UNSW SYDNEY

Individualising tacrolimus therapy in adult heart transplant recipients

Author:
Kirubakaran, Ranita

Publication Date:
2021

DOI:
https://doi.org/10.26190/unsworks/1623

License:
https://creativecommons.org/licenses/by/4.0/
Link to license to see what you are allowed to do with this resource.

Downloaded from http://hdl.handle.net/1959.4/100023 in https://unsworks.unsw.edu.au on 2024-03-29

UNSW
SYDNEY

ST VINCENT'S
HOSPITAL
SYDNEY

Individualising tacrolimus therapy in adult heart transplant recipients

Ranita Kirubakaran

BPharm (Hons), Master of Pharmacy (Clinical Pharmacy)

A thesis in fulfilment of the requirements for the degree of Doctor of Philosophy

St. Vincent's Clinical School

Faculty of Medicine and Health

University of New South Wales

Sydney, Australia

September 2021

Thesis submission for the degree of Doctor of Philosophy

Thesis Title and Abstract	Declarations	Inclusion of Publications Statement	Corrected Thesis and Responses

Thesis Title

Individualising tacrolimus therapy in adult heart transplant recipients

Thesis Abstract

Tacrolimus is the key immunosuppressant used in most solid-organ transplant recipients, including heart transplants, to prevent graft rejection. However, tacrolimus dosing strategies are complicated by the narrow therapeutic window and considerable pharmacokinetic variability. Individualising lifelong tacrolimus therapy to avoid graft rejection and minimise adverse effects is essential for heart transplant recipients. This thesis aimed to investigate the individualisation of tacrolimus therapy in adult heart transplant recipients using the pharmacokinetic modelling approach.

In Chapter 1, I present an overview of tacrolimus clinical pharmacology, including clinical factors influencing tacrolimus pharmacokinetics (e.g., concomitant azole antifungal therapy). In Chapter 2, I explore tacrolimus dosing and monitoring practices in heart transplant recipients (n=87) at St. Vincent's Hospital Sydney, a major heart transplant centre in Australia. Additionally, I assess the ability of a Bayesian dosing software, approved by the Therapeutic Goods Administration to predict tacrolimus concentrations in heart transplant recipients. Tacrolimus dosing and monitoring practices were discordant with the hospital guidelines. The population pharmacokinetic model integrated within the software was suitable in guiding tacrolimus dosing only after 11 days of therapy. This finding necessitated the identification of other model(s) that might be more suitable for use in heart transplant recipients, particularly for the immediate post-transplantation phase.

In Chapter 3, I conduct a systematic review summarising published population pharmacokinetic models of tacrolimus (n=69) developed from various organ transplant recipient populations. In Chapter 4, I select relevant tacrolimus models (n=17) from the systematic review and evaluated their predictive performance in heart transplant recipients (n=85). The evaluated models displayed poor predictive performances. This finding complements the work from Chapters 2 and 3 highlighting a tacrolimus model for heart transplant recipients is required.

In Chapter 5, I successfully develop a tacrolimus population pharmacokinetic model for heart transplant recipients. The model incorporated the effects of concomitant azole antifungal use, haematocrit, and body weight on tacrolimus pharmacokinetics. Model evaluation in an independent heart transplant recipient cohort displayed good model performance. The model can be implemented in clinical practice to individualise tacrolimus dosing in heart transplant recipients. In Chapter 6, I discuss the clinical implication of this work and recommendations for future research.

ORIGINALITY STATEMENT

☑ I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

COPYRIGHT STATEMENT

☑ I hereby grant the University of New South Wales or its agents a non-exclusive licence to archive and to make available (including to members of the public) my thesis or dissertation in whole or part in the University libraries in all forms of media, now or here after known. I acknowledge that I retain all intellectual property rights which subsist in my thesis or dissertation, such as copyright and patent rights, subject to applicable law. I also retain the right to use all or part of my thesis or dissertation in future works (such as articles or books).

For any substantial portions of copyright material used in this thesis, written permission for use has been obtained, or the copyright material is removed from the final public version of the thesis.

AUTHENTICITY STATEMENT

☑ I certify that the Library deposit digital copy is a direct equivalent of the final officially approved version of my thesis.

UNSW is supportive of candidates publishing their research results during their candidature as detailed in the UNSW Thesis Examination Procedure.

Publications can be used in the candidate's thesis in lieu of a Chapter provided:

- The candidate contributed **greater than 50%** of the content in the publication and are the "primary author", i.e. they were responsible primarily for the planning, execution and preparation of the work for publication.
- The candidate has obtained approval to include the publication in their thesis in lieu of a Chapter from their Supervisor and Postgraduate Coordinator.
- The publication is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in the thesis.

☑ The candidate has declared that **their thesis has publications - either published or submitted for publication - incorporated into it in lieu of a Chapter/s. Details of these publications are provided below.**.

Publication Details #1

Full Title:	Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software.
Authors:	Ranita Kirubakaran, Sophie L. Stocker, Lilibeth Carlos, Richard O. Day, and Jane E. Carland
Journal or Book Name:	Therapeutic Drug Monitoring
Volume/Page Numbers:	43[6]/736-774
Date Accepted/Published:	19/05/2021
Status:	published
The Candidate's Contribution to the Work:	Ms Kirubakaran designed the study, performed data collection, data analysis and was involved in the interpretation of data. Ms Kirubakaran drafted the initial manuscript, revised the manuscript, approved the final manuscript, and submitted it for publication. Ms Kirubakaran was responsible for making all changes requested by journal reviewers.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	Chapter 2: This chapter presents the (1) exploration of tacrolimus dosing and monitoring practices in heart transplant recipients at St. Vincent's Hospital Sydney, a major heart transplant centre in Australia and (2) assessment of the ability of a renal transplant-derived tacrolimus population pharmacokinetic model integrated within a Bayesian dosing software to predict tacrolimus concentrations in heart transplant recipients. The findings of this study necessitated the identification of other population pharmacokinetic model(s) that might be more suitable for use in heart transplant recipients, particularly for the immediate post-transplantation phase.

Publication Details #2

Full Title:	Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review
Authors:	Ranita Kirubakaran, Sophie L. Stocker, Stefanie Hennig, Richard O. Day, Jane E. Carland
Journal or Book Name:	Clinical Pharmacokinetics
Volume/Page Numbers:	59/1357-1392
Date Accepted/Published:	03/07/2020
Status:	published
The Candidate's Contribution to the Work:	Ms Kirubakaran designed and performed the literature research, analysed the data and was one of the independent reviewers for the systematic review. Ms Kirubakaran drafted the initial manuscript, revised the manuscript, approved the final manuscript, and submitted it for publication. Ms Kirubakaran was responsible for making all changes requested by journal reviewers.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	Chapter 3: This chapter is a systematic review summarising published population pharmacokinetic models of tacrolimus developed from various organ transplant recipient populations. The results of this study informed the selection of tacrolimus population pharmacokinetic models for subsequent evaluation of their predictive performance in heart transplant recipients.

Publication Details #3

Full Title:	Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients
Authors:	Ranita Kirubakaran, Stefanie Hennig, Ben Maslen, Richard O Day, Jane E Carland, Sophie L Stocker
Journal or Book Name:	British Journal of Clinical Pharmacology
Volume/Page Numbers:	
Date Accepted/Published:	14/09/2021
Status:	published
The Candidate's Contribution to the Work:	Ms Kirubakaran designed the study, performed data collection and pharmacometrics analysis. Ms Kirubakaran drafted the initial manuscript, revised the manuscript, approved the final manuscript, and submitted it for publication. Ms Kirubakaran was responsible for making all changes requested by journal reviewers.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	Chapter 4: This chapter presents the evaluation of the predictive performance of specific tacrolimus population pharmacokinetic models developed from various organ transplant recipient populations identified in Chapter 3 in adult heart transplant recipients. The results of this manuscript have guided the development of a tacrolimus population pharmacokinetic model to facilitate dose individualisation in heart transplant recipients.

Publication Details #4

Full Title:	Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients.
Authors:	Ranita Kirubakaran, David W Uster, Stefanie Hennig, Jane E Carland, Richard O Day, Sebastian G Wicha, Sophie L Stocker.
Journal or Book Name:	British Journal of Clinical Pharmacology
Volume/Page Numbers:	-
Date Accepted/Published:	-
Status:	submitted
The Candidate's Contribution to the Work:	Ms Kirubakaran designed the study, performed data collection and pharmacometrics analysis. Ms Kirubakaran drafted the initial manuscript, revised the manuscript, approved the final manuscript, and submitted it for publication.
Location of the work in the thesis and/or how the work is incorporated in the thesis:	Chapter 5: This chapter presents the development and evaluation of a population pharmacokinetic model for tacrolimus in heart transplant recipients, considering the tacrolimus-azole antifungal interaction.

Candidate's Declaration

I confirm that where I have used a publication in lieu of a chapter, the listed publication(s) above meet(s) the requirements to be included in the thesis. I also declare that I have complied with the Thesis Examination Procedure.

Copyright permission

I, Ranita Kirubakaran, hereby declare that I have obtained copyright permission from the respective journals for the publication of the following articles in this thesis:

1. Kirubakaran R, Hennig S, Maslen B, Day RO, Carland JE, Stocker SL. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients. *British Journal of Clinical Pharmacology*. 2021. DOI: 10.1111/bcp.15091.
2. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software. *Therapeutic Drug Monitoring*. 2021;43(6):736-746. DOI: 10.1097/FTD.0000000000000909.
3. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review. *Clinical Pharmacokinetics*. 2020;59(11):1357-1392. DOI: 10.1007/s40262-020-00922-x.

Acknowledgements

"Gratitude is when memory is stored in the heart and not in the mind."

The work presented in this thesis was performed from February 2018 to February 2021 at the Department of Clinical Pharmacology and Toxicology, St. Vincent's Hospital Sydney. I wish to express my gratitude to all those who have supported me throughout my PhD candidature:

First, thank you to my great team of supervisors: Professor Richard Day, Dr Jane Carland and Dr Sophie Stoker. I feel privileged to have had the opportunity to work with such an inspiring supervisory team, without which this thesis would not have been possible. Thank you for believing in me, for sharing your wealth of knowledge, for always keeping your doors open and replying quickly to all kinds of questions. Thank you for giving me both freedom and responsibility during these three years.

Secondly, thank you to Thuy Huynh (St. Vincent's Clinical School Administrative Officer) — you are one of the most remarkable people I have met. I will be forever thankful for the unwavering kindness and support that you have given me since I started my journey as a PhD student.

A love-filled thank you to my family and relatives for supporting and always being there for me. Mum and Dad, thank you for giving me the opportunity to undertake this PhD. I hope I have made you proud.

To my family in Sydney: Vasanthan Narayanasami, Akila Rengasamy and Adhitya Vasanthan. Thank you for the home-cooked food, festival celebrations, indoor games, and companionship in exploring several beautiful walking tracks in Sydney. I will forever cherish the quality time spent together.

A sincere thank you to the following co-authors and experts in the pharmacometrics field who I have had the privilege to work with over the past three years: Dr Stefanie Hennig (Certara, Australia®), Professor Sebastian G. Wicha (University of Hamburg, Germany), Dr Shaun Kumar (Parexel, Australia), Dr Yuwei Lin (Certara®, Australia), David William Uster (University of Hamburg, Germany) and Dr Ooi Qing Xi (Pharmetheus®, Sweden).

Thank you to my colleagues: Eindra Aung, Marcel Schulz, Kulanka Premachandra, Tamara Milder, Gina Chowdhury, Alice Staciwa, Olivia Ng, Nanda Lakhwani, Yokasundery Muniandy, and Guangda Ma for good company and support along my PhD journey.

Finally, I would like to thank all the heart transplant recipients who participated in my pharmacogenomic project.

I dedicate this thesis to our future generation: my nephews and niece; Sricharan, Divyasri and Taarun, to symbolise that determination and integrity will always triumph.

Abstract

Tacrolimus is the key immunosuppressant used in most solid-organ transplant recipients, including heart transplants, to prevent graft rejection. However, tacrolimus dosing strategies are complicated by the narrow therapeutic window and considerable pharmacokinetic variability. Individualising lifelong tacrolimus therapy to avoid graft rejection and minimise adverse effects is essential for heart transplant recipients. This thesis aimed to investigate the individualisation of tacrolimus therapy in adult heart transplant recipients using the pharmacokinetic modelling approach.

In Chapter 1, I present an overview of tacrolimus clinical pharmacology, including clinical factors influencing tacrolimus pharmacokinetics (e.g., concomitant azole antifungal therapy). In Chapter 2, I explore tacrolimus dosing and monitoring practices in heart transplant recipients (n=87) at St. Vincent's Hospital Sydney, a major heart transplant centre in Australia. Additionally, I assess the ability of a Bayesian dosing software, approved by the Therapeutic Goods Administration to predict tacrolimus concentrations in heart transplant recipients. Tacrolimus dosing and monitoring practices were discordant with the hospital guidelines. The population pharmacokinetic model integrated within the software was suitable in guiding tacrolimus dosing only after 11 days of therapy. This finding necessitated the identification of other model(s) that might be more suitable for use in heart transplant recipients, particularly for the immediate post-transplantation phase.

In Chapter 3, I conduct a systematic review summarising published population pharmacokinetic models of tacrolimus (n=69) developed from various organ transplant

recipient populations. In Chapter 4, I select relevant tacrolimus models (n=17) from the systematic review and evaluated their predictive performance in heart transplant recipients (n=85). The evaluated models displayed poor predictive performances. This finding complements the work from Chapters 2 and 3 highlighting a tacrolimus model for heart transplant recipients is required.

In Chapter 5, I successfully develop a tacrolimus population pharmacokinetic model for heart transplant recipients. The model incorporated the effects of concomitant azole antifungal use, haematocrit, and body weight on tacrolimus pharmacokinetics. Model evaluation in an independent heart transplant recipient cohort displayed good model performance. The model can be implemented in clinical practice to individualise tacrolimus dosing in heart transplant recipients. In Chapter 6, I discuss the clinical implication of this work and recommendations for future research.

List of publications

*Publications related to thesis.

A) Original papers

Under review:

1. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients [submitted 31 August 2021].

Accepted/published:

1. *Kirubakaran R, Hennig S, Maslen B, Day RO, Carland JE, Stocker SL. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients. *British Journal of Clinical Pharmacology*. 2021. DOI: 10.1111/bcp.15091.
2. *Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software. *Therapeutic Drug Monitoring*. 2021;43(6):736−746. DOI: 10.1097/FTD.0000000000000909.
3. Stocker SL, Carland JE, Reuter SE, Stacy AE, Schaffer AL, Kirubakaran R, *et al*. Evaluation of a pilot vancomycin precision dosing advisory service on target exposure attainment using an interrupted time series analysis. *Clinical Pharmacology and Therapeutics*. 2021;109(1):212−221. DOI: 10.1002/cpt.2113.
4. *Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic

review. *Clinical Pharmacokinetics*. 2020;59(11):1357−1392. DOI: 10.1007/s40262-020-00922-x.

B) Conference abstract

1. *Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. Can population pharmacokinetic models of tacrolimus be extrapolated between different transplant populations? *CPT: Pharmacometrics and Systems Pharmacology.* World Conference on Pharmacometrics (WCoP), South Africa. 2020; 9 (SUPPL 1): S21−S22.

Conference presentations

*Presentations related to thesis.

Invited presentation:

1. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Matters close to the heart: Adaptation of tacrolimus models to inform therapeutic drug monitoring using the PRIOR approach. Population Approach Group of Australia & New Zealand (PAGANZ) Virtual Scientific Meeting, 27−29 January 2021.

Oral presentations selected by abstract:

1. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Australasian Pharmaceutical Science Association (APSA) Annual Conference, virtual meeting, 6 − 8 December 2021.
2. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) Virtual Annual Scientific Meeting, 29 November − 2 December 2021.
3. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. ASCEPT Student Forum Symposium, virtual meeting, 7 October 2021.

4. Singh R, Kirubakaran R, Carland JE, Day RO, Stocker SL. Predictive performance of population pharmacokinetic models for tacrolimus in lung transplant recipients. ASCEPT−APSA Virtual Scientific Meeting, 24−26 November 2020.
5. *Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.
6. *Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. American Association of Pharmaceutical Scientists (AAPS) Symposium, Sydney, Australia, 22 November 2019.
7. *Kirubakaran R, Stocker SL, Day RO, Carland JE. Predictive performance of a Bayesian forecasting software for tacrolimus in adult heart transplant. PAGANZ, Auckland, New Zealand, 6−9 February 2019.

Poster presentations:

1. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. ASCEPT Virtual Annual Scientific Meeting, 29 November − 2 December 2021.
2. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Twelfth American Conference on Pharmacometrics (ACoP12), virtual meeting, 8−12 November 2021.

3. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. 19th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), virtual meeting, 19−22 September 2021.
4. *Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Population Approach Group Europe (PAGE), virtual meeting, 2−7 September 2021.
5. *Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. Pharmacometrics Japan Conference, Tokyo, Japan, 22−23 January 2020.
6. *Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.
7. *Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review. ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.
8. *Kirubakaran R, Stocker SL, Day RO, Carland JE. Tacrolimus dosing and monitoring in heart transplant: A retrospective observational study. ASCEPT Annual Scientific Meeting, Adelaide, Australia, 27−30 November 2018.

9. *Kirubakaran R, Stocker SL, Day RO, Carland JE. Predictive performance of a Bayesian forecasting software for tacrolimus in adult heart transplant. ASCEPT Annual Scientific Meeting, Adelaide, Australia, 27−30 November 2018.
10. Kim YJ, Kirubakaran R, Stocker SL, Day RO, Carland JE. Tacrolimus dosing and monitoring in lung transplant. ASCEPT Annual Scientific Meeting, Adelaide, Australia, 27−30 November 2018.

Other presentations related to thesis

1. Twelfth American Conference on Pharmacometrics (ACoP12) Trainee Communication Challenge (2021).
2. Twitter Presentation, St. Vincent's Campus Research Symposium (2021).
3. 3 Minutes Thesis Competition, UNSW heat, UNSW Sydney (2021).
4. 3 Minutes Thesis Competition, Faculty of Medicine and Health heat, UNSW Sydney (2021).
5. Fast Forward Presentation, St. Vincent's Campus Research Week, St. Vincent's Hospital Sydney (2019).
6. 3 Minutes Thesis Competition, St. Vincent's Clinical School Heat, UNSW Sydney (2019).
7. Therapeutic Drug Monitoring Meeting (Invited speaker), St. Vincent's Hospital Sydney (2019).

Grants and awards related to thesis

Grants and Scholarships:

Title	Funding Source	Year
ASCEPT Student Registration Grant	ASCEPT	2021
APSA Student Registration Grant	APSA	2021
Thesis Allowance	UNSW Sydney	2021
Higher Degree Research (HDR) Completion Scholarship	UNSW Sydney	2021
Postgraduate Research Student Support (PRSS), Virtual Conference Funding	UNSW Sydney	2021
International Postgraduate Award (UIPA), PhD Scholarship	UNSW Sydney	2018–2021
Applied Medical Research (AMR) PhD Scholarship Supplementation	St. Vincent's Centre for AMR, Sydney	2018–2021
Pharmacometrics Japan Conference International Travel Grant	Pharmacometrics Japan	2020
ASCEPT Travel Award (New Zealand)	ASCEPT	2019
PAGANZ Travel Award (New Zealand)	PAGANZ	2019
St. Vincent's Clinical School HDR Travel Award (New Zealand)	UNSW Sydney	2019

Awards and Prizes:

Title	Year
ASCEPT Twitter Competition – best "conference with a view"	2021
Twelfth American Conference on Pharmacometrics (ACoP12) Trainee Communication Challenge (Winner)	2021
ASCEPT Student Forum Symposium (Second prize)	2021
International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT) Best Young Scientist Poster Award	2021
Greening Hospitals Mini-Hackathon, UNSW Faculty of Medicine and Health (First prize)	2021
3 Minutes Thesis Competition, UNSW Faculty of Medicine and Health Heat (First Prize)	2021
UNSW International Student of the Year Award (Finalist)	2021
Australian Research Council (ARC) UNSW Postgraduate Research Student Award	2020
PAGANZ Nick Holford Prize for best oral presentation in Clinical Pharmacology and Pharmacometrics (New Zealand)	2019
ASCEPT Clinical Pharmacology Special Interest Group Prize for best poster presentation in Clinical Pharmacology (Australia)	2018
ASCEPT Neville Percy Prize Finalist for best poster communication (Australia)	2018

Contents

Copyright permission		ii
Acknowledgements		iii
Abstract		vi
List of publications		viii
Conference presentations		x
Other presentations related to thesis		xiv
Grants and awards related to thesis		xv
Contents		xvii
List of Abbreviations		xxii
List of Chapter Figures		xxiv
List of Chapter Tables and Appendices		xxvii
Chapter 1. Introduction		1
1.1	Current status of heart transplantation	2
1.1.1	Statistics based on the International Society for Heart and Lung Transplantation Registry	2
1.1.2	Statistics based on the Australia and New Zealand Cardiothoracic Organ Transplant Registry	3
1.1.3	Challenges following heart transplantation	3
1.2	Pharmacological immunosuppressive therapy	4
1.2.1	Induction therapy	4
1.2.2	Maintenance therapy	5
1.3	Tacrolimus	7
1.3.1	Mechanism of action	7
1.3.2	Clinical pharmacokinetics	8
1.3.3	Licensed indication in Australia	12
1.3.4	Formulations	13
1.4	Tacrolimus pharmacokinetic monitoring	15
1.4.1	Therapeutic drug monitoring	15
1.4.2	Trough concentration	16
1.4.3	Trough concentration therapeutic range	17
1.4.4	An alternative approach for drug exposure monitoring - area under the concentration-time curve	19
1.4.5	Methods to measure whole blood tacrolimus concentration	21
1.5	Tacrolimus dosing	22
1.5.1	Tacrolimus dose conversion	22
1.6	Tacrolimus adverse effects and toxicities	23

1.7 Invasive fungal infection following heart transplantation 25
1.7.1 Antifungal therapies 25
1.7.2 Tacrolimus-azole antifungal interaction 26
1.8 The utilisation of population pharmacokinetic model-based Bayesian estimation to inform tacrolimus therapy 27
1.8.1 Fundamental principles of pharmacokinetic modelling 27
1.8.2 Clinical utility of population pharmacokinetic model-based Bayesian estimation 28
1.9 Thesis aims 30
1.10 Addressing the research gaps 32
References 34

Chapter 2. Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software 46
2.1 Link to thesis 47
2.2 Abstract 48
2.3 Background 50
2.4 Materials and Methods 52
2.4.1 Subjects and data collection 52
2.4.2 Genotyping 53
2.4.3 Assessment of concordance of tacrolimus dosing with institutional guidelines 54
2.4.4 Assessment of concordance of tacrolimus monitoring with institutional guidelines 55
2.4.5 Evaluation of a Bayesian forecasting software 57
2.4.6 Statistical analysis 59
2.5 Results 59
2.5.1 Heart transplant recipient characteristics and concomitant medications 59
2.5.2 Guideline concordance of tacrolimus dosing 63
2.5.3 Guideline concordance of tacrolimus monitoring 63
2.5.4 Predictive performance of the Bayesian forecasting software 64
2.6 Discussion 69
2.7 Limitations 77
2.8 Conclusions 77
2.9 Acknowledgements 77
2.10 Conflict of interest and source of funding 78
References 79
Appendices 83

Chapter 3. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review 86
3.1 Link to thesis 87
3.2 Abstract 88
3.3 Introduction 89
3.4 Methods 91
3.5 Results 92
3.6 Discussion 129
3.7 Limitations 136
3.8 Conclusions 137
3.9 Study highlights 137
3.10 Acknowledgements 138
3.11 Conflict of interest 138
3.12 Author Contributions 138
References 139
Appendices 148

Chapter 4. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients 149
4.1 Link to thesis 150
4.2 Abstract 151
4.3 Introduction 153
4.4 Methods 155
4.4.1 Selection of published population pharmacokinetic models for evaluation 155
4.4.2 Subjects and data collection 155
4.4.3 Institutional immunosuppressive and antibiotic protocol for heart transplant recipients 157
4.4.4 Bioanalytical method 158
4.4.5 Race and genotyping 158
4.4.6 Model evaluation 159
4.5 Results 162
4.5.1 Characteristics of the models evaluated 162
4.5.2 Characteristics of the heart transplant recipients 171
4.5.3 Model evaluation 176
4.6 Discussion 186
4.7 Limitations 189
4.8 Conclusions 189
4.9 Study highlights 190
4.10 Acknowledgements 190

4.11 Funding 191
4.12 Conflict of interest 191
4.13 Author Contributions 191
References 192
Appendices 198

Chapter 5. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients 205
5.1 Link to thesis 206
5.2 Abstract 208
5.3 Introduction 209
5.4 Methods 211
5.4.1 Subjects and data collection 211
5.4.2 Institutional immunosuppressive and antibiotic protocol for heart transplant recipients 213
5.4.3 Bioanalytical method 213
5.4.4 Ethnicity and genotyping 213
5.4.5 Population pharmacokinetic modelling 214
5.5 Results 223
5.5.1 Characteristics of the heart transplant recipients 223
5.5.2 Model building dataset 226
5.5.3 Population pharmacokinetic analysis 228
5.6 Discussion 234
5.7 Limitations 238
5.8 Conclusions 238
5.9 Key points 239
5.10 Acknowledgements 239
5.11 Funding 240
5.12 Conflict of interest 240
5.13 Author Contributions 240
References 241
Appendices 247

Chapter 6. Discussion and conclusions 261
6.1 Summary 262
6.2 Overview of thesis and key findings 264
6.3 Strengths of the current research 273
6.4 Limitations of the current research 275
6.5 Implications for clinical practice 276
6.6 Future directions 277
6.7 Overall conclusions 280

References 282

Chapter 7. Thesis appendices 288

Appendix A. ASCEPT Student Forum Symposium (Second prize) 289

Appendix B. International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT) Best Young Scientist Poster Award 290

Appendix C. UNSW 3 Minutes Thesis Competition. 291

Appendix D. 3 Minutes Thesis Competition, UNSW Faculty of Medicine and Health Heat (First Prize). 292

Appendix E. UNSW International Student of the Year Award (Finalist). 293

Appendix F. Greening Hospitals Mini-Hackathon, UNSW Faculty of Medicine and Health (First prize). 294

Appendix G. Australian Research Council (ARC) UNSW Postgraduate Research Student Award. 295

Appendix H. PAGANZ Nick Holford Prize for best oral presentation in Clinical Pharmacology and Pharmacometrics. 296

Appendix I. St. Vincent's Clinical School HDR Travel Award. 297

Appendix J. ASCEPT Clinical Pharmacology Special Interest Group Prize for best poster presentation in Clinical Pharmacology. 298

List of Abbreviations

ABCB1	ATP binding cassette subfamily B member 1
ABCC2	ATP binding cassette subfamily C member 2
AUC	Area under the concentration-time curve
B_{max}	Maximum amount bound
BLQ	Below the limit of quantitation
BOV	Between-occasion variability
BSV	Between-subject variability
CI	Confidence interval
CL/F	Apparent clearance
CV	Coefficient of variation
CWRES	Conditional weighted residuals
CYP	Cytochrome P450
eGFR	Estimated glomerular filtration rate
ETA	Individual random effects
F	Bioavailability
FKBP12	FK506-binding protein 12
FOCE+I	First-order conditional estimation method with interaction
GOF	Goodness of fit
HCO-60	Polyoxyl-60-hydrogenated castor oil
ICU	Intensive care unit
IV	Intravenous
K_a	Absorption rate constant
KD	Apparent dissociation constant
LC-MS	Liquid chromatography-mass spectrometry
LSS	Limited sampling strategy
mRNA	messenger RNA
NFAT	Nuclear factor of activated T-cells
NONMEM	Nonlinear mixed-effects modelling

NPDE	Normalised prediction distribution errors
OFV	Objective function value
pcVPC	Prediction-corrected visual predictive check
*POR**28	P450 oxidoreductase*28
PsN	Perl-speaks-NONMEM
Q/F	Apparent intercompartmental clearance
RSE	Relative standard error
RUV	Random unexplained variability
SCM	Stepwise covariate modelling
SVHS	St. Vincent's Hospital Sydney
TDM	Therapeutic drug monitoring
TGA	Therapeutic Goods Administration
UHPLC-MS/MS	Ultra-high performance liquid chromatography-tandem mass spectroscopy
V_2/F	Apparent central volume of distribution
V_3/F	Apparent peripheral volume of distribution
VPC	Visual predictive check

List of Chapter Figures

Figure 1	Molecular pathway of tacrolimus mechanism of action.	8
Figure 2	Cartoon of tacrolimus absorption in the small intestine.	9
Figure 3	Simulated whole blood tacrolimus concentration-time profile for twelve heart transplant recipients with different pharmacokinetic characteristics (sex, age, weight, days post-transplant, haematocrit concentration, alkaline phosphatase concentration, aspartate aminotransferase concentration, CYP3A5 genotype, CYP3A4 genotype, ABCB1 genotype [encodes P-glycoprotein] and concomitant itraconazole use) showing a large variability in tacrolimus concentration despite receiving the same dose (0.5 mg) of the oral immediate-release formulation of tacrolimus.	15
Figure 4	The collection of multiple drug concentrations over a dosing interval for area under the concentration-time curve monitoring.	20
Figure 5	The principles of population pharmacokinetic model-based Bayesian estimation to individualise drug dosing and inform therapeutic drug monitoring.	29
Figure 6	Thesis flow chart.	32
Figure 7	Tacrolimus trough concentrations collected at steady-state from (a) day 1 – day 7, (b) day 8 − day 90 and (c) day 91 − day 137 post-heart transplant.	64
Figure 8	Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in heart transplant recipients (n=81).	66
Figure 9	Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in a subset of heart transplant recipients who were hospitalised >14 days (n=51).	68

Figure 10 The goodness of fit plot for observed tacrolimus concentrations against individual predicted concentrations by the software for the oral immediate-release and intravenous data. 69

Figure 11 A Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram detailing the number of studies identified from databases search, abstracts screened, and the full-text articles retrieved. 93

Figure 12 Dose-normalised tacrolimus concentrations against time after dose in adult heart transplant recipients (n=85), with concomitant azole antifungal therapy (n=1770 tacrolimus concentrations) and without concomitant azole antifungal therapy (n=617 tacrolimus concentrations). 175

Figure 13 Dose-normalised tacrolimus concentrations against days post-transplant in adult heart transplant recipients (n=52), stratified based on concomitant azole antifungal use and CYP3A5 genotype. 176

Figure 14 Model predicted tacrolimus concentration-time profiles following the first dose of 5 mg oral immediate-release formulation of tacrolimus administered on day 1 post-transplant to a typical heart transplant recipient. 177

Figure 15 The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=100; blinded to the model) by each model relative to the observed concentrations, with concomitant azole antifungal therapy. 179

Figure 16 The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=52; blinded to the model) by each model relative to the observed concentrations, without concomitant azole antifungal therapy. 182

Figure 17 Prediction-corrected visual predictive check (pcVPC) plots for all models (n=17) with concomitant azole antifungal therapy. 184

Figure 18 Prediction-corrected visual predictive check (pcVPC) plots for all models (n=17) without concomitant azole antifungal therapy. 185

Figure 19 Flow chart of the modelling approach. 216

Figure 20 Dose-normalised tacrolimus concentrations against time after dose in heart transplant recipients stratified based on sex, diabetes status, ethnicity (White and Asian), ABCB1 genotype (CC, CT, and TT) and CYP3A5 genotype (*1/*1, *1/*3, and 3/*3). 219

Figure 21 Dose-normalised tacrolimus concentrations against time after dose in heart transplant recipients (n=47), with concomitant azole antifungal therapy (759 tacrolimus concentrations) and without concomitant azole antifungal therapy (341 tacrolimus concentrations). 227

Figure 22 Prediction-corrected visual predictive check (pcVPC) plots for the updated and final population pharmacokinetic model for tacrolimus. 231

Figure 23 The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion (blinded to the model) by the final tacrolimus population pharmacokinetic model relative to the observed concentrations (40 transplant recipients, 87 dosing occasions were predicted across stable and unstable post-transplantation periods). 233

Figure 24 Overview of thesis chapters and key findings. 265

List of Chapter Tables and Appendices

Table 1	Summary of phases and types of immunosuppressive pharmacotherapy for heart transplant recipients.	4
Table 2	Tacrolimus formulations available at St. Vincent's Hospital Sydney.	13
Table 3	Tacrolimus AUC therapeutic ranges corresponding to trough concentration therapeutic ranges for adult renal transplant recipients.	20
Table 4	Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients.	56
Table 5	Heart transplant recipient characteristics (n=87) on admission and concomitant medications administered immediately post-transplant.	61
Table 6	Tacrolimus doses administered (oral only) and tacrolimus concentrations obtained for the first 3 weeks in heart transplant recipients (n=87).	62
Table 7	Overall mean bias and imprecision stratified based on transplant recipient characteristics (n=81) following the oral immediate-release formulation of tacrolimus.	67
Appendix 1	Pharmacokinetic parameters and covariate relationships of the tacrolimus model integrated within the Bayesian forecasting software.	83
Appendix 2	Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in heart transplant recipients (n=81).	84
Appendix 3	Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in a subset of heart transplant recipients who were hospitalised >14 days (n=51).	85

Table 8	Description of population pharmacokinetic studies of tacrolimus in adult transplant recipients.	94
Table 9	Summary of population pharmacokinetic models of tacrolimus in adult transplant recipients.	104
Table 10	Estimates of apparent clearance and volume of distribution of tacrolimus from published population pharmacokinetic models amongst different graft types.	127
Appendix 4	Estimates of absorption rate constant (K_a) of tacrolimus from published population pharmacokinetic models amongst different graft types.	148
Table 11	Summary of published population pharmacokinetic models of tacrolimus in adult transplant recipients retained for model evaluation.	165
Table 12	Heart transplant recipient characteristics (n=85) on admission and concomitant medications administered immediately post-heart transplant.	172
Table 13	Heart transplant recipient (n=50) CYP3A4, CYP3A5 and ABCB1 genotypes stratified based on race.	173
Table 14	The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations.	180
Appendix 5	Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients.	198
Appendix 6	The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=100; blinded to the model) by each model relative to the observed concentrations, with concomitant azole antifungal therapy.	199
Appendix 7	The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=52; blinded to the model) by each model relative to the	201

observed concentrations, without concomitant azole antifungal therapy.

Appendix 8 The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (blinded to the model) by models developed solely using data from heart transplant recipients relative to the observed concentrations. 203

Appendix 9 Prediction-corrected visual predictive check (pcVPC) plots for models developed solely using data from heart transplant recipients, stratified based on concomitant azole antifungal use. 204

Table 15 Heart transplant recipient characteristics on admission and concomitant medications administered immediately post-heart transplantation. 224

Table 16 Heart transplant recipient CYP3A4, CYP3A5 and ABCB1 genotypes stratified based on ethnicity. 226

Table 17 Pharmacokinetic parameter estimates and covariate relationships of the published, updated, and final population pharmacokinetic model for tacrolimus in heart transplant recipients. 229

Appendix 10 Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients. 247

Appendix 11 Stepwise covariate modelling (SCM) file. 248

Appendix 12 Goodness-of-fit plots of the updated and final population pharmacokinetic model for tacrolimus. 249

Appendix 13 Pharmacokinetic parameter estimates and covariate relationships of the updated tacrolimus population model, with and without |CWRES|>5 removed. 250

Appendix 14 Individual random effects (ETA) distribution for apparent tacrolimus clearance (CL/F) of the updated and final 251

population pharmacokinetic model for tacrolimus stratified based on concomitant azole antifungal use.

Appendix 15 NONMEM code of the final model. 252

Appendix 16 The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion (n=87; blinded to the model) by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations across various evaluation settings. 257

Appendix 17 The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion (blinded to the model) by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations across three-time points of post-operative days (POD). 258

Appendix 18 Boxplot showing the relative prediction errors of the individual predicted tacrolimus concentrations of the fourth dosing occasion (blinded to the model) by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations. 260

Chapter 1:

Introduction.

1.1 Current status of heart transplantation

1.1.1 Statistics based on the International Society for Heart and Lung Transplantation Registry

Heart transplantation is a surgical replacement of the heart in people with end-stage heart failure. From January 1992 to June 2018, 108,034 adults have undergone heart transplantations worldwide [1], with a steady increase in the number of transplantations performed annually [2]. The primary indications for a heart transplant are non-ischemic dilated cardiomyopathy (51%) and ischemic cardiomyopathy (32%) [3]. Post-transplant survival has improved over the years. The median survival following heart transplantations performed between the years 2002−2009 was 12.5 years in comparison to 10.5 years for transplantations performed between the years 1992−2001 [3]. This improvement reflects advancement in surgical techniques and medical management, better patient selection for transplant and discovery of more effective immunosuppressive therapies. The leading causes of long-term mortality among heart transplant recipients include graft failure, non-cytomegalovirus infection and multiple organ failure [3]. Mortality due to acute graft failure (40%) is highest in the first 30 days, while mortality attributed to infection (32%) is highest in the first year post-transplant [3]. The risk of developing malignancy, acute graft rejection, cardiac allograft vasculopathy, and renal failure mortality increase with days post-transplant [3]. The majority of chronic graft failure cases are likely due to cardiac allograft vasculopathy [3].

1.1.2 Statistics based on the Australia and New Zealand Cardiothoracic Organ Transplant Registry

There were 2452 adult heart transplantations performed in Australia between the years 1984−2018 and 43% of these were performed at St. Vincent's Hospital Sydney [4]. St. Vincent's Hospital Sydney is one of four adult heart transplant centres in Australia with approximately 50 heart transplant surgeries performed yearly [4]. This is followed by The Alfred Hospital Melbourne (30 heart transplant surgeries/year), Prince Charles Hospital Brisbane (20 heart transplant surgeries/year) and Fiona Stanley Hospital Perth (10 heart transplant surgeries/year) [4]. The two most common indications for a heart transplant in Australia are idiopathic dilated cardiomyopathy (42%) and ischemic heart disease (31%) [4]. The 1- and 5-year survival rates were 89% and 83%, respectively, following heart transplantation performed between the years 2010−2018 [4]. Similar to the international registry [3], the main causes of mortality within 5 years post-heart transplant were infection (23%) and graft rejection (16%) [4]. The primary causes of mortality beyond 5 years post-heart transplant are coronary artery disease (22%) and non-lymphoid malignancy (22%) [4].

1.1.3 Challenges following heart transplantation

At present, the main challenge experienced by transplant clinicians is to improve the long-term outcomes following transplantation, while maintaining the good short-term outcomes that have already been achieved. Complications resulting from graft rejection and immunosuppressive therapy, such as cardiac allograft vasculopathy, infection, and malignancies, continue to affect long-term survival [1, 3]. Unlike renal transplant failure which can be treated with dialysis, heart transplant failure results in death.

Immunosuppressive therapy is a modifiable factor in the long-term care of heart transplant recipients that can be improved. Therefore, a focus of current work is to optimise the lifelong immunosuppressive therapy which is essential to reduce the long-term complications resulting from the therapy.

1.2 Pharmacological immunosuppressive therapy

Immunosuppressive therapies for heart transplant recipients are categorised into induction and maintenance phases (Table 1).

Table 1. Summary of phases and types of immunosuppressive pharmacotherapy for heart transplant recipients.

Phases of therapy	Drug class	Example
Induction	Interleukin-2 receptor antagonist	Basiliximab
	Lymphocyte-depleting agent	Anti-thymocyte globulin
Maintenance	Calcineurin inhibitors	Cyclosporine
		Tacrolimus
	Anti-proliferative agent	Mycophenolate mofetil
		Mycophenolate sodium
		Azathioprine
	Mammalian target of rapamycin inhibitor	Sirolimus
		Everolimus
	Corticosteroid	Prednisolone

1.2.1 Induction therapy

Induction therapy aims to provide intense immunosuppression immediately post-transplant [5] to prevent acute graft rejection. It is primarily prescribed for heart transplant recipients with renal impairment (to delay the commencement of tacrolimus)

or those who received mechanical circulatory support (left ventricular, biventricular assist devices or total artificial hearts) [6]. Over 50% of heart transplant recipients worldwide received induction therapy at the time of transplantation between January 2010 and June 2018 [3]. However, no significant difference (p >0.05) in post-transplant survival based on the use or type of induction therapy was observed [3].

1.2.2 Maintenance therapy

Maintenance immunosuppressive therapy is prescribed lifelong to achieve a state of equilibrium whereby the immune system is adequately suppressed to minimise graft rejection, but remains sufficient active to fight infections [5]. At present, in most transplant centres, a triple maintenance immunosuppressive regimen is prescribed for heart transplant recipients, consisting of a combination of a calcineurin inhibitor, an anti-proliferative agent and a corticosteroid [3].

Tacrolimus is currently the first choice calcineurin inhibitor used in most organ transplant recipients, including heart transplants, to prevent graft rejection. In comparison to cyclosporine, tacrolimus is 10−100 times more potent [7], exhibits higher patient and graft survival rates [8, 9], reduces the incidence of acute graft rejection [10, 11], and has a lower incidence of hypertrichosis [10, 12], hypertension and hyperlipidaemia [10]. However, it has a higher incidence of post-transplant diabetes mellitus relative to cyclosporin [10]. Tacrolimus has been in use for over two decades and no new drug has emerged to replace tacrolimus.

Mycophenolate is the preferred anti-proliferative agent in heart transplants. It works by inhibiting lymphocyte proliferation [3]. In comparison to azathioprine, mycophenolate is associated with a significant reduction in graft rejection and mortality in the first-year post-heart transplantation [13, 14]. While mycophenolate mofetil is the commonly prescribed form, mycophenolate sodium (enteric-coated, extended-release formulation) is available as an alternative for transplant recipients experiencing intolerable gastrointestinal side effects due to mycophenolate mofetil [15]. In contrast to tacrolimus, mycophenolate has been shown to have nephroprotective effects [16].

Prednisolone is an anti-inflammatory agent largely used for the prevention and treatment of acute graft rejection [3, 7, 17]. However, if consumed long term, it can cause multiple adverse effects such as osteoporosis, new-onset diabetes mellitus, hypertension and the features seen in Cushing's syndrome. As such, many transplant centres use a relatively high dose of prednisolone early post-transplant and subsequently, taper down the dose and discontinue or maintain at the lowest possible dose over 6–12 months [7, 18].

In summary, tacrolimus, mycophenolate mofetil and prednisolone are the three key agents in the maintenance immunosuppressive strategies following heart transplantation [3]. This is also evident from the high global usage of tacrolimus and mycophenolate mofetil (90%) at 1-year follow-up post-heart transplantation with 80% of the transplant recipients remaining on prednisolone (follow-ups: January 2010−June 2018) [3]. Unlike mycophenolate and prednisolone, the narrow therapeutic range and considerable variability [19-25] in tacrolimus pharmacokinetics result in the difficulty in

dosing tacrolimus in clinical practice. Therefore, this thesis focuses on tacrolimus therapy, particularly investigating methods to enable precision dosing of tacrolimus in heart transplant recipients.

1.3 Tacrolimus

1.3.1 Mechanism of action

After entry into the cytoplasm of T-cells, the unbound tacrolimus binds to an intracellular protein, FK506-binding protein 12 (FKBP12) (Figure 1) [26, 27]. The tacrolimus–FKBP12 complex inhibits the activity of the enzyme calcineurin, thereby preventing the activation of nuclear factor of activated T-cells (NFAT), a transcription factor involved in the production of several cytokines, including interleukin-2 [26, 27]. These cytokines are involved in T-cell activation and proliferation [27], leading to acute graft rejection [28]. By suppressing the formation of these cytokines, tacrolimus prevents cell-mediated acute graft rejection [28]. Additionally, tacrolimus inhibits T-cell dependent B-cell activation, hence, preventing antibody-mediated graft rejection [29].

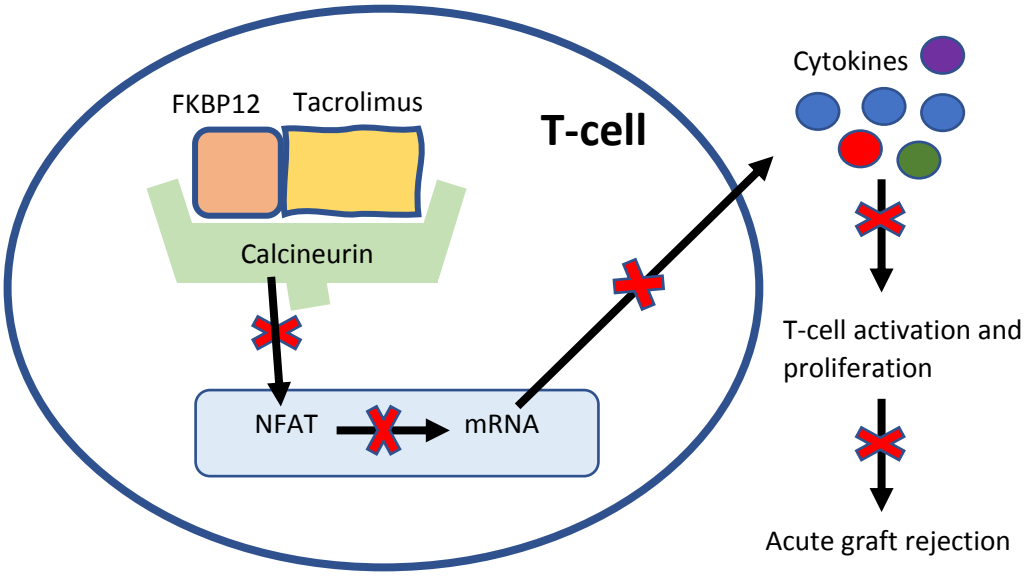


Figure 1. Molecular pathway of tacrolimus mechanism of action. In the cytoplasm of T-cells, tacrolimus binds to FK506-binding protein 12 (FKBP12) to form a complex that inhibits the activity of enzyme calcineurin. This inhibition prevents the activation of nuclear factor of activated T-cells (NFAT) in producing cytokines that are involved in the graft rejection process. *mRNA:* messenger RNA.

1.3.2 Clinical pharmacokinetics

Absorption

Tacrolimus is rapidly absorbed, with a peak concentration attained within 0.5–1 hour after oral administration [30, 31]. For some transplant recipients, tacrolimus absorption is delayed, resulting in a lag time [32] or secondary peaks [33]. The oral bioavailability of tacrolimus is poor (mean: 25%) [34, 35] and highly variable (5–93%) [36, 37]. Extensive pre-systemic metabolism by cytochrome P450 (CYP) 3A4 and 3A5 enzymes located in the intestinal cell [38-40] and efflux of tacrolimus back into the intestinal lumen by P-glycoprotein located in the apical membrane of the intestinal cell [38-40] (Figure 2) contribute to the poor oral absorption of tacrolimus. The fraction of tacrolimus escaping pre-systemic metabolism was estimated to be 0.14–0.26 [41, 42]. Of note, the functions

of CYP3A4 enzymes, CYP3A5 enzymes, and P-glycoproteins are determined by a complex interplay between genetic polymorphisms, the inductive or inhibitory effects of many concomitant medications, and endogenous substances such as uremic toxins in the case of end-stage renal disease [43]. The poor dissolution of tacrolimus in gastric juices (dissolution rate-limited absorption) [31], administration with food [44, 45], and alteration in gastrointestinal motility [31] have also been identified as factors contributing to the variability in tacrolimus absorption. The estimated absorption rate constant (K_a), as estimated from whole blood tacrolimus concentrations across various organ transplant recipient populations, is described in Chapter 3 (Appendix 4).

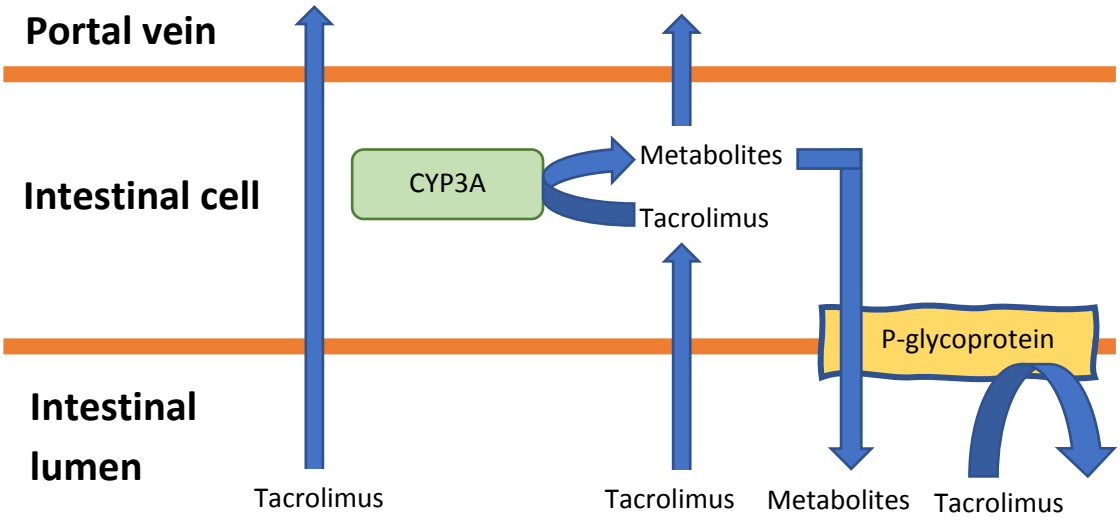


Figure 2. Cartoon of tacrolimus absorption in the small intestine. Tacrolimus is able to cross the intestinal wall due to its lipophilicity [46]. Once inside the intestinal cell, tacrolimus can either diffuse across the intestinal cell into the portal circulation or be metabolised by CYP3A enzymes. P-glycoprotein lowers systemic tacrolimus concentration by actively pumping the drug back into the intestinal lumen where it is transported into more distal segments of the intestine containing lower amounts of P-glycoproteins and CYP3A enzymes. Tacrolimus metabolites can also be pumped into the intestinal lumen by P-glycoprotein or diffuse into the portal circulation.

Distribution

Overall, there is an extensive distribution of tacrolimus in whole blood due to the presence of high concentrations of FKBP12 proteins within erythrocytes [36, 47]. This explains the 15-fold (range: 4−114) [34, 36] higher whole blood tacrolimus concentrations than those measured in plasma. In blood, tacrolimus is extensively bound to erythrocytes (85−95%) [48, 49], with maximum amount bound (B_{max}) of 418 ± 258 μg/L and apparent dissociation constant (KD) of 3.8 ± 4.7 μg/L in transplant recipients [31]. Within plasma, tacrolimus is approximately 99% bound to plasma proteins such as α-acid glycoprotein, lipoproteins, globulins and albumin [48]. The binding of tacrolimus by plasma proteins is not saturable at physiological drug concentrations [50].

The partitioning of tacrolimus between blood and plasma is influenced by haematocrit, temperature of the blood sample, plasma protein concentration and tacrolimus concentration [50]. An increase in haematocrit leads to an increase in the blood:plasma ratio of tacrolimus [50]. Of note, changes in haematocrit affect the whole blood tacrolimus concentrations without impacting the actual pharmacologically active unbound plasma concentrations [51]. When the blood sample temperature is >25°C, more drug partitions to the plasma compartment [50]. An increase in α-acid glycoprotein concentration in plasma leads to an increase in tacrolimus binding in plasma, hence, a decrease in the blood:plasma ratio of tacrolimus [31]. The uptake of tacrolimus by erythrocytes is concentration-dependent [50]. When whole blood tacrolimus concentration increases, the uptake of tacrolimus by erythrocytes is saturated, resulting in lower blood:plasma ratio of tacrolimus [50].

Animal studies indicate that tacrolimus is widely distributed into most tissues, including the lungs, spleen, heart, kidney, pancreas, brain, muscle and liver [31, 52]. In human studies, tacrolimus is able to cross the placenta and has been detected in breast milk [31, 52]. The estimated apparent volume of distribution, as estimated from whole blood tacrolimus concentrations across various organ transplant recipient populations, is described in Chapter 3 (Table 10).

Metabolism

Tacrolimus is extensively metabolised by CYP3A4 and CYP3A5 enzymes in the liver and intestine, with up to 15 metabolites produced [31]. These metabolites are classified as first-generation metabolites, formed from tacrolimus, and second-generation metabolites formed from first-generation metabolites [53]. The major metabolites of tacrolimus are 13-O-desmethyl tacrolimus and 15-O-desmethyl tacrolimus [54, 55]. The 13-O-desmethyl tacrolimus has 10% of the activity of the parent drug. Another metabolite, 31-O-desmethyl tacrolimus has similar activity to the parent drug [56] but the concentrations are low-to-not-detectable in transplant recipients [57]. Other metabolites had little or no activity [56].

Elimination

Based on whole blood tacrolimus concentration, tacrolimus is considered a low-clearance drug with total body clearance equivalent to 3% of liver blood flow (low-hepatic extraction-ratio drug) [30]. Thus, the total hepatic clearance of tacrolimus should be determined by the intrinsic clearance and the free fraction of tacrolimus in blood, based on the Well-Stirred model [58]. The low blood clearance of tacrolimus

suggests that the low bioavailability of tacrolimus could be due to poor oral absorption of the drug [31].

More than 95% of tacrolimus metabolites are excreted via the biliary route with negligible urine excretion (<3%) [30]. Less than 0.5% of the parent drug is excreted via urine or faeces [30]. The terminal elimination half-life of tacrolimus ranges between 11−16 hours in transplant recipients and is dependent on the clearance rate of tacrolimus [59]. The estimated apparent clearance, as estimated from whole blood tacrolimus concentrations across various organ transplant recipient populations, is described in Chapter 3 (Table 10). Important to note that transplant recipients who are CYP3A5 expressers (*1/*1 and *1/*3 genotypes) exhibit 1.5- to 2-fold higher tacrolimus apparent clearance than CYP3A5 non-expressers (*3/*3 genotype) [32, 60, 61].

Overall, one should be cautious when interpreting the pharmacokinetics of tacrolimus. Most pharmacokinetic studies have utilised immunoassay to measure tacrolimus concentrations. The cross-reactivity of the assay with tacrolimus metabolites results in spuriously higher tacrolimus concentration measurements [62] and consequently, incorrect pharmacokinetic parameter estimates than the observed values.

1.3.3 Licensed indication in Australia

The approved indications for tacrolimus use vary depending on the formulation and country [54]. Tacrolimus is currently licensed in Australia for use as an adjunct to liver, kidney, lung or heart allograft transplantation in adults and children [63].

1.3.4 Formulations

Tacrolimus is available in both oral (immediate-release and extended-release capsules) and intravenous formulations (Table 2). There is no commercially available oral suspension. However, formulas for extemporaneous compounding of the oral suspension are available using the oral immediate-release formulation of tacrolimus. This extemporaneous preparation is particularly important for transplant recipients who require nasogastric feeding or need doses that cannot be achieved by taking the whole capsule. Of note, since the patent protection of Prograf® expired, approximately 260 products have been registered worldwide, mostly immediate-release generic formulations of tacrolimus [54].

Table 2. Tacrolimus formulations available at St. Vincent's Hospital Sydney.

Formulation	**Strength**
Prograf® capsule (immediate-release)	0.5 mg, 1 mg, 5 mg
Prograf® XL capsule (extended-release)	0.5 mg, 1 mg, 5 mg
Prograf® injection	5 mg/mL

The commonly used oral immediate-release formulation of tacrolimus, Prograf®, is administered twice daily, while Prograf® XL is consumed once daily. The dose conversion ratio between Prograf® and Prograf® XL is 1:1 [59]. However, in clinical practice, an approximately 10% dose increase may be required for the extended-release formulation to achieve similar systemic drug exposure as the immediate-release formulation of tacrolimus [59]. Indications for the conversion from the oral immediate-release to the extended-release formulation of tacrolimus use include gastrointestinal disturbances, transplant recipients with suspected poor adherence and a large increase or decrease in

tacrolimus blood concentration with small dose changes of the oral immediate-release formulation. Of the two oral formulations, improved adherence to tacrolimus therapy has been observed in transplant recipients receiving the extended-release formulation, leading to a reduction in graft rejection and graft loss in long-term follow-ups [64].

In some cases, the immediate-release formulation of tacrolimus can be administered sublingually (capsule placed under the tongue and left to dissolve or content of the capsule placed under the tongue) for short term use in transplant recipients who are unable to take the immediate-release dosage form of tacrolimus orally [65]. However, a proportion of tacrolimus absorption from sublingual dosing might be from swallowed drug, rather than direct buccal absorption [66].

The intravenous route may be considered for a limited time if the oral route is not feasible (intubated and sedated transplant recipients). Prolonged intravenous tacrolimus use is associated with severe nephrotoxicity due to the presence of solvent polyoxyl-60-hydrogenated castor oil (HCO-60) in the formulation [67, 68]. The recommended method of administration of intravenous tacrolimus is continuous infusion over 24 hours and is not to be administered as a bolus, intermittent or short infusion [59]. This is because of higher rates of neurologic and renal toxicities (due to increased peak tacrolimus concentrations) seen when intravenous tacrolimus is administered by rapid or short infusions twice daily [69].

1.4 Tacrolimus pharmacokinetic monitoring

1.4.1 Therapeutic drug monitoring

Tacrolimus displays considerable pharmacokinetic variability [19-25]. This is evident from the poor correlation observed between tacrolimus dose and whole blood tacrolimus concentration (Figure 3). As a result, there is an increased risk of therapeutic failure if uniform doses of tacrolimus are prescribed to all transplant recipients. To overcome this, therapeutic drug monitoring (TDM) of tacrolimus concentrations is recommended to individualise tacrolimus therapy [54]. TDM is the standard approach used in most transplant centres to optimise the likelihood of achieving efficacious and safe use, and in assessing transplant recipient's adherence to tacrolimus therapy.

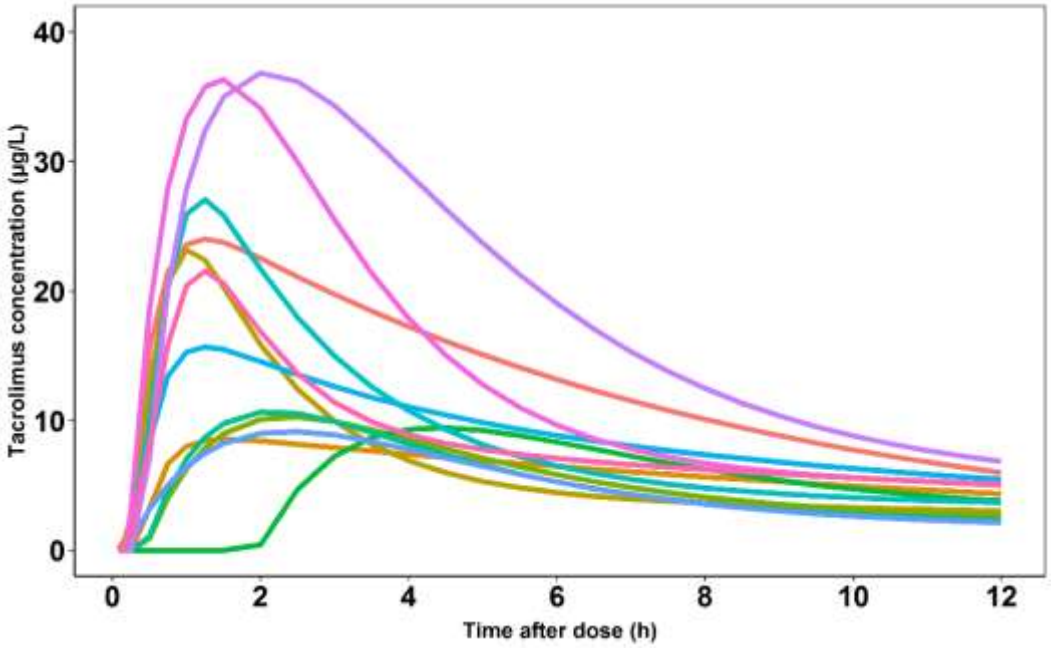


Figure 3. Simulated whole blood tacrolimus concentration-time profile for twelve heart transplant recipients with different pharmacokinetic characteristics (sex, age, weight, days post-transplant, haematocrit concentration, alkaline phosphatase concentration, aspartate aminotransferase concentration, CYP3A5 genotype, CYP3A4 genotype, ABCB1 genotype [encodes P-glycoprotein] and concomitant itraconazole use) showing a large variability in tacrolimus concentration despite receiving the same dose (0.5 mg) of the oral immediate-release formulation of tacrolimus.

Tacrolimus is highly bound to erythrocytes [48, 49]. Hence, tacrolimus dose recommendations are based on whole blood rather than plasma tacrolimus concentration. This is despite the unbound plasma concentration better reflect tacrolimus efficacy and toxicity [70-72]. Challenges in analysing unbound tacrolimus plasma concentrations include technical difficulties in quantification and limited information on the appropriate therapeutic range [73]. It should also be noted that tacrolimus concentration in the blood does not necessarily reflect cellular concentrations and hence, the extent of interaction with intracellular targets [52]. In this thesis, all data on the application of tacrolimus concentrations as a guide to therapy were based on whole blood measurements.

1.4.2 Trough concentration

TDM of whole blood trough concentrations is recommended to guide tacrolimus dosing decisions in transplant recipients [54]. This is due to the clinical convenience of collecting a single rather than multiple drug concentrations over a dosing interval. Trough concentration is the least variable timepoint in the drug concentration-time profile. This ensures consistent interpretation of drug exposure to facilitate dose individualisation in clinical practice.

Trough concentrations are sampled at steady-state within one hour before the next scheduled dose. A trough concentration is usually sampled just before the morning oral dose of tacrolimus. These morning trough concentrations have been used to develop the therapeutic range for tacrolimus [74]. However, it is unclear if there is a significant difference between trough concentrations sampled before the morning and evening

doses of the oral immediate-release formulation (administered every 12 hours) of tacrolimus [75, 76]. Despite widespread implementation, trough concentration-guided dosing remains a challenge in clinical practice due to the collection of poorly timed and non-steady-state trough concentrations [6, 77]. It is important to note that this monitoring parameter failed to show a relationship with acute graft rejection in a recent meta-analysis [78].

The frequency of tacrolimus TDM sampling post-transplant depends on centre-specific protocols and is adjusted based on the clinician's discretion and transplant recipient's clinical status. Generally, in the early post-transplant phase, TDM samples are collected at steady-state 2−5 times per week, subject to the transplant recipient's overall clinical assessment (e.g., renal function, graft function, rejection history, and hemodynamic status). TDM samples are collected once per month once stable whole blood tacrolimus concentrations have been achieved on the prescribed maintenance dose (personal communication).

1.4.3 Trough concentration therapeutic range

The trough concentration therapeutic range of tacrolimus varies between the type of organ transplant, days post-transplant (natural decrease in tacrolimus apparent clearance over time reaching a plateau) and tacrolimus formulation. Additionally, the range varies according to centre-specific protocols and is adjusted based on the clinician's experience and observation taking into consideration transplant recipient (1) clinical status, and (2) tacrolimus toxicity and graft rejection experienced.

Generally, the therapeutic range for tacrolimus whole blood concentrations is poorly defined in heart transplantation. The suggested therapeutic range is 15–20 µg/L for the first 2 months, 10–15 µg/L from months 3–6, and 8–10 µg/L from 6 months post-transplant and beyond [79]. This therapeutic range was proposed almost three decades ago [54]. It has not been subject to modification according to the type of (1) bioanalytical method or physiological matrices (whole blood versus plasma) used to determine tacrolimus concentration and (2) immunosuppressive regimen used alongside tacrolimus [54, 80]. While trough concentration therapeutic range describes the range that produces the best outcomes and least adverse events, it does not eliminate the risk of adverse events. This is because individual susceptibility differs between transplant recipients. A recent study has shown the risk of acute renal injury within the first two weeks after heart transplantation was significantly increased for trough concentrations >15 µg/L (therapeutic range is 15–20 µg/L) [81]. As a result, more work is required to enable the refinement of a therapeutic range for tacrolimus concerning clinical outcomes for heart transplant recipients.

Given the narrow therapeutic range, small variations in tacrolimus trough concentrations could affect graft and transplant recipient clinical outcomes. For example, subtherapeutic tacrolimus trough concentrations could lead to an increased risk of graft rejection. On the contrary, supratherapeutic trough concentrations could lead to tacrolimus toxicities, such as nephrotoxicity and neurotoxicity. Therefore, transplant clinicians aim to achieve tacrolimus trough concentrations within the therapeutic range. However, achieving and maintaining tacrolimus trough

concentrations within the narrow therapeutic range is a challenging task for transplant clinicians due to the highly variable and unpredictable pharmacokinetics of tacrolimus.

1.4.4 An alternative approach for drug exposure monitoring - area under the concentration-time curve

Instead of trough concentrations, the area under the concentration-time curve (AUC) is regarded as a better measure of tacrolimus exposure associated with treatment efficacy [54, 82, 83]. This follows from the poor correlation observed between tacrolimus trough concentrations and AUC_{0-12} [12, 54, 84, 85]. However, no prospective study has been conducted to investigate the potential for superior clinical outcomes for AUC- versus trough concentration-guided tacrolimus dosing in transplant recipients [54]. Further, AUC monitoring generally requires frequent TDM sampling (≥6 samples over a dosing interval) [86] (Figure 4) to enable AUC calculation using the trapezoidal rule (reference method). Hence, this approach is not widely practised due to cost and clinical constraints.

Although not formally defined, AUC therapeutic ranges corresponding to trough concentration therapeutic ranges have been suggested following the administration of the oral immediate-release [87] and extended-release [88] formulation of tacrolimus (Table 3). The AUC therapeutic ranges were proposed based on retrospective studies performed in adult renal transplant recipients [87, 88].

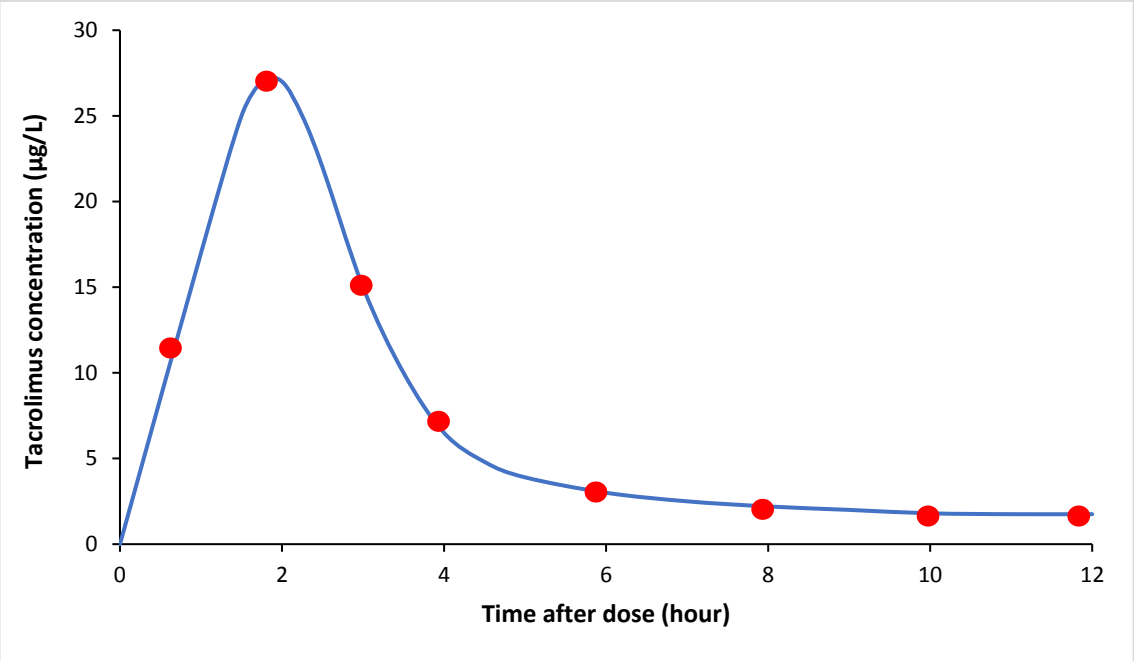


Figure 4. The collection of multiple drug concentrations (●) over a dosing interval for the area under the concentration-time curve monitoring.

Table 3. Tacrolimus AUC therapeutic ranges corresponding to trough concentration therapeutic ranges for adult renal transplant recipients [87, 88].

Therapeutic range of trough concentration (µg/L)	**AUC_{0-12} therapeutic range (µg*h/L) [oral immediate-release formulation]**	**AUC_{0-24} therapeutic range (µg*h/L) [oral extended-release formulation]**
3–7	75–140	130–275
5–10	100–190	180–350
8–12	140–210	260–400
10–15	180–270	310–475

In recent years, several studies have formulated a limited sampling strategy (LSS) to allow prediction of tacrolimus AUC in various organ transplant recipients. LSS involves the collection of 2−4 tacrolimus concentrations during the early phase within the dosing interval. In a study of 22 heart transplant recipients, a four-point abbreviated AUC, calculated using tacrolimus concentrations collected at 0, 1, 2, 4 hours, predicted AUC_{0-12} accurately (r^2=0.98) relative to the AUC_{0-12} calculated using the trapezoidal rule (reference method) [89]. However, the use of LSS for AUC estimation has not been firmly established because of the paucity of data demonstrating improved clinical outcomes for AUC compared to trough concentration monitoring. Before this new monitoring strategy can be implemented in clinical practice, more work is required to determine the relationship between AUC and tacrolimus efficacy and safety, and importantly, to establish an optimal AUC therapeutic range for heart transplant recipients.

1.4.5 Methods to measure whole blood tacrolimus concentration

Tacrolimus concentrations can be measured using immunoassay or liquid chromatography with mass spectrometry detection. In an international survey (14 countries) conducted in 2013, 53% of the laboratories used liquid chromatography-tandem mass spectrometry and 47% used immunoassays to analyse tacrolimus [90]. The ideal method is to utilise liquid chromatography with mass spectrometry detection, which is more specific and sensitive, and has a higher degree of precision [62, 91]. Immunoassay is not recommended as the reagents interact with tacrolimus metabolites, providing spuriously higher (5%−36% versus liquid chromatography with mass spectrometry detection [54, 92, 93]) tacrolimus concentration measurements [62]. Regardless of the method used, all are subject to intra-assay variation (estimated by

coefficient of variation) and inter-day variation (also estimated by coefficient of variation).

