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Pharmacokinetic Optimization of Immunosuppressive Therapy in Thoracic Transplantation

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Abstract

Although immunosuppressive treatments and therapeutic drug monitoring have significantly contributed to the increased success of thoracic transplantation, there is currently no consensus on the best immunosuppressive strategies. Maintenance therapy typically consists of a triple-drug regimen including corticosteroids, a calcineurin inhibitor (cyclosporine or tacrolimus) and either a purine synthesis antagonist (mycophenolate mofetil or azathioprine) or a mTOR inhibitor (sirolimus or everolimus). The incidence of acute and chronic rejection and of mortality after thoracic transplantation is still high compared to other types of solid organ transplantation. The high allogeneicity and immunogenicity of the lungs justify the use of higher doses of immunosuppressants, putting lung transplant recipients at a higher risk for drug-induced toxicities. All immunosuppressants are characterized by a large intra- and inter-individual variability of their pharmacokinetics and by a narrow therapeutic index. It is essential to know their pharmacokinetic properties and to use them for treatment individualization through therapeutic drug monitoring (TDM) in order to improve treatment outcome. Unlike the kidneys and the liver, the heart and the lungs are not directly involved in drug metabolism and elimination, which may be the cause of pharmacokinetic differences between patients from all these transplant groups.

TDM is mandatory for most immunosuppressants, and has become an integral part of immunosuppressive drug therapy. It is usually based on trough concentrations (C_0) monitoring, but other TDM tools include the area under the concentration-time curve over the dosing interval (AUC_{0-12}) or over the first 4 hours post-dose (AUC_{0-4}), as well as other single concentration-time points, such as the concentration 2 hours after dosing (C_2). Given the peculiarities of thoracic transplantation, a review of the pharmacokinetics and TDM of the main immunosuppressants used in thoracic transplantation is presented in this article. Even more so than in other solid organ transplant populations, their pharmacokinetics is characterized by wide inter- and intra-individual variability in thoracic transplant recipients. The pharmacokinetics of cyclosporine in heart and lung transplant recipients has been explored in a number of studies, but less is known about that of mycophenolate and tacrolimus in these populations, while there are also hardly any studies on the pharmacokinetics of sirolimus and everolimus. Given the increased use of these molecules in thoracic transplant recipients, their pharmacokinetics deserves to be explored more in depth. There is very little data, some of which is conflicting, on the practices and outcomes of the TDM of immunosuppressants after thoracic transplantation. The development of sophisticated

TDM tools dedicated to thoracic transplantation are awaited, in order to evaluate accurately and precisely patients' exposure to drugs in general and in particular, to immunosuppressants. Finally, large cohort TDM studies definitely need to be conducted in thoracic transplant patients, in order to identify the most predictive exposure indices, and their target values, and to validate the clinical usefulness of improved TDM in these conditions.

Keywords: Heart transplantation; lung transplantation; cyclosporine; tacrolimus; mycophenolate; sirolimus; everolimus; therapeutic drug monitoring; systemic exposure.

List of abbreviations

AcMPAG: acyl glucuronide of MPA – $AUC_{0-\tau}$: area under the concentration-time curve over the dosing interval – AZA: azathioprine – BOS: bronchiolitis obliterans syndrome – BPAR: biopsy-proven acute rejection – CAV: cardiac allograft vasculopathy – CF: cystic fibrosis – CL/F: apparent clearance – C_{max}: maximum concentration – CNI: calcineurin inhibitor – CYP: cytochrome P450 – D: dose – EMB: endomyocardial biopsy – FEV1: forced expiratory volume in one second – LVEF: left ventricular ejection fraction – MAT: mean absorption time – MMF: mycophenolate mofetil – MPA: mycophenolic acid – MPAG: 7-O-mycophenolic acid glucuronide – P-gp: P-glycoprotein – PD: pharmacodynamics – PK: pharmacokinetics – TDM: therapeutic drug monitoring – ISHLT: International Society for Heart and Lung Transplantation – $T_{1/2}$: elimination half-life – t_{max}: time to maximum concentration – UGT: uridine diphosphate glucuronyl transferase – V_1/F: apparent volume of the central compartment – V_d/F: apparent volume of distribution.

Introduction

Heart and lung transplantations are life-saving interventions for patients suffering from end-stage cardiac or pulmonary failure. Many fields have been improved since the first transplantations of these organs (in 1963 for the lung and 1967 for the heart): optimal patient selection, donor management, organ preservation, histocompatibility testing, surgical techniques, post-operative management and immunosuppressive treatment and monitoring have all significantly contributed to this progress.[1] In the past 20 years (1986 to 2005), a large increase has been observed in the number of heart (from 2,158 to 3,095 per year) and lung (from 1 to 2,169 per year) transplantations in adults, as reported by the Registry of the International Society for Heart and Lung Transplantation (ISHLT).[2,3] The survival of heart and lung transplant recipients has improved in parallel, with 1-year rates of 90 and 80%, respectively, and 5-year rates of approx. 70 and 50%, respectively.[2,3]

Heart and lung transplantations are not comparable with the transplantation of other solid organs in terms of morbidity and mortality. First, contrary to graft failure in kidney transplant recipients which can often be treated by dialysis, graft loss in heart or lung transplant patients generally results in death. Secondly, the lung is a highly allogeneic and immunogenic organ because it is in direct contact with the environment (airborne organisms, polluting particles) through respiration, and the lung tissue is highly lymphoid.[4] Thirdly, unlike the kidneys and the liver, the heart is not directly involved in drug metabolism and elimination, other than by providing blood supply to the other organs, while the lungs are rich in metabolism enzymes that participate in the elimination of certain drugs and are characterized by a very large contact surface area between the alveoli wall and the blood stream. These peculiarities may be the cause of pharmacokinetic (PK) differences between patients from all of these transplant groups.

The increasing success of thoracic transplantation is largely attributable to the development of effective immunosuppressive regimens.[5] The strategies used in thoracic transplantation were initially derived from clinical trials performed in other solid organ transplant settings, such as kidney transplantation. However, there is no consensus on immunosuppressive strategies after heart[2] or lung[3] transplantation to date. Maintenance therapy typically consists of a triple-drug regimen including corticosteroids, a calcineurin inhibitor (CNI, *i.e.* cyclosporine or tacrolimus) and either a purine synthesis antagonist (mycophenolate mofetil –MMF– or azathioprine –AZA) or a mTOR inhibitor (sirolimus or everolimus). Between 2002 and 2006, the maintenance regimen of approximately 75% of heart or lung transplant recipients at both 1 and 5 years after transplantation was comprised of a calcineurin inhibitor plus a purine synthesis antagonist. More precisely, the combination of tacrolimus and MMF has now become the most widely used combination internationally.[2,3]

Despite the improvement of immunosuppressive strategies, the incidence of acute and chronic rejection and of mortality after thoracic transplantation remains high compared to other types of solid organ transplantation. In 2007, 35 to 45% of heart[2] and 40 to 50% of lung[3] transplant recipients were treated for acute rejection during their first year post-transplantation. Moreover, many studies in lung transplantation have shown that acute rejection is a significant risk factor for the development of chronic rejection.[6,7] Chronic allograft rejection (in the form of cardiac allograft vasculopathy in heart, and of bronchiolitis obliterans syndrome in lung transplantation) and infections are the main threats to long-term survival and quality of life.[2,3,8-10] Therefore, further improvements in the treatment of heart and lung transplant recipients must be sought.

Optimal immunosuppression is essential to maintain a viable allograft. The well-known large intra- and inter-individual variability of the PK of the immunosuppressive agents as well as their narrow therapeutic index represent a challenge to the clinicians, who need to select the best treatment and the best dosage for a given patient. Knowing the PK properties of these drugs and using them for treatment individualization through therapeutic drug monitoring (TDM) is thus crucial to improve treatment outcome. Since the introduction of cyclosporine, TDM has become an integral part of immunosuppressive drug therapy, because of the high correlation between systemic exposure and clinical outcomes.[11] The pre-dose concentration (so-called trough level or C_0) is routinely used for individualizing the dose of most immunosuppressive drugs. For cyclosporine, the area under the concentration-time curve over the dosing interval (AUC_{0-12}) or over the first 4 hours post-dose (AUC_{0-4}) were also identified as good, or even better predictors of outcome in renal transplant patients.[12,13] TDM based on cyclosporine concentration 2 hours after dosing (C_2) has also been proposed, because it has been shown to be highly correlated with the AUC_{0-4} and with the incidence of acute rejection in kidney and liver transplant recipients.[14,15] Based on these observations, TDM has become mandatory for cyclosporine. It is also mandatory for tacrolimus, sirolimus and everolimus, for which it is usually based on C_0 monitoring. On the opposite, MMF is usually administered at a fixed dose, possibly adapted on clinical signs of inefficacy or toxicity. However, there is increasing evidence in renal transplantation that MMF TDM should also become standard clinical practice, particularly in the early post-transplantation period.[16]

Heart and lung transplant patients display particularities when compared to the other solid organ transplant patients. First, lung transplant recipients usually require higher maintenance immunosuppression as compared to recipients of heart, liver or kidney grafts because of the high immunogenicity of the lung tissue, putting them at a higher risk for immunosuppressant-induced toxicities. Secondly, given the potential PK differences between populations, the PK of immunosuppressants may be different in heart and lung transplant recipients from that of liver or kidney transplant recipients and thus deserves to be specifically studied. For instance, heart and lung transplant patients often suffer from gastroparesis as a result of their initial pathology (*e.g.*, cystic fibrosis) or of the transplantation procedure, which may modify the absorption and thus the PK profile of drugs.[17,18] More generally, the lung transplant population is very heterogeneic as a consequence or the diverse indications for lung transplantation, which is also in favour of individual therapy and dose adjustment. Finally, close TDM in thoracic transplant patients may be justified given the high morbidity and mortality rates in these populations.

