Should we abandon therapeutic drug monitoring of tacrolimus in whole blood and move to intracellular concentration measurements?

Udomkarnjananun, S.; Eiamsitrakoon, T.; Winter, B.C.M. de; Gelder, T. van; Hesselink, D.A.

Received: 25 August 2023 | Revised: 18 October 2023 | Accepted: 19 October 2023

DOI: 10.1111/bcp.15946

THEMED ISSUE REVIEW

Should we abandon therapeutic drug monitoring of tacrolimus in whole blood and move to intracellular concentration measurements?

Suwasin Udomkarnjananun[1,2,3,4] | Thanee Eiamsitrakoon[5] | Brenda C. M. de Winter[6,7] | Teun van Gelder[8] | Dennis A. Hesselink[7,9]

[1]Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand

[2]Excellence Center for Organ Transplantation (ECOT), King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand

[3]Renal Immunology and Transplantation Research Unit, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

[4]Center of Excellence on Translational Research in Inflammation and Immunology (CETRII), Department of Microbiology, Chulalongkorn University, Bangkok, Thailand

[5]Division of Nephrology, Department of Medicine, Faculty of Medicine, Thammasat University, Pathum Thani, Thailand

[6]Department of Hospital Pharmacy, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[7]Erasmus MC Transplant Institute, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

[8]Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, The Netherlands

[9]Department of Internal Medicine, Division of Nephrology and Transplantation, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Correspondence
Suwasin Udomkarnjananun, Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, 1873, Rama 4 road, Pathumwan, 10330 Bangkok, Thailand.
Email: suwasin.u@gmail.com

Funding information
National Research Council of Thailand, Grant/Award Number: N42A661041

The measurement of whole blood (WB) concentrations has been the primary method for therapeutic drug monitoring of tacrolimus since its introduction in the field of organ transplantation. However, >99% of tacrolimus measured in WB is bound to erythrocytes and plasma proteins, which are the pharmacologically inactive fractions. The pharmacologically active fractions, the free (or unbound) tacrolimus in plasma and the intracellular tacrolimus, make up 1% or less of the WB concentration. The mechanism of action of tacrolimus is to inhibit the enzyme calcineurin within T lymphocytes and, therefore, measuring the intralymphocytic tacrolimus concentration may better reflect its pharmacodynamic effects and better correlate with clinical outcomes. However, studies on intracellular tacrolimus concentrations have shown conflicting results. In this review, we argue that we need to overcome the analytical limitations of current assays for the measurement of intracellular tacrolimus before moving this technique into the clinical setting. The validity and standardization of the cell isolation process before the measurement of the intracellular tacrolimus concentration is as important as the measurement itself but has received little attention in our view. Recent evidence suggests that the addition of an inhibitor of P-glycoprotein, an efflux transporter expressed on lymphocytes, prevents the expulsion of tacrolimus during the cell isolation process. Refining the technique for the intracellular tacrolimus concentration measurement should be the focus followed by clinical evaluation of its association with rejection risk.

KEYWORDS
immunosuppressive drugs, intracellular concentration, peripheral blood mononuclear cells, T lymphocyte, tacrolimus, therapeutic drug monitoring, transplantation

1 | INTRODUCTION

Thirty-six years ago, **tacrolimus** was isolated from the fermentation broths of *Streptomyces tsukubaensis*, a bacterium found in the soil on the foothills of Mount Tsukuba in Ibaraki, Japan.[1,2] Originally identified as a macrolide, tacrolimus exhibited suppressive activity against alloreactive T lymphocytes in mixed lymphocyte reactions giving this *Mount Tsukuba macrolide immunosuppressant*, its name.[3] Interestingly, Mount Tsukuba is known in Japanese folklore for being blessed by a god, and it is described as a place with rich vegetation and abundant nature.[1] It is fascinating to consider a drug derived from an organism found on this god-blessed mountain, which has contributed so much to the success of solid organ transplantation.[4]

Since its discovery, tacrolimus rapidly became the cornerstone of maintenance immunosuppressive regimens in kidney transplantation. Numerous studies, including randomized controlled trials, registry studies and meta-analyses, consistently demonstrated the superiority of tacrolimus over its calcineurin (CN) inhibitor predecessor, **cyclosporine A**.[5–9] Tacrolimus has been shown to improve kidney allograft survival, reduce the incidence of acute rejection and better preserve kidney allograft function.[5–9] Tacrolimus withdrawal, avoidance or conversion is associated with an increased risk of allograft rejection.[10–14] Various modern studies now focus on low-exposure tacrolimus regimens rather than tacrolimus-free immunosuppression.[8,15–17] This further emphasizes the significance of tacrolimus in the current landscape of organ transplantation.

Despite its remarkable efficacy, tacrolimus' full potential may not have been realized yet. The high interpatient variability in tacrolimus exposure is 1 of the main reasons for performing therapeutic drug monitoring (TDM) of this drug. For this purpose, the whole blood (WB) concentration is routinely measured. With TDM, the tacrolimus dose is targeted to a *therapeutic* WB concentration range, but despite this, allograft rejection and toxicity still occur, even in patients who are *on target*.[18,19] In this paper, we argue that the outcomes of tacrolimus therapy may be improved further by performing TDM in a matrix other than WB, namely the intralymphocytic compartment.[20–22]

2 | THE CURRENT STATE AND LIMITATIONS OF TACROLIMUS TDM

The pharmacokinetic properties of tacrolimus make it a typical drug that benefits from TDM (Figure 1). The measurement of WB tacrolimus concentrations is the classic TDM approach to optimize the balance between toxicity and efficacy (i.e. the prevention of rejection). For this purpose, the tacrolimus predose (C_0) is routinely used, although some centres rely on more rich pharmacokinetic sampling.[20,23] Studies have consistently shown that higher WB predose concentrations of tacrolimus are associated with reduced risk of acute rejection but an increased risk of nephrotoxicity.[24–26] Conversely, lower tacrolimus predose WB concentrations are associated with a higher risk of acute rejection but lower risk of toxicity.[24–26] The therapeutic window for the WB predose tacrolimus concentration used to be wide, ranging from 5 to 15 ng/mL in the early phase after kidney transplantation.[24,25] Modern therapeutic targets have been adapted from the landmark ELITE-SYMPHONY study, which targeted

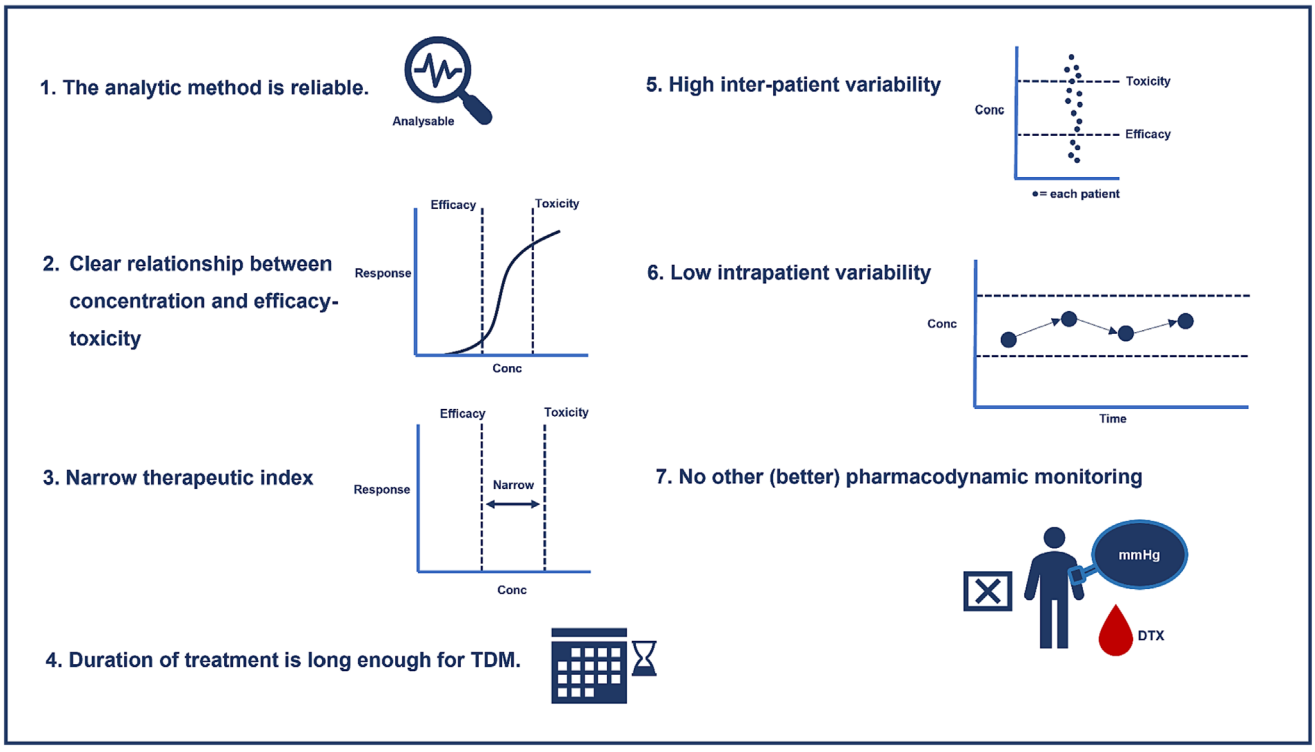

FIGURE 1 Concept of medications requiring therapeutic drug monitoring.

