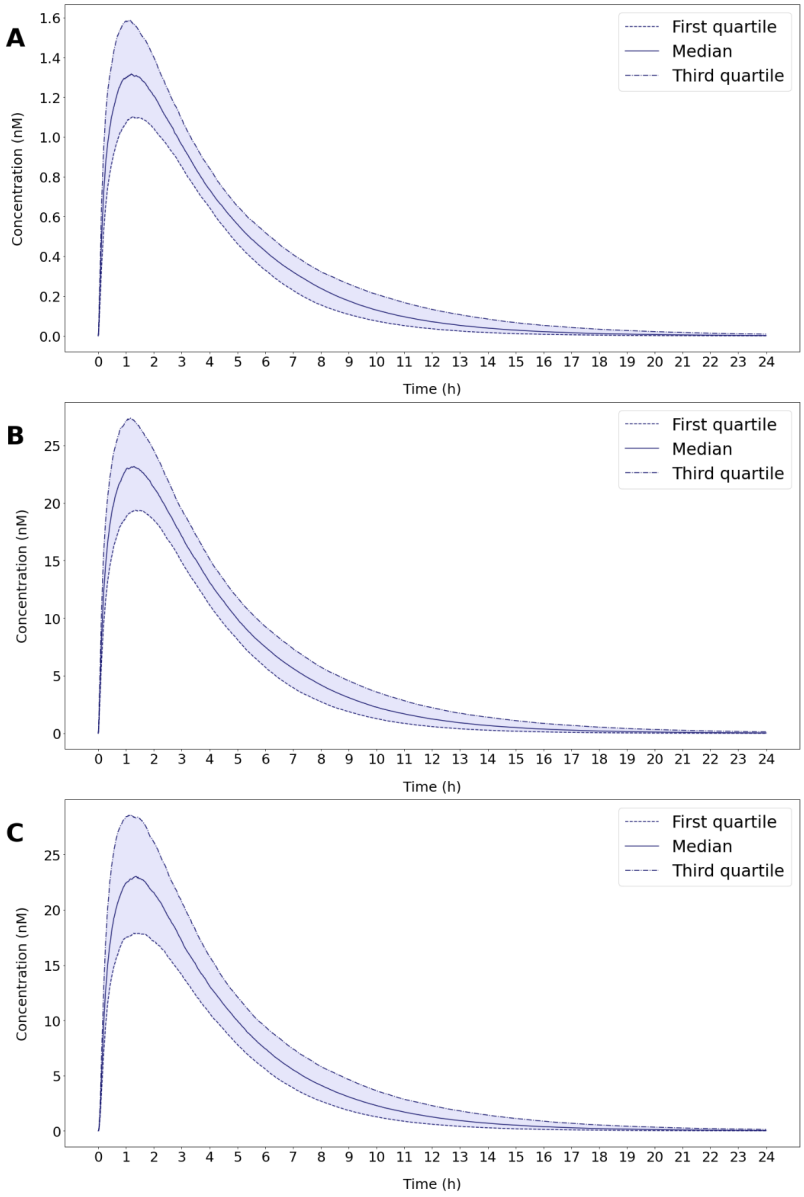


Figure 8.5.4 Predicted baricitinib concentrations in brain tissue versus time profiles following a single oral dose of 4mg. Concentration estimated from QSAR prediction (Prediction 1 [QSAR], [A]), concentration extrapolated from a *in vivo* study in mice (Prediction 2 [Mouse exp.], [B]), Concentration estimated by *in vitro-in vivo* scaling of blood brain barrier permeability (Prediction 3 [QIVIVE BBB], [C]). The shaded area encloses the first and third quartiles of the 1000 Monte Carlo simulations re-generating the sensitive parameters, while the solid line depicts the median simulation result. **Abbreviations**: BBB: Blood brain barrier; QSAR: Quantitative structure-activity relationship. QIVIVE: Quantitative *in vitro* to *in vivo* extrapolation.