The aim of the present article was therefore to review the state of the art on the PK and TDM of the main immunosuppressants used in thoracic transplantation, and to determine what fields need to be further explored in future PK and TDM studies in these conditions in order to improve transplantation success and patients survival. All numerical results presented in this review are expressed as mean±SD, unless otherwise specified.

In preparing this review, the authors conducted a search of Medline (January 1975 to June 2008) for English-language articles using the following search terms: “cyclosporine”, “cyclosporin”, “tacrolimus”, “calcineurin inhibitor”, “mycophenolate”, “mycophelonic acid”, “MMF”, “MPA”, “sirolimus”, “rapamycin”, “everolimus”, “mTOR inhibitor”, “heart transplantation”, “lung transplantation”, “thoracic transplantation”, “pharmacokinetics”, “metabolism”, “drug interactions”, and “therapeutic drug monitoring”. This search was supplemented by a bibliographic review of all the relevant articles. Given the notably high number of articles on cyclosporine, only those considered as fair to high quality were kept for the review. Those rejected either concerned a very limited number of patients, or only reported mean C0 values in a given population, without connection with clinical data or other exposure indices. On the contrary, as there were only a few articles on tacrolimus, MMF and mTOR inhibitors, almost all were considered. Overall, around 80% of the original list of cited articles were included in this review.

1 Calcineurin inhibitors

According to the ISHLT database, calcineurin inhibitors represent the cornerstone of immunosuppression after thoracic transplantation.[2,3]

1.1 Cyclosporine

Cyclosporine penetrates into the cells and forms complexes with intracytoplasmic proteins (cyclophilins), inhibiting calcineurin, hence IL-2 production and T-cell activation.

Cyclosporine has become an essential component of standard treatment after heart or lung transplantation, improving drastically patients’ survival and quality of life. In 2007, the ISHLT registry indicated that 21 and 26% of all lung transplant recipients received cyclosporine as part of their maintenance immunosuppressive regimen at 1 and 5 years post-transplantation, respectively.[3]

1.1.1 Pharmacokinetics

Cyclosporine PK can be affected by drug formulation (Neoral® vs Sandimmune®), drug-drug interactions, pre-existing conditions (age, ethnic origin, pathology, diet, activity of metabolic enzymes and drug transporters), clinical status (time after transplantation, gastro-intestinal motility and bile flow, blood lipoprotein content, haematocrit, etc.), potentially altering blood concentrations and total exposure, and thus the degree of immunosuppression.

The vast majority of PK studies performed in thoracic transplantation were descriptive, using model-independent PK methods, where mean and standard deviation of exposure indices, PK parameters and, to a lesser extent, factors of variability in the evaluated population were calculated from individual data (Tables I and II).[4,6,19-28] Some authors used the iterative two-stage (ITS) method, for which they either set up their own PK models or used already existing PK models in order to determine individual exposure indices and PK parameters and then to calculate mean PK parameters in the population.[29-32] To our knowledge, only one population PK (popPK) study was performed in heart transplant recipients[33] and one in heart-lung transplant recipients (Table III).[34]

In most cases, PK studies were performed in contexts where cyclosporine monitoring was based on C_0 or on C_2.

1.1.1.1 Absorption

Cyclosporine is a highly lipophilic molecule. Early PK studies with the first oil-based formulation Sandimmune® showed a poor and highly variable relative oral bioavailability. The microemulsion formulation (Neoral®) designed in a second time to increase the solubility of cyclosporine in the small bowel improved bioavailablity and decreased absorption variability, with a higher maximum concentration (C_{max}), shorter time to maximum concentration (t_{max}), higher AUC, and lower intra-patient variability on absorption than with Sandimmune®.[35]

An increased and more consistent exposure to cyclosporine after Neoral® than Sandimmune® administration was also reported in heart[29] and heart-lung[28] transplant recipients. As a consequence, Neoral® became the core maintenance immunosuppressant chosen by most thoracic transplantation centers worldwide.[1] However, the high intra- and inter-patient variability of Neoral® absorption was confirmed by Johnston et al in a study performed in 15 *de novo* heart transplant recipients, in which three PK profiles were collected during the first year post-transplantation. Intra-patient and inter-patient variability of cyclosporine concentrations was the highest within the first two hours post-dose.[36]

Cyclosporine absorption typically lasts 4 hours, as illustrated by many studies in thoracic transplantation where C_{max} usually occurred between 1 and 3 hours post-dose.[4,19,23-26,29,32] In a randomized study comparing Neoral® to Sandimmune® in 35 heart transplant recipients, absolute oral bioavailability based on AUC_{0-12} with respect to IV administration was 57±9% and 47±12%, respectively ($p = 0.02$), at 12 weeks post-transplantation.[23] A significant difference of absolute bioavailability between Neoral® and Sandimmune® was also reported by Baraldo et al in 20 stable heart transplant recipients on cyclosporine three times daily, 8 of whom were on diltiazem (75±19% for Neoral® vs 66±16% for Sandimmune; $p < 0.001$).[29]. However, these two studies employed a non-specific cyclosporine assay (FPIA for TDx), which cross-reacts with cyclosporine metabolites. Oral bioavailability might have been overestimated as blood concentrations of cyclosporine metabolites are higher after oral than after IV administration due to the first-pass hepatic and intestinal effects, which are particularly important for substrates of CYP3A isoforms such as cyclosporine.

The PK models developed for cyclosporine by three groups[29,33,34] described absorption as following a first-order process, however with inconsistent mean values for the absorption rate constant k_a. The first study[33] was a popPK study performed in 69 heart transplant patients on Sandimmune® during the first three months post-transplantation. Unfortunately, the fixed k_a value (0.3 h^{-1}) implemented in the model did not take into account the important variability of cyclosporine absorption, leading to an overestimation of cyclosporine concentrations by 150 to 400 µg/L. The second study[34] was a retrospective popPK analysis of data collected from 48 heart-lung transplant recipients, followed throughout their first year post-transplantation. Unfortunately, this study focused on apparent clearance (CL/F) and volume of distribution (V_d/F) and fixed k_a to values estimated by the same group in one of their previous works (0.25 and 1.35 h^{-1} for Sandimmune® and Neoral®, respectively).[19] The model chosen led to a large residual random variability of 44.0 % (proportional) + 76.4 µg/L (additive). As pointed out by Saint-Marcoux et al, this model systematically underestimated C_2 values, showing the limits of a linear model for cyclosporine absorption.[37] In the third study,[29] the PK of cyclosporine was investigated using the ITS-method in 20 stable heart transplant males grafted for more than 3 years, switched from Sandimmune® to Neoral®. The absorption rate constant was k_a = 1.99±1.42 h^{-1} and 2.71±1.47 h^{-1}, respectively ($p < 0.05$). It is therefore noticeable that k_a differed almost 8-fold between studies for Sandimmune®, and 2-fold for Neoral®.

Considering that model-independent PK methods or first-order absorption could not accurately describe the erratic and variable absorption of cyclosporine, our group used an original absorption model based on a Gamma distribution, initially developed for renal

transplant recipients.[38] In this model, the absorption rate is described as an asymmetrical peak and the absorbed fraction of the dose as a function of time as an S-shaped curve. This model was evaluated in a subpopulation of 14 *de novo* heart transplant recipients on Neoral® enrolled in study OLN351, in whom a PK profile of cyclosporine was drawn at three post-transplantation periods: the first week (W1), the third month (M3) and the end of the first year (Y1).[32] Population exposure indices and PK parameters were estimated using the standard ITS-method. The PK model fitted well with the concentration-time data at all periods, with an excellent correlation between observed and modelled concentration values. For each period, the mean relative bias on concentrations was less than 1% and precision was good (> 88%). This model was also tested successfully on a population of 19 stable lung and heart-lung transplant recipients, with or without cystic fibrosis (CF). Three consecutive profiles were collected in each patient within 5 days. Good correlation and non significant differences were observed between measured and modeled concentration values, in non-CF ($r^2 = 0.986$) as well as in CF patients ($r^2 = 0.976$), confirming the robustness of the Gamma model (Table I). The mean absorption time (MAT) was not different between the two groups, with corresponding CV% of 21% and 25% in CF and non-CF patients, respectively.