1.5 Tacrolimus dosing

The tacrolimus starting dose varies according to the type of organ transplanted and formulation. A starting dose of 0.075 mg/kg/day for oral tacrolimus and 0.01–0.02 mg/kg/day for a 24-hour continuous intravenous infusion is recommended in heart transplant recipients [59]. Subsequent maintenance doses are titrated intuitively by clinicians based on trough concentration(s), transplant recipient's overall clinical assessment and concomitant drug interactions. This intuitive dosing is further complicated by the variation in tacrolimus pharmacokinetics with days post-transplant, as observed by a continuous decrease in tacrolimus apparent clearance reaching a plateau during the first-year post-transplant [32, 94-96, 43]. This decrease is likely due to an improvement in haematocrit and serum albumin concentrations and tapering down of corticosteroid dose [32, 94, 96, 43].

1.5.1 Tacrolimus dose conversion

The recommendations for conversion from intravenous to oral and oral to sublingual vary according to the organ transplanted. In heart transplants, intravenous doses are usually converted to oral dose equivalents using a 1:5 (intravenous:oral) ratio [79]. This conversion ratio reflects the average oral bioavailability of tacrolimus which is between 20−25% [52]. There is scarce data to recommend dose conversion from oral to sublingual in heart transplants. However, a suggested conversion in solid organ transplant is to give

50% of the oral dose sublingually in the absence of interacting medications such as azole antifungals [65].

1.6 Tacrolimus adverse effects and toxicities

Increasing tacrolimus concentrations may lead to an increased risk of tacrolimus adverse effects and toxicities. However, it is difficult to establish a clear threshold due to substantial overlap between treatment efficacy and the appearance of adverse effects/toxicities [97]. Nonetheless, adverse events occur most frequently in the first few months post-transplantation and decline thereafter [52]. This possibly reflects the (1) clinical instability during the recovery period post-surgery and (2) more aggressive dosing required to achieve guideline-recommended therapeutic drug concentrations, that also decrease based on days post-transplant. The most common adverse effects of tacrolimus are nephrotoxicity, neurotoxicity, new-onset diabetes mellitus and hypertension [10, 11]. Other commonly documented adverse effects include gastrointestinal disturbances (nausea, constipation and diarrhoea), alopecia, electrolyte imbalance (hyperkalaemia and hypomagnesaemia) and hyperlipidaemia [10, 11].

Tacrolimus induced-nephrotoxicity is defined as the deterioration in renal function accompanied by a supratherapeutic tacrolimus trough concentration [81] when no other cause for the deterioration of renal function, such as prerenal (dehydration, heart failure, sepsis), renal (acute tubular necrosis, interstitial nephritis, glomerulonephritis), or postrenal (ureteric obstruction, renal vascular thrombosis) has been documented [98]. The incidence of tacrolimus induced-nephrotoxicity in heart transplant recipients varies substantially (10%–62%) depending on the (1) study design and (2) renal

biomarker (e.g.: serum creatinine concentration, estimated glomerular filtration rate or creatinine clearance) and its threshold defined as delineating nephrotoxicity [99]. The risk of tacrolimus-induced nephrotoxicity is increased in transplant recipients receiving other concomitant nephrotoxic drugs [10, 11] or with pre-existing renal impairment [59, 81]. To avoid tacrolimus-induced nephrotoxicity decreased doses and/or delayed commencement of tacrolimus therapy have been suggested [81].

Tacrolimus-induced neurotoxicity is usually caused by supratherapeutic tacrolimus concentrations [59]. Its manifestations include tremor, headache, paraesthesia, changes in mental status, insomnia, confusion, seizures, coma, delirium, blindness, and posterior reversible encephalopathy syndrome [97]. On the contrary, no clear relationship was identified between tacrolimus exposure and the development of post-transplant new-onset diabetes mellitus and hypertension [99]. Post-transplant new-onset diabetes mellitus is defined as the use of anti-diabetic therapy on day 30 post-transplantation in a transplant recipient not needing such treatment before transplantation [100]. In general, it is difficult to attribute causation of post-transplant new-onset diabetes mellitus and hypertension to tacrolimus therapy because other factors, such as concomitant use of prednisolone, may also predispose transplant recipients to these conditions. Studies with clear criteria for diagnosing adverse effects or toxicities due to tacrolimus are required to enhance the safety of tacrolimus use in heart transplant recipients [99].

1.7 Invasive fungal infection following heart transplantation

One complication following heart transplantation is invasive fungal infections. Invasive fungal infections are uncommon, with the cumulative incidence ranging from 4% to 11% (reporting years 2001−2012) during the first-year post-heart transplant [101, 102]. These infections are mainly caused by *Candida* and *Aspergillosis* species [101, 102]. If invasive fungal infection occurs, it is usually during the first 3−6 months post-transplant, and it is associated with significant morbidity and mortality [101, 103, 104]. Given the low incidence of invasive fungal infections, antifungal prophylaxis is not recommended routinely in heart transplant recipients [105]. However, heart transplant recipients who are at high risk (re-operation, cytomegalovirus infection, post-transplantation haemodialysis, use of broad-spectrum antibiotics, prolonged duration of antibiotic use, presence of central venous catheters, use of extracorporeal membrane oxygenation and exposure to high environmental load of *Aspergillus* species in the intensive care unit) of developing an invasive fungal infection are usually prescribed an antifungal [105, 106]. Of note, all heart transplant recipients at St. Vincent's Hospital Sydney are transferred to the intensive care unit for close monitoring following transplantation. Therefore, all of them receive prophylactic azole antifungal therapy attributed to the time spent in the intensive care unit.

1.7.1 Antifungal therapies

In the absence of randomised controlled trials, the best antifungal therapy, optimal dosing regimen and duration of dosing to prescribe heart transplant recipients in reducing the risk of invasive fungal infection post-transplantation remains unknown [104]. The choice and duration of antifungal therapy currently depend on the clinician's

discretion, local epidemiology or sensitivities of fungi, efficacy and toxicology profile of candidate antifungals, concomitant drug interactions, cost, and types of formulation available [104].

1.7.2 Tacrolimus-azole antifungal interaction

Tacrolimus is a substrate of CYP3A5, CYP3A4 and P-glycoprotein that are expressed in the intestine and liver. Therefore, tacrolimus is susceptible to drug-drug interactions with inducers and inhibitors of CYP3A5, CYP3A4, and P-glycoprotein that may significantly affect systemic tacrolimus concentration. One class of drugs that have demonstrated clinically significant interactions with tacrolimus are the oral azole antifungals [107-113]. In a recent multi-centre (n=150) study conducted in the United States in hospitalised patients, tacrolimus was in the top 15 of drugs that interacted with azole antifungals (voriconazole and posaconazole) [114].

Azole antifungals inhibit P-glycoprotein and CYP3A4 enzymes, thereby (1) increasing oral absorption of tacrolimus and (2) reducing the metabolism of tacrolimus in the intestine and liver [107, 108, 115]. This results in increased tacrolimus bioavailability [116] and reduced apparent clearance [117, 118]. Consequently, a profound increase in systemic tacrolimus concentration is observed, requiring a substantial tacrolimus dose reduction (20−80%) [109-113, 115, 117-120] to avoid toxicity in transplant recipients. The potency of CYP3A4 enzyme and P-glycoprotein inhibition varies between azole antifungals. Ketoconazole is the most potent inhibitor of CYP3A4 enzymes and P-glycoproteins, followed by itraconazole > voriconazole > fluconazole [119, 121-123].

The interaction between tacrolimus and azole antifungals is usually observed within 24 hours of co-administration [117, 120, 124]. The interaction persists for up to 7−10 days [117, 120, 125] once azole antifungal is discontinued due to the long half-life of some azole antifungals (e.g.: itraconazole). Therefore, one should also be cautious when withdrawing azole antifungals as tacrolimus concentrations may decrease rapidly, leading to acute graft rejection. Upon initiation and withdrawal of concomitant azole antifungal therapy, tacrolimus concentrations should be monitored 2−3 times for the first week [124], and as required for the subsequent weeks, to avoid over- and under-dosing of tacrolimus, respectively. The frequency of monitoring also depends on the transplant recipient's overall clinical assessment by the transplant clinician [124].

1.8 The utilisation of population pharmacokinetic model-based Bayesian estimation to inform tacrolimus therapy

1.8.1 Fundamental principles of pharmacokinetic modelling

Over the last two decades, population pharmacokinetic modelling has been performed to characterise tacrolimus pharmacokinetics in various organ transplant recipient populations. In population pharmacokinetic modelling, sparse (one or a few concentrations across a dosing interval) or intensive (≥6 samples over a dosing interval [86]) blood sampling data are collected from a group of subjects to estimate typical population pharmacokinetic parameters (e.g., clearance and volume of distribution), between-subject variability (BSV), between-occasion variability (BOV) and residual unexplained variability (RUV; e.g., analytical assay error, uncertain drug concentration sampling/dosing times, model misspecification) [126, 127]. The influence of clinical determinants (covariates) on pharmacokinetic parameters are assessed simultaneously

[128]. The final population pharmacokinetic model can then be used to facilitate individualised drug therapy that is predicted to achieve a target drug concentration. In short, a population pharmacokinetic model is a mathematical model, describing the pharmacokinetics of a drug consumed by a patient population and the relationship between drug concentration and drug doses in the body over time [129, 130].

1.8.2 Clinical utility of population pharmacokinetic model-based Bayesian estimation

Currently, the successful implementation of TDM is hampered by the difficulty in collecting trough concentrations. Alternatively, there is growing evidence in the literature to support the use of population pharmacokinetic model-based Bayesian estimation (1) for dose individualisation and (2) to facilitate the widespread implementation of TDM [131]. These studies have demonstrated improved achievement of target drug concentrations for immunosuppressants [100, 132-134]. The population pharmacokinetic model-based Bayesian estimation approach uses a population pharmacokinetic model together with patient-specific information (e.g., weight and height) and at least one drug concentration to provide individualised (1) estimates of pharmacokinetic parameters and (2) dose recommendations that are predicted to achieve target drug concentrations (Figure 5) [135-137].

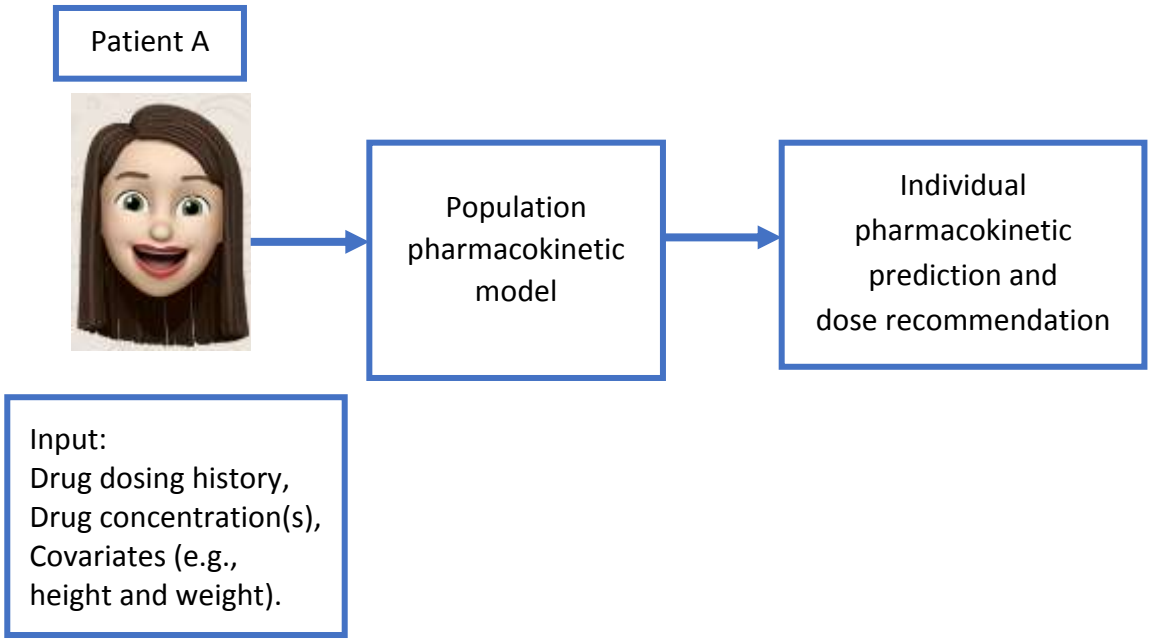


Figure 5. The principles of population pharmacokinetic model-based Bayesian estimation to individualise drug dosing and inform therapeutic drug monitoring. Patient-specific information and at least one drug concentration are inputted into the population pharmacokinetic model to estimate individual patient pharmacokinetic parameters. From this, an optimal drug dose predicted to achieve target drug concentrations is identified.

There are several advantages to using population pharmacokinetic model-based Bayesian estimation to guide tacrolimus dosing. Apart from providing flexible TDM sampling schemes [130], its use could assist in estimating tacrolimus trough concentrations, if the drug concentration collected is not a trough concentration. Additionally, the population pharmacokinetic model-based Bayesian estimation could predict the variation in tacrolimus pharmacokinetics over time provided the model included a time-varying covariate. Further, the population pharmacokinetic model-based Bayesian estimation can be used to simulate an alternative dosing regimen to achieve a pre-determined therapeutic range. Instead of collecting intensive blood

samples to calculate individual tacrolimus AUC_{0-12} using the trapezoidal rule (reference method), population pharmacokinetic model-based Bayesian estimation enables the estimation of individual AUC_{0-12} based on a limited number of drug concentrations collected over a dosing interval [80, 138]. This allows for the transition from trough concentration-guided dosing to AUC-guided dosing given that AUC is a better surrogate endpoint for treatment efficacy. With this promising dosing method, the optimal AUC therapeutic range could be achieved and the evidence for safer, more effective therapy investigated.

1.9 Thesis aims

A plethora (n=74) of population pharmacokinetic models of tacrolimus is available [32, 82, 139-142]. However, there are still unanswered questions related to their clinical use to guide tacrolimus dosing decisions, particularly in heart transplant recipients (less well-studied transplant recipient population [32]). While pharmacokinetic modelling methods for tacrolimus in transplantation are crucial for the quantitation and prediction for new clinical scenarios [54], most models were developed for descriptive purposes, without their predictive capability being reported [54]. The generalisability of existing tacrolimus population pharmacokinetic models to describe tacrolimus pharmacokinetics in heart transplant recipients, predictive performances of the models accommodating for the effect of tacrolimus-azole antifungal interaction in heart transplant recipients and improvement in long-term transplant recipient outcomes following the use of those models to guide tacrolimus dosing, are unknown. Therefore, this thesis aimed to answer some of these questions.

The overall aim of this thesis is to investigate the individualisation of tacrolimus therapy in heart transplant recipients using the pharmacokinetic modelling approach. Specifically, the following investigations have been undertaken:

1. Exploration of tacrolimus dosing and monitoring practices in heart transplant recipients (Chapter 2).
2. Evaluation of the predictive performance of a commercially available Bayesian dosing software to predict tacrolimus concentrations in adult heart transplant recipients (Chapter 2).
3. Identification and summary of published population pharmacokinetic models of tacrolimus developed from adult transplant recipients (Chapter 3).
4. Determination of the predictive performances of relevant population pharmacokinetic models of tacrolimus to predict tacrolimus concentrations in adult heart transplant recipients, stratified based on concomitant azole antifungal use (Chapter 4).
5. Development and evaluation of a population pharmacokinetic model for tacrolimus in heart transplant recipients, considering the effect of tacrolimus-azole antifungal interaction (Chapter 5).

A flow chart of this thesis is displayed in Figure 6.

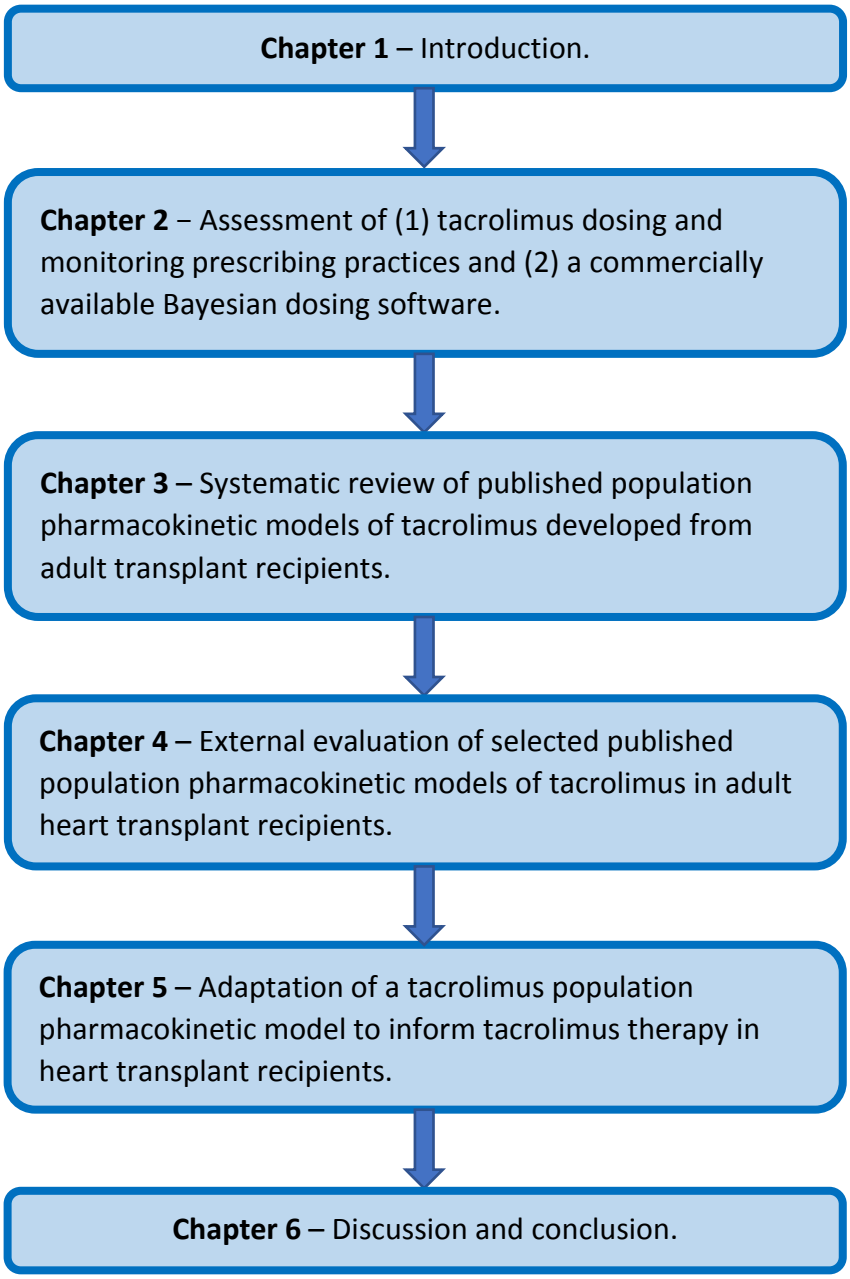


Figure 6. Thesis flow chart.

1.10 Addressing the research gaps

Current dosing and monitoring practices of tacrolimus in adult heart transplant recipients warrant further optimisation. To address this, I conducted a program of work using the pharmacokinetic modelling approach, including a systematic review. In the

following chapters, I present a sequence of studies that ultimately led to the development of a population pharmacokinetic model to facilitate tacrolimus dose individualisation in heart transplant recipients. This model is the first dosing support tool in the world that comprehensively describes tacrolimus pharmacokinetics in heart transplant recipients, considering clinically significant tacrolimus-azole antifungal interaction, over one-year post-transplant. I hope that the model will benefit (1) heart transplant recipients, leading to improved clinical outcomes and (2) transplant clinicians in overcoming the challenges with dosing tacrolimus, particularly in the presence of concomitant azole antifungal therapy.

References

1. Khush KK, Potena L, Cherikh WS, Chambers DC, Harhay MO, Hayes D, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: 37th adult heart transplantation report—2020; focus on deceased donor characteristics. J Heart Lung Transplant. 2020;39(10):1003-15.

2. Khush KK, Cherikh WS, Chambers DC, Goldfarb S, Hayes D, Kucheryavaya AY, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult heart transplantation report—2018; focus theme: multiorgan transplantation. J Heart Lung Transplant. 2018;37(10):1155-68.

3. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report—2019; focus theme: Donor and recipient size match. J Heart Lung Transplant. 2019;38(10):1056-66.

4. Keogh A, Williams T, Pettersson R. Australia and New Zealand Cardiothoracic Organ Transplant Registry: Twenty Third Annual Report 1984–2018. 2018:1-60.

5. Baron D, Giral M, Brouard S. Reconsidering the detection of tolerance to individualize immunosuppression minimization and to improve long-term kidney graft outcomes. Transpl Int. 2015;28(8):938-59.

6. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus Therapy in Adult Heart Transplant Recipients: Evaluation of a Bayesian Forecasting Software. Ther Drug Monit. 2021;43(6):736-46.

7. Scheffert JL, Raza K. Immunosuppression in lung transplantation. J Thorac Dis. 2014;6(8):1039-53.

8. Grimm M, Rinaldi M, Yonan N, Arpesella G, Arizón Del Prado J, Pulpón L, et al. Superior prevention of acute rejection by tacrolimus vs. cyclosporine in heart transplant recipients—a large European trial. Am J Transplant. 2006;6(6):1387-97.

9. Guethoff S, Meiser BM, Groetzner J, Eifert S, Grinninger C, Ueberfuhr P, et al. Ten-year results of a randomized trial comparing tacrolimus versus cyclosporine a in combination with mycophenolate mofetil after heart transplantation. Transplantation. 2013;95(4):629-34.

10. Dandel M, Lehmkuhl HB, Knosalla C, Hetzer R. Impact of different long-term maintenance immunosuppressive therapy strategies on patients' outcome after heart transplantation. Transpl Immunol. 2010;23(3):93-103.

11. Shuker N, van Gelder T, Hesselink DA. Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management. Transplant Rev. 2015;29(2):78-84.

12. Knoop C, Thiry P, Saint-Marcoux F, Rousseau A, Marquet P, Estenne M. Tacrolimus pharmacokinetics and dose monitoring after lung transplantation for cystic fibrosis and other conditions. Am J Transplant. 2005;5(6):1477-82.

13. Kobashigawa J, Miller L, Renlund D, Mentzer R, Alderman E, Bourge R, et al. A randomized active-controlled trial of mycophenolate mofetil in heart transplant recipients. Transplantation. 1998;66(4):507-15.

14. Kobashigawa JA, Renlund DG, Gerosa G, Almenar L, Eisen HJ, Keogh AM, et al. Similar efficacy and safety of enteric-coated mycophenolate sodium (EC-MPS, myfortic) compared with mycophenolate mofetil (MMF) in de novo heart transplant recipients: results of a 12-month, single-blind, randomized, parallel-group, multicenter study. J Heart Lung Transplant. 2006;25(8):935-41.

15. Gardiner KM, Tett SE, Staatz CE. Is conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium justifiable for gastrointestinal quality of life? Drugs R D. 2018;18(4):271-82.

16. Beckebaum S, Cicinnati VR. Conversion to combined mycophenolate mofetil and low-dose calcineurin inhibitor therapy for renal dysfunction in liver transplant patients: never too late? Dig Dis Sci. 2011;56(1):4-6.

17. Kim IC, Youn JC, Kobashigawa JA. The Past, Present and Future of Heart Transplantation. Korean Circ J. 2018;48(7):565-90.

18. Kittleson MM, Patel JK, JA. K. Chapter 72: cardiac transplantation. Hurst's the Heart. 2017;14th ed. New York (NY)(McGraw-Hill).

19. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

20. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

21. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

22. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

23. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

24. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

25. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

26. Thomson A, Bonham C, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. Ther Drug Monit. 1995;17(6):584-91.

27. Bowman LJ, Brennan DC. The role of tacrolimus in renal transplantation. Expert Opin Pharmacother. 2008;9(4):635-43.

28. Moreau A, Varey E, Anegon I, Cuturi M-C. Effector mechanisms of rejection. Cold Spring Harb Perspect Med. 2013;3(11):a015461.

29. Heidt S, Roelen DL, Eijsink C, Eikmans M, Van Kooten C, Claas FH, et al. Calcineurin inhibitors affect B cell antibody responses indirectly by interfering with T cell help. Clin Exp Immunol. 2010;159(2):199-207.

30. Möller A, Iwasaki K, Kawamura A, Teramura Y, Shiraga T, Hata T, et al. The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos. 1999;27(6):633-6.

31. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

32. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review. Clin Pharmacokinet. 2020;59(11):1357-92.

33. Saint-Marcoux F, Knoop C, Debord J, Thiry P, Rousseau A, Estenne M, et al. Pharmacokinetic study of tacrolimus in cystic fibrosis and non-cystic fibrosis lung transplant patients and design of Bayesian estimators using limited sampling strategies. Clin Pharmacokinet. 2005;44(12):1317-28.

34. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA, et al. Pharmacokinetics of tacrolimus in liver transplant patients. Clin Pharmacol Ther. 1995;57(3):281-90.

35. Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Furakawa H, Imventarza O, et al. Pharmacokinetics of FK 506 following oral administration: a comparison of FK 506 and cyclosporine. Transplant Proc. 1991;23(1 Pt 2):931-3.

36. Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Alessiani M, Lever J, et al. Pharmacokinetics of FK 506 in transplant patients. Transplant Proc. 1991;23(6):2736-40.

37. Jain A, Venkataramanan R, Todo S, Abu-Elmagd K, Fung J, Warty V, et al. Intravenous, oral pharmacokinetics, and oral dosing of FK 506 in small bowel transplant patients. Transplant Proc. 1992;24(3):1181-2.

38. Tuteja S, Alloway RR, Johnson JA, Gaber AO. The effect of gut metabolism on tacrolimus bioavailability in renal transplant recipients. Transplantation. 2001;71(9):1303-7.

39. Benet LZ. Impact of intraindividual variability of drugs on therapeutic outcomes: lessons from outside transplantation. Transplant Proc. 1998;30(5):1650-1.

40. Zhang Y, Benet LZ. The gut as a barrier to drug absorption. Clin Pharmacokinet. 2001;40(3):159-68.

41. Gertz M, Harrison A, Houston JB, Galetin A. Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. Drug Metab Dispos. 2010;38(7):1147-58.

42. Oda K, Yamano K. Effect of telaprevir on the metabolism and hepatic uptake of tacrolimus (FK506). Biopharm Drug Dispos. 2014;35(9):501-12.

43. Vanhove T, Annaert P, Kuypers DR. Clinical determinants of calcineurin inhibitor disposition: a mechanistic review. Drug Metab Rev. 2016;48(1):88-112.

44. Mekki Q, Lee C, Carrier S, Klintmaim G, Shaefer M, Burke P, et al. The effect of food on oral bioavailability of tacrolimus (FK506) in liver-transplant patients. Clin Pharmacol Ther. 1993;53(2):229.

45. Bekersky I, Dressler D, Mekki Q. Effect of time of meal consumption on bioavailability of a single oral 5 mg tacrolimus dose. J Clin Pharmacol. 2001;41(3):289-97.

46. Iwasaki K. Metabolism of tacrolimus (FK506) and recent topics in clinical pharmacokinetics. Drug Metab Pharmacokinet. 2007;22(5):328-35.

47. Marfo K, Altshuler J, Lu A. Tacrolimus pharmacokinetic and pharmacogenomic differences between adults and pediatric solid organ transplant recipients. Pharmaceutics. 2010;2(3):291-9.

48. Zahir H, Nand R, Brown K, Tattam B, McLachlan A. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. J Pharmacol Toxicol Methods. 2001;46(1):27-35.

49. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

50. Nagase K, Iwasaki K, Nozaki K, Noda K. Distribution and protein binding of FK506, a potent immunosuppressive macrolide lactone, in human blood and its uptake by erythrocytes. J Pharm Pharmacol. 1994;46(2):113-7.

51. Storset E, Holford N, Midtvedt K, Bremer S, Bergan S, Asberg A. Importance of hematocrit for a tacrolimus target concentration strategy. Eur J Clin Pharmacol. 2014;70(1):65-77.

52. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623-53.

53. Alak A, Moy S. Biological activity of tacrolimus (FK506) and its metabolites from whole blood of kidney transplant patients. Transplant Proc. 1997;29(5):2487-90.

54. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit. 2019;41(3):261-307.

55. Chitnis SD, Ogasawara K, Schniedewind B, Gohh RY, Christians U, Akhlaghi F. Concentration of tacrolimus and major metabolites in kidney transplant recipients as a function of diabetes mellitus and cytochrome P450 3A gene polymorphism. Xenobiotica. 2013;43(7):641-9.

56. Iwasaki K, Shiraga T, Nagase K, Tozuka Z, Noda K, Sakuma S, et al. Isolation, identification, and biological activities of oxidative metabolites of FK506, a potent immunosuppressive macrolide lactone. Drug Metab Dispos. 1993;21(6):971-7.

57. Zegarska J, Hryniewiecka E, Zochowska D, Samborowska E, Jazwiec R, Maciej K, et al. Evaluation of the relationship between concentrations of tacrolimus metabolites, 13-O-demethyl tacrolimus and 15-O-demethyl tacrolimus, and clinical and biochemical parameters in kidney transplant recipients. Transplant Proc. 2018;50(7):2235-9.

58. Benet LZ, Zia-Amirhosseini P. Basic principles of pharmacokinetics. Toxicologic pathology. 1995;23(2):115-23.

59. Prograf®. Northbrook, IL: AstellasPharma US, Inc. 2020.

60. Asberg A, Midtvedt K, van Guilder M, Storset E, Bremer S, Bergan S, et al. Inclusion of CYP3A5 genotyping in a nonparametric population model improves dosing of tacrolimus early after transplantation. Transpl Int. 2013;26(12):1198-207.

61. Birdwell KA, Decker B, Barbarino JM, Peterson JF, Stein CM, Sadee W, et al. Clinical pharmacogenetics implementation consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. Clin Pharmacol Ther. 2015;98(1):19-24.

62. Tszyrsznic W, Borowiec A, Pawlowska E, Jazwiec R, Zochowska D, Bartlomiejczyk I, et al. Two rapid ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) methods with common sample pretreatment for therapeutic drug monitoring of immunosuppressants compared to immunoassay. J Chromatogr B Analyt Technol Biomed Life Sci. 2013;928:9-15.

63. Therapeutic Goods Administration. Tacrolimus. Canberra: Therapeutic Goods Administration. 2010.

https://www.ebs.tga.gov.au/ebs/picmi/picmirepository.nsf/PICMI?OpenForm&t=&q=tacrolimus. Accessed 31 March 2021.

64. Denhaerynck K, Dobbels F, Cleemput I, Desmyttere A, Schäfer-Keller P, Schaub S, et al. Prevalence, consequences, and determinants of nonadherence in adult renal transplant patients: a literature review. Transpl Int. 2005;18(10):1121-33.

65. Pennington CA, Park JM. Sublingual tacrolimus as an alternative to oral administration for solid organ transplant recipients. Am J Health Syst Pharm. 2015;72(4):277-84.

66. Snell GI, Westall GP, Paraskeva MA. Immunosuppression and allograft rejection following lung transplantation: evidence to date. Drugs. 2013;73(16):1793-813.

67. Doligalski CT, Liu EC, Sammons CM, Silverman A, Logan AT. Sublingual administration of tacrolimus: current trends and available evidence. Pharmacotherapy. 2014;34(11):1209-19.

68. Porayko MK, Textor SC, Krom RA, Hay JE, Gores GJ, Richards TM, et al. Nephrotoxic effects of primary immunosuppression with FK-506 and cyclosporine regimens after liver transplantation. Mayo Clinic Proceedings. 1994;69(2):105-11.

69. Przepiorka D, Devine S, Fay J, Uberti J, Wingard J. Practical considerations in the use of tacrolimus for allogeneic marrow transplantation. Bone Marrow Transplant. 1999;24(10):1053-6.

70. Hebert MF, Zheng S, Hays K, Shen DD, Davis CL, Umans JG, et al. Interpreting tacrolimus concentrations during pregnancy and postpartum. Transplantation. 2013;95(7):908-15.

71. Zahir H, McCaughan G, Gleeson M, Nand R, McLachlan A. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. Br J Clin Pharmacol. 2004;57(3):298-309.

72. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Changes in tacrolimus distribution in blood and plasma protein binding following liver transplantation. Ther Drug Monit. 2004;26(5):506-15.

73. Sikma MA, Van Maarseveen EM, Hunault CC, Moreno JM, Van de Graaf EA, Kirkels JH, et al. Unbound Plasma, Total Plasma, and Whole-Blood Tacrolimus Pharmacokinetics Early After Thoracic Organ Transplantation. Clin Pharmacokinet. 2019;59(6):771–80.

74. Staatz CE, Tett SE. Clinical pharmacokinetics of once-daily tacrolimus in solid-organ transplant patients. Clin Pharmacokinet. 2015;54(10):993-1025.

75. Gustavsen MT, Midtvedt K, Robertsen I, Woillard JB, Debord J, Klaasen RA, et al. Fasting Status and Circadian Variation Must be Considered When Performing AUC-based Therapeutic Drug Monitoring of Tacrolimus in Renal Transplant Recipients. Clin Transl Sci. 2020;13(6):1327-35.

76. Fontova P, Colom H, Rigo-Bonnin R, van Merendonk LN, Vidal-Alabró A, Montero N, et al. Influence of the Circadian Timing System on Tacrolimus Pharmacokinetics and Pharmacodynamics After Kidney Transplantation. Front Pharmacol. 2021;12:326.

77. Dasari B, Hodson J, Nassir A, Widmer J, Isaac J, Mergentel H, et al. Variations in practice to therapeutic monitoring of tacrolimus following primary adult liver transplantation. Int J Organ Transplant Med. 2016;7(1):1-8.

78. Bouamar R, Shuker N, Hesselink DA, Weimar W, Ekberg H, Kaplan B, et al. Tacrolimus predose concentrations do not predict the risk of acute rejection after renal transplantation: a pooled analysis from three randomized-controlled clinical trials. Am J Transplant. 2013;13(5):1253-61.

79. Taylor DO, Barr ML, Meiser BM, Pham SM, Mentzer RM, Gass AL. Suggested guidelines for the use of tacrolimus in cardiac transplant recipients. J Heart Lung Transplant. 2001;20(7):734-8.

80. Woillard J-B, Saint-Marcoux F, Debord J, Åsberg A. Pharmacokinetic models to assist the prescriber in choosing the best tacrolimus dose. Pharmacol Res. 2018;130:316-21.

81. Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, de Lange DW. Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients. Eur J Drug Metab Pharmacokinet. 2018;43(3):311-20.

82. Marquet P, Destère A, Monchaud C, Rérolle J-P, Buchler M, Mazouz H, et al. Clinical Pharmacokinetics and Bayesian Estimators for the Individual Dose Adjustment of a Generic Formulation of Tacrolimus in Adult Kidney Transplant Recipients. Clin Pharmacokinet. 2021;60(5):611-22.

83. Decrocq-Rudler M-A, Meunier L, Fraisse J, Ursic-Bedoya J, Khier S. Can We Predict Individual Concentrations of Tacrolimus After Liver Transplantation? Application and Tweaking of a Published Population Pharmacokinetic Model in Clinical Practice. Ther Drug Monit. 2021;43(4):490-8.

84. Wong KM, Shek CC, Chau KF, Li CS. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. Am J Kidney Dis. 2000;35(4):660-6.

85. Tron C, Woillard J-B, Houssel-Debry P, David V, Jezequel C, Rayar M, et al. Pharmacogenetic—Whole blood and intracellular pharmacokinetic—Pharmacodynamic (PG-PK2-PD) relationship of tacrolimus in liver transplant recipients. PloS one. 2020;15(3):e0230195.

86. Kiang TK, Sherwin CM, Spigarelli MG, Ensom MH. Fundamentals of Population Pharmacokinetic Modelling. Clin Pharmacokinet. 2012;51(8):515-25.

87. Saint-Marcoux F, Woillard JB, Jurado C, Marquet P. Lessons from routine dose adjustment of tacrolimus in renal transplant patients based on global exposure. Ther Drug Monit. 2013;35(3):322-7.

88. Marquet P, Bedu A, Monchaud C, Saint-Marcoux F, Rerolle JP, Etienne I, et al. Pharmacokinetic Therapeutic Drug Monitoring of Advagraf in More Than 500 Adult Renal Transplant Patients, Using an Expert System Online. Ther Drug Monit. 2018;40(3):285-91.

89. Aumente MR, del Prado Arizón J, de Molina López MM, Cárdenas MA, Segura JS-G, López AG, et al. Clinical pharmacokinetics of tacrolimus in heart transplantation: new strategies of monitoring. Transplant Proc. 2003;35(5):1988-91.

90. Christians U, Vinks AA, Langman LJ, Clarke W, Wallemacq P, Van Gelder T, et al. Impact of laboratory practices on interlaboratory variability in therapeutic drug monitoring of immunosuppressive drugs. Ther Drug Monit. 2015;37(6):718-24.

91. Koster RA, Dijkers EC, Uges DR. Robust, high-throughput LC-MS/MS method for therapeutic drug monitoring of cyclosporine, tacrolimus, everolimus, and sirolimus in whole blood. Ther Drug Monit. 2009;31(1):116-25.

92. Cogill JL, Taylor PJ, Westley IS, Morris RG, Lynch SV, Johnson AG. Evaluation of the tacrolimus II microparticle enzyme immunoassay (MEIA II) in liver and renal transplant recipients. Clin Chem. 1998;44(9):1942-6.

93. Armstrong VW, Schuetz E, Zhang Q, Groothuisen S, Scholz C, Shipkova M, et al. Modified pentamer formation assay for measurement of tacrolimus and its active metabolites: comparison with liquid chromatography–tandem mass spectrometry and microparticle enzyme-linked immunoassay (MEIA-II). Clin Chem. 1998;44(12):2516-23.

94. Undre N, Schäfer A. Factors affecting the pharmacokinetics of tacrolimus in the first year after renal transplantation. Transplant Proc. 1998;30(4):1261-3.

95. Gong Y, Yang M, Sun Y, Li J, Lu Y, Li X. Population pharmacokinetic analysis of tacrolimus in Chinese cardiac transplant recipients. Eur J Hosp Pharm. 2020;27(e1):e012-18.

96. Christiaans M, Van Duijnhoven E, Beysens T, Undre N, Schäfer A. Effect of breakfast on the oral bioavailability of tacrolimus and changes in pharmacokinetics at different times posttransplant in renal transplant recipients. Transplant Proc. 1998;30(4):1271-3.

97. Wallemacq P, Armstrong VW, Brunet M, Haufroid V, Holt DW, Johnston A, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. Ther Drug Monit. 2009;31(2):139-52.

98. Randhawa PS, Starzl TE, Demetris AJ. Tacrolimus (FK506)-associated renal pathology. Adv Anat Pathol. 1997;4(4):265-76.

99. Braithwaite H, Darley D, Brett J, Day R, Carland J. Identifying the association between tacrolimus exposure and toxicity in heart and lung transplant recipients: A systematic review. Transplant Rev. 2021;35(2):100610.

100. Francke MI, Andrews LM, Le HL, van de Wetering J, Clahsen-van Groningen MC, van Gelder T, et al. Avoiding Tacrolimus Underexposure and Overexposure with a Dosing Algorithm for Renal Transplant Recipients: A Single Arm Prospective Intervention Trial. Clin Pharmacol Ther. 2021;110(1):169-78.

101. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). Clin Infect Dis. 2010;50(8):1101-11.

102. Echenique IA, Angarone MP, Gordon RA, Rich J, Anderson AS, McGee EC, et al. Invasive fungal infection after heart transplantation: A 7-year, single-center experience. Transpl Infect Dis. 2017;19(1):e12650.

103. Brett J, Chong O, Graham GG, Ray JE, Marriott D, Williams KM, et al. Antifungal use and therapeutic monitoring of plasma concentrations of itraconazole in heart and lung transplantation patients. Ther Drug Monit. 2013;35(1):133-6.

104. Husain S, Sole A, Alexander BD, Aslam S, Avery R, Benden C, et al. The 2015 International Society for Heart and Lung Transplantation Guidelines for the management of fungal infections in mechanical circulatory support and cardiothoracic organ transplant recipients: executive summary. J Heart Lung Transplant. 2016;35(3):261-82.

105. Muñoz P, Valerio M, Palomo J, Giannella M, Yañez JF, Desco M, et al. Targeted antifungal prophylaxis in heart transplant recipients. Transplantation. 2013;96(7):664-9.

106. Anesi JA, Baddley JW. Approach to the solid organ transplant patient with suspected fungal infection. Infect Dis Clin North Am. 2016;30(1):277-96.

107. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

108. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

109. Osowski CL, Dix SP, Lin LS, Mullins RE, Geller RB, Wingard JR. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. Transplantation. 1996;61(8):1268-72.

110. Mañez R, Martin M, Raman V, Silverman D, Jain A, Warty V, et al. Fluconazole therapy in transplant recipients receiving FK506. Transplantation. 1994;57(10):1521-3.

111. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic evaluation of the drug interaction between intravenous itraconazole and intravenous tacrolimus or

intravenous cyclosporin A in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2006;12(3):325-34.

112. Sansone-Parsons A, Krishna G, Martinho M, Kantesaria B, Gelone S, Mant TG. Effect of oral posaconazole on the pharmacokinetics of cyclosporine and tacrolimus. Pharmacotherapy. 2007;27(6):825-34.

113. Shitrit D, Ollech JE, Ollech A, Bakal I, Saute M, Sahar G, et al. Itraconazole prophylaxis in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. J Heart Lung Transplant. 2005;24(12):2148-52.

114. Andes D, Azie N, Yang H, Harrington R, Kelley C, Tan R-D, et al. Drug-drug interaction associated with mold-active triazoles among hospitalized patients. Antimicrob Agents Chemother. 2016;60(6):3398-406.

115. Gu TM, Lewis JS, Le H, Bubalo JS. Comparative effects of fluconazole, posaconazole, and isavuconazole upon tacrolimus and cyclosporine serum concentrations. J Oncol Pharm Pract. 2021:10781552211029046.

116. Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, et al. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. Clin Pharmacol Ther. 1997;62(1):41-9.

117. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

118. Darley D, Carlos L, Hennig S, Glanville A. Quantitation of the Effect of Azole Antifungals on Tacrolimus Clearance. J Heart Lung Transplant. 2016;35(4):S236.

119. Trofe-Clark J, Lemonovich T, Practice AIDCo. Interactions between anti-infective agents and immunosuppressants in solid organ transplantation. Am J Transplant. 2013;13(s4):318-26.

120. Moreno M, Latorre A, Manzanares C, Morales E, Herrero J, Dominguez-Gil B, et al. Clinical management of tacrolimus drug interactions in renal transplant patients. Transplant Proc. 1999;31(6):2252-3.

121. Zhang S, Pillai VC, Mada SR, Strom S, Venkataramanan R. Effect of voriconazole and other azole antifungal agents on CYP3A activity and metabolism of tacrolimus in human liver microsomes. Xenobiotica. 2012;42(5):409-16.

122. Kramer MR, Amital A, Fuks L, Shitrit D. Voriconazole and itraconazole in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. Clin Transplant. 2011;25(2):E163-7.

123. Kuypers DR, de Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. Pharmacogenet Genomics. 2008;18(10):861-8.

124. Page II RL, Mueller SW, Levi ME, Lindenfeld J. Pharmacokinetic drug–drug interactions between calcineurin inhibitors and proliferation signal inhibitors with antimicrobial agents: Implications for therapeutic drug monitoring. J Heart Lung Transplant. 2011;30(2):124-35.

125. Capone D, Gentile A, Imperatore P, Palmiero G, Basile V. Effects of itraconazole on tacrolimus blood concentrations in a renal transplant recipient. Ann Pharmacother. 1999;33(10):1124-5.

126. Mould D, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development—part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometr Syst Pharmacol. 2013;2(4):1-14.

127. Abrantes JA, Jönsson S, Karlsson MO, Nielsen EI. Handling interoccasion variability in model-based dose individualization using therapeutic drug monitoring data. Br J Clin Pharmacol. 2019;85(6):1326-36.

128. Aarons L. Population pharmacokinetics: theory and practice. Br J Clin Pharmacol. 1991;32(6):669-70.

129. Sheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters from routine clinical data. J Pharmacokinet Biopharm. 1977;5(5):445-79.

130. Zwart TC, Moes DJA, van der Boog PJ, van Erp NP, de Fijter JW, Guchelaar H-J, et al. Model-informed precision dosing of everolimus: external validation in adult renal transplant recipients. Clin Pharmacokinet. 2021;60(2):191-203.

131. Fuchs A, Csajka C, Thoma Y, Buclin T, Widmer N. Benchmarking therapeutic drug monitoring software: a review of available computer tools. Clin Pharmacokinet. 2013;52(1):9-22.

132. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

133. Fukudo M, Yano I, Shinsako K, Katsura T, Takada Y, Uemoto S, et al. Prospective evaluation of the bayesian method for individualizing tacrolimus dose early after living-donor liver transplantation. J Clin Pharmacol. 2009;49(7):789-97.

134. Åsberg A, Falck P, Undset LH, Dørje C, Holdaas H, Hartmann A, et al. Computer-assisted cyclosporine dosing performs better than traditional dosing in renal transplant recipients: results of a pilot study. Ther Drug Monit. 2010;32(2):152-8.

135. Meinshausen J, Bühl K, Engel G, Harings-Kaim A, Drewelow B, Klotz U. Generation of pharmacokinetic data during routine therapeutic drug monitoring: Bayesian approach vs. pharmacokinetic studies. Ther Drug Monit. 1993;15(4):281-8.

136. del Mar Fernández de Gatta M, Martin-Suarez A, Lanao JM. Approaches for dosage individualisation in critically ill patients. Expert Opin Drug Metab Toxicol. 2013;9(11):1481-93.

137. Sheiner LB, Beal S, Rosenberg B, Marathe VV. Forecasting individual pharmacokinetics. Clin Pharmacol Ther. 1979;26(3):294-305.

138. Campagne O, Mager DE, Tornatore KM. Population pharmacokinetics of tacrolimus in transplant recipients: what did we learn about sources of interindividual variabilities? J Clin Pharmacol. 2019;59(3):309-25.

139. Cai X, Song H, Jiao Z, Yang H, Zhu M, Wang C, et al. Population pharmacokinetics and dosing regimen optimization of tacrolimus in Chinese lung transplant recipients. Eur J Pharm Sci. 2020;152:105448.

140. Emoto C, Johnson TN, Hahn D, Christians U, Alloway RR, Vinks AA, et al. A Theoretical physiologically-based pharmacokinetic approach to ascertain covariates explaining the large interpatient variability in tacrolimus disposition. CPT Pharmacometr Syst Pharmacol. 2019;8(5):273-84.

141. Jing Y, Kong Y, Hou X, Liu H, Fu Q, Jiao Z, et al. Population pharmacokinetic analysis and dosing guidelines for tacrolimus co-administration with Wuzhi capsule in Chinese renal transplant recipients. J Clin Pharm Ther. 2021;46(4):1117-28.

142. Woillard JB, Labriffe M, Debord J, Marquet P. Tacrolimus exposure prediction using machine learning. Clin Pharmacol Ther. 2020.

Chapter 2:

Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software.

2.1 Link to thesis

In Chapter 1, I presented an overview of tacrolimus, including its pharmacokinetics and pharmacodynamics. I also discussed the challenges with current dosing and monitoring strategies for tacrolimus in transplant recipients. The population pharmacokinetic model-based Bayesian estimation approach provides an opportunity to overcome the known difficulties with current drug dosing and monitoring strategies, specifically (1) providing a flexible TDM sampling scheme and (2) selection of dosing regimen to achieve a pre-determined therapeutic range based on simulations. Prior to determining the suitability of a population pharmacokinetic model utilised by a Bayesian forecasting software to guide tacrolimus dosing in heart transplant recipients, it is important to understand the prescribing practices for tacrolimus dosing and monitoring at the transplant centre. In Chapter 2, I present (1) an exploration of tacrolimus dosing and monitoring practices in heart transplant recipients at St. Vincent's Hospital Sydney and (2) an assessment of a tacrolimus population pharmacokinetic model integrated within a Bayesian forecasting software to predict tacrolimus concentrations in the early post-heart transplantation phase. The ability of the model to adequately predict tacrolimus concentrations relative to the observed concentrations informs the suitability of the model to guide tacrolimus dosing in heart transplant recipients. To the best of my knowledge, no studies have been performed to (1) assess tacrolimus dosing and monitoring practices and (2) determine the ability of a Bayesian forecasting software to predict tacrolimus exposure in heart transplant recipients.

This work has been published:

Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus therapy in adult heart transplant recipients: Evaluation of a Bayesian forecasting software. *Therapeutic Drug Monitoring*. 2021;4(36):736−746.

This work has been presented at one international conference and two national conferences:

1. Kirubakaran R, Stocker SL, Day RO, Carland JE. Predictive performance of a Bayesian forecasting software for tacrolimus in adult heart transplant. Oral Presentation, PAGANZ, Auckland, New Zealand, 6−9 February 2019.
2. Kirubakaran R, Stocker SL, Day RO, Carland JE. Tacrolimus dosing and monitoring in heart transplant: A retrospective observational study. Poster Presentation, ASCEPT Annual Scientific Meeting, Adelaide, Australia, 27−30 November 2018.
3. Kirubakaran R, Stocker SL, Day RO, Carland JE. Predictive performance of a Bayesian forecasting software for tacrolimus in adult heart transplant. Poster Presentation, ASCEPT Annual Scientific Meeting, Adelaide, Australia, 27−30 November 2018.

2.2 Abstract

Background Therapeutic drug monitoring is recommended to guide tacrolimus dosing because of its narrow therapeutic window and considerable pharmacokinetic variability. This study assessed tacrolimus dosing and monitoring practices in heart transplant recipients and evaluated the predictive performance of a Bayesian forecasting software using a renal transplant-derived tacrolimus model to predict tacrolimus concentrations.

Methods A retrospective audit of heart transplant recipients (n=87) treated with tacrolimus was performed. Relevant data were collected from the time of transplant to discharge. The concordance of tacrolimus dosing and monitoring according to hospital guidelines was assessed. The observed and software-predicted tacrolimus concentrations (n=931) were compared for the first 3 weeks of oral immediate-release tacrolimus (Prograf®) therapy, and the predictive performance (bias and imprecision) of the software was evaluated.

Results The majority (96%) of initial oral tacrolimus doses were guideline concordant. Most initial intravenous doses (93%) were lower than the guideline recommendations. Overall, 36% of initial tacrolimus doses were administered to transplant recipients with an estimated glomerular filtration rate <60 mL/min/1.73 m^2 despite recommendations to delay the commencement of therapy. Of the tacrolimus concentrations collected during oral therapy (n=1,498), 25% were trough concentrations obtained at steady-state. The software displayed acceptable predictions of tacrolimus concentration from day 12 (bias: -6% [95% CI: -11.8 to 2.5], imprecision: 16% [95% CI: 8.7 to 24.3]) of therapy.

Conclusions Tacrolimus dosing and monitoring were discordant with the guidelines. The Bayesian forecasting software was suitable for guiding tacrolimus dosing after 11 days of therapy in heart transplant recipients. Understanding the factors contributing to the variability in tacrolimus pharmacokinetics immediately post-transplant may help improve software predictions.

2.3 Background

Tacrolimus is the first-choice calcineurin inhibitor used in most solid-organ transplant recipients, including heart transplants, to prevent graft rejection [1]. Due to its narrow therapeutic window [2] and considerable inter- and intra-patient pharmacokinetic variability [3-9], therapeutic drug monitoring (TDM) of steady-state trough concentrations is recommended to guide tacrolimus therapy. The requirement for the collection of timed trough concentrations is a barrier to achieving the maximum value from the implementation of tacrolimus TDM in routine clinical practice. This is mainly because of the difficulties in obtaining TDM samples at the required time within a dosing interval and at steady-state, possibly due to the busy clinical environment, particularly early after transplantation [10]. An audit of tacrolimus TDM practice in hospitalised adult liver transplant recipients observed that only 5% of tacrolimus concentrations were collected at the guideline-recommended time [11]. Incorrect timing of TDM samples wastes resources, inconveniences patients, and potentially results in the misinterpretation of tacrolimus concentrations and consequently, inappropriate dose recommendations [12]. The assessment of tacrolimus dosing and monitoring practices against guideline recommendations is important to ensure the safe and effective use of tacrolimus and to identify any necessary changes required to improve current practice. To the best of our knowledge, no studies have assessed this, particularly in the early post-transplant period in heart transplant recipients.

An alternative to the trough concentration-guided dosing approach is the use of Bayesian forecasting software to individualise tacrolimus therapy for transplant recipients. Bayesian forecasting is currently considered the gold standard approach to

TDM [13]. Bayesian forecasting uses patient demographics (e.g., sex, height, and weight), available drug concentration(s), and population pharmacokinetic model parameter estimates to provide individualised predictions of drug exposure and dose recommendations [14, 15]. Its ability to interpret drug concentrations collected at any time point, including before steady-state is achieved, can promote earlier achievement of target drug exposure with fewer dose adjustments [10]. Bayesian-guided tacrolimus dosing has been shown to significantly improve the achievement of target concentrations in renal transplant recipients in comparison to standard trough concentration-guided dosing in the first 8 weeks post-transplant [10]. Of note, Bayesian forecasting software is currently not used to guide tacrolimus dosing decisions at our institution.

Several commercially available Bayesian forecasting software programs have been developed to facilitate the practice of TDM and dose individualisation for immunosuppressants [13]. Of these, DoseMeRx™ (Moorestown, NJ, USA) is the only software that has been approved by the Therapeutic Goods Administration (TGA) in Australia for implementation into clinical practice. Only one population pharmacokinetic model for tacrolimus was integrated into the software. The model was developed from renal transplant recipients [16]. The model was constructed as a 2-compartment Erlang model with first-order elimination [16]. The model simultaneously described the pharmacokinetic disposition for the oral immediate-release (Prograf®, Levallois-Perret, France) and prolonged-release (Advagraf®, Levallois-Perret, France) formulations of tacrolimus [16], the two most common formulations used in clinical practice. The ability

of this software to predict tacrolimus exposure in heart transplant recipients has not yet been evaluated.

This study aimed to assess the concordance of tacrolimus dosing and monitoring for heart transplant recipients at St. Vincent's Hospital Sydney (SVHS) according to hospital guidelines, and second, to evaluate the predictive performance of a commercially available Bayesian forecasting software to predict oral immediate-release tacrolimus (Prograf®) concentrations in heart transplant recipients.

2.4 Materials and Methods

2.4.1 Subjects and data collection

This was a retrospective audit of tacrolimus therapy for patients who underwent heart transplant surgery between January 1, 2017, and December 31, 2018, at SVHS, one of four adult heart transplant centres in Australia. This study was approved by the SVHS Human Research Ethics Committee (LNR/18/SVH/178). A waiver of consent was obtained due to the retrospective nature of the study. Relevant data were collected from electronic medical records, including transplant recipient demographics (age, sex, weight, and height), length of hospital stay, concomitant medications (basiliximab, azole antifungals, corticosteroids, and mycophenolate), tacrolimus dosing and monitoring history (dosing regimen, dose administration times, tacrolimus concentrations, and TDM sample collection times), and laboratory investigations (haematocrit [fraction] and estimated glomerular filtration rate [eGFR]). The data were collected from immediately after heart transplant until discharge. Given that data were collected from hospitalised transplant recipients, adherence to tacrolimus therapy was not a concern. While being

an inpatient, nurses are largely responsible for administering medications to transplant recipients.

Whole blood tacrolimus concentrations were measured using an ultra-high performance liquid chromatography-tandem mass spectroscopy (UHPLC-MS/MS) assay. The assay range was between 2 and 50 µg/L, with an intra-assay coefficient of variation (CV) <8.5% and inter-day CV <10%.

2.4.2 Genotyping

Cytochrome P450 (CYP) *3A5**3 (rs776746) genotype information was prospectively collected to evaluate the commercially available Bayesian forecasting software. Heart transplant recipients who were alive at the time of the study were approached, and consent was obtained from those who agreed to participate in this study. This study was approved by the local governance and the SVHS Human Research Ethics Committee (2020/ETH00679).

Genomic DNA was extracted from buccal swabs using the Applied Biosystems DNA Multi-sample Ultra Kit on the Kingfisher 96 instrument (Thermo Fisher Scientific, Waltham MA, USA) and was stored at 4°C until analysis. The *CYP3A5**3 genotype was identified using specific TaqMan Real Time PCR assays on a Quantstudio 6 K instrument (Thermo Fisher Scientific).

2.4.3 Assessment of concordance of tacrolimus dosing with institutional guidelines

At the time of the study, hospital guidelines were available to inform tacrolimus dosing decisions (Table 4). Postoperatively, a fixed test dose of 0.5 mg oral immediate-release formulation of tacrolimus, Prograf® (Astellas, Macquarie Park, Australia), was administered via the oral route or a nasogastric tube unless the transplant recipient's eGFR was <60 mL/min/1.73 m^2. If the eGFR was <60 mL/min/1.73 m^2, the commencement of tacrolimus therapy was delayed until the eGFR was ≥60 mL/min/1.73 m^2. Subsequent tacrolimus doses were administered every 12 hours. These doses were adjusted based on tacrolimus steady-state trough concentrations and the overall clinical assessment (e.g., renal function, graft function, rejection history, and hemodynamic status) of the transplant recipient. If the transplant recipients were unable to commence oral therapy, intravenous (IV) tacrolimus (0.015 mg/kg/day) was infused over 24 hours and subsequent continuous IV infusion doses were adjusted based on steady-state tacrolimus concentrations and overall clinical assessment of the transplant recipient. IV regimens were continued until the transplant recipients were able to commence oral therapy. Concordance of tacrolimus dosing with hospital guidelines was assessed for (i) initial tacrolimus dose (oral test dose and IV) and (ii) initial tacrolimus dose relative to eGFR <60 mL/min/1.73 m^2. Transplant recipients who were on renal replacement therapy (e.g., dialysis) when tacrolimus therapy was initiated were excluded from the latter analysis.

2.4.4 Assessment of concordance of tacrolimus monitoring with institutional guidelines

Consistent with international guidelines, hospital guidelines recommend TDM of trough concentrations to guide tacrolimus dosing decisions for heart transplant recipients. Although the frequency of TDM sample collection is not explicitly stated in the hospital guidelines, daily tacrolimus concentrations are collected from heart transplant recipients, while in the intensive care unit (ICU). Once transferred to a general ward, TDM samples were collected at least twice weekly or based on the transplant recipient's overall clinical assessment.

Table 4. Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients.

Dosing		
	Formulation	
	Oral	**Intravenous***
Initial dose	0.5 mg (fixed test dose)	0.015 mg/kg/day infused over 24 hours
Maintenance dose	Administered every 12 hours. Dose adjusted based on steady-state trough concentration(s)** and transplant recipient's overall clinical assessment.	Administered as a continuous infusion over 24 hours. Dose adjusted based on steady-state tacrolimus concentration(s) and transplant recipient's overall clinical assessment.
Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2	Delay commencement of initial dose until eGFR is ≥60 mL/min/1.73 m^2.	
Monitoring		
Initial dose	Check trough concentration.	Check steady-state tacrolimus concentration.
Maintenance dose	Check trough concentration at steady-state**, frequency of monitoring based on transplant recipient's overall clinical assessment.	Check steady-state tacrolimus concentration twice weekly or based on transplant recipient's overall clinical assessment.
Therapeutic range of trough concentration (Time post-transplant)***	8−12 µg/L (day 1 – day 7) 10−15 µg/L (day 8 − 3 months) 8−12 µg/L (>3 − 12 months) 6−8 µg/L (>12 months)	

*In Australia, intravenous tacrolimus is licensed to be administered only as a continuous infusion and not to be administered as a bolus, intermittent, or short infusion [17].

**At least 48 hours after initiation of maintenance therapy or any dose adjustment.

***To achieve the same drug exposure, the drug concentration targeted while a transplant recipient is on continuous infusion of tacrolimus will be higher than the drug concentration targeted while a transplant recipient receives the oral twice-daily dosing. In our institute, clinicians target at least the higher end of the therapeutic range specified in the guideline table if a transplant recipient receives an intravenous infusion.

Concordance of tacrolimus TDM with hospital guidelines (Table 4) was assessed for courses of oral tacrolimus therapy administered to transplant recipients during their hospitalisation. Both the (i) concordance of TDM sample collection time with guidelines and (ii) percentage of trough concentrations collected at steady-state (at least 48 hours after initiation of maintenance therapy or any dose adjustment) were evaluated. Since the oral immediate-release formulation of tacrolimus was administered every 12 hours, a trough concentration was defined as the tacrolimus concentration collected between 11 and 15 hours after the last administered dose and just before the next administered dose. Trough concentrations obtained at steady-state were further assessed for the attainment of target drug concentrations. The desired therapeutic ranges of trough concentrations varied according to the time post-transplant (Table 4).

2.4.5 Evaluation of a Bayesian forecasting software

The commercially available Bayesian forecasting software, DoseMeRx™ (Moorestown, NJ, USA), was used to predict oral immediate-release tacrolimus (Prograf®) concentrations for the first 3 weeks of therapy. The population pharmacokinetic model for tacrolimus integrated within the software is an existing model [16] built by another research group using data collected from renal transplant recipients (Appendix 1).

The software does not accommodate IV formulations of tacrolimus. Therefore, all IV doses were converted to oral dose equivalents using a 1:5 (IV: oral) ratio [18] to enable input into the software. This conversion ratio reflects the average oral bioavailability of tacrolimus, which is between 20% and 25% [19]. The converted IV doses were inputted as a single dose. Transplant recipients were included in the evaluation if they had at least

two tacrolimus concentrations measured. Tacrolimus concentrations <2 µg/L were fixed at 2 µg/L, as this was the minimum concentration allowed by the software based on our institutional setting for assay quantification. The information required to generate individual pharmacokinetic parameter estimates of tacrolimus included age, sex, weight, height (optional), haematocrit, and recipient CYP3A5 genotype. The software limits for this model were age: 18−100 years, weight: 50−200 kg, and height: 150−220 cm. It was outside the scope of this study to adjust software limits. For transplant recipients whom the CYP3A5 genotype information was unavailable, the software automatically selected the default genotype *CYP3A5**3/*3 (non-expressers). Most of the transplant recipients in this study self-identified as Caucasian, therefore this assumption is consistent with the estimated allele frequency (0.82–0.95) of *CYP3A5**3/*3 in Caucasians [20, 21].

The predictive performance (bias and imprecision) of the software was evaluated by comparing the software-predicted tacrolimus concentrations to the observed concentrations. This was performed by inputting data up to the day of tacrolimus therapy before predicting the next drug concentration. This process was performed for all tacrolimus concentrations collected from each transplant recipient. Although all available drug concentrations were inputted into the software, the predictive performance of the software was evaluated based on predictions following the administration of oral tacrolimus dosing (drug concentration predictions following IV administration were excluded). The weighting of recent tacrolimus concentrations addressed the changes in tacrolimus pharmacokinetics over time.

Bias (median percentage predictive error, Equation 1) and imprecision (median absolute percentage predictive error, Equation 2) were calculated [22, 23] using R software (version 3.6.1; R Foundation for Statistical Computing, http://www.R-project.org). A positive or negative bias represents an over- or under-prediction of tacrolimus concentration using the software, respectively. Clinically acceptable bias was defined as within ±20% because this is the deviation from the targeted value that would clinically warrant a dose adjustment [24, 25]. The prediction was unbiased if the 95% confidence interval (CI) included zero. The clinically acceptable imprecision was ≤20%.

$$\text{Bias (\%)} = \text{Median} \sum \frac{(Individual\ predicted - Observed)}{Observed} \times 100 \qquad (\textit{Equation 1})$$

$$\text{Imprecision (\%)} = \text{Median} \sum \frac{|\ Individual\ predicted - Observed|}{Observed} \times 100 \qquad (\textit{Equation 2})$$

2.4.6 Statistical analysis

Descriptive statistics were used to summarise the data. Graphical plots were generated using R software. The 95% CI for the median was calculated via non-parametric bootstrapping of 1,000 datasets using the DescTools package (version 0.99.30) in R software.

2.5 Results

2.5.1 Heart transplant recipient characteristics and concomitant medications

In 2017 and 2018, 87 patients received heart transplant at SVHS and were treated with tacrolimus (Table 5). One transplant recipient had a history of solid organ transplantation. The median age was 54 years (range, 16–70 years), and most of the

transplant recipients were men (68%). The median length of hospital stay was 21 days (range, 9–137 days). All transplant recipients received triple maintenance immunosuppressive therapy comprising tacrolimus, mycophenolate mofetil, and corticosteroids following their heart transplant. Most heart transplant recipients (84%) commenced on the oral immediate-release formulation of tacrolimus immediately post-transplant, while the remaining transplant recipients (16%) received the IV formulation. Oral tacrolimus doses gradually increased, on average, from 0.007 mg/kg every 12 hours on day 1 of therapy to 0.015 mg/kg every 12 hours on day 6 (Table 6). IV basiliximab was administered as an induction therapy on the day of operation and day 4 post-heart transplant to 82% of transplant recipients with renal impairment (to delay the commencement of tacrolimus) or those who received mechanical circulatory support (left ventricular, biventricular assist devices or total artificial hearts). All heart transplant recipients received oral itraconazole (200 mg every 12 hours) immediately post-transplant as prophylaxis against *Aspergillus* fungal infection. This antifungal therapy was continued for up to 6 months post-transplant.

Table 5. Heart transplant recipient characteristics (n=87) on admission and concomitant medications administered immediately post-transplant.

Characteristics	Median (range)*
Male, n (%)	59 (68)
Age (years)	54 (16−70)
Weight (kg)	76 (40−111)
Height (cm)	175 (150−195)
Length of hospital stay (days)	21 (9−137)
Cytochrome (CYP) 3A5, n (%)	
*1/*1	1 (1)
*1/*3	16 (18)
*3/*3	36 (42)
Unknown	34 (39)
Haematocrit (fraction)**	0.27 (0.21−0.38)
Estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2)**	53 (18−137)
Tacrolimus formulation, n (%)	
Oral immediate-release (Prograf®)	73 (84)***
Intravenous	14 (16)
Concomitant immunosuppressants, n (%)	
Mycophenolate mofetil	87 (100)
Corticosteroids	87 (100)
Basiliximab	71 (82)
Concomitant azole antifungal, n (%)	
Itraconazole	87 (100)

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

*Data are presented in median (range) unless otherwise stated.

**Just before the first tacrolimus dose.

***One transplant recipient was administered the oral immediate-release formulation of tacrolimus sublingually.

Table 6. Tacrolimus doses administered (oral only) and tacrolimus concentrations obtained for the first 3 weeks in heart transplant recipients (n=87).

Day of tacrolimus therapy*	Tacrolimus doses			Tacrolimus concentrations***	
	Number**	Mean (SD) dose (mg)	Mean (SD) dose (mg/kg)	Number	Mean (SD) concentration (µg/L)
1	84	0.50 (0.09)	0.007 (0.001)	-	-
2	121	0.51 (0.13)	0.007 (0.002)	39	2.6 (1.7)
3	147	0.63 (0.29)	0.008 (0.004)	52	4.0 (3.0)
4	150	0.82 (0.42)	0.011 (0.006)	59	5.3 (3.9)
5	147	1.00 (0.54)	0.013 (0.007)	59	7.0 (3.9)
6	145	1.15 (0.67)	0.015 (0.008)	66	9.1 (5.0)
7	150	1.12 (0.66)	0.014 (0.008)	61	9.8 (4.5)
8	154	1.12 (0.62)	0.015 (0.008)	60	11.3 (4.9)
9	155	1.06 (0.63)	0.014 (0.008)	61	12.8 (5.6)
10	146	1.10 (0.65)	0.015 (0.011)	62	11.8 (4.3)
11	132	1.18 (0.71)	0.016 (0.012)	56	11.9 (4.4)
12	126	1.18 (0.73)	0.016 (0.011)	45	11.8 (3.6)
13	115	1.23 (0.80)	0.017 (0.012)	48	12.0 (5.0)
14	102	1.25 (0.89)	0.017 (0.013)	44	12.3 (4.7)
15	93	1.29 (0.97)	0.018 (0.015)	41	13.1 (5.0)
16	84	1.30 (1.05)	0.018 (0.016)	31	11.8 (3.7)
17	81	1.36 (1.14)	0.019 (0.017)	32	11.7 (4.7)
18	78	1.49 (1.34)	0.021 (0.021)	32	10.7 (4.0)
19	80	1.52 (1.40)	0.022 (0.022)	27	11.6 (4.1)
20	79	1.57 (1.45)	0.023 (0.023)	31	11.1 (4.6)
21	74	1.66 (1.63)	0.024 (0.027)	28	11.8 (3.8)

*The majority of heart transplant recipients received an initial oral tacrolimus dose on days 1 – 2 post-transplant. Several heart transplant recipients received an initial oral tacrolimus dose on days 3 – 4 (n=26), days 5 – 6 (n=6), day 7 (n=3), and day 10 post-transplant (n=1).

**The majority of transplant recipients received oral twice-daily dosing. However, some transplant recipients received only one oral tacrolimus dose per day, while others received no doses (when a dose was withheld due to supratherapeutic tacrolimus concentrations or acute kidney injury). Therefore, the number of oral tacrolimus doses per day was calculated.

***Concentrations collected any time within a dosing interval (concentrations may not be troughs). Tacrolimus concentrations <2 µg/L were excluded from the analysis because they would result in a discrepancy in the reported daily mean tacrolimus concentration.

2.5.2 Guideline concordance of tacrolimus dosing

The majority (96%, n=70/73) of initial oral tacrolimus doses were concordant with the hospital guideline of the initial dose recommendation (0.5 mg test dose). However, most (93%, n=13/14) initial IV doses were, on average, 55% lower than recommended. The mean initial IV dose administered to transplant recipients was 0.007 mg/kg/day (range, 0.002−0.015 mg/kg/day) compared to the guideline recommendation of 0.015 mg/kg/day. Overall, 36% (31/87) of initial tacrolimus doses were administered to transplant recipients with an eGFR of <60 mL/min/1.73 m^2 (range, 18−53 mL/min/1.73 m^2). None of these transplant recipients received renal replacement therapy at the commencement of tacrolimus therapy.

