Opportunities to Optimize Tacrolimus Therapy in Solid Organ Transplantation: Report of the European Consensus Conference

Pierre Wallemacq, PhD, Victor W. Armstrong, PhD,† Merce Brunet, PhD,‡ Vincent Haufroid, PhD,* David W. Holt, PhD,§ Atholl Johnston, PhD,¶ Dirk Kuypers, MD, PhD,‖ Yannick Le Meur, MD, PhD,# Pierre Marquet, MD, PhD,** Michael Oellerich, MD,† Eric Thervet, MD, PhD,†† Burkhand Toenshoff, MD, PhD,‡‡ Nas Undre, PhD,§§ Lutz T. Weber, MD,¶¶ Ian S. Westley, BMedSci,* and Michel Mourad MD, PhD‖‖*

Abstract: In 2007, a consortium of European experts on tacrolimus (TAC) met to discuss the most recent advances in the drug/dose optimization of TAC taking into account specific clinical situations and the analytical methods currently available and drew some recommendations and guidelines to help clinicians with the practical use of the drug. Pharmacokinetic, pharmacodynamic, and more recently pharmacogenetic approaches aid physicians to individualize long-term therapies as TAC demonstrates a high degree of both between- and within-individual variability, which may result in an increased risk of therapeutic failure if all patients are administered a uniform dose. TAC has undoubtedly benefited from therapeutic drug monitoring, but interpretation of the blood concentration is confounded by the relative differences between the assays. Single time points, limited sampling strategies, and area under concentration–time curve have all been considered to determine the most appropriate sampling procedure that correlates with efficacy. Therapeutic trough TAC concentration ranges have changed since the initial introduction of the drug, while still maintaining adequate immunosuppression and avoiding drug-related adverse effects. Pharmacodynamic markers have also been considered advantageous to the clinician, which may better reflect efficacy and safety, taking into account the between-individual variability rather than whole blood concentrations. The choice of method, differences between methods, and potential pitfalls of the method should all be considered when determining TAC concentrations. The recommendations of this consensus meeting regarding the analytical methods include the following: encourage the development and promote the use of analytical methods displaying a lower limit of quantification (1 ng/mL), perform careful validation when implementing a new analytical assay, participate in external proficiency testing programs, promote the use of certified material as calibrators in high-performance liquid chromatography with mass spectrometric detection methods, and take account of the assay and intermethod bias when comparing clinical trial outcomes. It is also important to consider that TAC concentrations may also be influenced by other factors such as specific pharmacokinetic characteristics associated with the population, drug interactions, pharmacogenetics, adverse events that may alter TAC concentrations, and any change in the oral formulation that may result in pharmacokinetic changes. This meeting emphasized the importance of obtaining multicenter prospective trials to assess the efficacy of alternative strategies to TAC trough concentrations whether it is other single time points or area under the concentration–time curve Bayesian estimation using limited sampling strategies and to select, standardize, and validate routine biomarkers of TAC pharmacodynamics.

Key Words: tacrolimus, transplantation, therapeutic drug monitoring, consensus

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From the *Department of Clinical Chemistry, Université Catholique de Louvain, Brussels, Belgium; †Department of Clinical Chemistry, Georg August University, Göttingen, Germany; ‡Farmacologia, Hospital Clinic Universitat de Barcelona, Barcelona, Spain; §Analytical Unit, St George's—University of London; ¶Clinical Pharmacology, Barts and The London, London, United Kingdom; ‖Department of Nephrology, University Hospitals Leuven, Leuven, Belgium; Departments of #Nephrology and **Pharmacology–Toxicology, University Hospital Dupuytren, Limoges; ††Kidney Transplantation, Hospital Necker, Paris, France; ‡‡Division of Pediatric Nephrology, University Children's Hospital, Heidelberg; §§Department of Pharmacokinetics, Astellas Pharma GmbH; ¶¶Pediatric Nephrology, Ludwig-Maximilians University, Munich, Germany; and ‖‖Abdominal Transplantation Unit, Université Catholique de Louvain, Brussels, Belgium.
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Correspondence: Prof Pierre Wallemacq, PhD, Department of Clinical Chemistry, Laboratory of Toxicology and Therapeutic Drug Monitoring, University Hospital St Luc, 10 Hippocrate Avenue, B-1200 Brussels, Belgium (e-mail: pierre.wallemacq@uclouvain.be).

INTRODUCTION

In May 2007, a panel of European experts on tacrolimus (TAC) met in Brussels, on individual author's initiative, to discuss the most recent advances in the drug/dose optimization of TAC taking into account specific clinical situations and the analytical methods currently available. This work summarizes the main topics discussed and draws some recommendations and guidelines to help clinicians with the practical use of the drug.

Optimal immunosuppressant drug therapy is essential for maintaining a viable organ allograft. Immunosuppressive

agents are critical dose drugs exhibiting the desired therapeutic effect with an acceptable tolerability within a narrow range of blood concentrations. Furthermore, they exhibit a high degree of between-individual pharmacokinetic and pharmacodynamic variability, which may result in an increased risk of therapeutic failure if these agents are used at uniform doses in all patients.[1] The correlation between blood drug concentrations and clinical outcomes is an important factor supporting the use of therapeutic drug monitoring (TDM).[1–5] Individualizing a patient's drug therapy to obtain the ideal balance between therapeutic efficacy and the occurrence of adverse events is the primary goal of the clinician. Each patient must be considered individually in the way he/she will respond to a drug due to differences in age, body weight, fat/lean tissue content, enzymatic activities, kidney or liver function, and concomitant therapies.[6,7] Pharmacokinetic, pharmacodynamic, and more recently pharmacogenetic approaches aid physicians to individualize long-term therapies. TAC was introduced in the early 1990s by Fujisawa (renamed Astellas after merging with Yamanouchi in 2005) and appears today as an effective immunosuppressive drug with a global widespread use. TAC, like other immunosuppressive drugs, has undoubtedly benefited from TDM as tremendous efforts have been dedicated to improve its use over the past decades.

Evidence-Based TDM for TAC

Factors limiting long-term allograft survival are still irreversible chronic rejection and adverse reactions to standard immunosuppression therapy. Chronic over or under immunosuppression is still common and should be avoided. A large number of studies, together with the pharmaceutical company Astellas itself, strongly recommend TAC TDM to optimize efficacy and prevent adverse reactions. These studies stress the importance of between- and within-patient variability in drug disposition, related to pharmacokinetics, pharmacogenetics, or pharmacodynamics. However, the relationship between TAC whole blood concentrations and efficacy or toxicity has not yet been fully established. Many factors may interfere with the efficacy, and indeed, TAC dose optimization does benefit from TDM. Among these factors are the type of organ transplanted, the age of the patient, the analytical method used to determine TAC whole blood concentrations, the marker of exposure monitored [trough concentration or limited sampling strategies (LSS)], the time after transplantation, the concomitant immunosuppressants, the study design and the definition, and recording of outcome (adverse effects and rejection score).