1.1.1.2 Distribution and elimination

Cyclosporine is mainly metabolized by the cytochrome P450 (CYP) 3A enzymes in the liver and the gut. It is also a substrate of P-glycoprotein (P-gp), particularly in the intestinal mucosa. Hence, it may interact with many of the substrates, inducers and inhibitors of both of these systems. V_d/F, CL/F and elimination half-life ($T_{1/2}$) of cyclosporine in thoracic transplantation were estimated in a few studies using non-compartmental analysis,[19,21] the ITS-method[29,32] or population PK analysis.[34]

Formal comparison of V_d/F between studies is not presented here because only four of them addressed this parameter, unfortunately with different models and units. Two studies used a 2-compartment model, one of which expressed the apparent volume of the central compartment (V_1/F) in liters,[32] the other in L/kg.[29] In the two remaining studies, V_d/F was expressed in L/kg.[21,27] The same issue was faced for clearance, which was reported in studies using two-, single- or non-compartment models and expressing clearance or CL/F in $L.h^{-1}$ or in $L.h^{-1}/kg$. In 15 stable heart transplant recipients on Neoral®, V_d/F was 2.42±0.95 L/kg, CL/F was 5.7±1.7 $mL.min^{-1}/kg$ (0.342±0.102 $L.h^{-1}/kg$) and $T_{1/2}$ was 5.0±1.3 h.[21] In 47 stable heart transplant adults switched from Sandimmune® to Neoral®, CL/F (as dose/AUC_{0-12}) was significantly lower with Neoral® in the two patient subgroups who were not on ketoconazole,

but there was no significant difference in the other two subgroups who received ketoconazole.[19] This is obviously due to improved bioavailability, as illustrated earlier. In 20 stable heart transplant patients who consecutively received Sandimmune®, IV cyclosporine then Neoral®, CL/F and the volume of distribution of the central compartment V_1/F were 0.20±0.04 $L.h^{-1}$/kg and 1.86±0.72 L/kg after IV infusion, 0.21±0.04 $L.h^{-1}$/kg and 1.00±0.43 L/kg after Neoral®. $T_{1/2}$ was longer than usually reported, ranging between 17.27±3.23 (Sandimmune®) and 18.83±3.58 h (Neoral®).[29] Finally, using the ITS method in 19 lung and heart-lung transplant patients (of whom 9 with CF) on Neoral®, CL/F was 50±20 and 50±14 L/h and V_1/F = 88±41 and 74±44 L in CF and non-CF patients, respectively (ns).[32]

In the popPK study in heart-lung (21) and lung transplant recipients (27) performed by Rosenbaum et al, using a one-compartment PK model, the results were expressed as pooled Sandimmune® and Neoral® data. V_d/F was 147 L (range, 130-164 L) and CL/F was 22.1 L/h (range, 19.5-24.7 L/h) on average.[34] The inter-patient variability on modelled clearance was 17.1%, but no intra-patient variability was reported for this PK parameter. The following factors were reported to influence CL/F: co-administration of itraconazole, weight, and CF. No inter- or intra-patient variability of, and no covariates on V_d/F were reported. In a popPK study performed in heart transplant recipients, Parke et al proposed an estimation of clearance based on body weight and the co-administration of diltiazem.[33] The inter-patient variability on clearance was 20.2%.

1.1.1.3 Drug-drug interactions

Lipid-lowering therapy is commonly administered to transplant patients to overcome hyperlipidemia and prevent coronary-artery disease.[39] Considering the affinity of cyclosporine for plasma lipoproteins, it was hypothesized that the modification of lipoprotein concentration may influence cyclosporine disposition and PK. Akhlaghi et al evaluated the effect of simvastatin on cyclosporine PK in heart transplant patients with a population approach using the P-PHARM program, though only to estimate cyclosporine elimination based on C_0, which might have limited the power of this study.[40] The authors found a moderate increase in cyclosporine CL/F in patients on lipid lowering agents. However, the difference in clearance before and after simvastatin administration did not reach statistical significance, whether patients received ketoconazole or not.

Diltiazem is another enzyme and transporter inhibitor often given early after heart transplantation to prevent coronary-artery narrowing and coronary artery disease on the transplant. Lung transplant patients often need azole antifungals such as ketoconazole,

itraconazole or voriconazole as prophylactic or curative treatments, because they risk developing invasive fungal infections of the graft. To our knowledge, the impact of enzyme or transporter inhibitors on the PK of cyclosporine after thoracic transplantation was evaluated in only two studies.[19,41] One hundred and eighty two patients receiving Sandimmune® as part of their immunosuppressive regimen after cardiothoracic transplantation were recruited in the first study.[41] The authors defined a linear relationship between cyclosporine dose rate (DR) and trough concentrations at steady-state ("Css_{trough}"). The relationship was described as follows: $DR = \theta \times Css_{trough}$, where θ was the dose rate-steady-state trough concentration ratio, assuming that the PK of cyclosporine is linear. Posterior Bayesian estimation using P-PHARM indicated that the concomitant administration of metabolic inhibitors influenced significantly θ, probably because of a change in CL/F (although a modification of cyclosporine distribution could not be excluded). A dramatic reduction (-82%) of θ was caused by the co-administration of ketoconazole and diltiazem, while co-administration of either ketoconazole, itraconazole or diltiazem resulted in 75%, 40% and 23% reduction, respectively. Consistently, patients who had been administered ketoconazole had a 75% reduction in cyclosporine dose and those on diltiazem a 23% decrease.

The 47 stable heart transplant recipients included in the second study were switched from Sandimmune® to Neoral® and were divided into four groups depending on the long-term (beyond two years) co-administration of enzyme inhibitors (diltiazem and/or ketoconazole or none).[19] Cyclosporine AUC was significantly higher after administration of Neoral® compared to Sandimmune® only in patients who were either receiving no metabolic inhibitors (4,911±935 vs 3,655±1,120 h.µg/L, $p < 0.05$) or diltiazem alone (4,747±923 vs 3,605±1,079 h.µg/L, $p < 0.05$), but not in those taking concomitant ketoconazole or a combination of ketoconazole and diltiazem. Similarly, cyclosporine CL/F was significantly lower with Neoral® formulation, but only for patients on diltiazem (26.8±9.0 vs 36.7±14.1 L/h, $p < 0.05$) or on no metabolic inhibitor (31.8±9.5 vs 46.7±21.1 L/h, $p < 0.05$). The authors hypothesized that in patients on ketoconazole, maximal cyclosporine absorption was already achieved, so that no further increase was possible by switching to Neoral®. The mechanism of metabolic inhibition by ketoconazole is not completely known, but it seems that it inhibits the metabolism and enhances the absorption of cyclosporine in the gastrointestinal mucosa by a complete blockage of prehepatic metabolism and inhibition of intestinal P-gp. On the other hand, diltiazem appeared here to have very little, if any, effect on cyclosporine PK.[19]

1.1.1.4 Special populations

1.1.1.4.1 Pediatric transplantation

According to the ISHLT registry, between January 1998 and June 2006, 32.8% of heart and heart-lung transplant recipients were aged less than 19 years at the time of transplantation.[3] Since 2001, there has been a slow rise in the total number of transplant procedures performed in children: 77 were reported in 2005 versus 59 in 2001. CF is the main indication for thoracic transplantation in children (69% of patients aged 12 to 17 years who underwent transplantation between January 1991 and June 2006).[42]

The PK of cyclosporine in paediatric patients is known to be highly variable,[43] and different from that of adults in terms of gastro-intestinal absorption, volume of distribution and systemic clearance (hepatic metabolism).[44,45]

To our knowledge, no study has been specifically designed to evaluate the PK of cyclosporine in children after thoracic transplantation. One study performed in both adults and children, in different types of transplantation (renal, cardiac, hepatic and bone marrow transplantation) reported PK parameters of cyclosporine in 12 heart-transplant patients (apparently mainly children).[46] Their mean V_d/F and mean residence time were 7.4±1.8 L/kg and 10.7±4.4 h respectively, with no significant difference compared to other types of transplantation.

Given the high proportion of children among patients benefiting from thoracic transplantation, more studies are definitely needed to better characterize cyclosporine PK in this population.

1.1.1.4.2 Cystic fibrosis

CF is a major indication for lung and heart-lung transplantation. Despite the administration of exogeneous pancreatic enzymes, patients with CF are poor absorbers (gastro-intestinal disorders and malabsorption of lipids owing to pancreatic insufficiency). They exhibit higher variability on absorption compared with non-CF patients, with erratic PK profiles and a delayed t_{max}, and consequenly require different dosage regimens.[8,25-27,47]

A study in 31 heart and lung transplant candidates (mainly adults) reported lower cyclosporine bioavailability, higher apparent oral clearance and shorter mean residence time in subjects with CF as compared to those with Eisenmenger's syndrome. The authors suggested that the difference on cyclosporine CL/F may be due to the lower bioavailability in CF patients.[47] In a retrospective case-control study, the same authors compared the PK of cyclosporine in CF patients (n = 11), vs non-CF patients (n = 11), following heart-lung transplantation. Patients' daily dose was 16.7±7.2 mg/kg vs 8.2±19 mg/kg, respectively ($p < 0.01$). Cyclosporine PK parameters were estimated using a Bayesian approach.[48] A

statistically significant difference on cyclosporine CL/F was found between CF and non-CF patients (3.5±1.3 vs 1.7±0.5 mL.min^{-1}/kg, respectively; p = 0.003), but not on V_d/F (7.2±5.3 vs 4.3±2.2 L/kg, respectively; ns).[27] The authors proposed that patients with CF be given 1.5 to 2 times higher oral doses of cyclosporine than patients without CF.[27,47]. Higher drug doses were required in 9 CF vs 41 non-CF patients ($p < 0.001$) on either Sandimmune® or Neoral®, resulting in lower dose-normalized AUC_{0-6} ($p < 0.001$).[28]