Maria Luisa Marques de Sá Faquetti

Education

ETH Zurich — *Sep 2019 - Sep 2023*, *Zurich, Switzerland*

Doctor of Science (Ph.D.) in Pharmacoepidemiology

Completion of the PhD programme in Epidemiology and Biostatistics at University of Zurich

ETH Zurich — *Sep 2016 - Feb 2018*, *Zurich, Switzerland*

M.Sc. Medicinal and Industrial Pharmaceutical Sciences

Universidade Federal de São Paulo — *Mar 2009 - Jul 2015*, *São Paulo, Brazil*

B.Sc. Pharmacy and Biochemistry

Experience

Quality Assurance Analyst II - Kivalita Consulting — *Mar 2019 - Aug 2019*, *São Paulo, Brazil*

- In charge of assembling the quality systems to Cipla Brazil, including preparation of quality system documents (e.g., SOPs, Analytical Methods, Specifications and other processes of GMP and GLP documentation)
- Managed QA documentation and Product Quality Reviews (PQR/APQR)
- Training for personnel

Mandatory Pharmacy degree internship (Pharmacist Assistant) - Malheiro Pharmacy — *Mar 2015 - Jun 2015*, *São Paulo, Brazil*

- Prepared formulations and inspections (active pharmaceutical ingredients and packaging materials)
- GLP and GMP (in accordance with licensed practitioner's prescriptions [USP], including herbal API)
- Patient counselling.

Internship in Research & Development - Fleury Group — *Feb 2014 - Feb 2015*, *São Paulo, Brazil*

- Routine use of spectroscopic and chromatographic methods covering the areas of analysis and techniques (HPLC, UPLC, GC-MS, tandem, UV spectroscopy and organic synthesis)

  Validated and qualified analytical chromatographic methods

Internship in Quality Assurance (Animal Health) - Novartis — *Nov 2011 - Apr 2012*, *São Paulo, Brazil*

- Implemented quality assurance standards (GMP, GLP, GDP)
- Assured ongoing compliance with quality and industry regulatory requirements
- Prepared Standard Operating Procedures (SOPs)
- Supported on-site audits conducted by external providers
- Investigated quality deviations

Teaching Experience

Pharmaceutical Analytics - ETH Zürich *2021 - 2022*

Practical course; Level: B.Sc. in Pharmacy *Zurich, Switzerland*

Pharmacoepidemiology and Drug Safety - ETH Zürich *2019 - 2022*

Level: M.Sc. in Pharmacy and Pharmaceutical Sciences *Zurich, Switzerland*

Summer Schools

Intermediate Pharmacoepidemiology - *Summer 2021*

Summer school focused on themes related to the exposure time-windows, the use of active drug comparators, latency and the application of lag periods, reverse causality, detection bias, methodological considerations in the assessment of acute versus insidious outcomes, new-user designs, healthy-user effects, and non-traditional study designs *Montreal, Canada*

Advanced Pharmacoepidemiology *Summer 2022*

Summer school focused on advanced methodological issues, study design, analysis and interpretation of results, selection bias, time-risk functions, and time-related biases *Montreal, Canada*

Other

Languages	Portuguese (native speaker)
	English (proficient)
	Spanish (basic command)
	German (basic command)
Software and Programming	R
	KNIME
	MestreNova
	PRISMA
	Microsoft Office Package skills

Volunteer Work

Head of the Pharmaceutical Scientists' Association (PSA) career team *2022 - 2023*

Academic association of the Institute of Pharmaceutical Sciences, ETH Zurich *Zurich, Switzerland*

Member of the Pharmaceutical Scientists' Association (PSA) career team *2019 - 2022*

Academic association of the Institute of Pharmaceutical Sciences, ETH Zürich *Zurich, Switzerland*

Membership

Swiss Society of Industrial Pharmacists (GSIA) *2019 - 2022*
Switzerland

Awards

Research project "Synthesis of a marker to new steroidomic approach" – winner in overall research project category in the XXIV Fleury group congress *2014*
São Paulo, Brazil