Unfortunately, only a few multicenter, prospective, concentration-controlled trials have been published to enable the establishment of target TAC concentrations in relation to clinical outcome. In kidney transplantation, a relationship has been demonstrated between TAC trough concentrations (C_0) and clinical outcome from both prospective and retrospective data. Using logistic regression analysis, Laskow et al[1] found a significant trend for increasing toxicity with increasing maximum TAC trough concentrations recorded within 7 days posttransplantation ($P = 0.01$) and for decreasing rates of rejection with increasing minimum trough TAC trough concentrations ($P = 0.021$). A significant relationship was found between low area under the concentration–time curve (AUC) and acute rejection on day 2, but no correlation could be demonstrated between rejection and low AUC at 2 weeks or 3 months posttransplantation.[2]

In liver transplantation, a significant linear relationship was found between TAC concentrations and adverse effects ($P = 0.001$) but not with organ rejection ($P = 0.323$).[3,4] Subsequently, a prospective multicenter trial demonstrated a significant relationship between increasing TAC concentrations and both a decreasing risk of acute rejection and increasing incidence of adverse effects ($P = 0.046$) using logistical regression analysis. In this study, the analytical method used to determine TAC whole blood concentrations achieved a limit of quantification (LOQ) of 1 ng/mL.[5] In addition, the relationships between adverse effects and whole blood TAC concentrations seem to be variable, depending on the adverse effect considered. Adverse effects, such as nephrotoxicity, hypertension, or neurotoxicity, seem to display a stronger relationship with blood concentrations than do diarrhea or the onset of diabetes.[8]

Only a few decades ago, the hurdle of early acute allograft rejection posed the biggest challenge for clinicians, whereas, nowadays, other clinical end points are considered equally or even more important. With the ability to better control acute allograft rejection, previous "surrogate end points" have become increasingly important. These may include graft function, tolerability, drug-related toxicity, recipient comorbidity, subclinical rejection, and chronic allograft damage. As early acute rejection rates are currently in the range of 8%–15% with many current triple and quadruple immunosuppressive regimens, it is becoming increasingly difficult to obtain further improvement. With the ever-increasing demand for organs, clinicians are more and more focused on prolonging graft survival and improving recipients' quality of life. New surrogate markers of graft function and long-term survival are therefore necessary to reach such improvements. Examples of the latter include the search for tolerance-permissive immunosuppressive drug combinations; increasing the use of invasive and noninvasive methods of graft monitoring using biomarkers such as protocol biopsies, proteomics, or microarray; and gene amplification techniques. This also includes the increasing importance of pharmacogenetics (-omics), the development of sophisticated monitoring tools for immune cell function, and further progression in the field of clinical pharmacokinetics and analytics.

TDM Strategies and Markers of TAC Exposure

Single Time Point

As previously discussed, the lack of prospective randomized studies makes it difficult to reach any firm conclusions regarding the advantage of one particular TDM strategy over another. Most transplantation centers are using the whole blood trough concentrations (C_0) to adjust the TAC dosage regimen, even though some controversies remain about the relationship between C_0 and clinical outcome. The AUC is generally considered as the best marker of drug exposure, but it is rather difficult to justify for financial and practical reasons (involving between 8 and 12 blood specimens).[9] For TAC, the correlation between C_0 and AUC_{0-12h} is a matter of debate,

with generally a better relationship during the early phase posttransplantation (first month) than later on. Several factors may influence C_0. The morning trough concentration may differ from the evening trough concentration. The dose-normalized C_0 tends to increase with time posttransplantation (day 7, month 6, and month 12). Moreover, C_0 is characterized by an important between- (20%–60%) and within-patient variability (10%–40%). As a consequence of these difficulties, other single time points have been studied. Contrary to cyclosporine, TAC concentrations 2 hours postdose (C_2) do not seem to correlate better with AUC than C_0 and are not used. Dansirikul et al[10] suggested a better correlation between TAC C_5 whole blood concentrations and $AUC_{0–6h}$. Several studies reported tighter relationships between TAC C_3 or C_4 whole blood concentrations and $AUC_{0–12h}$, in kidney, lung, and liver transplantation.[11–13] Prospective, multicenter, randomized trials would however be necessary to define the real benefit of these single point blood sampling strategies.

Limited Sampling Strategies and Area Under the Time–Concentration Curve

Ting et al[14] published a recent review on the different LSS approaches. Most LSS studies utilize blood sampling within the first 4 hours (C_2 and C_4) or the first 8 hours (C_1, C_4, and C_8) postdose with multiple regression analysis. Encouraging results were obtained, but these strategies need proper multicenter validation before clinical use. Another approach consists of analyzing and including the influence of covariates such as: hematocrit, time elapsed since onset of treatment, albumin, liver function tests, serum creatinine, age, body weight, and genetic polymorphisms in the calculation of $AUC_{0–12h}$.[15–17] Most of these covariant analysis studies are, however, characterized by large between-individual variability and residual random error and do not seem safe, validated, and practical enough on their own for a priori dose adjustment. Many of these covariates are however definitely related to TAC pharmacokinetics and are helpful in designing more efficient population pharmacokinetic models to describe and forecast TAC exposure. Furthermore, similar to trough concentrations (C_0), the dose-normalized TAC $AUC_{0–12h}$ increases with time after transplantation, up to 5 years, and the latter is dependent on the patient's cytochrome P450 (CYP) 3A5/CYP3A4 genotype.[18]

The Bayesian estimation of $AUC_{0–12h}$ using LSS is another interesting way to predict TAC exposure, but the prerequisite for efficient Bayesian estimation is the availability of an accurate pharmacokinetic model to obtain unbiased and precise estimates of the individual and population parameters. Several reports have identified accurate correlations using maximal a priori Bayesian estimation using LSS to estimate an individual's CsA pharmacokinetics in renal, heart, and lung transplant patients.[19–21]

Pharmacodynamic Biomarkers for TAC Monitoring

Parallel to the research into optimizing clinical pharmacokinetics and drug exposure as markers to forecast treatment efficiency and adverse effects, several studies have focused on pharmacodynamic markers, which may better reflect efficacy and safety taking into account the between-individual variability in the immunomodulatory effect of TAC rather than whole blood concentrations. Previous studies reported the development of bioassays such as the pentamer formation assay (TAC, calcineurin, calmodulin, FK-binding protein, and Ca^{2+}) that can be applied to the quantification of TAC in whole blood samples.[22,23] Results from previous studies have demonstrated the impact of TAC on calcineurin activity and cytokine synthesis.[24,25] A correlation between TAC concentration and calcineurin inhibition was found, and a significant inhibition of cytokine synthesis [interleukin 2 (IL-2) and interferon-γ] was observed in patients treated with TAC in comparison with that in healthy controls. Of particular interest is the measurement of intracellular or soluble cytokine showing a strong correlation with clinical outcome. In liver transplant recipients treated with anticalcineurin drugs, insufficient inhibition of peripheral $CD8^+$ T-cell IL-2 production was associated with acute allograft rejection.[26]