Several studies in limited numbers of mixed, adult and paediatric CF patients addressed the potential benefit of improved cyclosporine oral absorption after Neoral® rather than Sandimmune® administration. Three studies reported that Neoral® allowed a substantial increase in AUC_{0-12} (by 2.63; 1.44 and 1.48-fold, respectively) and C_{max} (by 2.28; 2.28 and 1.91, respectively) when compared to Sandimmune®, with inconsistent findings regarding C_0.[24,26,49] The intra-individual and inter-individual coefficient of variation on C_0 and on AUC_{0-12} were less with Neoral® than Sandimmune®, confirming improved PK consistency.[26] Increased exposure was attributed to higher bioavailability, with CL/F significantly and V_d/F numerically higher in CF than non-CF patients.[50]

The PK of cyclosporine was also studied in 10 CF and 10 non-CF clinically stable lung and heart-lung transplant recipients on Neoral® in an open-label trial, with a non-compartmental approach.[25] Cyclosporine exposure was similar in patients with and without CF, but exposure-per-milligram-dose was approx. 25% lower in CF patients, due to lower bioavailability. Rousseau et al conducted a retrospective study of the data collected from 19/20 patients included in this trial,[32] confirming that although CF patients received higher doses (250±76 mg BID vs 175±52 mg BID, respectively, $p < 0.025$), no statistically significant difference was found for measured C_2 values between the 2 groups, resulting in lower C_2/dose ratios in CF patients ($p < 0.001$). Similarly, lower AUC_{0-12}/dose and AUC_{0-4}/dose ratios were found in CF patients ($p < 0.005$). No significant difference was found between CF and non-CF patients, in V_1/F (88±41 and 74±44 L, respectively) and CL/F (50±20 and 50±14 L/h, respectively), maybe due to the small number of patients. The two elimination constants were significantly different between CF and non-CF patients, the first higher and the second lower, which is consistent with the similar CL/F values.[32]

1.1.2 Therapeutic drug monitoring

Cyclosporine is characterized by a marked inter- and intra-patient variability in its PK parameters and a narrow therapeutic index: subtherapeutic exposure may be associated with

rejection and supratherapeutic exposure with drug-induced toxicity (*e.g.*, nephrotoxicity: end-stage renal failure requiring dialysis occurs in up to 6.5% of heart transplant recipients[51]).

TDM of cyclosporine has been recognized as an essential tool in the management of allograft transplant recipients and has been performed routinely in transplantation for almost 20 years.[52,53] However, there is still no consensus about the optimal method for monitoring cyclosporine, and very little data is available on the practices and outcomes of cyclosporine monitoring after thoracic transplantation. Much of the available information has been extrapolated from data obtained in other types of organ transplantation. Table IV presents a summary of TDM methods together with their advantages and limitations, derived from reviews and tables by Fernandez de Gatta et al[43] and Dumont et al.[53]

1.1.2.1 Single concentrations monitoring

The traditional method for cyclosporine dose adjustment is trough concentration monitoring, and it is widely used in most clinical settings. However, many studies in thoracic transplantation have evidenced a poor correlation between C_0 and exposure to cyclosporine, expressed either by the full AUC over the dosing interval (AUC_{0-12}) or by an abbreviated AUC (AUC_{0-4}, AUC_{0-6}).

Correlation coefficients between C_0 and AUC_{0-12} or AUC_{0-4} reported in adult heart transplantation, ranged from 0.06 to 0.6, and from 0.16 to 0.4, respectively.[21,23,54-56] Similar results were reported after lung or heart-lung transplantation: in a study of 50 patients on cyclosporine after heart-lung transplantation, Trull et al found that C_0 was well correlated with AUC_{0-6} estimated from three time-points (C_0, C_2, C_6) using the linear-log trapezoidal rule (r^2 = 0.574 and 0.671 for Sandimmune® and Neoral® respectively).[28] Surprisingly, using the same database, apparently limited however to 48 patients, Akhlaghi et al reported a poor correlation between C_0 and AUC_{0-6} (r^2 = 0.267).[20] This may be due to the fact that Sandimmune® and Neoral® data were pooled and the linear rather than the linear-log trapezoidal rule employed. Jaksch et al[4] reported a correlation coefficient of 0.64 between C_0 and AUC_{0-4} in 20 lung transplant recipients, and Hangler et al[6] a correlation coefficient of 0.31 between C_0 and AUC_{0-6} in 12 lung transplant recipients.

As it was recognized that C_0 values were rather poorly correlated with the AUC,[52] monitoring, another single concentration was proposed as an alternative to C_0 monitoring. The immunosuppressive effect of cyclosporine is maximal and most consistent around t_{max}, which is usually within 2 hours post-dose. C_2 has been demonstrated as an excellent predictor of AUC_{0-4} in renal transplantation.[14,57] In thoracic transplantation, a series of studies have

shown that C_2 quite reliably predicts AUC_{0-12} and abbreviated AUC: coefficients of determination between C_2 and AUC_{0-12} ranged from 0.94 to 0.99 in heart transplant recipients;[21,54,55] lower coefficients of determination between C_2 and AUC_{0-12} were reported in one study in heart-lung transplantation (0.76 and 0.66 in CF and non-CF patients, respectively);[25] coefficients of determination reported between C_2 and AUC_{0-4} were comprised between 0.78 and 0.98 in all types of transplantation.[4,25,54,56]

Some authors also reported a good correlation between C_2 and AUC_{0-6} ($r^2 > 0.80$).[6,20,28] However, in two of them,[20,28] performed on the same database, AUC_{0-6} was estimated using only three time-points (C_0, C_2, C_6) and the trapezoidal rule, which necessarily implies a good correlation with each of the three points, particularly with the middle one which is used twice for the trapezes calculation.

In another paper analysing the same database of 47 heart transplant patients as Akhlaghi et al[19] (though with inconsistencies in patients' numbers and classification, as well as AUC values despite the use of identical samples, analytical method and method of calculation), Ray et al considered AUC_{0-5} and concluded that in general, it was poorly predicted by both C_2 (r^2 = 0.197 to 0.898) and C_0 (r^2 = 0.022 to 0.710), but that C_0 did better than C_2 in patients on cyclosporine alone ($r^2 = 0.710$, $p = 0.001$, vs 0.197), while C_2 did better than C_0 in patients co-administered ketoconazole (± ditiazem) ($r^2 = 0.870$, $p < 10^{-4}$, vs 0.176 and 0.898, $p < 10^{-4}$, vs 0.022) and both were weak predictors in patients on cyclosporine and ditiazem (but no ketoconazole) ($r^2 = 0.65$ for both).[58] However, these conclusions were drawn from very small numbers of patients in each group.

In conclusion, C_2 seems to be a better single-time-point surrogate marker than C_0 for cyclosporine AUC_{0-12}, and is now strongly encouraged. It seems to be of particular interest in CF patients and in patients in whom cyclosporine-induced toxicity is suspected, despite acceptable C_0.[9] Unfortunately, the optimal concentration range for cyclosporine C_2 has not been established for thoracic transplant recipients. Moreover, rigid collection timing is a drawback of C_2 monitoring, and the pertinence of C_2 may be compromised by all the factors of variability of cyclosporine absorption or exposure, including CF (see above).[30,58]

Alternatives to C_0 or C_2 monitoring are other single concentrations measured at specific times post-dose. Several time-points have been proposed, based on the results of linear regression analyses intended for developing sparse sampling strategies.[53] C_6 was proposed for heart transplant recipients, based on efficacy, toxicity and cost in 20 patients.[59] A good correlation was found between C_4 and AUC_{0-12} ($r^2 = 0.95$) in 15 heart transplant patients.[21] In heart-lung

transplant recipients, the best correlation was between C_3 and AUC_{0-12}, in CF as well as in non-CF patients ($r^2 = 0.87$ and 0.82, respectively).[25]

Single concentration monitoring (whether based on C_0, C_2, C_3 or C_6) is widely used in clinical routine. However, as further discussed in paragraph 1.1.2.3.1 ("C_0 monitoring"), robust data on the correlation between such concentrations and overall exposure to cyclosporine and target values is still lacking, especially in populations such as children or CF patients. Further exploration is still needed, and a consensus on such tools, with the definition of target concentrations, is awaited.

1.1.2.2 AUC monitoring

1.1.2.2.1 Full AUC

The inter-dose AUC over the dosing interval ($AUC_{0-\tau}$) is believed to be a better surrogate marker of cyclosporine efficacy and toxicity than single concentrations, as it gives a complete picture of its absorption and elimination and is proportional to the mean concentration over the dosing interval ($AUC = C_{ss} \times \tau$). Indeed, in the absence of any evidence that cyclosporine effects are linked to its maximal or minimal blood levels, the mean concentration is supposed to better reflect its activity on calcineurin.[60,61] As a consequence, AUC monitoring has become the gold standard for the TDM of cyclosporine.[62] However, proper calculation of AUC requires repeated blood sampling, followed by the calculation of AUC from cyclosporine blood concentrations using the trapezoidal method.[53] As a consequence of the intensive sampling strategy, it is uncomfortable for the patients, inconvenient for the clinical team and costly to the health-care provider, which explains why it is only performed infrequently.