Scholarship

International Society for Pharmacoepidemiology (ISPE) Scholarship to attend ICPE 2022 (Denmark, August 24-28, 2022)

Publications

Faquetti ML, Slappendel L, Bigonne H, Aichinger G, Grisoni F, Schneider P, Schneider G, Sturla S, Burden AM. Baricitinib and Tofacitinib *Off-Target Profile, with a Focus on Alzheimer's Disease.* ChemRxiv. Preprint published online August 3, 2023. doi.org/10.26434/chemrxiv-2023-b108s.

Faquetti ML, Vallejo-Yagüe E, Cordtz R, Dreyer L, Burden AM. *JAK-inhibitors and risk on serious viral infection, venous thromboembolism and cardiac events in patients with rheumatoid arthritis: A protocol for a prevalent new-user cohort study using the Danish nationwide DANBIO register.* PLOS ONE. 2023;18(7):e0288757. doi:10.1371/journal.pone.0288757

Faquetti ML, Frey G, Stämpfli D, Weiler S, Burden AM. *Examining inappropriate medication in UK primary care for type 2 diabetes patients with polypharmacy.* medRxiv. Preprint published online May 28, 2023. doi.org/10.1101/2023.05.24.23290466

Faquetti ML, la Torre AMD, Burkard T, Obozinski G, Burden AM. *Identification of polypharmacy patterns in new-users of metformin using the Apriori algorithm: A novel framework for investigating concomitant drug utilization through association rule mining.* Pharmacoepidemiology & Drug Safety. 2023;32(3):366-381. doi:10.1002/pds.5583

Faquetti ML, Grisoni F, Schneider P, Schneider G, Burden AM. *Identification of novel off targets of baricitinib and tofacitinib by machine learning with a focus on thrombosis and viral infection*. Scientific Reports. 2022;12(1):7843. doi:10.1038/s41598-022-11879-1

la Torre AMD, **Faquetti ML**, Perez-Cruz F, Meier C, Weiler S, Burden AM. *A Data-Driven Approach to Identify Clusters of HbA1c Longitudinal Trajectories and Associated Outcomes in Type 2 Diabetes Mellitus: A Large Population-Based Cohort Study.* medRxiv. 2022; doi:10.1101/2022.06.14.22276398

Published conference abstracts as presenting author

ICPE 2022 – Two posters

Faquetti MLMDS, Burkard T, De la Torre AM, Burden A. Age and sex differences in polypharmacy patterns in new users of metformin: A UK-based descriptive study. ICPE All Access conference abstracts. Pharmacoepidemiol Drug Saf 2022; 31: 294-295

Faquetti MLMDS, Burkard T, De la Torre AM, Burden A. Personalized prescribing in type 2 diabetes following the 2015 UK treatment guidelines: Description of non-insulin anti-diabetic drug (niad) utilization and polypharmacy patterns. ICPE All Access conference abstracts. Pharmacoepidemiol Drug Saf 2022; 31: 542-543

EULAR 2021 – Poster

Faquetti ML, Grisoni F, Schneider P, Schneider G, Burden AM. POS0091 Off-target profiling of Janus Kinase (JAK) inhibitors in rheumatoid arthritis: a computer-based approach for drug safety studies and repurposing. Ann Rheum Dis. 2021;80(1):255.2-255.

SCS Fall Meeting 2021 – Poster

Faquetti ML, Grisoni F, Schneider P, Schneider G, Burden AM. MC-107 Off-target profiling of Janus Kinase (JAK) inhibitors in rheumatoid arthritis: a computer-based approach for drug safety studies and repurposing. SCS Fall Meeting 2021 all abstracts. CHIMIA. 2021;75: 7-8

Abbreviations: *ICPE: International Society for Pharmacoepidemiology (ISPE) Annual Conference; EULAR: Annual European congress of Rheumatology; SCS: Swiss Chemical Society.*