Over the last 5 years, several methods have been evaluated and validated for the measurement of biomarkers: lymphocyte proliferation (by proliferating cell nuclear antigen expression), T-cell surface antigen expression (CD25, CD26, CD71, CD54, CD95, and CD134),[27,28] and intracellular cytokine synthesis (IL-2, tumor necrosis factor-α, and interferon-γ) in stimulated whole blood from treated transplant recipients. Knowledge from these previous studies in solid organ transplant recipients with combined mycophenolate mofetil (MMF) and low TAC concentrations demonstrates the ability not only to prevent drug toxicity (mainly nephrotoxicity due to TAC) but also to improve efficacy by synergistic effect between TAC and MMF.[25,29] Another marker of interest could be the intralymphocyte adenosine triphosphate concentration in $CD4^+$ T cells.[30,31] The interest of this marker should however be further investigated in properly designed studies. Based on the preliminary studies performed to date, no conclusions can be drawn as to which are the most appropriate biomarkers to use for preventing or determining organ rejection or adverse effects for 3 main reasons. First, these studies included a small number of patients. Second, there were no standardized analytical protocols to analyze biomarkers, thus hampering comparison of results obtained by different centers. Third, these studies were usually performed at specific time points, which did not allow changes in these biomarkers to be evaluated throughout treatment (sequential pharmacodynamic monitoring). For all these reasons, multicenter clinical trials with a large number of patients are required. Pretransplant measurement of appropriate pharmacodynamic parameters may identify patients at risk for early acute rejection. Such patients would probably need more potent immunosuppression. Although it is unlikely that a single biomarker may be sufficient for identifying tolerance because of the complexity of the involved mechanisms, the number of regulatory T cells $CD4^+$, $CD25^+$, and Fox $P3^+$ may be an interesting candidate for tolerance.[32,33]

Target Ranges for Efficacy

Whereas in the early years of its use, initial TAC target ranges were relatively broad, ranging between 5 and 40 ng/mL,[1,3,34–36] subsequently, lower trough concentrations

were adopted, varying between 10 and 20 ng/mL.[2,37] Interestingly, despite these high target ranges, acute rejection rates ranged between 41% and 45% of patients in these early trials,[34,38] which can possibly be explained by the limited practical experience with the drug and the concomitant use of azathioprine instead of MMF and/or the lack of induction therapy.[1,3,34,35,38]

The first consensus conference on TAC optimization was held at Lake Louise in 1995.[39] This consensus document concluded that TAC whole blood concentrations were targeted between 5 and 20 ng/mL for all transplant populations. An update of this consensus document was published in 1998,[40] and the recommendation was then made specific for adult kidney,[41,42] kidney–pancreas,[43] liver,[44] heart,[45] and lung[46] transplant patients. The manufacturer's recommendations are available on the Astellas Web site (http://www.prograf.com/professionals_dosing.php).

The gain in clinical experience together with the introduction of MMF and induction therapies [IL-2 monoclonal antibodies (mAb): daclizumab and basiliximab] led to a dramatic reduction of rejection rates to between 8% and 20%,[47–53] simultaneously with a reduction in the target whole blood TAC concentrations to 10–15 ng/mL[47–51] or even 8–12 ng/mL[52,53] and with some improvement in the identification of surrogate end points like allograft function, side effects, adverse events, and quality of life. Recently, important data were obtained from the Symphony study, the largest multicenter trial to date addressing the issue of further reducing TAC exposure (in a randomized setting compared with standard and reduced dose cyclosporine (CsA) and reduced dose sirolimus) to obtain better allograft function and to possibly reduce drug-related adverse effects while maintaining efficacy.[54] In the Symphony study, comprising more than 1600 patients, predefined trough TAC concentrations targeted between 3 and 7 ng/mL (the mean concentrations actually achieved were 8.0 ± 2.2 ng/mL, decreasing to 6.4 ± 1.4 ng/mL at 12-month posttransplantation) were associated with the lowest acute rejection rate (11.2%) and the best allograft function (65 mL/min creatinine clearance) after 1 year.[54] This study demonstrated that reduced TAC exposure yielded sufficient immunosuppression when combined with MMF (2 g/d) and IL-2 mAb induction therapy.[54,55] Irrespective of the long-term results, this large trial demonstrated the ability to maintain low acute rejection rates with lower TAC exposure together with improved graft function, thereby confirming earlier findings of small and uncontrolled studies.[56,57] Recent reviews however suggest, on the long term, a reduction of the initial benefit observed in the Symphony trial with the TAC minimization scheme, regarding the kidney function.[58,59]

Careful interpretation of recent clinical data, albeit without performing a formal systematic review, allows us to roughly position current effective target trough TAC concentrations between 5 and 10 ng/mL, at least in the first year after transplantation provided that TAC is incorporated in an immunosuppressant regime with mycophenolate, corticosteroids, and anti-IL-2 mAb induction (Tables 1 and 2). It is clear that these target ranges are in no way validated guidelines but a mere observation of what has been evaluated in today's clinical studies. All target ranges mentioned above refer to determinations with immunoassays. Prospective studies are necessary to evaluate the therapeutic ranges used for liquid chromatography-mass spectrometry (LC-MS/MS).

Concentration Ranges Associated With Adverse Events

Although it is obvious that increasing TAC concentrations may lead to an increase in drug-related adverse effects, it is difficult to establish a clear cutoff due to a substantial overlap between concentration ranges of increased efficacy and the appearance of adverse effects. Patients with low TAC exposure displayed a better 1-year graft function, probably both as a result of reduced cumulative alloimmune injury and less drug-related nephrotoxicity. The combination of TAC, MMF, and corticosteroids still retained its characteristic toxicity, resulting in more diarrhea (27.4%) and new-onset diabetes mellitus after transplantation (NODAT) (10.6%).[8] Moreover, drug-related toxicity remains often difficult to quantify due to differences in study design, definition of toxicity, diagnosis and registration of adverse events, differences in nonimmunosuppressive concomitant medication, length of follow-up, and study populations. One way to evaluate drug-related adverse events is to standardize diagnostic criteria for identifying specific adverse effects (eg, NODAT, nephrotoxicity, and diarrhea) to enable true comparisons across clinical studies. For example, the fixed dose versus concentration controlled (FDCC) trial, a large randomized study, assessed adverse reactions by comparing patients with concentration-controlled mycophenolic acid (MPA) dosing with fixed MMF dosing in calcineurin inhibitor–treated patients.[60] In this 12-month study, TAC exposure was significantly higher than in the Symphony study (mean trough concentrations started at 12.2 ± 4.4 ng/mL and decreased to 8.0 ± 3.1 ng/mL over 12 months). Despite this, the reported

TABLE 1. Proposed Target TAC C_0 Concentration (ng/mL)* Guidelines for Kidney Transplantation[41]

	Without Induction	IL-2R Antibody	Polyclonal Antibodies/HRI	MMF/Steroid Minimization	mTOR Inhibitors
Time (mo)		Tritherapy			
0–3	10–15	3–7†	5–10/10–15	10–15	3–7
3–12	5–15	3–7†	5–10/10–15	8–12	3–7
>12	5–10	3–7†	5–10/8–12	5–10	3–7

HRI, anti-human rabbit immunoglobulin.

*Proposed concentration range applied for the MEIA assay.

†Three to 7 ng/mL was the target concentration range in the Symphony trial. Actually reached TAC concentrations started at 8 ± 2.2 ng/mL and decreased to 6.4 ± 1.4 ng/mL over 12-month time.[54]

TABLE 2. Proposed Target TAC C_0 Concentration (ng/mL)* Guidelines for Heart and Liver Transplantation[6,44,45]

Adult Transplant Recipient		
Organ	**Time Period†**	**Target Concentration**
Heart	Days 0–60	15–20
	Days 60–180	10–15
	After 6 months	8–10
	>6–9 months in stable patients	5–10
Liver	0–1 month	10–20
	1–3 months	5–15
	>3 months	5–10

*Proposed concentration range applied for the MEIA assay.
†Primary therapy.

incidence of diarrhea and NODAT did not differ significantly from the Symphony study, whereas efficacy was comparable.[54–57,60] Potential differences in capturing or defining drug-related adverse effects could explain, at least in part, this apparent similarity between the 2 studies.