2.5.3 Guideline concordance of tacrolimus monitoring

A total of 1,498 TDM samples were collected from inpatients following the initiation of oral tacrolimus therapy. Samples were collected between 2 and 111 days after heart transplant. The median number of TDM samples collected per transplant recipient was 13 (range, 3–64). Of the samples collected, 3% (51/1498) were below the limit of quantitation (BLQ) and 46% (686/1498) were trough concentrations (not collected at steady-state). Only 25% (375/1498) of the samples were steady-state trough concentrations. During the first week post-heart transplant, 29% (5/17) of the steady-state trough concentrations were within the therapeutic range, and 71% (12/17) were sub-therapeutic (Figure 7). From day 8 to day 90 post-heart transplant, 51% (183/355) of the steady-state trough concentrations were within the therapeutic range, 35% (123/355) were sub-therapeutic, and 14% (49/355) were supra-therapeutic. From day

91 to day 137 post-heart transplant, 67% (2/3) of the steady-state trough concentrations were within the therapeutic range, and 33% (1/3) were sub-therapeutic.

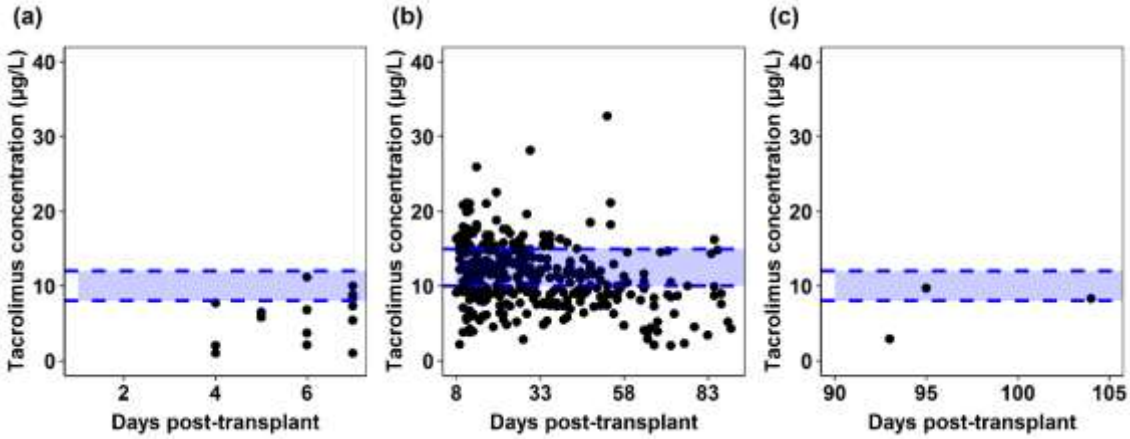


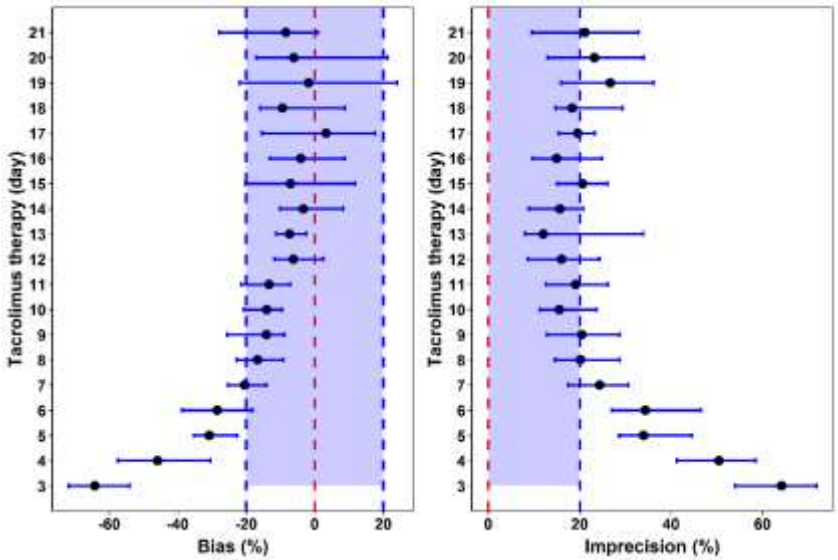
Figure 7. Tacrolimus trough concentrations collected at steady-state from (a) day 1 – day 7, (b) day 8 – day 90 and (c) day 91 – day 137 post-heart transplant. The blue shading depicts the therapeutic range of tacrolimus trough concentrations.

2.5.4 Predictive performance of the Bayesian forecasting software

Overall, 1,009 tacrolimus concentrations collected from 81 heart transplant recipients during the first 3 weeks of oral immediate-release (931 concentrations, 92%) and IV (78 concentrations, 8%) tacrolimus therapy were analysed using the Bayesian forecasting software. Six of the 87 transplant recipients (age <18 years, n=2; weight <45 kg, n=4) were excluded from the assessment because their characteristics were outside the software limits for the model. All transplant recipients had at least two tacrolimus concentrations available for evaluation. Seventy-nine IV doses were converted to oral dose equivalents, and 49 tacrolimus concentrations (5%) were fixed at 2 µg/L.

The predictive performance of the software improved as the duration of tacrolimus therapy increased. Bias and imprecision improved with the inclusion of additional tacrolimus concentration data. Bias improved from -64% (day 3 of tacrolimus therapy) to -6% (day 12), and imprecision improved from 64% (day 3) to 16% (day 12) (Figure 8, Appendix 2). Overall, the software tended to underpredict tacrolimus concentrations for the first 3 weeks of tacrolimus therapy. The software displayed clinically acceptable performances from day 12 (bias: -6% [95% CI: -11.8 to 2.5], imprecision: 16% [95% CI: 8.7−24.3]) of tacrolimus therapy.

The overall bias and imprecision values were stratified based on transplant recipient characteristics (Table 7), none of which had a major discrepant influence. Sensitivity analysis was performed for a subset of heart transplant recipients (63%) who were hospitalised for >14 days, resulting in the software displaying acceptable performance from day 12 of tacrolimus therapy (Figure 9, Appendix 3). There was no major discrepancy between the oral and IV tacrolimus concentration predictions by the software based on the assessment of bioavailability (Figure 10).

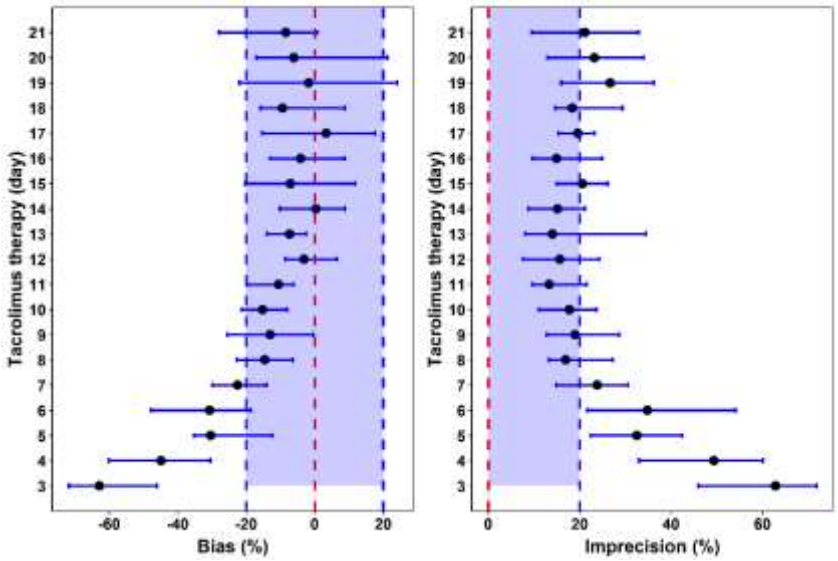


Number of TDM samples
26
29
26
30
30
28
40
42
45
42
52
60
60
56
59
64
58
62
50

Figure 8. Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in heart transplant recipients (n=81). The panel on the left shows the median and 95% CI of the bias. The panel on the right shows the median and 95% CI of the imprecision. The blue shading depicts the clinically acceptable range for bias (left panel) and imprecision (right panel). The software displays clinically acceptable drug concentration predictions if the bias and imprecision are within the clinically acceptable range and the 95% CI of bias crosses zero. Software performance is high when the bias and imprecision are close to zero (dotted red lines). The number of observed concentrations included for software predictions for each day of tacrolimus therapy is summarised in the column on the right. The software-predicted tacrolimus concentrations on day 2 of therapy were not displayed in the figure as most concentrations were population predicted and not individual predicted concentrations.

Table 7. Overall mean bias and imprecision stratified based on transplant recipient characteristics (n=81) following the oral immediate-release formulation of tacrolimus.

Characteristics	Mean bias (%)	Mean imprecision (%)
Sex		
Male	-15	31
Female	-12	36
Age (years)		
19 – 44	-12	34
45 – 70	-15	32
Weight (kg)		
46 – 79	-17	36
80 – 113	-11	30
Height (cm)		
150 – 172	-11	33
173 – 195	-16	33
Cytochrome P450 3A5		
*1/*1, *1/*3	-17	31
*3/*3	-16	37
Unknown	-10	30
Haematocrit		
0.20 – 0.32	-15	33
0.33 – 0.45	-11	32



Number of TDM samples
26
29
26
30
30
28
40
39
41
35
38
41
38
37
39
39
37
38
29

Figure 9. Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in a subset of heart transplant recipients who were hospitalised >14 days (n=51). The panel on the left shows the median and 95% CI of the bias. The panel on the right shows the median and 95% CI of the imprecision. The blue shading depicts the clinically acceptable range for bias (left panel) and imprecision (right panel). The software displays clinically acceptable drug concentration predictions if the bias and imprecision are within the clinically acceptable range and the 95% CI of bias crosses zero. Software performance is high when the bias and imprecision are close to zero (dotted red lines). The number of observed concentrations included for software predictions for each day of tacrolimus therapy is summarised in the column on the right. The software-predicted tacrolimus concentrations on day 2 of therapy were not displayed in the figure as most concentrations were population predicted and not individual predicted concentrations.

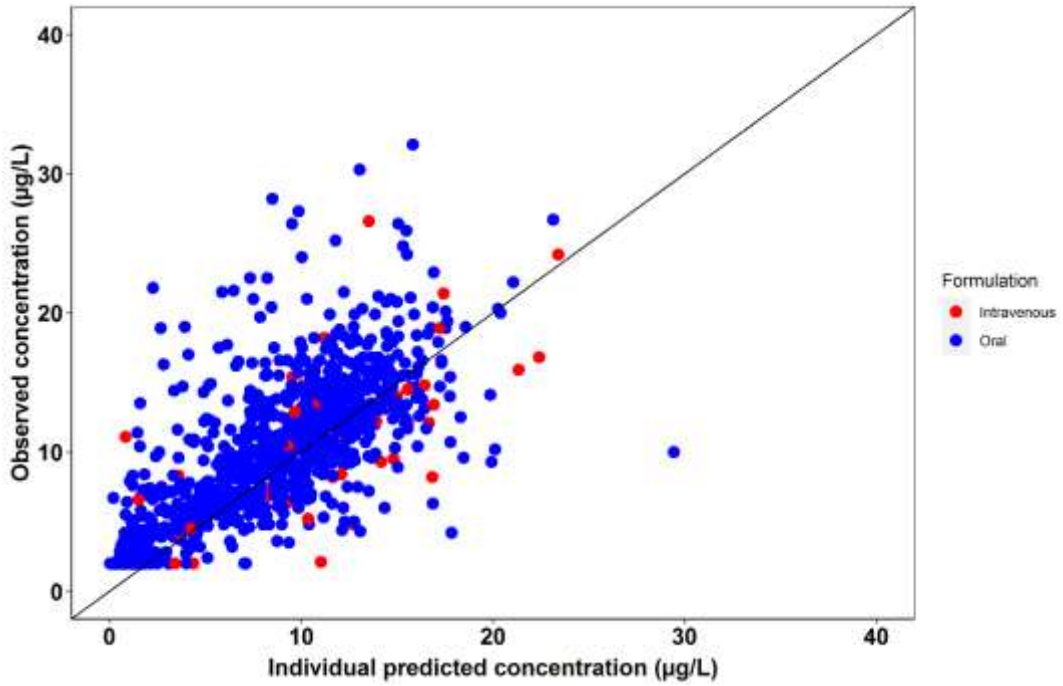


Figure 10. The goodness of fit plot for observed tacrolimus concentrations against individual predicted concentrations by the software for the oral immediate-release and intravenous data.

2.6 Discussion

Tacrolimus dosing strategies are complicated by their narrow therapeutic range and considerable pharmacokinetic variability. Therefore, guidelines have been developed to inform dosing and monitoring practices to ensure the safe and effective use of tacrolimus. Consistent with a previous study conducted in liver transplant recipients [11], compliance with tacrolimus dosing and monitoring guidelines in heart transplant recipients was generally poor. The evaluation of a commercially available Bayesian forecasting software that integrated a model developed from renal transplant recipients highlighted that this approach was able to adequately predict tacrolimus concentrations and therefore guide tacrolimus dosing in heart transplant recipients, but only after 11 days of therapy.

Underdosing of initial tacrolimus therapy was particularly common with the IV formulation. Prescribers' concerns about drug-related toxicities contributed to the observed cautious dosing of tacrolimus at the initiation of IV therapy (personal communication). IV tacrolimus, administered as a continuous infusion over 24 hours, is associated with severe nephrotoxicity and neurotoxicity due to the presence of the solvent polyoxyl 60 hydrogenated castor oil (HCO-60) in its formulation [26, 27]. In addition, tacrolimus dosing decisions are influenced by the presence of concomitant azole antifungal, itraconazole. Itraconazole inhibits CYP3A4 enzymes, thereby reducing the metabolism of tacrolimus in the gut and liver [28, 29], resulting in increased tacrolimus oral bioavailability [30] and reduced apparent clearance [31]. Consequently, a reduction in tacrolimus dose is required to avoid drug toxicity in heart transplant recipients. The recommended initial IV dose in the hospital guideline was based on international guidelines [18] which, unfortunately, do not provide guidance on the required adjustments of tacrolimus doses in the setting of concomitant azole antifungal therapy. In the context of concomitant azole antifungal therapy, although initial IV tacrolimus doses were 58% (range, 22%–85%) lower than the guideline recommendations, they reflect the "correct" adaptation of dosing practices to avoid supratherapeutic tacrolimus concentrations. In view of this, the hospital guidelines for the initial IV tacrolimus dose should be updated to reflect this practice. In contrast to IV dosing, almost all the initial oral tacrolimus doses were guideline concordant. This is likely because the guidelines recommend a conservative fixed test dose (0.5 mg); hence, no dose selection decisions were required. This fixed test dose approach, rather than the commonly used weight-based dose of 0.075 mg/kg/day [17], was used to gauge tacrolimus metabolism in each transplant recipient and to guarantee renal safety [32].

Renal impairment is a limiting factor in the initiation of tacrolimus therapy due to the increased risk of nephrotoxicity [17, 33]. The reduction of tacrolimus doses compared to the guidelines and delayed initiation of tacrolimus therapy have been suggested as strategies to mitigate this risk [33]. In some heart transplant recipients with renal impairment, consistent with the guidelines, initiation of tacrolimus therapy was delayed (between day 2 and day 7 post-transplant). In contrast, some transplant recipients received an initial tacrolimus dose despite their impaired renal function (eGFR <60 mL/min/1.73 m^2). These transplant recipients did not receive renal replacement therapy at the commencement of tacrolimus therapy. The reasons for this practice are difficult to infer; however, in some cases, the prescribers may have decided that the risk of graft rejection outweighed the risk of nephrotoxicity. A greater understanding of the factors influencing prescribers' decision-making regarding the initiation of tacrolimus therapy is required, particularly in transplant recipients with renal impairment.

Monitoring trough concentrations is currently recommended to guide tacrolimus dosing decisions for heart transplant recipients. As observed in other studies [34-36], the majority of tacrolimus samples collected in the present study were not steady-state trough concentrations. The busy clinical environment and potential critical condition of heart transplant recipients, especially early after transplantation, could be associated with a failure to collect trough concentrations and inappropriate TDM requests (collection of non-steady-state concentrations). Further, despite clear instructions, some transplant recipients might forget to delay the consumption of their tacrolimus dose until a TDM sample is collected. It is unclear how prescribers interpret non-trough tacrolimus concentrations when making dosing decisions or even if they are aware that

the sample is not a trough concentration. Anecdotal reports at our institution suggest that prescribers usually disregard a non-trough concentration and request a new TDM sample to be collected the next day if they are aware that the sample taken was not a trough concentration. However, if they are not aware that the TDM sample taken was not a trough concentration, the sample will be misinterpreted as a trough concentration, leading to inappropriate dosage adjustments. An important caveat of this is the accuracy of the time of tacrolimus dose administration and TDM sample collection reported in the electronic medical record relative to the actual time of administration or collection [37]. The discrepancy between the actual and reported time further complicates the interpretation of TDM results.

Overall, 49% of the steady-state trough concentrations identified in our study were outside the therapeutic range (sub-therapeutic, 36%; supra-therapeutic, 13%). This is consistent with a previous study conducted in adult liver transplant recipients [38]. However, it must be noted that some transplant recipients in this study had an extended post-transplant hospital stay (up to 137 days). In these transplant recipients, postoperative complications or infections often occur, in response to which prescribers may prefer to dose tacrolimus more cautiously (personal communication). Therefore, it may be that the “under-exposure” reported in this study was intentional.

Bayesian-guided dosing provides an attractive alternative approach to inform tacrolimus dosing decisions, which overcomes the reliance on the collection of specifically timed steady-state trough concentrations. Ideally, evaluation of the predictive performance of a Bayesian forecasting software should occur prior to the implementation of the

software in routine clinical practice. This is to determine if the software is suitable for estimating tacrolimus exposure in the population where it will be applied to guide dosing. The Bayesian forecasting software package approved for use in Australia employs a tacrolimus population pharmacokinetic model developed from renal transplant recipients [16]. Model selection likely reflects that the majority of population pharmacokinetic models for tacrolimus have been developed from renal (55%) and liver (30%) transplant recipients, with few models developed in heart, lung, and heart and lung transplant recipients [12]. To date, no work has been undertaken to determine the generalisability of a tacrolimus model developed from renal transplant recipients integrated within a Bayesian forecasting software in heart transplant recipients. The outcomes of this study, therefore, provide essential evidence to inform decisions regarding the implementation of the Bayesian approach to support prescribing practices across recipients of different solid organ transplants.

The evaluated Bayesian forecasting software demonstrated clinically acceptable predictions of tacrolimus concentrations for heart transplant recipients from day 12 of tacrolimus therapy. This outcome is more promising than a previous study indicating that the predictive performance of another renal model was only clinically acceptable after day 21 of tacrolimus therapy in renal transplant recipients [39]. The delay in the achievement of clinically acceptable predictions was not due to a paucity of data points or the length of hospitalisation (Figure 9, Appendix 3) but to the reported instability of tacrolimus pharmacokinetics during the recovery period post-surgery, where anaemia, organ dysfunction, and ischemia-reperfusion injury impact the pharmacokinetics of tacrolimus [1]. Indeed, software performance remained clinically acceptable from day

12 of tacrolimus therapy without the inclusion of tacrolimus concentration data obtained during the first 11 days of tacrolimus therapy (data not shown). The influence of concomitant itraconazole therapy on the variability of tacrolimus pharmacokinetics during the post-heart transplant period must also be considered. Although few models [40-42] have quantified the effect of azole antifungals on the apparent clearance of tacrolimus, the software evaluated did not have concomitant azole antifungal therapy use as a covariate. It is suggested that it may take at least a week for the effect of this interaction on tacrolimus pharmacokinetics to stabilise [43]. Additionally, in the first week post-heart transplant, approximately 95% of transplant recipients in this study received high doses of a proton pump inhibitor, which may reduce the absorption of itraconazole [44] and thereby contribute to a variability in the effect of drug-drug interaction on tacrolimus pharmacokinetics. Consistent with other studies [1, 45], the present study (Table 6) demonstrated that the dynamic physiological changes that occur in heart transplant recipients in the first 1 to 2 weeks post-transplant make predicting tacrolimus drug exposure during this period very difficult. Further work is required to examine whether these temporal changes can be systematically quantified to improve the prediction of tacrolimus concentrations using the Bayesian software during the first 11 days of tacrolimus therapy.

The Bayesian forecasting software only allowed for the input of oral doses. Therefore, the conversion of a continuous IV infusion dose to a single oral dose at a 1:5 ratio was the most pragmatic approach to accommodate this dose in the present study. This is despite the differences in the pharmacokinetic properties between the two formulations. Excluding IV doses from the current analysis will result in the software

underpredicting tacrolimus concentrations and will not account for drug accumulation. Only 8% of drug concentration data aligned with IV doses; hence, the influence of dose conversion on software performance is expected to be minimal (Figure 10). Of note, there are currently no IV models available for tacrolimus [12]. Setting tacrolimus concentrations BLQ of 2 µg/L to half of the BLQ value (1 µg/L) instead of 2 µg/L would have been a better approach to evaluate software performance. However, only 5% of the tacrolimus concentrations recorded were concentrations BLQ, which would not have significantly affected the results of this study. Haematocrit, which influences tacrolimus apparent clearance, was the only time-varying covariate in the model. The software allowed input of haematocrit value only once, at the initiation of therapy. As a result, this could not account for the changes in tacrolimus apparent clearance over time. The model integrated within the software was able to simultaneously describe tacrolimus pharmacokinetics for the oral immediate-release (Prograf®) and prolonged-release (Advagraf®) formulations of tacrolimus. The software did not permit the conversion between formulations within a course of tacrolimus therapy.

Determining the correlation between transplant recipient characteristics/frequency of TDM sampling and time to achieve clinically acceptable predictions would be interesting to investigate. However, the predictive performance of the software varied within an individual over time. For example, for one transplant recipient, the software prediction may be acceptable on days 12 and 14−18 but not on days 13 and 19. Given the variability in software performance within an individual over time, it was considered inappropriate to assess this correlation. Comparison with other Bayesian forecasting software was not undertaken, as only DoseMeRx™ is approved by the TGA in Australia for

implementation in clinical practice. Nevertheless, the choice of software should be considered as variations in predictions of drug exposure have been observed when the same population pharmacokinetic model was integrated within different Bayesian forecasting software [46]. Since we evaluated the performance of the software (plus model) as a whole entity, it was not possible to generate a visual predictive check (VPC) or normalised prediction distribution errors (NPDE) plot to describe software-predicted tacrolimus concentration over time for our study population.

Currently, all tacrolimus models have been developed to support oral tacrolimus dosing. Therefore, it may not be plausible to use existing models to guide IV tacrolimus dosing. Additionally, the presence of clinically significant drug-drug interactions may hinder the generalisability of the model, particularly if the evaluated model does not account for the interaction. However, our work shows that a renal model can be extrapolated for use in a heart transplant recipient population. We believe that these models are a tool to help clinicians improve dosing, yet they do not stand alone. The outcome of the present study suggests that tacrolimus dose recommendations should be based on tacrolimus steady-state trough concentrations and the overall clinical assessment of the transplant recipient for the first 11 days of tacrolimus therapy. Beyond 11 days, however, the model provides recommendations that, when used alongside clinical judgment and available drug concentration(s), could help optimise tacrolimus therapy in heart transplant recipients.

2.7 Limitations

Due to the retrospective nature of the study, the accuracy of tacrolimus administration and TDM sampling times recorded in electronic medical records cannot be guaranteed. It is important to note that software predictions might have been closer to the "real" concentrations at the time they were actually collected in relation to the dose when they were actually administered.

2.8 Conclusions

This study assessed tacrolimus dosing and monitoring practices in heart transplant recipients and evaluated the predictive performance of a commercially available Bayesian forecasting software to predict tacrolimus concentrations. It was found that tacrolimus dosing and monitoring were discordant with the guidelines. A better understanding of the factors influencing prescribers' decisions regarding the dosing and monitoring practices of tacrolimus is required. The Bayesian forecasting software, which integrated a model developed from renal transplant recipients, was able to predict tacrolimus concentrations in heart transplant recipients after 11 days of therapy. More research is required to understand the temporal changes that contribute to the variability in tacrolimus pharmacokinetics, particularly within the first 11 days post-transplant, to improve the predictions of tacrolimus concentrations using the Bayesian forecasting software.

2.9 Acknowledgements

We would like to thank Professor Peter Macdonald, senior staff cardiologist of the Heart Transplant Unit at St. Vincent's Hospital Sydney for providing valuable feedback on the

manuscript. Ms. Ranita Kirubakaran is a recipient of the Faculty of Medicine and Health, University of New South Wales, Sydney, Doctoral Scholarship funded by the Australian Government, and St. Vincent's Centre for Applied Medical Research Student Scholarship Supplementation Scheme.

2.10 Conflict of interest and source of funding

Ranita Kirubakaran, Sophie L. Stocker, Lilibeth Carlos, Richard O. Day, and Jane E. Carland have no conflicts of interest relevant to the content of this manuscript. DoseMeRx™ (Moorestown, NJ, USA) was not involved in funding this study or its design, data collection, data analysis, interpretation of data, and writing of the manuscript. DoseMeRx™ was provided with a copy of the draft manuscript prior to submission and was invited to comment. The authors retained full control and responsibility for all aspects of this study. This work was supported by the St. Vincent's Curran Foundation Grant.

References

1. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

2. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

3. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

4. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

5. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

6. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

7. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

8. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

9. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

10. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

11. Dasari B, Hodson J, Nassir A, Widmer J, Isaac J, Mergentel H, et al. Variations in practice to therapeutic monitoring of tacrolimus following primary adult liver transplantation. Int J Organ Transplant Med. 2016;7(1):1-8.

12. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review. Clin Pharmacokinet. 2020;59(11):1357-92.

13. Fuchs A, Csajka C, Thoma Y, Buclin T, Widmer N. Benchmarking therapeutic drug monitoring software: a review of available computer tools. Clin Pharmacokinet. 2013;52(1):9-22.

14. Meinshausen J, Bühl K, Engel G, Harings-Kaim A, Drewelow B, Klotz U. Generation of pharmacokinetic data during routine therapeutic drug monitoring: Bayesian approach vs. pharmacokinetic studies. Ther Drug Monit. 1993;15(4):281-8.

15. del Mar Fernández de Gatta M, Martin-Suarez A, Lanao JM. Approaches for dosage individualisation in critically ill patients. Expert Opin Drug Metab Toxicol. 2013;9(11):1481-93.

16. Woillard JB, de Winter BC, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations--twice daily Prograf and once daily Advagraf. Br J Clin Pharmacol. 2011;71(3):391-402.

17. Prograf®. Northbrook, IL: AstellasPharma US, Inc. 2020.

18. Taylor DO, Barr ML, Meiser BM, Pham SM, Mentzer RM, Gass AL. Suggested guidelines for the use of tacrolimus in cardiac transplant recipients. J Heart Lung Transplant. 2001;20(7):734-8.

19. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623-53.

20. Kurose K, Sugiyama E, Saito Y. Population differences in major functional polymorphisms of pharmacokinetics/pharmacodynamics-related genes in Eastern Asians and Europeans: implications in the clinical trials for novel drug development. Drug Metab Pharmacokinet. 2012;27(1):9-54.

21. Lee S-J, Usmani KA, Chanas B, Ghanayem B, Xi T, Hodgson E, et al. Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenet Genom. 2003;13(8):461-72.

22. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm. 1981;9(4):503-12.

23. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Phar. 1982;10(2):229-.

24. Dansirikul C, Morris RG, Tett SE, Duffull SB. A Bayesian approach for population pharmacokinetic modelling of sirolimus. Br J of Clin Pharmacol. 2006;62(4):420-34.

25. David OJ, Johnston A. Limited sampling strategies for estimating cyclosporin area under the concentration–time curve: review of current algorithms. Ther Drug Monit. 2001;23(2):100-14.

26. Porayko MK, Textor SC, Krom RA, Hay JE, Gores GJ, Richards TM, et al. Nephrotoxic effects of primary immunosuppression with FK-506 and cyclosporine regimens after liver transplantation. Mayo Clinic Proceedings. 1994;69(2):105-11.

27. McDiarmid SV, Klintmalm GB, Busuttil RW. FK506 conversion for intractable rejection of the liver allograft. Transplant Int. 1993;6(6):305-12.

28. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

29. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

30. Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, et al. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. Clin Pharmacol Ther. 1997;62(1):41-9.

31. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

32. Baran DA, Galin I, Sandler D, Segura L, Cheng J, Courtney MC, et al. Tacrolimus in cardiac transplantation: efficacy and safety of a novel dosing protocol. Transplantation. 2002;74(8):1136-41.

33. Sikma MA, Hunault CC, Kirkels JH, Verhaar MC, Kesecioglu J, de Lange DW. Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients. Eur J Drug Metab Pharmacokinet. 2018;43(3):311-20.

34. Al Za'abi M, Al Muqbali J, Al-Waili K. Sampling time and indications appropriateness for therapeutically monitored drugs at a teaching university hospital in Oman. Saudi Pharm J. 2015;23(4):458-62.

35. Al-Sulaiti FK, Nader A, El-Mekaty E, Elewa H, El-Badriyeh D, El-Zubair A, et al. Vancomycin Therapeutic Drug Monitoring Service Quality Indices and Clinical Effectiveness Outcomes: A Retrospective Cohort and Clinical Audit. J Am Coll Clin Pharm. 2020;3(4):778-85.

36. Chaudhri K, Stocker SL, Williams KM, McLeay RC, Marriott DJ, Di Tanna GL, et al. Voriconazole: an audit of hospital-based dosing and monitoring and evaluation of the predictive performance of a dose-prediction software package. J Antimicrob Chemother. 2020;75(7):1981-4.

37. Roydhouse SA, Carland JE, Debono DS, Baysari MT, Reuter SE, Staciwa AJ, et al. Accuracy of documented administration times for intravenous antimicrobial drugs and impact on dosing decisions. Br J Clin Pharmacol. 2021.

38. Liu Z, Cheng J, Powell E, Macdonald G, Fawcett J, Lynch S, et al. Weight-based tacrolimus trough concentrations post liver transplant. Intern Med J. 2019;49(1):79-83.

39. Antignac M, Fernandez C, Barrou B, Roca M, Favrat JL, Urien S, et al. Prediction tacrolimus blood levels based on the Bayesian method in adult kidney transplant patients. Eur J Drug Metab Pharmacokinet. 2011;36(1):25-33.

40. Zahir H, McLachlan AJ, Nelson A, McCaughan G, Gleeson M, Akhlaghi F. Population pharmacokinetic estimation of tacrolimus apparent clearance in adult liver transplant recipients. Ther Drug Monit. 2005;27(4):422-30.

41. Zhou H, Cai J, Huang J, Zhang J, Shi SJ, Liu YN, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: A population pharmacokinetic approach. Pharmacogenomics. 2019;20(1):21-35.

42. Cai X, Song H, Jiao Z, Yang H, Zhu M, Wang C, et al. Population pharmacokinetics and dosing regimen optimization of tacrolimus in Chinese lung transplant recipients. Eur J Pharm Sci. 2020;152:105448.

43. Capone D, Gentile A, Imperatore P, Palmiero G, Basile V. Effects of itraconazole on tacrolimus blood concentrations in a renal transplant recipient. Ann Pharmacother. 1999;33(10):1124-5.

44. Jaruratanasirikul S, Sriwiriyajan S. Effect of omeprazole on the pharmacokinetics of itraconazole. Eur J Clin Pharmacol. 1998;54(2):159-61.

45. Sikma MA, Hunault CC, Van Maarseveen EM, Huitema AD, Van de Graaf EA, Kirkels JH, et al. High variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Eur J Drug Metab Pharmacokinet. 2020;45(1):123-34.

46. Shingde RV, Reuter SE, Graham GG, Carland JE, Williams KM, Day RO, et al. Assessing the accuracy of two Bayesian forecasting programs in estimating vancomycin drug exposure. J Antimicrob Chemother. 2020;75(11):3293-302.

Appendices

Appendix 1. Pharmacokinetic parameters and covariate relationships of the tacrolimus model [16] integrated within the Bayesian forecasting software.

Pharmacokinetic parameters and covariate relationships		BSV (%)	BOV (%)
Ktr	$= 3.34*(1.53^{FORM})\ h^{-1}$	24	33
CL/F	$= 21.2*[(HCT/35)^{-1.14}]*(2^{CYP3A5})$ L/h	28	31
V_2/F	$= 486*(0.29^{FORM})$ L	31	75
Q/F	= 79 L/h	54	
V_3/F	= 271 L	60	
Prop RUV	= 11.3%		
Add RUV	= 0.71 ng/mL		

FORM = 0 if transplant recipient received Advagraf, otherwise FORM = 1.

CYP3A5 = 0 if transplant recipient is a CYP3A5 non-expresser (*3/*3), otherwise CYP3A5 = 1 (*1/*1, *1/*3).

Add RUV additive residual unexplained variability; *BOV* between-occasion variability; *BSV* between-subject variability; *CL/F* apparent clearance; *HCT* haematocrit; *Ktr* transfer rate constant; *Prop RUV* proportional residual unexplained variability; *Q/F* apparent intercompartmental clearance; V_2/F apparent central volume of distribution; V_3/F apparent peripheral volume of distribution.

Appendix 2. Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in heart transplant recipients (n=81).

Day starting tacrolimus therapy	Number of TDM samples	Bias		Imprecision	
		%	95% CI	%	95% CI
3	50	-64.2	-71.82, -54.00	64.2	54.00, 71.82
4	62	-45.9	-57.50, -30.51	50.5	41.16, 58.50
5	58	-30.8	-35.32, -22.74	34.0	28.62, 44.56
6	64	-28.5	-38.73, -18.16	34.4	26.93, 46.57
7	59	-20.4	-25.45, -14.11	24.4	17.44, 30.56
8	56	-16.7	-22.81, -9.18	20.2	14.59, 28.68
9	60	-14.1	-25.58, -8.90	20.5	12.75, 28.70
10	60	-14.0	-20.71, -9.57	15.6	11.24, 23.70
11	52	-13.3	-21.51, -7.13	19.1	12.56, 26.21
12	42	-6.3	-11.83, 2.46	16.1	8.65, 24.29
13	45	-7.3	-11.28, -2.42	12.1	8.02, 33.87
14	42	-3.3	-10.20, 8.32	15.7	8.83, 20.80
15	40	-7.0	-20.33, 11.88	20.6	15.00, 26.11
16	28	-4.1	-13.14, 8.72	15.0	9.70, 24.90
17	30	3.3	-15.38, 17.65	19.6	15.38, 23.28
18	30	-9.4	-15.90, 8.88	18.4	14.66, 29.38
19	26	-1.8	-21.96, 24.00	26.7	15.93, 36.17
20	29	-6.1	-17.06, 21.25	23.3	12.93, 33.98
21	26	-8.4	-28.00, 0.83	21.2	9.48, 32.87

Appendix 3. Software performance of individual predicted tacrolimus concentrations relative to the observed concentrations following the oral immediate-release formulation of tacrolimus in a subset of heart transplant recipients who were hospitalised >14 days (n=51).

Day starting tacrolimus therapy	Number of TDM samples	Bias		Imprecision	
		%	95% CI	%	95% CI
3	29	-62.9	-71.82, -46.00	62.9	46.00, 71.82
4	38	-44.8	-60.14, -30.51	49.4	33.00, 60.14
5	37	-30.4	-35.32, -12.47	32.5	22.42, 42.44
6	39	-30.7	-47.87, -18.64	34.9	21.67, 54.13
7	39	-22.5	-29.85, -14.11	23.8	14.81, 30.56
8	37	-14.6	-22.81, -6.37	17.0	13.22, 27.24
9	38	-13.0	-25.58, -0.43	19.0	12.75, 28.70
10	41	-15.2	-21.31, -8.13	17.8	11.06, 23.70
11	38	-10.5	-19.87, -6.18	13.3	9.67, 21.51
12	35	-3.1	-8.60, 6.57	15.7	7.50, 24.29
13	41	-7.3	-14.04, -2.42	14.0	8.02, 34.55
14	39	0.3	-10.20, 8.83	15.2	8.78, 21.13
15	40	-7.0	-20.33, 11.88	20.6	15.00, 26.11
16	28	-4.1	-13.14, 8.72	15.0	9.70, 24.90
17	30	3.3	-15.38, 17.65	19.6	15.38, 23.28
18	30	-9.4	-15.90, 8.88	18.4	14.66, 29.38
19	26	-1.8	-21.96, 24.00	26.7	15.93, 36.17
20	29	-6.1	-17.06, 21.25	23.3	12.93, 33.98
21	26	-8.4	-28.00, 0.83	21.2	9.48, 32.87

Chapter 3:

Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review.

3.1 Link to thesis

In Chapter 2, I presented the findings following a clinical audit of tacrolimus therapy in heart transplant recipients, along with an assessment of a Bayesian forecasting software to predict tacrolimus concentrations. Tacrolimus dosing and monitoring were discordant with hospital guidelines. The renal transplant-derived tacrolimus population pharmacokinetic model integrated within the software performed poorly in the early post-heart transplantation period. The model was suitable for guiding tacrolimus dosing only after 11 days of therapy. This finding necessitated the identification of other model(s) that might be more suitable for use in heart transplant recipients, particularly for the immediate post-transplantation phase. A systematic review was undertaken to inform model selection for subsequent evaluation of their predictive performance in heart transplant recipients. The review summarised published population pharmacokinetic models of tacrolimus developed from various organ transplant recipient populations. In addition, clinical factors influencing tacrolimus pharmacokinetics were determined. Areas requiring additional research to facilitate the use of population pharmacokinetic models to guide tacrolimus dosing decisions in transplant recipients were also identified. These additional aims were important to inform model development. This chapter presents this systematic review.

This work has been published:

Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review. *Clinical Pharmacokinetics*. 2020;59(11):1357-1392.

This work has been presented at one international conference:

Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population pharmacokinetic models of tacrolimus in adult transplant recipients: A systematic review. Poster Presentation, ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.

3.2 Abstract

Background and Objectives Numerous population pharmacokinetic models of tacrolimus in adult transplant recipients have been published to characterise tacrolimus pharmacokinetics and facilitate dose individualisation. This study aimed to (1) investigate clinical determinants influencing tacrolimus pharmacokinetics and (2) identify areas requiring additional research to facilitate the use of population pharmacokinetic models to guide tacrolimus dosing decisions.

Methods MEDLINE, EMBASE and the reference lists of all articles were searched to identify population pharmacokinetic models of tacrolimus developed from adult transplant recipients published from the inception of databases to 29 February 2020.

Results Of the 69 studies identified, 55% were developed from kidney and 30% from liver transplant recipients. Most studies (91%) investigated the oral immediate-release formulation of tacrolimus. Few studies (17%) explained the effect of drug-drug interactions on tacrolimus pharmacokinetics. Only 35% of the studies performed an external evaluation to assess the generalisability of the models. Studies related variability in tacrolimus whole blood clearance amongst transplant recipients to either cytochrome P450 (CYP) 3A5 genotype (41%), days post-transplant (30%) or haematocrit

(29%). Variability in the central volume of distribution was mainly explained by body weight (20% of studies).

Conclusions The effect of clinically significant drug-drug interactions and, different formulations and brands of tacrolimus should be considered for any future tacrolimus population pharmacokinetic model development. Further work is required to assess the generalisability of existing models and identify key factors that influence both initial and maintenance doses of tacrolimus, particularly in heart and lung transplant recipients.

3.3 Introduction

The immunosuppressant, tacrolimus, is the first choice calcineurin inhibitor used in most solid-organ transplantation [1]. Compared to cyclosporine, tacrolimus is associated with improved graft survival rates [1] and lower acute rejection rates [2]. The predominant characteristics of tacrolimus pharmacokinetics are its poor and variable oral bioavailability (5−93%) [3, 4] and extensive binding to erythrocytes (85−95%) [1, 5]. Furthermore, tacrolimus is extensively metabolised by cytochrome P450 (CYP) 3A isoenzymes in the liver and gut, and mainly excreted as metabolites (99%) via the biliary route [6].

Tacrolimus is a candidate for therapeutic drug monitoring (TDM), owing to its narrow therapeutic window [7] and considerable inter- and intra-patient pharmacokinetic variability [8-14]. At present, monitoring whole blood trough concentrations is recommended to guide tacrolimus dosing [15]. However, monitoring trough concentrations requires the collection of strictly timed blood samples (usually within 60 minutes prior to the next administered dose), which is difficult to achieve in a busy

clinical environment. In adult heart and liver transplant recipients, only 46% and 5%, respectively, of TDM samples collected were trough concentrations [16, 17]. Incorrect timing of TDM sample collection wastes resources, inconveniences patients and could result in erroneous interpretation of tacrolimus concentrations and consequently, inappropriate dose recommendations. Given the difficulty of successfully implementing trough concentration-guided dosing for tacrolimus, a population pharmacokinetic-guided approach presents a possible solution for individualised dosing advice in transplant recipients [18].

In population pharmacokinetic analysis, sparse (one or a few drug concentrations across a dosing interval) or intensive (≥6 drug concentrations over a dosing interval [19]) blood sampling data are collected from a group of subjects to estimate typical population pharmacokinetic parameters, between-subject variability (BSV), between-occasion variability (BOV) and residual unexplained variability (RUV) [20]. The influence of clinical determinants (covariates) on pharmacokinetic parameters are assessed simultaneously [21]. The final population pharmacokinetic model can then be integrated within a Bayesian forecasting program to provide individualised dose predictions. Bayesian forecasting program facilitates TDM processes by removing the need for specifically timed blood samples as required when collecting trough concentrations. Its ability to interpret drug concentrations collected at any time point(s) (i.e., steady-state conditions are not necessary), can reduce the number of TDM samples needed to achieve target drug exposure.

Selecting an appropriate and reliable population pharmacokinetic model within a Bayesian forecasting program to guide tacrolimus dosing is essential prior to the implementation of the program into routine clinical practice. To date, two reviews of population pharmacokinetic models of tacrolimus have been published. Brookes *et al.* [22] provided a summary of available models, while Nanga *et al.* [23] used tacrolimus pharmacokinetic parameter estimates to develop a meta-model. The current, comprehensive review aimed to (1) investigate clinical determinants influencing tacrolimus pharmacokinetics and (2) identify areas requiring additional research to facilitate the use of population pharmacokinetic models to guide tacrolimus dosing decisions.

3.4 Methods

A systematic review using MEDLINE and EMBASE databases from their inception to February 29, 2020 was undertaken to identify population pharmacokinetic models of tacrolimus employing data from adult transplant recipients. Reference lists of all articles were also searched for additional studies. Search terms included 'tacrolimus' AND 'predict' AND 'Bayesian' AND 'population pharmacokinetic*' AND 'transplant'; 'tacrolimus' AND 'Bayesian' AND 'transplant'; 'tacrolimus' AND 'population pharmacokinetic*' AND 'transplant'. Studies published in English and conducted in patients ≥18 years old were included. Two independent reviewers (RK, JEC) screened the titles and abstracts of each article and read full-text articles to determine eligibility, resolving any discrepancies of opinion by consensus.

A standardised data collection form was used to extract relevant information from each eligible study including country of study, type of organ transplant, tacrolimus formulation, concomitant immunosuppressant(s), number of transplant recipients and tacrolimus concentrations used for model building, tacrolimus concentration sampling time(s), time post-transplant, bioanalytical method and biological fluid containing tacrolimus sample used to analyse drug concentrations, software used for pharmacokinetic analysis, model evaluation approach, model structure, tacrolimus pharmacokinetic parameter estimates and covariate relationships, BSV, BOV and RUV. Descriptive statistics were used to summarise the data. The risk of bias assessment was not undertaken as the aim of this study was not to evaluate the quality of data presented in each study.

3.5 Results

A total of 69 population pharmacokinetic models were evaluated (Figure 11). Of the studies identified, 42% were undertaken in Europe and 30% in Asian countries (Table 8). More than half of the studies (55%) explored tacrolimus pharmacokinetics in kidney transplant recipients, with 30% of the studies conducted in liver transplant recipients. Only two studies were conducted in heart [24, 25], lung [26, 27], and heart and lung [28, 29] transplant recipients, respectively, with one study conducted in hematopoietic stem cell transplant recipients [30].

Although the study period varied greatly, from immediate up to a few years post-transplant, most studies (67%) were performed within the first year following transplantation. The median (range) number of transplant recipients used for model

building was 69 (12−681), with 70% of the studies including less than 100 recipients. The median (range) number of concentrations per transplant recipient was 13 (2–57).

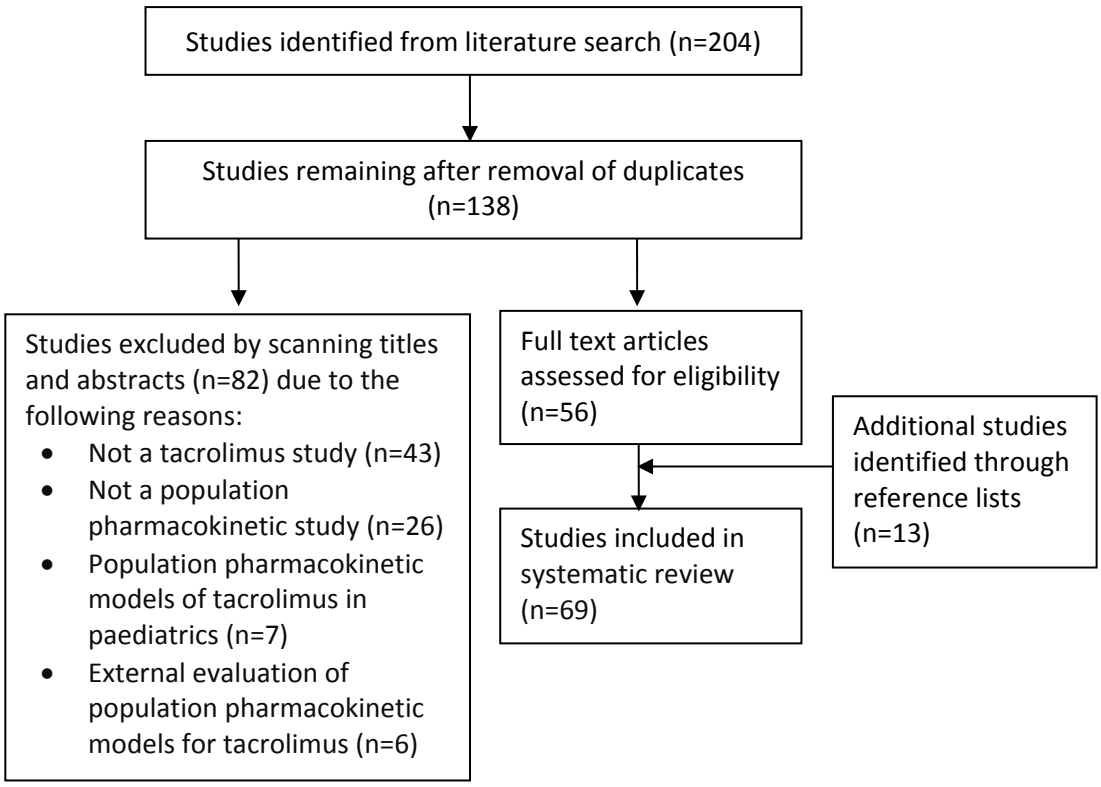


Figure 11. A Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram detailing the number of studies identified from databases search, abstracts screened, and the full-text articles retrieved.

Table 8. Description of population pharmacokinetic studies of tacrolimus in adult transplant recipients.

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Staatz et al. [8]	Australia (Oceania)	Single	Kidney	Prograf® [po]	Azathioprine or MMF + corticosteroids	70	1060	Pre-dose	2-1475 days	LC-MS/MS	whole blood
Scholten et al. [31]	Netherlands (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab	17	160	Pre-dose, 1, 2, 3, 4, 6, 8, 12	2–52 weeks	MEIA	whole blood
Antignac et al. [32]	France (Europe)	Single	Kidney	Prograf® [iv/po]	MMF + corticosteroids	83	1589	Pre-dose	1-158 days	MEIA	whole blood
Benkali et al. [33]	France (Europe)	Multi	Kidney	Prograf® [po]	MMF + corticosteroids	32	NS	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9 at 5 transplant periods, 12 h (Week 1 and 2)	1 week-6 months	TFC-MS/MS	whole blood
Press et al. [34]	Netherlands (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab	31	NS	Full profiles: Pre-dose, 1, 2, 3, 4, 6, 12; Limited profiles: Pre-dose, 2, 3	2–52 weeks	MEIA	whole blood
Benkali et al. [35]	France (Europe)	Single	Kidney	Advagraf® [po]	MMF ± corticosteroids	41	492	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9, 12, 24	>12 months	TFC-MS/MS	whole blood
Saint-Marcoux et al. [36]	NS (Europe)	Multi	Kidney	Advagraf® [po]	NS	12	408	17 concentration data points over 24 hours	Day 14 and 42	HPLC-MS	whole blood
Velickovic-Radovanovic et al. [37]	Serbia (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	46	77	Pre-dose	7–2882 days	MEIA	whole blood
Woillard et al. [38]	France (Europe)	Multi	Kidney	Prograf® [po] (n=32)	MMF + corticosteroids	73	NS	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9 at 5 transplant periods, 12 h (Week 1 and 2)	1 week-6 months	TFC-MS/MS	whole blood
				Advagraf® [po] (n=41)				Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9, 12, 24	>12 months		

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Saint-Marcoux et al. [39]	France (Europe)	Single	Kidney	Advagraf® [po]	NS	30	NS	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9, 12, 24	>12 months	TFC-MS/MS, EMIT, CMIA	whole blood
Grover et al. [40]	USA (North America)	Single	Kidney	Prograf® [po]	NS	24	NS	Pre-dose, 0.5, 1, 2, 4, 6, 8, and 12	Mean(SD) = 30(23) months	CMIA	whole blood
Passey et al. [41]	USA, Canada (North America)	Multi	Kidney	Prograf® [po]	NS	681	11823	Pre-dose	First 6 months	LC-MS	whole blood
Musuamba et al. [42]	Belgium (Europe)	Single	Kidney	tacrolimus IR[a] [po]	MMF or EC-MPS + corticosteroids	65	NS	Pre-dose; 6 samples post-dose (based on a D-optimal study design with sampling windows around optimal times: 0.248, 0.64, 0.98, 1.37, 2.38, 11.03)	Day 15	MEIA	whole blood
Musuamba et al. [10]	Belgium (Europe)	Single	Kidney	tacrolimus IR[a] [po]	MMF or EC-MPS + corticosteroids	65 plus simulated data generated from 4 previous models	NS	Pre-dose, 0.5, 1.5, 3, 4, 8, 12	Day 15; Simulated data: 3 days – 1 month	MEIA	whole blood
Han et al. [43]	Korea (Asia)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	80	2788	Pre-dose	0–400 days	MEIA	whole blood
Ogasawara et al. [11]	USA (North America)	Single	Kidney	Prograf® [po]	MPA + corticosteroids	102	500	31 patients: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12; 71 patients: Pre-dose, 2	2–123 months	HPLC-MS/MS	whole blood
Zuo et al. [44]	China (Asia)	Single	Kidney	Prograf® [po]	Mycophenolate + corticosteroids	161	873	Pre-dose	0–95 days	MEIA	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Asberg et al. [45]	Norway (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab; high-risk patients: human immunoglobulins and rituximab	69	1546	29 patients: ''Intensely sampled''; 40 patients: Pre-dose	1 to >921 days	HPLC-MS/MS	whole blood
Zhao et al. [46]	France (Europe)	Single	Kidney	Advagraf® [po]	MMF or MPA (Myfortic®)	22 (paediatrics and adolescents)	171	Pre-dose, 1, 2, 3, 6, 12, 16, 24	193-4983 days	EMIT	whole blood
Gaies et al. [47]	Tunisia (North Africa)	Single	Kidney	Prograf® [po]	MMF	20	200	Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12	NS	CMIA	whole blood
Storset et al. [48]	Norway (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab; high-risk patients: humane immune globulins and rituximab. Substudies: prednisolone	69	1546	29 patients (substudies): Pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 23, 24; 44 patients: Pre-dose	First 70 days. Substudies: 26 days-18 years	MEIA, CMIA, LC-MS/MS	whole blood
Bergmann et al. [49]	Australia (Oceania)	Single	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab	173	1554	20 patients: Pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 9, 12; 153 patients: Pre-dose, 1, 2, 4	4 days–6.5 years	HPLC-MS/MS	whole blood
Golubovic et al. [50]	Serbia (Europe)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	105	1999	Pre-dose	0–206 days	CMIA	whole blood
Storset et al. [51]	Norway (Europe), Australia (Oceania)	Multi	Kidney	Prograf® [po]	MMF + corticosteroids + basiliximab	242	3100	All patients: Pre-dose; Some patients from full pharmacokinetic profiling and some from limited pharmacokinetic profiling	4 days-15 years	MEIA, CMIA, LC-MS/MS	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Han et al. [52]	Korea (Asia)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	102	1626	All patients: pre-dose; 55 patients: 0.5, 1, 2, 4, 6, 8, 12	2-21 days	LC-MS/MS	whole blood
Andreu et al. [53]	NS	Multi	Kidney	Prograf® [po]	MMF + corticosteroids + daclizumab	16	594	Pre-dose, 0.33, 0.66, 1.25, 2, 3, 4, 6, 8, 10, 12	7–365 days	EMIT	whole blood
Zhang et al. [54]	China (Asia)	Single	Kidney	tacrolimus IR[a] [po]	MMF + corticosteroids	83	246	Pre-dose	Mean(SD) = 39.8(26.1) months	EIA	whole blood
Robles-Piedras et al. [55]	Mexico (North America)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	36	592	Pre-dose	2-574 days	CMIA	whole blood
Fredj et al. [56]	Tunisia (North Africa)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	50	306	Pre-dose	0 to >12 months	EMIT	whole blood
Vadcharavivad et al. [57]	Thailand (Asia)	Single	Kidney	tacrolimus IR[a] [po]	Mycophenolate + corticosteroids	96	1183	All patients: Pre-dose; 26 patients: 1, 2, 4, 6, 8, 12	7-6284 days	CMIA	whole blood
Zhang et al. [12]	China (Asia)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	83	2109	Pre-dose	≤1 year	EMIT	whole blood
Andreu et al. [58]	Spain (Europe)	Single	Kidney	Prograf® [po]	MMF	304	1891	All patients: Pre-dose; 7 patients: 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12	Day 7-12 months	EMIT, UPLC-MS	whole blood
Woillard et al. [59]	Belgium (Europe)	Single	Kidney	tacrolimus IR[a] [po]	MMF or MPS + corticosteroids	59	NS	Pre-dose, 0.5, 1.5, 3, 4, 8, 12	NS	CMIA	whole blood
Campagne et al. [13]	USA (North America)	Single	Kidney	Prograf® [po]	EC-MPS	67	594	Pre-dose, 1, 2, 3, 4, 6, 8, 10, 12	≥6 months	CMIA	whole blood
Rong et al. [60]	Canada (North America)	Single	Kidney	tacrolimus IR[a] [po] (Generic)	MMF (corticosteroid free regimen)	49	320	21 patients: Pre-dose, 2; 28 patients: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 10, 12	Mean(SD): 525(683) days	LC-MS	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Zhu et al. [61]	China (Asia)	Single	Kidney	Prograf® [po]	MMF + corticosteroids	141	1232	Pre-dose	Mean(SD): 310.33 (601.82) days	CMIA	whole blood
Resendiz-Galvan et al. [62]	Mexico (North America)	Single	Kidney	Limustin®/Framebin® [po] (Generic IR)	MMF + corticosteroids	52	600	Pre-dose	4-2730 days	CMIA	whole blood
Andrews et al. [63]	Netherlands (Europe)	Single	Kidney	Prograf® [po]	MPA	337	4527	Pre-dose, 1, 2, 3, 4, 5, 6	0-100 days	EMIT, ACMIA, LC-MS/MS	whole blood
Woillard et al. [64]	NS	Multi	Kidney and liver	Envarsus® [po]	NS	106 (liver=57, kidney=49)	NS	Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 20, 24	0.50-14.25 years	LC-MS/MS	whole blood
Nanga et al. [23]	NS (Europe)	Multi	Kidney and liver	Prograf® [po]	NS	281 (adults and paediatrics; liver=201, kidney=80)	NS	3 studies: Pre-dose; 4 studies: intensive sampling	1–394 days	MEIA, CMIA, LC-MS/MS	whole blood
Macchi-Andanson et al. [65]	France (Europe)	Single	Liver	Prograf® [po]	NS	40	NS	Pre-dose	1–14 days	MEIA	whole blood
Fukatsu et al. [66]	Japan (Asia)	Single	Liver (LDLT)	Prograf® [iv/po]	Corticosteroids	35	824	Pre-dose	≤30 days	MEIA	whole blood
Fukudo et al. [67]	Japan (Asia)	Single	Liver (LDLT)	Prograf® [iv/po]	Corticosteroids	82	1846	Pre-dose	≤30 days	MEIA	whole blood
Staatz et al. [9]	Australia (Oceania)	Single	Liver	Prograf® [po]	MMF or azathioprine + corticosteroids	68	1742	All patients: Pre-dose; 36 patients: 1, 2, 4, 6 or 8	6–2115 days	LC-MS/MS	whole blood
Antignac et al. [68]	France (Europe)	Single	Liver	tacrolimus IR[a] [po]	MMF + corticosteroids	37	728	Pre-dose	11–66 days	MEIA	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Zahir et al. [69]	Australia (Oceania)	Single	Liver	Prograf® [po]	Corticosteroids ± azathioprine ± sirolimus ± MMF ± Muromonab	67	694	Pre-dose	14–94 days	MEIA	whole blood
Lee et al. [14]	Korea (Asia)	Single	Liver	tacrolimus IR[a] [po]	Corticosteroids ± MMF	51	1775	Pre-dose	24-308 days	MEIA	whole blood
Sam et al. [70]	Singapore (Asia)	Single	Liver	Prograf® [po]	Adult=Basiliximab + corticosteroids; Paediatric=corticosteroids	31 (16 adults, 15 paediatrics) 29 (15 adults, 14 paediatrics)	213 157	‘‘Serial samples within a dosing interval of 12h’’	‘‘Immediate post-transplant period’’	HPLC-MS/MS	whole blood plasma
Li et al. [71]	China (Asia)	Single	Liver	Prograf® [po]	MMF + corticosteroids	72	703	Pre-dose	1-88.5 days	MEIA	whole blood
Blanchet et al. [72]	France (Europe)	Single	Liver	Prograf® [po]	Corticosteroids	14	NS	Pre-dose, 2, 3, 4, 6, 9	6-97 days	EMIT	whole blood
Zhang et al. [73]	China (Asia)	Single	Liver	Prograf® [po]	MMF + corticosteroids	262	3763	Pre-dose	2–1941 days	MEIA	whole blood
Oteo et al. [74]	Spain (Europe)	Single	Liver	Prograf® [po]	Corticosteroids ± azathioprine	75	335	Pre-dose	0–15 days	MEIA	whole blood
Valdivieso et al. [75]	Spain (Europe)	Single	Liver	Prograf® [po]	Corticosteroids	150	NS	Pre-dose	0–15 days	MEIA	whole blood
Zhu et al. [76]	China (Asia)	Single	Liver	tacrolimus IR[a] [po]	MMF + corticosteroids	47	435	Most patients: Pre-dose, 0.3, 1, 1.5, 2, 4, 6, 8, 12; Remainder: Pre-dose only	2–85 days	MEIA	whole blood
Zhu et al. [77]	China (Asia)	Single	Liver	tacrolimus IR[a] [po]	MMF + corticosteroids	95	2285	Pre-dose	1–341 days	MEIA	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Moes et al. [78]	Netherlands (Europe)	Single	Liver	Advagraf® [po]	NS	49	282	Pre-dose, 1, 2, 3, 4, 6	≥3 months	LC-MS/MS	whole blood
Chen et al. [79]	China (Asia)	Single	Liver	Prograf® [po]	MMF or EC-MPS + corticosteroids	125	1704	All patients: Pre-dose; 28 patients: 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12	3-89 days	LC-MS/MS, MEIA	whole blood
Ji et al. [80]	Korea (Asia)	Single	Liver (LDLT)	Prograf® [po]	Corticosteroids ± MMF	58	605	Pre-dose	0-14 days	EIA	whole blood
Riff et al. [81]	NS	Multi	Liver	Prograf® [po] or Advagraf® [po]	Corticosteroids	80	1654	Prograf®: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12; Advagraf®: Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 12.5, 13, 14, 15, 16, 18, 20, 24	Day 7 and Week 6 (± 7 days)	HPLC-MS/MS	whole blood
Lu et al. [82]	China (Asia)	Single	Liver + healthy male volunteers	Prograf® [po]	MMF + corticosteroids	152 (liver=112, healthy=40)	liver=1100; healthy=851	Liver: Pre-dose; Healthy: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 12, 24, 48, 72	2–137 days	MEIA (liver transplant), HPLC-MS (healthy)	whole blood
Shao et al. [83]	China (Asia)	Single	Liver	Prograf® [po]	Mycophenolate + corticosteroids	43	721	Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12	2–90 days	CMIA	whole blood
Saint-Marcoux et al. [27]	France (Europe)	Single	Lung	tacrolimus IR[a] [po]	MMF or azathioprine	22 (11 CF)	NS	Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12	≥3 months	MEIA	whole blood
Monchaud et al. [26]	France, Belgium (Europe)	Multi	Lung	Prograf® [po]	Corticosteroid + azathioprine or MMF	78	2113	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 10, 12	≤1 year	TFC-MS/MS	whole blood

Reference	Country of study (Continent)	Single- or multi-centre	Type of transplant	Tacrolimus formulation	Concomitant immunosuppressant drugs	Number of transplant recipients	Number of concentrations	Sampling schedules (h)	Post-transplant time	Bioanalytical method	Biological fluid
Sikma et al. [29]	Netherlands (Europe)	Single	Heart and lung	Prograf® [po]	Heart: MMF + corticosteroids; Lung: MMF + corticosteroids + basiliximab	30 (10 heart, 20 lung)	Whole blood: 119; Plasma: 90	Whole blood: Pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12; Plasma: 0, 2, 3 (if CF), 6, 12	First 6 days	HPLC-MS/MS	Whole blood and plasma (total & unbound)
Sikma et al. [28]	Netherlands (Europe)	Single	Heart and lung	Prograf® [po]	Heart: MMF + corticosteroids; Lung: MMF + corticosteroids + basiliximab	30 (10 heart, 20 lung)	119	Pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12	First 6 days	HPLC-MS/MS	Whole blood
Gong et al. [25]	China (Asia)	Single	Heart	Prograf® [po]	Mycophenolate + corticosteroids	146	891	Pre-dose	1-96 days	EMIT	whole blood
Zhou et al. [24]	China (Asia)	Single	Heart	Prograf® [po]	MMF + corticosteroids + basiliximab	107	707	All patients: pre-dose. 7 patients: 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12	3-41 days	EMIT (predose), ECLIA (intensive sampling)	whole blood
Lu et al. [84]	Europe, USA, Canada (North America), Korea (Asia), South Africa	Multi	Liver, kidney, heart	Prograf® [po], Advagraf® [po], Astagraf XL® [po]	NS	408 (391 adults and 17 paediatrics)	23176	Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 12.5, 13, 14, 15, 16, 18, 20, 24	NS	HPLC-MS/MS, MEIA	whole blood
Jacobson et al. [30]	USA	Single	Hematopoietic cell	tacrolimus IR[a] [iv/po]	± Methotrexate	122	1625	Pre-dose	6-122 days	MEIA	whole blood

ACMIA antibody-conjugated magnetic immunoassay; *CF* cystic fibrosis; *CMIA* chemiluminescent microparticle immunoassay; *ECLIA* electrochemiluminescence immunoassays; *EC-MPS* enteric-coated mycophenolate sodium; *EIA* enzyme immunoassay; *EMIT* enzyme-multiplied immunoassay technique; *HPLC-MS* high-performance liquid chromatography-mass spectrometry; *HPLC-MS/MS* high-performance liquid chromatography-tandem mass spectrometry; *IR* immediate-release; *iv* intravenous; *LC-MS/MS* liquid chromatography-tandem mass spectrometry; *LC-MS* liquid chromatography-mass spectrometry; *LDLT* living donor liver transplant; *MEIA* microparticle enzyme immunoassay; *MMF* mycophenolate mofetil; *MPA* mycophenolic acid; *MPS* mycophenolate sodium; *NS* not stated; *po* oral; *TFC-MS/MS* turbulence flow chromatography-tandem mass spectrometry; *UPLC-MS* ultra-performance liquid chromatography-mass spectrometry.
[a]Models did not specify what brand was used.

Most studies (91%) investigated the oral immediate-release formulation of tacrolimus. Only four studies had intravenous data [30, 32, 66, 67]. Two studies looked at the generic oral immediate-release formulation of tacrolimus [60, 62]. Two studies simultaneously described the pharmacokinetic disposition for both the immediate- and prolonged-release formulations [38, 84]. Four studies explored both the intravenous and oral immediate-release formulation to estimate oral bioavailability [30, 32, 66, 67]. Several studies (17%), whilst reporting the formulation, did not stipulate the brand used [10, 14, 27, 30, 42, 54, 57, 59, 60, 68, 76, 77]. More than half of the studies (55%) involved transplant recipients who received triple maintenance immunosuppressant regimen – tacrolimus, mycophenolate, and corticosteroid.

Models were developed using intensive sampling data in 61% of the studies, while the remainder used trough concentrations alone. Most studies (59%) used immunoassays to determine tacrolimus concentrations, followed by liquid chromatography-mass spectrometry (28%) or a combination of both bioanalytical methods (13%). All studies described tacrolimus pharmacokinetics based on whole blood concentrations. Two studies compared the difference in pharmacokinetic parameter estimates between two physiological matrices, whole blood, and plasma concentrations [29, 70].

Most of the models (81%) were developed using NONMEM®, a parametric population modelling software (Table 9). Some studies utilised non-parametric software, such as Pmetrics (7%) [45, 56, 59, 64, 81]. Diagnostic goodness-of-fit plots to assess model improvement during model building were generated by 86% of the studies. Final model accuracy and robustness were evaluated using bootstrapping (57%) and/or visual

predictive check (51%). Only 35% of the studies performed an external evaluation using an independent dataset. Evaluation of these models was limited to the same type of organ transplant population used to develop the models. Four studies did not describe the model evaluation approach used [30, 36, 72, 75].

The pharmacokinetics of tacrolimus were described using a one-compartment model in 54% of studies and a two-compartment model in 43% of studies, all with first-order elimination. All studies that used only trough concentration data resulted in one-compartment models. Despite the availability of intensive sampling data (≥6 concentrations), several studies (29%) developed one-compartment models instead of two-compartment models. First-order absorption was displayed by 43% of the models. Several studies (28%) used first-order absorption with a lag time to describe the delayed drug absorption phase. More complex models were used by few studies (7%) to describe the absorption phase, such as the Erlang model [26, 33, 35, 38, 47] with few transit compartments to characterise the highly variable mean absorption time of tacrolimus between patients [35]. Six studies (9%) described a double gamma distribution of tacrolimus [27, 36, 39, 59, 64, 81] to explain the second peak concentration seen in some patients [27].

Table 9. Summary of population pharmacokinetic models of tacrolimus in adult transplant recipients.