tacrolimus to 3–7 ng/mL.[8] This study demonstrated the superiority of tacrolimus over cyclosporine A and sirolimus-based regimens in terms of kidney allograft survival and acute rejection incidence. However, careful interpretation of this target range is essential since the actual mean predose tacrolimus concentration throughout the study period was ~6–7 ng/mL, which was at the upper end of the target range. Subsequent studies confirmed that WB predose concentrations <5–6 ng/mL are associated with allograft rejection and the formation of de novo donor-specific anti-human leucocyte antigen antibodies, while concentrations >8–10 ng/mL were associated with increased infection risk, neurotoxicity, nephrotoxicity and post-transplant diabetes mellitus.[27–32] The Consensus on Managing Modifiable Risk in Transplantation Group recommended a predose tacrolimus WB concentration of 5–10 ng/mL in the first year after transplantation when used together with **mycophenolic acid** (MPA).[33]

Nonetheless, kidney transplant recipients on tacrolimus therapy may still experience acute rejection despite having an exposure within the recommended 5–10 ng/mL target concentration range. This phenomenon was highlighted by Bouamar *et al*., where combined data from 3 large, randomized, controlled trials were analysed (the ELITE-SYMPHONY, FDCC and OPTICEPT studies).[34] In these trials, all kidney transplant recipients were on a regimen of tacrolimus and MPA, and the median tacrolimus predose concentrations were within the 6–11 ng/mL range. There was no significant difference in tacrolimus concentrations between recipients who experienced acute rejection and those who did not.

This study provided important insights into the complexities of tacrolimus therapy and TDM in kidney transplant recipients. While target predose tacrolimus concentrations <5 ng/mL are associated with an increased risk of rejection and concentrations >10 ng/mL may lead to toxicity, the study highlights the limitations of relying solely on these *coarse* adjustments within the so-called *therapeutic range* (5–10 ng/mL). The *fine adjustment* for tacrolimus exposure within the therapeutic range remains poorly understood. For instance, the question arises whether a tacrolimus concentration of 6 ng/mL would result in a similar incidence of allograft rejection compared to a level of 9 ng/mL.

Moreover, evidence has shown that identical tacrolimus predose WB concentrations do not necessarily indicate comparable total drug exposure, as measured by the area under the concentration *vs*. time curve (AUC), among different individuals. This phenomenon is particularly observed in recipients exhibiting a flat peak concentration pattern of the tacrolimus AUC, which contrasts with the normal peak pattern observed in the majority of recipients.[35] In addition, the same predose concentrations of tacrolimus may lead to varying exposure among recipients with high intrapatient variability. In recipients with high intrapatient variability, there is a possibility of unrecognized drops in tacrolimus concentrations, thereby increasing the risk of acute rejection, despite apparent concentrations remaining within the therapeutic range during each hospital visit.[20,29,36,37] Certain recipients, who require a high dose of tacrolimus to reach the desired target C_0, exhibit a low C_0/tacrolimus dose ratio. This pharmacokinetic profile leads to elevated peak concentrations compared to recipients with a high C_0/tacrolimus dose ratio. This increased tacrolimus peak concentration is associated with an increased risk of nephrotoxicity, BK virus infection and graft loss.[38] These studies have only begun to uncover the complexities surrounding the estimation of tacrolimus exposure and the importance of TDM.

Based on the available evidence, it is undeniable that monitoring WB tacrolimus has considerably improved the outcomes of tacrolimus therapy. Nonetheless, it remains imperfect. Shifting our focus to another dimension of TDM, the integration of pharmacodynamic monitoring of tacrolimus could potentially emerge as a pivotal tool for refining the TDM process of this drug. By supplementing predose concentration measurements, this approach has the potential to provide invaluable insights into how an individual patient responds to the medication, thus enabling the customization of treatment protocols accordingly.[39–41] Nevertheless, it is crucial to acknowledge the existing limitations within the current landscape of pharmacodynamic monitoring of tacrolimus, which presently limit its integration into routine clinical practice.

3 | LIMITATIONS OF PHARMACODYNAMIC MONITORING AND ALTERNATIVES

Treatment with several drugs can be monitored by measuring their pharmacodynamic activity. For instance, the dose of vitamin K antagonists (such as warfarin) can be based on the international normalized ratio, insulin treatment can be guided by blood glucose and HbA1c concentrations, and antihypertensive drug therapy can be guided by individual blood pressure measurements. Regrettably, immunosuppressive agents currently lack reliable and reproducible pharmacodynamic monitoring tools that provide feedback on a recipients' immune status, enabling dosage adjustments before rejection occurs. There remains a critical need for the development of pharmacodynamic monitoring tools to enhance therapeutic precision and patient outcomes of immunosuppressive therapy.

Examples of approaches for pharmacodynamic monitoring of tacrolimus include monitoring of CN phosphatase activity, nuclear translocation of nuclear factor of activated T lymphocytes (NFAT) or NFAT-regulated gene expression, and of less specific immunological biomarkers such as T lymphocyte-produced cytokines (e.g. interleukin [IL]-2 production), the expression of T lymphocyte surface activation markers (e.g. CD25, CD134, CD137 or CD154) and intracellular adenosine triphosphate changes.[39,40,42,43] Although most studies linking the pharmacodynamic biomarkers of tacrolimus with clinical outcome were small and explorative in nature, some studies were able to demonstrate such an association more reliably. For example, the NFAT-regulated gene expressions of IL-2, interferon-γ and granulocyte–macrophage colony-stimulating factor were shown to correlate with acute rejection and recurrent infections in kidney transplant recipients receiving tacrolimus. Recipients with biopsy-proven acute rejection showed significantly higher levels of residual NFAT-regulated gene expression, and vice versa for recipients with recurrent infection.[44]

Another study in liver transplant recipients demonstrated a significantly higher CN activity in recipients with acute rejection compared to recipients without rejection.[45] However, despite these promising findings, these pharmacodynamic biomarker studies have not been incorporated into routine monitoring due to their lack of external validation, labour intensiveness and the requirement for expensive instruments. Furthermore, the sample sizes of these biomarker studies have mainly been limited to proof-of-concept analyses, and they have not been sufficient to demonstrate an impact on clinical practice. Furthermore, the protocols for measurement and analyses of these biomarkers are complex and vary across different transplantation laboratories, requiring standardization for broader application.

Nonetheless, the potential benefits of pharmacodynamic biomarkers are significant. If these biomarkers are validated and become available in the future, tacrolimus dose adjustments could be made proactively as soon as signs of rejection begin to emerge. This proactive approach would allow for fine-tuning of the drug before allograft injury occurs, potentially leading to improved outcomes and a reduction in rejection episodes.