It is clear that with increasing clinical experience and a subsequent reduction in the very wide initial target concentration ranges used over the last decade, specific TAC-related toxicity has improved in recent years as demonstrated by the reduction in onset of diabetes mellitus,[61–63] neurotoxicity,[47–51] and, to a lesser extent, nephrotoxicity in the transplant population.[54,56,57] This last observation could well imply that current TAC target concentration ranges for both efficacy and toxicity have almost reached their optimal clinical potential within the framework of the currently available immunosuppressive armamentarium and within the relatively low margins set by recent trials. New combinations including the use of novel immunosuppressive compounds might allow further optimization of both therapeutic and toxic TAC target concentration ranges. When relating this to the observed current target ranges for efficacy, between 5 and 10 ng/mL (see above), new drug combinations could potentially be employed to explore even lower effective TAC target concentration ranges in the future.

What Should the AUC Targets Be?

Some authors recommend the use of TAC exposure (AUC) as the index for TDM. AUC is widely considered as the best exposure index, but its use is limited by the number of blood samples necessary for its calculation. The interest is reinforced by the controversial relationships between trough concentrations (C_0) and AUC, with coefficients of determination (r^2) varying between 0.11 and 0.93, and by the poor performance of the trough concentrations in predicting treatment efficacy. The extremely wide range of r^2 could be partly explained by different study conditions (time posttransplantation, organ transplanted, age of the patient, specificity or sensitivity of the analytical method used). However, due to the limited number of studies recommending a target AUC, it is difficult to reach any consensus. Undre et al[2] and Squifflet et al[51] suggested an AUC >200 $ng \cdot h^{-1} \cdot mL^{-1}$ to be highly discriminatory for the risk of acute rejection. Uchida et al[9] used AUC_{0-4} for TAC dose adjustment in a group of 12 kidney graft recipients. They chose the following targets: 150 $ng \cdot h^{-1} \cdot mL^{-1}$ for the first 4 weeks and 120 $ng \cdot h^{-1} \cdot mL^{-1}$ for weeks 4–8, with good clinical outcome. Finally, the most convincing study was published in 2005 by Scholten et al.[52] They first established a population-based pharmacokinetic model for TAC and developed a Bayesian estimator based on an LSS of 2 points (C_0 plus any other single point between C_2 and C_4). Then, they performed an AUC-guided dosing study in 15 renal transplant recipients. Targets for AUC were as follows: 210 $ng \cdot h^{-1} \cdot mL^{-1}$ for weeks 2–6 (corresponding to a C_0 of 12.5 ng/mL) and 125 $ng \cdot h^{-1} \cdot mL^{-1}$ for weeks 6–52 (corresponding to a C_0 of 7.5 ng/mL). AUC calculations were performed at weeks 2, 4, 6, 8, 10, 12, 17, 21, 26, 39, and 52, and TAC dose was adjusted according to TAC exposure. They demonstrated good clinical results, even if the number of AUC calculations and dose adjustments were hardly compatible with the every day clinical life in most centers. In summary, an AUC target between 150 and 200 $ng \cdot h^{-1} \cdot mL^{-1}$ is probably appropriate. Further prospective studies analyzing the interest in TAC AUC–based TDM are required.

Methods to Measure TAC

Since its introduction, measurement of TAC as a guide to therapy has been advocated.[39] Initially, as for the measurement of CsA, the situation was complicated by the choice of the appropriate matrix for the determination of TAC concentrations. Because the concentration of TAC is high within red blood cells, it proved impractical to attempt to measure the drug in plasma or serum, and all our current data on the application of TAC concentrations as a guide to therapy are based on whole blood measurements. The review of methods shown below focuses on changes in assay methodology since the introduction of the drug and issues relating to assay calibration and specificity, which impact on target concentration ranges and sharing data between clinical centers. Much of the data are drawn from the records of the International Tacrolimus Proficiency Testing Scheme (http://www.bioanalytics.co.uk).[64]

Assay Methods

The routine measurement of TAC has been dominated by 1 particular immunoassay technique, the microparticle enzyme immunoassay (MEIA, Abbott Diagnostics, Chicago, IL), performed on the IMx analytical platform. Initially, few centers could analyze this drug using high-performance liquid chromatography because the molecule has a very poor ultraviolet absorption spectrum. As a result, the only practical chromatographic technique that can be used is high-performance liquid chromatography with mass spectrometric detection (HPLC-MS), a technique beyond the resources of many laboratories until relatively recently. Table 3, obtained from the Tacrolimus International Proficiency Testing Scheme, illustrates the changes in methodology and demonstrates that, currently (September 2008), HPLC-MS is performed by a much larger proportion of laboratories (24%) than was the case in 1999 (2%) and that there have been changes in the immunoassays available. Although the MEIA assay still dominates the market, other immunoassay methods have appeared—the

TABLE 3. Analytical Methods Reported to the Tacrolimus International Proficiency Testing Scheme in July 1999 and September 2008 and to the College of American Pathologists Proficiency Testing Scheme, April 2007

	Tacrolimus International Proficiency Testing Scheme (%)		CAP
Method	July 1999	September 2008	April 2007
MEIA	96	33.2	86
EMIT	—	15.9	4
HPLC-MS	2	24	10
ACMIA	—	16.7	—
CMIA	—	6.9	—
Other	2	3.2	—
n	211	376	455

n = number of centers reporting results.
ACMIA, affinity column–mediated immunoassay; CAP, College of American Pathologists.

enzyme multiplied immunoassay technique (EMIT, Dade Behring, Glasgow, DE), the antibody-conjugated magnetic immunoassay (ACMIA, Dade Behring-Siemens, Deerfield, IL),[65,66] the cloned enzyme donor immunoassay (CEDIA, Microgenics, Fremont, CA),[67] and the recent chemiluminescence microparticle immunoassay (CMIA, Abbott Diagnostics).[68,69] An earlier immunoassay, an enzyme-linked immunosorbent assay (ELISA, DiaSorin, Stillwater, MN), did not achieve significant market penetration and is now used by very few centers. As not all assays are available throughout the world, their use does vary. The 2007 situation in the United States is reflected in Table 3, based on information supplied by the College of American Pathology proficiency testing scheme (S. Soldin, PhD, written communication, April 2007).

Another change underlies these data. The original MEIA kit was configured to measure TAC concentrations over a range of about 5–40 ng/mL, reflecting the target ranges in use at that time. As experience with the use of the drug increased and as it was combined with other immunosuppressive therapies, TAC target concentration ranges were reduced and the assay was no longer able to measure the lower concentrations accurately as the new ranges required. The manufacturers eventually reconfigured the method (Tacrolimus II assay), such that it was capable of providing adequate precision at concentrations of about 3 ng/mL.[70] Most other immunoassays have an LOQ between 2 and 4 ng/mL, except the new CMIA, developed by Abbott Diagnostics on the Architect platform, which displays a functional sensitivity below 1 ng/mL.