1.1.2.2.2 Abbreviated AUC

As full AUC_{0-12} is impractical to obtain, abbreviated AUCs have been tested. The 4-hour AUC (AUC_{0-4}) has been validated as a reliable estimate of AUC_{0-12}.[57,63] Indeed, the largest part of cyclosporine AUC_{0-12} variability occurs in the first 4 hours following administration. The AUC_{0-4} reflects the highly variable absorption profile of cyclosporine, suggesting it is a more accurate measure of exposure than C_0.[36]

A retrospective study of 156 *de novo* renal graft recipients showed that cyclosporine AUC_{0-4} (sampling at 0, 1, 2, 3, 4h) measured in the first 2-4 days post-transplantation (but not C_0) was lower in patients who experienced acute rejection at 3 months than those who did not.[12] In addition, AUC_{0-4} was reported to be a sensitive marker of nephrotoxicity (contrary to C_0). In a

second time, the same authors performed a prospective, non comparative study in 59 *de novo* renal transplant patients, with cyclosporine dose adjustment based on AUC_{0-4} (target range: 4,400 – 5,500 h.µg/L).[64] They found that the incidence of acute rejection was 3% when AUC_{0-4} at day 3 was > 4,400 h.µg/L (n = 33) vs 45% when it was < 4,400 h.µg/L (n = 22) ($p = 2.10^{-4}$). Again, they did not note any nephrotoxicity episodes when AUC was < 5,500 h.µg/L.

Unfortunately, no equivalent or better designed studies were conducted in thoracic transplant patients.

1.1.2.2.3 Sparse sampling strategies

Rather than replacing AUC_{0-12} monitoring by the determination of the abbreviated AUC_{0-4}, some authors sought for other clinically applicable methods to accurately estimate AUC_{0-12}. This led to the development of sparse sampling strategies, in which specific algorithms allow AUC estimation from a limited number of blood samples collected at precisely defined times. A sparse sampling strategy is developed using full AUC values in a sample population. Stepwise multiple regression (MLR) analysis is then performed on the concentration-time points sampled. The points that do not correlate well with AUC are removed until a regression equation consisting of 2 or 3 concentration-time points is left. The AUC estimate obtained using the retained equation must have a high coefficient of correlation with the full AUC, calculated using a reference method. A good predictive performance (bias and precision) of the sparse sampling strategy is also mandatory.

To our knowledge only a few studies have developed such sparse sampling strategies and MLR equations for thoracic transplant recipients (Table V). Sparse sampling strategies simplify AUC monitoring but there are some limitations that need to be pointed out. First, the sampling strategies that were originally developed in renal transplantation proposed impracticable collection times in an outpatient setting.[65] To be clinically applicable, such strategies should be limited to the first 4 hours post dose. This prerequisite was fulfilled by most sparse sampling strategies developed in thoracic transplantation.[6,21,66] Secondly, sparse sampling strategies developed using Sandimmune® are no longer applicable because of the PK differences between Neoral® and Sandimmune®.[53] Fortunately, all the sparse sampling strategies developed in thoracic transplantation were developed using Neoral®. Thirdly, no study has been conducted to date to demonstrate the superiority in terms of outcome of sparse sampling strategies over C_0 or C_2 monitoring. As a consequence, there are no evidence-based data justifying the use of a sparse sampling strategy which requires 2 or 3 blood samples,

rather than a single concentration. In addition, although few blood samples are needed, multiple linear regression requires a strict respect of sampling times, which is not easily feasible in a clinical setting. Moreover, in most cases, sparse sampling strategies were chosen solely on the basis of high coefficients of correlation[6,21] rather than looking for other criteria such as bias or prediction error.[53,66,67] In addition, to be applicable routinely, sparse sampling strategies should be validated in external populations, which is seldom the case.[6,21] Finally, the algorithm proposed should allow AUC calculation over an appropriate dosing interval (AUC_{0-12} or AUC_{0-8} for patients receiving cyclosporine two or three times daily, respectively); unfortunately, the actual dosing interval was only reported in 2/4 studies.[21,68] The algorithm proposed by Hangler et al[6] allows the determination of AUC_{0-6} which is of a limited interest, as the clinical pertinence of AUC_{0-6} in patients dosed twice daily has never been demonstrated, nor have any targets been proposed. The algorithm proposed by Dumont et al[66] also presents major drawbacks rendering it useless: first of all, there is no indication on the type of lung transplant patients (CF or not) in whom the strategy was developed; secondly, the algorithm allows the calculation of "$AUC_{0-\tau}$" without any precision on the value of τ.

In brief, only one published sparse sampling strategy seems to be clinically applicable in heart transplantation (*i.e.*, with a maximum of 3 samples collected within the first 4 hours post-dose, actually C_1 and C_4), although it still needs external validation.[21] There is currently no applicable sparse sampling algorithm for lung transplant patients.

1.1.2.2.4 Bayesian forecasting

Maximum *a posteriori* (MAP) Bayesian forecasting based on a population model allows simultaneous estimation of individual PK parameters and exposure indices, which helps define the dose and administration schedule needed to achieve the desired exposure in a given patient, whatever the targeted exposure index (single concentration value, full AUC or abbreviated AUC).[32] Bayesian forecasting of cyclosoporine AUC_{0-12} has been proposed as a TDM tool in renal transplantation,[69-72] but seldom in thoracic transplantation.

In heart transplantation, the first such study intended to develop a Bayesian estimator based on a routinely applicable sparse sampling strategy to accurately estimate cyclosporine AUC_{0-12}.[31] Concentration-time data was obtained from 14 heart transplant recipients at three periods during the first year post-transplantation. The popPK parameters were evaluated using the standard two-stage method. The accuracy of the model was evaluated using the Jackknife method. A MAP-Bayesian estimator was built using the distribution of the population parameters, and all sparse sampling strategies combining a maximum of three sampling times

within 4 hours post-dose were tested. The best sampling time combination, with respect to its predictive performance for AUC_{0-12} estimation and to the D-optimality criterion (a statistical test) applied to Bayesian estimation, was found to be T_0, T_{1h} and T_{3h}, whatever the period post-transplantation, yielding excellent correlation with the "reference" AUC_{0-12} calculated using the linear trapezoidal rule: $r^2 = 0.887$ to 0.998, bias = -0.20 to +3.06%.

In a second study performed in 31 heart transplant recipients, cyclosporine AUC_{0-12} was calculated with Bayesian estimation and multiple linear regression in parallel, using previously reported estimator and equations.[54] The Bayesian estimator used for this purpose was originally designed for renal transplant recipients.[69] The authors investigated the correlation between AUC_{0-12} Bayesian estimates and measured C_0 ($r^2 = 0.43$) or C_2 ($r^2 = 0.85$) values, confirming that C_2 was a better surrogate marker of AUC_{0-12}. Unfortunately, as they did not compare the values of AUC_{0-12} obtained using the different methods, Bayesian estimation could not be validated, neither vs multiple linear regression nor vs the linear trapezoidal rule.[54]

The third study[30] was performed on a database of cyclosporine concentrations previously collected from 47 adult heart transplant recipients (although presenting discrepancies with the original description of the population, notably on patient numbers and classification as already mentioned).[19] Patients were divided into four groups based on the long-term concomitant administration of enzyme inhibitors (no enzyme inhibitor, diltiazem and/or ketoconazole). The structural PK model was selected from the literature,[73] and parameters for model building were selected from a previously published PK study in stable heart transplant recipients on Neoral®.[29] Of note, the authors added a "disposition factor" of 0.76 on V_d for patients on ketoconazole + diltiazem and of 0.5 on CL for patients on ketoconazole with or without diltiazem, thus improving the model and AUC estimation.[30] The Bayesian models tested included one, two or three concentrations out of four (C_0, C_1, C_2 and C_7). Unfortunately, no other sampling times were tested, although $C_{0.5}$, C_3 and C_5 were available. AUC_{0-12} Bayesian estimates were compared to the "reference" AUC_{0-12} calculated with the linear trapezoidal rule, based on linear regression analysis, bias and precision RMSE%. The best prediction of AUC_{0-12} was obtained with samples collected at 0, 1 and 2 hours after cyclosporine administration in the four groups of patients. In patients who did not receive concomitant enzyme inhibitors, a good correlation was observed ($r^2 = 0.871$), with a bias of 11.7% and a RMSE% of 13.4%. However, the predictive performance was not so good for patients on enzyme inhibitors, with a bias ranging between -14.2% for patients on ketoconazole and diltiazem and +19.0% for patients on diltiazem only. In these groups,

squared correlation coefficients were 0.818 and 0.791 and RMSE% 16.9 and 22%, respectively.[30]

In heart-lung transplantation, Bayesian estimators for cyclosporine AUC_{0-12} forecasting and dose adjustment using a limited number of blood samples were also developed by our team,[32] using data from a previous trial where repeated cyclosporine profiles were collected from 19 stable transplant patients (9 CF patients).[25] Three PK models and Bayesian estimators were designed using a similar method as that presented for heart transplant patients above. The best sampling strategy was also 0, 1 and 3 hours in both groups of patients (CF and non-CF), allowing accurate estimation of AUC_{0-12} in all patients. The comparison between AUC_{0-12} calculated by non-linear regression (PK modelling using all available time points) and AUC_{0-12} calculated using the Bayesian method found bias values of +3.0% (ns) and +5.3% ($p < 0.05$) in CF and non-CF patients, respectively, with a good precision in both groups (RMSE% = 8.7% and 10.0%, respectively). In non-CF patients (where bias was statistically significant, though small), the examination of individual AUC_{0-12} showed relative estimation errors in the range of -20.2 to +23.7%, of which only 3 were outside the ±20% interval.[32]

In conclusion, blood sampling schedules such as T_0, T_{1h} and T_{2h} or T_{0h}, T_{1h} and T_{3h} can easily be integrated into clinical practice because they do not require a prolonged hospital stay. Moreover, as opposed to multiple linear regression and single concentrations monitoring such as C_2, Bayesian forecasting is characterized by its flexibility in sampling times, as long as the true sampling times are reported to the pharmacologists. Another advantage is that such a method can estimate several PK parameters and exposure indices simultaneously. Therefore, it could help to identify absorption and elimination problems in diabetic or CF patients, for instance.