4 | ALTERNATIVES FOR WB TDM OF TACROLIMUS

Given the shortcomings of traditional WB-based TDM and in the absence of reliable pharmacodynamic biomarkers for tacrolimus, other strategies for TDM have been explored. When tacrolimus is considered in WB, approximately 80–85% of the drug binds to erythrocytes, as they contain abundant intracellular **FK-binding protein**, the target protein of tacrolimus.[20,46,47] The remaining 15% of tacrolimus in WB is bound to plasma proteins.[47,48] However, tacrolimus bound to erythrocytes and plasma proteins is pharmacologically inactive, and only the unbound fraction and the tacrolimus present within the leucocyte compartment are pharmacologically active. As a result, this active component (i.e. the intracellular tacrolimus concentration) accounts for merely 1% (with variability among patients) of the WB tacrolimus concentration measured in clinical practice.

The correlation between intracellular drug concentrations and clinical efficacy is perhaps best demonstrated in studies of antiretroviral therapy.[49] A study of zidovudine and lamivudine showed that intracellular concentrations of these drugs were significantly correlated with changes in human immunodeficiency virus (HIV) viral load, while there was no association between their plasma concentrations and efficacy.[50] The median time to suppressed viral load was significantly shorter in patients with HIV infection who had intracellular zidovudine concentration higher than 30 fmol/million cells, compared to those with intracellular zidovudine concentration below this threshold.[51]

Given that the mechanism of action of tacrolimus involves inhibiting CN activity within T lymphocytes, the intracellular tacrolimus concentration may better represent the pharmacologically active component compared to WB concentration.[52,53] Recent studies conducted by a Spanish group demonstrated a significantly negative correlation between the peak concentration of intracellular tacrolimus and the maximum inhibition of CN in stable kidney transplant recipients receiving extended-release tacrolimus.[54] However, the same correlation could not be established between the WB concentration and CN activity, suggesting that the intracellular tacrolimus concentration may provide a more accurate explanation of the pharmacodynamic action at the intracellular level.[52,55] Other studies have also shown an association between intracellular tacrolimus concentrations and pharmacodynamic parameters including CN inhibition and intracellular IL-2 and interferon-γ production.[56–58]

5 | PROGRESS IN INTRACELLULAR TACROLIMUS CONCENTRATION MEASUREMENT

The first study to demonstrate the potential of the intracellular tacrolimus concentration for clinical practice was conducted in liver transplant recipients. The authors reported a significant association between decreased intracellular tacrolimus concentrations in peripheral blood mononuclear cells (PBMCs) and higher Banff scores for acute liver allograft rejection.[59] Conversely, they did not observe any correlations between WB tacrolimus concentrations and Banff scores for acute rejection. This pioneering study paved the way for subsequent research exploring the use of intracellular tacrolimus concentration in predicting and diagnosing allograft rejection. However, subsequent studies have not replicated the correlation between intracellular tacrolimus concentration and acute rejection, leading to conflicting results.[59–65] The summary of characteristics of studies investigating intracellular tacrolimus concentrations and clinical outcomes in solid organ transplantation is shown in Table 1.

Several aspects require careful consideration when interpreting the conflicting results of the above-mentioned studies on the intracellular tacrolimus concentration. First, the study by Capron *et al*., which demonstrated the association between acute rejection and intracellular tacrolimus concentration, was conducted in liver transplant recipients who received tacrolimus monotherapy.[59] Consequently, the association between tacrolimus and the risk of rejection was not interfered with by other immunosuppressive medications, unlike in later studies conducted in solid organ transplant recipients receiving standard, triple immunosuppression. Second, the timing of intracellular tacrolimus measurements is crucial. For instance, in protocols that involve a single time-point measurement of the intracellular tacrolimus concentration, the correlation with acute rejection may be less when the measurement is performed relatively remote from the clinical event.[60,62] Third, to ensure accurate measurement of intracellular tacrolimus concentration and avoid falsely elevated results, a red blood cell lysis step is necessary to purify PBMCs and eliminate erythrocytes from the sample. Previous studies have clearly shown that without red blood cell lysis and washing steps, intracellular tacrolimus concentrations in PBMCs can be 38–58% higher, compared to when the red blood cell lysis steps is applied.[66] The red blood cell lysis buffer was incubated with PBMCs and subsequently washed.

TABLE 1 Summary of studies exploring the association between intracellular tacrolimus concentrations in PBMCs and clinical outcomes of solid organ transplantations.

Authors	Transplant organ	Number of intra-TAC samples and number of recipients	Cell population	Sampling times	Red blood cell lysis used	WB-intracellular tacrolimus correlation	Clinical outcome associations	Other findings
Capron et al. 2012 (ref. 59)	Liver	360 samples from 90 recipients	PBMCs	Day 1, 3, 5 and 7 post-transplant (C_0)	No	$r^2 = 0.01$	Recipients with rejection had lower intra-TAC at Days 3, 5 and 7 compared to recipients without rejection	• No correlation between WB tacrolimus and rejection • Intrahepatic tacrolimus at Day 7 was significantly lower in recipients with rejection
Klaasen et al. 2018 (ref [60])	Kidney	87 samples from 29 recipients	PBMCs	Week 1, 6 and 52 post-transplant (C_0 and $C_{1.5}$)	No	$r = 0.59$, -0.32 and 0.53 for C_0 $r = 0.82$, 0.40 and 0.79 for $C_{1.5}$	No association between intra-TAC and rejection	• Intra-TAC increased from predose to 1.5-h postdose the first week after transplantation, but not at 6-week or 1-year post-transplant
Rayer et al. 2018 (ref. [61])	Liver	181 samples from 41 recipients	PBMCs	Daily for the first 7 days post-transplant (C_0)	No	$r = 0.53$	No association between intra-TAC and LFT or neurotoxicity	• Tacrolimus concentration in bile correlated with LFT and neurotoxicity
Francke et al. 2020 (ref. [62])	Kidney	358 samples from 175 recipients	PBMCs	Month 3, 6 and 12 post-transplant (C_0)	No	$r = 0.31$, 0.41 and 0.61, respectively	No association between intra-TAC and rejection or nephrotoxicity	• Intra-TAC-to-WB ratio was associated with age, albumin and haematocrit
Francke et al. 2022 (ref. [63])	Kidney	83 samples from 44 recipients	PBMCs	Day 3 and 10 post-transplant, and on the same morning of for-cause biopsy (C_0)	Yes	$r = 0.56$ and 0.20 on Day 3 and 10 post-transplant	No association between intra-TAC and rejection	• Median interoccasion variability 19.4% (dose-corrected intra-TAC)
Udomkarnjananun et al. 2022 (ref. [64])	Kidney	61 samples from 53 recipients	PBMCs, T lymphocytes, monocytes	On the same morning of for-cause biopsy (C_0)	Yes	$r = 0.27$ for PBMC $r = 0.43$ for T lymphocytes, $r = 0.58$ for monocytes	No association between intra-TAC and rejection (PBMCs, T lymphocytes and monocytes)	• T lymphocytes had much lower intra-TAC than monocytes. • Cryopreserved samples had lower intra-TAC than freshly isolated samples

(Continues)

TABLE 1 (Continued)

Authors	Transplant organ	Number of intra-TAC samples and number of recipients	Cell population	Sampling times	Red blood cell lysis used	WB-intracellular tacrolimus correlation	Clinical outcome associations	Other findings
Coste et al. 2023 (ref. [65])	Heart	97 samples from 22 recipients	PBMCs	Routine out-patient follow-up (C_0) (median time 5.3 months post-transplant)	No	$r^2 = 0.28$	No association between intra-TAC and rejection	• Coefficient variability (CV%) = 65.2% (raw intra-TAC)

Abbreviations: C_0, predose concentration; intra-TAC, intracellular tacrolimus concentration; LFT, liver function test; PBMCs, peripheral blood mononuclear cells; *r*, Pearson's or Spearman's correlation coefficient; r^2, coefficient of determination; WB, whole blood.