Assay Differences

TAC is metabolized extensively, so it is no surprise that immunoassays for the drug could be biased by cross-reactivity with TAC metabolites. As the International Proficiency Testing Scheme circulates pooled samples from patients receiving the drug, it is possible to make an estimate of this bias in clinical samples. However, the relative differences between the assays are, in part, also due to differences in assay calibration. Using samples spiked with TAC in the range of 3–30 ng/mL, data collected over the last 2 years from the scheme have been presented recently.[71] There were insufficient data for the ACMIA, CEDIA, or CMIA assays over this period for inclusion. The data showed that:

- Spiking accuracy for these samples, when referenced to the mean result for HPLC-MS, was excellent. The mean (95% confidence interval) bias was −0.06% (−0.67% to 0.54%).
- For MEIA, there was a negative bias, −8.9% (−9.5% to −8.4%), whereas for EMIT, there was a positive bias, 7.9% (7.3%–8.5%).

Similarly, the results for 22 pooled samples from kidney transplant patients were compared by analysis of variance and demonstrated that both the MEIA and EMIT assays were significantly different from the results produced by HPLC-MS. The percent differences for the methods' means were 105% (94%–115%) for MEIA and 117% (106%–127%) for EMIT. Thus, both immunoassays had a positive bias compared with HPLC-MS. However, this difference is influenced by calibration inaccuracy. Both immunoassays have similar biases, but the bias of the MEIA is reduced by its negative calibration bias and that for the EMIT is enhanced by its positive calibration bias. It must be remembered that the differences shown here are mean values for pooled samples collected in 1 transplant setting. Results of individual patient sample could differ substantially from those produced by HPLC-MS if the relative proportion of metabolites is much higher.[72] This is a situation that could arise if a patient had poor liver function, as would be common after liver transplantation. Thus, no formula can be applied to "correct" an immunoassay result to that produced by a chromatographic technique. However, it should be appreciated that the mean relative differences between these assays mask substantial between-center differences in assay performance. This is illustrated in Figure 1 that shows the spread of results for each of 3 assay methods for one of the pooled samples from kidney transplant patients. No single method could be said to be superior on the basis of these results.

The interlaboratory variability and imprecision data of current methods are displayed in Figure 2, based on the

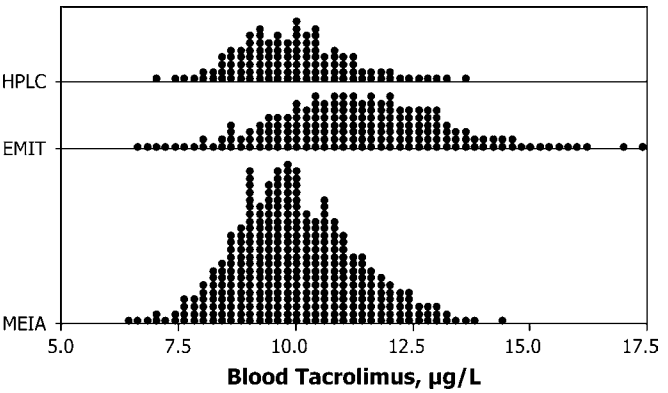

FIGURE 1. Range of values reported for a pool of blood samples from patients receiving TAC after kidney transplantation, by method of analysis. The mean concentrations were as follows: HPLC-MS 9.8 ng/mL, EMIT 11.2 ng/mL, and MEIA 9.9 ng/mL. HPLC-MS, high-performance liquid chromatography with mass spectrometric detector; EMIT, enzyme multiplied immunoassay technique; MEIA, microparticle enzyme immunoassay.

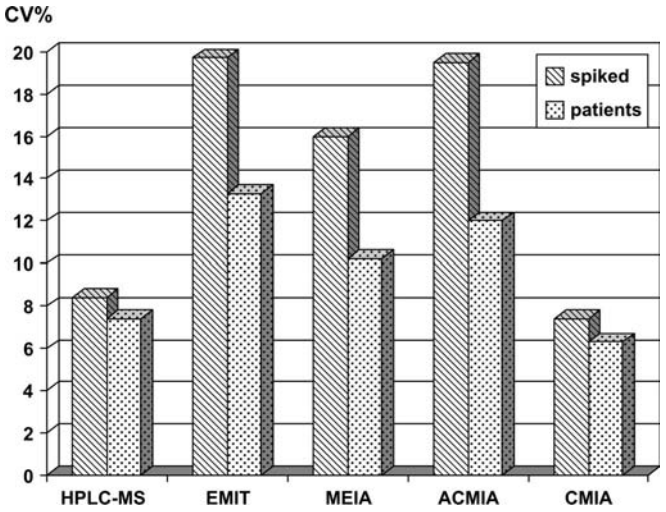

FIGURE 2. Interlaboratory variability and imprecision of current analytical methods, expressed as mean CV recorded from the Tacrolimus International Proficiency Testing Scheme between March and August 2008. Data are displayed separately for patient samples (concentration range: 7.7–10.4 ng/mL) and spiked samples (concentration range: 3–20 ng/mL). HPLC-MS, high-performance liquid chromatography with mass spectrometric detector; EMIT, enzyme multiplied immunoassay technique; MEIA, microparticle enzyme immunoassay; ACMIA, affinity column–mediated immunoassay; CMIA, chemiluminescent microparticles immunoassay.

coefficient of variation (CV) observed between March and August 2008 from the Tacrolimus International Proficiency Testing Scheme. For the patient samples (TAC concentrations ranging from 7.7 to 10.4 ng/mL), the interlaboratory mean CVs ranged from 6.3% to 13.3%, according to the method. Three methods displayed higher CVs with spiked TAC samples. This could be explained either by some matrix effects of the reconstituted samples or by the wider concentration range proposed (3–20 ng/mL), including some low-concentration samples.

Choice of the Assay Method

Do these assay differences matter? Feedback from our clinicians suggests that they are more interested in consistency of the results rather than the absolute number produced by the laboratory. This is because they are content to use method-related target ranges, which reflect the biases of individual methods, and because prescription is often modified in response to changes in TAC concentrations. However, if we are to make any meaningful comparison of data between centers, for instance in multicenter studies, then we should strive to produce an absolute value that is comparable between methods. This is becoming increasingly difficult as the number of assay methods proliferate and as clinicians and laboratories are not always aware of the method differences.[73]

Even though HPLC-MS has the potential to be the reference method, its application in individual centers initially produced highly disparate results. In part, this was because of inadequate upfront chromatography and difficulties in preparing in-house calibrator/control material, whereas users of immunoassays have a common material provided by the manufacturer of their kit. It should be noted that according to more recent data observed from the International Proficiency Testing Scheme, the interlaboratory HPLC-MS variability remains within the therapeutic range, similar to that of immunoassays, and in the low-concentration range, much better than that of most immunoassays. Indeed, the availability of commercial calibrators and quality control material for HPLC-MS, although expensive, may provide an alternative to in-house calibrator and control materials. The initial disparate results could also be the result of a poor understanding of HPLC-MS technology, including matrix effects and the absence of a deuterated TAC internal standard that would do much to resolve these problems (see below).[74]

The choice of which assay method a laboratory will adopt rests on a variety of factors. These include the expertise and equipment available within the laboratory and the cost of the technique. Many centers assay too few TAC samples to justify the investment in HPLC-MS, unless they can use the equipment to measure other drugs, including other immunosuppressive agents or other biochemical markers. Current data suggest that immunoassays will remain the backbone of assay services for TAC, so we must ensure that they are being used correctly and that the data are useful for clinical practice. This can be achieved by constant surveillance of laboratory performance, usually by external proficiency testing, and by constantly reassessing the relationship between clinical efficacy and TAC concentration data. This is particularly important in the light of changing patterns in the prescription of TAC, resulting in relatively low target concentration ranges between 3 and 7 ng/mL. Thus, it may be necessary to recommend that assays in use demonstrate the ability to measure these low concentrations and that their LOQ is close to 1 ng/mL. Although most clinicians would be reluctant to prescribe this drug without TDM, users of these assays should be aware that there are no randomized concentration-controlled data to show that TAC measurement is of undisputed benefit. Indeed, concentration efficacy studies for TAC do not reach the standards achieved for the drugs developed later, sirolimus and everolimus.[75,76] It is evident that the bulk of the data on which our current target concentration ranges are based were developed using the original MEIA kit, and none are based on the EMIT assay or the immunoassays that have appeared later.