1.1.2.3 Impact of cyclosporine TDM on patient outcome

Studies on Bayesian forecasting for cyclosporine after thoracic transplantation have not evaluated patient and graft survival, and only a few have looked for relationships between global exposure as expressed by the AUC and surrogate markers of efficacy or toxicity. A number of studies describing other TDM tools, particularly C_0 and C_2, collected indicators of efficacy (*e.g.*, acute rejection, graft loss, left ventricular ejection fraction (LVEF), graft atherosclerosis, mortality, forced expiratory volume in one second (FEV1), depending on the type of graft) (Table VI) or toxicity (renal dysfunction, infections) (Table VII).

1.1.2.3.1 C_0 monitoring

Various observational studies sought for a relationship between C_0 and clinical outcome, with contrasted conclusions (Tables VI to VIII).

A significant relationship between C_0 and clinical outcome was established in a series of studies.

In a study in 48 adult heart transplant recipients, on a steroid-free regimen containing Sandimmune® and azathioprine, who were at least 8 months beyond transplantation, an inverse relationship was found between cyclosporine C_0 and the probability of cellular rejection ($p < 0.03$):[74] C_0 = 211 µg/L was associated with a 2.5% probability of rejection ≥ grade 1B and a 1.1% probability of rejection ≥ grade 3A. The lowest probability of acute cellular rejection was obtained with C_0 > 300 µg/L. On the other hand, no correlation was found between cyclosporine C_0 and simultaneously obtained serum creatinine measurements, but this may well be due to the partly cumulative mechanism of cyclosporine nephrotoxicity.

Results from a retrospective analysis of 1,407 endomyocardial biopsies (EMB) and cyclosporine concentrations in 105 heart transplant adults (time post transplantation not reported) showed significantly lower C_0 in patients with grade 3A cellular rejection compared to patients with no rejection (173 [95%CI = 148-197] vs 206 [95%CI = 182-230] µg/L, $p = 0.005$).[75] Again, no correlation was found between cyclosporine C_0 and serum creatinine levels.

In a randomized clinical trial performed in 50 lung or heart-lung transplant recipients, the mean C_0 observed in patients who did not experience acute rejection was 449 vs 394 µg/L in patients who had at least one rejection episode ($p = 0.042$).[28] Once again, C_0 did not correlate with any of the markers of nephrotoxicity. Based on those results, the authors recommended the use of C_0 rather than C_2 as a monitoring strategy. However, this study was not designed to establish a potential relationship between exposure parameters and effects but to compare clinical outcomes between patients on Sandimmune® and patients on Neoral®. In a retrospective analysis of 32 heart or heart-lung transplant recipients in their first three months post-transplantation, the same authors had previously reported a relationship between $\log(C_0)$ and renal function (1/Cr) evaluated at the same time or in the subsequent 5-day period, showing $r^2 = -0.33$ and -0.69, respectively.[76,77]

In a recent study in 70 *de novo* heart transplant adults, mean C_0 levels over the first week post-transplantation were significantly lower in patients with vs without acute rejection during the first year post-transplantation (126±56 vs 169±48 µg/L, $p = 0.003$).[78] ROC curve analysis

showed that patients whose mean C_0 during the first week was > 150 µg/L had a significantly lower incidence of acute rejection (30.3 vs 64.9%, p = 0.009).

In a small study performed in 15 adult lung transplant recipients on cyclosporine (dose-adjusted on C_0, same target whatever the period) during the first year post-transplantation, significantly higher C_0 were reported in patients who developed CMV infection or disease than in patients who did not (289±93 vs 222±82 µg/L, $p < 0.05$).[79] C_0 and C_2 displayed the same sensitivity (84.6%) but C_0 yielded better specificity (66.7%) than C_2 (57.1%).

Cyclosporine C_0 may not always be an appropriate TDM tool, however, as shown by a series of studies in which there was no correlation between C_0 and clinical outcomes.

In 31 heart-lung transplanted adults followed during the first 3 months after transplantation, no relationship was found between C_0 and subsequent rejection. The authors put more emphasis on the evidence of C_0 variability as a risk factor for subsequent rejection, with intra-individual CV > 40% resulting in a relative risk of 1.51 (95%CI = 1.01-2.27) for rejection.[80]

In a more recent study in 48 patients who underwent lung or heart-lung transplantation, treated by either Sandimmune® or Neoral® and followed for one year after transplantation, no statistically significant difference on C_0 was found between patients who experienced 2 acute graft rejection episodes or more, and patients who experienced less than 2 episodes. No relationship was found either between C_0 and infections or the deterioration of renal function.[20]

The same observations were made in another study performed in 31 heart transplant recipients between 3 weeks and 2 years post-transplantation, cyclosporine dose-adjusted on C_0, and in whom no relationship was found between C_0 and acute graft rejection or impairment of renal function.[54]

1.1.2.3.2 C_2 monitoring

A significant number of observational studies demonstrated that C_2 monitoring is an efficient TDM tool in solid organ transplant patients receiving cyclosporine: it allows the reduction of incidence of acute and chronic rejection and of hypertension after transplantation. Unfortunately, most of these studies were performed in hepatic or renal transplantation.[81-83]

As a consequence, although C_2 targets were validated in various other solid organ transplant settings,[81-83] there is no published consensus to date for C_2 target levels after heart or lung transplantation.

In thoracic transplantation, a number of authors succeeded in establishing a relationship between C_2 and clinical outcomes and thus advocated C_2 monitoring.[10,56,84-88]

C_2 was an excellent tool to predict AUC_{0-12} and AUC_{0-4} in CF and non-CF patients in small single-center studies.[4,6,21,25,54] A longitudinal follow-up was conducted in 114 adults who had undergone heart transplantation more than one year prior to the study.[10] Follow-up was performed in two phases: cyclosporine dose was adjusted on C_2 during the first phase (target: 300 to 600 µg/L) then adjusted on C_0 during the second phase (target: 100 to 200 µg/L). The primary endpoint was the clinical benefit, defined by the authors as a composite criterion comprised of positive cardiac outcomes (no mortality, no acute rejection, no decrease in LVEF > 10%) and positive renal outcomes (absence of increase in serum creatinine > 10%). There was no difference between the two phases in terms of incidence of acute rejection or mortality, but there was a significant clinical benefit of C_2 monitoring vs C_0 monitoring, with a relative "risk" of positive outcome of 1.6 ($p = 10^{-5}$). Moreover, there was a lower incidence of serum creatinine increase > 10% during phase 1, with a relative risk of 0.37 ($p < 10^{-4}$). However, one limitation of this study is that it was a non-randomized sequential assessment of 2 strategies for Neoral® dose adjustment: as this concerns a chronic, progressive pathology, there is no certainty that patients were comparable between phases I and II in terms of both cardiac and renal functions.

Another prospective study was performed in 30 stable heart transplant adults who were randomized to either C_0 or C_2 monitoring.[56] None of the patients experienced biopsy-proven acute rejection ≥ grade 2 according to the ISHLT classification. As a consequence, this study could not report any relationship between any monitoring tool and the clinical outcome, and was rather a study on the correlation between single concentration time points and AUC_{0-4}.

In a similar prospective study, Delgado et al performed parallel determination of C_0 and C_2 levels in 58 adults with orthotopic heart transplantation.[86] Follow-up consisted of two phases: during phase 1 (6-months follow-up on C_0), the authors found no significant difference on C_0 between patients who experienced rejection and those who did not (195±121 vs 197±100 µg/L, p = 0.96) but C_2 was lower in patients with rejection than in patients without (777±326 vs 1,015±422 µg/L, p = 0.022). During phase 2 (6-months follow-up on C_2), no significant difference was found between C_0 levels (204±85 vs 209±138 µg/L, p = 0.88), whereas rejection was associated with lower C_2 levels (765±297 vs 967±470 µg/L, p = 0.03). There was no difference on serum creatinine, creatinine clearance or infections between the two phases. As a consequence, the authors concluded that higher C_2 levels were associated with significantly fewer rejection episodes and that C_2 monitoring was safe in terms of preservation of renal function and infection rates.