Following this washing process, the PBMC pellet became clear, devoid of any red colour, in contrast to its appearance before incubation with the solution. A single washing step using the red blood cell lysis buffer proved sufficient to purify the PBMC pellet. This method offers greater reliability in eliminating erythrocytes and the associated contamination of intracellular tacrolimus in PBMCs, in comparison to the traditional method relying on a visual scale for assessing the redness of the cell pellet.[62,66] Finally, since the action of tacrolimus is to inhibit CN activity within T lymphocytes and the proportion of T lymphocytes in PBMCs can vary among individuals, using total PBMCs as the matrix of measurement could lead to additional confounding from irrelevant cells.[67]

To address these limitations, we recently performed a study to overcome some of these limitations.[64] In this study, PBMCs, T lymphocytes and monocytes were used as the matrices for measuring intracellular tacrolimus, and additional red blood cell lysis steps were incorporated to ensure accurate measurement.[64] The timing of sample collection was synchronized with the predose tacrolimus concentration sampling and was performed on the same day as the kidney allograft biopsy. Again, this study did not demonstrate a significant association between intracellular tacrolimus concentrations in T lymphocytes, monocytes or PBMCs and acute rejection. However, the study did reveal 2 important findings. First, the intracellular tacrolimus concentrations in T lymphocytes were found to be much lower than in monocytes, with median values of 12.8 and 81.6 pg/million cells, respectively. Second, cryopreserved samples from retrospectively enrolled recipients exhibited significantly lower intracellular tacrolimus concentrations compared to samples that did not undergo the freeze and thawing processes. This observation suggested that tacrolimus may be lost during the freeze–thaw process.

To gain a deeper understanding of the differences in intracellular tacrolimus concentrations between T lymphocytes and monocytes, a subsequent study delved into the underlying mechanisms.[68] Through the use of flow cytometric analyses for cell surface markers and intracellular cytokines, western blotting and rhodamine-123 activity measurement, the authors confirmed that T lymphocytes possess higher activity of **P-glycoprotein** (P-gp),[69] which serves as the efflux transporter of tacrolimus,[70,71] and lower FK-binding protein (the tacrolimus receptor) levels compared to monocytes. These molecular differences explain why in T lymphocytes intracellular tacrolimus concentrations are lower than in monocytes. Furthermore, the study revealed that when the cell isolation process is conducted without the use of a P-gp inhibitor (**verapamil**), a significant amount of tacrolimus is lost from T lymphocytes via the P-gp-mediated efflux. In this study, verapamil was promptly added to the blood immediately after collection and to each solution used in the cell isolation process, ensuring the preservation of intracellular tacrolimus. We believe these findings once again highlight the importance of optimization of the intracellular tacrolimus assay and that this can be made more accurate by incorporating a P-gp inhibitor during the cell isolation process.[68] Notably, the effectiveness of a selective P-gp inhibitor, **PSC833**, in preventing tacrolimus efflux during cell isolation has not been assessed. Consequently, it would be intriguing to explore the impact of different P-gp

inhibitors by comparing PSC833 with verapamil in preserving intracellular tacrolimus during the cell isolation process.[72]

Cell isolation under cold (4°C) temperature was proposed as another method to prevent tacrolimus efflux by P-gp. However, it is important to note that various steps of the process require a consistent temperature to obtain an adequate number of T lymphocytes for measuring intracellular tacrolimus concentration. Altering the temperature during these steps can lead to reduced cell yield, lower cell viability, and increased contamination by granulocytes and erythrocytes (Ficoll-Paque Plus, GE Healthcare, Uppsala, Sweden, and pan T cell isolation kits, Miltenyi Biotec, Bergisch Gladbach, Germany). This can result in an insufficient number of T lymphocytes for the measurement of intracellular tacrolimus concentration. Therefore, we recommend the use of a P-gp inhibitor over cold centrifugation, as the latter may lead to decreased cell isolation yield or necessitate the collection of a larger blood volume.

6 | WHAT NEEDS TO BE DONE BEFORE IMPLEMENTATION OF INTRACELLULAR TACROLIMUS CONCENTRATION MEASUREMENT INTO CLINICAL PRACTICE?

Although the expert consensus from the International Association of Therapeutic Drug Monitoring and Clinical Toxicology provides detailed guidelines on the cell isolation process and the method for measuring intracellular tacrolimus concentration,[53] several questions and challenges remain to be answered before the measurement of the intracellular tacrolimus concentration can be implemented in routine TDM.

The first challenge involves the technical intricacies of the cell isolation process and the use of drug transporter inhibition. As tacrolimus in WB of recipients is in steady state, the goal is to capture a *snapshot* of the intracellular tacrolimus concentration ex vivo to reflect its in vivo state. Failure to add a P-gp inhibitor during the cell isolation process may result in the loss of intracellular tacrolimus from T lymphocytes and PBMCs, leading to less precise measurements. While the impact of genetic polymorphisms of ***ABCB1***,[69] the gene that encodes P-gp, on the need for P-gp inhibitors during cell isolation remains unexplored, it is possible that recipients with specific *ABCB1* alleles that decrease P-gp expression may not require such inhibitors.[73] Nonetheless, current evidence supports the empirical practice of adding P-gp inhibitors to every sample, as it has shown no adverse effects. The requirement of adding a P-gp inhibitor to the blood sample immediately after it is drawn and the need for the cell isolation process to be completed as soon as possible (to prevent potential loss of tacrolimus from the cells) are labour-intensive steps that may limit the feasibility of this approach in routine clinical practice.

Indeed, beyond P-gp, there is a possibility that the intracellular tacrolimus concentration is affected by other unidentified and relevant efflux transporters. However, the current evidence indicates that P-gp is the only relevant efflux transporter for tacrolimus in PBMCs.[52,68,74] The role of *influx* transporters in mediating the uptake of tacrolimus into cells remains an area of active research and is not yet fully elucidated.[75] In a recent French study, several potential influx transporters were investigated and these included organic anion transporter, organic anion transporting polypeptide, concentrative nucleoside transporter 3 (CNT3) and equilibrative nucleoside transporter 1 (ENT1), as candidates for tacrolimus uptake into cells. The experiments involving organic anion transporter and organic anion transporting polypeptide did not show a significant influence on the uptake of tacrolimus in PBMCs, suggesting that these transporters do not play a major role in facilitating the entry of tacrolimus into cells.[76] The experiments of CNT3 and ENT1 showed some correlation between protein expression and the intracellular tacrolimus-to-WB concentration ratio. However, the overexpressing cellular models used in this study could not conclusively demonstrate the definitive roles of CNT3 and ENT1 in tacrolimus uptake.[74] Further research is needed to determine whether these transporters indeed contribute significantly to the influx of tacrolimus. When contemplating the involvement of influx transporters in the cell isolation process, wherein cells are isolated in a tacrolimus-free medium, the significance of influx transporters may be less pronounced in comparison to the efflux transporters.

The intracellular tacrolimus concentration is typically expressed as a unit of mass per a specific number of cells, such as pg/million cells. However, there is currently no specific recommendation for the method of cell counting, whether it involves automated machinery or manual counting.[53] The use of automated counting systems offers the advantage of increased reproducibility and reduced variability across different centres. However, their precision diminishes when the cell count falls below the lower limit of detection, which is not an uncommon scenario for transplant recipients. Additionally, these systems provide information on mean cell volume, which can be valuable for normalizing intracellular tacrolimus concentrations in matrices containing diverse cell types, such as PBMCs.[77] Future research should investigate the comparison between different units of measurement (e.g. pg/million cells *vs.* pg/mL normalized with mean cell volume) and their associations with clinical outcomes. The use of highly sensitive assays, such as liquid chromatography–tandem mass spectrometry, for measuring intracellular tacrolimus presents a challenge due to its cost and availability, which may impact its widespread adoption in standard clinical practice.[53,66]

Another aspect that requires consideration is the choice of matrix for measuring the intracellular tacrolimus concentration. Initially, the intracellular tacrolimus concentration in PBMCs appeared to be a more reliable representation than WB concentration. However, since tacrolimus functions by inhibiting CN activity within T lymphocytes, utilizing T lymphocytes as a matrix for measuring intracellular tacrolimus concentration might enhance the clinical correlation compared to PBMC concentration. Other studies have demonstrated that B-cell development and their immunological response are also influenced by the CN phosphatase complex and NFAT.[78,79] This suggests that not only T lymphocytes but other cell types may also be affected by tacrolimus. The correlation between intracellular tacrolimus concentrations in each PBMC subpopulation and their corresponding

pharmacodynamic effects needs to be evaluated. Nevertheless, the correlation between intracellular tacrolimus concentration in each cell type and its pharmacodynamic effects might not be linear and may vary among cell types. Consequently, a pharmacokinetic model at the cellular level for each subpopulation in PBMCs (T lymphocytes, B lymphocytes, monocytes and natural killer lymphocytes) might be necessary. However, determining such a model would require a large volume of blood to extract sufficient amounts of each cell type, which is currently not feasible with the available cell isolation technology.