For a variety of reasons, these assays do not give the same numerical value for the measurement of the drug. Users need to be aware of these differences and how they could impact on the clinical interpretation of the result. There is a need for outcome studies that are based on the changing patterns of prescription of the drug and the different assay techniques now in use, a challenge the diagnostics industry should address.

Analytical Pitfalls

In addition to the differences of concentrations observed by these analytical methods, one should be aware of potential pitfalls affecting some of these techniques. Reduced hematocrit values (<30%) have been noted as the cause of falsely

increased TAC concentrations (10%–40% overestimation by MEIA as compared with HPLC-MS).[77,78] Conversely, increased hematocrit can result in underestimation of TAC concentrations between 0% and 20% obtained by MEIA. Similar observations were made for reduced albumin concentrations (<30 g/L), resulting in a positive bias in TAC concentrations by MEIA (by 5%–50%).[79] From the current information available, these interferences were not observed using the newer techniques such as ACMIA or CMIA.[80]

A major issue that should be addressed by HPLC-MS users is the possible presence of a matrix effect or ion suppression effect. This effect is particularly important with electrospray interface and is caused by coeluting molecules altering the ionization efficiency. This could result in erratic underestimation (or overestimation) of TAC concentrations as this effect may vary from one clinical sample to another. All laboratories using HPLC-MS should carefully assess this possible effect either by postextraction addition or by postcolumn infusion as described by Taylor[81] or Peters et al.[82] With increasing experience in this technique, a general recommendation is to minimize the ion suppression effect by improving the extraction/separation efficiency or to avoid early elution of the analytes of interest. The use of stable isotope (deuterated) internal standards, if available, can counterbalance this effect and improve this analytical approach dramatically. Unfortunately, to date, deuterated TAC is not yet available, and some centers use ascomycin as an internal standard in its absence. When developing an assay by HPLC-MS, it is therefore mandatory to ensure a correct validation of the method including assessment of carryover, of late-eluting compounds progressively suppressing TAC, or internal standard signal, which may be overcome by increasing the time between injections. It is also important to assess potential interferences caused by silicone-coated tubes or the stability of TAC and/or internal standard stock solutions in solvents such as methanol.

Pharmacogenetics

The daily practice of drug monitoring reveals large between-individual variability in TAC pharmacokinetics and particularly in the dose required to achieve target blood concentrations.[83] Among several factors investigated, polymorphisms in genes coding for biotransformation enzymes (CYP isoenzymes 3A4 and 3A5) and drug transporters (ABCB1, previously known as MDR1) have received much attention to date. In contrast to CYP3A4, expression of CYP3A5 has been found to be largely determined by genetic polymorphisms, with only 15%–25% of whites expressing CYP3A5 (*CYP3A5*1/*1* or *CYP3A5*1/*3*) at a detectable level.[84] Only individuals with at least 1 *CYP3A5*1* allele (defined as the "wild-type" allele) are classified as CYP3A5 expressors. Another rare single-nucleotide polymorphism (SNP) located in exon 7 (*CYP3A5*6* allele) has also been shown to be associated with CYP3A5 polymorphism.[84] As for CYP3A4 and 3A5, several SNPs have been identified in *ABCB1* [the gene coding for the P-glycoprotein (P-gp)], some of which affect P-gp expression and/or function.[85] To date, the most studied polymorphism that affects P-gp expression in human tissues is the silent mutation in exon 26 (3435C>T). In the initial study reporting an effect of this polymorphism, homozygous carriers of the 3435T allele had on average more than 2-fold lower intestinal ABCB1 expression compared with 3435C homozygous carriers.[86] Interestingly, this SNP is in strong linkage disequilibrium with 2 other SNPs located in exon 12 (1236C>T, silent mutation) and exon 21 (2677G>T/A, nonsynonymous, Ala893Ser/Thr), respectively, and recent studies have emphasized the importance of assessing haplotypes in *ABCB1* rather than selected genotypes if meaningful genotype–phenotype correlations need to be determined.[85]

It is now well established that whole blood TAC concentrations and dose requirements are strongly associated with the *CYP3A5* polymorphism. Indeed, numerous reports[85] have confirmed that patients carrying at least 1 *CYP3A5*1* allele have a lower TAC concentration to dose ratio when compared with nonexpressors (*CYP3A5*3/*3*) in kidney, liver, lung, and heart transplant recipients. In a cohort of kidney transplant recipients, TAC dose–adjusted trough concentrations were 5.8-fold lower in *CYP3A5*1/*1* patients than *CYP3A5*3/*3* patients.[87] There was a 2.3-fold difference in the daily dose required to maintain target blood concentrations between *CYP3A5*3/*3* and *CYP3A5*1/*1* patients. As TAC biotransformation occurs in the liver and also in the intestine, in the specific case of liver transplantation, both recipient and donor *CYP3A5* genotypes have to be considered to explain TAC blood concentrations and dose requirements.[88]

The impact of *CYP3A5* polymorphism on TAC pharmacokinetics was also studied in the early phase post kidney transplantation.[89–91] A significant delay in reaching target trough TAC concentrations was observed during the first 2 weeks after kidney transplantation in CYP3A5 expressors.[89] In this study, the rate of biopsy-proven acute rejection was not different but rejection episodes occurred earlier when the *CYP3A5*1* allele was present. The influence of *CYP3A5* polymorphism also applies to the starting dose after kidney transplantation.[90] Among CYP3A5 nonexpressors, 65% of patients receiving the standard initial dose of TAC (0.1 mg/kg body weight, twice a day) had TAC concentrations above the therapeutic window (>15 ng/mL) compared with just 20% of CYP3A5 expressors.

More recently, a prospective study assessed the effect of *CYP3A5* polymorphism on TAC pharmacokinetics in a group of potential candidates for renal transplantation who were selected on the basis of their genotype.[92] Median $AUC_{0-\infty}$ and trough concentrations obtained after the first administration of TAC were found to be, respectively, 2.6-fold and 5-fold higher in CYP3A5 nonexpressors. This study provided evidence for individualizing the first oral dose and proposed guidelines for the loading dose based on CYP3A5 genotype. CYP3A5 expressors would benefit from an increased loading dose (up to 0.15 mg/kg body weight, twice a day) to reach the target concentration range rapidly. A decreased loading dose (ie, 0.075 mg/kg body weight, twice a day) was also suggested for nonexpressors to avoid early trough concentrations that may be potentially toxic. It should be noted that CYP3A5 polymorphisms occur at very different frequencies according to ethnicity. For example, the *CYP3A5*3* allele occurs homozygously (CYP3A5 nonexpressors) in 80% of whites but only in

30% of African Americans.[85] The CYP3A5*6 allele was found in 3 of 20 African Americans but only in 1 of 500 whites. Such differences in *CYP3A5* allele frequencies could explain a significant part of the variability observed in TAC pharmacokinetic parameters between whites and African Americans. A recent report from Hesselink et al[93] confirmed that patients carrying at least 1 CYP3A5*1 allele had lower TAC trough concentrations. However, they could not identify any impact on the biopsy-proven acute rejection rate between CYP3A5 expressors and nonexpressors, possibly due to the correcting action of TDM in reaching target blood concentrations within the first week.