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Staatz et al. [8]	NONMEM®	Goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 23.6 + (31.9/DOT) + (76.7/AST) L/h V/F = 1070 L	 42 111		Add RRE = 3.7 ng/mL
Scholten et al. [31]	MW/Pharm	Examined in a separate cohort	2-CMT First-order absorption, with a lag time and first-order elimination	F = 0.23 (fixed) Ka = 0.58 h^{-1} Lag time = 0.956 h Ke = 0.517 h^{-1} V_1 = (0.18*WT) L K_{12} = 2.85 h^{-1} K_{21} = 0.384 h^{-1} CL= 28.3 L/h			
Antignac et al. [32]	NONMEM®	Bootstrap, goodness-of-fit plot	1-CMT First-order absorption and elimination	F = 0.137 Ka = 4.5 h^{-1} (fixed) CL = 1.81*[1 + ($POD^{2.54}$/($POD^{2.54}$ + $3.81^{2.54}$))]*PREDDOSE L/h PREDDOSE = 1.575 if prednisone dosage >25 mg, otherwise PREDDOSE = 1 V = (98.4*WT) L CL_{min} = Minimum CL value when POD=0	32 CL_{min} =31 79		Prop RRE = 18.6% Add RRE = 0.96 ng/ml
Benkali et al. [33]	NONMEM®	Bootstrap, data splitting (cross-validation), goodness-of-fit plot	2-CMT Erlang model with 3 transit compartments and first-order elimination	Ktr = 6.5 h^{-1} CL/F = (863/HCT) L/h V_1/F = 147 L Q/F = 60 L/h V_2/F = 500 L (fixed)	15 30 26 63	24 27 71	Prop RRE = 10% Add RRE = 1.5 ng/ml
Benkali et al. [35]	NONMEM®	Bootstrap, VPC, examined in a separate cohort, goodness-of-fit plot	2-CMT Erlang model with 3 transit compartments and first-order elimination	Ktr = 3.3 h^{-1} CL/F = 19*(2.15^{CYP3A5}) L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1 V_1/F = 486 L K_{12} = 0.13 h^{-1} K_{21} = 0.09 h^{-1}	52 35 53 54		Prop RRE = 8% Add RRE = 0.7 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Press et al. [34]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption and elimination	F = 0.23*[1 - (DD/(25 + DD))]*PREDDOSE PREDDOSE = 1 if prednisolone dose ≤10 mg, otherwise PREDDOSE = 0.85 Ka = 3.7 h^{-1} (once-daily regimen) Ka = 1.6 h^{-1} (twice-daily regimen) CL = 3.7 L/h (CYP3A5*3/*3 genotype) CL = 5.5 L/h (CYP3A5*1/*3 genotype) V_1 = 61 L (once-daily regimen) V_1 = 42 L (twice-daily regimen) Q = 10 L/h V_2 = V1	19 28	22	Prop RRE = 23%
Saint-Marcoux et al. [36]	ITSIM	NS	1-CMT open model Double gamma distribution and first-order elimination	**Day 14 / Day 42** C_0 = 0.816 ± 0.441 **/** 0.883 ± 0.441 a_1 = 13.358 ± 6.182 **/** 13.880 ± 6.182 b_1 = 20.414 ± 6.564 **/** 13.495 ± 6.564 a_2 = 7.397 ± 1.581 **/** 4.428 ± 1.581 b_2 = 2.183 ± 0.273 **/** 0.719 ± 0.273 r = 0.684 ± 0.146 **/** 0.627 ± 0.146 F*A_IV = 1.846 ± 1.359 **/** 2.946 ± 1.359 α = 0.199 ± 0.178 **/** 0.349 ± 0.178 Values expressed as mean values ± SD; C_0 is trough concentration. a_1, b_1, a_2 and b_2 are the parameters of the gamma distribution, r is the fraction of drug absorbed through the first route, F is the bioavailability, A_IV is the initial concentration, α is the apparent elimination rate after intravenous bolus administration of a unit dose.			
Velickovic-Radovanovic et al. [37]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	F = 1.18 Ka = 4.5 h^{-1} (fixed) CL = 0.862 + 0.32*DD + 1.16*PREDDOSE L/h PREDDOSE = 1 if prednisolone dose >25 mg, otherwise PREDDOSE = 0 V = 166 L			Prop RRE = 30.95%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Woillard et al. [38]	NONMEM®	Examined in a separate cohort, VPC, goodness-of-fit plot	2-CMT Erlang model with 3 transit compartments and first-order elimination	Ktr = 3.34*(1.53^{FORM}) h^{-1} CL/F = 21.2*[(HCT/35)$^{-1.14}$]*(2^{CYP3A5}) L/h V_1/F = 486*(0.29^{FORM}) L Q/F = 79 L/h V_2/F = 271 L FORM = 0 if patient received Advagraf®, otherwise FORM = 1 CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1	24 28 31 54 60	33 31 75	Prop RRE = 11.3% Add RRE = 0.71 ng/mL
Saint-Marcoux et al. [39]	In-house program	Examined in a separate cohort	1-CMT Gamma distribution, with 2 parallel absorption routes and first-order elimination	CL/F = 26.3 ± 12.2 L/h (LC-MS/MS) CL/F = 23.4 ± 11.4 L/h (CMIA) CL/F = 23.4 ± 12.1 L/h (EMIT) V/F = 405 ± 171 L (LC-MS/MS)* V/F = 321 ± 168 L (CMIA) V/F = 264 ± 125 L (EMIT)* Values expressed as mean values ± SD; *Variance of log-transformed parameter (approximately equal to the coefficient of variation squared), unit-less.			
Grover et al. [40]	NONMEM®	Goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 1.38 h^{-1} Lag time = 0.573 h CL/F = 10.1 L/h V_1/F = 73.3 L Q/F = 27.1 L/h V_{ss}/F = 462 L	46 13.3 43.5 36.9 50.4		
Passey et al. [41]	NONMEM®	Bootstrap, goodness-of-fit plot	NS	CL/F = 38.4*[1 (if POD 3–5) or 0.86 (if POD 6–10) or 0.71 (if POD 11–180)]*[1.69 (if CYP3A5*1/*3 genotype) or 2.00 (if CYP3A5*1/*1 genotype) or 1 (if CYP3A5*3/*3 genotype)]*[0.7 (if steroid sparing centre), otherwise = 1]*[(Age/50)$^{-0.4}$]*[0.94 (if CCB is present), otherwise = 1] L/h	40.1		Add RRE = 3.19 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Musuamba et al. [42]	NONMEM®	Bootstrap, case deletion diagnostics, cross-validation, VPC, goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 0.45 h^{-1} Lag time = 0.1 h CL/F = 16.3 + CYP3A5 + ABCB1 + (20.6*HCT/21) L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 15.4 ABCB1 = 7.6 if patient a CC-GG-CC carrier at ABCB1 exons 12-21-26, otherwise ABCB1 = 0 V_1/F = 86.4 L Q/F = 58.2 L/h V_2/F = 1115 L	91 61 32 55 48		Prop RRE = 13% Add RRE = 0.88 ng/mL
Musuamba et al. [10]	NONMEM®	Goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 1.68 h^{-1} Lag time = 0.045 h CL/F = 3.85 L/h V_1/F = 221 L Q/F = 21.9 L/h V_2/F = 520.8 L	199 350 185 133 144		Prop RRE = 1% Add RRE = 0.97 ng/mL
Han et al. [43]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.5 h^{-1} (fixed) CL/F = 22.9*[EXP(0.170 (If CYP3A5*1/*3) or 0.0525 (If CYP3A5*3/*3) or 0.111 (If CYP3A5*1/*1))]*[EXP(0.297 (if HCT≤33) or 0.117 (if HCT >33))]*($POD^{-0.00762}$) L/h V/F = 716*[EXP(0.355*WT/59.025)] L	 49.8 48.7		Prop RRE = 40% Add RRE = 0.4 ng/mL
Ogasawara et al. [11]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 0.544 h^{-1} Lag time = 0.183*(2.60^{DM}) h DM = 1 if diabetic, otherwise DM = 0 CL/F = 20.7*[$(Age/50)^{-0.78}$]*[2.03^{CYP3A5}]*[1.40^{ABCC2}] L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1; ABCC2 = 1 if patient is ABCC2 haplotype H2/H2 or H1/H2, otherwise ABCC2 = 0 V_1/F = 234 L Q/F = 70.7 L/h V_2/F = 1319 L	 43.9 157		Prop RRE = 18.4%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Zuo et al. [44]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 3.09 h^{-1} (fixed) CL/F = 26.6*[(HCT/27.9)$^{-0.451}$]*CYP3A L/h CYP3A = 1.21 if patient has CYP3A5*1/*1 or CYP3A5*1/*3, CYP3A4*1/*1G or CYP3A4*1G/*1G genotype; CYP3A = 0.982 if patient has CYP3A5*1/*1, CYP3A5*1/*3 or CYP3A4*1/*1 genotype; CYP3A = 0.77 if patient has CYP3A5*3/*3, CYP3A4*1/*1G or CYP3A4*1G/*1G genotype; CYP3A = 0.577 if patient has CYP3A5*3/*3 or CYP3A4*1/*1 genotype V/F = 1020 L	 24.2 58.5		Prop RRE = 19.8% Add RRE = 1.47 ng/mL
Asberg et al. [45]	Pmetrics	Examined in a separate cohort, goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	F = 0.63 (CYP3A5 expressers) or 1 (CYP3A5 non-expressers) Ka = 1.04 h^{-1} Lag time = 1.0 h (first week) Lag time = 0.15 h (week 2–4) Lag time = 0.59 h (after first month) CL/F = 26.7*[(FFM/59)$^{0.75}$] L/h (CYP3A5 expressers) CL/F = 21.2*[(FFM/59)$^{0.75}$] L/h (CYP3A5 non-expressers) V_1/F = 177*(BMI/26) L Q/F = 19.5*[(FFM/59)$^{0.75}$] L/h V_2/F = 3707*(FFM/59) L Not clear how HCT was modelled on CL/F, V1/F or V2/F			
Zhao et al. [46]	NONMEM®	Bootstrap, VPC, NPDE, goodness-of-fit plot	1-CMT First-order absorption with a lag time and first-order elimination	Ka = 8.34 h^{-1} Lag time = 0.872 h CL/F = 30.6*[(WT/70)$^{0.75}$]*CYP3A5 L/h CYP3A5 = 1 if patient is a CYP3A5*3/*3, CYP3A5 = 1.66 if patient is a CYP3A5*1/*3 V/F = 1100*(WT/70) L	150 34.6 52.1		Prop RRE =22.1%
Gaies et al. [47]	NONMEM®	Bootstrap, jackknife, goodness-of-fit plot	2-CMT Erlang model with 3 transit compartments and first-order elimination	Ktr = 3.77 h^{-1} CL/F = 22.6 L/h V_1/F = 84.2*(WT/72)$^{1.77}$ L Q/F = 50.4 L/h V_2/F = 300 L (fixed)	11.66 41.83 7.69 100 BSV on WT=41.8%		Prop RRE = 16% Add RRE = 0.71 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Storset et al. [48]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption, with a lag time and first-order elimination	$F = [1.87 + (1 – 1.87)/(1 + (POD/2.6)^{-10}))]*[1 + 0.27/(1 + (POD/30)^{-2.3})]*[Fmin,age + (1 - Fmin,age)/(1 + (AGE/44)^{-12.2})]*CYP3A5$ CYP3A5 = 0.51 if patient is a CYP3A5 expresser, otherwise CYP3A5 = 1. Fmin,age = 0.44 (females) or 0.68 (males). $Ka = 1.14\ h^{-1}$ Ka, substudy 2 = $0.37\ h^{-1}$ Lag time = 0.21 h Lag time, substudy 2 = 0.82 h $CL/Fn = 20.5*[(FFM/60)0.75]$ L/h $V_1/Fn = 105*(FFM/60)$ L $Q/Fn = 35.8*[(FFM/60)0.75]$ L/h $V_2/Fn = 450*(FFM/60)$ L Correlations (CL/Fn, Q/Fn) = 0.75 Pharmacokinetic parameters were standardised to a 40-year-old male, CYP3A5 non-expresser with FFM of 60 kg and HCT of 45% at a time point with the lowest estimated bioavailability (Fn, POD 5)	BSV Fmax,late = 117 33 14 91 52	Fn = 16 63	Prop RRE = 16.7% Study 2 factor = 0.57 Study 3 factor = 0.73
Bergmann et al. [49]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption, with a lag-time and first-order elimination	$Ka = 0.35\ h^{-1}$ Lag time = 0.44 h $CL/F = 25.5*(1.6^{CYP3A5})*[(1 –1.01*(HCT - 0.33))]*[(WT/70)^{0.75}]*[(1 – 0.0021*(POD - 22.7))]$ L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1 POD is capped at 180 days $V_1/F = 113*[(1 - 0.0028*(Pred_{Cmax,unbound} - 155.5))$ L $Pred_{Cmax,unbound}$ is maximum concentration value for free prednisolone (nmol/L) Q/F = 67.9 L/h $V_2/F = 1060$ L Correlations: V_1/F, Ka = 0.677 V_1/F, V_2/F = –0.049 Ka, V_2/F = –0.013	47.6 29.5 46.8 89.4	 29.9 126.5	Prop RRE = 18.3%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Golubovic et al. [50]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 1.3 h^{-1} (fixed) CL/F = 10.017*[(POD/47)$^{-0.0283}$]*[(WT/68)$^{0.869}$]*[(TP/63)$^{0.161}$]*[1 – 0.086*(AST – 15)]*[1 – 0.831*(HCT – 0.31)] L/h V/F = [0.68*WT] L (fixed)	 15.2		Add RRE = 4.07 ng/mL
Storset et al. [51]	NONMEM®	Bootstrap, VPC, examine in separate cohort	2-CMT First-order absorption with a lag time and first-order elimination	F = 1*[1 – (0.67*PREDDOSE)/(35 + PREDDOSE)]*[2.68 (If POD 1), otherwise = 1]*[0.82 (If CYP3A5 expresser), otherwise = 1] CL_p/F = 811*[(FFM/60)$^{0.75}$]*[1.3 (If CYP3A5 expresser) or 1 (If CYP3A5 non-expresser)] L/h V_{1p}/F = 6290*FFM/60 L Q_p/F = 1200*[(FFM/60)$^{0.75}$] L/h V_{2p}/F = 32100*FFM/60 L Ka = 1.01 h^{-1} Lag time = 0.41 h CL/F (HCT 45%, FFM 60 kg) = 16.1 L/h V_1/F (HCT 45%, FFM 60 kg) = 125 L Q/F (HCT 45%, FFM 60 kg) = 23.8 L/h V_2/F (HCT 45%, FFM 60 kg) = 636 L Correlation: V_1/F, CL/F = 0.43 CL/F, Q /F = 0.62	FPOD1 = 57 40 54 63	23 120	Prop RRE = 14.9%
Han et al. [52]	NONMEM®	Bootstrap, VPC, examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption, with a lag time and first-order elimination	Ka = 3.43 h^{-1} Lag time = 0.25 h (fixed) CL/F = 21.9*(1 + 0.0119*(POD - 9.6))*[0.816^{CYP3A5}] L/h CYP3A5 = 1 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 0 V/F = 205 L	158.9 42.6 64.6		Prop RRE = 5.4% Add RRE = 1.94 ng/mL
Robles-Piedras et al. [55]	NONMEM®	Examine in a separate cohort, goodness-of-fit plot	1-CMT open model First-order absorption and elimination	Ka = 4.5 h^{-1} (fixed) CL/F = 22.5 L/h V/F = 812.7 L Factor for CCB = 7.6[a] Factor for HCT = 10.2[a]	 52.9 82.1		Add RRE = 15.2 ng/mL

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Andreu et al. [53]	NONMEM®	Bootstrap, VPC, NPDE, goodness-of-fit plot, examine in a separate cohort	2-CMT First-order absorption with 3 transit compartments in the absorption phase and first-order elimination	Ka = 0.47 h^{-1} Mean transit time (MTT) = 0.83 h Number of transit compartments (NN) = 3 (fixed) Ktr = 3.61 h CL/F = 16.5 L/h V_1/F = 9.89 L Q/F = 35.56 L/h V_2/F = 526.03 L	35 32 39	 29	Prop RRE = 21%
Zhang et al. [54]	NONMEM®	Bootstrap, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = NS CL/F = 23.3*(1.04*gene 1 + 0.83*gene 2 + 0.62*gene 3) L/h If CYP3A5 genotype was *1/*1 or *1/*3 and POR*28 genotype was CC: gene 1 = 0, gene 2 = 1 and gene 3 = 0; If CYP3A5 genotype was *1/*1 or *1/*3 and POR*28 genotype was CT or TT: gene 1 = 1, gene 2 = 0 and gene 3 = 0; If CYP3A5 genotype was *3/*3: gene 1 = 0, gene 2 = 0 and gene 3 = 1 V/F = 240 L (fixed)	 26.3 10 (fixed)		Prop RRE = 21.1% Add RRE = 0.81 ng/mL
Fredj et al. [56]	Pmetrics	Examine in a separate cohort, goodness-of-fit plot, VPC	1-CMT	CL/F = 3.6 L/h V/F = 9.9 L			
Vadcharavivad et al. [57]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 7.06 h^{-1} (fixed) CL/F = 21.5*[EXP(-0.05*(HB - 11.8))]*[(DOT/125)$^{-0.06}$] L/h V/F = 333 L	 36.8 63.64		Prop RRE = 23.17 % Add RRE = 0.88 ng/ml
Zhang et al. [12]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.5 h^{-1} (fixed) CL/F = 22.4*[EXP(-0.0526*83/POD)]*[(39.1/HCT)$^{0.548}$]*[EXP(-0.32*CYP3A5)] L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1 V/F = 179*[POD$^{0.842}$] L	 50 60.4		Add RRE = 2.33 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Andreu et al. [58]	NONMEM®	Bootstrap, VPC, examine in a separate cohort	2-CMT First-order absorption, with a lag-time and first-order elimination	Ka = 0.138 h^{-1} Lag time = 0.243 h CL = 20.5*(1 - 0.205*AGE) L/h (CYP3A4*22 non-carriers with the CYP3A5*1 carrier) CL = 12.5*(1 - 0.205*AGE) L/h (CYP3A4*22 non-carriers with the CYP3A5*3/*3 genotype or CYP3A4*22 carriers with the CYP3A5*1/*1 genotype) CL = 9.1*(1 - 0.205*AGE) L/h (CYP3A4*22 carriers with the CYP3A5*3/*3 genotype) AGE = 1 if patient's age ≥63 years old, otherwise AGE = 0 V_1 = 5.02 L Q = 4.2 L/h V_2 = 526 L (fixed) Tacrolimus whole blood concentrations standardised to an HCT of 45%.	27.8	33.3	Prop RRE = 25%
Woillard et al. [59]	Pmetrics	NPDE, VPC, goodness-of-fit plot	1-CMT Double gamma distribution and first-order elimination	C_0 = 2.94 a_1 = 12.33 b_1 = 20.36 a_2 = 15.19 b_2 = 5.05 r = 0.46 F*AIV = 24.52 α = 1.52 θ_{CYP3A} = 0.77 Values expressed as mean; C_0 = the model estimated tacrolimus trough level for a theoretical dose of 1000 mg (the real trough level can be calculated by dividing this value by 1000 and multiplying by the patient dose); a_1, b_1, a_2 and b_2 = parameters of the gamma distributions; r = the fraction of dose absorbed following the first gamma function; F = bioavailability coefficient; α = elimination parameter; AIV = initial blood concentration obtained after a bolus IV injection; θ_{CYP3A} = effect of CYP3A on the typical value of tacrolimus blood concentrations			

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Campagne et al. [13]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 4.21 h^{-1} Lag time = 0.828 h CL/F = 19.7*1.45^{H1}*2.25^{H2} L/h H1 = 1 for CYP3A5*3*6*7 (intermediate metaboliser), otherwise H1 = 0; H2 = 1 for CYP3A5*3*6*7 (extensive metaboliser), otherwise H2 = 0 V_1/F = 234*(WT/85.9) L Q/F = 52.6 L/h V_2/F = 403 L	69.4 37 76.7 48.6		Prop RRE = 9%
Rong et al. [60]	Monolix	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 2.2 h^{-1} Lag time = 0.554 h CL/F = 17.9*[(eGFR/56)$^{-0.885}$] L/h V_1/F = 150*[(eGFR/56)$^{-2.13}$] L Q/F = 53.7 L/h V_2/F = 700 L	57.3 51.9 34.6 80.8 55.8 106		Add RRE = 0.771 ng/ml
Zhu et al. [61]	NONMEM®	Bootstrap, examine in separate cohort, VPC, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 3.09 h^{-1} (fixed) CL/F = 27.2*[(WT/70)$^{0.75}$]*[(HCT/0.35)$^{-0.501}$]*[(POD/180)$^{0.0306}$]*CYP3A5 L/h CYP3A5 = 0.753 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1 V/F = 240 L (fixed)	 28.8	 9.6	Prop RRE = 18.8% Add RRE = 2.5 ng/ml
Resendiz-Galvan et al. [62]	NONMEM®	Bootstrap, examine in separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 1.04 h^{-1} (fixed) $F_{Limustin®}$ = 0.53 $F_{Framebin®}$ = 1 CL/F = 12.3*[(HCT/39.2)$^{-0.32}$]*(1 + [1.12 (if CYP3A5*1/*1 genotype) or 0.531 (if CYP3A5*1/*3 genotype) or 0 (if CYP3A5*3/*3 genotype)]) L/h V/F = 604 L	 33 63.3		Add RRE = 2.5 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Andrews et al. [63]	NONMEM®	VPC, goodness-of-fit plot, NPDE, examine in a separate cohort	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 3.58 h^{-1} Lag time = 0.38 h CL/F = 23*[1.0 (if CYP3A5*3/*3) or 1.631 (if CYP3A5*1/*3 or CYP3A5*1/*1)]*[1.0 (if CYP3A4*1 or unknown) or 0.8 (if CYP3A4*22)]* $[(Age/56)^{-0.43}]*[(ALB/42)^{0.43}]*[(BSA/1.93)^{0.88}]*[(SCR/135)^{-0.14}]*[(HCT/0.34)^{-0.76}]$ L/h $V_1/F = 692*[(LBW/58.94)^{1.52}]$ L Q = 11.6 L/h V_2/F = 5340 L	38.6 49.2 78.7 53	13.6	Prop RRE = 17.7% (immunoassay); 24.5% (HPLC-MS/MS) Add RRE = 0.88 ug/L (immunoassay)
Woillard et al. [64]	Pmetrics & ITSIM	VPC, goodness-of-fit plot	1-CMT Double gamma distribution and first-order elimination	**Kidney – ITSIM / Pmetrics** C_0 = 1.7 (0.3-5.2) **/** 1.7 (0.3–4.9) mg/L FAIV = 2.8 (0.7–6.1) **/** 2.3 (0.5–9.9) µg/L a_1 = 7.9 (1.0–23.3) **/** 19.4 (1.2–39.8) b_1 = 5.6 (1.2–11.0) **/** 5.9 (0.2–29.8) h-1 a_2 = 13.8 (1.0–33.8) **/** 20.7 (4.3–39.8) b_2 = 2.3 (0.3–5.7) **/** 6.8 (0.8–29.8) h-1 r = 0.4 (0.0–1.0) **/** 0.6 (0.0–0.9) α = 0.17 (0.05–0.45) **/** 0.15 (0.01–0.99) h-1 C_{max} = 12.1 (5.4–31.5) **/** 12.4 (5.4–39.6) µg/L T_{max} = 6.3 (1.3–12.7) **/** 5.4 (1.0–10.2) h **Liver – ITSIM / Pmetrics** C_0 = 1.3 (0.3–3.4) **/** 1.4 (0.4–3.4) mg/L FAIV = 2.6 (34%) **/** 2.0 (0.7–19.1) µg/L a_1 = 5.2 (1.0–11.9) **/** 27.2 (4.2–49.7) b_1 = 5.5 (1.8–12.8) **/** 5.5 (0.3–48.7) h-1 a_2 = 14.3 (4.1–30.9) **/** 20.8 (1.2–49.7) b_2 = 2.9 (0.6–7.3) **/** 8.6 (0.5–29.8) h-1 r = 0.21 (0.0–0.64) **/** 0.62 (0.11–0.99) α = 0.18 (0.05–0.43) **/** 0.18 (0.02–1.49) h-1 C_{max} = 11.7 (3.70–30.6) **/** 12.5 (4.2–29.5) µg/L T_{max} = 5.5 (1.2–15.0) **/** 5.6 (0.8–20.2) h			

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
				Values expressed as median (min-max); C_0 is the model estimated trough level for a theoretical dose of 1000 mg (the real trough level can be calculated by dividing this value by 1000 and multiplying by the patient dose); FAIV is the estimated, absolute bioavailability factor with respect to the intravenous route; a_1, b_1, a_2, b_2 are the shape and scale of the two gamma functions; r is the fraction of dose absorbed following the first gamma function; α is the elimination parameter; C_{max} is maximum concentration; T_{max} is time to C_{max}; C_{max} and T_{max} were estimated from the fitted pharmacokinetic profiles.			
Nanga et al. [23]	NONMEM®	VPC, goodness-of-fit plot, bootstrap, examine in a separate cohort	2-CMT First-order absorption with a lag time and first-order, time-varying elimination	F = 0.53 (syrup formulation) Ka = 3.37 h^{-1} (fixed) Lag time = 0.32 h (fixed) CL = 22.5*[(WT/50)$^{0.61}$]*[0.38$^{(1 - TRANS)}$]*(1 + TICL) L/h TRANS = 0 if liver transplant, otherwise TRANS = 1 TICL = (POD$^{8.88}$)/[POD$^{8.88}$ + 6.12$^{8.88}$] if liver transplant, otherwise TICL = 0 V_1 = 246.2*[(WT/50)$^{0.53}$] L Q = 24.2 L/h V_2 = 109.9 L	 59.4 133.2		Prop RRE = 24.32% Add RRE = 3.22 ng/mL
Macchi-Andanson et al. [65]	NPEM2	Cross-validation, goodness-of-fit plot	1-CMT First-order absorption and elimination	F = 0.25 (fixed) Ka = 4.5 h^{-1} (fixed) V = 1.58*WT (POD 1–4), 2.06*WT (POD 5–7), 3.45*WT (POD 8–11), 2.56*WT (POD 12–14) L Ke = 0.07 (POD 1–4), 0.11 (POD 5–7), 0.09 (POD 8–11), 0.09 (POD 12–14) h-1	 79 -124 89 - 150		
Fukatsu et al. [66]	NONMEM®	Goodness-of-fit plot, examined in a separate cohort[67]	1-CMT No absorption process, First-order elimination	F = 0.0677 CL = (0.737 + 0.0134*POD)*[0.728^{BILI}]*[0.809^{SCR}]*(HW/600) L/h BILI = 1 if total bilirubin >2.5mg/dL, otherwise BILI = 0; SCR = 1 if serum creatinine >1 mg/dL, otherwise SCR = 0 V = (1.52*WT) L Correlation (CL, F) = 0.776	63.0 57.4 39.7		Add RRE = 2.9 ng/mL

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Fukudo et al. [67]	NONMEM®	Goodness-of-fit plot	1-CMT No absorption process, First-order elimination	F = 0.0732 CL = (0.743 + 0.0157*POD)*[0.792^{BILI}]*[0.810^{SCR}]*(HW/600) L/h BILI = 1 if total bilirubin >2.5 mg/dL, otherwise BILI = 0; SCR = 1 if serum creatinine >1 mg/dL, otherwise SCR = 0 V = (1.64*WT) L Correlation (CL, F) = 0.770	71.2 60 35.4		Add RRE = 2.57 ng/mL
Staatz et al. [9]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 29.6 L/h (AST <70 U/L) CL/F = 24.0 L/h (AST >70 U/L) V/F = 601*(WT/72.1) L	 43 93		Add RRE = 3.3 ng/mL
Antignac et al. [68]	NONMEM®	Bootstrap, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = (CL/F_{max}*$POD^{4.9}$)/($TCL_{50}^{4.9}$ + $POD^{4.9}$) L/h CL/F_{max} = 36*[$(ALB/38)^{-0.64}$] L/h CL/F_{max} is the maximum CL value at plateau TCL_{50} = 6.3*[$(AST/46)^{0.28}$] TCL_{50} is the time needed to obtain 50% of maximum CL V/F = 1870 L Correlation (CL/F_{max}, V/F) = 0.55	 43.6 33.2 49.0		Add RRE = 3.07 ng/mL
Zahir et al. [69]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.5 h-1 (fixed) CL/F = 21.3 + 9.8*(1 - HCT) + 3.4*(1 - ALB) - 2.1*(1 - DIL) - 7.4*(1 – FLU) L/h HCT = 0 if haematocrit <0.35, otherwise HCT = 1; ALB = 0 if albumin <3.5 g/dL, otherwise ALB = 1; DIL = 0 if diltiazem co-administered, otherwise DIL = 1; FLU = 0 if fluconazole co-administered, otherwise FLU = 1 V/F = 314 L	 31.6		Prop RRE = 24.3%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Lee et al. [14]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	CL/F = (0.36 + (2.01/POD)*LPOD)*[BILI$^{-0.23}$]*[0.49^{EPOD}]*[0.75^{INR}]*[0.86^{GWBWR}]*WT L/h LPOD = 1 if POD >35 days, otherwise LPOD = 0; BILI = 1 if total bilirubin level ≤1.2 mg/dL, otherwise BILI = total bilirubin level; EPOD = 1 if POD ≤3 days, otherwise EPOD = 0; INR = 1 if INR >1.4, otherwise INR = 0; GWBWR = 1 if graft: recipient body weight ratio ≤1.25%, otherwise GWBWR = 0	35.35		Add RRE = 3.14 ng/mL
				V/F = 568 L	68.12		
Sam et al. [70]	NONMEM®	Case deletion diagnostics or cross-validation, goodness-of-fit plot	1-CMT First-order absorption and elimination	**Whole blood**			Prop RRE = 34.8 %
				Ka = 2.08 h^{-1}			
				CL/F = 14.1 + 0.237*(WT-55) – 2.93*Y – 0.0801*(SCR-60) L/h	65.7		
				Y=0 for ALP<200 U/L; Otherwise, Y=1			
				V/F = 217 – [7.83*(HCT-31.1)] + [179*(HT-1.61)] L	63.8		
				Plasma			Add RRE = 0.548 ng/mL
				Ka = 5.21 h^{-1}			
				CL_p/F = 537 + 10.5*(WT-55) L/h	96		
				V_p/F = 563 + 5380$^{Ce/Cp}$ L	105.4		
				Ce/Cp = 1 if erythrocyte-to-plasma ratio of concentration ≥68, otherwise Ce/Cp = 0			
Li et al. [71]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed)			Add RRE = 2.81 ng/mL
				CL/F = 15.9 – 1.88*BILI + 7.65*CYPD + 7*CYPR L/h	31.2		
				BILI = 0, if total bilirubin level ≤25.7 µmol/L			
				BILI = 1, if total bilirubin level 25.8 – 51.4 µmol/L			
				BILI = 2, if total bilirubin level 51.5 – 77.1 µmol/L			
				BILI = 3, if total bilirubin level 77.2 – 128.5 µmol/L			
				BILI = 4, if total bilirubin level >128.5 µmol/L			
				CYPD = 0 if donor CYP3A5*3/*3, otherwise CYPD = 1			
				CYPR = 0 if recipient CYP3A5*3/*3, otherwise CYPR = 1			
				V/F = 620 L	55		

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Blanchet et al. [72]	WINBUGS	NS	2-CMT First-order absorption and elimination	Ka = 4.03 h^{-1} CL/F = 2.85*[$0.36^{W/S}$]*[1.026^{FV}] L/h W/S and FV were expressed as 0 or 1 V_1/F = 87 L Q/F = 22 L/h V_2/F = 1290 L *Variance of the log-transformed parameter (approximately equal to the coefficient of variation squared), unit-less. Covariates between parameters were close to 0. **Approximately equal to the coefficient of variation squared, unit-less.	0.44* 0.23* 0.44* 0.39* 0.36*	13.1* 0.30* 0.74* 0.85* 1.35*	Prop RRE = 0.012**
Zhang et al. [73]	NONMEM®	Bootstrap, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.0 h^{-1} (fixed) CL/F = 20.9*[$(DD/4)^{0.582}$]*[$(HCT/35.4)^{0.418}$]*[$(TP/69.1)^{0.780}$]*[0.841^{SU}] L/h SU = 1 if sulfonylurea co-administered, otherwise SU = 0 V/F = 808*[$(HCT/35.4)^{1.52}$]*[$(TP/69.1)^{1.81}$]	 23.8 70.4		Prop RRE = 33.6% Add RRE = 0.96 ng/mL
Oteo et al. [74]	NONMEM®	Examined in a separate cohort, goodness-of-fit plot	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F (POD: 0–3) = 11.1 L/h, if AST <500 U/L and rapid recovery CL/F (POD: 0–3) = 8.04 L/h, if AST ≥500 U/L or slow recovery CL/F (POD: 4–15) = 17.8 L/h, if AST <500 U/L and rapid recovery CL/F (POD: 4–15) = 24.5 L/h, if ALB <2.5 g/dL and HCT <28% V/F (POD: 0–3) = 328 L V/F (POD: 4–15) = 568 L	 45.93 (POD: 0–3) 36.74 (POD: 4–15) 52.15 20.2		Prop RRE = 28.04%
Valdivieso et al. [75]	NONMEM®	NS	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F (POD: 0–3) = 14.5 L/h CL/F (POD: 0–3) = 10.1 L/h (high AST) CL/F (POD: 4–15) = 19.3 L/h CL/F (POD: 4–15) = 23.8 L/h (low HCT and ALB) V/F (POD: 0–3) = 365 L V/F (POD: 4–15) = 597 L	 54.3 (POD: 0–3) 36.74 (POD: 4–15) 54.49 30.09		Add RRE = 3.04 ng/mL
Zhu et al. [76]	NONMEM®	Bootstrap, VPC, goodness-of-fit plot	2-CMT First-order absorption and elimination	Ka = 0.723 h^{-1} CL/F = 11.2*[$DD^{0.371}$]*[$POD^{0.127}$] L/h V_1/F = 406 L Q/F = 57.3 L/h V_2/F = 503 L	74.3 16.2 163 19.7 199		Prop RRE = 26.54%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Zhu et al. [77]	NONMEM®	Bootstrap, goodness-of-fit plot, examined in a separate cohort	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 17.6 L/h Factor for POD on CL/F = 0.205[a] Factor for BUN on CL/F = -0.116[a] Factor for ALP on CL/F = 0.165[a] Factor for total BILI on CL/F = -0.142[a] Factor for HCT on CL/F = -0.789[a] Factor for CYP3A5 on CL/F = 0.661[a] V/F = 225 L Factor for POD on V/F = 0.852[a] Factor for HB on V/F = -0.813[a]	 53.9 68.0		Prop RRE = 28.4% Add RRE = 0.606 ng/mL
Moes et al. [78]	NONMEM®	Bootstrap, VPC	2-CMT First-order absorption with 3 transit compartments and first-order elimination	Ka = 3.76 h^{-1} F = 0.23 (fixed) CL = 4.21*(1 + COMB) L/h COMB = 0, if donor and recipient are CYP3A5*1 non-carriers COMB = 0.33, if recipient is CYP3A5*1 carrier and donor is non-carrier COMB = 0.33, if recipient is CYP3A5*1 non-carrier and donor is carrier COMB = 0.71, if both donor and recipient are CYP3A5*1 carriers V_1 = 88.3 L Q = 14 L/h V_2 = 145 L	65.9 42.8 86.3		Prop RRE = 13%
Ji et al. [80]	NONMEM®	Bootstrap, VPC, goodness-of-fit	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 6.33*[$POD^{0.257}$]*[2.314 (if CYP3A5 expresser recipient grafted from CYP3A5 expresser donor) or 1.523 (if CYP3A5 expresser recipient grafted from CYP3A5 non-expresser donor)] L/h V/F =465*[$POD^{0.322}$] L	 34.2 45.5		Prop RRE = 42.7% Add RRE = 0.838 ng/ml

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Chen et al. [79]	NONMEM®	Bootstrap, VPC, goodness-of-fit	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 0.55 h^{-1} Lag time = 1.96 h CL/F = 21.9*[EXP(0.01*POD)]*[EXP(0.26*CrCL/103.1)]*[EXP(-0.15*ABCB1)] L/h V_1/F =284*[EXP(-0.13*ABCB1)] L Q/F = 62.1 L/h V_2/F = 710 L ABCB1 = 0 if patient is a 3435 CC, ABCB1 = 1 if patient is a 3435 CT, ABCB1 = 2 if patient is a 3435 TT	56.7 36.3 89.4		Prop RRE = 33.3%
Riff et al. [81]	Pmetrics & ITSIM	VPC, data-splitting, goodness-of-fit	1-CMT Double gamma distribution and first-order elimination	**ITSIM - Prograf® [oral] / Advagraf® [oral] (Day 7)** C_0 = 1.27 (0.53–3.53) **/** 0.57 (0.1–1.23) mg/L a_1 = 10.26 (3.08–15.74) **/** 4.88 (1.59–9.89) b_1 = 17 (7.17–24.64) **/** 6.33 (1.36–12.48) h-1 a_2 = 11.83 (7.95–14.28) **/** 15.57 (2.98–31.52) b_2 = 9.25 (2.56–20.77) **/** 2.36 (0.37–5.18) h-1 r = 0.58 (0.01–1.00) **/** 0.71 (0.39–1) FAIV = 3.12 (1.27-9.96) **/** 1.93 (1.00 – 5.65) ug/L α = 0.39 (0.10–0.68) **/** 0.26 (0.07–1.40) h-1 V/F = 321 (100-787) **/** 515 (177-1000) L CL/F = 40.1 (20.1-75.9) **/** 48.5 (19.2-91.7) L/h **ITSIM - Prograf® [oral] / Advagraf® [oral] Week 6** C_0 = 1.66 (0.83–8.52) **/** 0.73 (0.29–2.32) mg/L a_1 = 12.64 (6.03–18.33) **/** 6 (1.00–12.05) b_1 = 17.93 (12.18–23.19) **/** 6.82 (1.52–14.48) h-1 a_2 = 10.25 (5.04–15.23) **/** 10.82 (3.9–35.32) b_2 = 7.24 (1.89–10.07) **/** 1.78 (0.28–4.63) h-1 r = 0.74 (0.06–1.00) **/** 0.66 (0.01–1) FAIV = 4.98 (0.97-9.53) **/** 2.43 (1.11– 6) ug/L α = 0.44 (0.25–0.77) **/** 0.25 (0.13–0.43) h-1 V/F = 214 (105-1030) **/** 412 (167-901) L CL/F = 34.1 (9.0-78.7) **/** 35.1 (15.8-80.1) L/h			

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
				Pmetrics - Prograf® [oral] / Advagraf® [oral] Day 7 C_0 = 1.3 (0.62–3.46) **/** 0.57 (0.06–1.21) mg/L a_1 = 11.9 (0.09–16.92) **/** 7.26 (0.06–11.94) b_1 = 12.25 (0.88–24.88) **/** 13.8 (0.23–14.93) h-1 a_2 = 13.1 (5.05–14.95) **/** 9.52 (0.27–34.83) b_2 = 12.81 (3.09–20.91) **/** 5.19 (0.09–5.97) h-1 r = 0.55 (0.07–1) **/** 0.58 (0.02–0.91) FAIV = 3.6 (2.25–9.95) **/** 1.7 (0.35–2.99) µg/L α = 0.44 (0.21–1) **/** 0.20 (0.08–0.83) h-1 V/F = 278 (101-444) **/** 589 (335-2857) L CL/F = 39.3 (20.1-75.6) **/** 48.7 (18.9-91.3) L/h **Pmetrics - Prograf® [oral] / Advagraf® [oral] W6** C_0 = 1.6 (0.75–8.65) **/** 0.73(0.29–2.39) mg/L a_1 = 10.68 (1.89–18.91) **/** 7.00 (0.56–12.94) b_1 = 13.38 (1.38–24.88) **/** 9.49 (0.57–14.93) h-1 a_2 = 8.17 (1.73–14.93) **/** 10.80 (1.18–35.83) b_2 = 5.9 (0.35–9.95) h-1 **/** 1.83 (0.04–4.98) h-1 r = 0.56 (0.06–1) **/** 0.53 (0.01–0.87) FAIV = 6.3 (1.75–9.95) **/** 3.18 (0.99–5.97) µg/L α = 0.55 (0.26–1) **/** 0.27 (0.10–0.76) h-1 V/F = 159 (100-571) **/** 314 (167-1010) L CL/F = 35.1 (9-73) **/** 35.3 (15.7-81.5) L/h Values expressed as median (min-max); C_0 is the model estimated trough level for a theoretical dose of 1000 mg; a_1, b_1, a_2, b_2 are the shape and scale of the two gamma functions; r is the fraction of dose absorbed following the first gamma distribution; FAIV is the absolute bioavailability factor with respect to the intravenous route multiplied by the maximum concentration which would have been reached after a theoretical bolus IV of the reference dose); α is the elimination parameter.			

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Lu et al. [82]	NONMEM®	Bootstrap, VPC, goodness-of-fit	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 0.419 h^{-1} Lag time = 0.404 h CL/F = 32.8 L/h (healthy volunteers) CL/F = 32.8*0.562*[EXP(ALT*(-0.0237)/40)] L/h (liver transplant) V_1/F = 22.7 L Q/F = 76.3 L/h V_2/F = 916 L (fixed)	0 (fixed) 46.6 57.3 46.0 93.5		Prop RRE = 39.8%
Shao et al. [83]	NONMEM®	Bootstrap, NPDE, goodness-of-fit	2-CMT First-order absorption and elimination	Ka = 0.163 h^{-1} CL/F = 18.1*(1.29^{CYP345_D})*(1.32^{CYP345_R}) L/h CYP345_D = 1 if CYP345*1 carrier donor, otherwise CYP345_D = 0 CYP345_R = 1 if CYP345*1 carrier recipient, otherwise CYP345_R = 0 V_1/F = 72.7 L Q/F = 76.3 L/h (fixed) V_2/F = 412 L	120 18.4 0 (fixed) 147		Prop RRE = 13.2%
Saint-Marcoux et al. [27]	ITSIM	Jackknife	1-CMT Double gamma distribution and first-order elimination	**Cystic fibrosis / Non-cystic fibrosis** MAT1 = 1.10 h **/** 0.92 h MAT2 = 5.14 h **/** 5.47 h A_IV = 4.03 h-1 **/** 9.36 h-1 λ = 0.64 h-1 **/** 0.80 h-1 r = 0.58 **/** 0.62 Cˆ0 = 0.91 mg/L **/** 1.81 mg/L CL/F = 68.22 L/h **/** 36.49 L/h V/F = 2011 L **/** 444 L Values expressed as mean; MAT1 is the mean absorption time associated with the first absorption phase; MAT2 is the mean absorption time associated with the second absorption phase; A_IV is the intravenous disposition coefficient; λ is the disposition rate constant; r is the fraction of the dose absorbed by the faster phase; Cˆ0 is the theoretical residual concentration.			

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Monchaud et al. [26]	NONMEM®	Bootstrap, VPC, goodness-of-fit	2-CMT Erlang with 4 transit compartments and first-order elimination	Ktr = 7.06*[0.47^{CF}] h^{-1} F = 0.63^{CF} CL/F = 17.5*[1.4^{CYP3A5}] L/h V_1/F = 136 L Q/F = 41.1 L/h V_2/F = 529 L CF = 0 in non-cystic fibrosis patients, otherwise CF = 1; CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1	42.7 53.7 41.0 71.7 126	45.8 46.8 75.4	Prop RRE = 6.9% Add RRE = 1.6 ng/mL
Sikma et al. [29]	NONMEM®	Goodness-of-fit	2-CMT Mixed zero- and first-order absorption	Ka = 0.579 h^{-1} (fixed) F = 1 (fixed) CL/F = 20.9 L/h V_1/F = 220 L Q/F = 72 L/h V_2/F = 469 L B_{max} (WBC), (pg/mL) = 2700 K_d (WBC), (pg/mL) = 0.142 N_{plasma} = 137 B_{max}: Maximum binding capacity WBC: Whole-blood concentration K_d: Equilibrium dissociation constant N_{plasma}: Non-specific binding constant TPC: Total tacrolimus plasma concentrations UPC: Unbound tacrolimus plasma concentrations	10 (fixed) 10 (fixed) 42.1 10 (fixed) 10 (fixed) 10 (fixed) 27 3 (fixed) 29	98.3 (fixed) 65	Prop RRE (WBC) = 16.7% Prop RRE (UPC) = 36.3% Prop RRE (TPC) = 31.6% Correlation (WBC, UPC) = 0.26 Correlation (WBC, TPC) = 0.51 Correlation (TPC, UPC) = 0.51
Sikma et al. [28]	NONMEM®	Goodness-of-fit	2-CMT Mixed zero- and first-order absorption	Ka = 0.579 h^{-1} F = 1 (fixed) CL/F = 19.6 L/h V_1/F = 231 L Q/F = 58.2 L/h V_2/F = 521 L	 34.6	98.3 55 29.5 35.1	Prop RRE = 14%

Reference	Software	Model evaluation	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Gong et al. [25]	Phoenix NLME 8.0	Bootstrap, VPC, goodness-of-fit	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 14.23*[(POD/12)$^{-0.12}$]*[1.477 (if concomitant wuzhi tablet), otherwise=1] L/h V/F = 846.91*[(WT/66)$^{2.06}$] L	 38.89 85.95		Prop RRE = 30%
Zhou et al. [24]	NONMEM®	Bootstrap, VPC, goodness-of-fit, examined in a separate cohort	1-CMT First-order absorption and elimination	Ka = 3.8 h^{-1} (fixed) CL/F = 13.7*[1.91^{CYP3A5}]*[0.608^{AFDs}]*[0.664^{WZ}] L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1; AFDs = 1 if concomitant with fluconazole, AFDs = 2 if concomitant with voriconazole, otherwise AFDs = 0; WZ = 1 if concomitant with wuzhi supplement, otherwise WZ = 0; Concentrations were normalised to their corresponding equivalents of a LC-MS method according to the reported bioassays formulae. V/F = 791 L	 36.9		Prop RRE = 23.3% Add RRE = 0.84 ng/ml
Lu et al. [84]	NONMEM®	Bootstrap, VPC, goodness-of-fit	2-CMT First order absorption with a lag time and first-order elimination	F = (1 + [0.25 (if Asians) or -0.433 (if Blacks) or 1 (if White)]*[1.51^{FORM}]*[(ALB/39)$^{1.04}$]*[(LAST/3.15)$^{0.74}$] FORM= 1 (immediate-release), FORM = 0 (prolonged-release) Ka = 0.375 h^{-1} (immediate-release) Ka = 0.499 h^{-1} (prolonged-release) Lag time = 0.44 h CL/F = 44.3*(1 + 0.59*RACE)*[(LAST/3.15)$^{-0.318}$] L/h RACE = 1 if Asians, otherwise RACE = 0 V_1/F = 110*(1-0.446*SEX)*[(ALB/39)$^{1.03}$]*[(LAST/3.15)$^{1.73}$] L SEX = 1 if Female, SEX = 0 if Male Q/F = 131 L/h V_2/F = 3180*[(LAST/3.15)$^{-0.945}$] L	30.5 35.5 30.9 106 39.3 99	59.9	Prop RRE = 21.1% (immunoassay); 15.8% (HPLC-MS/MS)
Jacobson et al. [30]	NONMEM®	NS	Non-compartmental approach	F = 0.28 CL/F = 5.22*[0.797 (if total BILI 2 - 9.9 mg/dL) or 0.581 (if total BILI ≥10 mg/dL) or 1 (if total BILI <2 mg/dL)]*[0.587 (if SCR ≥2 mg/dL) or 1 (if SCR <2 mg/dL)]*[0.814 (if grade III-IV GVHD) or 1 (if grade 0 - II GVHD)]*[0.814 (if VOD present or previously diagnosed) or 1 (if VOD absent)] L/h	44.3 33		Prop RRE = 27.5% (tacrolimus concentration = 10 µg/L); 16.8%

(tacrolimus concentration = 20 µg/L)

Add RRE additive residual random error; *ALB* albumin; *ALP* alkaline phosphatase; *ALT* alanine transaminase; *AST* aspartate transaminase; *BILI* bilirubin; *BMI* body mass index; *BOV* between-occasion variability; *BSA* body surface area; *BSV* between-subject variability; *BUN* blood urea nitrogen; *CCB* calcium channel blocker; *CMIA* chemiluminescent microparticle immunoassay; *CMT* compartment; *CL* clearance; *CL/F* apparent oral clearance; CL_p/F apparent oral clearance from plasma; *CrCL* creatinine clearance; *CYP* cytochrome P450; *DD* tacrolimus daily dose; *DOT* days of tacrolimus therapy; *eGFR* estimated glomerular filtration rate; *EMIT* enzyme-multiplied immunoassay technique; *EPOD* early post-operative day; *EXP* exponential; *F* bioavailability; *FFM* fat-free mass; *FV* coagulation factor V; *GVHD* graft-versus-host disease; *HB* Haemoglobin; *HCT* haematocrit; *HT* height; *HW* graft hepatic weight; *INR* international normalised ratio; *ITSIM* interactive 2 stage Bayesian modelling; K_{12} transfer rate constant from the central compartment to the peripheral compartment; K_{21} transfer rate constant from the peripheral compartment to the central compartment; *Ka* absorption rate constant; *Ke* elimination rate constant; *Ktr* transfer rate constant; *LAST* log transformed aspartate transaminase; *LBW* lean body weight; *LC-MS/MS* liquid chromatography–tandem mass spectrometry; *LPOD* late post-operative day; *NONMEM* Non-linear Mixed Effects Modelling; *NPDE* Normalised Prediction Distribution Errors; *NPEM2* Nonparametric expectation maximisation; *NS* not stated; *POD* post-operative days; *PREDDOSE* prednisone or prednisolone dose; *Prop RRE* proportional residual random error; *Q* intercompartmental clearance; *Q/F* apparent oral intercompartmental clearance; Q_p/F apparent oral intercompartmental clearance in plasma; *RUV* random unexplained variability; *SD* standard deviation; *SCR* serum creatinine; *TP* total plasma protein; *V* volume of distribution; V_1 central volume of distribution; V_2 peripheral volume of distribution; *V/F* apparent oral volume of distribution; V_1/F apparent oral central volume of distribution; V_2/F apparent oral peripheral volume of distribution; V_p/F apparent oral volume of distribution in plasma; V_{1p}/F apparent oral central volume of distribution in plasma; V_{2p}/F apparent oral peripheral volume of distribution in plasma; V_{ss}/F steady-state distribution volume; *VOD* veno-occlusive disease; *VPC* visual predictive check; *WT* weight; *W/S* whole/split cadaveric liver.

[a]Not clear how modelled.

Based on tacrolimus model pharmacokinetic parameter estimates (Table 10, Appendix 4), on average, higher apparent whole blood clearance was observed among kidney (0.36 L/kg/h, range: 0.24–0.55) and lung (0.34 L/kg/h) transplant recipients in comparison to liver (0.20 L/kg/h, range: 0.10–0.27) and heart (0.21 L/kg/h) transplant recipients. Recipient CYP3A5 expressers (*1/*1 and *1/*3 genotypes) displayed approximately 1.5- to 2-fold higher apparent clearance in comparison to non-expressers (*3/*3 genotype), irrespective of transplant type.

In terms of covariate analysis, the median (range) number of covariates investigated per study was 13 (0–29). The most common covariates investigated were body weight (total/lean/ideal) (84%), age (81%), sex (78%), days post-transplant (67%) and haematocrit (65%). Of all the studies that incorporated recipient CYP3A5 genotype assessments (43%) during model building, 97% were successfully included in the final model. Some studies (17%) explained the effect of drug-drug interactions on tacrolimus pharmacokinetics [24, 25, 32, 34, 37, 41, 49, 51, 55, 69, 73]. Of the two studies [23, 84] that investigated the type of organ transplant during model building, only one [23] found significant differences in tacrolimus clearance between a liver and non-liver transplant population. Three studies did not clearly report tacrolimus pharmacokinetic parameter-covariate relationships [45, 55, 77].

Table 10. Estimates of apparent clearance and volume of distribution of tacrolimus from published population pharmacokinetic models amongst different graft types.

Transplant population	Time post-transplant	Number of subjects	Pharmacokinetic parameters, mean or median (range)		References
			CL/F (L/kg/h)	V/F (L/kg)	
Kidney	Various	78	CYP3A5 expresser: 0.61 (0.35–0.81)	11.68 (6.34–18.23)	[11, 35, 38, 42, 46, 51]
		390	CYP3A5 non-expresser: 0.36 (0.24–0.55)		
		356	0.34 (0.24–0.45)*		[8, 31-33, 47, 48, 53, 60]
Liver	First 3 months	80	CYP3A5 expresser: 0.29 (0.16–0.36)	8.81 (4.04–15.63)	[71, 80, 83]
		94	CYP3A5 non-expresser: 0.20 (0.10–0.27)		
		223	0.29 (0.26–0.34)*		[69, 70, 79]
Lung	≥ 1 month	11	Non-cystic fibrosis: 0.63	7.65	[27]
		11	Cystic fibrosis: 1.18	34.7	
		12	CYP3A5 expresser: 0.48	13.04	[26]
		66	CYP3A5 non-expresser: 0.34		
Heart	First 3 months	56	CYP3A5 expresser: 0.40	12.50 (12.17–12.83)	[24]
		51	CYP3A5 non-expresser: 0.21		
		146	0.22*		[25]
Hematopoietic stem cell	First 4 months	122	0.08*	Not estimated	[30]

CL/F apparent whole blood clearance; *V/F* apparent volume of distribution, or the sum of the apparent volume of the central compartment and the peripheral compartment in the two-compartment models; *CYP3A5 expresser (recipient) CYP3A5**1/*1, *CYP3A5**1/*3; *CYP3A5 non-expresser (recipient) CYP3A5**3/*3.

*Recipient CYP3A5 genotype status is unknown.

Studies related variability in tacrolimus whole blood clearance amongst transplant recipients to either CYP3A5 genotype (41%), days post-transplant (30%) or haematocrit (29%). Models developed from liver transplant recipients tend to have days post-transplant (52%) as the most significant covariate affecting tacrolimus whole blood

clearance. CYP3A5 genotype (55%) and haematocrit (39%) were covariates commonly included in models developed from kidney transplant recipients. In liver transplant, the effects of CYP3A5 genotype of the donor and graft recipient on tacrolimus disposition should be considered separately. Only four studies [71, 78, 80, 83] accommodated both these effects on tacrolimus clearance. Clearance increased with days post-transplant in 71% of the studies. By contrast, clearance decreased with the increase in haematocrit concentration in 90% of the studies. Five studies [12, 43, 49, 61, 77] had all three covariates: recipient CYP3A5 genotype, days post-transplant and haematocrit affecting tacrolimus clearance, while three studies [50, 74, 75] had both days post-transplant and haematocrit. Studies related variability in the central volume of distribution to body weight (20%). Approximately half of the studies (52%) did not have any covariates affecting the central volume of distribution.

BSV of clearance and central volume of distribution of the models was described in 56 (81%) and 50 (72%) studies, respectively. The median (range) BSV of clearance reported was 37% (15−185) and central volume of distribution was 57% (0−163). Three studies showed the occurrence of a relatively large (>45%) BSV of tacrolimus clearance and central volume of distribution immediately post-transplant, with a decrease in these variations over time [65, 74, 75]. Ten studies (14%) reported the BOV of clearance and the median (range) was 30% (10−47). Of these, two studies reported BOV greater than BSV [38, 58].

RUV of the population pharmacokinetic models was described using proportional error in 35% of the studies, combined proportional and additive error in 29% of the studies

and additive error in 20% of the studies. The median (range) proportional error across the studies was 21% (1−43), while the additive error was 1.6 µg/L (0.4−15.2).

3.6 Discussion

Numerous population pharmacokinetic models for tacrolimus have been developed across five transplant populations. The properties of the models and covariates identified vary, reflecting the diverse characteristics of transplant populations used for model building and the variability in tacrolimus pharmacokinetics. Across the models identified, variability in tacrolimus central volume of distribution was largely explained by body weight – heavier subjects have a larger body surface area for tacrolimus distribution. Variability in clearance was mostly related to either recipient CYP3A5 genotype, days post-transplant or haematocrit. The mechanism by which these factors contribute to variability in tacrolimus clearance is briefly described.

Pharmacogenomics information, particularly CYP3A5 genotype, is important to guide initial dose selection for tacrolimus in the absence of concentration data [45]. Recipient CYP3A5 expressers (*1/*1 and *1/*3 genotypes) require an approximately 1.5- to 2-fold higher starting dose (due to 1.5- to 2-fold higher clearance) than CYP3A5 non-expressers (*3/*3 genotype) to attain similar target drug exposure [45]. Some studies suggest that *CYP3A**22 [58, 59, 63], ATP binding cassette subfamily B member 1 (ABCB1) [79] and P450 oxidoreductase *28 (*POR**28) [54] may also influence tacrolimus initial dose requirements. The recent TDM of Tacrolimus Second Consensus Report recommended integrating both *CYP3A5**3 and *CYP3A4**22 genotype information in any future tacrolimus population pharmacokinetic model development to optimise initial dosing

[15]. Current evidence is insufficient to support pharmacogenomic-guided tacrolimus therapy beyond the initial dose. No prospective studies have been conducted to determine if guiding initial tacrolimus doses by a pharmacogenetic-based rather than a weight-based approach is associated with improved treatment outcomes post-transplantation [15]. While it is likely that pharmacogenomic information, if available before commencing tacrolimus therapy, might enable achievement of target tacrolimus exposure faster after the initial dose, it remains unclear if tacrolimus concentrations collected early in the course of therapy (i.e., within the first 24 hours of dosing) are a suitable alternative to guide initial dosing. Given the cost of pharmacogenomic analysis, studies determining the influence of pharmacogenomic information on initial dosing decisions, and the transplant recipients who would likely benefit, are required.

Days post-transplant was identified as a factor affecting tacrolimus clearance in many of the studies. While some studies report that tacrolimus clearance increases with days post-transplant and then reaches a plateau [12, 23, 32, 52, 61, 66-68, 74-77, 79, 80] other studies report a decrease in tacrolimus clearance [41, 43, 49, 50]. These discrepancies could be due to the direct or indirect effect of the type of organ transplant on tacrolimus clearance given that days post-transplant was identified as the most common covariate affecting tacrolimus clearance in models developed from liver transplant recipients (direct effect), while haematocrit was commonly included in models developed from kidney transplant recipients (indirect effect). All models developed from liver transplant recipients reported an increase in tacrolimus clearance with days post-transplant [66-68, 74-77, 79, 80]. This change is likely related to the improvement in metabolic function with the regeneration of the grafted liver [32, 85,

86]. On the other hand, some models developed from kidney transplant recipients demonstrated a decrease in tacrolimus clearance [41, 43, 49, 50] with days post-transplant while some models demonstrated the contrary [12, 32, 52, 61]. These variations could be attributed to the changes in tacrolimus binding resulting from variations in haematocrit which indirectly affects tacrolimus metabolism. There is an inverse correlation between haematocrit and tacrolimus clearance. If the haematocrit concentration is low, a reduced fraction of tacrolimus is bound to erythrocytes, enabling the drug to be readily metabolised by the liver [33, 44]. Hence, higher tacrolimus clearance will be observed and vice-versa. Although days post-transplant is an important surrogate marker of variation in tacrolimus clearance, whether incorporation of this covariate improves the accuracy of model predicted tacrolimus concentrations remains unclear.

While some studies identified haematocrit as an important determinant affecting tacrolimus clearance, other studies could not establish this relationship. This may, in part, be because the effect of haematocrit may have been confounded by days post-transplant [44, 69]. Future studies in a large population are necessary to determine if haematocrit and days post-transplant simultaneously affect tacrolimus clearance [44] or whether it is sufficient to have either one time-dependent covariate as a predictor of tacrolimus pharmacokinetic variability.

Despite model heterogeneity, several areas requiring further research to facilitate the use of tacrolimus population pharmacokinetic models to guide dosing have been

identified. These areas are not limited to strategies to better utilise existing models but also additional consideration for the development of new models.

In total, 69 population pharmacokinetic models have been developed in adult transplant recipients. Given the range of models available, could existing information be utilised to develop a more comprehensive understanding of tacrolimus pharmacokinetics in different transplant populations without the need to collect new data? Development of a hybrid population pharmacokinetic model using the model averaging approach by combining information from appropriate or relevant existing models in a biologically plausible manner [87] could serve as an alternative approach to model building. Nanga *et al.* [23] recently developed a meta-model to facilitate optimal tacrolimus dosing in different indications and patient populations. Unfortunately, the predictive performance of the meta-model was inconsistent across the dataset. Therefore, further optimisation of this meta-model is required prior to implementation into clinical practice. Regardless, consideration of alternative approaches to model development is important especially given the resource requirements and ethical considerations associated with the development of models from new datasets, including patient recruitment and blood sample collections.

Although many population pharmacokinetic models of tacrolimus in adult transplant recipients have been published, limited external evaluation [88-91] of the models has been performed. This has hampered the implementation of Bayesian-guided tacrolimus dosing into routine clinical practice. Evaluation of models using a dataset not included in the model building process enables determination of generalisability of the model(s),

an important consideration when selecting a model to be used in clinical practice. Brooks et al [22] recently conducted a review, summarising the predictive performances of studies that have performed Maximum *A Posteriori* Bayesian estimation to predict tacrolimus exposure. Although bias was within the clinically acceptable range across all the studies, there was some level of imprecision for some studies. Therefore, assessment of the predictive performance of the models currently available is required to help transplant centres select the most appropriate model(s) to accurately inform tacrolimus dosing decisions for their transplant recipient populations. In terms of clinical application, the ImmunoSuppressant Bayesian dose Adjustment (ISBA) web portal (https://pharmaco.chu-limoges.fr/) and a commercially available Bayesian forecasting software, DoseMeRx® have been used to guide dose individualisation for several drugs, including tacrolimus in Europe and America. Furthermore, the implementation of a population pharmacokinetic model [45] in routine clinical practice to inform tacrolimus dosing decisions has been assessed by Storset *et al* [92], indicating a significant improvement in the achievement of target concentration in kidney transplant recipients in comparison to standard trough concentration-guided dosing [92]. To date, population pharmacokinetic models of tacrolimus have predominately been developed in kidney and liver transplant recipients. Few models have been developed in heart, lung, and hematopoietic stem cell transplant recipients. Determining the generalisability of existing models would reveal if organ transplant specific models are needed. The mean apparent clearance of tacrolimus reported in kidney and lung transplant recipient populations, as well as in liver and heart transplant recipient populations is similar (Table 10). This suggests that models may be interchangeable between specific transplant recipient populations. However, one [23] of the two population pharmacokinetic models

[23, 84] that considered type of organ transplant, identified distinct differences in tacrolimus clearance between a liver and non-liver transplant recipient population. This highlights that further evaluation of the generalisability of existing population pharmacokinetic models of tacrolimus is required to inform the need for additional data collection in less well-studied transplant recipient populations.

Most studies modelled the pharmacokinetics of the oral immediate-release formulation of tacrolimus (Prograf®). Although the immediate- and prolonged-release formulations of tacrolimus are not bioequivalent [74], only two studies have simultaneously described the pharmacokinetics for both formulations [38, 84]. Given that other formulations, such as intravenous, are available, a model that can estimate exposure for more than one formulation may be more suitable for clinical practice. Additionally, switching between different brands of tacrolimus even of the same formulation results in significant changes in tacrolimus exposure [62]. Further, the incidence of graft rejection during the first year post-kidney transplant is increased when patients transitioned from branded to generic tacrolimus (10.2% vs. 23.1%; p=0.024) [93], reflecting a 20% decrease in tacrolimus exposure. Hence, studies investigating the pharmacokinetics of different formulations and brands of tacrolimus, including generic brands, are required to establish models which can provide reliable dosing advice.

Although co-medication is an important clinical determinant affecting the pharmacokinetics of tacrolimus, most population pharmacokinetic models did not investigate the influence of CYP3A inhibitors or inducers. Azole antifungals, commonly prescribed in thoracic transplant recipients as a prophylaxis or treatment for invasive

fungal infection, demonstrate clinically significant interaction with tacrolimus [94, 95]. Azole antifungals inhibit CYP3A4 metabolism of tacrolimus in the intestine and liver [94, 95], resulting in increased tacrolimus bioavailability [96] and reduced apparent clearance [97]. Two models [24, 69] which accommodated for the effect of azole antifungal therapy (fluconazole [24, 69], voriconazole [24]), reported a reduction in tacrolimus clearance between 20% and 80%. This observation has significant clinical implication for dosing tacrolimus. While proton pump inhibitors have been thought previously to influence tacrolimus pharmacokinetics, recent work has shown concomitant administration of proton pump inhibitors with tacrolimus did not influence tacrolimus pharmacokinetics [98, 99]. This finding is consistent with concomitant proton pump inhibitor use not being identified as a covariate in any model. In contrast, two models included calcium channel blockers (diltiazem) as a covariate [41, 69]. While calcium channel blockers are known to inhibit CYP3A metabolism of tacrolimus and may increase tacrolimus exposure [100], this interaction is considered unlikely to be of clinical importance [101]. Therefore, incorporating data that adequately describes common and clinically significant drug-drug interactions during model building may improve the clinical utility of Bayesian dose recommendations in transplant recipients co-prescribed with metabolic inhibitors or inducers.

Several studies have identified other factors affecting tacrolimus pharmacokinetics, including circadian variation and the administration of food. All population pharmacokinetic models for tacrolimus developed so far were based on daytime-only sampling, thus, did not account for circadian variation. It should be noted that no significant difference between trough concentrations collected prior to administration

of morning or evening doses of oral tacrolimus was reported [102]. Further, continuous intravenous infusion of tacrolimus is able to maintain adequate therapeutic blood concentrations over 24 hours [103]. By contrast, the presence of food decreases tacrolimus bioavailability by 27% [104] and if patients choose to take tacrolimus with food, they are counselled to always take it with food to avoid fluctuation in tacrolimus concentrations [100]. However, a study in 27 kidney transplant recipients concluded that conversion to non-fasting ingestion of tacrolimus, without dosage adjustments, did not significantly change tacrolimus trough concentrations in stable patients [105]. Therefore, neither circadian variation nor food was investigated or identified as covariates during model development perhaps reflecting that they do not impact tacrolimus dosing decisions.

3.7 Limitations

As the literature search in this study was restricted to English language, studies published in other languages may have been missed. Further, model comparison was hindered as some details (e.g., tacrolimus brand, model evaluation approach, parameter-covariate relationship) were missing from some publications. Authors were contacted for further clarification to overcome this limitation. While this review attempts to identify areas requiring additional research to facilitate the use of population pharmacokinetic models in Bayesian forecasting software to inform tacrolimus dosing decision tools, it does not provide an insight into the quality of models developed.

3.8 Conclusions

Numerous population pharmacokinetic models for tacrolimus use in adult transplant recipients have been published. Comprehensive and systematic external evaluation of relevant models is required to assess the generalisability of these models and subsequently, facilitate the implementation of accurate and reliable Bayesian-guided tacrolimus dosing decisions across different transplant recipient populations. The effect of clinically significant drug-drug interactions and, different formulations and brands of tacrolimus should be considered for any future tacrolimus population pharmacokinetic model development. Further work is required to identify key factors that influence both initial and maintenance doses of tacrolimus, particularly in heart and lung transplant recipients.

3.9 Study highlights

- Sixty-nine population pharmacokinetic models of tacrolimus in adult transplant recipients have been published.
- Recipient cytochrome P450 3A5 genotype, days post-transplant, haematocrit and body weight were identified as significant clinical determinants influencing tacrolimus pharmacokinetics.
- Strategies to better utilise existing models and additional consideration for the development of new models, including external evaluation, model averaging (combining information from published models in a biologically plausible manner), drug-drug interactions and different formulations and brands of tacrolimus, to facilitate Bayesian-guided tacrolimus dosing decisions have been identified.

3.10 Acknowledgements

The authors would like to thank Elisabet Storset (Norway), Bing Chen (China), Franc Andreu Solduga (Spain), Ken Ogasawara (USA), Tom M Nanga (France) and Zheng Lu (USA) for corresponding to queries regarding their publication. We would also like to thank Dr Shaun Kumar (Model Answers, Brisbane) for providing valuable input on the manuscript. Ranita Kirubakaran is a recipient of the Faculty of Medicine and Health, University of New South Wales, Sydney, Doctoral Scholarship funded by the Australian Government, and St. Vincent's Centre for Applied Medical Research Student Scholarship Supplementation Scheme.

3.11 Conflict of interest

All authors have no conflicts of interest that are relevant to the content of this review.

3.12 Author Contributions

Ranita Kirubakaran designed and performed the literature research, analysed the data, and wrote the manuscript. Ranita Kirubakaran and Jane E. Carland were the independent reviewers for the systematic review. All authors critically reviewed, edited, and approved the final manuscript.

References

1. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

2. Christie JD, Edwards LB, Kucheryavaya AY, Benden C, Dipchand AI, Dobbels F, et al. The Registry of the International Society for Heart and Lung Transplantation: 29th adult lung and heart-lung transplant report—2012. J Heart Lung Transplant. 2012;31(10):1073-86.

3. Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Alessiani M, Lever J, et al. Pharmacokinetics of FK 506 in transplant patients. Transplant Proc. 1991;23(6):2736-40.

4. Jain A, Venkataramanan R, Todo S, Abu-Elmagd K, Fung J, Warty V, et al. Intravenous, oral pharmacokinetics, and oral dosing of FK 506 in small bowel transplant patients. Transplant Proc. 1992;24(3):1181-2.

5. Zahir H, Nand R, Brown K, Tattam B, McLachlan A. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. J Pharmacol Toxicol Methods. 2001;46(1):27-35.

6. Möller A, Iwasaki K, Kawamura A, Teramura Y, Shiraga T, Hata T, et al. The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos. 1999;27(6):633-6.

7. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

8. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

9. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

10. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

11. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

12. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

13. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

14. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

15. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit. 2019;41(3):261-307.

16. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus Therapy in Adult Heart Transplant Recipients: Evaluation of a Bayesian Forecasting Software. Ther Drug Monit. 2021;43(6):736-46.

17. Dasari B, Hodson J, Nassir A, Widmer J, Isaac J, Mergentel H, et al. Variations in practice to therapeutic monitoring of tacrolimus following primary adult liver transplantation. Int J Organ Transplant Med. 2016;7(1):1-8.

18. Tett SE, Holford NH, McLachlan AJ. Population pharmacokinetics and pharmacodynamics: an underutilized resource. Drug Inf J. 1998;32(3):693-710.

19. Kiang TK, Sherwin CM, Spigarelli MG, Ensom MH. Fundamentals of Population Pharmacokinetic Modelling. Clin Pharmacokinet. 2012;51(8):515-25.

20. Mould D, Upton RN. Basic concepts in population modeling, simulation, and model-based drug development—part 2: introduction to pharmacokinetic modeling methods. CPT Pharmacometr Syst Pharmacol. 2013;2(4):1-14.

21. Aarons L. Population pharmacokinetics: theory and practice. Br J Clin Pharmacol. 1991;32(6):669-70.

22. Brooks E, Tett SE, Isbel NM, Staatz CE. Population pharmacokinetic modelling and Bayesian estimation of tacrolimus exposure: is this clinically useful for dosage prediction yet? Clin Pharmacokinet. 2016;55(11):1295-335.

23. Nanga TM, Doan TT, Marquet P, Musuamba FT. Toward a robust tool for pharmacokinetic-based personalization of treatment with tacrolimus in solid organ transplantation: a model-based meta-analysis approach. Br J Clin Pharmacol. 2019;85(12):2793-823.

24. Zhou H, Cai J, Huang J, Zhang J, Shi SJ, Liu YN, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: A population pharmacokinetic approach. Pharmacogenomics. 2019;20(1):21-35.

25. Gong Y, Yang M, Sun Y, Li J, Lu Y, Li X. Population pharmacokinetic analysis of tacrolimus in Chinese cardiac transplant recipients. Eur J Hosp Pharm. 2020;27(e1):e012-18.

26. Monchaud C, de Winter BC, Knoop C, Estenne M, Reynaud-Gaubert M, Pison C, et al. Population pharmacokinetic modelling and design of a Bayesian estimator for therapeutic drug monitoring of tacrolimus in lung transplantation. Clin Pharmacokinet. 2012;51(3):175-86.