The second issue related to the measurement of intracellular tacrolimus concentration is its clinical application. We conducted a systematic review on PubMed, utilizing the following MeSH term: ('Leukocytes, Mononuclear'[Mesh]) AND 'Tacrolimus'[Mesh], to identify prior studies that employed intracellular tacrolimus concentration at the predose timepoint for assessing its correlation with acute rejection episodes (Table 1). It was recently demonstrated that a strong correlation exists between the intracellular tacrolimus predose concentration and its AUC up to 24 h (Pearson's correlation coefficient = 0.927).[54] This finding should, however, be confirmed in future studies. It is possible that other concentrations, such as peak concentration, could also serve as surrogates for total exposure of intracellular tacrolimus concentration. Determining the optimal timepoint for measurement is another critical consideration. Potential timepoints include measuring the intracellular concentration at the time of allograft dysfunction (either on the same day or in the previous week), exploring the time-average chronic exposure (e.g. median or mean intracellular tacrolimus concentration in the past 6 months before rejection occurs), and assessing the intrapatient variability of intracellular tacrolimus concentration.

If a correlation between efficacy, toxicity and intracellular tacrolimus concentration is established and the target concentration range is known, a pharmacokinetic model for predicting intracellular tacrolimus concentration from WB concentration would also be valuable. This is especially important considering that the process of cell isolation and the measurement technique can be complex and may not be available in every transplant centre. One study described the steady-state WB-to-intracellular concentration ratio of tacrolimus in PBMCs using an effect compartment. The authors found that lean body weight and haematocrit were associated with the ratio, and by incorporating these variables into the model, the WB-to-intracellular concentration ratio can be accurately predicted.[80] Additionally, it is important to investigate whether the ratio of WB-to-intracellular concentration remains stable over time after transplantation. If this ratio proves to be consistently stable, a single measurement of this ratio during the early post-transplantation period could hold valuable benefits. This measurement could then serve as a reference value for each individual patient.

It is important to acknowledge that transplant recipients not only receive tacrolimus but also other immunosuppressive medication such as MPA or **mammalian target of rapamycin** inhibitor. Both of these drugs have pharmacologically active and inactive components in WB or plasma concentrations.[81–83] Similar to tacrolimus, the pharmacologically active part of mammalian target of rapamycin inhibitor and MPA (unbound form or intracellular compartment) should be targeted for possible improvement in TDM that better correlates to their pharmacodynamics. The absence of a correlation between WB tacrolimus concentration and allograft rejection may be attributed to suboptimal active components of other immunosuppressive medications, such as

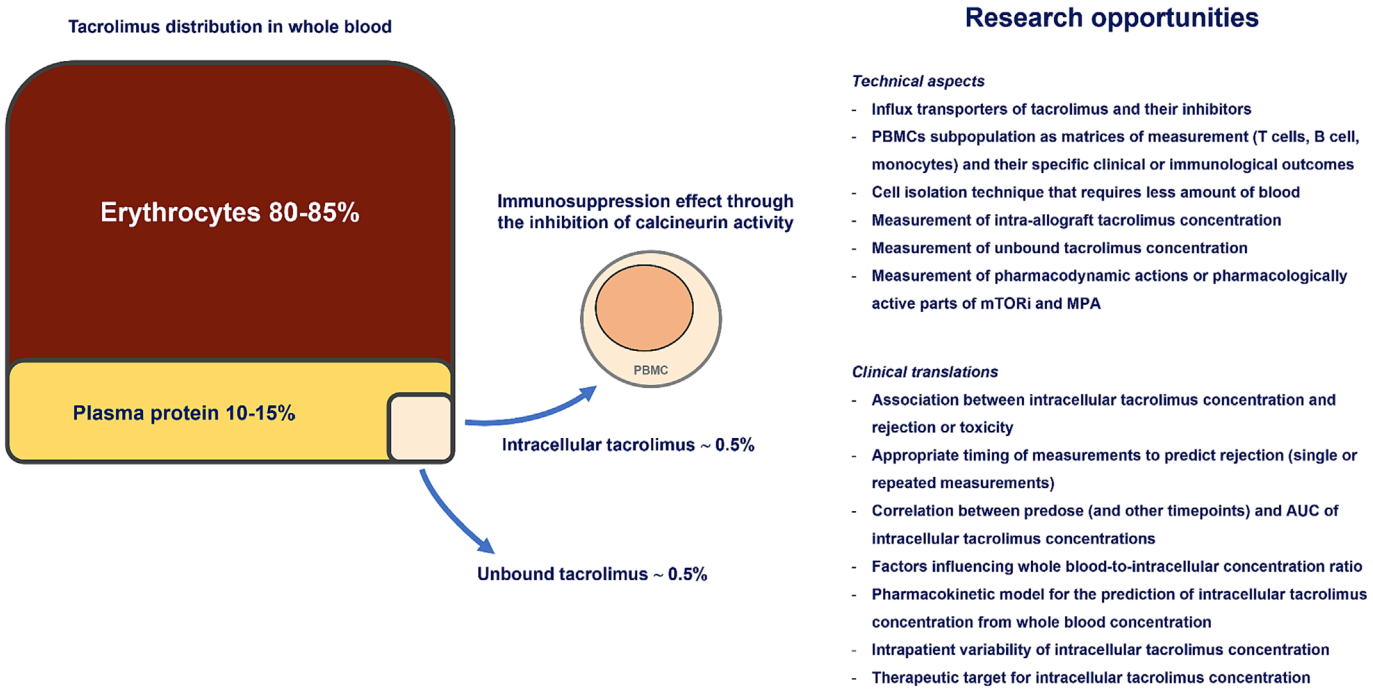

FIGURE 2 Current concept and research opportunities for intracellular tacrolimus concentration. AUC, area under the curve; MPA, mycophenolic acid; mTORi, mammalian target of rapamycin inhibitor; PBMC, peripheral blood mononuclear cell.

MPA or corticosteroids, which should also be a focal point for the measurement of their pharmacologically active constituents. Furthermore, certain pharmacodynamic biomarkers, such as IL-2 or CD25, lack specificity for any particular immunosuppressants.[84] Additional investigations are warranted to establish the association between intracellular or active components of immunosuppressive medications and their specific pharmacodynamic biomarkers. Detailed information on this subject is extensively covered in the review article and consensus guideline.[20,39] Figure 2 illustrates the concept and current limitations of intracellular tacrolimus concentration measurement.

7 | CONCLUSIONS: SHOULD WE ABANDON TACROLIMUS WB TDM AND MOVE TO INTRACELLULAR CONCENTRATION MEASUREMENT?

The answer is *not yet* for now but might become *yes* in the not-so-distant future. The measurement of the intracellular tacrolimus concentration is still in its early stages of development and not ready for routine clinical implementation. However, the potential benefits and promising findings from initial studies suggest that further exploration and well-designed studies are warranted to address the technical issues and clinical applications of this novel approach.[85] We will need more than a single study to answer all of the relevant questions. As we continue to refine and optimize the measurement techniques, address technical challenges and conduct further studies, we move closer to unlocking the full potential of intracellular tacrolimus monitoring. Future advancements in cell isolation technology may also help streamline the process and allow for more precise analysis with reduced blood volume requirements, making it more feasible for routine clinical practice. Moreover, the techniques established for the measurement of intracellular tacrolimus concentration in PBMCs may be applicable to the measurement of intragraft tacrolimus concentrations, as studies have shown evidence that local tacrolimus concentrations in the graft might be associated with rejection and nephrotoxicity.[59,86]

Currently, the WB concentration remains the primary and most widely used method for TDM of tacrolimus in clinical practice. At present, TDM of intracellular tacrolimus concentration is still in the analytical validation phase. Once the measurement of intracellular tacrolimus concentration becomes available and its technical aspects are refined, the next step would be to establish its association with clinical outcomes, during the clinical validation phase. During this period, it will be essential to establish a target therapeutic concentration range that correlates with efficacy and toxicity through a concentration-controlled study. The therapeutic target of intracellular tacrolimus concentration might fall somewhere within the recommended ranges of WB concentration that have been established based on the current use of tacrolimus in modern immunosuppressive regimens. Finally, it will be necessary to demonstrate the clinical utility of intracellular tacrolimus concentration measurement, whether it is better than WB concentration monitoring, in a randomized, controlled trial.[85] Alternatively, the combination of traditional WB concentration-based TDM with intracellular concentration monitoring might serve as a fine-tuning tool for tacrolimus therapy, providing clinicians with additional information to optimize immunosuppressive regimens for transplant recipients. We feel that at present, it is too early to replace traditional WB TDM with the intracellular tacrolimus concentration measurement as there are still many steps to go through, most importantly the clinical validation, before implementing into clinical practice. However, the future holds many research opportunities in this area.