The association between TAC pharmacokinetic parameters and the *ABCB1* genotype/haplotype is still a matter of debate. Indeed, most of the studies failed to demonstrate any association between *ABCB1* polymorphisms and TAC dose requirements,[85] whereas only a few reports suggested a significant relationship between *ABCB1* polymorphisms and TAC dose requirements in heart[94] and kidney[95–97] transplant recipients. Confounding factors may play a major role in these conflicting results including the CYP3A5 genetic status. The independent effect of *ABCB1* polymorphisms should be evaluated in CYP3A5 nonexpressors. In a pilot study, TAC concentrations in hepatic tissue have been shown to better correlate with acute rejection score (Banff, Alberta) than whole blood concentrations in adult liver–transplanted recipients from whom protocol biopsies were obtained.[98] In the same population, a significant relationship was observed between intrahepatic TAC concentrations and *ABCB1* polymorphisms, whereas no correlation was seen when TAC whole blood concentrations were considered independently.[99] This suggests that *ABCB1* genotypes may influence TAC biliary excretion and/or tissue distribution, but further studies are still needed to confirm these findings. Preliminary unpublished data obtained by the same group in determining TAC concentrations in lymphocytes suggest similar conclusions, which could open new perspectives in TDM.

Finally, regarding the clinical impact of the *MDR1* gene, the role of the MDR1 donor genotype has been evaluated with reference to the incidence of nephrotoxicity. The results demonstrate that the 3435>T polymorphism of kidney donors was significantly overexpressed in those patients with CsA nephrotoxicity (no published data available for TAC).[100]

Interestingly, persistent diarrhea has been found to increase TAC exposure, leading to possible side effects. This increased exposure observed in case of chronic diarrhea is due to a higher oral bioavailability of TAC due to a reduction in intestinal CYP3A and P-gp activity.[101]

Drug Interactions

The frequency of drug–drug interactions is high in most solid organ transplant recipients because of polypharmacy. These interactions (mostly inducers or inhibitors) involve, predominantly, the CYP enzymes and the P-gp, occurring at either the hepatic or intestinal levels. In clinical studies, CYP3A/P-glycoprotein inhibitors and inducers primarily affect oral bioavailability of TAC rather than its clearance, indicating a key role of intestinal P-gp and CYPA3. TDM to guide dosage adjustments of TAC is an efficient tool to manage drug interactions. Different types of interactions are reported as follows: pharmacokinetic drug interactions, with either effects of other drugs on TAC pharmacokinetics or effects of TAC on pharmacokinetics of other drugs, and pharmacodynamic drug interactions. Several reviews summarize most drug interactions.[7,102] It is also important to distinguish in vitro assessment of drug interactions and clinically relevant reported drug interactions. Based on the last criteria, increase of TAC blood levels has been reported with chloramphenicol, clarithromycin, clotrimazole, cyclosporine, danazol, diltiazem, erythromycin, fluconazole, itraconazole, ketoconazole, methylprednisolone, mibefradil, nefazodone, human immunodeficiency virus protease inhibitors, theophylline, and grapefruit juice (naringenin and dihydroxybergamottin).[103–105] Other drugs have been suggested to cause an increase in TAC levels based on in vitro or animal studies: bromocriptine, cimetidine, cisapride, metoclopramide, nicardipine, nifedipine, verapamil, and troleandomycin.

Decrease of TAC blood levels has been clinically described with rifampicin. In vitro or animal studies suggest some reduction of TAC exposure after well-known CYP3A inducers (carbamazepine, dexamethasone, phenobarbital, and phenytoin) or antiacids (magnesium oxide and aluminum hydroxide) or sodium bicarbonate intake due to physical adsorption or pH-mediated degradation.[83] Long-term consumption of St John's Wort (*Hypericum*), used as herbal preparation for antidepressive properties, has been associated to a reduction of TAC blood levels.[106]

Unlike cyclosporine, TAC does not inhibit the active secretion of the glucuronide metabolite of mycophenolic acid (MPAG) through the hepatic canalicules pathway into bile, resulting in elevated MPAG concentrations in the gut lumen, hydrolyzed back to MPA, giving a secondary recirculation plasma MPA peak and higher oral bioavailability.

Pharmacodynamic drug interactions have been reported after aminoglycosides, amphotericin B, cisplatin, angiotensin-converting enzyme inhibitors, cyclosporine, or ibuprofen intake (additive or synergistic nephrotoxicity). The neurotoxicity of ganciclovir may be aggravated if combined with TAC.[102]

Use of TAC in Pediatric Populations

The North American Pediatric Renal Transplant Cooperative Study registry (2006) reports that TAC became the most prescribed immunosuppressive in pediatric solid organ transplant recipients with 63.2% of pediatric patients receiving TAC compared with 14.5% in 1997.[107] The preferred use of TAC versus CsA is based on several, randomized, multicenter, pediatric trials that compared their efficacy and safety. There is evidence that TAC is superior to CsA (conventional or microemulsion form) in preventing acute rejection and in improving long-term graft survival.[108] Indeed, long-term graft survival is even more important for this population than it is for adults, and the availability of a pediatric-adapted formulation is mandatory.

The pharmacokinetics of TAC in pediatric patients differs from those observed in the adult population by several characteristics. TAC total body clearance is higher in pediatric patients than in adult patients, 2–3 versus 1–2 $mL \cdot min^{-1} \cdot kg^{-1}$,

respectively.[109,110] This indicates that pediatric patients require approximately 1.5–2 times the dose of TAC given to adults to achieve similar systemic exposure.[109–111] The increased total body clearance of TAC is most likely due to a larger relative liver size in pediatric patients, together with an increased proportional activity of CYP3A4 during the first years of life.

Published data recommend that pediatric renal transplant recipients receive an initial TAC dose of 0.15 mg/kg twice a day, to achieve trough blood concentrations between 10 and 20 ng/mL during the first 2 months after transplantation and between 5 and 10 ng/mL thereafter.[112] Unfortunately, these dosing recommendations do not take into account the wide variability in total body clearance within the pediatric age group. For example, an initial relative TAC daily dose of 0.3 mg/kg body weight in an adolescent patient of 80 kg converts to an absolute dose of 24 mg/d, which may lead to overexposure and toxicity in adolescent pediatric patients.[113]

TDM is definitely recommended in the pediatric population, due to even higher between- and within-patient variability in pharmacokinetics than in adults. Due to the potential limitations of trough concentration monitoring, various abbreviated AUCs have been proposed for a more reliable estimation of TAC exposure, based on an LSS of either 2 (C_0 and C_2)[114] or 4 (C_0, C_1, C_2, and C_4)[115] sampling time points, as discussed previously. However, target AUCs for the optimization of TAC exposure, which also takes into account the concomitant immunosuppressive medication, have not been published for pediatric patients and should be the focus of future research. A moderate correlation between TAC whole blood trough concentrations and the corresponding AUC (r = 0.74; r = 0.745)[114,115] has been observed in pediatric renal transplant recipients in the initial period after transplantation. In a study involving 20 pediatric renal transplant recipients, the correlation between TAC whole blood trough concentrations and the corresponding AUC was poor in the cohort receiving steroids (r = 0.53, n = 15) compared with those on a steroid minimization regimen (r = 0.91, n = 5) in the first month posttransplantation, although, at subsequent data points, the correlations between AUC and troughs were comparable in both groups (r = 0.87 and 0.88, respectively).[116]

There is clearly a need for further pharmacokinetic, pharmacodynamic, and pharmacogenetic studies in pediatric transplant recipients to establish more precise dosing recommendations for different age and body size subgroups within the pediatric patient population.