27. Saint-Marcoux F, Knoop C, Debord J, Thiry P, Rousseau A, Estenne M, et al. Pharmacokinetic study of tacrolimus in cystic fibrosis and non-cystic fibrosis lung transplant patients and design of Bayesian estimators using limited sampling strategies. Clin Pharmacokinet. 2005;44(12):1317-28.

28. Sikma MA, Hunault CC, Van Maarseveen EM, Huitema AD, Van de Graaf EA, Kirkels JH, et al. High variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Eur J Drug Metab Pharmacokinet. 2020;45(1):123-34.

29. Sikma MA, Van Maarseveen EM, Hunault CC, Moreno JM, Van de Graaf EA, Kirkels JH, et al. Unbound Plasma, Total Plasma, and Whole-Blood Tacrolimus Pharmacokinetics Early After Thoracic Organ Transplantation. Clin Pharmacokinet. 2019;59(6):771–80.

30. Jacobson P, Ng J, Ratanatharathorn V, Uberti J, Brundage RC. Factors affecting the pharmacokinetics of tacrolimus (FK506) in hematopoietic cell transplant (HCT) patients. Bone Marrow Transplant. 2001;28(8):753-8.

31. Scholten EM, Cremers SC, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, et al. AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. Kidney Int. 2005;67(6):2440-7.

32. Antignac M, Barrou B, Farinotti R, Lechat P, Urien S. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. Br J Clin Pharmacol. 2007;64(6):750-7.

33. Benkali K, Premaud A, Picard N, Rerolle JP, Toupance O, Hoizey G, et al. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet. 2009;48(12):805-16.

34. Press RR, Ploeger BA, den Hartigh J, van der Straaten T, van Pelt J, Danhof M, et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Ther Drug Monit. 2009;31(2):187-97.

35. Benkali K, Rostaing L, Premaud A, Woillard JB, Saint-Marcoux F, Urien S, et al. Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in renal transplant recipients on a new once-daily formulation. Clin Pharmacokinet. 2010;49(10):683-92.

36. Saint-Marcoux F, Debord J, Undre N, Rousseau A, Marquet P. Pharmacokinetic modeling and development of bayesian estimators in kidney transplant patients receiving the tacrolimus once-daily formulation. Ther Drug Monit. 2010;32(2):129-35.

37. Velickovic-Radovanovic R, Catic-Djordjevic A, Milovanovic JR, Djordjevic V, Paunovic G, Jankovic SM. Population pharmacokinetics of tacrolimus in kidney transplant patients. Int J Clin Pharmacol Ther. 2010;48(6):375-82.

38. Woillard JB, de Winter BC, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations--twice daily Prograf and once daily Advagraf. Br J Clin Pharmacol. 2011;71(3):391-402.

39. Saint-Marcoux F, Debord J, Parant F, Labalette M, Kamar N, Rostaing L, et al. Development and evaluation of a simulation procedure to take into account various assays for the Bayesian dose adjustment of tacrolimus. Ther Drug Monit. 2011;33(2):171-7.

40. Grover A, Frassetto LA, Benet LZ, Chakkera HA. Pharmacokinetic differences corroborate observed low tacrolimus dosage in Native American renal transplant patients. Drug Metab Dispos. 2011;39(11):2017-9.

41. Passey C, Birnbaum AK, Brundage RC, Oetting WS, Israni AK, Jacobson PA. Dosing equation for tacrolimus using genetic variants and clinical factors. Br J Clin Pharmacol. 2011;72(6):948-57.

42. Musuamba FT, Mourad M, Haufroid V, Demeyer M, Capron A, Delattre IK, et al. A simultaneous d-optimal designed study for population pharmacokinetic analyses of mycophenolic acid and tacrolimus early after renal transplantation. J Clin Pharmacol. 2012;52(12):1833-43.

43. Han N, Yun HY, Hong JY, Kim IW, Ji E, Hong SH, et al. Prediction of the tacrolimus population pharmacokinetic parameters according to CYP3A5 genotype and clinical factors using NONMEM in adult kidney transplant recipients. Eur J Clin Pharmacol. 2013;69(1):53-63.

44. Zuo XC, Ng CM, Barrett JS, Luo AJ, Zhang BK, Deng CH, et al. Effects of CYP3A4 and CYP3A5 polymorphisms on tacrolimus pharmacokinetics in Chinese adult renal transplant recipients: a population pharmacokinetic analysis. Pharmacogenet Genomics. 2013;23(5):251-61.

45. Asberg A, Midtvedt K, van Guilder M, Storset E, Bremer S, Bergan S, et al. Inclusion of CYP3A5 genotyping in a nonparametric population model improves dosing of tacrolimus early after transplantation. Transpl Int. 2013;26(12):1198-207.

46. Zhao W, Fakhoury M, Baudouin V, Storme T, Maisin A, Deschenes G, et al. Population pharmacokinetics and pharmacogenetics of once daily prolonged-release formulation of tacrolimus in pediatric and adolescent kidney transplant recipients. Eur J Clin Pharmacol. 2013;69(2):189-95.

47. Gaies E, Bacha MM, Woillard JB, Eljebari H, Helal I, Abderrahim E, et al. Tacrolimus population pharmacokinetics and Bayesian estimation in Tunisian renal transplant recipients. Int J Pharm Pharm Sci. 2013;5:108-15.

48. Storset E, Holford N, Midtvedt K, Bremer S, Bergan S, Asberg A. Importance of hematocrit for a tacrolimus target concentration strategy. Eur J Clin Pharmacol. 2014;70(1):65-77.

49. Bergmann TK, Hennig S, Barraclough KA, Isbel NM, Staatz CE. Population pharmacokinetics of tacrolimus in adult kidney transplant patients: impact of CYP3A5 genotype on starting dose. Ther Drug Monit. 2014;36(1):62-70.

50. Golubovic B, Vucicevic K, Radivojevic D, Kovacevic SV, Prostran M, Miljkovic B. Total plasma protein effect on tacrolimus elimination in kidney transplant patients--population pharmacokinetic approach. Eur J Pharm Sci. 2014;52:34-40.

51. Storset E, Holford N, Hennig S, Bergmann TK, Bergan S, Bremer S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. Br J Clin Pharmacol. 2014;78(3):509-23.

52. Han N, Ha S, Yun Hy, Kim MG, Min SI, Ha J, et al. Population pharmacokinetic–pharmacogenetic model of tacrolimus in the early period after kidney transplantation. Basic Clin Pharmacol Toxicol. 2014;114(5):400-6.

53. Andreu F, Colom H, Grinyo JM, Torras J, Cruzado JM, Lloberas N. Development of a population PK model of tacrolimus for adaptive dosage control in stable kidney transplant patients. Ther Drug Monit. 2015;37(2):246-55.

54. Zhang JJ, Liu SB, Xue L, Ding XL, Zhang H, Miao LY. The genetic polymorphisms of POR*28 and CYP3A5*3 significantly influence the pharmacokinetics of tacrolimus in Chinese renal transplant recipients. Int J Clin Pharmacol Ther. 2015;53(9):728-36.

55. Robles-Piedras AL, Romano-Moreno S, Fuentes-Noriega I, Mancilla-Urrea E, Castro-Torres NN, Dominguez-Ramirez AM. Population pharmacokinetic analysis of Tacrolimus in adult Mexican patients with renal transplant. Biomed & Pharmacol J. 2015;8(1):47-56.

56. Fredj NB, Woillard JB, Debord J, Chaabane A, Boughattas N, Marquet P, et al. Modeling of tacrolimus exposure in kidney transplant according to posttransplant time based on routine trough concentration data. Exp Clin Transplant. 2016;14(4):394-400.

57. Vadcharavivad S, Praisuwan S, Techawathanawanna N, Treyaprasert W, Avihingsanon Y. Population pharmacokinetics of tacrolimus in Thai kidney transplant patients: comparison with similar data from other populations. J Clin Pharm Ther. 2016;41(3):310-28.

58. Andreu F, Colom H, Elens L, van Gelder T, van Schaik RHN, Hesselink DA, et al. A New CYP3A5*3 and CYP3A4*22 Cluster Influencing Tacrolimus Target Concentrations: A Population Approach. Clin Pharmacokinet. 2017;56(8):963-75.

59. Woillard JB, Mourad M, Neely M, Capron A, van Schaik RH, van Gelder T, et al. Tacrolimus Updated Guidelines through popPK Modeling: How to Benefit More from

CYP3A Pre-emptive Genotyping Prior to Kidney Transplantation. Front Pharmacol. 2017;8:358.

60. Rong Y, Mayo P, Ensom MHH, Kiang TKL. Population Pharmacokinetic Analysis of Immediate-Release Oral Tacrolimus Co-administered with Mycophenolate Mofetil in Corticosteroid-Free Adult Kidney Transplant Recipients. Eur J Drug Metab Pharmacokinet. 2018;44(3):409-22.

61. Zhu W, Xue L, Peng H, Duan Z, Zheng X, Cao D, et al. Tacrolimus population pharmacokinetic models according to CYP3A5/CYP3A4/POR genotypes in Chinese Han renal transplant patients. Pharmacogenomics. 2018;19(13):1013-25.

62. Resendiz-Galvan JE, Medellin-Garibay SE, Milan-Segovia RDC, Nino-Moreno PDC, Isordia-Segovia J, Romano-Moreno S. Dosing recommendations based on population pharmacokinetics of tacrolimus in Mexican adult patients with kidney transplant. Basic Clin Pharmacol Toxicol. 2019;124(3):303-11.

63. Andrews LM, Hesselink DA, van Schaik RHN, van Gelder T, de Fijter JW, Lloberas N, et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2019;85(3):601-15.

64. Woillard JB, Debord J, Monchaud C, Saint-Marcoux F, Marquet P. Population Pharmacokinetics and Bayesian Estimators for Refined Dose Adjustment of a New Tacrolimus Formulation in Kidney and Liver Transplant Patients. Clin Pharmacokinet. 2017;56(12):1491-8.

65. Macchi-Andanson M, Charpiat B, Jelliffe RW, Ducerf C, Fourcade N, Baulieux J. Failure of traditional trough levels to predict tacrolimus concentrations. Ther Drug Monit. 2001;23(2):129-33.

66. Fukatsu S, Yano I, Igarashi T, Hashida T, Takayanagi K, Saito H, et al. Population pharmacokinetics of tacrolimus in adult recipients receiving living-donor liver transplantation. Eur J Clin Pharmacol. 2001;57(6):479-84.

67. Fukudo M, Yano I, Fukatsu S, Saito H, Uemoto S, Kiuchi T, et al. Forecasting of blood tacrolimus concentrations based on the Bayesian method in adult patients receiving living-donor liver transplantation. Clin Pharmacokinet. 2003;42(13):1161-78.

68. Antignac M, Hulot JS, Boleslawski E, Hannoun L, Touitou Y, Farinotti R, et al. Population pharmacokinetics of tacrolimus in full liver transplant patients: Modelling of the post-operative clearance. Eur J Clin Pharmacol. 2005;61(5):409-16.

69. Zahir H, McLachlan AJ, Nelson A, McCaughan G, Gleeson M, Akhlaghi F. Population pharmacokinetic estimation of tacrolimus apparent clearance in adult liver transplant recipients. Ther Drug Monit. 2005;27(4):422-30.

70. Sam WJ, Tham LS, Holmes MJ, Aw M, Quak SH, Lee KH, et al. Population pharmacokinetics of tacrolimus in whole blood and plasma in asian liver transplant patients. Clin Pharmacokinet. 2006;45(1):59-75.

71. Li D, Lu W, Zhu JY, Gao J, Lou YQ, Zhang GL. Population pharmacokinetics of tacrolimus and CYP3A5, MDR1 and IL-10 polymorphisms in adult liver transplant patients. J Clin Pharm Ther. 2007;32(5):505-15.

72. Blanchet B, Duvoux C, Costentin CE, Barrault C, Ghaleh B, Salvat A, et al. Pharmacokinetic-pharmacodynamic assessment of tacrolimus in liver-transplant recipients during the early post-transplantation period. Ther Drug Monit. 2008;30(4):412-8.

73. Zhang XQ, Wang ZW, Fan JW, Li YP, Jiao Z, Gao JW, et al. The impact of sulfonylureas on tacrolimus apparent clearance revealed by a population pharmacokinetics analysis in Chinese adult liver-transplant patients. Ther Drug Monit. 2012;34(2):126-33.

74. Oteo I, Lukas JC, Leal N, Suarez E, Valdivieso A, Gastaca M, et al. Tacrolimus pharmacokinetics in the early post-liver transplantation period and clinical applicability via Bayesian prediction. Eur J Clin Pharmacol. 2013;69(1):65-74.

75. Valdivieso N, Oteo I, Valdivieso A, Lukas JC, Leal N, Gastaca M, et al. Tacrolimus dose individualization in" de novo" patients after 10 years of experience in liver transplantation: pharmacokinetic considerations and patient pathophysiology. Int J Clin Pharmacol Ther. 2013;51(7):606-14.

76. Zhu L, Wang H, Sun X, Rao W, Qu W, Zhang Y, et al. The population pharmacokinetic models of tacrolimus in Chinese adult liver transplantation patients. J Pharm (Cairo). 2014;2014:713650.

77. Zhu L, Yang J, Jing Y, Zhang Y, Li G. Effects of CYP3A5 genotypes, ABCB1 C3435T and G2677T/A polymorphism on pharmacokinetics of Tacrolimus in Chinese adult liver transplant patients. Xenobiotica. 2015;45(9):840-6.

78. Moes DJ, van der Bent SA, Swen JJ, van der Straaten T, Inderson A, Olofsen E, et al. Population pharmacokinetics and pharmacogenetics of once daily tacrolimus formulation in stable liver transplant recipients. Eur J Clin Pharmacol. 2016;72(2):163-74.

79. Chen B, Shi HQ, Liu XX, Zhang WX, Lu JQ, Xu BM, et al. Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in Chinese liver transplant patients. J Clin Pharm Ther. 2017;42(6):679-88.

80. Ji E, Kim MG, Oh JM. CYP3A5 genotype-based model to predict tacrolimus dosage in the early postoperative period after living donor liver transplantation. Ther Clin Risk Manag. 2018;14:2119-26.

81. Riff C, Debord J, Monchaud C, Marquet P, Woillard JB. Population pharmacokinetic model and Bayesian estimator for 2 tacrolimus formulations in adult liver transplant patients. Br J Clin Pharmacol. 2019;85(8):1740-50.

82. Lu YX, Su QH, Wu KH, Ren YP, Li L, Zhou TY, et al. A population pharmacokinetic study of tacrolimus in healthy Chinese volunteers and liver transplant patients. Acta Pharmacol Sin. 2015;36(2):281-8.

83. Shao J, Wang C, Fu P, Chen F, Zhang Y, Wei J. Impact of Donor and Recipient CYP3A5* 3 Genotype on Tacrolimus Population Pharmacokinetics in Chinese Adult Liver Transplant Recipients. Ann Pharmacother. 2020;54(7):652-61.

84. Lu Z, Bonate P, Keirns J. Population pharmacokinetics of immediate- and prolonged-release tacrolimus formulations in liver, kidney and heart transplant recipients. Br J Clin Pharmacol. 2019;85(8):1692-703.

85. Kawasaki S, Makuuchi M, Ishizone S, Matsunami H, Terada M, Kawarazaki H. Liver regeneration in recipients and donors after transplantation. Lancet. 1992;339(8793):580-1.

86. Tuteja S, Alloway RR, Johnson JA, Gaber AO. The effect of gut metabolism on tacrolimus bioavailability in renal transplant recipients. Transplantation. 2001;71(9):1303-7.

87. McDougall DA, Martin J, Playford EG, Green B. Determination of a suitable voriconazole pharmacokinetic model for personalised dosing. J Pharmacokinet Pharmacodyn. 2016;43(2):165-77.

88. Cai X, Li R, Sheng C, Tao Y, Zhang Q, Zhang X, et al. Systematic external evaluation of published population pharmacokinetic models for tacrolimus in adult liver transplant recipients. Eur J Pharm Sci. 2020;145:105237.

89. Hu C, Yin WJ, Li DY, Ding JJ, Zhou LY, Wang JL, et al. Evaluating tacrolimus pharmacokinetic models in adult renal transplant recipients with different CYP3A5 genotypes. Eur J Clin Pharmacol. 2018;74(11):1437-47.

90. Zhao CY, Jiao Z, Mao JJ, Qiu XY. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2016;81(5):891-907.

91. Willis C, Staatz CE, Tett SE. Bayesian forecasting and prediction of tacrolimus concentrations in pediatric liver and adult renal transplant recipients. Ther Drug Monit. 2003;25(2):158-66.

92. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

93. Hauch A, John M, Smith A, Dortonne I, Patel U, Kandil E, et al. Generics: Are all immunosuppression agents created equally? Surgery. 2015;158(4):1049-55.

94. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

95. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

96. Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, et al. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. Clin Pharmacol Ther. 1997;62(1):41-9.

97. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

98. Katsakiori PF, Papapetrou EP, Goumenos DS, Nikiforidis GC, Flordellis CS. Investigation of clinical interaction between omeprazole and tacrolimus in CYP3A5 non-expressors, renal transplant recipients. Ther Clin Risk Manag. 2010;6:265-9.

99. Bremer SC, Reinhardt L, Sobotta M, Hasselluhn MC, Lorf T, Ellenrieder V, et al. Pantoprazole does not affect serum trough levels of tacrolimus and everolimus in liver transplant recipients. Front Med (Lausanne). 2018;5:320.

100. Prograf®. Northbrook, IL: AstellasPharma US, Inc. 2020.

101. Chow F-S, Jusko WJ. Immunosuppressive interactions among calcium channel antagonists and selected corticosteroids and macrolides using human whole blood lymphocytes. Drug Metab Pharmacokinet. 2004;19(6):413-21.

102. Min DI, Chen H-Y, Fabrega A, Ukah FO, Wu Y-M, Corwin C, et al. Circardian variation of tacrolimus disposition in liver allograft recipients. Transplantation. 1996;62(8):1190-2.

103. Satoh S, Tada H, Tachiki Y, Tsuchiya N, Shimoda N, Akao T, et al. Chrono and clinical pharmacokinetic study of tacrolimus in continuous intravenous administration. Int J Urol. 2001;8(7):353-8.

104. Mekki Q, Lee C, Carrier S, Klintmaim G, Shaefer M, Burke P, et al. The effect of food on oral bioavailability of tacrolimus (FK506) in liver-transplant patients. Clin Pharmacol Ther. 1993;53(2):229.

105. van Duijnhoven E, Christiaans M, Boots J, van Hooff J. Evidence that fasting does not significantly affect trough levels of tacrolimus in stable renal transplant recipients. Transplant Proc. 2002;34(5):1723-5.

Appendices

Appendix 4. Estimates of absorption rate constant (K_a) of tacrolimus from published population pharmacokinetic models amongst different graft types.

Transplant population	Number of subjects	K_a (h^{-1}), Median (range)*	References
Kidney	1553	1.04 (0.14 – 4.21)	[11, 13, 31, 34, 40, 43, 45, 48, 49, 51, 53, 58, 63]
Liver	393	1.32 (0.16 – 4.48)	[9, 70, 72, 79, 82, 83]
Kidney and liver	281	3.37	[23]
Kidney, liver, and heart	408	0.38	[84]
Heart and Lung	30	0.58	[28, 29]
Heart	107	3.80	[24]

*Values obtained from model developed from intensive sampling dataset and are based on the oral immediate-release formulation of tacrolimus (Prograf®).

Chapter 4:

Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients.

4.1 Link to thesis

In Chapter 3, I presented a systematic review summarising published population pharmacokinetic models of tacrolimus developed from adult transplant recipients. The review aimed to inform the selection of models for subsequent evaluation of their predictive performance in heart transplant recipients. The goal is that the best performing model will be integrated within a Bayesian dosing software to facilitate dose individualisation in heart transplant recipients at St. Vincent's Hospital Sydney. In Chapter 4, I evaluated the predictive performance of specific tacrolimus models developed from various organ transplant recipient populations identified in Chapter 3 in adult heart transplant recipients. The evaluation was stratified based on concomitant azole antifungal use because concomitant azole antifungal therapy alters tacrolimus pharmacokinetics substantially necessitating dose adjustments. To the best of my knowledge, this is the first comprehensive evaluation of existing models to determine their suitability in guiding tacrolimus dosing decisions in adult heart transplant recipients.

This work has been published:

Kirubakaran R, Hennig S, Maslen B, Day RO, Carland JE, Stocker SL. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients. *British Journal of Clinical Pharmacology*. 2021.

I have presented this work at three international conferences and one national symposium:

1. Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. Poster Presentation, Pharmacometrics Japan Conference, Tokyo, Japan, 22−23 January 2020.
2. Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. Oral Presentation, ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.
3. Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. Poster Presentation, ASCEPT−PAGANZ Joint-Scientific Meeting, Queenstown, New Zealand, 25−29 November 2019.
4. Kirubakaran R, Hennig S, Carland JE, Day RO, Stocker SL. External evaluation of population pharmacokinetic models of tacrolimus in adult heart transplant recipients. Oral Presentation, American Association of Pharmaceutical Scientists (AAPS) Symposium, Sydney, Australia, 22 November 2019.

4.2 Abstract

Background and Aim Identification of the most appropriate population pharmacokinetic model-based Bayesian estimation is required prior to its implementation in routine clinical practice to inform tacrolimus dosing decisions in transplant recipients. This study aimed to determine the predictive performances of relevant population

pharmacokinetic models of tacrolimus developed from various solid organ transplant recipient populations in adult heart transplant recipients, stratified based on concomitant azole antifungal use. Concomitant azole antifungal therapy alters tacrolimus pharmacokinetics substantially necessitating dose adjustments.

Methods Population pharmacokinetic models of tacrolimus were selected (n=17) for evaluation from a recent systematic review. The models were transcribed and implemented in NONMEM version 7.4.3. Data from 85 heart transplant recipients (2387 tacrolimus concentrations) administered the oral immediate-release formulation of tacrolimus (Prograf®) were obtained up to 391 days post-transplant. The performance of each model was evaluated using (1) prediction-based assessment (bias and imprecision) of the individual predicted tacrolimus concentration of the fourth dosing occasion (MAXEVAL=0, FOCE-I) from 1−3 prior dosing occasions and (2) simulation-based assessment (prediction-corrected visual predictive check, pcVPC). Both assessments were stratified based on concomitant azole antifungal use.

Results Regardless of the number of prior dosing occasions (1−3) and concomitant azole antifungal use, all models demonstrated unacceptable individual predicted tacrolimus concentration of the fourth dosing occasion (n=152). The pcVPC graphics indicated these models inadequately predicted observed tacrolimus concentrations.

Conclusions All models evaluated were unable to adequately describe tacrolimus pharmacokinetics in adult heart transplant recipients included in this study. Further work is required to describe tacrolimus pharmacokinetics for our heart transplant recipient cohort.

4.3 Introduction

Tacrolimus is the cornerstone immunosuppressant used to prevent graft rejection in most solid-organ transplant recipients [1], including heart transplants. However, tacrolimus dosing strategies are complicated by the narrow therapeutic range [2] and considerable inter- and intra-patient pharmacokinetic variability [3-9]. Two key challenges when dosing tacrolimus are (i) choosing the right starting dose and (ii) making appropriate dose adjustments to compensate for the variation in tacrolimus pharmacokinetics in an individual over time. Individualising lifelong tacrolimus therapy to avoid allograft rejection and minimise adverse effects is essential for heart transplant recipients.

Monitoring whole blood trough concentrations is recommended to individualise tacrolimus therapy [10]. However, the collection of blood samples at a specific time is often challenging, particularly in a busy clinical environment but also in the outpatient setting. In hospitalised adult heart [11] and liver [12] transplant recipients, only 46% and 5%, respectively, of blood samples collected were at trough concentration-time point. Incorrect timing of these samples can result in erroneous interpretation of tacrolimus concentrations and consequently, inappropriate dose recommendations.

Given the challenges in successfully implementing trough concentration-guided dosing, population pharmacokinetic model-based Bayesian estimation presents an alternative solution for individualised tacrolimus dosing advice in transplant recipients. The Bayesian approach uses patient-specific information (e.g., sex, height, and weight), at least one drug concentration and a population pharmacokinetic model to estimate

individual patient pharmacokinetic parameters [13-15]. From this, an optimal drug dose predicted to achieve target drug concentrations can be identified. While the Bayesian approach provides flexible blood sampling schemes [16], its utilisation can also reduce the number of blood samples needed to achieve target drug concentrations [17]. The accuracy of predicted drug concentrations, that are used to inform the recommended dosing regimen, is dependent on the suitability of the underlying population pharmacokinetic model and the accuracy of patient data inputted into the model [18].

At least 70 population pharmacokinetic models of tacrolimus in adult transplant recipients have been published to characterise tacrolimus pharmacokinetics [19-21]. However, very few models reflect current clinical practice as only three tacrolimus models [20, 22, 23] accounted for concomitant azole antifungal use, despite the clinically significant drug-drug interaction [24-30]. Azole antifungals are commonly prescribed in thoracic transplant recipients as a prophylaxis, or for treatment, against invasive fungal infection [11, 31]. Only two population pharmacokinetic models for tacrolimus [22, 32] have been developed solely using data obtained from heart transplant recipients. One of these incorporated pharmacokinetic changes with concomitant azole antifungal therapy [22]. Given ethical considerations associated with developing a new model, including patient recruitment and intensive blood sample collections, existing tacrolimus models developed from various solid organ transplant recipient populations should be evaluated, at least initially, to assess their suitability to predict tacrolimus concentrations in heart transplant recipients.

This study aimed to determine the performances of relevant population pharmacokinetic models of tacrolimus developed from various solid organ transplant recipient populations in predicting tacrolimus concentrations in adult heart transplant recipients, with and without concomitant azole antifungal therapy. The overarching aim was to inform model selection for use within a Bayesian dosing software to provide individualised tacrolimus dosing recommendations for adult heart transplant recipients.

4.4 Methods

4.4.1 Selection of published population pharmacokinetic models for evaluation

Population pharmacokinetic models of tacrolimus developed from adult transplant recipients were selected from a recent systematic review [19] for evaluation. Models were included if the (1) parametric non-linear mixed effect approach was used for pharmacokinetic modelling, (2) models were developed from intensive sampling data, (3) oral immediate-release formulation of tacrolimus (Prograf®) was investigated, and (4) at least liquid chromatography-mass spectrometry (LC-MS) was used to analyse tacrolimus concentrations.

4.4.2 Subjects and data collection

Patients ≥18 years old who underwent heart transplant surgery between 1 January 2017 and 31 December 2018 at St. Vincent's Hospital Sydney (SVHS) and were administered the oral immediate-release formulation of tacrolimus, Prograf® (Astellas, Macquarie Park, Australia) were included. This study was approved by the local governance and SVHS Human Research Ethics Committee (LNR/18/SVH/178). A waiver of consent was received due to the retrospective nature of this study.

Data collected retrospectively from electronic and paper medical records included transplant recipient demographics (age, sex, weight, and height), days post-transplant (a value of 1 corresponds to the first day after transplantation), comorbidities (diabetes mellitus and cystic fibrosis), concomitant medications (basiliximab, mycophenolate, corticosteroids, and azole antifungals), tacrolimus dosing and monitoring history (dosing regimen, dose administration times, tacrolimus concentrations and their times of sample collection), days of tacrolimus therapy and laboratory investigations (haematocrit, serum creatinine concentration, albumin, aspartate aminotransferase, and alkaline phosphatase). Creatinine clearance was calculated using the Cockcroft-Gault formula [33]. Data were collected for each heart transplant recipient, from immediately post-transplantation up to one-year post-transplantation (i.e., included both inpatient and outpatient data).

Covariates with time-dependent values (weight, haematocrit, serum creatinine concentration, creatinine clearance, albumin, aspartate aminotransferase, and alkaline phosphatase) were updated for each recorded tacrolimus concentration. These covariates, if missing at any time point (5% of the data), were inputted as the last observation carried forward for a maximum time of 1 month.

In the inpatient setting, the time of tacrolimus administration by transplant recipients was obtained from the electronic medication management system. In the outpatient setting, the time of tacrolimus administration was assumed to be 8.00 am and 8.00 pm. Full compliance with tacrolimus dosing was presumed unless the missed dose was recorded. Full compliance was considered reasonable as pharmacists counsel transplant

recipients on the timing of tacrolimus administration and the importance of adherence to immunosuppressive therapies. The time of the last administered tacrolimus dose prior to the collection of a blood sample for the measurement of tacrolimus concentration was obtained from the pathology request form. When details of tacrolimus dose were not documented, the last recorded dose was carried forward for a maximum time of 1 month. Under such circumstances, tacrolimus concentrations (5% of the data) were excluded from analysis as there was uncertainty around tacrolimus dosing information.

4.4.3 Institutional immunosuppressive and antibiotic protocol for heart transplant recipients

The dosing and monitoring of tacrolimus and intravenous (IV) basiliximab in heart transplant recipients have been described previously [11] (Appendix 5). Pre-operatively, oral mycophenolate mofetil (1.5 g), IV cephazolin (2 g) and IV methylprednisolone (500 mg) were administered. Post-operatively, IV cephazolin 2 g (2 doses; 8 hours apart) and IV methylprednisolone 125 mg (3 doses; 8 hours apart) were administered. This was followed by maintenance oral prednisolone 1 mg/kg administered in 2 divided doses, tapered by 5 mg every day to 0.20 mg/kg/day, with a complete cessation by month 3 post-transplant. The tapering of prednisolone doses also depended on the endomyocardial biopsy results. The maintenance (oral/IV) mycophenolate mofetil 1 g was administered every 12 hours.

4.4.4 Bioanalytical method

Whole blood tacrolimus concentrations were measured by ultra-high performance liquid chromatography-tandem mass spectroscopy (UHPLC-MS/MS) assay. The assay range was between 2 and 50 µg/L, with an intra-assay coefficient of variation (CV) <8.5% and inter-day CV <10%. Tacrolimus concentrations obtained from external pathology services (5% of the data) were excluded from analysis because the bioanalytical techniques used were unknown.

4.4.5 Race and genotyping

Race (White [Caucasian]/ Black/ Asian) and genotype information (Cytochrome P450 (CYP) *3A5**3 (rs776746), *CYP3A4**22 (rs35599367) and *ABCB1* (ATP Binding Cassette Subfamily B Member 1; encodes P-glycoprotein; rs1045642; C3435T)) were collected prospectively for model evaluation. Heart transplant recipients who were alive at the time of the study were approached, and consent was obtained from those who agreed to participate in this study. This prospective study was approved by the local governance and SVHS Human Research Ethics Committee (2020/ETH00679).

Genomic DNA was extracted from buccal swabs using the Applied Biosystems DNA Multi-sample Ultra Kit on the Kingfisher 96 instrument (Thermo Fisher Scientific, Waltham MA, USA) and was stored at 4°C until analysis. Genotypes of *CYP3A5**3, *CYP3A4**22 and *ABCB1* were identified using specific Taqman Real Time PCR assays on a Quantstudio 6 K instrument (Thermo Fisher Scientific).

For transplant recipients for whom the genotype information was unavailable (39% of the cohort), the most common polymorphism among Caucasians was assumed as the majority self-identified as Caucasian. Specifically, CYP3A5 genotype was assigned to *CYP3A5**3/*3, CYP3A4 allele to *CYP3A4**1, *ABCC2* (ATP Binding Cassette Subfamily C Member 2) haplotype to H1/H1 and *ABCB1* C3435T genotype to CT. Transplant recipients for whom race information was unavailable (40% of the cohort), were assumed to be Caucasian.

4.4.6 Model evaluation

4.4.6.1 Model implementation

Models were transcribed and implemented in NONMEM® version 7.4.3 (ICON Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation method with interaction (FOCE+I) was employed. Pharmacokinetic parameters and covariate relationships for each tacrolimus model were fixed to the values reported. One covariate (prednisolone dose) was excluded from model implementation [34, 35] because this information was unavailable. Of note, the maintenance oral prednisolone protocol used at SVHS differs from that used in previous studies [34, 35] where prednisolone dose was included as a covariate in the model. Authors were contacted if inadequate information was provided in the publication to enable model implementation. Tacrolimus concentrations below the limit of quantification of the assay (3%, 85/2472) were excluded from the analysis. The remaining tacrolimus concentrations were used for model predictions (Method 1) [36].

4.4.6.2 Simulated concentration-time profiles

Tacrolimus concentration-time profiles for each tacrolimus model were generated for comparison, representing a typical heart transplant recipient administered a single dose of 5 mg oral immediate-release formulation of tacrolimus on day 1 post-transplant. The simulation was performed using RxODE package (version 0.9.1-7) in R software (version 3.6.1; R Foundation for Statistical Computing, http://www.R-project.org). The typical heart transplant recipient was defined as a 40-year-old Caucasian male, body weight: 60 kg, height: 170 cm, non-diabetic, non-cystic fibrosis, haematocrit fraction: 0.30, serum creatinine concentration: 110 µmol/L, albumin: 35 g/L, alkaline phosphatase: 50 U/L, aspartate aminotransferase: 90 U/L, *CYP3A5**3/*3 genotype, *CYP3A4**1 allele, *ABCC2* haplotype H1/H1 and *ABCB1* C3435T CT genotype. Expected tacrolimus trough concentrations (11.5 hours after the administered dose) and area under the concentration-time curve, AUC_{0-12} were determined based on each model for the typical heart transplant recipient.

4.4.6.3 Predictive performance metrics

Model performances were evaluated using prediction- and simulation-based assessment.

a) Prediction-based assessment

Models were tested on the dataset using the Bayesian approach (MAXEVAL=0). One to three previous dosing occasions for which an observed tacrolimus concentration was available were used to predict the individual predicted tacrolimus concentration of the fourth dosing occasion. That is, the (1) third dosing occasion was used to predict

tacrolimus concentration of the fourth dosing occasion, (2) second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion, and (3) first, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion. Of note, tacrolimus concentrations in the fourth dosing occasion were blinded to the model (MDV = 1, EVID=2). Model predictions were assessed with and without concomitant azole antifungal therapy. This is because concomitant azole antifungals significantly reduce tacrolimus apparent clearance with a required tacrolimus dose reduction of approximately 20%–80% [24, 25, 28, 37-43]. The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations, with and without concomitant azole antifungal therapy were also determined.

The bias (median percentage predictive error, Equation 1) and imprecision (median absolute percentage predictive error, Equation 2) were calculated by comparing individual predicted tacrolimus concentrations of the fourth dosing occasion by the models to the observed concentrations [44, 45] for each of the evaluation settings described above.

$$\text{Bias (\%)} = \text{Median} \sum \frac{(Individual\ predicted - Observed)}{Observed} \times 100 \qquad \text{(Equation 1)}$$

$$\text{Imprecision (\%)} = \text{Median} \sum \frac{|\ Individual\ predicted - Observed|}{Observed} \times 100 \qquad \text{(Equation 2)}$$

A positive or negative bias represents an over- or under-prediction of tacrolimus concentrations by the model, respectively. Clinically acceptable bias was defined as

within ±20%. The ±20% cut off corresponds to the 80−125% bioequivalence criterion [46] that suggests differences in systemic drug exposure up to 20% are not clinically significant. The prediction was unbiased if the 95% confidence interval (CI) included zero. Clinically acceptable imprecision was ≤20%. The 95% CI of the median was calculated via non-parametric bootstrapping of 1,000 datasets using DescTools package (version 0.99.30) in R software. Model performances were considered high when bias and imprecision are close to zero. Results were graphically summarised and displayed using R software.

b) Simulation-based assessment

Prediction-corrected visual predictive checks (pcVPC) were performed using Perl-speaks-NONMEM® (PsN; version 5.0.0) for all models. Graphics, overlaying 5th, median (50th), and 95th percentiles of the observed tacrolimus concentrations on top of the model-simulated concentration-time profiles with 95% prediction intervals based on 1,000 simulations using each model, were generated. If the model adequately describes the data, the 5th, 50th and 95th percentile of the simulated tacrolimus concentration-time profiles should overlay with the 5th, 50th and 95th percentile of the observed tacrolimus concentrations.

4.5 Results

4.5.1 Characteristics of the models evaluated

A total of 17 population pharmacokinetic models of tacrolimus were evaluated (Table 11). Models were predominately (n=9, 53%) developed from renal transplant recipients. Three models were developed from heart transplant recipients in combination with liver

and renal [47] or lung [48, 49] transplant recipients. The median (range) number of transplant recipients used for model development was 102 (30−408), with 47% of the studies including less than 100 recipients. The median (range) number of concentrations per transplant recipient was 13 (4–57). Most studies (63%) collected transplant recipient data during the first year following transplantation. This included five studies with data collected ≤3 months post-transplantation. Of note, tacrolimus models that had azole antifungal use as a covariate [20, 22, 23] or were developed solely using heart transplant recipient data [22, 32] were not evaluated as their study design did not fulfil the model selection criteria.

The pharmacokinetics of tacrolimus was described using 1- and 2-compartment models, all with first-order elimination, in 18% and 82% of studies, respectively. Six models [35, 47-51] estimated oral bioavailability, with the population average value fixed to one in these studies. More than half of the studies (59%) used first-order absorption with a lag time to describe the delayed drug absorption phase. More complex models were also used by a few studies (18%) [50, 52, 53], such as the Erlang model, with several transit compartments.

The most common covariates identified to be influential on whole blood tacrolimus clearance across the 17 models were CYP3A5 genotype (47% of studies) [6, 34, 35, 50, 52, 54-56], haematocrit (41% of studies) [34, 35, 51-53, 55, 56] and days post-transplant (24% of studies) [34, 54, 57, 58]. Covariates explaining the variability around central volume of distribution were haematocrit (24%) [35, 51, 56, 59] and total or lean body weight (18%) [4, 55, 58]. Between subject variability (BSV) and between occasion

variability (BOV) on pharmacokinetic parameters, and random unexplained variability (RUV) estimates for each model are described in Table 11.

Table 11. Summary of published population pharmacokinetic models of tacrolimus in adult transplant recipients retained for model evaluation

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Andreu et al. [56] [M1]	Renal	304	1891	Day 7-12 months	All patients: Pre-dose; 7 patients: 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12	EMIT, UPLC-MS	2-CMT First-order absorption, with a lag-time and first-order elimination	Ka = 0.138 h^{-1} Lag time = 0.243 h CL = 20.5*(1 - 0.205*AGE) L/h (CYP3A4*22 non-carriers with the CYP3A5*1 genotype) CL = 12.5*(1 - 0.205*AGE) L/h (CYP3A4*22 non-carriers with the CYP3A5*3/*3 genotype or CYP3A4*22 carriers with the CYP3A5*1/*1 genotype) CL = 9.1*(1 - 0.205*AGE) L/h (CYP3A4*22 carriers with the CYP3A5*3/*3 genotype) AGE = 1 if patient's age ≥63 years old, otherwise AGE = 0 V_1 = 5.02 L Q = 4.2 L/h V_2 = 526 L (fixed) Tacrolimus whole blood concentrations standardised to a HCT of 45%	27.8	33.3	Prop RRE = 25%
Andrews et al. [55] [M2]	Renal	337	4527	0-100 days	Pre-dose, 1, 2, 3, 4, 5, 6	EMIT, ACMIA, LC-MS/MS	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 3.58 h^{-1} Lag time = 0.38 h CL/F = 23*[1.0 (if CYP3A5*3/*3) or 1.631 (if CYP3A5*1/*3 or CYP3A5*1/*1)]*[1.0 (if CYP3A4*1 or unknown) or 0.8 (if CYP3A4*22)]*$[(Age/56)^{0.43}]$*$[(ALB/42)^{0.43}]$*$[(BSA/1.93)^{0.88}]$*$[(SCR/135)^{-0.14}]$*$[(HCT/0.34)^{-0.76}]$ L/h V_1/F = 692*$[(LBW/58.94)^{1.52}]$ L Q/F = 11.6 L/h V_2/F = 5340 L	38.6 49.2 78.7 53	13.6	Prop RRE = 17.7% (immunoassay); 24.5% (HPLC-MS/MS) Add RRE = 0.88 ug/L (immunoassay)
Benkali et al. [53] [M3]	Renal	32	NS	1 week-6 months	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9 at 5 transplant periods, 12 h (Week 1 and 2)	TFC-MS/MS	2-CMT. Erlang model with 3 transit CMT and first-order elimination	Ktr = 6.5 h^{-1} CL/F = (863/HCT) L/h V_1/F = 147 L Q/F = 60 L/h V_2/F = 500 L (fixed)	15 30 26 63	24 27 71	Prop RRE = 10% Add RRE = 1.5 ng/ml

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Bergmann et al. [34][M4]	Renal	173	1554	4 days–6.5 years	20 patients: Pre-dose, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 9, 12; 153 patients: Pre-dose, 1, 2, 4	HPLC-MS/MS	2-CMT First-order absorption, with a lag-time and first-order elimination	Ka = 0.35 h^{-1} Lag time = 0.44 h CL/F = 25.5*(1.6^{CYP3A5})*[(1 –1.01*(HCT - 0.33))]* [(WT/70)$^{0.75}$]*[(1 – 0.0021*(POD - 22.7))] L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1 POD is capped at 180 days V_1/F = 113*[(1 - 0.0028*(Pred$_{Cmax,unbound}$ - 155.5)) L Pred$C_{max,unbound}$ is maximum concentration value for free prednisolone (nmol/L) Q/F = 67.9 L/h V_2/F = 1060 L Correlations: V_1/F, Ka = 0.677 V_1/F, V_2/F = –0.049 Ka, V_2/F = –0.013	47.6 29.5 46.8 89.4	29.9 126.5	Prop RRE = 18.3%
Chen et al. [57] [M5]	Liver	125	1704	3-89 days	All patients: Pre-dose; 28 patients: 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12	LC-MS/MS, MEIA	2-CMT First-order absorption with a lag time and first-order elimination	Ka = 0.55 h^{-1} Lag time = 1.96 h CL/F = 21.9*[EXP(0.01*POD)]*[EXP(0.26*CrCL/103.1)]* [EXP(-0.15*ABCB1)] L/h V_1/F =284*[EXP(-0.13*ABCB1)] L Q/F = 62.1 L/h V_2/F = 710 L ABCB1 = 0 if patient is a 3435 CC, ABCB1 = 1 if patient is a 3435 CT, ABCB1 = 2 if patient is a 3435 TT	56.7 36.3 89.4		Prop RRE = 33.3%
Han et al. [54] [M6]	Renal	102	1626	2-21 days	All patients: pre-dose; 55 patients: 0.5, 1, 2, 4, 6, 8, 12	LC-MS/MS	1-CMT First-order absorption, with a lag time and first-order elimination	Ka = 3.43 h^{-1} Lag time = 0.25 h (fixed) CL/F = 21.9*(1 + 0.0119*(POD - 9.6))*[0.816^{CYP3A5}] L/h CYP3A5 = 1 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 0 V/F = 205 L	158.9 42.6 64.6		Prop RRE = 5.4% Add RRE = 1.94 ng/mL

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Lu et al. [47] [M7]	Renal, liver, heart	408	23176	NS	Pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 12.5, 13, 14, 15, 16, 18, 20, 24	HPLC-MS/MS, MEIA	2-CMT First order absorption with a lag time and first-order elimination	$F = (1 + [0.25 \text{ (if Asians) or } -0.433 \text{ (if Blacks) or } 1 \text{ (if White)}]*[1.51^{FORM}]*[(ALB/39)^{1.04}]*[(LAST/3.15)^{0.74}]$ FORM= 1 (immediate-release), FORM = 0 (prolonged-release) $Ka = 0.375\ h^{-1}$ (immediate-release) $Ka = 0.499\ h^{-1}$ (prolonged-release) Lag time = 0.44 h $CL/F = 44.3*(1 + 0.59*RACE)*[(LAST/3.15)^{-0.318}]$ L/h RACE = 1 if Asians, otherwise RACE = 0 $V_1/F = 110*(1-0.446*SEX)*[(ALB/39)^{1.03}]*[(LAST/3.15)^{1.73}]$ L SEX = 1 if Female, SEX = 0 if Male Q/F = 131 L/h $V_2/F = 3180*[(LAST/3.15)^{-0.945}]$ L	30.5 35.5 30.9 106 39.3 99	59.9	Prop RRE = 21.1% (immunoassay); 15.8% (HPLC-MS/MS)
Monchaud et al. [50] [M8]	Lung	78	2113	≤1 year	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 8, 10, 12	TFC-MS/MS	2-CMT Erlang with 4 transit CMT and first-order elimination	$Ktr = 7.06*[0.47^{CF}]\ h^{-1}$ $F = 0.63^{CF}$ $CL/F = 17.5*[1.4^{CYP3A5}]$ L/h $V_1/F = 136$ L Q/F = 41.1 L/h $V_2/F = 529$ L CF = 0 in non-cystic fibrosis patients, otherwise CF = 1; CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1	42.7 53.7 41.0 71.7 126	45.8 46.8 75.4	Prop RRE = 6.9% Add RRE = 1.6 ng/mL
Nanga et al. [58] [M9]	Renal and liver	281	NS	1–394 days	3 studies: Pre-dose; 4 studies: intensive sampling	MEIA, CMIA, LC-MS/MS	2-CMT First-order absorption with a lag time and first-order, time-varying elimination	F = 0.53 (syrup formulation) $Ka = 3.37\ h^{-1}$ (fixed) Lag time = 0.32 h (fixed) $CL = 22.5*[(WT/50)^{0.61}]*[0.38^{(1 - TRANS)}]*(1 + TICL)$ L/h TRANS = 0 if liver transplant, otherwise TRANS = 1 $TICL = (POD^{8.88})/[POD^{8.88} + 6.12^{8.88}]$ if liver transplant, otherwise TICL = 0 $V_1 = 246.2*[(WT/50)^{0.53}]$ L Q = 24.2 L/h $V_2 = 109.9$ L	59.4 133.2		Prop RRE = 24.32% Add RRE = 3.22 ng/mL

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Ogasawara et al. [6] [M10]	Renal	102	500	2–123 months	31 patients: Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12; 71 patients: Pre-dose, 2	HPLC-MS/MS	2-CMT First-order absorption, with a lag time and first-order elimination	Ka = 0.544 h^{-1} Lag time = 0.183*(2.60^{DM}) h DM=1 if diabetic, otherwise DM =0 CL/F = 20.7*[$(Age/50)^{-0.78}$]*[2.03^{CYP3A5}]*[1.40^{ABCC2}] L/h CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1; ABCC2 = 1 if patient is ABCC2 haplotype H2/H2 or H1/H2, otherwise ABCC2 = 0 V_1/F = 234 L Q/F = 70.7 L/h V_2/F = 1319 L	 43.9 157		Prop RRE = 18.4%
Sam et al. [59] [M11]	Liver	31	213	''Immediate post-transplant period''	''Serial samples within a dosing interval of 12h''	HPLC-MS/MS	1-CMT First-order absorption and elimination	Ka = 2.08 h^{-1} CL/F = 14.1 + 0.237*(WT-55) – 2.93*Y – 0.0801*(SCR-60) L/h Y=0 for ALP<200 U/L; Otherwise, Y=1 V/F = 217 – [7.83*(HCT-31.1)] + [179*(HT-1.61)] L	 65.7 63.8		Prop RRE = 34.8 %
Sikma et al. [49] [M12]	Heart and lung	30 (10 heart, 20 lung)	119	First 6 days	Pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12	HPLC-MS/MS	2-CMT Mixed zero- and first-order absorption	Ka = 0.579 h^{-1} (fixed) F = 1 (fixed) CL/F = 20.9 L/h V_1/F = 220 L Q/F = 72 L/h V_2/F = 469 L B_{max} (WBC), (pg/mL) = 2700 K_d (WBC), (pg/mL) = 0.142 N_{plasma} = 137	10 (fixed) 10 (fixed) 42.1 10 (fixed) 10 (fixed) 10 (fixed) 27 3 (fixed) 29	98.3 (fixed) 65	Prop RRE: WBC = 16.7% UPC = 36.3% TPC = 31.6% Correlation: WBC, UPC = 0.26 WBC, TPC = 0.51 TPC, UPC = 0.51

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
Sikma et al. [48] [M13]	Heart and lung	30 (10 heart, 20 lung)	119	First 6 days	Pre-dose, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12	HPLC-MS/MS	2-CMT Mixed zero- and first-order absorption	Ka = 0.579 h^{-1} F = 1 (fixed) CL/F = 19.6 L/h V_1/F = 231 L Q/F = 58.2 L/h V_2/F = 521 L	 34.6	98.3 55 29.5 35.1	Prop RRE = 14%
Staatz et al. [4] [M14]	Liver	68	1742	6–2115 days	All patients: Pre-dose; 36 patients: 1, 2, 4, 6 or 8	LC-MS/MS	1-CMT First-order absorption and elimination	Ka = 4.48 h^{-1} (fixed) CL/F = 29.6 L/h (AST <70 U/L) CL/F = 24.0 L/h (AST >70 U/L) V/F = 601*(WT/72.1) L	 43 93		Add RRE = 3.3 ng/mL
Storset et al. [35] [M15]	Renal	242	3100	4 days-15 years	All patients: Pre-dose; Some patients from full PK profiling and some from limited PK profiling	MEIA, CMIA, LC-MS/MS	2-CMT First-order absorption with a lag time and first-order elimination	F = 1*[1 – (0.67*PREDDOSE)/(35 + PREDDOSE)]*[2.68 (If POD1), otherwise = 1]*[0.82 (If CYP3A5 expresser), otherwise = 1] CL_p/F = 811*[(FFM/60)$^{0.75}$]*[1.3 (If CYP3A5 expresser) or 1 (If CYP3A5 non-expresser)] L/h V_{1p}/F = 6290*FFM/60 L Q_p/F = 1200*[(FFM/60)$^{0.75}$] L/h V_{2p}/F = 32100*FFM/60 L Ka = 1.01 h^{-1} Lag time = 0.41 h CL/F (HCT 45%, FFM 60 kg) = 16.1 L/h V_1/F (HCT 45%, FFM 60 kg) = 125 L V_2/F (HCT 45%, FFM 60 kg) = 636 L Correlation: V_1/F, CL/F = 0.43 CL/F, Q /F = 0.62	F_{POD1} = 57 40 54 63	23 120	Prop RRE = 14.9%
Storset et al. [51] [M16]	Renal	69	1546	First 70 days. Substudies: 26 days-18 years	29 patients (substudies): Pre-dose, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 23, 24; 44 patients: Pre-dose	MEIA, CMIA, LC-MS/MS	2-CMT First-order absorption, with a lag time and first-order elimination	F = [1.87 + (1 – 1.87)/(1 + (POD/2.6)$^{-10}$))]* [1 + 0.27/(1 + (POD/30)$^{-2.3}$))]* [$F_{min,age}$ + (1 - $F_{min,age}$)/(1 + (AGE/44)$^{-12.2}$)]*CYP3A5 CYP3A5 = 0.51 if patient is a CYP3A5 expresser, otherwise CYP3A5 = 1. $F_{min,age}$ = 0.44 (females) or 0.68 (males). Ka = 1.14 h^{-1} Ka, substudy 2 = 0.37 h^{-1} Lag time = 0.21 h Lag time, substudy 2 = 0.82 h CL/Fn = 20.5*[(FFM/60)$^{0.75}$] L/h	BSV $F_{max,late}$ = 117 33	Fn = 16 63	Prop RRE = 16.7% Study 2 factor = 0.57 Study 3 factor = 0.73

Reference [Model number]	Type of transplant	Number of subjects	Number of concentrations	Post-transplant time	Sampling schedules (h)	Bioanalytical method	Model structure	Pharmacokinetic parameters and covariate relationships	BSV (%)	BOV (%)	RUV
								V_1/Fn = 105*(FFM/60) L Q/Fn = 35.8*[(FFM/60)$^{0.75}$] L/h V_2/Fn = 450*(FFM/60) L Correlation (CL/Fn, Q/Fn) = 0.75 PK parameters were standardised to a 40-year-old male, CYP3A5 non-expresser with FFM of 60 kg and HCT of 45% at a time point with the lowest estimated bioavailability (Fn, POD 5)	14 91 52		
Woillard et al. [52] [M17]	Renal	73	NS	1 week-6 months	Pre-dose, 0.33, 0.66, 1, 1.5, 2, 3, 4, 6, 9 at 5 transplant periods, 12 h (Week 1 and 2)	TFC-MS/MS	2-CMT . Erlang model with 3 transit CMT and first-order elimination	Ktr = 3.34*(1.53^{FORM}) h^{-1} CL/F = 21.2*[(HCT/35)$^{-1.14}$]*(2^{CYP3A5}) L/h V_1/F = 486*(0.29^{FORM}) L Q/F = 79 L/h V_2/F = 271 L FORM = 0 if patient received Advagraf®, otherwise FORM = 1 CYP3A5 = 0 if patient is a CYP3A5 non-expresser, otherwise CYP3A5 = 1	24 28 31 54 60	33 31 75	Prop RRE = 11.3% Add RRE = 0.71 ng/mL

ACMIA antibody-conjugated magnetic immunoassay; *Add RRE* additive residual random error; *ALB* albumin; *ALP* alkaline phosphatase; *AST* aspartate transaminase; B_{max} Maximum binding capacity; *BOV* between-occasion variability; *BSA* body surface area; *BSV* between-subject variability; *CL* clearance; *CL/F* apparent oral clearance; *CLp/F* apparent oral clearance from plasma; *CMIA* chemiluminescent microparticle immunoassay; *CMT* compartment; *CrCL* creatinine clearance; *CYP* cytochrome P450; *EMIT* enzyme-multiplied immunoassay technique; *F* bioavailability; *FFM* fat-free mass; *HCT* haematocrit; *HPLC-MS/MS* high-performance liquid chromatography-tandem mass spectrometry; *HT* height; *Ka* absorption rate constant; *Ktr* transfer rate constant; *LAST* log transformed aspartate transaminase; *LBW* lean body weight; *LC-MS/MS* liquid chromatography-tandem mass spectrometry; *MEIA* microparticle enzyme immunoassay; N_{plasma} Non-specific binding constant; *NS* not stated; *POD* post-operative days; *PREDDOSE* prednisone or prednisolone dose; *Prop RRE* proportional residual random error; *Q* intercompartmental clearance; *Q/F* apparent oral intercompartmental clearance; *Qp/F* apparent oral intercompartmental clearance in plasma; *RUV* random unexplained variability; *SCR* serum creatinine; *TFC-MS/MS* turbulence flow chromatography-tandem mass spectrometry; *TPC* Total tacrolimus plasma concentrations; *UPC* Unbound tacrolimus plasma concentrations; *UPLC-MS* ultra-performance liquid chromatography-mass spectrometry; V_1 central volume of distribution; V_2 peripheral volume of distribution; *V/F* apparent oral volume of distribution; V_1/F apparent oral central volume of distribution; V_2/F apparent oral peripheral volume of distribution; V_{1p}/F apparent oral central volume of distribution in plasma; V_{2p}/F apparent oral peripheral volume of distribution in plasma; *WBC* Whole-blood concentration; *WT* weight.

4.5.2 Characteristics of the heart transplant recipients

Data from 85 heart transplant recipients were collected for model evaluation (Table 12 and 13). One transplant recipient had a history of organ transplantation. None of the transplant recipients had cystic fibrosis. The median (range) age was 55 (19−70) years, and the majority were male (67%). All heart transplant recipients received triple maintenance immunosuppressive therapy comprising tacrolimus, mycophenolate mofetil and corticosteroids following transplantation. Intravenous basiliximab was administered as an induction therapy on the day of operation and day 4 post-heart transplant to 81% of transplant recipients with renal impairment (to delay the commencement of tacrolimus) or those who received mechanical circulatory support (left ventricular, biventricular assist devices or total artificial hearts). All heart transplant recipients received oral itraconazole (200 mg 12-hourly) immediately post-transplantation as prophylaxis against *Aspergillus* fungal infection. The antifungal therapy was continued for up to 6 months post-transplantation unless contraindicated due to deranged liver enzymes. During azole antifungal therapy, eleven heart transplant recipients were switched from itraconazole to a different azole antifungal (fluconazole; n=6, voriconazole; n=4, posaconazole; n=1) based on the fungal culture and sensitivity results.

Table 12. Heart transplant recipient characteristics (n=85) on admission and concomitant medications administered immediately post-heart transplant.

Characteristics	**Median (range)***
Male, n (%)	57 (67)
Age (years)	55 (19−70)
Weight (kg)	77 (40−111)
Height (cm)	175 (150−195)
Race, n (%)	
White/Caucasian	41 (48)
Asian	10 (12)
Unknown	34 (40)
Diabetes mellitus, n (%)	29 (34)
CYP3A5 (rs776746), n (%)	
*1/*1	1 (1)
*1/*3	16 (19)
*3/*3	35 (41)
Unknown	33 (39)
CYP3A4 (rs35599367), n (%)	
*1/*1	48 (56)
*1/*22	4 (5)
Unknown	33 (39)
CYP3A phenotype$^{\Delta}$	
Extensive metabolisers	15 (18)
Intermediate metabolisers	35 (41)
Poor metabolisers	2 (2)
Unknown	33 (39)
ABCB1 (rs1045642), n (%)	
CC	11 (13)
CT	28 (33)
TT	13 (15)
Unknown	33 (39)

Haematocrit (fraction)**	0.27 (0.21−0.38)
Albumin (g/L)**	35 (20−45)
Serum creatinine concentration (µmol/L)**	115 (39−293)
Creatinine clearance (mL/min)**	63 (25−172)
Aspartate aminotransferase (U/L)**	73 (14−1390)
Alkaline phosphatase (U/L)**	53 (21−249)
Concomitant immunosuppressants, n (%)	
Mycophenolate mofetil	85 (100)
Corticosteroids	85 (100)
Basiliximab	69 (81)
Concomitant azole antifungal, n (%)	
Itraconazole	85 (100)

Creatinine clearance was calculated using the Cockcroft-Gault formula [33].

*Data are presented as median (range) unless otherwise stated.

**Just before the first tacrolimus dose.

$^{\Delta}$CYP3A phenotype: poor metabolisers (*CYP3A4**22 carriers with the *CYP3A5**3/*3 genotype), intermediate metabolisers (*CYP3A4**22 non-carriers with the *CYP3A5**3/*3 genotype or *CYP3A4**22 carriers with the *CYP3A5**1/*1 genotype), and extensive metabolisers (*CYP3A4**22 non-carriers and *CYP3A5**1 carriers).

Table 13. Heart transplant recipient (n=50) CYP3A4, CYP3A5 and ABCB1 genotypes stratified based on race.

Race	**CYP3A5**			**CYP3A4**		**ABCB1**		
	***3/*3**	***1/*3**	***1/*1**	***1/*1**	***1/*22**	**CC**	**CT**	**TT**
Asian* (n=10)	6	3	1	10	0	2	7	1
White/ Caucasian (n=40)	28	12	0	36	4	9	20	11

Race data was not available for two transplant recipients with genotype information.

*South-east Asian (n=2), North-east Asian (n=5), Southern and Central Asian (n=3).

A total of 2387 tacrolimus concentrations, including 1770 with concomitant azole antifungal and 617 without concomitant azole antifungal were collected between 2- and 391-days post-transplant. Most (52%; 1234/2387) tacrolimus concentrations were collected in the outpatient setting with the remaining concentrations obtained whilst an inpatient. The median (range) number of tacrolimus concentrations collected per transplant recipient was 25 (1−66). The median (range) time after tacrolimus dose administration was 12.61 (6.05–94.78) hours.

The median (range) tacrolimus dose administered by heart transplant recipient while receiving concomitant azole antifungal therapy was 0.50 mg 12-hourly (0.05 mg to 8.00 mg 12-hourly) and without concomitant azole antifungal therapy was 3.00 mg 12-hourly (0.10 mg to 12.00 mg 12-hourly). Dose-normalised tacrolimus concentrations were higher when transplant recipients were receiving concomitant azole antifungal therapy (Figure 12). CYP3A5 expressers (*CYP3A5**1 carriers) were less susceptible to the inhibitory effects of concomitant azole antifungal on tacrolimus metabolism in comparison to CYP3A5 non-expressers (Figure 13).

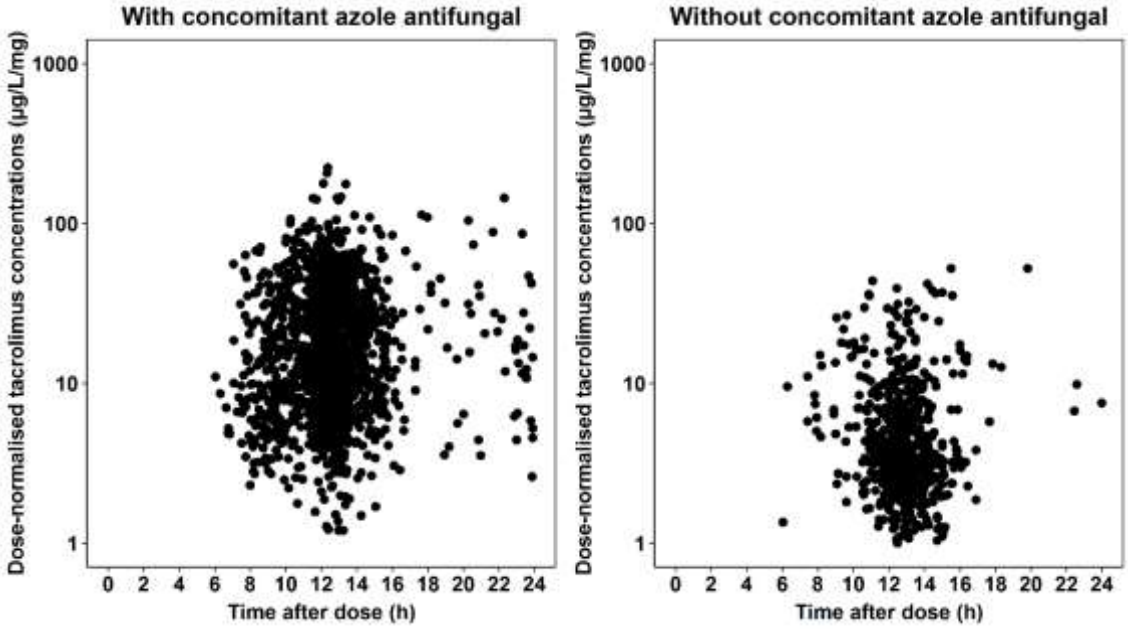


Figure 12. Dose-normalised tacrolimus concentrations against time after dose in adult heart transplant recipients (n=85), with concomitant azole antifungal therapy (n=1770 tacrolimus concentrations) and without concomitant azole antifungal therapy (n=617 tacrolimus concentrations). Y-axis is on a log10 scale. The delay in tacrolimus concentration collection up to 16 hours after the last administered dose was due to the busy clinical environment and critical condition of heart transplant recipients. Some tacrolimus doses were withheld due to supratherapeutic tacrolimus concentration(s) or acute kidney injury. Due to these clinical conditions, tacrolimus concentrations are monitored daily until the condition resolves, explaining the reported time after tacrolimus dose of greater than 16 hours.

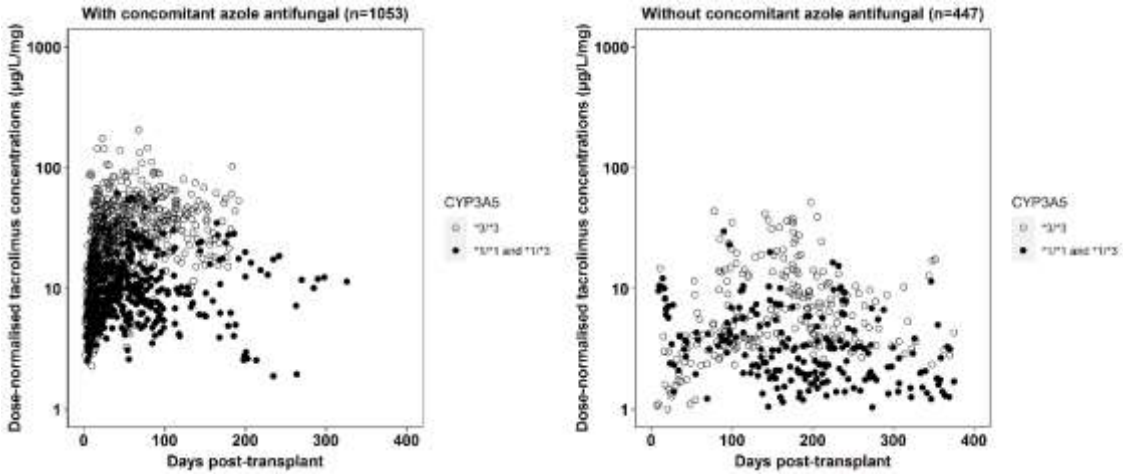


Figure 13. Dose-normalised tacrolimus concentrations against days post-transplant in adult heart transplant recipients (n=52), stratified based on concomitant azole antifungal use and CYP3A5 genotype. Y-axis is on a log10 scale.

4.5.3 Model evaluation

4.5.3.1 Simulated concentration-time profiles

The simulated tacrolimus concentration-time profiles of a typical heart transplant recipient obtained from each of the 17 population pharmacokinetic models showed large variability in the predicted concentration-time profiles regardless of type of organ transplanted and model structure (Figure 14). Predicted trough concentrations for the typical transplant recipient ranged from 2.2 to 10.4 µg/L, while $AUC_{0\text{-}12}$ ranged between 61 and 275 µg*h/L after a single dose of 5 mg oral immediate-release formulation of tacrolimus.

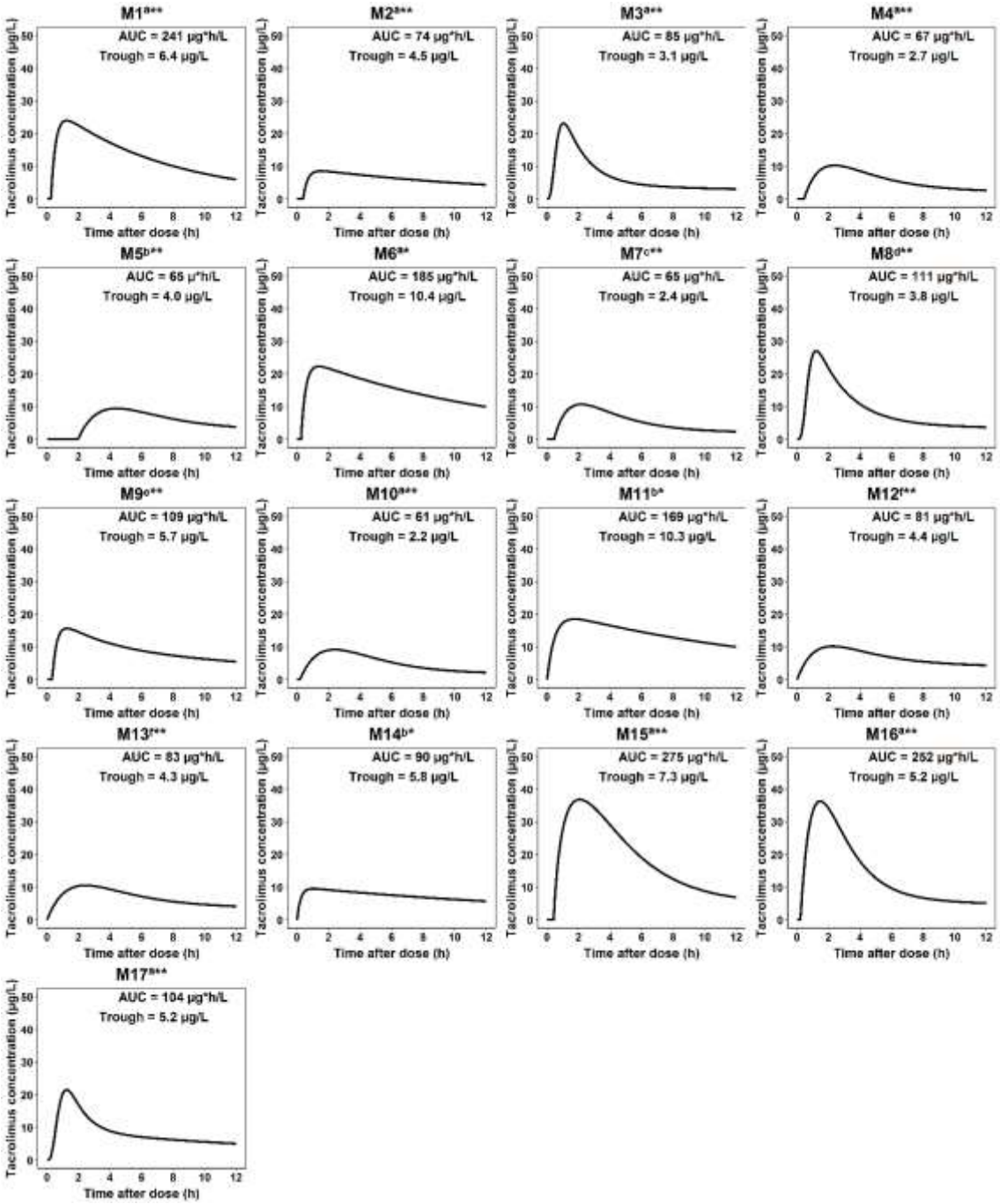


Figure 14. Model predicted tacrolimus concentration-time profiles following the first dose of 5 mg oral immediate-release formulation of tacrolimus administered on day 1 post-transplant to a typical heart transplant recipient. A typical heart transplant recipient is defined here as a 40-year-old Caucasian male, with a body weight of 60 kg, height: 170 cm, haematocrit fraction: 0.30, serum creatinine concentration: 110 µmol/L, albumin: 35 g/L, alkaline phosphatase: 50 U/L, aspartate aminotransferase: 90 U/L, non-diabetic, non-cystic fibrosis, *CYP3A5**3/*3 genotype, *CYP3A4**1 allele, *ABCC2* haplotype H1/H1 and *ABCB1* C3435T CT genotype]. (*) represents a 1-compartment model and

(**) is a 2-compartment model. ([a]) represents model developed from renal transplant recipients, ([b]) model developed from liver transplant recipients, ([c]) model developed from renal, liver and heart transplant recipients, ([d]) model developed from lung transplant recipients, ([e]) model developed from renal and liver transplant recipients, and ([f]) model developed from heart and lung transplant recipients.