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in. http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[69]

AUTHOR CONTRIBUTIONS

Suwasin Udomkarnjananun involved with study design, manuscript first draft, data reviewing and flow of the review. Thanee Eiamsitrakoon involved with manuscript review. Brenda C.M. de Winter and Teun van Gelder involved with manuscript review and edit. Dennis A. Hesselink involved with study design, manuscript review and edit.

ACKNOWLEDGEMENTS

The authors thank to the staffs and technicians at Transplant Institute of Erasmus MC, Rotterdam, the Netherlands, and King Chulalongkorn Memorial Hospital, Bangkok, Thailand, for providing great supports for the work of intracellular tacrolimus concentration measurement. This study was supported by the National Research Council of Thailand: N42A661041.

CONFLICT OF INTEREST STATEMENT

T. van Gelder has received lecture fees and consulting fees from Roche Diagnostics, Thermo Fisher, Vitaeris, CSL Behring, Astellas and Aurinia Pharma. In all cases, money has been transferred to hospital accounts, and none has been paid to his personal bank accounts. T. van Gelder does not have employment or stock ownership at any of these companies, and neither does he have patents or patent applications. D.A. Hesselink has received lecture fees and consulting fees from Astellas Pharma, Astra Zeneca, Chiesi Pharma, Medincell, Novartis Pharma, Sangamo Therapeutics and Vifor Pharma. He has received grant support from Astellas Pharma, Bristol-Myers Squibb and Chiesi Pharma (paid to his institution). D.A. Hesselink does not have employment or stock ownership at any of these companies, and neither does he have patents or patent applications. Other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data relevant to this study have been included in the manuscript.

ORCID

Suwasin Udomkarnjananun https://orcid.org/0000-0003-4087-9032

Teun van Gelder https://orcid.org/0000-0001-5980-6947

REFERENCES

1. Ng W, Ikeda S. Mount Tsukuba and the origin of tacrolimus. *Arch Dermatol*. 2009;145(3):284. doi:10.1001/archdermatol.2009.11
2. Tanaka H, Nakahara K, Hatanaka H, Inamura N, Kuroda A. Discovery and development of a novel immunosuppressant, tacrolimus hydrate. *Yakugaku Zasshi*. 1997;117(8):542-554. doi:10.1248/yakushi1947.117.8_542
3. Kino T, Hatanaka H, Hashimoto M, et al. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. *J Antibiot (Tokyo)*. 1987;40(9):1249-1255. doi:10.7164/antibiotics.40.1249
4. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. *Lancet*. 1989;2(8670):1000-1004. doi:10.1016/S0140-6736(89)91014-3
5. Yang K, Zhang M, Zhang B, Zhang Y, Zhao Q. Systematic review and meta-analysis of calcineurin inhibitors on long-term prognosis of renal transplant patients. *Transpl Immunol*. 2022;75:101741. doi:10.1016/j.trim.2022.101741
6. Webster A, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus cyclosporin as primary immunosuppression for kidney transplant recipients. *Cochrane Database Syst Rev*. 2005;(4):Cd003961. doi:10.1002/14651858.CD003961.pub2
7. McAlister VC, Haddad E, Renouf E, Malthaner RA, Kjaer MS, Gluud LL. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am J Transplant*. 2006;6(7):1578-1585. doi:10.1111/j.1600-6143.2006.01360.x
8. Ekberg H, Tedesco-Silva H, Demirbas A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med*. 2007;357(25):2562-2575. doi:10.1056/NEJMoa067411
9. Udomkarnjananun S, Townamchai N, Kerr SJ, et al. The first Asian kidney transplantation prediction models for long-term patient and allograft survival. *Transplantation*. 2020;104(5):1048-1057. doi:10.1097/TP.0000000000002918
10. Zeng J, Zhong Q, Feng X, et al. Conversion from Calcineurin inhibitors to mammalian target of rapamycin inhibitors in kidney transplant recipients: a systematic review and meta-analysis of randomized controlled trials. *Front Immunol*. 2021;12:663602. doi:10.3389/fimmu.2021.663602
11. Sharif A, Shabir S, Chand S, Cockwell P, Ball S, Borrows R. Meta-analysis of calcineurin-inhibitor-sparing regimens in kidney transplantation. *J am Soc Nephrol*. 2011;22(11):2107-2118. doi:10.1681/ASN.2010111160
12. Lim WH, Eris J, Kanellis J, et al. A systematic review of conversion from calcineurin inhibitor to mammalian target of rapamycin inhibitors for maintenance immunosuppression in kidney transplant recipients. *Am J Transplant*. 2014;14(9):2106-2119. doi:10.1111/ajt.12795
13. Sawinski D, Trofe-Clark J, Leas B, et al. Calcineurin inhibitor minimization, conversion, withdrawal, and avoidance strategies in renal transplantation: a systematic review and meta-analysis. *Am J Transplant*. 2016;16(7):2117-2138. doi:10.1111/ajt.13710
14. Vincenti F, Rostaing L, Grinyo J, et al. Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med*. 2016;374(4):333-343. doi:10.1056/NEJMoa1506027
15. Pascual J, Berger SP, Witzke O, et al. Everolimus with reduced calcineurin inhibitor exposure in renal transplantation. *J am Soc Nephrol*. 2018;29(7):1979-1991. doi:10.1681/ASN.2018010009
16. Bouamar R, Shuker N, Osinga JAJ, et al. Conversion from tacrolimus to everolimus with complete and early glucocorticoid withdrawal after kidney transplantation: a randomised trial. *Neth J Med*. 2018;76(1):14-26.
17. de Boer SE, Sanders JSF, Bemelman FJ, et al. Rationale and design of the OPTIMIZE trial: OPen label multicenter randomized trial comparing standard IMmunosuppression with tacrolimus and mycophenolate mofetil with a low exposure tacrolimus regimen in combination with everolimus in de novo renal transplantation in elderly patients. *BMC Nephrol*. 2021;22(1):208. doi:10.1186/s12882-021-02409-8
18. Holford N, Ma G, Metz D. TDM is dead. Long live TCI! *Br J Clin Pharmacol*. 2022;88(4):1406-1413. doi:10.1111/bcp.14434
19. Lemaitre F, Hesselink DA. TDM is alive and kicking! *Ther Drug Monit*. 2023;45(1):3-5. doi:10.1097/FTD.0000000000001034
20. Udomkarnjananun S, Francke MI, De Winter BCM, et al. Therapeutic drug monitoring of immunosuppressive drugs in hepatology and gastroenterology. *Best Pract Res Clin Gastroenterol*. 2021;54-55:101756. doi:10.1016/j.bpg.2021.101756
21. Ihara H, Shinkuma D, Ichikawa Y, Nojima M, Nagano S, Ikoma F. Intra- and interindividual variation in the pharmacokinetics of tacrolimus (FK506) in kidney transplant recipients--importance of trough level as a practical indicator. *Int J Urol*. 1995;2(3):151-155. doi:10.1111/j.1442-2042.1995.tb00444.x
22. Ong SC, Gaston RS. Thirty years of Tacrolimus in clinical practice. *Transplantation*. 2021;105(3):484-495. doi:10.1097/TP.0000000000003350
23. Scholten EM, Cremers SC, Schoemaker RC, et al. AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. *Kidney Int*. 2005;67(6):2440-2447. doi:10.1111/j.1523-1755.2005.00352.x
24. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation*. 1996;62(7):920-926. doi:10.1097/00007890-199610150-00009
25. Laskow DA, Vincenti F, Neylan JF, Mendez R, Matas AJ. An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: a report of the United States multicenter FK506 kidney transplant group. *Transplantation*. 1996;62(7):900-905. doi:10.1097/00007890-199610150-00005
26. Böttiger Y, Brattström C, Tydén G, Säwe J, Groth CG. Tacrolimus whole blood concentrations correlate closely to side-effects in renal transplant recipients. *Br J Clin Pharmacol*. 1999;48(3):445-448. doi:10.1046/j.1365-2125.1999.00007.x
27. Béland MA, Lapointe I, Noël R, et al. Higher calcineurin inhibitor levels predict better kidney graft survival in patients with de novo donor-specific anti-HLA antibodies: a cohort study. *Transpl Int*. 2017;30(5):502-509. doi:10.1111/tri.12934
28. Wiebe C, Rush DN, Nevins TE, et al. Class II Eplet mismatch modulates Tacrolimus trough levels required to prevent donor-specific antibody development. *J am Soc Nephrol*. 2017;28(11):3353-3362. doi:10.1681/ASN.2017030287
29. Davis S, Gralla J, Klem P, et al. Lower tacrolimus exposure and time in therapeutic range increase the risk of de novo donor-specific antibodies in the first year of kidney transplantation. *Am J Transplant*. 2018;18(4):907-915. doi:10.1111/ajt.14504
30. Yin S, Song T, Jiang Y, Li X, Fan Y, Lin T. Tacrolimus trough level at the first month may predict renal transplantation outcomes among living Chinese kidney transplant patients: a propensity score-matched analysis. *Ther Drug Monit*. 2019;41(3):308-316. doi:10.1097/FTD.0000000000000593
31. Ajabnoor A, Nasser M, Khan N, Habhab W. Evaluation of tacrolimus trough level in patients who developed post-transplant diabetes mellitus after kidney transplantation: a retrospective single-center study in Saudi Arabia. *Transplant Proc*. 2020;52(10):3160-3167. doi:10.1016/j.transproceed.2020.05.014