In lung transplantation, Glanville et al conducted a single-arm, single-center pilot study on 15 stable transplanted adults with renal dysfunction.[87] Cyclosporine monitoring was switched from C_0 to C_2 (C_2 target: 300 to 600 µg/L), resulting in cyclosporine dosage being divided by two within three months, with a significant improvement in renal function. Lung function remained stable in all patients, except for one acute rejection episode. Based on these results, the authors concluded that C_2 monitoring allows safe dose reductions in patients with altered renal function, in cases where AUC monitoring is not feasible.

The same team performed a study in 50 *de novo* lung or heart-lung transplant patients, 20 of whom with CF, using 338 (73 CF) historic controls.[88] The authors concluded that C_2 monitoring and dose adjustment on C_2 brought a clinical benefit compared to C_0 monitoring in terms of survival, incidence of acute rejection, occurrence of BOS and renal dysfunction. Unfortunately, the comparison with historic controls is of limited interest given the significant time bias and the lower proportion of patients with CF or bilateral lung transplant in the control group.

In another study, Caforio et al retrospectively compared C_0 vs C_2 as predictors of rejection and renal dysfunction in 269 adults after 1 year post-heart transplantation.[85] Dose adjustment was based on C_0 (target: 100 to 250 µg/L). Patients with higher C_2 levels (> 740 µg/L) had higher severe rejection score at 2 years than patients with lower C_2 ($p = 0.02$), whereas no significant association was found with C_0. However, based on the rejection history of the patients, the authors used higher cyclosporine dose and C_0 targets even in the long term in patients considered high rejectors vs the low rejectors, which probably accounts for this quite unexpected result.

Finally, Baraldo et al compared the distribution and variability of C_2 values after Neoral® twice daily (BID) or three times daily (TID) between 2 groups of 25 stable heart transplant recipients followed for one year.[84] Cyclosporine dose adjustment was based on C_0 (target: 250 to 350 µg/L). Significantly higher C_2 variability was observed in the BID than in the TID regimen (CV = 25.3 vs 15.1%, $p < 0.001$), but it is not quite clear whether the authors referred to intra-patient or inter-patient variability or a mixture of the two (overall C_2 variability). There was no significant difference in rejection incidence or cardiac function (LVEF) between the 2 groups, but the TID regimen led to a lower incidence of nephrotoxicity. Based on these results, the authors suggested that in stable heart transplant patients, cyclosporine TID would lead to similar (or better, considering a lesser variability on cyclosporine concentrations) cardiac outcomes than BID, with decreased nephrotoxicity (decreased C_2 and

decreased variability on C_2), with a target range for C_2 of 650 to 850 µg/L. However, these statements are speculative and have never been confirmed by a prospective trial.

To our knowledge, only one prospective study compared C_0 to C_2 monitoring, in 50 *de novo* pediatric heart transplant recipients.[55] No significant difference was found on C_0 between patients with or without acute graft rejection, but C_2 was significantly higher in children who did not experience acute rejection, whatever the post-transplantation period. The ROC curve analysis for C_0 and C_2 found better sensitivity and specificity for C_2, with respective values of 100% and 83% for a C_2 target of 600 µg/L.

On the other hand, many observational studies failed to evidence a relationship between C_2 and clinical outcome. In 7 consecutive male adults on Neoral® followed for one year after heart transplantation, no statistically significant difference in C_2 was found between patients with and without acute rejection, which might be due to the fact that cyclosporine dose was adjusted in all the patients according to C_2 (target range: 300-600 µg/L)[89] and, above all, that too few patients were included in order to perform a powerful analysis.

In another retrospective study, Cantarovich et al analyzed the results of 517 EMB from 39 adult heart transplant recipients during the first year post-transplantation.[90] When EMB were split into 2 groups based on the degree of acute rejection (≤ 2 or ≥ 3A according to the ISHLT classification), no statistically significant difference in C_2 values was found. However, cyclosporine monitoring was not homogeneous in all patients, being based on C_0 in 13 of them and on C_2 in the 26 others, and cyclosporine was assayed using different analytical methods throughout the study (EMIT or CEDIA), which may have introduced confusing factors in the analysis. In another study, a prospective analysis of 2 consecutive periods was performed in 22 *de novo* heart transplant patients.[22] In group I, follow-up was based on C_0 whereas in group II, follow-up was based on C_2 (targets depending on time post-transplantation). Of note, all patients received MMF but the dose was different between the groups (1 g twice daily in group I vs 1.5 twice daily in group II). No significant difference was found between the 2 groups on the following: the incidence of acute rejection episodes ≥ grade 3A according to the ISHLT classification, time to acute rejection, death, LVEF, incidence of graft atherosclerosis, nephrotoxicity or infections.

More recently, Cantarovich et al performed a multi-centre randomized open-label study in 87 *de novo* adult heart transplant recipients, with the objective of determining the minimal effective exposure to cyclosporine with C_2 monitoring.[91] Patients were stratified into 2 cohorts based on renal function, and three C_2 ranges were defined (high, intermediate and low C_2), which varied with time post-transplantation. Again, the study failed to show any

statistically significant difference between groups, in terms of acute rejection incidence or renal function. The authors finally concluded that it was safe to monitor Neoral® with a "low" C_2 range (< M1: 1,200-1,400 µg/L; M2-M3: 1,000-1,200 µg/L; M4-M5: 800-1,000 µg/L; M6-M12: 700-900), preserving renal function without increasing the risk of acute rejection.

The studies performed by Trull et al[28] and Akhlaghi et al[20] in 50 lung or heart-lung transplant recipients during the first year post-transplantation did not show any relationship between C_2 and the incidence of treated acute rejection episodes, and C_2 did not correlate with any of the markers of cyclosporine nephrotoxicity.

A prospective randomized controlled study, the aim of which was to compare C_0 (target: 80 to 120 µg/L) to C_2 (target: 300 to 600 µg/L) monitoring in terms of clinical outcomes, was performed in 125 heart transplant recipients after 1 year post-transplantation, followed-up for 6 months.[51] The study failed to detect a possible effect of C_2 vs C_0 monitoring on acute rejection, infections or nephrotoxicity, probably because it was not powered to detect a small effect. However, a significant difference between the two groups was found for the primary endpoint: cyclosporine daily dose decreased by 26 mg over the follow-up period in patients monitored on C_2, vs 11 mg in patients monitored on C_0 ($p = 0.0025$).

In lung transplantation, a study was performed on 2 sequential groups of 18 *de novo* bilateral lung transplant patients, 17 of whom had CF.[92] In the first group, cyclosporine dose was adjusted on C_0 (target at W1: 450 µg/L; at M3: 250 µg/L) whereas in the second group, dose was adjusted on C_2 (target at W1: 1200 µg/L; at M3: 800 µg/L). Acute rejection rates were similar in both groups and there was no statistically significant difference on FEV1 and on the incidence of infections between the two groups of patients. A greater increase of serum creatinine from baseline was observed in the C_0 group. However, these two groups were not comparable as patients in the C_0 group were older and their baseline serum creatinine was lower (no mention was made of creatinine clearance). Moreover, the associated immunosuppressive treatment was different, as 14 of the 18 patients in the C_0 group were on azathioprine whereas 16 of the 18 patients in the C_2 group were on MMF.

1.1.2.3.3 C_6 monitoring

One study in heart transplantation investigated the use of C_6 as monitoring tool. In this prospective study, 20 adults were randomized into 2 groups: in the first group, monitoring was based on C_0 (target: 150 to 250 µg/L) whereas in the second group, cyclosporine dose was adjusted on C_6 (same target). The two groups were similar in terms of efficacy and toxicity, but patients of group II received a significantly lower dose of cyclosporine (2.6±0.6

vs 3.5±1 mg/kg/day, p = 0.002), with a significantly lower total cyclosporine cost (3,589±1,116 vs 5,106±1,045 CDN $, p = 0.005).[59] The result is however evident and directly depends on the protocol, but in no way guarantees that C_6 is as safe and as efficient as C_0 monitoring on the long term.

1.1.2.3.4 AUC monitoring

Our literature search allowed us to identify only three studies in which a potential relationship between AUC and clinical outcomes was evaluated (Tables VI & VII).

Thirty-one patients were included in the first study, in which the main objective was to determine the clinical significance of C_2 compared with C_0 following heart transplantation.[54] No significant difference on AUC_{0-12} and AUC_{0-4} was found between patients who experienced acute rejection and those who did not (whether these AUCs were calculated by Bayesian estimation or using sparse-sampling algorithms).

The aim of the second study, performed in 50 children, was to assess the relative value of C_0 and C_2 monitoring to prevent acute rejection in pediatric *de novo* heart transplant patients.[55] A subgroup analysis was performed in 10 children, 5 with and the other 5 without acute rejection. A statistically significant difference was found between the two groups on AUC_{0-12} (rejectors vs non-rejectors: 3,615±508 vs 5,530±889, $p < 0.001$) and on AUC_{0-4} (rejectors vs non-rejectors: 1,498±132 vs 2,713±536, $p < 0.001$), both calculated using the linear trapezoidal rule. However, this is only a case-control study in a very small number of patients, which does not permit definitive conclusions to be drawn, even more so in that the first study (also conducted in a small population) did not confirm this finding.