4.5.3.2 Predictive performance metrics

a) Prediction-based assessment

With concomitant azole antifungal therapy

Regardless of the number of prior dosing occasions (1−3), all models underpredicted (bias <0%) tacrolimus concentrations of the fourth dosing occasion (n=100) in the presence of concomitant azole antifungal therapy (Figure 15, Appendix 6). None of the models had a 95% CI of bias that included zero and imprecision ≤20% for all evaluation settings. Therefore, none of the models was considered clinically acceptable in the presence of concomitant azole antifungal therapy. The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations were ≤40% in all evaluation settings (Table 14).

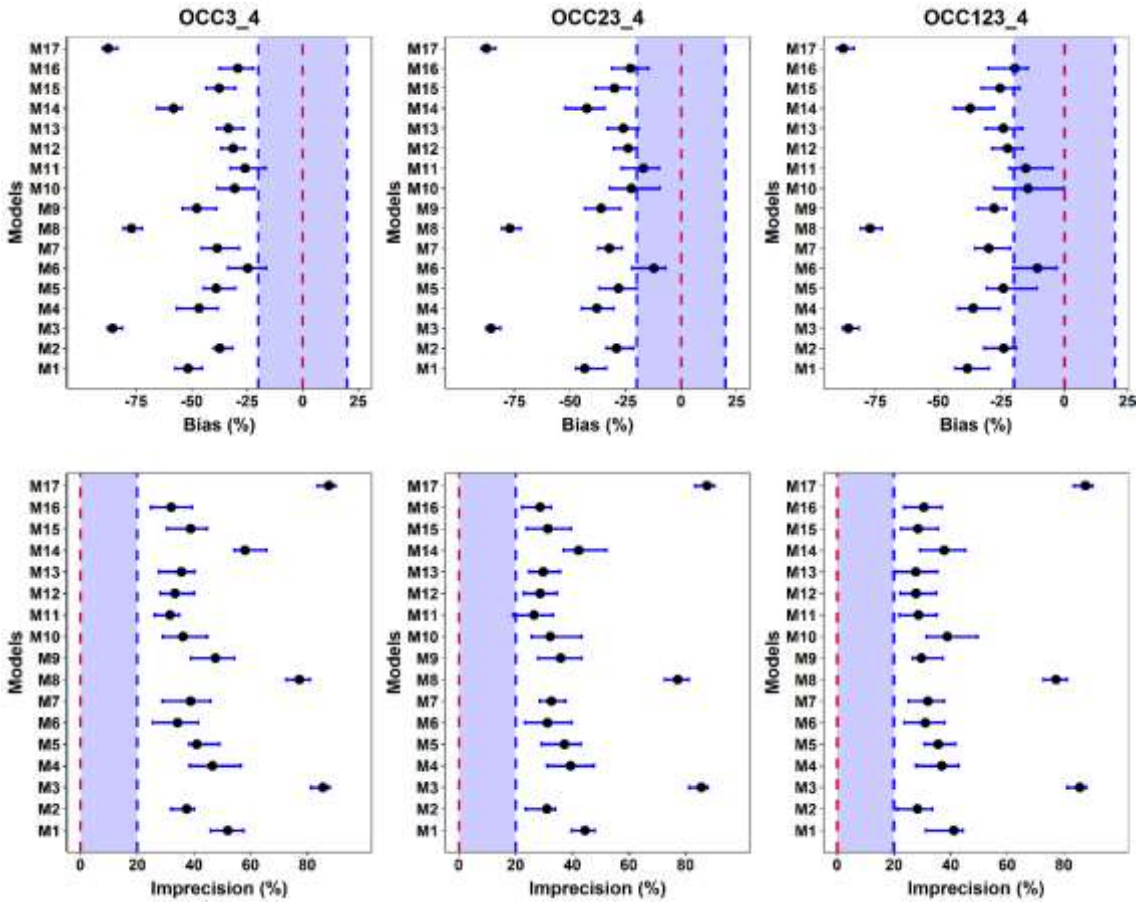


Figure 15. The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=100; blinded to the model) by each model relative to the observed concentrations, with concomitant azole antifungal therapy. The first-row panels show the median bias and its 95% CI. The second-row panels show the median imprecision and its 95% CI. The blue shading depicts the clinically acceptable range for bias (first-row panels) and imprecision (second-row panels). A model is clinically acceptable if bias and imprecision are within the clinically acceptable range, and the 95% CI of bias crosses zero (dashed red lines). *OCC3_4* Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

Table 14. The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations.

Model	**The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations**		
	OCC3_4	**OCC23_4**	**OCC123_4**
With concomitant azole antifungal therapy (n=100)			
M1	15 (15/100)	14 (14/100)	20 (20/100)
M2	16 (16/100)	32 (32/100)	35(35/100)
M3	1 (1/100)	1 (1/100)	1 (1/100)
M4	18 (18/100)	18 (18/100)	27 (27/100)
M5	22 (22/100)	25 (25/100)	23 (23/100)
M6	32 (32/100)	35 (35/100)	34 (34/100)
M7	20 (20/100)	26 (26/100)	31 (31/100)
M8	0 (0/100)	0 (0/100)	0 (0/100)
M9	9 (9/100)	28 (28/100)	27 (27/100)
M10	27 (27/100)	29 (29/100)	24 (24/100)
M11	30 (30/100)	40 (40/100)	36 (36/100)
M12	24 (24/100)	31 (31/100)	36 (36/100)
M13	26 (26/100)	31 (31/100)	39 (39/100)
M14	12 (12/100)	21 (21/100)	27 (27/100)
M15	24 (24/100)	32 (32/100)	38 (38/100)
M16	32 (32/100)	35 (35/100)	34 (34/100)
M17	1 (1/100)	1 (1/100)	1 (1/100)
Without concomitant azole antifungal therapy (n=52)			
M1	29 (15/52)	23 (12/52)	12 (6/52)
M2	21 (11/52)	19 (10/52)	12 (6/52)
M3	12 (6/52)	12 (6/52)	12 (6/52)
M4	35 (18/52)	37 (19/52)	31 (16/52)
M5	27 (14/52)	37 (19/52)	25 (13/52)
M6	33 (17/52)	29 (15/52)	27 (14/52)

M7	37 (19/52)	37 (19/52)	33 (17/52)
M8	27 (14/52)	27 (14/52)	27 (14/52)
M9	40 (21/52)	33 (17/52)	27 (14/52)
M10	21 (11/52)	8 (4/52)	4 (2/52)
M11	23 (12/52)	17 (9/52)	13 (7/52)
M12	46 (24/52)	38 (20/52)	33 (17/52)
M13	44 (23/52)	37 (19/52)	31 (16/52)
M14	29 (15/52)	29 (15/52)	21 (11/52)
M15	31 (16/52)	23 (12/52)	13 (7/52)
M16	25 (13/52)	17 (9/52)	12 (6/52)
M17	21 (11/52)	21 (11/52)	21 (11/52)

OCC3_4 Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

Without concomitant azole antifungal therapy

Model performances varied substantially for individual predicted tacrolimus concentration of the fourth dosing occasion (n=52) without concomitant azole antifungal therapy (Figure 16, Appendix 7). None of the models had an imprecision of ≤20% for all evaluation settings. Therefore, none of the models was considered clinically acceptable in the absence of concomitant azole antifungal therapy. The percentage of individual predicted tacrolimus concentrations of the fourth dosing occasion that were predicted within ±20% of the observed concentrations were <50% in all evaluation settings (Table 14).

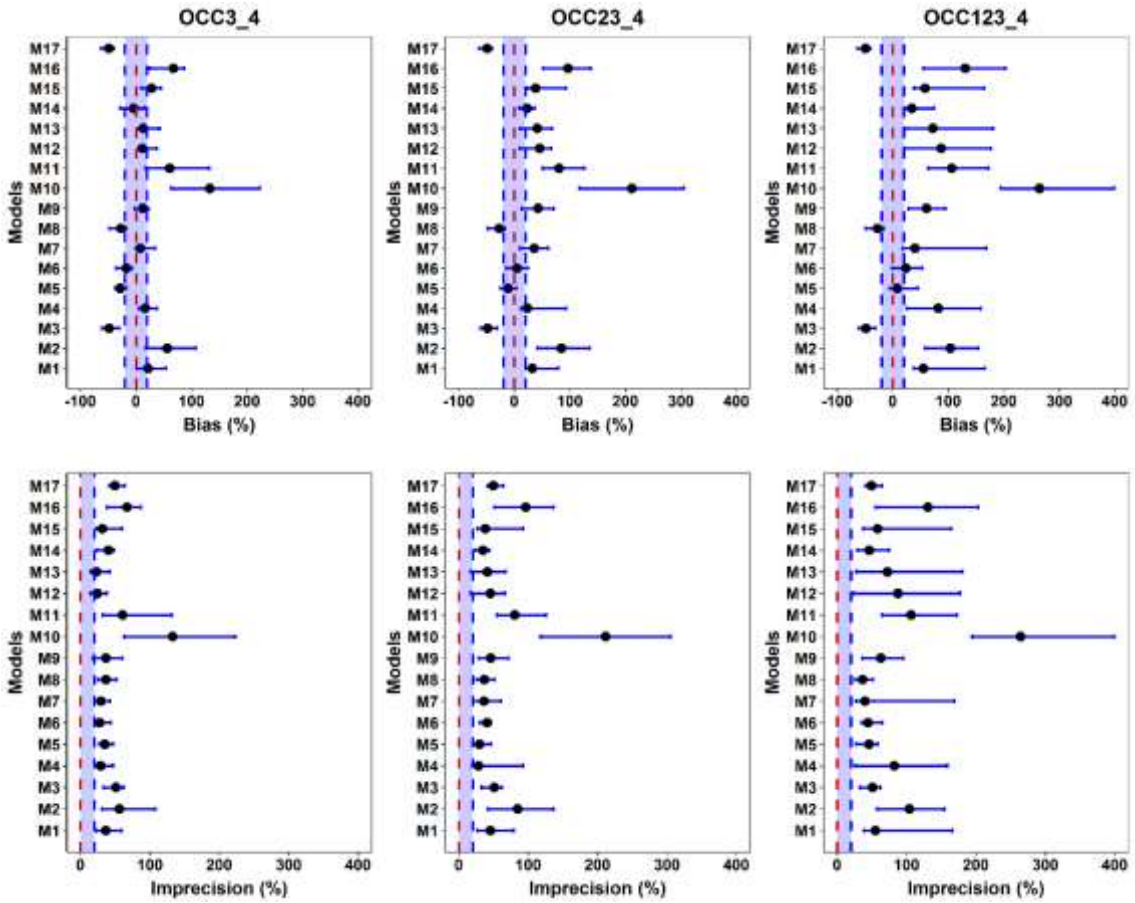


Figure 16. The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=52; blinded to the model) by each model relative to the observed concentrations, without concomitant azole antifungal therapy. The first-row panels show the median bias and its 95% CI. The second-row panels show the median imprecision and its 95% CI. The blue shading depicts the clinically acceptable range for bias (first-row panels) and imprecision (second-row panels). A model is clinically acceptable if bias and imprecision are within the clinically acceptable range, and the 95% CI of bias crosses zero (dashed red lines). *OCC3_4* Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

b) Simulation-based assessment

The poor overlap between the predicted and observed percentiles indicated all models inadequately described the data in the presence (Figure 17) and absence (Figure 18) of concomitant azole antifungal therapy. Specifically, the observed data were higher than the simulated data across all models in the presence of concomitant azole antifungal therapy.

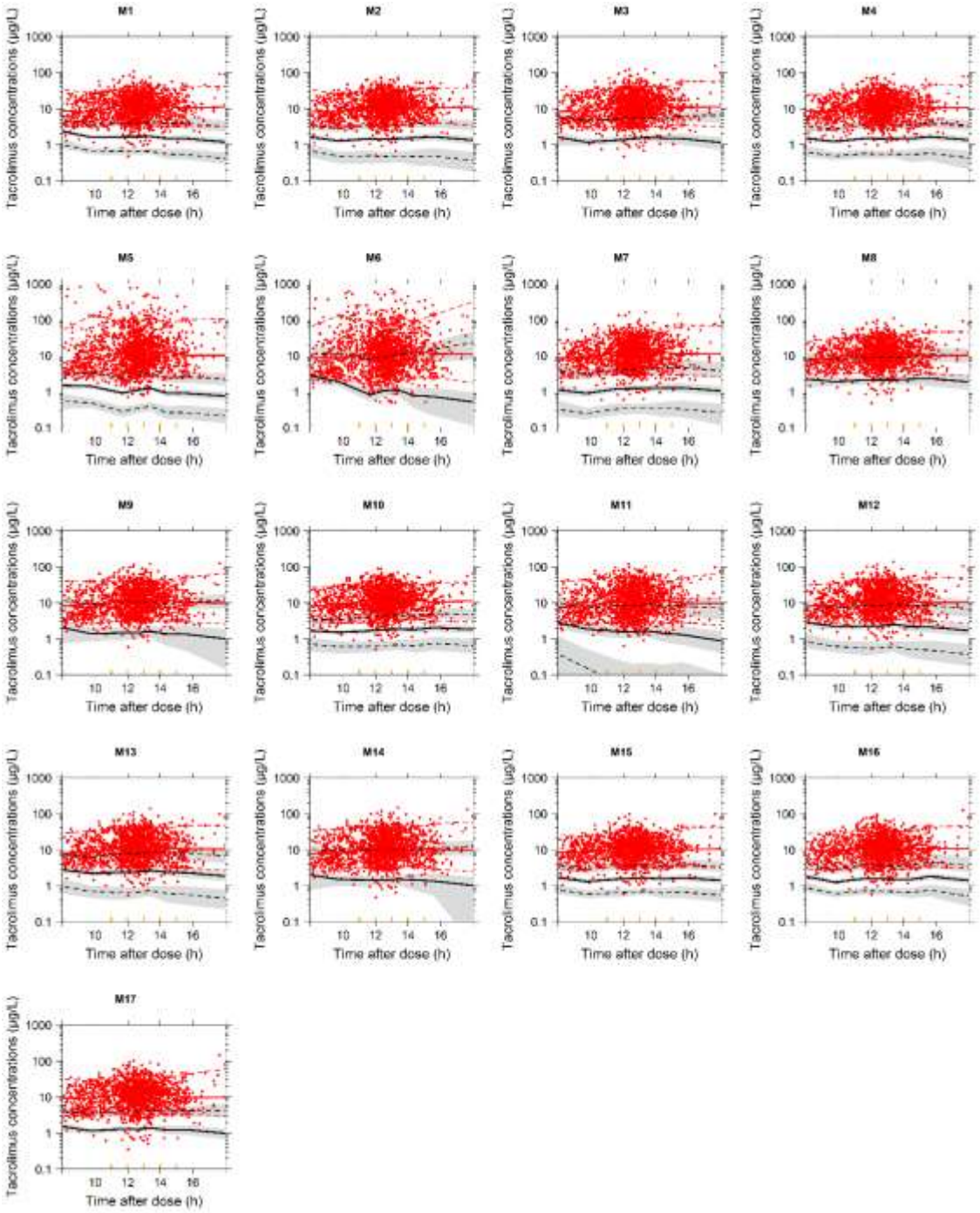


Figure 17. Prediction-corrected visual predictive check (pcVPC) plots for all models (n=17) with concomitant azole antifungal therapy. Y-axis is on a log10 scale. The observed concentrations (solid red dots), median observed (solid red line) and predicted (solid black line), 5th and 95th percentiles of the observations (dashed red line), corresponding prediction percentiles (dashed black line) and 95% prediction intervals for the predicted percentiles (grey band) are shown. The vertical dashes at the bottom of the plots separate the bins.

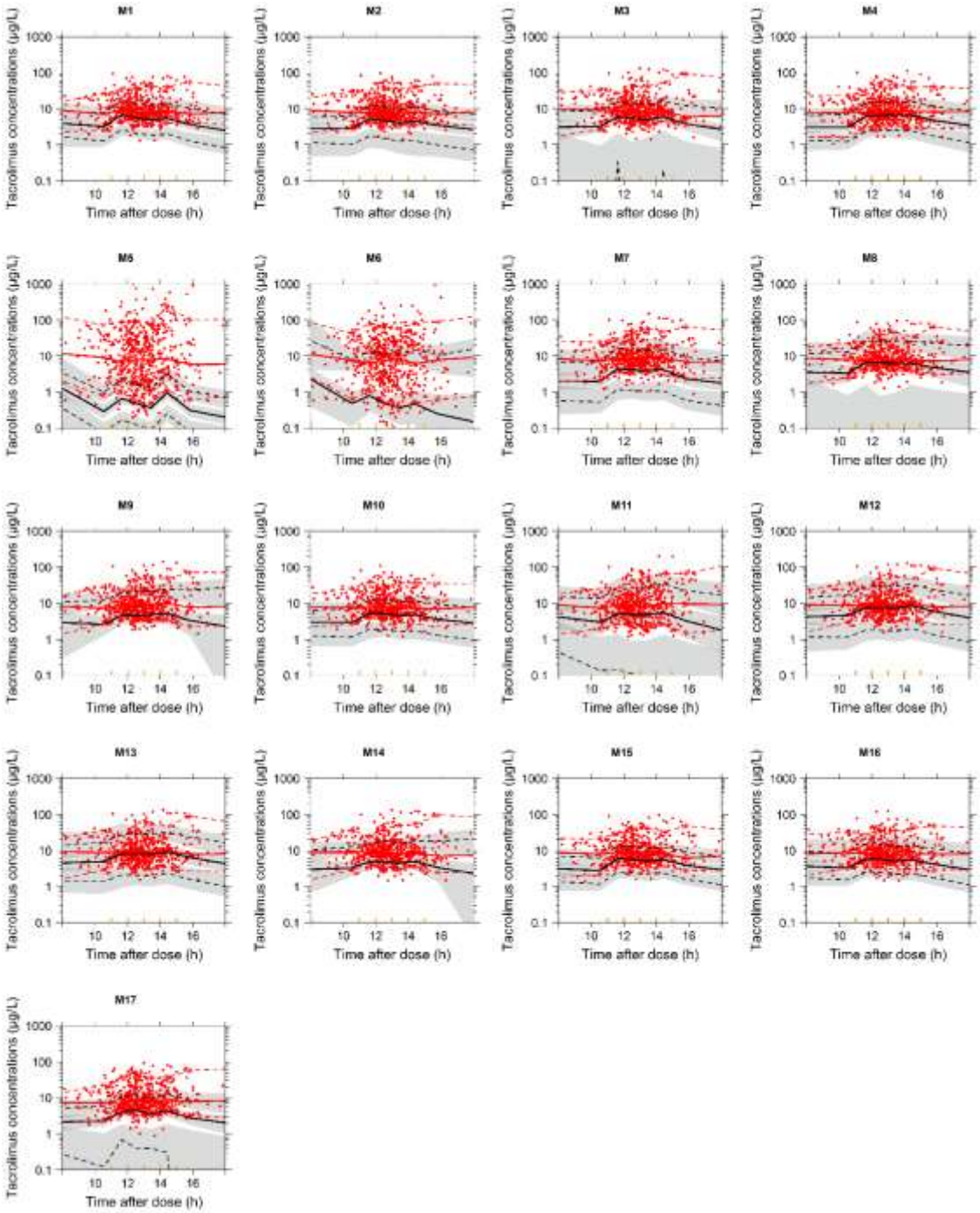


Figure 18. Prediction-corrected visual predictive check (pcVPC) plots for all models (n=17) without concomitant azole antifungal therapy. Y-axis is on a log10 scale. The observed concentrations (solid red dots), median observed (solid red line) and predicted (solid black line), 5th and 95th percentiles of the observations (dashed red line), corresponding prediction percentiles (dashed black line) and 95% prediction intervals for the predicted percentiles (grey band) are shown. The vertical dashes at the bottom of the plots separate the bins.

4.6 Discussion

Over 70 population pharmacokinetic models of tacrolimus in adult transplant recipients have been published [19]. Identifying an appropriate model(s) to be integrated within a Bayesian dosing software is essential prior to its implementation in routine clinical practice to ensure optimal dose recommendations for the targeted population [60]. To the best of our knowledge, this is the first comprehensive evaluation of the performances of existing models to predict tacrolimus concentrations in adult heart transplant recipients receiving and not receiving concomitant azole antifungal therapy.

Consistent with the large variability in tacrolimus pharmacokinetics, the resulting simulated concentration-time profiles of all evaluated models (Figure 14) showed large variability in the predicted tacrolimus concentration-time profiles following the same tacrolimus dose administered to a typical heart transplant recipients with the same characteristics. The variable exposure could be due to the differences in transplant recipient characteristics used for model building, study design, model structures and tacrolimus parameter-covariate relationships. The large variability in tacrolimus exposure across the models highlights the lack of model generalisability not only between different but also within the same solid organ transplant recipient populations. The requirement to use different models in specific transplant recipient populations is cumbersome and likely poses a significant barrier to the implementation of Bayesian approach to guide tacrolimus dosing decisions.

Mimicking clinical practice, the ability of tacrolimus models to predict tacrolimus concentrations of the fourth dosing occasion (blinded to the model) from 1−3 prior

dosing occasions was evaluated. Tacrolimus models developed from various solid organ transplant recipient populations, including thoracic transplant, were unsatisfactory in predicting tacrolimus concentrations of the fourth dosing in adult heart transplant recipients. This is regardless of (1) the number of prior dosing occasions (1−3) included for model predictions and (2) concomitant azole antifungal use. While studies [11, 61-66] have demonstrated an improvement in individual predicted tacrolimus concentration considerably by models with input of at least one prior tacrolimus concentration, this was not the case in the present study. Given the evaluated models were developed from transplant recipient populations where concomitant azole antifungal use was either prohibited or uncommon, the models are anticipated to reasonably predict tacrolimus concentration in the setting of without concomitant azole antifungal therapy in comparison to with concomitant azole antifungal therapy. The reason for the contrary outcome remains unclear. Due to poor model predictions, the models are deemed inappropriate to be used clinically to guide tacrolimus dosing decisions.

Three studies [61, 62, 67] have performed an external evaluation of several existing tacrolimus models (n=8 to 16) in transplant recipients. Consistent with the present study, external evaluation of liver [62] and renal [61] tacrolimus models performed in the respective transplant recipient populations displayed poor pcVPC. Another study evaluated renal-derived tacrolimus models in renal transplant recipients, stratified based on CYP3A5 genotype [67]. Unfortunately, most models displayed acceptable pcVPC only for CYP3A5 extensive metabolisers [67]. It is important to note that two [61, 62] of the three studies recommended the incorporation of non-linear pharmacokinetics

during pharmacokinetic modelling to improve model predictability. Considering this recommendation, further optimisation of tacrolimus models is required to enable model extrapolation not only between same but different organ transplant recipient populations.

Relevant models developed from non-heart transplant recipients were included for evaluation. This is because recent data indicate that tacrolimus models can be extrapolated across various organ transplant recipient populations [11, 65]. Further, only one [58] of two tacrolimus population pharmacokinetic models [47, 58] that considered type of organ transplant, identified distinct differences in tacrolimus clearance between a liver and non-liver transplant populations. With increasing evidence, this suggests the potential for extrapolation of tacrolimus models across different organ transplant recipient populations.

Five tacrolimus population pharmacokinetic models have been developed using data from heart transplant recipients [19]. While three of them (M7, M12, M13) were evaluated in the present study, the remaining two models [22, 32] were not evaluated as their study design did not fulfil the model selection criteria. Nonetheless, we did attempt to evaluate both models (Appendix 8 and Appendix 9). Unfortunately, they were unable to adequately describe tacrolimus pharmacokinetics in our study population, with and without concomitant azole antifungal. This concludes that none of the existing tacrolimus models developed from heart transplant recipients is suitable for use for our study population. Further work is required to modify or develop a population

pharmacokinetic model to describe tacrolimus pharmacokinetics for our heart transplant recipient cohort.

4.7 Limitations

Collection of prednisolone doses in the outpatient setting was not possible as they were not consistently documented in the paper medical record. High doses of corticosteroid therapy [9] administered in the early phase post-transplant may increase tacrolimus clearance and hence, models may overpredict tacrolimus concentrations. Due to the retrospective nature of the study, the time of tacrolimus administration in the outpatient setting cannot be guaranteed. This may impact the accuracy of data used for model prediction. Possible discrepancies between the actual and published model codes could have influenced model performances. Race, CYP3A5 genotype, CYP3A4 genotype and ABCB1 genotype have been shown to influence tacrolimus apparent clearance [10, 19, 47, 68]. These data were unavailable in approximately 40% of the heart transplant recipients included in this study. This could have influenced the performances of models that had any of these covariates in the model.

4.8 Conclusions

Seventeen published population pharmacokinetic models of tacrolimus developed from various solid organ transplant recipient populations were evaluated using the Bayesian approach in adult heart transplant recipients. These models were unable to adequately describe tacrolimus pharmacokinetics in heart transplant recipients included in this study. Further work is required to describe tacrolimus pharmacokinetics for our heart transplant recipient cohort.

4.9 Study highlights

What is already known about this subject?

- Numerous population pharmacokinetic models of tacrolimus in adult transplant recipients have been published to characterise tacrolimus pharmacokinetics.
- Identifying an appropriate tacrolimus model to be integrated within a Bayesian dosing software for specific patient sub-groups is essential prior to its implementation in routine clinical practice to facilitate dose individualisation.

What this study adds?

- A large number of population pharmacokinetic models of tacrolimus evaluated were unable to adequately describe tacrolimus pharmacokinetics in heart transplant recipients regardless of concomitant azole antifungal therapy.
- More work is required to adequately characterise tacrolimus pharmacokinetics in heart transplant recipients.

4.10 Acknowledgements

We would like to thank (a) The Australian Centre of Pharmacometrics for the NONMEM® software license, (b) Clinical Pharmacology laboratory, Sydpath, St. Vincent's Hospital Sydney for access to tacrolimus monitoring request forms, (c) Heart transplant clinic, St. Vincent's Hospital Sydney for access to transplant recipient medical records, (d) Dr Shaun Kumar (Parexel, Australia) for providing valuable input in terms of pharmacometrics analysis, and (e) All authors who have shared their NONMEM model codes: Elisabet Storset (Norway), Bing Chen (China), Christine Staatz (Australia), Franc Andreu Solduga (Spain), Jean Woillard (France), Khaled Benkali (France), Louise M.

Andrews (Netherlands), Maaike A Sikma (Netherlands), Tom M Nanga (France) and Zheng Lu (USA). Ms Ranita Kirubakaran is a recipient of the Faculty of Medicine and Health, University of New South Wales, Sydney, Doctoral Scholarship funded by the Australian Government, and St. Vincent's Centre for Applied Medical Research Student Scholarship Supplementation Scheme.

4.11 Funding

This work was supported by a National Health and Medical Research Council (NHMRC) APP1054146 Program Grant; Creating safe, effective systems of care: The translational challenge.

4.12 Conflict of interest

Ranita Kirubakaran, Stefanie Hennig, Ben Maslan, Richard O. Day, Jane E. Carland and Sophie L. Stocker have no conflicts of interest to declare that are relevant to the content of this article.

4.13 Author Contributions

RK, SLS, SH and JEC designed the study. RK performed data collection and manuscript writing. RK and SH performed pharmacometric analysis. BM assisted with R scripts. All authors were involved in the interpretation of data and critically reviewed, edited, and approved the final manuscript.

References

1. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

2. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

3. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

4. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

5. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

6. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

7. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

8. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

9. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

10. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit. 2019;41(3):261-307.

11. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus Therapy in Adult Heart Transplant Recipients: Evaluation of a Bayesian Forecasting Software. Ther Drug Monit. 2021;43(6):736-46.

12. Dasari B, Hodson J, Nassir A, Widmer J, Isaac J, Mergentel H, et al. Variations in practice to therapeutic monitoring of tacrolimus following primary adult liver transplantation. Int J Organ Transplant Med. 2016;7(1):1-8.

13. Sheiner LB, Beal S, Rosenberg B, Marathe VV. Forecasting individual pharmacokinetics. Clin Pharmacol Ther. 1979;26(3):294-305.

14. del Mar Fernández de Gatta M, Martin-Suarez A, Lanao JM. Approaches for dosage individualisation in critically ill patients. Expert Opin Drug Metab Toxicol. 2013;9(11):1481-93.

15. Meinshausen J, Bühl K, Engel G, Harings-Kaim A, Drewelow B, Klotz U. Generation of pharmacokinetic data during routine therapeutic drug monitoring: Bayesian approach vs. pharmacokinetic studies. Ther Drug Monit. 1993;15(4):281-8.

16. Gao Y, Hennig S, Barras M. Monitoring of tobramycin exposure: what is the best estimation method and sampling time for clinical practice? Clin Pharmacokinet. 2019;58(3):389-99.

17. Fuchs A, Csajka C, Thoma Y, Buclin T, Widmer N. Benchmarking therapeutic drug monitoring software: a review of available computer tools. Clin Pharmacokinet. 2013;52(1):9-22.

18. Roydhouse SA, Carland JE, Debono DS, Baysari MT, Reuter SE, Staciwa AJ, et al. Accuracy of documented administration times for intravenous antimicrobial drugs and impact on dosing decisions. Br J Clin Pharmacol. 2021.

19. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review. Clin Pharmacokinet. 2020;59(11):1357-92.

20. Cai X, Song H, Jiao Z, Yang H, Zhu M, Wang C, et al. Population pharmacokinetics and dosing regimen optimization of tacrolimus in Chinese lung transplant recipients. Eur J Pharm Sci. 2020;152:105448.

21. Marquet P, Destère A, Monchaud C, Rérolle J-P, Buchler M, Mazouz H, et al. Clinical Pharmacokinetics and Bayesian Estimators for the Individual Dose Adjustment of a Generic Formulation of Tacrolimus in Adult Kidney Transplant Recipients. Clin Pharmacokinet. 2021;60(5):611-22.

22. Zhou H, Cai J, Huang J, Zhang J, Shi SJ, Liu YN, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: A population pharmacokinetic approach. Pharmacogenomics. 2019;20(1):21-35.

23. Zahir H, McLachlan AJ, Nelson A, McCaughan G, Gleeson M, Akhlaghi F. Population pharmacokinetic estimation of tacrolimus apparent clearance in adult liver transplant recipients. Ther Drug Monit. 2005;27(4):422-30.

24. Darley D, Carlos L, Hennig S, Glanville A. Quantitation of the Effect of Azole Antifungals on Tacrolimus Clearance. J Heart Lung Transplant. 2016;35(4):S236.

25. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

26. He J, Yu Y, Yin C, Liu H, Zou H, Ma J, et al. Clinically significant drug-drug interaction between tacrolimus and fluconazole in stable renal transplant recipient and literature review. J Clin Pharm Ther. 2019;45(2):264-9.

27. Gaïes E, Salouage I, Sahnoun R, Trabelsi S, Jebabli N, Lakhal M, et al. Interaction between azole antifugals drugs and tacrolimus in four kidney transplant patients. J Mycol Med. 2010;21(1):46-50.

28. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic evaluation of the drug interaction between intravenous itraconazole and intravenous tacrolimus or intravenous cyclosporin A in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2006;12(3):325-34.

29. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

30. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

31. Muñoz P, Valerio M, Palomo J, Giannella M, Yañez JF, Desco M, et al. Targeted antifungal prophylaxis in heart transplant recipients. Transplantation. 2013;96(7):664-9.

32. Gong Y, Yang M, Sun Y, Li J, Lu Y, Li X. Population pharmacokinetic analysis of tacrolimus in Chinese cardiac transplant recipients. Eur J Hosp Pharm. 2020;27(e1):e012-18.

33. Cockcroft DW, Gault H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

34. Bergmann TK, Hennig S, Barraclough KA, Isbel NM, Staatz CE. Population pharmacokinetics of tacrolimus in adult kidney transplant patients: impact of CYP3A5 genotype on starting dose. Ther Drug Monit. 2014;36(1):62-70.

35. Storset E, Holford N, Hennig S, Bergmann TK, Bergan S, Bremer S, et al. Improved prediction of tacrolimus concentrations early after kidney transplantation using theory-based pharmacokinetic modelling. Br J Clin Pharmacol. 2014;78(3):509-23.

36. Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn. 2001;28(5):481-504.

37. Osowski CL, Dix SP, Lin LS, Mullins RE, Geller RB, Wingard JR. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. Transplantation. 1996;61(8):1268-72.

38. Mañez R, Martin M, Raman V, Silverman D, Jain A, Warty V, et al. Fluconazole therapy in transplant recipients receiving FK506. Transplantation. 1994;57(10):1521-3.

39. Sansone-Parsons A, Krishna G, Martinho M, Kantesaria B, Gelone S, Mant TG. Effect of oral posaconazole on the pharmacokinetics of cyclosporine and tacrolimus. Pharmacotherapy. 2007;27(6):825-34.

40. Shitrit D, Ollech JE, Ollech A, Bakal I, Saute M, Sahar G, et al. Itraconazole prophylaxis in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. J Heart Lung Transplant. 2005;24(12):2148-52.

41. Trofe-Clark J, Lemonovich T, Practice AIDCo. Interactions between anti-infective agents and immunosuppressants in solid organ transplantation. Am J Transplant. 2013;13(s4):318-26.

42. Moreno M, Latorre A, Manzanares C, Morales E, Herrero J, Dominguez-Gil B, et al. Clinical management of tacrolimus drug interactions in renal transplant patients. Transplant Proc. 1999;31(6):2252-3.

43. Gu TM, Lewis JS, Le H, Bubalo JS. Comparative effects of fluconazole, posaconazole, and isavuconazole upon tacrolimus and cyclosporine serum concentrations. J Oncol Pharm Pract. 2021:10781552211029046.

44. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm. 1981;9(4):503-12.

45. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Phar. 1982;10(2):229-.

46. Holford NH, Buclin T. Safe and effective variability—a criterion for dose individualization. Ther Drug Monit. 2012;34(5):565-8.

47. Lu Z, Bonate P, Keirns J. Population pharmacokinetics of immediate- and prolonged-release tacrolimus formulations in liver, kidney and heart transplant recipients. Br J Clin Pharmacol. 2019;85(8):1692-703.

48. Sikma MA, Hunault CC, Van Maarseveen EM, Huitema AD, Van de Graaf EA, Kirkels JH, et al. High variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Eur J Drug Metab Pharmacokinet. 2020;45(1):123-34.

49. Sikma MA, Van Maarseveen EM, Hunault CC, Moreno JM, Van de Graaf EA, Kirkels JH, et al. Unbound Plasma, Total Plasma, and Whole-Blood Tacrolimus Pharmacokinetics Early After Thoracic Organ Transplantation. Clin Pharmacokinet. 2019;59(6):771–80.

50. Monchaud C, de Winter BC, Knoop C, Estenne M, Reynaud-Gaubert M, Pison C, et al. Population pharmacokinetic modelling and design of a Bayesian estimator for

therapeutic drug monitoring of tacrolimus in lung transplantation. Clin Pharmacokinet. 2012;51(3):175-86.

51. Storset E, Holford N, Midtvedt K, Bremer S, Bergan S, Asberg A. Importance of hematocrit for a tacrolimus target concentration strategy. Eur J Clin Pharmacol. 2014;70(1):65-77.

52. Woillard JB, de Winter BC, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations--twice daily Prograf and once daily Advagraf. Br J Clin Pharmacol. 2011;71(3):391-402.

53. Benkali K, Premaud A, Picard N, Rerolle JP, Toupance O, Hoizey G, et al. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet. 2009;48(12):805-16.

54. Han N, Ha S, Yun Hy, Kim MG, Min SI, Ha J, et al. Population pharmacokinetic–pharmacogenetic model of tacrolimus in the early period after kidney transplantation. Basic Clin Pharmacol Toxicol. 2014;114(5):400-6.

55. Andrews LM, Hesselink DA, van Schaik RHN, van Gelder T, de Fijter JW, Lloberas N, et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2019;85(3):601-15.

56. Andreu F, Colom H, Elens L, van Gelder T, van Schaik RHN, Hesselink DA, et al. A New CYP3A5*3 and CYP3A4*22 Cluster Influencing Tacrolimus Target Concentrations: A Population Approach. Clin Pharmacokinet. 2017;56(8):963-75.

57. Chen B, Shi HQ, Liu XX, Zhang WX, Lu JQ, Xu BM, et al. Population pharmacokinetics and Bayesian estimation of tacrolimus exposure in Chinese liver transplant patients. J Clin Pharm Ther. 2017;42(6):679-88.

58. Nanga TM, Doan TT, Marquet P, Musuamba FT. Toward a robust tool for pharmacokinetic-based personalization of treatment with tacrolimus in solid organ transplantation: a model-based meta-analysis approach. Br J Clin Pharmacol. 2019;85(12):2793-823.

59. Sam WJ, Tham LS, Holmes MJ, Aw M, Quak SH, Lee KH, et al. Population pharmacokinetics of tacrolimus in whole blood and plasma in asian liver transplant patients. Clin Pharmacokinet. 2006;45(1):59-75.

60. Uster DW, Stocker SL, Carland JE, Brett J, Marriott DJ, Day RO, et al. A model averaging/selection approach improves the predictive performance of model-informed precision dosing: vancomycin as a case study. Clin Pharmacol Ther. 2021;109(1):175-83.

61. Zhao CY, Jiao Z, Mao JJ, Qiu XY. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2016;81(5):891-907.

62. Cai X, Li R, Sheng C, Tao Y, Zhang Q, Zhang X, et al. Systematic external evaluation of published population pharmacokinetic models for tacrolimus in adult liver transplant recipients. Eur J Pharm Sci. 2020;145:105237.

63. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

64. Fukudo M, Yano I, Shinsako K, Katsura T, Takada Y, Uemoto S, et al. Prospective evaluation of the bayesian method for individualizing tacrolimus dose early after living-donor liver transplantation. J Clin Pharmacol. 2009;49(7):789-97.

65. Zhu J, Campagne O, Torrice CD, Flynn G, Miller JA, Patel T, et al. Evaluation of the performance of a prior tacrolimus population pharmacokinetic kidney transplant model among adult allogeneic hematopoietic stem cell transplant patients. Clin Transl Sci. 2021;14(3):908-18.

66. Decrocq-Rudler M-A, Meunier L, Fraisse J, Ursic-Bedoya J, Khier S. Can We Predict Individual Concentrations of Tacrolimus After Liver Transplantation? Application and Tweaking of a Published Population Pharmacokinetic Model in Clinical Practice. Ther Drug Monit. 2021;43(4):490-8.

67. Hu C, Yin WJ, Li DY, Ding JJ, Zhou LY, Wang JL, et al. Evaluating tacrolimus pharmacokinetic models in adult renal transplant recipients with different CYP3A5 genotypes. Eur J Clin Pharmacol. 2018;74(11):1437-47.

68. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623-53.

69. Prograf®. Northbrook, IL: AstellasPharma US, Inc. 2020.

Appendices

Appendix 5. Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients.

Dosing		
	Formulation	
	Oral	**Intravenous***
Initial dose	0.5 mg (fixed test dose)	0.015 mg/kg/day infused over 24 hours
Maintenance dose	Administered 12-hourly. Dose adjusted based on steady-state** trough concentration(s) and transplant recipient's overall clinical assessment.	Administered as a continuous infusion over 24 hours. Dose adjusted based on steady-state tacrolimus concentration(s) and transplant recipient's overall clinical assessment.
Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2	Delay commencement of initial dose until eGFR is ≥60 mL/min/1.73 m^2.	
Monitoring		
Initial dose	Check trough concentration.	Check steady-state tacrolimus concentration.
Maintenance dose	Check trough concentration at steady-state**, frequency of monitoring based on transplant recipient's overall clinical assessment.	Check steady-state tacrolimus concentration twice weekly or based on transplant recipient's overall clinical assessment.
Therapeutic range of trough concentration (Time post-transplant)***	8−12 µg/L (day 1 – day 7) 10−15 µg/L (day 8 − 3 months) 8−12 µg/L (>3 − 12 months) 6−8 µg/L (>12 months)	

*In Australia, intravenous tacrolimus is licensed to be administered only as a continuous infusion and not to be administered as a bolus, intermittent, or short infusion [69].

**At least 48 hours after initiation of maintenance therapy or any dose adjustment.

***To achieve the same drug exposure, the drug concentration targeted while a transplant recipient is on continuous infusion of tacrolimus will be higher than the drug concentration targeted while a transplant recipient receives the oral twice-daily dosing. In our institute, clinicians target at least the higher end of the therapeutic range specified in the guideline table if a transplant recipient receives an intravenous infusion.

Appendix 6. The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=100; blinded to the model) by each model relative to the observed concentrations, with concomitant azole antifungal therapy.

Model	Bias		Imprecision	
	%	95% CI	%	95% CI
OCC3_4				
M1	-51.6	-57.59, -45.46	52.0	45.78, 57.59
M2	-37.4	-39.97, -31.48	37.5	32.02, 39.97
M3	-85.6	-87.84, -81.31	85.6	81.31, 87.84
M4	-46.5	-56.61, -38.30	46.5	38.47, 56.61
M5	-38.9	-44.64, -30.26	41.1	38.20, 48.98
M6	-24.7	-33.64, -16.24	34.3	25.52, 41.50
M7	-38.5	-45.48, -28.59	38.9	28.87, 45.87
M8	-77.1	-80.66, -72.21	77.3	72.67, 81.01
M9	-47.6	-54.20, -38.79	47.6	38.91, 54.20
M10	-30.6	-38.51, -21.65	36.2	28.96, 44.51
M11	-25.9	-32.61, -16.78	31.6	26.20, 34.87
M12	-31.3	-36.61, -25.97	33.3	28.12, 40.10
M13	-33.4	-38.55, -26.52	35.7	27.55, 40.18
M14	-58.0	-65.59, -54.32	58.0	54.32, 65.59
M15	-37.5	-43.42, -30.29	38.8	30.50, 44.66
M16	-29.1	-37.53, -22.37	32.0	24.74, 39.43
M17	-87.6	-90.02, -83.53	87.6	83.53, 90.02
OCC23_4				
M1	-43.2	-47.39, -33.77	44.6	39.67, 48.00
M2	-29.0	-33.64, -21.43	31.1	23.58, 33.95
M3	-85.6	-87.84, -81.31	85.6	81.31, 87.84
M4	-37.9	-44.83, -30.32	39.5	31.08, 47.65
M5	-28.2	-36.84, -19.94	37.3	29.16, 42.97
M6	-12.2	-21.97, -7.13	31.2	23.24, 39.68
M7	-32.2	-37.47, -26.69	32.7	28.46, 37.64
M8	-77.1	-80.66, -72.21	77.3	72.67, 81.01
M9	-35.9	-43.26, -27.17	35.9	27.82, 43.26
M10	-22.3	-31.97, -9.68	32.2	25.51, 43.21

M11	-16.9	-26.74, -9.82	26.5	19.06, 33.12
M12	-23.8	-30.27, -20.11	28.7	22.85, 34.52
M13	-26.0	-33.07, -19.08	29.7	24.81, 35.72
M14	-42.3	-52.01, -34.32	42.3	36.95, 52.01
M15	-29.9	-38.62, -23.01	31.4	23.77, 39.50
M16	-22.8	-31.19, -14.75	28.7	22.25, 32.44
M17	-87.6	-90.02, -83.53	87.6	83.53, 90.02
OCC123_4				
M1	-38.4	-43.16, -29.76	41.2	31.25, 44.36
M2	-24.0	-31.96, -19.05	28.4	21.19, 33.57
M3	-85.6	-87.84, -81.31	85.6	81.31, 87.84
M4	-36.1	-42.13, -25.79	36.9	27.87, 42.89
M5	-24.1	-30.77, -11.06	35.7	30.73, 41.69
M6	-10.7	-20.45, -2.96	31.1	23.56, 37.83
M7	-30.0	-35.41, -21.24	32.0	25.11, 37.67
M8	-77.1	-80.66, -72.21	77.3	72.67, 81.01
M9	-27.8	-34.30, -23.04	29.7	26.59, 37.35
M10	-14.5	-27.81, -0.14	38.8	31.48, 49.58
M11	-15.3	-21.82, -4.81	28.7	21.82, 35.09
M12	-22.5	-28.70, -16.62	27.8	22.23, 34.97
M13	-24.2	-31.32, -16.69	27.8	20.49, 35.40
M14	-37.3	-43.95, -27.98	37.8	29.10, 45.13
M15	-25.5	-32.80, -17.67	28.5	22.41, 35.52
M16	-19.6	-30.15, -14.55	30.6	23.53, 36.86
M17	-87.6	-90.02, -83.53	87.6	83.53, 90.02

OCC3_4 Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

Appendix 7. The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (n=52; blinded to the model) by each model relative to the observed concentrations, without concomitant azole antifungal therapy.

Model	Bias		Imprecision	
	%	95% CI	%	95% CI
OCC3_4				
M1	21.7	0.47, 53.36	36.4	22.33, 59.17
M2	56.1	16.64, 107.64	56.1	30.48, 107.64
M3	-48.3	-61.23, -30.96	50.9	32.67, 62.00
M4	16.6	4.55, 37.47	29.2	21.15, 46.43
M5	-28.7	-37.51, -17.18	35.1	26.86, 46.44
M6	-17.5	-36.56, -6.89	27.6	20.16, 43.62
M7	7.6	-0.48, 33.88	29.5	19.73, 42.39
M8	-27.2	-48.80, -17.18	36.6	24.92, 51.35
M9	12.3	-2.64, 23.04	36.6	18.02, 60.03
M10	132.8	62.57, 223.20	132.8	62.57, 223.20
M11	60.6	17.48, 131.48	60.6	31.88, 131.48
M12	11.5	4.05, 37.31	23.9	14.28, 38.10
M13	13.2	3.48, 42.42	22.7	13.95, 42.42
M14	-4.2	-29.42, 15.31	40.0	23.66, 48.26
M15	27.9	9.09, 44.07	31.7	22.64, 59.61
M16	67.1	24.78, 86.67	67.1	38.05, 86.67
M17	-48.9	-63.72, -40.32	49.6	41.07, 63.98
OCC23_4				
M1	32.4	20.12, 78.78	45.6	26.02, 78.78
M2	84.9	41.05, 136.33	84.9	41.89, 136.33
M3	-48.3	-61.23, -30.96	50.9	32.67, 62.00
M4	23.2	11.92, 92.42	28.5	18.60, 92.42
M5	-11.5	-25.86, 3.61	29.9	19.55, 46.57
M6	4.7	-15.40, 24.37	40.7	30.29, 45.84
M7	35.9	9.28, 60.96	36.3	19.81, 60.96
M8	-27.2	-48.80, -17.18	36.6	24.92, 51.35
M9	42.6	12.66, 71.25	45.8	28.87, 71.25
M10	211.5	117.13, 305.19	211.5	117.13, 305.19

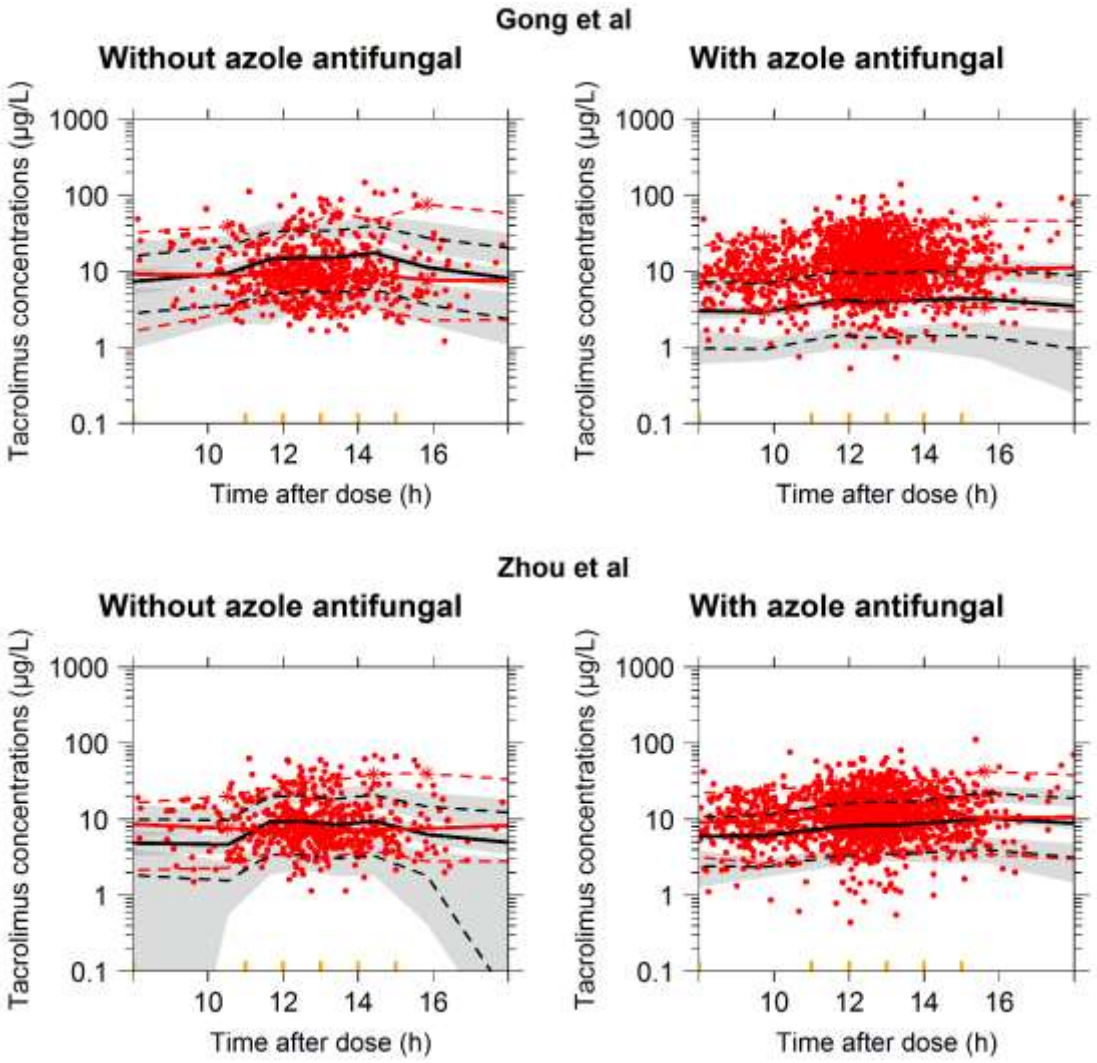
M11	80.6	50.46, 125.90	80.6	54.52, 125.90
M12	45.4	9.10, 66.28	45.4	17.06, 66.28
M13	41.1	9.67, 67.36	41.1	17.03, 67.36
M14	23.1	8.91, 36.58	34.5	22.44, 43.59
M15	38.1	23.19, 92.98	38.1	26.21, 92.98
M16	96.4	51.47, 136.56	96.4	51.47, 136.56
M17	-48.9	-63.72, -40.32	49.6	41.07, 63.98
OCC123_4				
M1	55.2	37.15, 165.70	55.2	38.13, 165.70
M2	104.0	57.65, 154.70	104.0	57.65, 154.70
M3	-48.3	-61.23, -30.96	50.9	32.67, 62.00
M4	82.3	24.99, 158.51	82.3	25.54, 158.51
M5	8.0	-5.43, 45.03	45.9	26.44, 58.22
M6	23.6	-0.86, 53.05	44.5	35.38, 65.07
M7	40.1	17.18, 168.90	40.1	26.43, 168.90
M8	-27.2	-48.80, -17.18	36.6	24.92, 51.35
M9	60.8	28.57, 94.86	62.9	35.48, 94.86
M10	264.3	194.36, 399.00	264.3	194.36, 399.00
M11	106.4	64.44, 172.60	106.4	64.44, 172.60
M12	87.3	20.29, 176.75	87.3	23.39, 176.75
M13	72.4	21.03, 180.42	72.4	27.29, 180.42
M14	34.5	22.32, 74.46	46.0	28.83, 74.46
M15	58.2	37.85, 164.34	58.2	37.85, 164.34
M16	130.6	54.86, 202.88	130.6	54.86, 202.88
M17	-48.9	-63.72, -40.32	49.6	41.07, 63.98

OCC3_4 Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

Appendix 8. The bias and imprecision of the individual predicted tacrolimus concentration of the fourth dosing occasion (blinded to the model) by models developed solely using data from heart transplant recipients relative to the observed concentrations.

Model	Bias		Imprecision	
	%	95% CI	%	95% CI
Gong *et al* [32]				
With concomitant azole antifungal therapy (n=100)				
OCC3_4	-27.3	-31.1, -21.9	28.9	25.3, 33.0
OCC23_4	-21.0	-25.5, -14.7	25.0	20.7, 27.7
OCC123_4	-16.5	-22.2, -6.7	24.9	21.2, 28.1
Without concomitant azole antifungal therapy (n=52)				
OCC3_4	99.9	45.2, 210.0	99.9	45.2, 210.0
OCC23_4	147.0	65.4, 241.0	147.0	65.4, 241.0
OCC123_4	180.0	97.8, 255.0	180.0	97.8, 255.0
Zhou *et al* [22]				
With concomitant azole antifungal therapy (n=100)				
OCC3_4	-11.4	-20.4, -3.15	29.8	20.4, 36.5
OCC23_4	-9.8	-17.2, -1.42	28.9	20.4, 37.3
OCC123_4	-4.7	-14.1, 1.66	28.7	21.4, 37.8
Without concomitant azole antifungal therapy (n=52)				
OCC3_4	11.7	0.7, 20.6	20.2	13.8, 33.4
OCC23_4	19.2	7.7, 36.3	28.4	16.0, 39.9
OCC123_4	32.2	11.5, 68.2	35.4	18.8, 68.2

OCC3_4 Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.



Appendix 9. Prediction-corrected visual predictive check (pcVPC) plots for models developed solely using data from heart transplant recipients, stratified based on concomitant azole antifungal use. Y-axis is on a log10 scale. The observed concentrations (solid red dots), median observed (solid red line) and predicted (solid black line), 5th and 95th percentiles of the observations (dashed red line), corresponding prediction percentiles (dashed black line) and 95% prediction intervals for the predicted percentiles (grey band) are shown. The vertical dashes at the bottom of the plots separate the bins.

Chapter 5:

Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients.

5.1 Link to thesis

In Chapter 4, I presented an evaluation of the predictive performance of selected tacrolimus population pharmacokinetic models developed from various organ transplant recipient populations in adult heart transplant recipients. The evaluation was stratified based on concomitant azole antifungal use. Unfortunately, none of the models displayed acceptable predictive performance. Together, the findings from Chapters 2, 3 and 4 highlight that a population pharmacokinetic model is required to describe tacrolimus pharmacokinetics in heart transplant recipients receiving and not receiving concomitant azole antifungal therapy. In Chapter 5, I develop and evaluate a population pharmacokinetic model for tacrolimus in heart transplant recipients that considers the tacrolimus-azole antifungal interaction. Routine drug monitoring data, principally pre-dose tacrolimus concentrations were available for model development. Therefore, a previously published model was employed to inform the estimation of some pharmacokinetic parameters of the model. Subsequently, the model was externally evaluated using the Bayesian forecasting approach to mimic clinical practice. To the best of my knowledge, this is the first population pharmacokinetic model to comprehensively describe tacrolimus pharmacokinetics in heart transplant recipients, considering the clinically significant tacrolimus-azole antifungal interaction, over one-year post-transplant.

This manuscript is currently under review:

Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Adaptation of a population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients [submitted 31 August 2021].

I have presented this work at one national symposium, two national conferences and four international conferences:

1. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Oral Presentation, Australasian Pharmaceutical Science Association (APSA) Annual Conference, 6 – 8 December 2021.
2. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Oral and Poster Presentation, Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) Virtual Annual Scientific Meeting, 29 November – 2 December 2021.
3. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Oral Presentation ASCEPT Student Forum Symposium, virtual meeting, 7 October 2021.
4. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Poster Presentation, Twelfth American Conference on Pharmacometrics (ACoP12), virtual meeting, 8–12 November 2021.
5. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Poster Presentation, 19th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology (IATDMCT), virtual meeting, 19–22 September 2021.

6. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. A population pharmacokinetic model to inform tacrolimus therapy in heart transplant recipients. Poster Presentation, Population Approach Group Europe (PAGE), virtual meeting, 2−7 September 2021.
7. Kirubakaran R, Uster DW, Hennig S, Carland JE, Day RO, Wicha SG, *et al*. Matters close to the heart: Adaptation of a tacrolimus model to inform therapeutic drug monitoring using the PRIOR approach. Oral Presentation, Population Approach Group of Australia & New Zealand (PAGANZ), virtual meeting, 27−29 January 2021.

5.2 Abstract

Background and Objective. Existing tacrolimus population pharmacokinetic models are unsuitable for guiding tacrolimus dosing in heart transplant recipients. This study aimed to develop and evaluate a population pharmacokinetic model for tacrolimus in heart transplant recipients that considers the tacrolimus-azole antifungal interaction.

Methods. Data from heart transplant recipients (n=87) administered the oral immediate-release formulation of tacrolimus (Prograf®) were collected. Routine drug monitoring data, principally trough concentrations, were used for model building (n=1100). A published tacrolimus model was used to inform the estimation of K_a, V_2/F, Q/F, and V_3/F. Body weight was implemented as a covariate on CL/F, V_2/F, V_3/F and Q/F on an allometry scale. The effect of concomitant azole antifungal use on tacrolimus CL/F was quantified. Subsequently, stepwise covariate modelling was performed. Significant covariates influencing tacrolimus CL/F were included in the final model. Robustness of the final model was confirmed using prediction-corrected visual predictive check

(pcVPC). The final model was externally evaluated for prediction of tacrolimus concentrations of the fourth dosing occasion (n=87) from 1−3 prior dosing occasions.

Results. Concomitant azole antifungal therapy reduced tacrolimus CL/F by 80%. Haematocrit (ΔOFV = -33, $p<0.001$) was included in the final model. The pcVPC of the final model displayed good model adequacy. One recent drug concentration is sufficient for the model to guide tacrolimus dosing.

Conclusion. A population pharmacokinetic model that adequately describes tacrolimus pharmacokinetics in heart transplant recipients, considering the tacrolimus-azole antifungal interaction was developed. Prospective evaluation is required to assess its clinical utility to improve transplant recipient clinical outcomes.

5.3 Introduction

Tacrolimus is the cornerstone immunosuppressant used extensively in most solid-organ transplant recipients [1], including heart transplants, to prevent graft rejection. Due to its narrow therapeutic range [2] and considerable pharmacokinetic variability [3-9], monitoring whole blood trough concentrations is recommended to individualise tacrolimus therapy [10]. However, the collection of appropriately timed trough concentrations rarely occurs in clinical settings [11]. This hampers the successful implementation of trough concentration-guided dosing in routine clinical practice.

Population pharmacokinetic model-based Bayesian estimation presents as an alternative approach to conventional trough concentrations in guiding tacrolimus dosing, providing flexible blood sampling schemes [10]. The Bayesian approach utilises a population pharmacokinetic model together with patient-specific information and at

least one drug concentration to estimate individual patient pharmacokinetic parameters [12-14]. From this, an optimal drug dose predicted to achieve target drug concentration can be identified. Implementation of the Bayesian approach to support decision-making in clinical practice has been shown to improve the attainment of target tacrolimus concentrations in renal and liver transplant recipients in the early post-transplantation period [15-17].

Prior to implementing the Bayesian approach in routine clinical practice, a suitable population pharmacokinetic model is required. External evaluation of existing population pharmacokinetic models using data from the patient population in which it is intended to be used is performed to identify the most suitable model to facilitate dose individualisation [18]. Evaluation of a number (n=19) of existing tacrolimus models developed from various solid organ transplant recipient populations in heart transplant recipients resulted in poor predictive performances (bias, imprecision, and visual predictive checks) [19]. Consequently, none of the 19 models was suitable in guiding tacrolimus dosing decisions in adult heart transplant recipients [19]. It is well known that concomitant azole antifungal therapy alters tacrolimus pharmacokinetics substantially [20-24] necessitating dose adjustments [20, 24-32]. Despite this, only one [33] of these models had accounted for concomitant azole antifungal use on tacrolimus pharmacokinetics. Given institutional guidelines recommend the use of concomitant azole antifungal therapy for the first six months post-heart transplantation, a model that describes tacrolimus pharmacokinetics both in the presence and absence of concomitant azole antifungal therapy is required.

This study aimed to (1) develop a population pharmacokinetic model for tacrolimus in heart transplant recipients, considering the drug-drug interaction with azole antifungals and (2) externally evaluate the model using the Bayesian approach.

5.4 Methods

5.4.1 Subjects and data collection

Subjects who underwent heart transplant surgery between January 1, 2017 and December 31, 2018 at St. Vincent's Hospital Sydney (SVHS) and were administered the oral immediate-release formulation of tacrolimus, Prograf® (Astellas, Macquarie Park, Australia) were included. This study was approved by the local governance and SVHS Human Research Ethics Committee (LNR/18/SVH/178). A waiver of consent was received due to the retrospective nature of this study.

Data collected retrospectively from electronic and paper medical records included transplant recipient demographics (age, sex, weight, and height), days post-transplant (a value of 1 corresponds to the first day after transplantation), comorbidities (diabetes mellitus and cystic fibrosis), concomitant medications (basiliximab, mycophenolate, corticosteroids, and azole antifungals), tacrolimus dosing and monitoring history (dosing regimen, dose administration times, tacrolimus concentrations and time of sample collection), days of tacrolimus therapy and laboratory investigations (haematocrit, serum creatinine concentration, albumin, aspartate aminotransferase and alkaline phosphatase). Creatinine clearance was calculated using the Cockcroft-Gault formula [34]. Data were collected for each heart transplant recipient, from immediately post-

transplantation up to approximately 1-year post-transplantation (i.e., included both inpatient and outpatient data).

Covariates with time-dependent values (weight, haematocrit, serum creatinine concentration, creatinine clearance, albumin, aspartate aminotransferase, and alkaline phosphatase) were updated for each recorded tacrolimus concentration. These covariates, if missing at any time point (5% of the data), were inputted as the last observation carried forward for a maximum time of 1 month.

In the inpatient setting, the time of tacrolimus administration was obtained from the electronic medication management system. In the outpatient setting, the time of tacrolimus administration was assumed to be 8.00 am and 8.00 pm. Full compliance with tacrolimus dosing was presumed unless the missed dose was recorded. Full compliance was considered reasonable as pharmacists intensively counsel transplant recipients on the timing of tacrolimus administration and the importance of adherence to immunosuppressive therapies. The time of the last administered tacrolimus dose before the collection of a blood sample for the measurement of tacrolimus concentration was obtained from the pathology request form. When details of tacrolimus dose were not documented, the last recorded dose was carried forward for a maximum time of 1 month. Under such circumstances, tacrolimus concentrations (5% of the data) were excluded from analysis as there was uncertainty around tacrolimus dosing information.

5.4.2 Institutional immunosuppressive and antibiotic protocol for heart transplant recipients

The dosing and monitoring of the oral immediate-release formulation of tacrolimus and intravenous (IV) basiliximab in heart transplant recipients have been described previously [11] (Appendix 10). Pre-operatively, oral mycophenolate mofetil (1.5 g), IV cephazolin (2 g) and IV methylprednisolone (500 mg) were administered. Post-operatively, IV cephazolin 2 g (2 doses; 8 hours apart) and IV methylprednisolone 125 mg (3 doses; 8 hours apart) were administered. This was followed by maintenance oral prednisolone 1 mg/kg administered in 2 divided doses, tapered down by 5 mg every day to 0.20 mg/kg/day, with a complete cessation by month 3 post-transplant. The tapering of prednisolone doses also depended on the endomyocardial biopsy results. The maintenance (oral/IV) mycophenolate mofetil 1 g was administered every 12 hours.

5.4.3 Bioanalytical method

Whole blood tacrolimus concentrations were measured by ultra-high performance liquid chromatography-tandem mass spectroscopy (UHPLC-MS/MS) assay. The assay range was between 2 and 50 µg/L, with an intra-assay coefficient of variation (CV) <8.5% and inter-day CV <10%. Tacrolimus concentrations obtained from external pathology services (5% of the data) were excluded from analysis because the bioanalytical techniques used were unknown.

5.4.4 Ethnicity and genotyping

Ethnicity (White [Caucasian]/ Black/ Asian) and genotype information (Cytochrome P450 (CYP) *3A5**3 (rs776746), *CYP3A4**22 (rs35599367) and *ABCB1* (ATP Binding

Cassette Subfamily B Member 1; encodes P-glycoprotein; rs1045642; C3435T)) were collected prospectively. Heart transplant recipients who were alive at the time of the study were approached, and consent was obtained from those who agreed to participate. This prospective study was approved by the local governance and SVHS Human Research Ethics Committee (2020/ETH00679).

Genomic DNA was extracted from buccal swabs using the Applied Biosystems DNA Multi-sample Ultra Kit on the Kingfisher 96 instrument (Thermo Fisher Scientific, Waltham MA, USA) and was stored at 4°C until analysis. Genotypes of *CYP3A5**3, *CYP3A4**22 and *ABCB1* were identified using specific Taqman Real Time PCR assays on a Quantstudio 6 K instrument (Thermo Fisher Scientific).

For transplant recipients for whom the genotype information was unavailable (49% of the cohort), the most common polymorphism among Caucasians was assumed as the majority self-identified as Caucasian. Specifically, CYP3A5 genotype was assigned to *CYP3A5**3/*3, CYP3A4 allele to *CYP3A4**1, and *ABCB1* C3435T genotype to CT. Transplant recipients for whom ethnicity information was unavailable (49% of the cohort), were assumed to be Caucasian.

5.4.5 Population pharmacokinetic modelling

Pharmacokinetic analysis was performed using nonlinear mixed-effects modelling software NONMEM® (version 7.4.3; ICON Development Solutions, Ellicott City, MD, USA). The first-order conditional estimation method with interaction (FOCE+I) was employed. Tacrolimus concentrations below the limit of quantification of the assay (4%

of the data, 41/1141) were excluded from the analysis. The remaining tacrolimus concentrations were used to estimate the pharmacokinetic parameters as if the concentrations came from a full distribution (Method 1) [35]. Data handling, visualisation and statistics were performed in R software (version 3.6.1; R Foundation for Statistical Computing; http://www.R-project.org).

The model building dataset consisted of data collected from heart transplant recipients in 2018 (n=47). The external evaluation dataset consisted of data collected from heart transplant recipients in 2017 (n=40). Only pre-dose, principally trough concentrations collected from routine drug monitoring were available for model building and external evaluation. The availability of limited drug concentrations over a dosing interval (at most one tacrolimus concentration over a dosing interval was available) may not support the estimation of all pharmacokinetic parameters during model building. Therefore, the PRIOR approach (PRIOR subroutine in NONMEM®) [36] was employed to support or stabilise the estimation of some pharmacokinetic parameters of the model using values from a published model. This approach not only provides an opportunity to generalise the use of published models in clinical practice but has been shown to improve the predictive performance of the model [37].

5.4.5.1 Model building (Figure 19)

Selection of published model

The published two-compartment linear model with first-order oral absorption by Sikma *et al*. [38] was selected as the prior model. This model was selected because it was developed using data from thoracic transplant recipients (n=30) and did not include

covariates, reducing the complexity of the model. The model was developed using intensive sampling pharmacokinetic data (10 tacrolimus concentration-time points over the 12 hours dosing interval). Details of concomitant azole antifungal use were not provided in the publication.

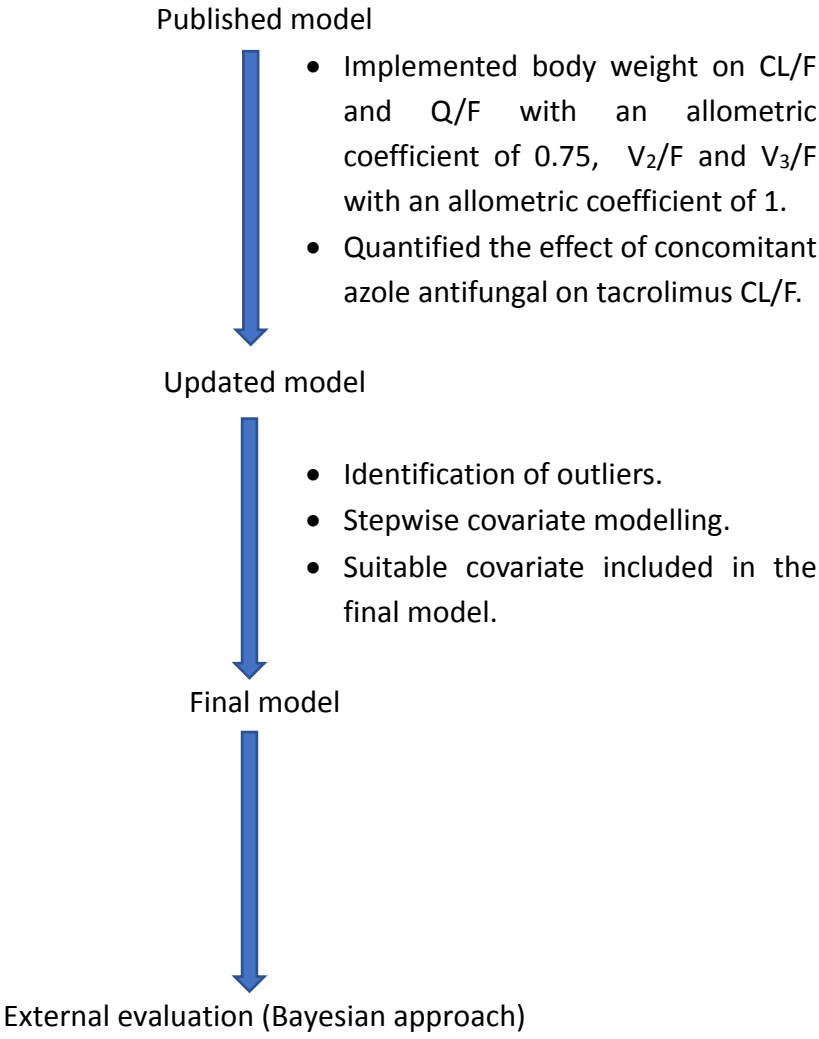


Figure 19. Flow chart of the modelling approach.