32. Israni AK, Riad SM, Leduc R, et al. Tacrolimus trough levels after month 3 as a predictor of acute rejection following kidney transplantation: a lesson learned from DeKAF genomics. *Transpl Int*. 2013;26(10):982-989. doi:10.1111/tri.12155
33. Neuberger JM, Bechstein WO, Kuypers DR, et al. Practical recommendations for long-term management of modifiable risks in kidney and liver transplant recipients: a guidance report and clinical checklist by the consensus on managing modifiable risk in transplantation (COMMIT) group. *Transplantation*. 2017;101(4S):1-56. doi:10.1097/TP.0000000000001651
34. Bouamar R, Shuker N, Hesselink DA, 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):1253-1261. doi:10.1111/ajt.12191
35. Kim SM, Lim Y, Min S, et al. Flat pattern peaks of tacrolimus absorption and associated Pharmacogenomic variants in kidney transplantation recipients. *J Korean Med Sci*. 2022;37(5):e33. doi:10.3346/jkms.2022.37.e33
36. Kuypers DRJ. Intrapatient variability of Tacrolimus exposure in solid organ transplantation: a novel marker for clinical outcome. *Clin Pharmacol Ther*. 2020;107(2):347-358. doi:10.1002/cpt.1618
37. Shuker N, Shuker L, van Rosmalen J, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. *Transpl Int*. 2016;29(11):1158-1167. doi:10.1111/tri.12798
38. van Gelder T, Meziyerh S, Swen JJ, de Vries APJ, Moes D. The clinical impact of the C(0)/D ratio and the CYP3A5 genotype on outcome in tacrolimus treated kidney transplant recipients. *Front Pharmacol*. 2020;11:1142. doi:10.3389/fphar.2020.01142
39. Brunet M, Shipkova M, van Gelder T, et al. Barcelona consensus on biomarker-based immunosuppressive drugs management in solid organ transplantation. *Ther Drug Monit*. 2016;38(Suppl 1):S1-S20. doi:10.1097/FTD.0000000000000287
40. Brunet M, van Gelder T, Åsberg A, et al. Therapeutic drug monitoring of tacrolimus-personalized therapy: second consensus report. *Ther Drug Monit*. 2019;41(3):261-307. doi:10.1097/FTD.0000000000000640
41. Shipkova M, Christians U. Improving therapeutic decisions: pharmacodynamic monitoring as an integral part of therapeutic drug monitoring. *Ther Drug Monit*. 2019;41(2):111-114. doi:10.1097/FTD.0000000000000627
42. van Rossum HH, de Fijter JW, van Pelt J. Pharmacodynamic monitoring of calcineurin inhibition therapy: principles, performance, and perspectives. *Ther Drug Monit*. 2010;32(1):3-10. doi:10.1097/FTD.0b013e3181c0eecb
43. He J, Li Y, Zhang H, et al. Immune function assay (ImmuKnow) as a predictor of allograft rejection and infection in kidney transplantation. *Clin Transplant*. 2013;27(4):E351-E358. doi:10.1111/ctr.12134
44. Sommerer C, Zeier M, Meuer S, Giese T. Individualized monitoring of nuclear factor of activated T cells-regulated gene expression in FK506-treated kidney transplant recipients. *Transplantation*. 2010;89(11):1417-1423. doi:10.1097/TP.0b013e3181dc13b6
45. Fukudo M, Yano I, Masuda S, et al. Pharmacodynamic analysis of tacrolimus and cyclosporine in living-donor liver transplant patients. *Clin Pharmacol Ther*. 2005;78(2):168-181. doi:10.1016/j.clpt.2005.04.008
46. Yoshikawa N, Yokota T, Matsuo A, Matsumoto N, Iwakiri T, Ikeda R. Role of FK506 binding protein on tacrolimus distribution in red blood cells. *Pharm Res*. 2020;37(7):143. doi:10.1007/s11095-020-02875-z
47. Zahir H, McCaughan G, Gleeson M, Nand RA, McLachlan AJ. Factors affecting variability in distribution of tacrolimus in liver transplant recipients. *Br J Clin Pharmacol*. 2004;57(3):298-309. doi:10.1046/j.1365-2125.2003.02008.x
48. 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):506-515. doi:10.1097/00007691-200410000-00008
49. Bazzoli C, Jullien V, Le Tiec C, Rey E, Mentré F, Taburet AM. Intracellular pharmacokinetics of antiretroviral drugs in HIV-infected patients, and their correlation with drug action. *Clin Pharmacokinet*. 2010;49(1):17-45. doi:10.2165/11318110-000000000-00000
50. Fletcher CV, Kawle SP, Kakuda TN, et al. Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. *Aids*. 2000;14(14):2137-2144. doi:10.1097/00002030-200009290-00010
51. Anderson PL, Kakuda TN, Kawle S, Fletcher CV. Antiviral dynamics and sex differences of zidovudine and lamivudine triphosphate concentrations in HIV-infected individuals. *Aids*. 2003;17(15):2159-2168. doi:10.1097/00002030-200310170-00003
52. Sallustio BC. Monitoring intra-cellular tacrolimus concentrations in solid organ transplantation: use of peripheral blood mononuclear cells and graft biopsy tissue. *Front Pharmacol*. 2021;12(2767):733285. doi:10.3389/fphar.2021.733285
53. Lemaitre F, Vethe NT, D'Avolio A, et al. Measuring intracellular concentrations of calcineurin inhibitors: expert consensus from the International Association of Therapeutic Drug Monitoring and Clinical Toxicology expert panel. *Ther Drug Monit*. 2020;42(5):665-670. doi:10.1097/FTD.0000000000000780
54. Fontova P, van Merendonk LN, Vidal-Alabró A, et al. The effect of intracellular tacrolimus exposure on calcineurin inhibition in immediate- and extended-release tacrolimus formulations. *Pharmaceutics*. 2023;15(5):1481. doi:10.3390/pharmaceutics15051481
55. Fontova P, Colom H, Rigo-Bonnin R, et al. Sustained inhibition of calcineurin activity with a melt-dose once-daily tacrolimus formulation in renal transplant recipients. *Clin Pharmacol Ther*. 2021;110(1):238-247. doi:10.1002/cpt.2220
56. Lemaitre F, Blanchet B, Latournerie M, et al. Pharmacokinetics and pharmacodynamics of tacrolimus in liver transplant recipients: inside the white blood cells. *Clin Biochem*. 2015;48(6):406-411. doi:10.1016/j.clinbiochem.2014.12.018
57. Han SS, Yang SH, Kim MC, et al. Monitoring the intracellular tacrolimus concentration in kidney transplant recipients with stable graft function. *PLoS ONE*. 2016;11(4):e0153491. doi:10.1371/journal.pone.0153491
58. Tron C, Woillard J-B, Houssel-Debry P, 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. doi:10.1371/journal.pone.0230195
59. Capron A, Lerut J, Latinne D, Rahier J, Haufroid V, Wallemacq P. Correlation of tacrolimus levels in peripheral blood mononuclear cells with histological staging of rejection after liver transplantation: preliminary results of a prospective study. *Transpl Int*. 2012;25(1):41-47. doi:10.1111/j.1432-2277.2011.01365.x
60. Klaasen RA, Bergan S, Bremer S, et al. Longitudinal study of tacrolimus in lymphocytes during the first year after kidney transplantation. *Ther Drug Monit*. 2018;40(5):558-566. doi:10.1097/FTD.0000000000000539
61. Rayar M, Tron C, Locher C, et al. Tacrolimus concentrations measured in excreted bile in liver transplant recipients: the STABILE study. *Clin Ther*. 2018;40(12):2088-2098. doi:10.1016/j.clinthera.2018.10.015
62. Francke MI, Hesselink DA, Li Y, et al. Monitoring the tacrolimus concentration in peripheral blood mononuclear cells of kidney transplant recipients. *Br J Clin Pharmacol*. 2020;87(4):1918-1929. doi:10.1111/bcp.14585
63. Francke MI, Andrews LM, Lan Le H, et al. Monitoring intracellular tacrolimus concentrations and its relationship with rejection in the early phase after renal transplantation. *Clin Biochem*. 2022;101:9-15. doi:10.1016/j.clinbiochem.2021.12.002