Generic and New Oral Formulations

Because immunosuppressive drugs are critical dose drugs, one should keep in mind that the effective and safe use of any generic formulation should require careful scientific studies, experienced medical management, careful TDM, and a close balance between cost/benefit analysis before implementation into clinical practice.

In a Korean study, the generic formulation TacroBell has been compared with Prograf as requested by the bioequivalence guidelines. In these healthy Korean adults, there were no significant differences between the pharmacokinetic parameters of both formulations including t_{max}, $t_{½}$, and $AUC_{0–24}$; however, the formulations were not bioequivalent. The 90% confidence interval for the ratio TacroBell to Prograf (PRO) was 119%–161% for C_{max} and 102%–139% for $AUC_{0–24}$.[117] Moreover, the relationship between predose concentration and AUC is not identical between both formulations. TacroBell predose level is associated with a larger AUC as compared with Prograf.

Recently, Astellas introduced a new prolonged release formulation of TAC in the market as a once daily formulation (Advagraf, ADV), approved in the European Union for prophylaxis of rejection in liver and kidney transplant recipients and in Canada for the prophylaxis of rejection in kidney transplant recipients. It was also recently approved in Japan for prophylaxis of rejection in liver, kidney, heart, lung, and pancreas transplant recipients and for the treatment of graft versus host disease in bone marrow transplantation. It is currently under review in the United States. Studies with ADV have been performed in de novo kidney and liver transplant recipients.[118] Conversion studies have been performed in stable adult kidney and liver transplant recipients and stable pediatric liver transplant recipients who were converted on a milligram to milligram basis from twice daily TAC (Prograf, PRO) to a single morning dose of the new formulation (Advagraf). Results of these studies have established the safety and efficacy of this once daily dosing alternative.[119] After conversion from PRO to ADV on a 1:1 (milligram to milligram) total daily dose basis, the systemic exposure to TAC ($AUC_{0–24}$) for ADV was approximately 10% lower than that for PRO. Similar trend was observed with trough levels. However, more importantly, the relationship between TAC trough levels (C_{24}) and systemic exposure ($AUC_{0–24}$) for ADV is similar to that of PRO. Therefore, when converting from PRO capsules to ADV, TAC predose levels should be measured before and after conversion to ensure that similar systemic exposure is maintained. Therapeutic regimens for transplant recipients are often complex, contributing to a high incidence of medication noncompliance and its consequences of increased mortality and morbidity. The TAC once daily dosing regimen may improve compliance while enabling the use of the same patient care strategies, total daily dose, target trough concentrations, and the same TDM methods as currently used with the twice-a-day formulation of TAC. The first studies investigating the use of ADV in de novo setting displayed TAC first dose $AUC_{0–24h}$ between 30% and 50% lower compared with PRO in kidney and liver transplant recipients, respectively.[119] Further studies are however needed to gain experience with this formulation, and all pharmacokinetic models based on the PRO formulation should be reassessed with the new formulation before being used in the LSS protocols.

CONCLUSIONS

During the European Consensus Conference on TAC optimization, it has been clearly confirmed that TDM of TAC remains a major support to patient management, to assess compliance, to prevent adverse events, and in the detection of drug interactions or unexpected pharmacogenetic influences. Trough concentration monitoring, although not the ideal marker, is still widely used as a guide to individualizing TAC

dose requirements. The relationship between high trough concentrations and toxicity has been demonstrated in various studies, and its relationship with graft rejection (incidence and severity) seems more controversial. A significant relationship between low trough concentrations and acute rejection has been shown in some studies but not in others. It should be noted that whole blood TAC concentrations, depending on individual pharmacokinetics and pharmacogenetics, do not necessarily reflect its cellular concentration and hence its ability to interact with intracellular targets. There is a general consensus about the efficacy of AUC and some of the LSS as markers of exposure, which could be considered for routine use after some multicenter prospective validation. To improve the prediction of efficacy, other approaches are very much needed. Among these approaches, monitoring of pharmacodynamic markers seems promising, such as intracellular IL-2 contents in $CD8^+$ T cells, after having resolved both the analytical and standardization challenges and subsequent, prospective, multicenter validation trials. The recent literature clearly emphasizes new trends toward lower TAC exposure and trough concentrations (depending on concomitant therapies). The recent Symphony study,[54] demonstrated that, at least during the first year posttransplantation, the optimal efficacy versus side effects ratio was observed with a regimen involving low-dose TAC in association with other immunosuppressive drugs, resulting in actually reached trough blood concentrations as low as 8 ± 2.2 ng/mL (6.4 ± 1.4 ng/mL at 12 months). The clinical benefit of minimizing TAC therapies should however be reassessed carefully in the long term. Important progress has been observed in the analytical methods available to quantify TAC both in terms of specificity and sensitivity. Immunoassays are still predominantly used in routine laboratories, but the use of HPLC-MS has increased in frequency and importance and is considered as the reference method even though it is not devoid of pitfalls such as matrix or ion suppression effects or interlaboratory variability, due to the use of in-house calibration standards. Taking into account the trend to use lower TAC doses and the emergence of new immunoassays, the recommendations of this consensus meeting regarding the analytical methods are the following:

1. Encourage the development and promote the use of analytical methods displaying a lower LOQ, ideally around 1 ng/mL.
2. Always perform careful validation when implementing a new analytical assay (immunoassay or HPLC-MS).
3. Participate in external proficiency testing programs.
4. Promote the use of certified material as calibrators in HPLC-MS methods.
5. Take account of the assay and intermethod bias when comparing clinical trial outcomes.

When interpreting whole blood TAC concentrations, clinicians should keep in mind that besides analytical aspects, other factors may influence the result such as:

1. Specific pharmacokinetic characteristics associated with the pediatric population (eg, higher dosage requirements).
2. The existence of a large number of drug interactions.
3. The existence of a significant impact of pharmacogenetics on drug disposition (CYP3A5 expressors need higher doses). The clinical role of this impact remains however unclear, and further large-scale trials are needed before reaching relevant recommendations.
4. Effect of chronic diarrhea by altering P-gp and increasing TAC levels.
5. Any change in the oral formulation of TAC (generic or prolonged release) may result in pharmacokinetic changes and should be well studied and documented.

This meeting emphasized the urgent need to obtain multicenter prospective trials to assess the efficacy of alternative strategies to C_0 (other single time points, AUC Bayesian estimation using LSS) and to select, standardize, and validate routine markers of TAC pharmacodynamics.

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