Updated model

The published model by Sikma *et al*. [38] was implemented and tested on the model building dataset using the PRIOR NWPRI subroutine in NONMEM®. Values from the published model were used to support the estimation of absorption rate constant [K_a], apparent central volume of distribution [V_2/F], apparent intercompartmental clearance [Q/F], apparent peripheral volume of distribution [V_3/F] during model building. The population average oral bioavailability [F] was fixed to one (1) [38]. All between-occasion variabilities (BOV) on apparent clearance [CL/F], V_2/F, K_a and F reported for the published model were fixed to zero to reduce model flexibility. The exclusion of BOVs does not significantly affect model performance if drug concentrations from more than one dosing occasion are available [39].

Body weight was implemented as a covariate on CL/F and Q/F with an allometric coefficient of 0.75 as previously reported [40]. Body weight was also implemented as a covariate on V_2/F and V_3/F with an allometric coefficient of 1 [40]. Median weight (73.5 kg) reported in the published study [38] was employed for this parameter-covariate relationship. The published model was extended to quantify the effect of concomitant azole antifungal therapy on tacrolimus CL/F.

The final stable model with a successful variance-covariance ($COV) step output, lowest objective function value (OFV), good visual inspection of goodness of fit (GOF) plots and good predictive ability with prediction-corrected visual predictive check (pcVPC; stratified based on concomitant azole antifungal use) was selected as the updated model. This updated model was carried forward to assess for outliers.

Outliers

Outliers were identified following the development of the updated model using the absolute value of conditional weighted residuals (CWRES) criteria, |CWRES|>5. Sensitivity analysis was performed to determine outliers' influence on tacrolimus pharmacokinetic parameter estimates. If tacrolimus pharmacokinetic parameter estimates were similar with and without the inclusion of the outliers, the outliers were retained in the model building dataset. Otherwise, the outliers were excluded from the model building dataset. If removal of outliers was required, the updated model with tacrolimus pharmacokinetic parameters and their variability estimated after the outliers were removed from the model building dataset was carried forward to the next stage of covariate analysis.

Inclusions of covariates using stepwise covariate modelling analysis

All biologically plausible covariates (age, ethnicity, days post-transplant, days of tacrolimus therapy, CYP3A5 genotype, ABCB1 genotype, haematocrit, albumin, aspartate aminotransferase, and alkaline phosphatase) influencing tacrolimus CL/F were included for covariate testing. Serum creatinine concentration and creatinine clearance were excluded as <1% of tacrolimus is renally excreted [41]. Given the small number of transplant recipients with *CYP3A4**1/*22 allele (n=2), CYP3A4 genotype was not tested. The dose-normalised tacrolimus concentrations were similar across different sex (sex effect is usually embedded with the total body weight effect) [42-44] and diabetes status [45] (Figure 20) hence, they were also not tested.

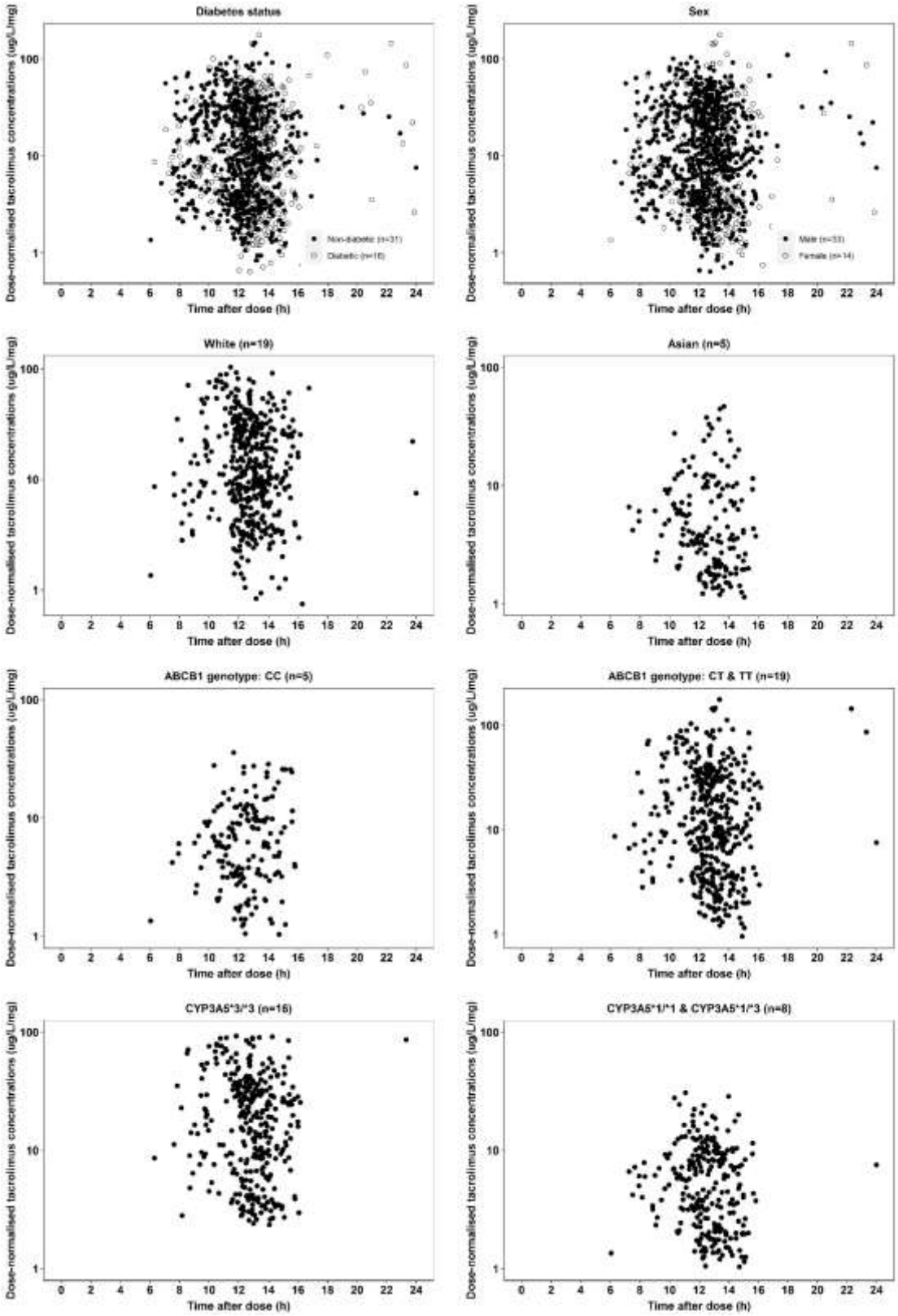


Figure 20. Dose-normalised tacrolimus concentrations against time after dose in heart transplant recipients stratified based on sex, diabetes status, ethnicity (White and Asian), ABCB1 genotype (CC, CT, and TT) and CYP3A5 genotype (*1/*1, *1/*3, and 3/*3). Y-axis is on a log10 scale; n = the number of heart transplant recipients.

The covariates were tested on the updated model for significance on tacrolimus CL/F using the stepwise covariate modelling (SCM) application in Perl-speaks-NONMEM® (PsN; version 5.0.0). The model was subjected to a forward inclusion step with a statistical significance criterion of α = 0.01 and a backward elimination step with a statistical significance criterion of α = 0.001 (Appendix 11). Categorical covariates were included using a linear function and continuous covariates using the power function. The covariate(s) that significantly ($p<0.05$) reduced the OFV, had successful $COV step output and for which the parameter-covariate relationship was biologically plausible were included in the final model. The final model was subjected to visual inspection of the GOF plots and predictive ability with pcVPC (stratified based on concomitant azole antifungal use).

Goodness of fit (GOF) plots

Plots of observed concentrations versus population predictions/individual predictions by the updated and final model were evaluated for randomness around the line of unity. Plots of CWRES versus time after dose/population predictions were evaluated for randomness around the zero line.

Prediction-corrected visual predictive check (pcVPC)

The pcVPC was performed using PsN. Graphics, overlaying 5th, median (50th), and 95th percentiles of the observed tacrolimus concentrations on top of the model-simulated concentration-time profiles with 95% prediction intervals based on 1,000 simulations using each model, were generated. If the model adequately describes the data, the 5th,

50th and 95th percentile of the simulated tacrolimus concentration-time profiles should overlay with the 5th, 50th and 95th percentile of the observed tacrolimus concentrations.

Shrinkage

If individual random effects (ETA) CL/F of the updated and final model displayed a reasonable shrinkage value (≤30%), the distribution of ETAs was checked to ensure normal distribution.

5.4.5.2 External evaluation

The final model was tested on an external evaluation dataset using the Bayesian approach (MAXEVAL=0). One to three previous dosing occasions for which an observed tacrolimus concentration was available were used to predict the individual predicted tacrolimus concentration of the fourth dosing occasion. That is, the (1) third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion, (2) second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion, and (3) first, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion. Of note, tacrolimus concentrations in the fourth dosing occasion were blinded to the model (MDV=1, EVID=2). Additionally, *a posteriori* (general model fit) was performed to determine model prediction of tacrolimus concentrations in the fourth dosing occasion (not blinded to the model; MDV=0, EVID=0). *A posteriori* was performed to determine the ability of the model to inform about past drug exposure. Regardless of concomitant azole antifungal use, the prediction of tacrolimus concentrations in the fourth dosing occasion by the model was performed for (1) all heart transplant recipients in the

unstable post-transplant period (immediately post-transplant) and (2) those who were still receiving continuation of care at SVHS in the stable post-transplant period (≥90 days post-transplant), for each of the evaluation settings described above.

The bias (median percentage predictive error, Equation 1) and imprecision (median absolute percentage predictive error, Equation 2) were calculated by comparing individual predicted tacrolimus concentrations of the fourth dosing occasion by the model to the observed concentrations [46, 47] for each of the evaluation settings.

$$\text{Bias (\%)} = \text{Median} \sum \frac{(Individual\ predicted - Observed)}{Observed} \times 100 \qquad \text{(Equation 1)}$$

$$\text{Imprecision (\%)} = \text{Median} \sum \frac{|\ Individual\ predicted - Observed|}{Observed} \times 100 \qquad \text{(Equation 2)}$$

A positive or negative bias represents an over- or under-prediction of tacrolimus concentrations by the model, respectively. Clinically acceptable bias was defined as within ±20%. The ±20% cut off corresponds to the 80−125% bioequivalence criterion [48] that suggests differences in systemic drug exposure up to 20% are not clinically significant. Moreover, the prediction was considered unbiased if the 95% confidence interval (CI) included zero. Clinically acceptable imprecision was ≤20%. The 95% CI of the median bias and imprecision was calculated via non-parametric bootstrapping of 1,000 datasets using DescTools package (version 0.99.30) in R software. Model performance was considered high when bias and imprecision are close to zero.

5.5 Results

5.5.1 Characteristics of the heart transplant recipients

Data from 87 heart transplant recipients were collected for model building (n=47; transplant received in 2018) and evaluation (n=40; transplant received in 2017) [Table 15 and 16]. None of the transplant recipients had cystic fibrosis. All heart transplant recipients received oral itraconazole (200 mg 12-hourly) immediately post-transplantation as prophylaxis against *Aspergillus* fungal infection. The antifungal therapy was continued for up to 6 months post-transplantation unless contraindicated due to deranged liver enzymes. In the model building dataset, during azole antifungal therapy, eight heart transplant recipients were switched from itraconazole to a different azole antifungal (fluconazole; n=5, voriconazole; n=3) based on the fungal culture and sensitivity results.

Table 15. Heart transplant recipient characteristics on admission and concomitant medications administered immediately post-heart transplantation.

Characteristics	**Median (range)***	
	Model building dataset (n=47)**	**External evaluation dataset (n=40)**
Male, n (%)	33 (70)	26 (65)
Age (years)	53 (16–70)	56 (16–69)
Weight (kg)	77 (40–107)	75 (45–111)
Height (cm)	175 (154–190)	175 (150–195)
Ethnicity, n (%)		
White/Caucasian	19 (40)	23 (58)
Asian	5 (11)	5 (13)
Unknown	23 (49)	12 (30)
Diabetes mellitus, n (%)	16 (34)	13 (33)
CYP3A5 (rs776746), n (%)		
*1/*1	1 (2)	0 (0)
*1/*3	7 (15)	9 (23)
*3/*3	16 (34)	20 (50)
Unknown	23 (49)	11 (28)
CYP3A4 (rs35599367), n (%)		
*1/*1	22 (47)	27 (68)
*1/*22	2 (4)	2 (5)
Unknown	23 (49)	11 (28)
CYP3A phenotype$^{\Delta}$		
Extensive metabolisers	8 (17)	7 (18)
Intermediate metabolisers	14 (30)	22 (55)
Poor metabolisers	2 (4)	0 (0)
Unknown	23 (49)	11 (27)
ABCB1 (rs1045642), n (%)		
CC	5 (11)	7 (17)
CT	12 (25)	16 (40)
TT	7 (15)	6 (15)
Unknown	23 (49)	11 (28)
Haematocrit (fraction)$^{\Delta\Delta}$	0.26 (0.21–0.38)	0.28 (0.21–0.37)

Albumin (g/L)[ΔΔ]	34 (20–43)	36 (30–45)
Serum creatinine concentration (μmol/L)[ΔΔ]	131 (39–276)	111 (70–293)
Creatinine clearance (mL/min)[ΔΔ]	63 (25–172)	64 (30–151)
Aspartate aminotransferase (U/L)[ΔΔ]	57 (14–1390)	87 (18–305)
Alkaline phosphatase (U/L)[ΔΔ]	56 (31–249)	48 (21–143)
Concomitant immunosuppressants, n (%)		
Mycophenolate mofetil	47 (100)	40 (100)
Corticosteroid	47 (100)	40 (100)
Basiliximab[⌂]	40 (85)	31 (78)
Concomitant azole antifungal, n (%)		
Itraconazole	47 (100)	40 (100)

Creatinine clearance was calculated using the Cockcroft-Gault formula [34].

*Data are presented as median (range) unless otherwise stated.

**One transplant recipient had a history of solid organ transplantation.

[Δ]CYP3A phenotype: poor metabolisers (*CYP3A4**22 carriers with the *CYP3A5**3/*3 genotype), intermediate metabolisers (*CYP3A4**22 non-carriers with the *CYP3A5**3/*3 genotype or *CYP3A4**22 carriers with the *CYP3A5**1/*1 genotype), and extensive metabolisers (*CYP3A4**22 non-carriers and *CYP3A5**1 carriers).

[ΔΔ]Just before the first tacrolimus dose.

[⌂]Intravenous basiliximab was administered as an induction therapy on the day of operation and day 4 post-transplantation to heart transplant recipients with renal impairment (to delay the commencement of tacrolimus) or those who received mechanical circulatory support (left ventricular, biventricular assist devices or total artificial hearts).

Table 16. Heart transplant recipient CYP3A4, CYP3A5 and ABCB1 genotypes stratified based on ethnicity.

Dataset	Ethnicity	CYP3A5			CYP3A4		ABCB1		
		*3/*3	*1/*3	*1/*1	*1/*1	*1/*22	CC	CT	TT
Model building	Asian* (n=5)	1	3	1	5	0	2	3	0
	White/ Caucasian (n=19)	15	4	0	17	2	3	9	7
External evaluation [Δ]	Asian** (n=5)	5	0	0	5	0	0	4	1
	White/ Caucasian (n=23)	15	8	0	21	2	7	11	5

*South-East Asian (n=1), North-East Asian (n=3), Southern and Central Asian (n=1).

**South-East Asian (n=1), North-East Asian (n=2), Southern and Central Asian (n=2).

[Δ]Ethnicity was not available for one transplant recipient with genotype information.

5.5.2 Model building dataset

A total of 1100 tacrolimus concentrations, including 759 with concomitant azole antifungal (itraconazole=715, voriconazole=23, fluconazole=21) and 341 without concomitant azole antifungal, collected between 2- and 391-days post-heart transplantation were used for model building. Most (58%; 643/1100) tacrolimus concentrations were collected in the outpatient setting with the remaining concentrations obtained whilst an inpatient. The median (range) number of tacrolimus concentrations collected per transplant recipient was 21 (1−61). The median (range) time after tacrolimus dose administration was 12.6 (6.05–93.62) hours.

The median (range) tacrolimus dose administered by heart transplant recipients while receiving concomitant azole antifungal therapy was 0.50 mg 12-hourly (0.05 mg to 8.00 mg 12-hourly) and without concomitant azole antifungal therapy was 3.00 mg 12-hourly (0.25 mg to 12.00 mg 12-hourly). Dose-normalised tacrolimus concentrations were higher when transplant recipients were receiving concomitant azole antifungal therapy (Figure 21).

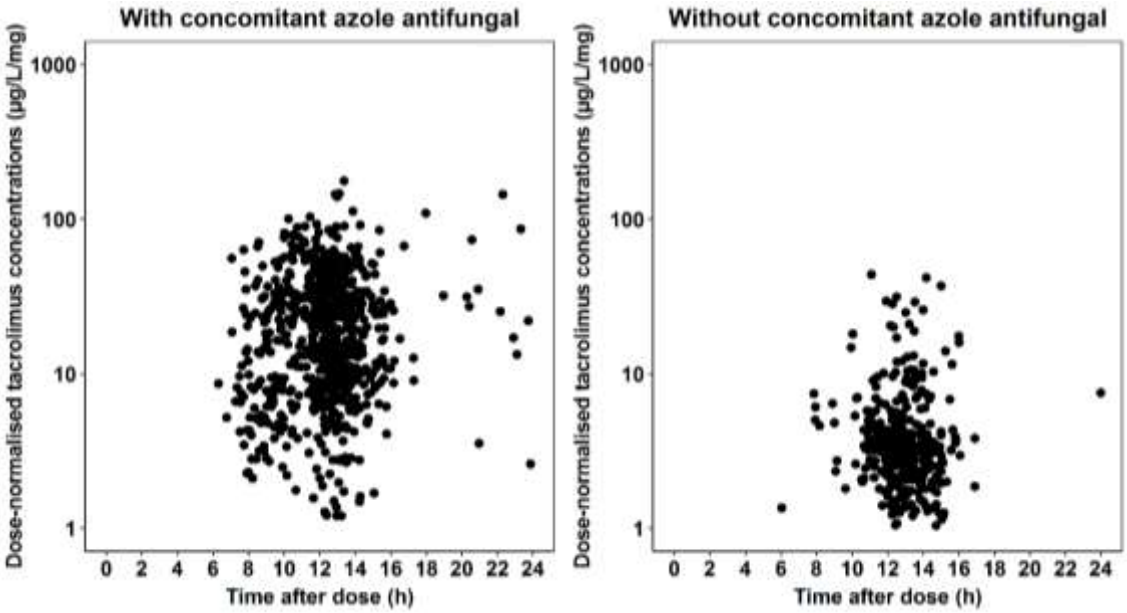


Figure 21. Dose-normalised tacrolimus concentrations against time after dose in heart transplant recipients (n=47), with concomitant azole antifungal therapy (759 tacrolimus concentrations) and without concomitant azole antifungal therapy (341 tacrolimus concentrations). Y-axis is on a log10 scale. The infrequent drug monitoring 6 months post-transplant, transfer of care to other healthcare facilities, conversion to the extended-release formulation of tacrolimus, and death explains the reduced number of tacrolimus concentrations available for analysis for the period of study following the cessation of azole antifungal therapy. The delay in tacrolimus concentration collection up to 16 hours after the last administered dose was due to the busy clinical environment and critical condition of heart transplant recipients. Some tacrolimus doses were withheld due to supratherapeutic tacrolimus concentration(s) or acute kidney injury. Due to these clinical conditions, tacrolimus concentrations are monitored daily until the condition resolves, explaining the reported time after tacrolimus dose of greater than 16 hours.

5.5.3 Population pharmacokinetic analysis

5.5.3.1 Outliers

Only one tacrolimus concentration had a |CWRES|>5 (Appendix 12). The exclusion of this concentration did not influence tacrolimus pharmacokinetic parameter estimates (Appendix 13). Consequently, this tacrolimus concentration was retained in the model building dataset.

5.5.3.2 Identification of significant covariates following stepwise covariate modelling analysis

Haematocrit was identified as a covariate influencing tacrolimus CL/F with an OFV reduction of 33 (degree of freedom = 1, p=1.44E-14) [Table 17]. The inverse correlation between haematocrit and tacrolimus CL/F (-0.89) was biologically plausible and was included in the final model.

Table 17. Pharmacokinetic parameter estimates and covariate relationships of the published, updated, and final population pharmacokinetic model for tacrolimus in heart transplant recipients.

Parameter	Unit	Published model$^{\Delta}$	Updated model$^{\Delta}$	Final model$^{\Delta}$
		Population mean estimate (RSE)		
K_a	h^{-1}	0.579 (78%)	0.282 (15%)	0.273 (14%)
F	-	1*	1*	1*
V_2/F	L	231 (41%)	231 (0%)	231 (0%)
CL/F (without azole)	L/h	19.6 (48%)	16.4 (13%)	18.6 (14%)
CL/F (with azole)	L/h	-	3.6 (12%)	3.7 (13%)
Q/F	L/h	58.2 (47%)	58.1 (1%)	58.1 (1%)
V_3/F	L	521 (52%)	521 (0%)	521 (0%)
Haematocrit effect on CL/F	-	-	-	-0.89 (17%)
		CV (RSE)[shrinkage]		
BSV CL/F (without azole)	%	35.7 (99%)	78.7 (16%)[25%]	83.1 (17%)[24%]
BSV CL/F (with azole)	%	-	93.0 (12%)[8%]	97.0 (12%)[8%]
BOV CL/F	%	30.2 (86%)	0*	0*
BOV K_a	%	127.6 (57%)	0*	0*
BOV F	%	59.4 (40%)	0*	0*
BOV V_2/F	%	36.2 (84%)	0*	0*
Proportional RUV	%	14 (13%)	-	-
Exponential RUV	%	-	46 (2%)	46 (2%)
OFV	-	-	-420	-453
Conditional number	-	-	1.5	1.7

The approximate percentage coefficient of variation (CV) was reported as $(\sqrt{\exp(\text{omega})^2}-1)*100$.

*Fixed value.

$^{\Delta}$Published model: Model by Sikma *et al*. [38]. Updated model: Final stable model after the inclusion of the effect of concomitant azole antifungal use and body weight on tacrolimus pharmacokinetics. Final model: Final stable model after the inclusion of haematocrit following stepwise covariate modelling.

BSV Between-subject variability*; BOV* Between-occasion variability*; CL/F* Apparent clearance*; F* Bioavailability*; K_a* Absorption rate constant; *OFV* Objective function value; *Q/F* Apparent intercompartmental clearance; *RSE* Relative standard error; *RUV* Residual unexplained variability; *V_2/F* Apparent central volume of distribution; *V_3/F* Apparent peripheral volume of distribution.

5.5.3.3 Final model

The pharmacokinetic parameter estimates of the final model were similar to the values of the published model except for K_a (0.579 vs. 0.273 h^{-1}; published vs. final model) [Table 17]. Body weight was successfully implemented as a covariate on CL/F, Q/F, V_2/F and V_3/F based on the reported allometry scale [40]. Concomitant azole antifungal therapy reduced tacrolimus CL/F by 80%. Between-subject variability (BSV) on CL/F was estimated both with (97%) and without azole antifungal therapy (83%). The BSV on CL/F displayed a reasonable shrinkage value (<25%). The ETA (BSV) CL/F distribution without concomitant azole antifungal therapy of the final model was minimally skewed (Appendix 14). An exponential residual unexplained variability (RUV, 46%) best described residual variability. Comparison of the observed and individual predicted tacrolimus concentrations by the final model (including the updated model) did not show significant misspecification or bias (Appendix 12). The pcVPC plots showed a good model fit to the dataset (Figure 22). The final tacrolimus model pharmacokinetic parameter estimates and covariate relationships (Appendix 15) are described below:

$CL/F = 18.6 * (WT/73.5)^{0.75} * (HCT/0.31)^{-0.89}$ L/h (without concomitant azole antifungal)

$CL/F = 3.7 * (WT/73.5)^{0.75} * (HCT/0.31)^{-0.89}$ L/h (with concomitant azole antifungal)

$V_2/F = 231 * (WT/73.5)$ L

$Q/F = 58.1 * (WT/73.5)^{0.75}$ L/h

$V_3/F = 521 * (WT/73.5)$ L

HCT Haematocrit*; WT* Body weight.

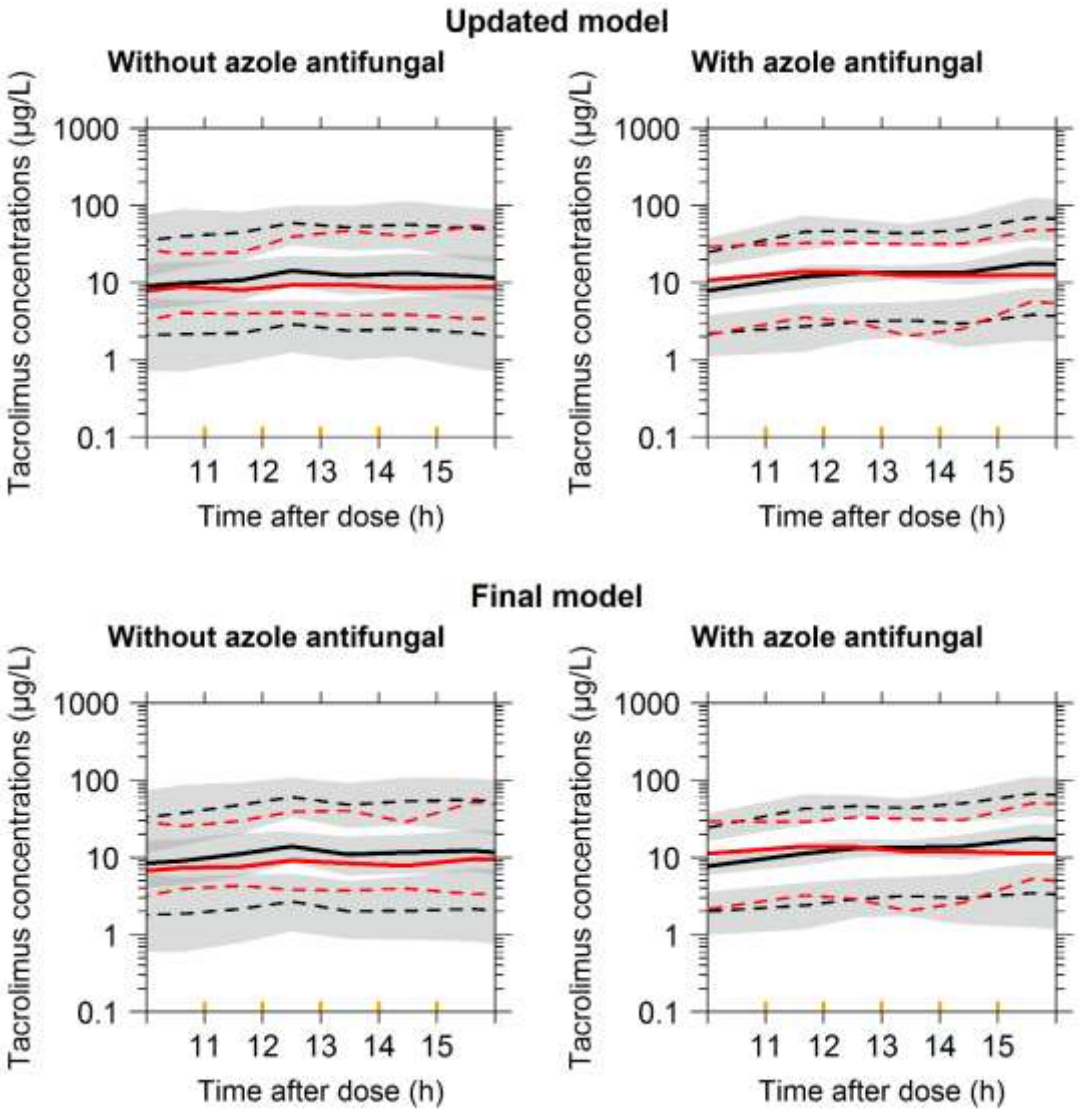


Figure 22. Prediction-corrected visual predictive check (pcVPC) plots for the updated and final population pharmacokinetic model for tacrolimus. Y-axis is on a log10 scale. The median observed (solid red line) and predicted (solid black line), 5th and 95th percentiles of the observations (dashed red line), corresponding prediction percentiles (dashed black line) and 95% prediction intervals for the predicted percentiles (grey band) are shown. The vertical dashes at the bottom of the plots separate the bins.

5.5.3.4 External evaluation

The external evaluation dataset included 40 heart transplant recipients and 348 tacrolimus concentrations (87 concentrations for each of the four dosing occasions) collected between 2- and 254-days post-heart transplantation. The final model demonstrated clinically acceptable individual predicted tacrolimus concentrations of the fourth dosing occasion relative to the observed concentrations irrespective of post-transplantation periods (stable/unstable) and number (1−3) of prior dosing occasions (Figure 23, Appendix 16, Appendix 17, and Appendix 18). Regardless of the number of prior dosing occasions (1−3), the bias of the individual predicted tacrolimus concentration of the fourth dosing occasion relative to the observed concentrations ranged from -1.3% to 0.6% and the 95% CI of bias included zero (Figure 23). The imprecision ranged between 11% and 13%. With the inclusion of tacrolimus concentrations in the fourth dosing occasion (*a posteriori*), model prediction of the fourth dosing occasion relative to the observed concentrations was clinically acceptable [bias: 0.4% (95% CI: -2.79 to 4.61); imprecision: 9.4% (95% CI: 5.92 to 11.47)].

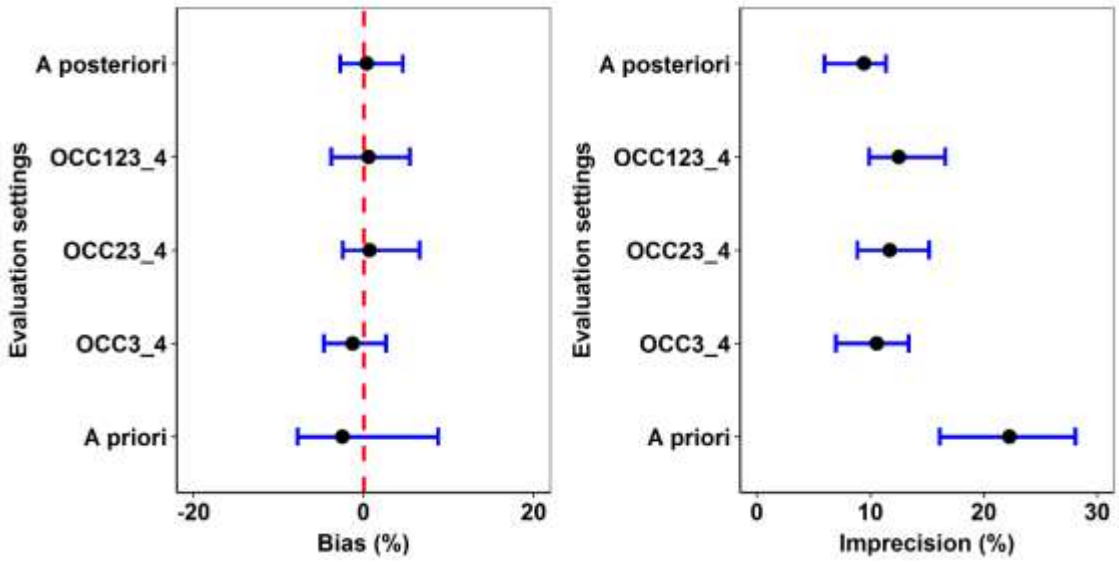


Figure 23. The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion by the final tacrolimus population pharmacokinetic model relative to the observed concentrations (40 transplant recipients, 87 dosing occasions were predicted across stable and unstable post-transplantation periods). The panel on the left shows the median bias and its 95% CI. The panel on the right shows the median imprecision and its 95% CI. *A priori* model prediction based on population pharmacokinetic parameter estimates, covariates, and dosing information; *A posteriori* model prediction based on the inclusion of tacrolimus concentrations in the fourth dosing occasion; *OCC3_4* Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

5.6 Discussion

A previously published tacrolimus population pharmacokinetic model provided a platform to establish a model for use in heart transplant recipients, considering the drug-drug interaction with azole antifungals. The model developed included important time-varying covariates such as body weight and haematocrit. External evaluation using the Bayesian approach indicated one recent tacrolimus concentration is sufficient for the model to adequately predict an individual's tacrolimus pharmacokinetic parameters. Overall, the model is suitable to guide tacrolimus dosing decisions in heart transplant recipients. To the best of our knowledge, this is the first population pharmacokinetic model to comprehensively describe tacrolimus pharmacokinetics in heart transplant recipients, considering the clinically significant tacrolimus-azole antifungal interaction, over one-year post-transplantation.

Only pre-dose tacrolimus concentrations were available for model building. These data are suitable to describe the elimination phase of tacrolimus but not the absorption or distribution phase. Therefore, tacrolimus CL/F was estimated independently from the published model. The estimated CL/F (without concomitant azole antifungal therapy) of the final model, 0.25 L/h/kg was similar to that reported previously [33, 49] in heart transplant recipients (0.21−0.22 L/h/kg). Although a prior value for K_a was implemented, the estimated K_a of the final model differed from the published model. The reason for this is unclear as ideally, the values should be similar. The exponential RUV was high (46%). This reflects the use of tacrolimus concentrations obtained from routine clinical monitoring as opposed to prospective studies where drug administration and blood sample collection is protocolised. Further, given that all BOVs were fixed to zero, any

variability in these values was distributed to BSV on CL/F and exponential RUV. Given that the oral bioavailability of tacrolimus is highly variable (5−93%) [50, 51], the BSV on tacrolimus CL/F could potentially reflect variability in bioavailability rather than CL [44, 52]. Limited tacrolimus concentrations were available following intravenous tacrolimus administration, hence the BSV in CL and bioavailability could not be separated.

Biologically plausible relationships between tacrolimus concentrations and (1) body weight and (2) concomitant azole antifungal use were incorporated in the updated model prior to performing SCM. The CL/F, Q/F, V_2/F and V_3/F are all weight-dependent pharmacokinetic parameters and therefore, body weight was included in the model based on the reported allometry scale [40]. For heavier individuals, there is an increase in extracellular fluid and they have a larger body surface area available for drug distribution [53].

Azole antifungals have demonstrated a clinically significant pharmacokinetic interaction with tacrolimus [21, 22, 25-29]. There is no standard practice for adjusting tacrolimus doses upon initiation or cessation of concomitant azole antifungal therapy [30]. One should be cautious when (1) initiating azole antifungal therapy as systemic tacrolimus concentrations may increase profoundly, leading to toxicity and (2) withdrawing azole antifungals as tacrolimus concentrations may decrease rapidly, leading to acute graft rejection. Considering this significant drug-drug interaction, the effect of concomitant itraconazole was quantified on tacrolimus CL/F. The reduction of tacrolimus CL/F by 80% (BSV with concomitant azole antifungal therapy = 97% vs. without concomitant azole antifungal therapy = 83%) is consistent with a previous study suggesting a 70-80%

tacrolimus dose reduction is required when initiating itraconazole [20]. The estimated CL/F with concomitant itraconazole therapy of the final model was 0.05 L/h/kg. This is lower than that reported previously in heart transplant recipients receiving concomitant voriconazole (0.08 L/h/kg) and or fluconazole (0.13 L/h/kg) [33]. This difference is consistent with the potency of inhibition of CYP3A4 enzymes and P-glycoproteins where itraconazole is a more potent inhibitor, followed by voriconazole and then, fluconazole [31, 54-56]. Of note, although tacrolimus concentrations obtained during concomitant fluconazole and voriconazole were included during model building, these drugs were co-administered briefly (not as prophylaxis therapy) and accounted for only 3% of tacrolimus concentrations. Hence, the inclusion of this pharmacokinetic data is unlikely to influence the reported tacrolimus pharmacokinetic parameter values.

Consistent with a recent review [18], haematocrit was found to be an important clinical determinant of tacrolimus CL/F. The correlation quantified (-0.89) was congruent with the value reported in a recent model developed using data from lung transplant recipients (-0.87) [57]. There is an inverse correlation between haematocrit and tacrolimus CL/F. In blood, tacrolimus is extensively bound to erythrocytes (85–95%) [1, 58]. If the haematocrit concentration is low, a reduced fraction of tacrolimus is bound to erythrocytes, enabling the drug to be readily metabolised by the liver [59, 60]. Hence, tacrolimus CL/F will be higher when haematocrit concentrations are low and vice-versa. Days post-transplant was not identified as a significant covariate despite previous studies [18] identifying this as an important time-varying covariate influencing tacrolimus CL/F. This finding is likely because the effect of days post-transplant is confounded by haematocrit as haematocrit concentrations vary over time. Interestingly,

although tacrolimus is predominantly metabolised by the liver, hepatic impairment (assessed by aspartate aminotransferase and alkaline phosphatase concentrations) was not identified as a significant covariate. This is despite the large range of aspartate aminotransferase and alkaline phosphatase concentrations reported. By contrast, either aspartate aminotransferase or alkaline phosphatase was included as a covariate affecting tacrolimus CL/F in some models developed from kidney and liver transplant recipients [3, 4, 61-67]. Ethnicity, CYP3A5 genotype, CYP3A4 genotype, and ABCB1 genotype are known to influence tacrolimus CL/F [10, 18, 61, 68]. Appropriate distribution of these variables within the population is important to accurately describe the final ethnicity/allele variant relationship to pharmacokinetic endpoints in a model [44]. Ethnicity, CYP3A5 genotype, and ABCB1 genotype data were unavailable in approximately 50% of the heart transplant recipients used for model building. This could have influenced the selection of covariates during SCM. Given the small number of transplant recipients with *CYP3A4**1/*22 allele (n=2), CYP3A4 genotype was unable to be tested as a covariate. Prednisolone doses were not well documented in the clinical notes. Given that steroids are known to induce CYP3A and ABCB1 expression [69, 70], it would have been valuable to assess the influence of this covariate on tacrolimus pharmacokinetics.

The population pharmacokinetic model-based Bayesian estimation recommends an optimal drug dose regimen following estimation of individual pharmacokinetic parameters using patient-specific characteristics (covariates) and drug concentration(s). Generally, drug concentration data are more informative than covariate data to enable a model to accurately predict individual pharmacokinetic parameters [12, 37, 71].

Consistent with this, model predictions improved with at least one tacrolimus concentration relative to no drug concentration (*a priori*) included in the model. Given that tacrolimus displays large pharmacokinetic variability, it is essential to include at least one tacrolimus concentration into the model to facilitate dose individualisation. Consistent with a recent study in hematopoietic stem cell transplant recipients [72], the present study suggests one recent tacrolimus concentration (obtained within 1−37 days) is sufficient for the model to accurately guide tacrolimus dosing. The requirement for a recent concentration also reflects the variation in tacrolimus pharmacokinetics over time. Using a recent tacrolimus concentration to predict individual pharmacokinetic parameters prevents the introduction of bias from BSV from additional historical drug concentrations [73]. Consequently, the number or frequency of tacrolimus concentration collection does not influence the accuracy of model prediction. This suggests that once transplant recipients are stable, less frequent monitoring of tacrolimus is acceptable if the model is used as an adjunct to inform tacrolimus dosing decisions.

5.7 Limitations

Due to the retrospective nature of the study, the time of tacrolimus administration in the outpatient setting cannot be guaranteed. This may impact the accuracy of data used for model building and evaluation.

5.8 Conclusions

A population pharmacokinetic model for tacrolimus in heart transplant recipients was successfully developed. The model incorporated the effects of body weight,

concomitant azole antifungal use, and haematocrit on tacrolimus pharmacokinetics. Mimicking clinical practice, the model was able to accurately predict individual tacrolimus pharmacokinetic parameters throughout the first-year post-heart transplantation. One recent tacrolimus concentration is sufficient for the model to inform tacrolimus dosing decisions suggesting less frequent monitoring in stable heart transplant recipients is possible. Prospective evaluation is required to access the clinical utility of the model to guide tacrolimus dose individualisation in heart transplant recipients and the potential impact of this approach on not only the attainment of target drug exposure but also the incidence of tacrolimus toxicity and/or organ rejection.

5.9 Key points

- A model that comprehensively described tacrolimus pharmacokinetics in heart transplant recipients, considering tacrolimus-azole antifungal interaction, over one-year post-transplantation was developed.
- The model was externally evaluated and is suitable to facilitate dose individualisation in heart transplant recipients using one recent tacrolimus concentration.

5.10 Acknowledgements

We would like to thank (a) The Australian Centre of Pharmacometrics for the NONMEM® software license, (b) Clinical Pharmacology laboratory, Sydpath, St. Vincent's Hospital Sydney for access to tacrolimus monitoring request forms, (c) Heart transplant clinic, St. Vincent's Hospital Sydney for access to transplant recipient medical records, (d) Dr Ooi Qing Xi (Pharmetheus, Sweden), Dr Shaun Kumar (Parexel, Australia) and Dr Yuwei Lin (Certara®, Australia) for providing valuable input on the pharmacometric analysis, (e)

Maaike A Sikma (Netherlands) for sharing the NONMEM model code, and (f) myDNA Life Australia Pty Ltd for performing pharmacogenomic analysis. Ms Ranita Kirubakaran is a recipient of the Faculty of Medicine and Health, University of New South Wales, Sydney, Doctoral Scholarship funded by the Australian Government, and St. Vincent's Centre for Applied Medical Research Student Scholarship Supplementation Scheme.

5.11 Funding

This work was supported by a National Health and Medical Research Council (NHMRC) APP1054146 Program Grant; Creating safe, effective systems of care: the translational challenge.

5.12 Conflict of interest

Ranita Kirubakaran, David W. Uster, Stefanie Hennig, Richard O. Day, Jane E. Carland, Sebastian G. Wicha and Sophie L. Stocker have no conflicts of interest to declare that are relevant to the content of this article.

5.13 Author Contributions

RK, DWU, SLS, SH and SGW designed the study. RK performed data collection and manuscript writing. RK and DWU performed pharmacometric analysis. All authors were involved in the interpretation of data and critically reviewed, edited, and approved the final manuscript.

References

1. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

2. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

3. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

4. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

5. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

6. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

7. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

8. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

9. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

10. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit. 2019;41(3):261-307.

11. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus Therapy in Adult Heart Transplant Recipients: Evaluation of a Bayesian Forecasting Software. Ther Drug Monit. 2021;43(6):736-46.

12. Sheiner LB, Beal S, Rosenberg B, Marathe VV. Forecasting individual pharmacokinetics. Clin Pharmacol Ther. 1979;26(3):294-305.

13. del Mar Fernández de Gatta M, Martin-Suarez A, Lanao JM. Approaches for dosage individualisation in critically ill patients. Expert Opin Drug Metab Toxicol. 2013;9(11):1481-93.

14. Meinshausen J, Bühl K, Engel G, Harings-Kaim A, Drewelow B, Klotz U. Generation of pharmacokinetic data during routine therapeutic drug monitoring: Bayesian approach vs. pharmacokinetic studies. Ther Drug Monit. 1993;15(4):281-8.

15. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

16. Fukudo M, Yano I, Shinsako K, Katsura T, Takada Y, Uemoto S, et al. Prospective evaluation of the bayesian method for individualizing tacrolimus dose early after living-donor liver transplantation. J Clin Pharmacol. 2009;49(7):789-97.

17. Francke MI, Andrews LM, Le HL, van de Wetering J, Clahsen-van Groningen MC, van Gelder T, et al. Avoiding Tacrolimus Underexposure and Overexposure with a Dosing Algorithm for Renal Transplant Recipients: A Single Arm Prospective Intervention Trial. Clin Pharmacol Ther. 2021;110(1):169-78.

18. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review. Clin Pharmacokinet. 2020;59(11):1357-92.

19. Kirubakaran R, Hennig S, Maslen B, Day RO, Carland JE, Stocker SL. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients. Br J Clin Pharmacol. 2021.

20. Darley D, Carlos L, Hennig S, Glanville A. Quantitation of the Effect of Azole Antifungals on Tacrolimus Clearance. J Heart Lung Transplant. 2016;35(4):S236.

21. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

22. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

23. Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, et al. Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. Clin Pharmacol Ther. 1997;62(1):41-9.

24. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

25. Osowski CL, Dix SP, Lin LS, Mullins RE, Geller RB, Wingard JR. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. Transplantation. 1996;61(8):1268-72.

26. Mañez R, Martin M, Raman V, Silverman D, Jain A, Warty V, et al. Fluconazole therapy in transplant recipients receiving FK506. Transplantation. 1994;57(10):1521-3.

27. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic evaluation of the drug interaction between intravenous itraconazole and intravenous tacrolimus or intravenous cyclosporin A in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2006;12(3):325-34.

28. Sansone-Parsons A, Krishna G, Martinho M, Kantesaria B, Gelone S, Mant TG. Effect of oral posaconazole on the pharmacokinetics of cyclosporine and tacrolimus. Pharmacotherapy. 2007;27(6):825-34.

29. Shitrit D, Ollech JE, Ollech A, Bakal I, Saute M, Sahar G, et al. Itraconazole prophylaxis in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. J Heart Lung Transplant. 2005;24(12):2148-52.

30. Gu TM, Lewis JS, Le H, Bubalo JS. Comparative effects of fluconazole, posaconazole, and isavuconazole upon tacrolimus and cyclosporine serum concentrations. J Oncol Pharm Pract. 2021:10781552211029046.

31. Trofe-Clark J, Lemonovich T, Practice AIDCo. Interactions between anti-infective agents and immunosuppressants in solid organ transplantation. Am J Transplant. 2013;13(s4):318-26.

32. Moreno M, Latorre A, Manzanares C, Morales E, Herrero J, Dominguez-Gil B, et al. Clinical management of tacrolimus drug interactions in renal transplant patients. Transplant Proc. 1999;31(6):2252-3.

33. Zhou H, Cai J, Huang J, Zhang J, Shi SJ, Liu YN, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: A population pharmacokinetic approach. Pharmacogenomics. 2019;20(1):21-35.

34. Cockcroft DW, Gault H. Prediction of creatinine clearance from serum creatinine. Nephron. 1976;16(1):31-41.

35. Beal SL. Ways to fit a PK model with some data below the quantification limit. J Pharmacokinet Pharmacodyn. 2001;28(5):481-504.

36. Anna H-X, Calvier EA, Fabre D, Gattacceca F, Khier S. Prior information for population pharmacokinetic and pharmacokinetic/pharmacodynamic analysis: overview and guidance with a focus on the NONMEM PRIOR subroutine. J Pharmacokinet Pharmacodyn. 2020(47):1-16.

37. Decrocq-Rudler M-A, Meunier L, Fraisse J, Ursic-Bedoya J, Khier S. Can We Predict Individual Concentrations of Tacrolimus After Liver Transplantation? Application and

Tweaking of a Published Population Pharmacokinetic Model in Clinical Practice. Ther Drug Monit. 2021;43(4):490-8.

38. Sikma MA, Hunault CC, Van Maarseveen EM, Huitema AD, Van de Graaf EA, Kirkels JH, et al. High variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Eur J Drug Metab Pharmacokinet. 2020;45(1):123-34.

39. Abrantes JA, Jönsson S, Karlsson MO, Nielsen EI. Handling interoccasion variability in model-based dose individualization using therapeutic drug monitoring data. Br J Clin Pharmacol. 2019;85(6):1326-36.

40. Anderson BJ, Holford NH. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol. 2008;48:303-32.

41. Möller A, Iwasaki K, Kawamura A, Teramura Y, Shiraga T, Hata T, et al. The disposition of 14C-labeled tacrolimus after intravenous and oral administration in healthy human subjects. Drug Metab Dispos. 1999;27(6):633-6.

42. Fitzsimmons W, Bekersky I, Dressler D, Raye K, Hodosh E, Mekki Q. Demographic considerations in tacrolimus pharmacokinetics. Transplant Proc. 1998;4(30):1359-64.

43. Press RR, Ploeger BA, den Hartigh J, van der Straaten T, van Pelt J, Danhof M, et al. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Ther Drug Monit. 2009;31(2):187-97.

44. Campagne O, Mager DE, Tornatore KM. Population pharmacokinetics of tacrolimus in transplant recipients: what did we learn about sources of interindividual variabilities? J Clin Pharmacol. 2019;59(3):309-25.

45. Marquet P, Albano L, Woillard JB, Rostaing L, Kamar N, Sakarovitch C, et al. Comparative clinical trial of the variability factors of the exposure indices used for the drug monitoring of two tacrolimus formulations in kidney transplant recipients. Pharmacol Res. 2018;129:84-94.

46. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm. 1981;9(4):503-12.

47. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J Pharmacokinet Phar. 1982;10(2):229-.

48. Holford NH, Buclin T. Safe and effective variability—a criterion for dose individualization. Ther Drug Monit. 2012;34(5):565-8.

49. Gong Y, Yang M, Sun Y, Li J, Lu Y, Li X. Population pharmacokinetic analysis of tacrolimus in Chinese cardiac transplant recipients. Eur J Hosp Pharm. 2020;27(e1):e012-18.

50. Venkataramanan R, Jain A, Warty V, Abu-Elmagd K, Alessiani M, Lever J, et al. Pharmacokinetics of FK 506 in transplant patients. Transplant Proc. 1991;23(6):2736-40.

51. Jain A, Venkataramanan R, Todo S, Abu-Elmagd K, Fung J, Warty V, et al. Intravenous, oral pharmacokinetics, and oral dosing of FK 506 in small bowel transplant patients. Transplant Proc. 1992;24(3):1181-2.

52. Jusko WJ, Piekoszewski W, Klintmalm GB, Shaefer MS, Hebert MF, Piergies AA, et al. Pharmacokinetics of tacrolimus in liver transplant patients. Clin Pharmacol Ther. 1995;57(3):281-90.

53. Brooks E, Tett SE, Isbel NM, Staatz CE. Population pharmacokinetic modelling and Bayesian estimation of tacrolimus exposure: is this clinically useful for dosage prediction yet? Clin Pharmacokinet. 2016;55(11):1295-335.

54. Zhang S, Pillai VC, Mada SR, Strom S, Venkataramanan R. Effect of voriconazole and other azole antifungal agents on CYP3A activity and metabolism of tacrolimus in human liver microsomes. Xenobiotica. 2012;42(5):409-16.

55. Kramer MR, Amital A, Fuks L, Shitrit D. Voriconazole and itraconazole in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. Clin Transplant. 2011;25(2):E163-7.

56. Kuypers DR, de Jonge H, Naesens M, Vanrenterghem Y. Effects of CYP3A5 and MDR1 single nucleotide polymorphisms on drug interactions between tacrolimus and fluconazole in renal allograft recipients. Pharmacogenet Genomics. 2008;18(10):861-8.

57. Cai X, Song H, Jiao Z, Yang H, Zhu M, Wang C, et al. Population pharmacokinetics and dosing regimen optimization of tacrolimus in Chinese lung transplant recipients. Eur J Pharm Sci. 2020;152:105448.

58. Zahir H, Nand R, Brown K, Tattam B, McLachlan A. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. J Pharmacol Toxicol Methods. 2001;46(1):27-35.

59. Benkali K, Premaud A, Picard N, Rerolle JP, Toupance O, Hoizey G, et al. Tacrolimus population pharmacokinetic-pharmacogenetic analysis and Bayesian estimation in renal transplant recipients. Clin Pharmacokinet. 2009;48(12):805-16.

60. Zuo XC, Ng CM, Barrett JS, Luo AJ, Zhang BK, Deng CH, et al. Effects of CYP3A4 and CYP3A5 polymorphisms on tacrolimus pharmacokinetics in Chinese adult renal transplant recipients: a population pharmacokinetic analysis. Pharmacogenet Genomics. 2013;23(5):251-61.

61. Lu Z, Bonate P, Keirns J. Population pharmacokinetics of immediate- and prolonged-release tacrolimus formulations in liver, kidney and heart transplant recipients. Br J Clin Pharmacol. 2019;85(8):1692-703.

62. Golubovic B, Vucicevic K, Radivojevic D, Kovacevic SV, Prostran M, Miljkovic B. Total plasma protein effect on tacrolimus elimination in kidney transplant patients--population pharmacokinetic approach. Eur J Pharm Sci. 2014;52:34-40.

63. Antignac M, Hulot JS, Boleslawski E, Hannoun L, Touitou Y, Farinotti R, et al. Population pharmacokinetics of tacrolimus in full liver transplant patients: Modelling of the post-operative clearance. Eur J Clin Pharmacol. 2005;61(5):409-16.

64. Sam WJ, Tham LS, Holmes MJ, Aw M, Quak SH, Lee KH, et al. Population pharmacokinetics of tacrolimus in whole blood and plasma in asian liver transplant patients. Clin Pharmacokinet. 2006;45(1):59-75.

65. Valdivieso N, Oteo I, Valdivieso A, Lukas JC, Leal N, Gastaca M, et al. Tacrolimus dose individualization in" de novo" patients after 10 years of experience in liver transplantation: pharmacokinetic considerations and patient pathophysiology. Int J Clin Pharmacol Ther. 2013;51(7):606-14.

66. Oteo I, Lukas JC, Leal N, Suarez E, Valdivieso A, Gastaca M, et al. Tacrolimus pharmacokinetics in the early post-liver transplantation period and clinical applicability via Bayesian prediction. Eur J Clin Pharmacol. 2013;69(1):65-74.

67. Zhu L, Yang J, Jing Y, Zhang Y, Li G. Effects of CYP3A5 genotypes, ABCB1 C3435T and G2677T/A polymorphism on pharmacokinetics of Tacrolimus in Chinese adult liver transplant patients. Xenobiotica. 2015;45(9):840-6.

68. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623-53.

69. Lam S, Partovi N, Ting LS, Ensom MH. Corticosteroid interactions with cyclosporine, tacrolimus, mycophenolate, and sirolimus: fact or fiction? Ann Pharmacother. 2008;42(7):1037-47.

70. Tron C, Woillard J-B, Houssel-Debry P, David V, Jezequel C, Rayar M, et al. Pharmacogenetic—Whole blood and intracellular pharmacokinetic—Pharmacodynamic (PG-PK2-PD) relationship of tacrolimus in liver transplant recipients. PloS one. 2020;15(3):e0230195.

71. Cai X, Li R, Sheng C, Tao Y, Zhang Q, Zhang X, et al. Systematic external evaluation of published population pharmacokinetic models for tacrolimus in adult liver transplant recipients. Eur J Pharm Sci. 2020;145:105237.

72. Zhu J, Campagne O, Torrice CD, Flynn G, Miller JA, Patel T, et al. Evaluation of the performance of a prior tacrolimus population pharmacokinetic kidney transplant model among adult allogeneic hematopoietic stem cell transplant patients. Clin Transl Sci. 2021;14(3):908-18.

73. Zwart TC, Moes DJA, van der Boog PJ, van Erp NP, de Fijter JW, Guchelaar H-J, et al. Model-informed precision dosing of everolimus: external validation in adult renal transplant recipients. Clin Pharmacokinet. 2021;60(2):191-203.

74. Prograf®. Northbrook, IL: AstellasPharma US, Inc. 2020.

Appendices

Appendix 10. Institutional tacrolimus dosing and monitoring guidelines for post-heart transplant recipients.

Dosing		
	Formulation	
	Oral	**Intravenous***
Initial dose	0.5 mg (fixed test dose)	0.015 mg/kg/day infused over 24 hours.
Maintenance dose	Administered 12-hourly. Dose adjusted based on steady-state** trough concentration(s) and transplant recipient's overall clinical assessment.	Administered as a continuous infusion over 24 hours. Dose adjusted based on steady-state tacrolimus concentration(s) and transplant recipient's overall clinical assessment.
Estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2	Delay commencement of initial dose until eGFR is ≥60 mL/min/1.73 m^2.	
Monitoring		
Initial dose	Check trough concentration.	Check steady-state tacrolimus concentration.
Maintenance dose	Check trough concentration at steady-state**, frequency of monitoring based on transplant recipient's overall clinical assessment.	Check steady-state tacrolimus concentration twice weekly or based on transplant recipient's overall clinical assessment.
Therapeutic range of trough concentration (Time post-transplant)***	8−12 µg/L (day 1 – day 7) 10−15 µg/L (day 8 − 3 months) 8−12 µg/L (>3 − 12 months) 6−8 µg/L (>12 months)	

*In Australia, intravenous tacrolimus is licensed to be administered only as a continuous infusion and not to be administered as a bolus, intermittent, or short infusion [74].

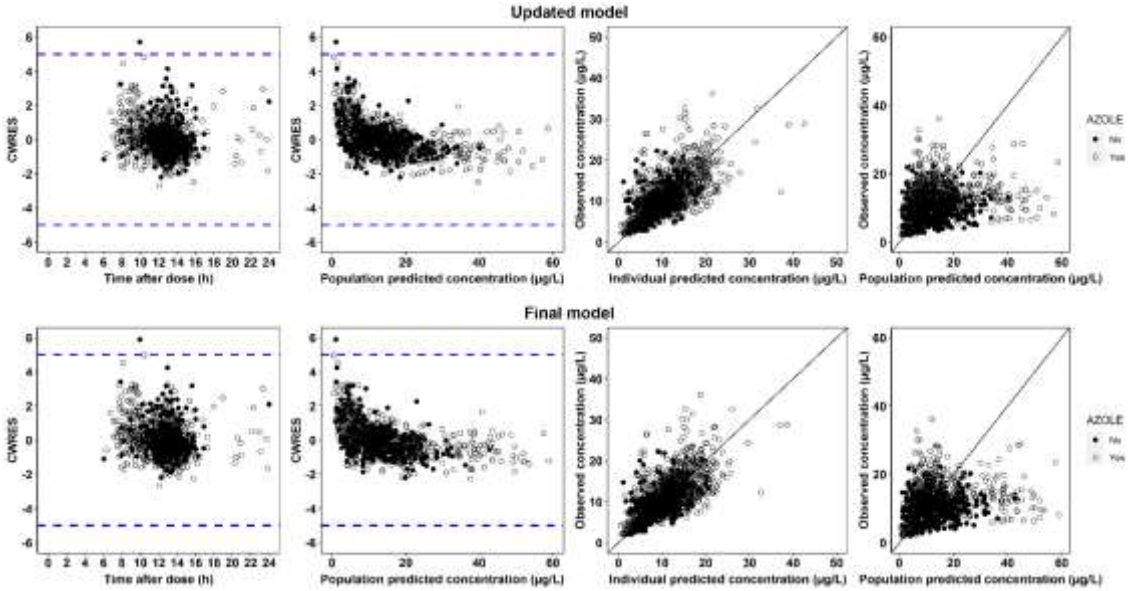
**At least 48 hours after initiation of maintenance therapy or any dose adjustment.

***To achieve the same drug exposure, the drug concentration targeted while a transplant recipient is on continuous infusion of tacrolimus will be higher than the drug concentration targeted while a transplant recipient receives the oral twice-daily dosing. In our institute, clinicians target at least the higher end of the therapeutic range specified in the guideline table if a transplant recipient receives an intravenous infusion.

Appendix 11. Stepwise covariate modelling (SCM) file

```
model=run003.mod
directory=scm_model_base
search_direction=both
p_forward  = 0.01
p_backward = 0.001
continuous_covariates  = AGE,POD,DST,HCT,ALB,AST,ALP
categorical_covariates = CYP3A5,RACE,ABCB1
time_varying = POD,DST,HCT,ALB,AST,ALP
do_not_drop = AGE,POD,DST,HCT,ALB,AST,ALP,CYP3A5,RACE,ABCB1
missing_data_token = -99
linearize=0
nm_version=default
[test_relations]
CL=POD,CYP3A5,DST,AGE,HCT,ALB,AST,ALP,RACE,ABCB1
[valid_states]
continuous=1,5
categorical=1,2
```

ALB Albumin*; ALP* Alkaline phosphatase; *AST* Aspartate aminotransferase; *DST* Days starting tacrolimus; *HCT* Haematocrit; *POD* Post-operative days.



Appendix 12. Goodness-of-fit plots of the updated and final population pharmacokinetic model for tacrolimus. *CWRES* conditional weighted residuals. Dashed blue lines show the cut off for |CWRES|>5.

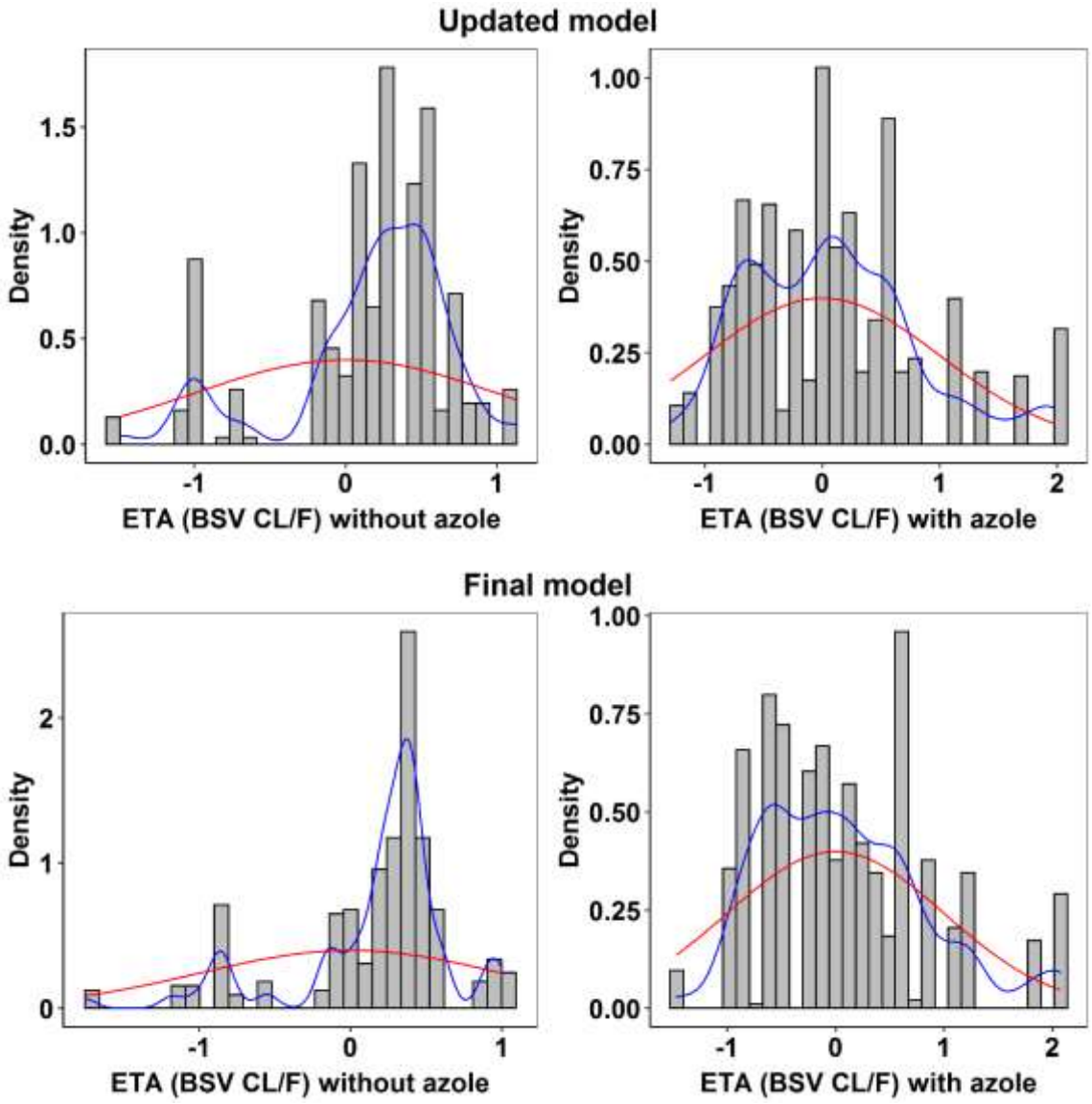
Appendix 13. Pharmacokinetic parameter estimates and covariate relationships of the updated tacrolimus population pharmacokinetic model, with and without |CWRES|>5 removed.

Parameter	**Unit**	**With |CWRES|>5**	**Without |CWRES|>5**
		Population mean estimate (RSE)	
K_a	h^{-1}	0.282 (15%)	0.287 (16%)
F	-	1*	1*
V_2/F	L	231 (0%)	231 (0%)
CL/F (without azole)	L/h	16.4 (13%)	16.3 (14%)
CL/F (with azole)	L/h	3.6 (12%)	3.6 (13%)
Q/F	L/h	58.1 (1%)	58.1 (1%)
V_3/F	L	521 (0%)	521 (0%)
		CV (RSE)[shrinkage]	
BSV CL/F (without azole)	%	78.7 (16%)[25%]	79.1 (16%)[24%]
BSV CL/F (with azole)	%	93.0 (12%)[8%]	93.3 (12%)[8%]
BOV CL/F	%	0*	0*
BOV K_a	%	0*	0*
BOV F	%	0*	0*
BOV V_2/F	%	0*	0*
Exponential RUV	%	46 (2%)	46 (2%)
OFV	-	-420	-452
Conditional number	-	1.5	1.5

The approximate percentage coefficient of variation (CV) was reported as $(\sqrt{\exp(\text{omega})^2}-1)*100$.

*Fixed value.

BSV Between-subject variability*; BOV* Between-occasion variability*; CL/F* Apparent clearance*; CWRES* Conditional weighted residuals; *F* Bioavailability*;* K_a Absorption rate constant; *OFV* Objective function value; *Q/F* Apparent intercompartmental clearance; *RSE* Relative standard error; *RUV* Residual unexplained variability; V_2/F Apparent central volume of distribution; V_3/F Apparent peripheral volume of distribution.



Appendix 14. Individual random effects (ETA) distribution for apparent tacrolimus clearance (CL/F) of the updated and final population pharmacokinetic model for tacrolimus stratified based on concomitant azole antifungal use. The histogram shows the ETA distribution of the heart transplant recipients (n=47) and the solid blue line is the loess smooth curve for the histogram. Theoretical ETA distribution based on the model is depicted by the solid red line.