64. Udomkarnjananun S, Francke MI, Dieterich M, et al. The association between the intracellular tacrolimus concentration in $CD3^+$ T lymphocytes and $CD14^+$ monocytes and acute kidney transplant rejection. *Ther Drug Monit*. 2022;44(5):625-632. doi:10.1097/FTD.0000000000000982
65. Coste G, Chabanne C, Tron C, et al. Blood, cellular, and tissular calcineurin inhibitors pharmacokinetic-pharmacodynamic relationship in heart transplant recipients: the INTRACAR study. *Ther Drug Monit*. 2023;45(2):229-235. doi:10.1097/FTD.0000000000001025
66. Bahmany S, de Wit LEA, Hesselink DA, et al. Highly sensitive and rapid determination of tacrolimus in peripheral blood mononuclear cells by liquid chromatography-tandem mass spectrometry. *Biomed Chromatogr*. 2019;33(1):e4416. doi:10.1002/bmc.4416
67. Udomkarnjananun S, Dieterich M, Boer K, Hesselink DA, Baan CC. Immune subsets from Ficoll density gradient separation in kidney transplant recipients. Transplant. *Director*. 2022;8(5):e1319. doi:10.1097/TXD.0000000000001319
68. Udomkarnjananun S, Francke MI, Dieterich M, et al. P-glycoprotein, FK-binding protein-12, and the intracellular tacrolimus concentration in T-lymphocytes and monocytes of kidney transplant recipients. *Transplantation*. 2023;107(2):382-391. doi:10.1097/TP.0000000000004287
69. Alexander SPH, Kelly E, Mathie A, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: transporters. *Br J Pharmacol*. 2021;178(S1):S412-S513. doi:10.1111/bph.15540
70. Pendse SS, Briscoe DM, Frank MH. P-glycoprotein and alloimmune T-cell activation. *Clin Appl Immunol Rev*. 2003;4(1):3-14. doi:10.1016/S1529-1049(03)00007-2
71. Vafadari R, Bouamar R, Hesselink DA, et al. Genetic polymorphisms in ABCB1 influence the pharmacodynamics of tacrolimus. *Ther Drug Monit*. 2013;35(4):459-465. doi:10.1097/FTD.0b013e31828c1581
72. Atadja P, Watanabe T, Xu H, Cohen D. PSC-833, a frontier in modulation of P-glycoprotein mediated multidrug resistance. *Cancer Metastasis Rev*. 1998;17(2):163-168. doi:10.1023/A:1006046201497
73. Ieiri I, Takane H, Otsubo K. The MDR1 (ABCB1) gene polymorphism and its clinical implications. *Clin Pharmacokinet*. 2004;43(9):553-576. doi:10.2165/00003088-200443090-00001
74. Coste G, Robin F, Chemouny J, et al. Drug transporters are implicated in the diffusion of tacrolimus into the T lymphocyte in kidney and liver transplant recipients: genetic, mRNA, protein expression, and functionality. *Drug Metab Pharmacokinet*. 2022;47:100473. doi:10.1016/j.dmpk.2022.100473
75. Brunet M, Pastor-Anglada M. Insights into the pharmacogenetics of tacrolimus pharmacokinetics and pharmacodynamics. *Pharmaceutics*. 2022;14(9):1755. doi:10.3390/pharmaceutics14091755
76. Tron C, Allard M, Petitcollin A, et al. Tacrolimus diffusion across the peripheral mononuclear blood cell membrane: impact of drug transporters. *Fundam Clin Pharmacol*. 2019;33(1):113-121. doi:10.1111/fcp.12412
77. Simiele M, D'Avolio A, Baietto L, et al. Evaluation of the mean corpuscular volume of peripheral blood mononuclear cells of HIV patients by a coulter counter to determine intracellular drug concentrations. *Antimicrob Agents Chemother*. 2011;55(6):2976-2978. doi:10.1128/AAC.01236-10
78. Winslow MM, Gallo EM, Neilson JR, Crabtree GR. The calcineurin phosphatase complex modulates immunogenic B cell responses. *Immunity*. 2006;24(2):141-152. doi:10.1016/j.immuni.2005.12.013
79. Giampaolo S, Wójcik G, Klein-Hessling S, Serfling E, Patra AK. B cell development is critically dependent on NFATc1 activity. *Cell Mol Immunol*. 2019;16(5):508-520. doi:10.1038/s41423-018-0052-9
80. Franken LG, Francke MI, Andrews LM, et al. A population pharmacokinetic model of whole-blood and intracellular tacrolimus in kidney transplant recipients. *Eur J Drug Metab Pharmacokinet*. 2022;47(4):523-535. doi:10.1007/s13318-022-00767-8
81. Budde K, Fritsche L, Waiser J, Glander P, Slowinski T, Neumayer HH. Pharmacokinetics of the immunosuppressant everolimus in maintenance renal transplant patients. *Eur J Med Res*. 2005;10(4):169-174.
82. Klawitter J, Nashan B, Christians U. Everolimus and sirolimus in transplantation-related but different. *Expert Opin Drug Saf*. 2015;14(7):1055-1070. doi:10.1517/14740338.2015.1040388
83. Lamba V, Sangkuhl K, Sanghavi K, Fish A, Altman RB, Klein TE. PharmGKB summary: mycophenolic acid pathway. *Pharmacogenet Genomics*. 2014;24(1):73-79. doi:10.1097/FPC.0000000000000010
84. Ross SH, Cantrell DA. Signaling and function of Interleukin-2 in T lymphocytes. *Annu Rev Immunol*. 2018;36(1):411-433. doi:10.1146/annurev-immunol-042617-053352
85. van Gelder T. The appropriately designed TDM clinical trial: endpoints, pitfalls, and perspectives. *Ther Drug Monit*. 2023;45(1):6-10. doi:10.1097/FTD.0000000000001010
86. Udomkarnjananun S, Townamchai N, Chariyavilaskul P, et al. The cytochrome P450 3A5 non-expressor kidney allograft as a risk factor for calcineurin inhibitor nephrotoxicity. *Am J Nephrol*. 2018;47(3):182-190. doi:10.1159/000487857

How to cite this article: Udomkarnjananun S, Eiamsitrakoon T, de Winter BCM, van Gelder T, Hesselink DA. Should we abandon therapeutic drug monitoring of tacrolimus in whole blood and move to intracellular concentration measurements? *Br J Clin Pharmacol*. 2023;1-12. doi:10.1111/bcp.15946