Appendix 15. NONMEM code of the final model

```
$SIZES      PD=-1000 LVR=-150 LTH=-200
$PROBLEM    HTX_2018
$ABBREVIATED DERIV2=NO
$INPUT   COMB ID TIME TIMEC DST POD DUR AMT AMTT DV BLQ OCC MDV EVID CMT SEX RACE
AGE HT WT HCT SCR CLCR ALB AST ALP CYP3A5 CYP3A4 CLUSTER ABCB1 DM AZOLE AZOLET
AZOLED

$PRIOR     NWPRI
$DATA      NONMEM_HTX_COMBINE_LN.csv
           IGNORE=#
           IGNORE=(AMTT.EQ.1,AMTT.EQ.4, BLQ.EQ.1)
$SUBROUTINE ADVAN4

$PK
;------DOSE-----------------------------------------------------
IF(NEWIND.LE.1)THEN
DOSE=0
DOSEMK=0
ENDIF
IF(AMT.GT.0)DOSE=AMT
IF(AMT.GT.0)DOSEMK=AMT/WT

IF(AMT.GT.0)TDOSE=TIME
TAD=TIME-TDOSE

;------  BOV-------------------------------------------------------
IF(OCC.EQ.1) BOVCL=ETA(2)
IF(OCC.EQ.2) BOVCL=ETA(3)
IF(OCC.EQ.3) BOVCL=ETA(4)
IF(OCC.EQ.4) BOVCL=ETA(5)
IF(OCC.EQ.5) BOVCL=ETA(6)
IF(OCC.EQ.6) BOVCL=ETA(7)
IF(OCC.EQ.7) BOVCL=ETA(8)
IF(OCC.EQ.8) BOVCL=ETA(9)
IF(OCC.EQ.9) BOVCL=ETA(10)
IF(OCC.EQ.10) BOVCL=ETA(11)
IF(OCC.EQ.11) BOVCL=ETA(12)
IF(OCC.GE.12) BOVCL=ETA(13)

IF(OCC.EQ.1) BOVV2=ETA(14)
IF(OCC.EQ.2) BOVV2=ETA(15)
IF(OCC.EQ.3) BOVV2=ETA(16)
```

```
IF(OCC.EQ.4) BOVV2=ETA(17)
IF(OCC.EQ.5) BOVV2=ETA(18)
IF(OCC.EQ.6) BOVV2=ETA(19)
IF(OCC.EQ.7) BOVV2=ETA(20)
IF(OCC.EQ.8) BOVV2=ETA(21)
IF(OCC.EQ.9) BOVV2=ETA(22)
IF(OCC.EQ.10) BOVV2=ETA(23)
IF(OCC.EQ.11) BOVV2=ETA(24)
IF(OCC.GE.12) BOVV2=ETA(25)

IF(OCC.EQ.1) BOVKA=ETA(26)
IF(OCC.EQ.2) BOVKA=ETA(27)
IF(OCC.EQ.3) BOVKA=ETA(28)
IF(OCC.EQ.4) BOVKA=ETA(29)
IF(OCC.EQ.5) BOVKA=ETA(30)
IF(OCC.EQ.6) BOVKA=ETA(31)
IF(OCC.EQ.7) BOVKA=ETA(32)
IF(OCC.EQ.8) BOVKA=ETA(33)
IF(OCC.EQ.9) BOVKA=ETA(34)
IF(OCC.EQ.10) BOVKA=ETA(35)
IF(OCC.EQ.11) BOVKA=ETA(36)
IF(OCC.GE.12) BOVKA=ETA(37)

IF(OCC.EQ.1) BOVF1=ETA(38)
IF(OCC.EQ.2) BOVF1=ETA(39)
IF(OCC.EQ.3) BOVF1=ETA(40)
IF(OCC.EQ.4) BOVF1=ETA(41)
IF(OCC.EQ.5) BOVF1=ETA(42)
IF(OCC.EQ.6) BOVF1=ETA(43)
IF(OCC.EQ.7) BOVF1=ETA(44)
IF(OCC.EQ.8) BOVF1=ETA(45)
IF(OCC.EQ.9) BOVF1=ETA(46)
IF(OCC.EQ.10) BOVF1=ETA(47)
IF(OCC.EQ.11) BOVF1=ETA(48)
IF(OCC.GE.12) BOVF1=ETA(49)

;--------Population average PK parameters and covariates------------------
HCTCL = ((HCT/0.31)**THETA(10))

NOTUSED=THETA(1)
TVV2    = LOG(THETA(2))
TVKA    = LOG(THETA(3))
TVQ     = LOG(THETA(4))
TVV3    = LOG(THETA(5))
TVF1    = LOG(THETA(6))
```

```
;--------Individual PK parameters, BSV & BOV on the TV parameters---------
IF(AZOLE.EQ.0)
CL = (EXP(LOG(THETA(8)))*EXP(ETA(1)+ BOVCL))*((WT/73.5)**0.75)*HCTCL

IF(AZOLE.EQ.1)
CL = (EXP(LOG(THETA(9)))*EXP(ETA(50)+ BOVCL))*((WT/73.5)**0.75)*HCTCL

V2    = (EXP(TVV2)*EXP(BOVV2))*((WT/73.5))
KA    = EXP(TVKA)*EXP(BOVKA)
Q     = (EXP(TVQ))*((WT/73.5)**0.75)
V3    = (EXP(TVV3))*((WT/73.5))
F1    = EXP(TVF1)*EXP(BOVF1)

K   = CL/V2
S2  = V2
K23 = Q/V2
K32 = Q/V3

$ERROR
IPRED = LOG(F + 0.000001)
IRES  = DV - IPRED
W     = THETA(7)
IWRES = IRES/W
Y     = IPRED + W*EPS(1)

$THETA
1 FIX ; 1 CL
231 ; 2 V2
0.273 ; 3 KA
58.1; 4 Q
521; 5 V3
1 FIX ; 6 F1
(0,0.442) ; 7 RUV
(0,18.6) ; 8 CL no azole
(0,3.7) ; 9 CL with azole
 (-2,-0.888,10) ; 10 HCTCL

; $THETA PRIOR (FIXED)
$THETAP  1 FIX ; 1 CL
$THETAP  231 FIX ; 2 V2
$THETAP  0.579 FIX ; 3 KA
$THETAP  58.2 FIX ; 4 Q
$THETAP  521 FIX ; 5 V3
```

```
;$OMEGA USUAL RECORDS
$OMEGA  0.525  ; BSV CL without azole

$OMEGA  BLOCK(1) FIX 0  ;     BOVCL
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) FIX 0  ;     BOVV2
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) FIX 0  ;     BOVKA
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME

$OMEGA  BLOCK(1) FIX 0  ;     BOVF1
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
```

```
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME
$OMEGA  BLOCK(1) SAME

$OMEGA  0.663  ; BSV CL with azole

;PRIOR THETA VARIANCE
$THETAPV  0             FIX  ;     1 CL
$THETAPV  0.169178  FIX  ;      2 V2
$THETAPV  0.603815  FIX  ;      3 KA
$THETAPV  0.221419  FIX  ;       4 Q
$THETAPV  0.267910  FIX  ;      5 V3

$SIGMA  1  FIX
$ESTIMATION METHOD=1 INTER MAXEVAL=9999 NOABORT SIG=3 PRINT=1 POSTHOC
$COVARIANCE MATRIX=R PRINT=E
$TABLE    ID TIME TIMEC TAD DST POD AMT AMTT DOSE DOSEMK DV BLQ OCC MDV EVID CMT
SEX RACE AGE HT WT HCT SCR CLCR ALB AST ALP CYP3A5 CYP3A4 CLUSTER ABCB1 DM AZOLE
AZOLET AZOLED
PRED IPRED CWRES Y IWRES IRES W ETAS(1:LAST)
CL V2 Q V3 KA F1 CLHCT ONEHEADER NOPRINT FILE=alltab005
```

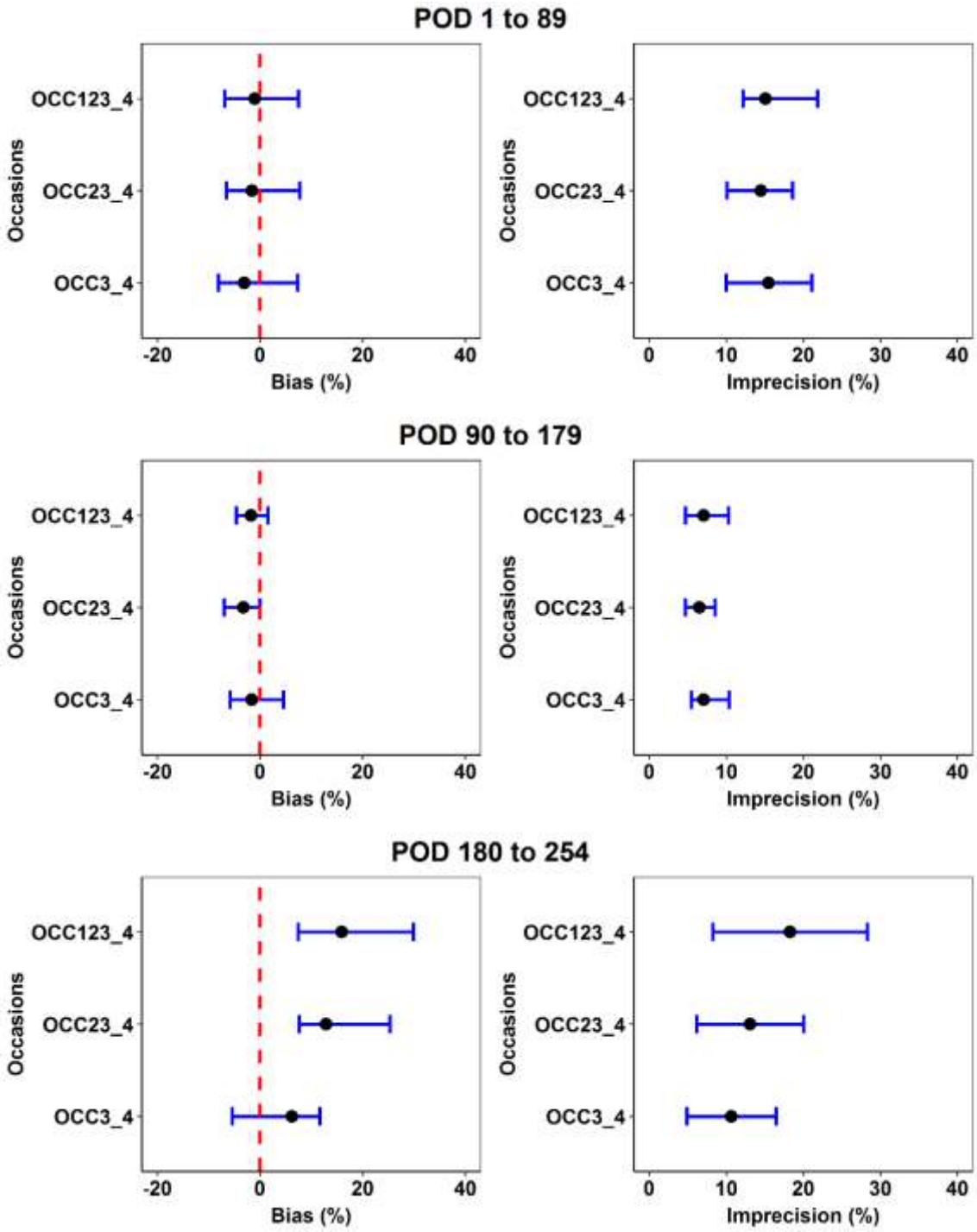
Appendix 16. The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion (n=87; blinded to the model) by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations across various evaluation settings.

	Bias		Imprecision	
Evaluation setting	**%**	**95% CI**	**%**	**95% CI**
*A priori**	-2.5	-7.98, 8.75	22.3	16.18, 27.63
Bayesian prediction (Figure 23)				
OCC3_4	-1.3	-4.67, 2.54	10.6	7.48, 13.36
OCC23_4	0.7	-3.03, 6.72	11.7	7.87, 15.16
OCC123_4	0.6	-3.44, 5.44	12.5	8.39, 16.58
Bayesian prediction (Appendix 17)				
POD 1−89 (n=40)				
OCC3_4	-3.1	-8.41, 6.72	15.5	9.56, 21.10
OCC23_4	-1.6	-6.51, 8.62	14.5	10.32, 18.73
OCC123_4	-1.0	-7.19, 7.49	15.1	12.18, 21.85
POD 90−179 (n=31)				
OCC3_4	-1.6	-5.84, 4.54	7.1	5.45, 10.37
OCC23_4	-3.3	-7.00, -0.19	6.5	4.66, 8.54
OCC123_4	-1.7	-4.64, 1.52	7.1	4.71, 10.28
POD ≥180 (n=16)				
OCC3_4	6.2	-5.39, 11.68	10.7	4.86, 16.49
OCC23_4	12.9	7.67, 25.33	13.1	6.14, 20.05
OCC123_4	15.9	7.40, 29.85	18.3	8.28, 28.32
*A posteriori***	0.4	-2.79, 4.61	9.4	5.92, 11.47

*Model prediction based on population pharmacokinetic parameter estimates, covariates, and dosing information.

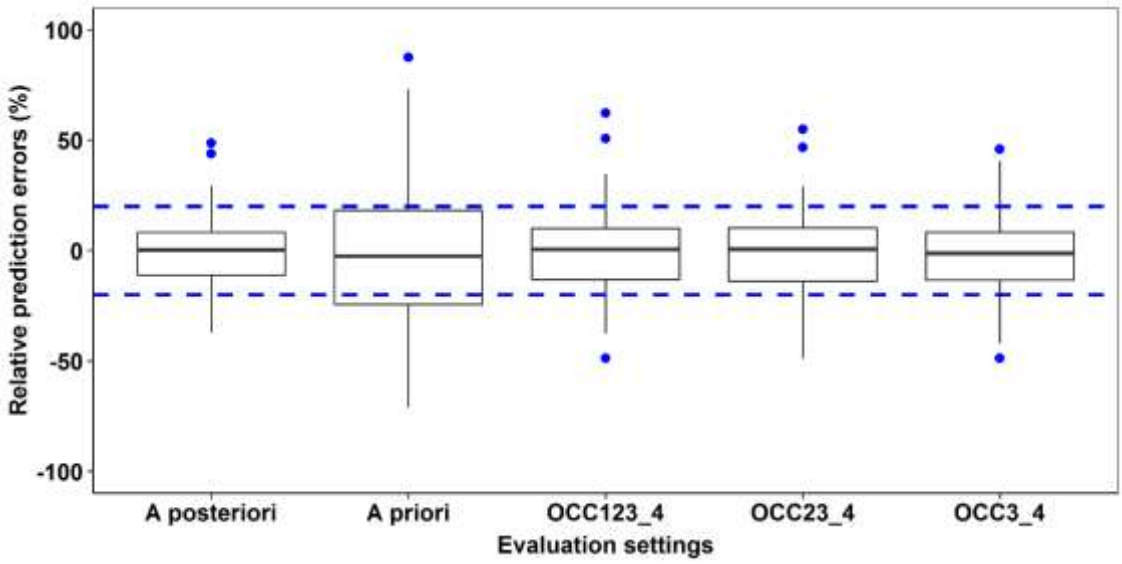
**Model prediction based on the inclusion of tacrolimus concentration in the fourth dosing occasion.

OCC3_4 Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *POD* Post-operative day.



Appendix 17. The bias and imprecision of the individual predicted tacrolimus concentrations of the fourth dosing occasion (blinded to the model) by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations across three-time points of post-operative days (POD) [POD 1−89 days; n=40 transplant recipients, POD 90−179; n=31, POD 180−254; n=16]. The panel on the

left shows the median bias and its 95% CI. The panel on the right shows the median imprecision and its 95% CI. The infrequent drug monitoring 6 months post-transplant, transfer of care to other healthcare facilities, conversion to the extended-release formulation of tacrolimus, and death explains the reduced number of drug concentrations available for analysis for POD ≥180. *OCC3_4* Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.



Appendix 18. Boxplot showing the relative prediction errors of the individual predicted tacrolimus concentrations of the fourth dosing occasion by the final population pharmacokinetic model for tacrolimus relative to the observed concentrations. The dashed blue lines depict the clinically acceptable range (±20%) for the relative prediction errors. *A priori* model prediction based on population pharmacokinetic parameter estimates, covariates, and dosing information; *A posteriori* model prediction based on the inclusion of tacrolimus concentration in the fourth dosing occasion; *OCC3_4* Third dosing occasion was used to predict tacrolimus concentration of the fourth dosing occasion; *OCC23_4* Second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion; *OCC123_4* First, second and third dosing occasions were used to predict tacrolimus concentration of the fourth dosing occasion.

Chapter 6:

Discussion and Conclusions.

6.1 Summary

Globally, approximately 110,000 adults (from January 1992 to June 2018) have undergone heart transplantations [1]. There is a steady increase in the number of transplantations performed annually [2], mainly due to advancements in surgical techniques and medical management, better patient selection for transplant and the use of more effective immunosuppressive therapies. Unfortunately, complications resulting from immunosuppressive therapy, such as cardiac allograft vasculopathy, infection, and malignancies, continue to affect long-term survival in heart transplant recipients [1, 3]. Therefore, the focus of the current work was to optimise the use of tacrolimus, one of the cornerstones lifelong immunosuppressive therapies required by heart transplant recipients. Optimising tacrolimus therapy is essential to reduce the long-term complications resulting from its use.

Tacrolimus is currently the first choice calcineurin inhibitor used in most solid organ transplant recipients, including heart transplants, to prevent graft rejection. Given the considerable pharmacokinetic variability [4-10], narrow therapeutic range [11], and non-linear pharmacokinetics [12-14], monitoring whole blood trough concentrations is recommended to individualise tacrolimus therapy in transplant recipients [15]. Individualisation of tacrolimus therapy in transplant recipients is complicated because (1) the optimal trough concentration and AUC therapeutic ranges in heart transplant recipients have not been clearly defined, (2) unbound plasma tacrolimus concentrations may better reflect tacrolimus efficacy and toxicity than whole blood tacrolimus concentrations, (3) collecting timed trough concentrations is difficult in clinical practice,

and (4) criteria for diagnosing adverse effects or toxicities due to tacrolimus have not been clearly defined.

Population pharmacokinetic model-based Bayesian estimation presents as an alternative approach for individualising tacrolimus therapy in transplant recipients [15]. This Bayesian approach utilises a population pharmacokinetic model together with patient-specific information, and at least one drug concentration, to provide individualised dosing recommendations. Its utilisation not only enables a flexible TDM sampling scheme but also facilitates the (1) estimation of AUC from at least one drug concentration, and (2) simulation of alternative dosing regimen(s) to achieve a pre-determined therapeutic range. Importantly, the Bayesian approach has been shown to improve the attainment of target tacrolimus concentrations in renal and liver transplant recipients in the early post-transplantation period [16-18]. Improved achievement of target tacrolimus concentrations likely translates into improved long-term graft and transplant recipient survival by reducing the risk of acute graft rejection and tacrolimus-related adverse effects or toxicities. Based on these clinical reasonings, this thesis described a program of work undertaken to identify a suitable tacrolimus population pharmacokinetic model to inform individualised dosing advice in heart transplant recipients.

The overall aim of this thesis was to investigate the individualisation of tacrolimus therapy in heart transplant recipients using the pharmacokinetic modelling approach. The specific aims were to:

1. Explore tacrolimus dosing and monitoring practices in heart transplant recipients (Chapter 2).
2. Evaluate the predictive performance of a commercially available Bayesian dosing software to predict tacrolimus concentrations in adult heart transplant recipients (Chapter 2).
3. Identify and summarise published population pharmacokinetic models of tacrolimus developed from adult transplant recipients (Chapter 3).
4. Determine the predictive performances of relevant population pharmacokinetic models of tacrolimus to predict tacrolimus concentrations in adult heart transplant recipients, stratified based on concomitant azole antifungal therapy (Chapter 4).
5. Develop and evaluate a population pharmacokinetic model for tacrolimus in heart transplant recipients, considering the effect of tacrolimus-azole antifungal drug interaction (Chapter 5).

6.2 Overview of thesis and key findings

I began my thesis with Chapter 1, providing a broad overview of tacrolimus clinical pharmacology, including clinical factors influencing tacrolimus pharmacokinetics (e.g., concomitant azole antifungal). I also discussed the challenges with current dosing and monitoring strategies for tacrolimus in transplant recipients. This introductory chapter highlighted a clear need for an alternative approach to the collection of timed trough concentrations to inform tacrolimus dosing decisions in transplant recipients. In the subsequent chapters, I presented the preparatory studies that ultimately led to the development and evaluation of a tacrolimus population pharmacokinetic model to facilitate dose individualisation in heart transplant recipients (Figure 24).

Chapter 1 – Thesis Introduction

Chapter 2 – *Clinical audit of tacrolimus dosing and monitoring practices.*

- Tacrolimus dosing and monitoring were discordant with hospital guidelines.

Chapter 2 – *Evaluation of a commercially available Bayesian dosing software.*

- The software was suitable for guiding tacrolimus dosing only after 11 days of therapy.

Chapter 3 – *Systematic review of available tacrolimus models (n=69).*

- Recipient CYP3A5 genotype, days post-transplant, haematocrit and body weight were identified as significant clinical determinants influencing tacrolimus pharmacokinetics.
- Strategies to better utilise existing models and additional consideration for the development of new models, to facilitate Bayesian-guided tacrolimus dosing decisions were identified.

Chapter 4 – *External evaluation of selected tacrolimus models (n=17).*

- None of the evaluated models adequately described tacrolimus pharmacokinetics in adult heart transplant recipients, stratified based on concomitant azole antifungal use.

Chapter 5 – *Development and evaluation of a tacrolimus model.*

- A model that comprehensively described tacrolimus pharmacokinetics in heart transplant recipients, considering tacrolimus-azole antifungal interaction, over one-year post-transplantation was developed.
- The model was externally evaluated and is suitable to facilitate dose individualisation in heart transplant recipients using one recent tacrolimus concentration.

Chapter 6 – Thesis discussion and conclusion

Figure 24. Overview of thesis chapters and key findings.

Assessment of tacrolimus dosing and monitoring practices against guideline recommendations is important. This is to ensure the safe and effective use of tacrolimus and to identify any necessary changes required to improve current practice. In **Chapter 2 (published in *Therapeutic Drug Monitoring*)**, an audit of tacrolimus therapy in hospitalised heart transplant recipients (n=87) at St. Vincent's Hospital Sydney, a major heart transplant centre in Australia, was undertaken. Consistent with a previous study conducted in liver transplant recipients [19], compliance with tacrolimus dosing and monitoring guidelines in heart transplant recipients was generally poor [20]. Specifically, the underdosing of initial IV doses and collection of TDM samples that were not steady-state trough concentrations were commonly observed. A greater understanding of the factors influencing prescribers' decision-making regarding dosing tacrolimus is required. The poorly timed tacrolimus concentrations reiterate the difficulties in dosing tacrolimus based on trough concentrations. It is important to note that adhering to the requirement of measuring timed trough concentrations is often not practical on busy wards [21]. This leads to delays or omission of the next drug dose while waiting for trough blood sample to be collected [21]. Findings from this chapter contribute to the literature as no studies have assessed the compliance of tacrolimus therapy with guidelines in heart transplant recipients.

Unlike in Europe and America, population pharmacokinetic model-based Bayesian estimation is not used to inform tacrolimus dosing decisions in transplant recipients in Australia. Therefore, the first step to consider when implementing the Bayesian approach is which model should be selected. How should one navigate the numerous tacrolimus models (n=74; [12, 22-26]) and covariates included in the models? Ideally,

the model should (a) capture essential drug disposition (e.g., a 2-compartment model), (b) show the distribution of transplant recipient covariates to be representative of the target transplant recipient population, and (c) include well-established covariates (e.g., haematocrit) [27]. The second step is to assess the robustness of the selected model(s) for use in the target transplant recipient population. This step is undertaken by determining the ability of the model(s) to accurately predict tacrolimus concentrations relative to the observed concentration. If the majority of model-predicted tacrolimus concentrations are within ±20% of the observed concentrations, the model is considered suitable to guide tacrolimus dosing in the target transplant recipient population. Consequently, the model can be integrated within a Bayesian dosing software to facilitate dose individualisation in the target transplant recipient population.

Several commercially available Bayesian dosing software (BestDose®, NextDose®, DoseMeRX™, MwPharm 1.82, and ImmunoSuppressants Bayesian dose Adjustment [IBSA]) have been developed to facilitate the practice of TDM and dose individualisation for tacrolimus [17, 28-30]. Despite the advantages of the Bayesian approach (Section 1.8.2), the implementation of Bayesian dosing software in healthcare settings has been limited. Potential barriers include the absence of high level pharmacokinetic and technical expertise at practice sites, lack of user-friendly software programs and difficulty with validating licensed programs in clinical settings [31, 32].

Evaluation of Bayesian dosing software for tacrolimus in the literature has been limited to date [30]. In **Chapter 2 (published in *Therapeutic Drug Monitoring*)**, a commercially available Bayesian dosing software approved for use in Australia was evaluated [20]. The

software uses a tacrolimus population pharmacokinetic model derived from renal transplant recipients [33]. Prior to Chapter 2, the ability of this model to predict tacrolimus exposure in heart transplant recipients had not been evaluated. The results of this chapter highlighted that the model was able to adequately predict tacrolimus concentration (n=931) after 11 days of tacrolimus therapy in adult heart transplant recipients (n=81) [20]. The delay in the achievement of acceptable model predictions likely reflects the instability of tacrolimus pharmacokinetics in the early post-transplantation period [34, 35] and the stabilisation of the clinically significant tacrolimus-azole antifungal interaction [36-42]. The delay observed in the present study mirrored the predictive performance of other models that were externally evaluated using the Bayesian approach [43-45]. These models, however, were evaluated in the same type of organ transplant recipient population used to develop the models. It is important for the model to adequately predict tacrolimus concentrations prior to day 12 of therapy as there is an increased risk of acute graft rejection in the first-week post-transplantation. The finding of Chapter 2 illustrates the need for more work to systematically quantify the large variability of tacrolimus pharmacokinetics early after transplantation to improve model predictions.

Determining the generalisability of existing tacrolimus population pharmacokinetic models would reveal if organ transplant specific models are needed to guide tacrolimus dosing decisions. Only one [46] of two existing tacrolimus population pharmacokinetic models [47, 46] that considered type of organ transplant, identified distinct differences in tacrolimus clearance between liver and non-liver transplant recipient populations. Congruent with Chapter 2 and the recent finding of Zhu *et al* [48], it may be possible to

extrapolate tacrolimus models across different organ transplant recipient populations. This is an important finding given the resource requirements and ethical considerations associated with obtaining relevant clinical data to develop new models, including patient recruitment and blood sample collections. The findings of Chapter 2 added to the scarce evidence in the literature regarding the possibility of using population pharmacokinetic model(s) to support tacrolimus prescribing practices across different organ transplant recipient populations.

Results from Chapter 2 emphasised the need to evaluate existing tacrolimus population pharmacokinetic models to identify one (or more) model(s) that might be more suitable for use in heart transplant recipients, particularly for the immediate post-transplantation phase. A systematic review was performed **(Chapter 3, published in *Clinical Pharmacokinetics*)** to inform the selection of models for subsequent evaluation of their predictive performance in adult heart transplant recipients. A total of 69 tacrolimus population pharmacokinetic models were identified and summarised [26]. The properties of the models and covariates identified varied, reflecting the diverse characteristics of the transplant recipient populations used for model building and the variability in tacrolimus pharmacokinetics [26]. In line with previous studies [27, 49], recipient CYP3A5, haematocrit, body weight and days post-transplant were identified as factors influencing tacrolimus pharmacokinetics [26]. This chapter contributes to the literature by providing numerous strategies to better utilise existing models (external evaluation and model averaging) and additional consideration for the development of new models (drug-drug interactions, and different formulations and brands of tacrolimus) to guide tacrolimus dosing decisions in transplant recipients [26]. The

content of Chapter 3 has proven useful for several clinical studies that have cited this Chapter [16, 20, 50-54].

Several studies have performed an external evaluation of existing tacrolimus population pharmacokinetic models (n=8−16) to predict tacrolimus exposure in renal [13, 55] and liver [14] transplant recipients. These studies were performed using an independent dataset – a dataset collected from a different transplant institute, as opposed to both model building and external evaluation datasets being collected from the same transplant institute. An external evaluation using an independent dataset collected from a different institute has not been performed on heart transplant recipients. Whilst preliminary evidence suggests that tacrolimus models can be extrapolated across different organ transplant recipient populations, there is still a need to externally evaluate the models in heart transplant recipients. This follows from the need to investigate model performances (a) in the immediate post-transplantation phase that inadvertently coincides with the initiation of concomitant azole antifungal therapy and (b) without concomitant azole antifungal therapy. Given institutional guidelines recommend the use of concomitant azole antifungal therapy for the first six months post-heart transplantation, a model was required to describe tacrolimus pharmacokinetics both in the presence and absence of concomitant azole antifungal therapy. Concomitant azole antifungal therapy alters tacrolimus pharmacokinetics substantially, necessitating dose adjustments [38-42, 56-60].

In **Chapter 4 (published in *British Journal of Clinical Pharmacology*)** [53], relevant tacrolimus models (n=19) developed from various organ transplant recipient

populations were selected from the systematic review (Chapter 3) [26] for the evaluation of their predictive performance in heart transplant recipients. Model evaluation was stratified based on concomitant azole antifungal use. The models were tested on data collected from 85 heart transplant recipients from immediately post-transplantation up to 391 days post-transplantation. Mimicking clinical practice, the models were unable to predict future tacrolimus concentrations given known tacrolimus concentrations, dosing information and transplant recipient factors. Further, all models displayed poor pcVPC. Overall, poor model performance was observed with and without concomitant azole antifungal. Therefore, all 19 models, including those developed using data obtained from heart transplant recipients, were inappropriate for use clinically to guide tacrolimus dosing decisions in heart transplant recipients at St. Vincent's Hospital Sydney. The findings of Chapter 4 contribute to the literature regarding the requirement for further work to describe tacrolimus pharmacokinetics in heart transplant recipients accommodating for concomitant azole antifungal use.

Findings from Chapters 2, 3 and 4 served to guide the development of a tacrolimus population pharmacokinetic model for heart transplant recipients, considering the drug-drug interaction with azole antifungals. In **Chapter 5 (*under review*)**, a tacrolimus model for heart transplant recipients was successfully developed. The model was developed using 1100 tacrolimus concentrations collected between 2- and 391-days post-heart transplantation. The model incorporated the effects of body weight, haematocrit, and concomitant azole antifungal use on tacrolimus pharmacokinetics. Consistent with previous reviews [26, 27, 49], these covariates are known to significantly influence tacrolimus pharmacokinetics. Additionally, the pcVPC showed a good model fit to the

model building dataset regardless of concomitant azole antifungal use. Mimicking clinical practice, the model was able to predict future tacrolimus concentrations given a known (recent) tacrolimus concentration, dosing information and transplant recipient factors in an independent heart transplant recipient cohort (n=40). This suggests that less frequent TDM monitoring of tacrolimus is acceptable once transplant recipients are stable. In comparison to the evaluation of the model integrated within the Bayesian dosing software (Chapter 2), the present model was able to display excellent predictive performance in both stable and unstable post-heart transplantation phases. As a result, the model can be implemented in clinical practice to individualise tacrolimus therapy in heart transplant recipients.

Ideally, model development requires the prospective collection of intensive sampled pharmacokinetic data under a protocolised setting. However, consideration of alternative approaches to model development is important given the resource requirements and ethical considerations associated with the development of new models. In Chapter 5, only pre-dose tacrolimus concentrations (at most one tacrolimus concentration over a dosing interval was available) collected from routine drug monitoring were available. Therefore, pharmacokinetic parameter values from a previously published tacrolimus model [35] were used to inform the estimation of some pharmacokinetic parameters during model building. This methodological approach is of particular interest given the difficulties in building models for less well-studied transplant recipient populations (e.g., heart, lung, and allogeneic hematopoietic stem cell transplant recipients), owing to data sparseness. Unfortunately, the utilisation of this methodological approach for model building is uncommon [61]. Nonetheless, a

recent study demonstrated an improvement in model predictive performance using this approach [62]. The findings from Chapter 5 adds further to the literature by providing similar evidence.

Existing tacrolimus models for use in heart transplant recipients (n=5) were developed using data collected in the early post-transplantation period (≤100 days) [35, 47, 63-65]. One of them had incorporated pharmacokinetic changes with concomitant azole antifungal use (voriconazole and fluconazole) [63]. Therefore, Chapter 5 contributes to the literature by being the first population pharmacokinetic model to comprehensively describe tacrolimus pharmacokinetics in heart transplant recipients, considering the clinically significant tacrolimus-itraconazole interaction, over one-year post-transplantation, including both stable and unstable phases of transplant recipient's clinical courses.

6.3 Strengths of the current research

This thesis presents a program of work that has led to the development and evaluation of a population pharmacokinetic model for tacrolimus in heart transplant recipients. To my knowledge, this model is the first to describe tacrolimus pharmacokinetics in heart transplant recipients, considering the clinically significant tacrolimus-azole antifungal interaction, over one-year post-transplantation. The overall aim of this thesis was to investigate the individualisation of tacrolimus therapy in heart transplant recipients using the pharmacokinetic modelling approach. This aim was accomplished, with studies leading to the development of a tacrolimus model. The model will be able to help transplant clinicians choose optimal tacrolimus doses and allow simulations of

alternative doses to compare potential pharmacokinetic outcomes. Important to note that any dosing recommendations by the model will only augment rather than replace the final clinical decision.

A major strength of this thesis is that the model developed provides an evidence base to implement the Bayesian approach for heart transplant recipients at St. Vincent's Hospital Sydney. The model incorporated the effect of concomitant azole antifungal use on tacrolimus pharmacokinetics. This was particularly important given the difficulties in dosing tacrolimus for the first two weeks upon initiation and cessation of concomitant azole antifungal therapy. The cessation of concomitant azole antifungal therapy happens much later during tacrolimus therapy. The model incorporated time-varying covariates (body weight and haematocrit) that are commonly collected as part of routine care. These covariates account for changes in tacrolimus pharmacokinetics over time. Consequently, the same model can be used to accurately inform tacrolimus dosing both in the early post-transplantation phase and throughout the lifelong course of tacrolimus therapy.

An additional strength of this thesis is the diverse heart transplant recipients (n=87) included in the studies. The study population is representative of "real-life" heart transplant recipients. The dosage of tacrolimus given to different transplant recipients (and during an individual's therapy) varied considerably. Thus, findings from this thesis should be applicable to a larger heart transplant recipient community. Given the heterogeneity of the study population, the tacrolimus model developed fulfilled high-performance expectations. While at one transplant centre - this centre performs the

highest number of heart transplantation in Australia. Thus, it is anticipated that the model will perform equally well if used in other heart transplant centres in Australia or even overseas.

Finally, the variety of methods applied to investigate the research questions is a strength of this thesis. Methods used included a systematic review and a comprehensive pharmacokinetic modelling approach (model building, model evaluation and simulations). Although tacrolimus dose individualisation was the focus of this thesis, the approaches used are applicable to a wide range of drug therapies.

6.4 Limitations of the current research

The limitations of individual studies are addressed within each chapter, but several limitations of the thesis as a whole warrant further discussion. Due to the retrospective nature of the study, the time of tacrolimus administration and TDM sample collection cannot be guaranteed. This may impact the accuracy of data used for model building and evaluation. The accuracy of these data is variable in clinical practice, particularly if electronic systems are used to capture the data [66]. An example is a recent study highlighting the observed discrepancies in vancomycin administration time that resulted in a different dose recommendation in 57% of cases [66]. It is important to note that this is the reality of clinical research, the same patient data are available to clinicians in their day-to-day workflow.

Another limitation is the dataset used for the individual studies. High doses of corticosteroid therapy administered in the early phase post-transplantation may

increase tacrolimus apparent clearance [10]. Unfortunately, the collection of prednisolone doses in the outpatient setting was not possible as they were not consistently documented in the paper medical record. Due to this, the performance of models that incorporated prednisolone dose as a covariate could not be assessed (Chapter 4) and prednisolone dose could not be tested as a covariate during model building (Chapter 5). Race, CYP3A5 genotype, CYP3A4 genotype and ABCB1 genotype have been shown to significantly influence tacrolimus apparent clearance [15, 26, 47, 67]. These data were unavailable in approximately 40% of the heart transplant recipients included in this study. This could have also influenced the predictive performances of models that had any of these as covariates in the model (Chapter 2 and 4) and inclusion as covariates following stepwise covariate modelling (Chapter 5).

6.5 Implications for clinical practice

Individualising tacrolimus therapy is crucial in achieving prolonged transplant recipient and graft survival with improved quality of life. However, the findings from my thesis (Chapter 2) and in the international literature [15, 19] indicate that tacrolimus dose individualisation is challenging given the difficulty in collecting timed trough concentrations. Nonetheless, this can now be overcome using the tacrolimus population pharmacokinetic model developed in Chapter 5 as it allows for the interpretation of non-trough concentrations. This clinical decision support tool used alongside clinical judgement will help inform tacrolimus dosing decisions in heart transplant recipients both in the inpatient and outpatient settings. Further, transplant clinicians will be able to visualise the distribution of expected tacrolimus concentration following acceptance of a dose recommendation(s). Using the “right” population pharmacokinetic model-

based Bayesian estimation has been shown to improve the attainment of target tacrolimus concentrations in renal and liver transplant recipients in the early post-transplantation period [16-18]. Therefore, it is hypothesised that the utilisation of the model developed in Chapter 5 will (1) improve the current standard of care and transplant recipients' clinical outcomes (safety/efficacy) and (2) shorten the time to reach therapeutic range of tacrolimus exposure.

As highlighted in Chapters 2, 3 and 4, it is difficult to dose tacrolimus in the presence of concomitant azole antifungal therapy. If tacrolimus dosing is not adjusted appropriately, transplant recipients will be at risk of either acute graft rejection or tacrolimus adverse effects/toxicities. To date, no tacrolimus model has quantified the effect of itraconazole on tacrolimus pharmacokinetics in heart transplant recipients. To cater for this, the model developed in Chapter 5 was adapted to quantify the effect of concomitant itraconazole on tacrolimus CL/F and BSV on CL/F. Concomitant itraconazole was found to reduce tacrolimus CL/F by 80%. Even without the use of the Bayesian approach, clinicians can use this knowledge and reduce tacrolimus doses by 80% if heart transplant recipients are co-prescribed with itraconazole.

6.6 Future directions

The present thesis highlighted several potential directions for future research, focusing on the model developed in Chapter 5. This includes prospective evaluation of the model to access its clinical utility, identification of optimal TDM sampling time, determination of the ability of the model to predict AUC_{0-12}, the establishment of optimal tacrolimus therapeutic ranges for both trough concentrations and AUC_{0-12} in heart transplant

recipients, assessment of model generalisability, and determination of model performances using tacrolimus concentrations measured by dried blood spot.

Despite the success of the development and evaluation of a tacrolimus population pharmacokinetic model to inform tacrolimus dosing in heart transplant recipients, prospective evaluation to access its clinical utility is required. While it is expected that population pharmacokinetic model-based Bayesian estimation yields improved target attainment of tacrolimus trough concentration, it is important to evaluate its clinical feasibility, clinical outcomes (graft rejection rates and incidence of tacrolimus-induced toxicities/adverse effects), and cost-effectiveness. This could be performed through a randomised controlled trial that is powered to compare clinical outcomes, comparing the usual practice of dosing tacrolimus (control group) versus population pharmacokinetic model-based Bayesian estimation-guided tacrolimus dosing (intervention group).

The tacrolimus population pharmacokinetic model developed could be used to design optimal TDM sampling time strategies. This strategy is important to identify the most informative time within a dosing interval to facilitate TDM blood sample collection. The optimal TDM sampling time can then be embedded into national dosing guidelines to facilitate tacrolimus dose individualisation in heart transplant recipients. A simulation study could be performed to assess the difference in tacrolimus dose recommendations using optimal and non-optimal sampling times.

Although practice guidelines currently advocate the use of trough concentrations to guide tacrolimus dosing, there is increasing evidence that AUC is a better surrogate for treatment efficacy [15, 22, 62]. Therefore, evaluation of the ability of the model to predict AUC_{0-12} is required prior to recommending the use of AUC_{0-12} to guide tacrolimus dosing in heart transplant recipients. Further study is required to compare model-predicted AUC_{0-12} to AUC_{0-12} calculated using the trapezoidal rule (reference method). For this study to be possible intensive sampling pharmacokinetic data collection is required to facilitate AUC_{0-12} calculation using the trapezoidal rule.

The model could be used to establish the optimal therapeutic ranges for both trough concentrations and AUC_{0-12} in heart transplant recipients. The refinement of therapeutic ranges for tacrolimus using this model would prove useful in clinical practice since (1) the model was developed using tacrolimus concentrations measured by high-performance liquid chromatography-tandem mass spectrometry - a bioanalytical method with a high degree of sensitivity, specificity and precision and (2) mycophenolate mofetil and prednisolone were used alongside tacrolimus – the three drugs commonly used combination of maintenance therapy in heart transplant recipients. The refinement of therapeutic ranges for trough concentrations and AUC_{0-12} could be performed by investigating tacrolimus concentrations/AUC that correspond to acute graft rejection and tacrolimus adverse effects/toxicities.

It is important to assess the generalisability of the developed model in (a) other heart transplant recipient populations and (b) other solid organ transplant recipient populations. St. Vincent's Hospital Sydney is a thoracic transplant centre. Given the

scope of this thesis, model performance in lung transplant recipients was not undertaken. However, the model is currently being evaluated in lung transplant recipients. The primary aim of this work is to evaluate the predictive performance of the model stratified based on cystic fibrosis status and concomitant azole antifungal use. Further, the AUC_{0-12} predicted by the model will be compared to AUC_{0-12} calculated using the trapezoidal rule. I hypothesise that the model will be able to be extrapolated for use in lung transplant recipients.

The use of dried blood spots has been identified as a new monitoring strategy to measure whole blood tacrolimus concentrations [15]. This approach is user friendly and provides both cost and time savings. It can be implemented at home and requires a very small amount of blood sample collection to determine tacrolimus concentration [15]. Comparing model performances using tacrolimus concentrations measured by dried blood spot to whole blood tacrolimus concentrations analysed in the laboratory could provide preliminary evidence for the use of dried blood spot alongside the model and clinical judgement to guide tacrolimus dosing in heart transplant recipients.

6.7 Overall conclusions

There is a clear need to establish an alternative approach to the collection of timed trough concentrations to enable precision dosing of tacrolimus in heart transplant recipients. This thesis presents the utilisation of a pharmacokinetic modelling approach in individualising tacrolimus therapy in heart transplant recipients and has led to the development of a tacrolimus population pharmacokinetic model. This model is the first dosing support tool that considered the clinically significant tacrolimus-azole antifungal

interaction, over one-year post-transplantation. This model will be able to assist transplant clinicians in prescribing tacrolimus in heart transplant recipients. Prospective evaluation of the model in clinical practice is a priority. My goal is to integrate this model in a Bayesian dosing software for implementation in routine clinical practice across transplant centres worldwide. Given that St. Vincent's Hospital Sydney is also a lung transplant centre, I aim to investigate the extrapolation of this model to lung transplant recipients.

References

1. Khush KK, Potena L, Cherikh WS, Chambers DC, Harhay MO, Hayes D, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: 37th adult heart transplantation report—2020; focus on deceased donor characteristics. J Heart Lung Transplant. 2020;39(10):1003-15.

2. Khush KK, Cherikh WS, Chambers DC, Goldfarb S, Hayes D, Kucheryavaya AY, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult heart transplantation report—2018; focus theme: multiorgan transplantation. J Heart Lung Transplant. 2018;37(10):1155-68.

3. Khush KK, Cherikh WS, Chambers DC, Harhay MO, Hayes D, Hsich E, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult heart transplantation report—2019; focus theme: Donor and recipient size match. J Heart Lung Transplant. 2019;38(10):1056-66.

4. Staatz CE, Willis C, Taylor PJ, Tett SE. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. Clin Pharmacol Ther. 2002;72(6):660-9.

5. Staatz CE, Willis C, Taylor PJ, Lynch SV, Tett SE. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. Liver Transpl. 2003;9(2):130-7.

6. Musuamba FT, Mourad M, Haufroid V, De Meyer M, Capron A, Delattre IK, et al. Statistical tools for dose individualization of mycophenolic acid and tacrolimus co-administered during the first month after renal transplantation. Br J Clin Pharmacol. 2013;75(5):1277-88.

7. Ogasawara K, Chitnis SD, Gohh RY, Christians U, Akhlaghi F. Multidrug resistance-associated protein 2 (MRP2/ABCC2) haplotypes significantly affect the pharmacokinetics of tacrolimus in kidney transplant recipients. Clin Pharmacokinet. 2013;52(9):751-62.

8. Zhang HJ, Li DY, Zhu HJ, Fang Y, Liu TS. Tacrolimus population pharmacokinetics according to CYP3A5 genotype and clinical factors in Chinese adult kidney transplant recipients. J Clin Pharm Ther. 2017;42(4):425-32.

9. Campagne O, Mager DE, Brazeau D, Venuto RC, Tornatore KM. Tacrolimus Population Pharmacokinetics and Multiple CYP3A5 Genotypes in Black and White Renal Transplant Recipients. J Clin Pharmacol. 2018;58(9):1184-95.

10. Lee JY, Hahn H, Son IJ, Suh KS, Yi NJ, Oh JM, et al. Factors affecting the apparent clearance of tacrolimus in Korean adult liver transplant recipients. Pharmacotherapy. 2006;26(8):1069-77.

11. Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet. 1995;29(6):404-30.

12. Cai X, Song H, Jiao Z, Yang H, Zhu M, Wang C, et al. Population pharmacokinetics and dosing regimen optimization of tacrolimus in Chinese lung transplant recipients. Eur J Pharm Sci. 2020;152:105448.

13. Zhao CY, Jiao Z, Mao JJ, Qiu XY. External evaluation of published population pharmacokinetic models of tacrolimus in adult renal transplant recipients. Br J Clin Pharmacol. 2016;81(5):891-907.

14. Cai X, Li R, Sheng C, Tao Y, Zhang Q, Zhang X, et al. Systematic external evaluation of published population pharmacokinetic models for tacrolimus in adult liver transplant recipients. Eur J Pharm Sci. 2020;145:105237.

15. Brunet M, van Gelder T, Asberg A, Haufroid V, Hesselink DA, Langman L, et al. Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report. Ther Drug Monit. 2019;41(3):261-307.

16. Francke MI, Andrews LM, Le HL, van de Wetering J, Clahsen-van Groningen MC, van Gelder T, et al. Avoiding Tacrolimus Underexposure and Overexposure with a Dosing Algorithm for Renal Transplant Recipients: A Single Arm Prospective Intervention Trial. Clin Pharmacol Ther. 2021;110(1):169-78.

17. Størset E, Åsberg A, Skauby M, Neely M, Bergan S, Bremer S, et al. Improved tacrolimus target concentration achievement using computerized dosing in renal transplant recipients–a prospective, randomized study. Transplantation. 2015;99(10):2158-66.

18. Fukudo M, Yano I, Shinsako K, Katsura T, Takada Y, Uemoto S, et al. Prospective evaluation of the bayesian method for individualizing tacrolimus dose early after living-donor liver transplantation. J Clin Pharmacol. 2009;49(7):789-97.

19. Dasari B, Hodson J, Nassir A, Widmer J, Isaac J, Mergentel H, et al. Variations in practice to therapeutic monitoring of tacrolimus following primary adult liver transplantation. Int J Organ Transplant Med. 2016;7(1):1-8.

20. Kirubakaran R, Stocker SL, Carlos L, Day RO, Carland JE. Tacrolimus Therapy in Adult Heart Transplant Recipients: Evaluation of a Bayesian Forecasting Software. Ther Drug Monit. 2021;43(6):736-46.

21. Vali L, Jenkins DR, Vaja R, Mulla H. Personalised dosing of vancomycin: A prospective and retrospective comparative quasi-experimental study. Br J Clin Pharmacol. 2021;87(2):506-15.

22. Marquet P, Destère A, Monchaud C, Rérolle J-P, Buchler M, Mazouz H, et al. Clinical Pharmacokinetics and Bayesian Estimators for the Individual Dose Adjustment of a Generic Formulation of Tacrolimus in Adult Kidney Transplant Recipients. Clin Pharmacokinet. 2021;60(5):611-22.

23. Emoto C, Johnson TN, Hahn D, Christians U, Alloway RR, Vinks AA, et al. A Theoretical physiologically-based pharmacokinetic approach to ascertain covariates explaining the

large interpatient variability in tacrolimus disposition. CPT Pharmacometr Syst Pharmacol. 2019;8(5):273-84.

24. Jing Y, Kong Y, Hou X, Liu H, Fu Q, Jiao Z, et al. Population pharmacokinetic analysis and dosing guidelines for tacrolimus co-administration with Wuzhi capsule in Chinese renal transplant recipients. J Clin Pharm Ther. 2021;46(4):1117-28.

25. Woillard JB, Labriffe M, Debord J, Marquet P. Tacrolimus exposure prediction using machine learning. Clin Pharmacol Ther. 2020.

26. Kirubakaran R, Stocker SL, Hennig S, Day RO, Carland JE. Population Pharmacokinetic Models of Tacrolimus in Adult Transplant Recipients: A Systematic Review. Clin Pharmacokinet. 2020;59(11):1357-92.

27. Campagne O, Mager DE, Tornatore KM. Population pharmacokinetics of tacrolimus in transplant recipients: what did we learn about sources of interindividual variabilities? J Clin Pharmacol. 2019;59(3):309-25.

28. Fuchs A, Csajka C, Thoma Y, Buclin T, Widmer N. Benchmarking therapeutic drug monitoring software: a review of available computer tools. Clin Pharmacokinet. 2013;52(1):9-22.

29. Woillard J-B, Saint-Marcoux F, Debord J, Åsberg A. Pharmacokinetic models to assist the prescriber in choosing the best tacrolimus dose. Pharmacol Res. 2018;130:316-21.

30. Brooks E, Tett SE, Isbel NM, McWhinney B, Staatz CE. Evaluation of Bayesian forecasting methods for prediction of tacrolimus exposure using samples taken on two occasions in adult kidney transplant recipients. Ther Drug Monit. 2021;43(2):238-46.

31. Darwich A, Ogungbenro K, Vinks AA, Powell JR, Reny JL, Marsousi N, et al. Why has model-informed precision dosing not yet become common clinical reality? Lessons from the past and a roadmap for the future. Clin Pharmacol Ther. 2017;101(5):646-56.

32. Donagher J, Martin JH, Barras MA. Individualised medicine: why we need Bayesian dosing. Intern Med J. 2017;47(5):593-600.

33. Woillard JB, de Winter BC, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations--twice daily Prograf and once daily Advagraf. Br J Clin Pharmacol. 2011;71(3):391-402.

34. Sikma M, Van Maarseveen E, Van De Graaf E, Kirkels J, Verhaar M, Donker D, et al. Pharmacokinetics and toxicity of tacrolimus early after heart and lung transplantation. Am J Transplant. 2015;15(9):2301-13.

35. Sikma MA, Hunault CC, Van Maarseveen EM, Huitema AD, Van de Graaf EA, Kirkels JH, et al. High variability of whole-blood tacrolimus pharmacokinetics early after thoracic organ transplantation. Eur J Drug Metab Pharmacokinet. 2020;45(1):123-34.

36. Groll AH, Townsend R, Desai A, Azie N, Jones M, Engelhardt M, et al. Drug-drug interactions between triazole antifungal agents used to treat invasive aspergillosis and immunosuppressants metabolized by cytochrome P450 3A4. Transpl Infect Dis. 2017;19(5):e12751.

37. Vanhove T, Bouwsma H, Hilbrands L, Swen JJ, Spriet I, Annaert P, et al. Determinants of the magnitude of interaction between tacrolimus and voriconazole/posaconazole in solid organ recipients. Am J Transplant. 2017;17(9):2372-80.

38. Osowski CL, Dix SP, Lin LS, Mullins RE, Geller RB, Wingard JR. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. Transplantation. 1996;61(8):1268-72.

39. Mañez R, Martin M, Raman V, Silverman D, Jain A, Warty V, et al. Fluconazole therapy in transplant recipients receiving FK506. Transplantation. 1994;57(10):1521-3.

40. Leather H, Boyette RM, Tian L, Wingard JR. Pharmacokinetic evaluation of the drug interaction between intravenous itraconazole and intravenous tacrolimus or intravenous cyclosporin A in allogeneic hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant. 2006;12(3):325-34.

41. Sansone-Parsons A, Krishna G, Martinho M, Kantesaria B, Gelone S, Mant TG. Effect of oral posaconazole on the pharmacokinetics of cyclosporine and tacrolimus. Pharmacotherapy. 2007;27(6):825-34.

42. Shitrit D, Ollech JE, Ollech A, Bakal I, Saute M, Sahar G, et al. Itraconazole prophylaxis in lung transplant recipients receiving tacrolimus (FK 506): efficacy and drug interaction. J Heart Lung Transplant. 2005;24(12):2148-52.

43. Willis C, Staatz CE, Tett SE. Bayesian forecasting and prediction of tacrolimus concentrations in pediatric liver and adult renal transplant recipients. Ther Drug Monit. 2003;25(2):158-66.

44. Antignac M, Fernandez C, Barrou B, Roca M, Favrat JL, Urien S, et al. Prediction tacrolimus blood levels based on the Bayesian method in adult kidney transplant patients. Eur J Drug Metab Pharmacokinet. 2011;36(1):25-33.

45. Fukudo M, Yano I, Fukatsu S, Saito H, Uemoto S, Kiuchi T, et al. Forecasting of blood tacrolimus concentrations based on the Bayesian method in adult patients receiving living-donor liver transplantation. Clin Pharmacokinet. 2003;42(13):1161-78.

46. Nanga TM, Doan TT, Marquet P, Musuamba FT. Toward a robust tool for pharmacokinetic-based personalization of treatment with tacrolimus in solid organ transplantation: a model-based meta-analysis approach. Br J Clin Pharmacol. 2019;85(12):2793-823.

47. Lu Z, Bonate P, Keirns J. Population pharmacokinetics of immediate- and prolonged-release tacrolimus formulations in liver, kidney and heart transplant recipients. Br J Clin Pharmacol. 2019;85(8):1692-703.

48. Zhu J, Campagne O, Torrice CD, Flynn G, Miller JA, Patel T, et al. Evaluation of the performance of a prior tacrolimus population pharmacokinetic kidney transplant model among adult allogeneic hematopoietic stem cell transplant patients. Clin Transl Sci. 2021;14(3):908-18.

49. Brooks E, Tett SE, Isbel NM, Staatz CE. Population pharmacokinetic modelling and Bayesian estimation of tacrolimus exposure: is this clinically useful for dosage prediction yet? Clin Pharmacokinet. 2016;55(11):1295-335.

50. Lu Y, Xu L, Cui J, Shen S, Li X. Effects of Postoperative Day and NR1I2 on Tacrolimus Clearance in Chinese Liver Transplant Recipients—A Population Model Approach. Clin Pharmacol Drug Dev. 2021.

51. Braithwaite H, Darley D, Brett J, Day R, Carland J. Identifying the association between tacrolimus exposure and toxicity in heart and lung transplant recipients: A systematic review. Transplant Rev. 2021;35(2):100610.

52. Quintairos L, Colom H, Millán O, Fortuna V, Espinosa C, Guirado L, et al. Early prognostic performance of miR155-5p monitoring for the risk of rejection: Logistic regression with a population pharmacokinetic approach in adult kidney transplant patients. PloS one. 2021;16(1):e0245880.

53. Kirubakaran R, Hennig S, Maslen B, Day RO, Carland JE, Stocker SL. Evaluation of published population pharmacokinetic models to inform tacrolimus dosing in adult heart transplant recipients. Br J Clin Pharmacol. 2021.

54. Henin E, Govoni M, Cella M, Laveille C, Piotti G. Therapeutic Drug Monitoring Strategies for Envarsus in De Novo Kidney Transplant Patients Using Population Modelling and Simulations. Adv Ther. 2021.

55. Hu C, Yin WJ, Li DY, Ding JJ, Zhou LY, Wang JL, et al. Evaluating tacrolimus pharmacokinetic models in adult renal transplant recipients with different CYP3A5 genotypes. Eur J Clin Pharmacol. 2018;74(11):1437-47.

56. Gu TM, Lewis JS, Le H, Bubalo JS. Comparative effects of fluconazole, posaconazole, and isavuconazole upon tacrolimus and cyclosporine serum concentrations. J Oncol Pharm Pract. 2021:10781552211029046.

57. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. Pharmacotherapy. 2006;26(12):1730-44.

58. Darley D, Carlos L, Hennig S, Glanville A. Quantitation of the Effect of Azole Antifungals on Tacrolimus Clearance. J Heart Lung Transplant. 2016;35(4):S236.

59. Trofe-Clark J, Lemonovich T, Practice AIDCo. Interactions between anti-infective agents and immunosuppressants in solid organ transplantation. Am J Transplant. 2013;13(s4):318-26.

60. Moreno M, Latorre A, Manzanares C, Morales E, Herrero J, Dominguez-Gil B, et al. Clinical management of tacrolimus drug interactions in renal transplant patients. Transplant Proc. 1999;31(6):2252-3.

61. Anna H-X, Calvier EA, Fabre D, Gattacceca F, Khier S. Prior information for population pharmacokinetic and pharmacokinetic/pharmacodynamic analysis: overview and guidance with a focus on the NONMEM PRIOR subroutine. J Pharmacokinet Pharmacodyn. 2020(47):1-16.

62. Decrocq-Rudler M-A, Meunier L, Fraisse J, Ursic-Bedoya J, Khier S. Can We Predict Individual Concentrations of Tacrolimus After Liver Transplantation? Application and Tweaking of a Published Population Pharmacokinetic Model in Clinical Practice. Ther Drug Monit. 2021;43(4):490-8.

63. Zhou H, Cai J, Huang J, Zhang J, Shi SJ, Liu YN, et al. Prediction of tacrolimus dosage in the early period after heart transplantation: A population pharmacokinetic approach. Pharmacogenomics. 2019;20(1):21-35.

64. Gong Y, Yang M, Sun Y, Li J, Lu Y, Li X. Population pharmacokinetic analysis of tacrolimus in Chinese cardiac transplant recipients. Eur J Hosp Pharm. 2020;27(e1):e012-18.

65. Sikma MA, Van Maarseveen EM, Hunault CC, Moreno JM, Van de Graaf EA, Kirkels JH, et al. Unbound Plasma, Total Plasma, and Whole-Blood Tacrolimus Pharmacokinetics Early After Thoracic Organ Transplantation. Clin Pharmacokinet. 2019;59(6):771–80.

66. Roydhouse SA, Carland JE, Debono DS, Baysari MT, Reuter SE, Staciwa AJ, et al. Accuracy of documented administration times for intravenous antimicrobial drugs and impact on dosing decisions. Br J Clin Pharmacol. 2021.

67. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. Clin Pharmacokinet. 2004;43(10):623-53.

Chapter 7.

Thesis appendices.

Appendix A. ASCEPT Student Forum Symposium (Second prize)

ASCEPT

AUSTRALASIAN SOCIETY OF CLINICAL AND EXPERIMENTAL PHARMACOLOGISTS AND TOXICOLOGISTS

Second Place

awarded to

Ranita Kirubakaran

at the

Student Forum SIG Virtual Symposium

7 October 2021

Appendix B. IATDMCT 2021 Best Young Scientist Poster Award.

Appendix C. UNSW 3 Minutes Thesis Competition.

UNSW
SYDNEY

6 September 2021

Ranita Kirubakaran
Faculty of Medicine & Health

Dear Ranita

Congratulations on your outstanding presentation for the 2021 UNSW 3 Minute Thesis (3MT) Competition.

Your talk *A Gift of Life: Every HEART Matters!* was engaging, and a testament to both your hard work and your passion for the research you are undertaking.

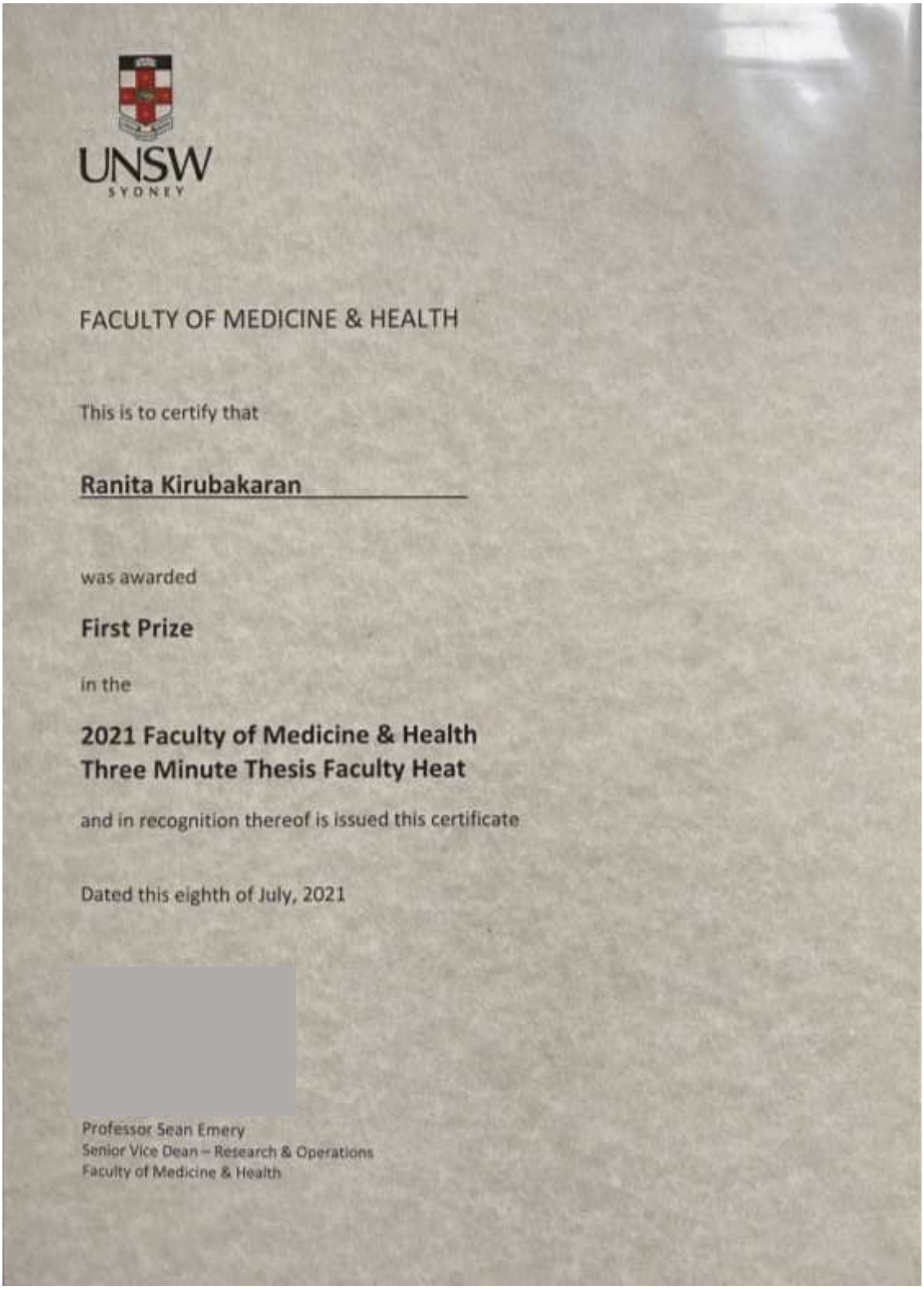
I wish you all the best for your future endeavours and look forward to hearing more about your research as your candidature progresses.

Sincerely,

Professor Jonathan Morris
Pro Vice-Chancellor of Research Training & Entrepreneurship and Dean of Graduate Research

UNSW SYDNEY NSW 2052 AUSTRALIA
T +61 (2) 9385 1000 | F +61 (2) 9385 0000 | ABN 57 195 873 179 | CRICOS Provider Code 00098G

Appendix D. 3 Minutes Thesis Competition, UNSW Faculty of Medicine and Health Heat (First Prize).



UNSW
SYDNEY

FACULTY OF MEDICINE & HEALTH

This is to certify that

Ranita Kirubakaran

was awarded

First Prize

in the

2021 Faculty of Medicine & Health
Three Minute Thesis Faculty Heat

and in recognition thereof is issued this certificate

Dated this eighth of July, 2021

Professor Sean Emery
Senior Vice Dean – Research & Operations
Faculty of Medicine & Health

Appendix E. UNSW International Student of the Year Award (Finalist).

UNSW SYDNEY

Ranita Kirubakaran

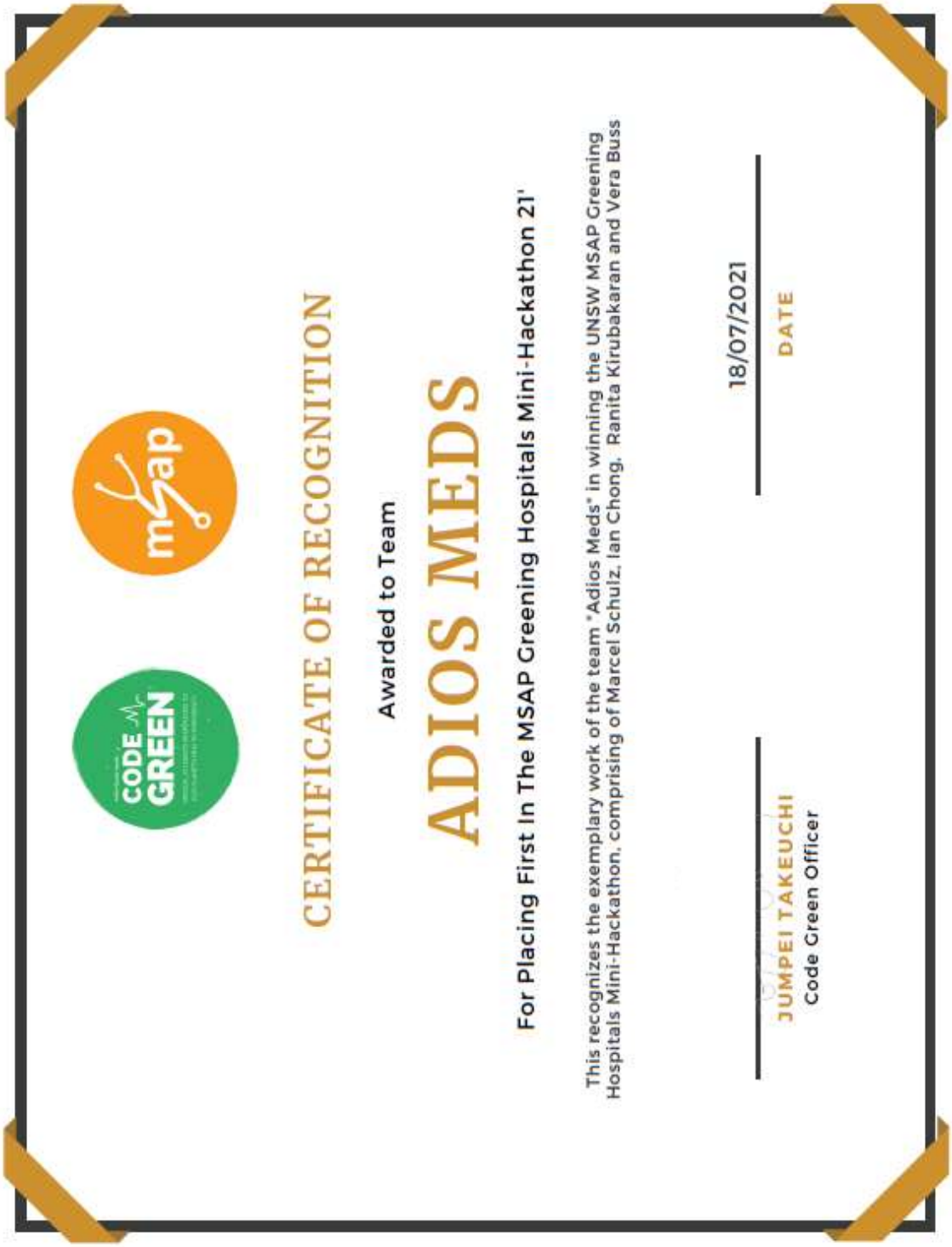
PhD (Medicine)

Malaysia

Nominated for outstanding contributions in:

- Active representative of the Faculty's Candidate Higher Degree Committee (CHDC) managing a HDR Faculty wide survey on enrolment, career development needs and mental health and wellbeing. Recipient of ARC Postgraduate Research Student Award in 2020
- Senior Cultural Mentor contributing to the international student experience through workshops, mentoring, organising online events and more. Aspire Ambassador serving students from diverse cultural and social backgrounds
- Tutor for junior medical and pharmacy students contributing to feedback on active learning situations and assessments
- Active contributor to the pharmacometrics community and part of St Vincent's Symposium organising committee. Faculty's winner of the 3-Minute Thesis (3MT) competition in 2021

Appendix F. Greening Hospitals Mini-Hackathon, UNSW Faculty of Medicine and Health (First prize).



Appendix G. Australian Research Council (ARC) UNSW Postgraduate Research Student Award.

UNSW SYDNEY

RESEARCH STUDENT AWARD

The Arc Postgraduate Council wish to acknowledge and thank

Ranita Kirubakaran

In recognition of your outstanding contribution to your research environment and higher degree research community.

This Award is also endorsed by the Dean of Graduate Research, Professor Jonathan Morris.

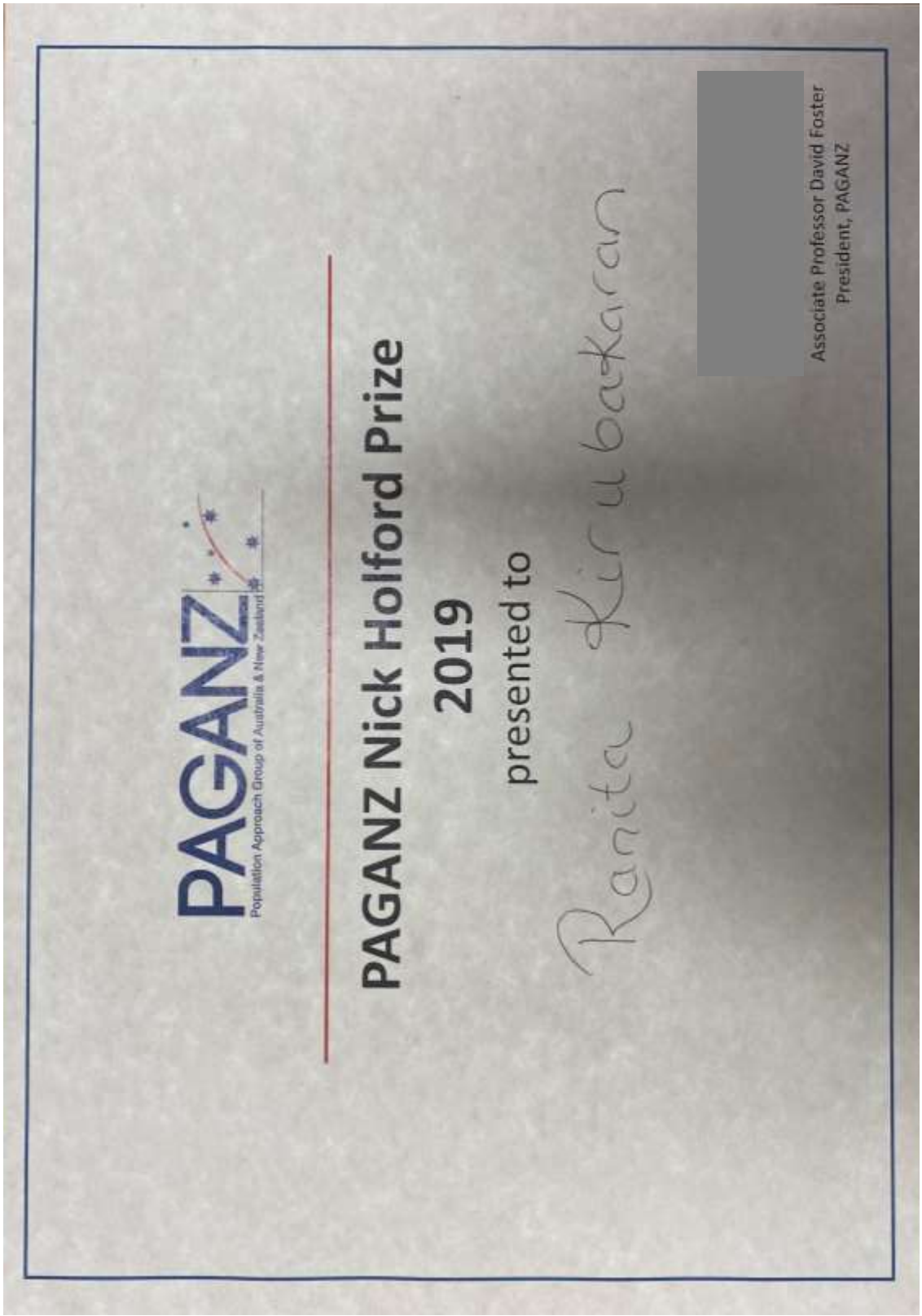
Dated 11 November 2020

Postgraduate Council UNSW

UNSW Student Life

UNSW SYDNEY | Graduate Research School

Appendix H. PAGANZ Nick Holford Prize for best oral presentation in Clinical Pharmacology and Pharmacometrics.



Appendix I. St. Vincent's Clinical School HDR Travel Award.

UNSW SYDNEY | Australia's Global University

St Vincent's Clinical School

2019 Travel Award

awarded to

Ranita Kirubakaran

dated this 23rd January 2019

Professor J Greenfield
Clinical Associate Dean
St Vincent's Clinical School

Appendix J. ASCEPT Clinical Pharmacology Special Interest Group Prize for best poster presentation in Clinical Pharmacology.