The third study enrolled 60 heart transplant patients, who were divided in 2 groups: "constant absorbers" (group A, CV of mean individual AUC < 15%); and "inconstant absorbers" (group B, CV of mean individual AUC > 15%).[93] Mean AUC levels were not different between the 2 groups (5,585 vs 5,772 h.µg/L), but patients with more variable absorption profiles were at higher risk for rejection (rejection rate of 19% vs 41% in groups A and B, respectively; $p < 0.05$).

In conclusion, the vast majority of these studies may have been of sufficient size to study the correlation between C_0 or C_2 and AUC, but underpowered for studying the relationship between drug concentrations and clinical outcome. Also, we did not find any study designed to evaluate the relationship between exposure measured by the AUC and efficacy (acute graft rejection, graft loss) or cyclosporine toxicity. Despite more than 20 years of use, no

prospective trial compared cyclosporine dose adjustment based on full AUC and C_0 or C_2 in any type of allograft transplantation, let alone thoracic transplantation.[53]

Available data has to be expanded to propose recommendations for optimal cyclosporine TDM in thoracic transplant recipients, including the identification and validation of the best exposure index and the definition of optimized target alues. Further investigations in special populations such as CF patients with gastroparesis are also awaited.

1.2 Tacrolimus

Tacrolimus (FK506, Prograf®) is a 10-100 times more potent immunosuppressant than cyclosporine *in vitro*.[8,9,94] Despite a chemical structure different from that of cyclosporine, tacrolimus suppresses the immune system by similar mechanisms: it inhibits the synthesis of IL-2 through the binding of a specific immunophilin, FK506 binding protein (FKBP12) and inhibition of calcineurin by this complex.[9]

Tacrolimus was proposed as an alternative to cyclosporine in combination with AZA or MMF after thoracic transplantation, leading to lower rates of acute rejection, similar infection rates and slightly higher incidence of new onset diabetes mellitus compared to cyclosporine-based therapy.[95-97] There has been a clear trend over the past decade to use tacrolimus instead of cyclosporine as part of maintenance immunosuppressive regimens after thoracic transplantation.[3] Tacrolimus is now the main calcineurin inhibitor given to adult and paediatric thoracic transplant recipients (approx. 60% of the patients).[2,3,42]

Some guidelines have been proposed for the use of tacrolimus after thoracic transplantation, with a recommended initial oral tacrolimus dose of 0.05 to 0.15 mg/kg/day in heart transplant patients.[95] and of 0.1 to 0.15 mg/day in lung transplant recipients.[98] Administration of tacrolimus sublingually or via a naso-gastric tube may be useful in some specific situations, such as in patients with severe gastro-intestinal troubles. In the early post-transplantation period, IV tacrolimus may be used when necessary[99] (for instance, during the very first days post-transplantation, or to treat resistant rejection), via continuous infusion over 24 hours, with a dose ranging between 0.015 and 0.10 mg/kg/day. Nevertheless, there are some concerns regarding neurotoxicity and nephrotoxicity when it is administered by this route.[9]

1.2.1 Pharmacokinetics

The PK of tacrolimus has been previously described in healthy volunteers and in diverse solid organ transplant settings (kidney, liver).[100] As with cyclosporine, it is characterized by a large inter-individual and intra-individual variability.[101,102] However, PK and variability data

on tacrolimus used after thoracic transplantation is relatively scarce and inconsistent (Tables IX & X). In many published studies, exposure indices and PK parameters were considered after the first dose.[100,103-105] Except for two studies[100,103] in which tacrolimus was assayed by HPLC-MS/MS, the PK studies we identified used immunoassays (mostly MEIA) to measure tacrolimus concentrations. Such assays (also including ELISA, EMIT, ACMIA, CEDIA and CMIA technologies) all lack specificity because of cross-reactivity of the assay antibody with metabolites, hence overestimating the actual blood levels (as assessed using the reference technique HPLC-MS/MS). Moreover, immunoassays may interact with haemoglobin: some authors observed a negative correlation between haematocrit and tacrolimus concentrations determined by MEIA.[106] This observation was confirmed in subsequent publications, which reported that both low haematocrit values and low albumin concentrations are likely to show artificially high concentrations of tacrolimus when measured by MEIA.[107,108] Finally, the majority of the PK studies performed in thoracic transplantation were descriptive in nature and used model-independent PK methods, where mean and standard deviation of exposure indices and PK parameters in the evaluated population were calculated from individual data.[100,102,103,105,109-111] One used the iterative two-stage (ITS) method, for which a specific PK model was set up in order to determine individual exposure indices and PK parameters and thus to calculate mean PK parameters in the population.[112] We could not find any popPK study of tacrolimus in thoracic transplantation.

The intra- and inter-individual variability of tacrolimus PK is related to factors such as erratic bioavailability, time post-transplantation, concomitant pathologies and drug-drug interactions, which unfortunately were rarely explored.

1.2.1.1 Absorption

Generally, the bioavailability of tacrolimus after oral administration is poor, averaging 25%, but is also characterized by a large inter-individual and intra-individual variability, ranging from 5% to 93% depending on the reports.[8,52,94,100,101] This low and variable bioavailability is caused by an extensive presystemic metabolism by CYP3A isoenzymes and by active transport by the trans-membrane efflux pump P-gp, all localized in the gut wall in addition to the liver.[8,101] Poor aqueous solubility and alterations in gut motility (*e.g.* in CF) may also be responsible for poor and erratic drug uptake.[101] Tacrolimus bioavailability can be reduced when the molecule is administered with low-fat food.[8,101] On the contrary, some authors reported an increased bioavailability in patients with persisting diarrhea, resulting in an

increase in tacrolimus trough levels.[113] However, in contrast to cyclosporine, tacrolimus is absorbed in a completely bile-independent manner.[8,94,101]

In most subjects, tacrolimus is rapidly absorbed following oral administration, t_{max} occuring usually around 2 hours after single administration[102,103,105] and between 0.5 and 1.5 hours at steady-state (Table IX).[52,100,101,103,111] However, drug uptake can also last longer, resulting in a flat absorption profile, an extended lag-time or secondary peaks, with maximum blood concentrations occurring up to 8 hours after dosing.[8,94,100,101,105,109,110,112]

With oral dosing, steady-state blood concentrations are usually achieved within approximately 48 to 72 hours.[95] In heart transplant patients, C_{max} was characterized by a coefficient of variation ranging between 41 and 56% after single oral administration,[103,105] and between 36 and 49% at steady-state[100,103,105,109] and between 53 and 68% in lung and heart-lung transplant patients.[112,114]

The rate of absorption of tacrolimus has been reported to be highly variable.[101] In heart transplant patients, absorption was characterized by a rate constant of absorption k_a ranging from 0.13 to 1.76 h^{-1} (CV > 40%) and a lag-time comprised between 0.1 and 0.5 hours.[115] In stable lung transplant recipients (with or without CF), tacrolimus absorption was best fitted using two Gamma distributions (double, sigmoid absorption profile with two different velocities), describing an early peak and a possible secondary peak of absorption.[112] This resulted in the calculation of two mean absorption times (MAT_1 and MAT_2), with similar values in CF and non-CF patients (MAT_1 = 1.10±0.68 h and 0.92±0.43 h; MAT_2 = 5.14±2.11 h and 5.47±2.30 h, respectively).

1.2.1.2 Distribution

Tacrolimus is highly lipophilic and binds extensively to erythrocytes, with higher blood than plasma concentrations. In plasma, it binds strongly to proteins (99%), mainly α_1-acid glycoprotein, lipoproteins, globulins and albumin.[101] After IV administration, tacrolimus concentration undergoes a rapid initial decline due to distribution, which slows down over the next 24 hours after reaching distribution equilibrium.[94] To our knowledge, tacrolimus V_d/F in thoracic transplant recipients was reported in only three studies, in two of which tacrolimus PK was fitted with a one-compartment model. In the first one,[112] V_d/F was determined at steady-state in 22 heart-lung transplant patients, with a significant difference between patients with and those without CF (2,011±1,740 L vs 444±326 L, respectively; $p < 0.01$), whereas in the second one[102] V_d/F ranged between 1.3 and 3.9 L/kg after single dose administration in 14 heart transplant recipients. In the third study,[115] performed in 19 heart transplant

recipients, the authors used a 2-compartment model and reported a mean V_1/F = 1.69±0.61 L/kg and 1.13±0.38 L/kg at ten days and two months after transplantation, respectively ($p < 0.01$). These rather inconsistent results might be partly explained by the different study conditions, the obviously large inter-patient variability (in the 3 studies, V_d/F coefficients of variation ranged from 33 to 86%) as well as by the generally suboptimal estimation of V_d/F by classical PK models, which usually estimate clearance more accurately than volume of distribution.

1.2.1.3 Metabolism and elimination

Like cyclosporine, tacrolimus is extensively metabolized (mainly by CYP3A4 and CYP3A5 isoenzymes in the liver and intestinal wall). More than 95% of tacrolimus metabolites are excreted in the bile, and less than 0.5% of tacrolimus dose appears unchanged in the urine or faeces.[52,94,101] As a consequence, its potential for drug-drug interactions seems similar to that of cyclosporine. One exception is that tacrolimus does not seem to interact with MMF PK like cyclosporine does, leading to greater MPA exposure when tacrolimus and MMF are used in combination.[52]

A few studies reported tacrolimus clearance values in thoracic transplant patients. In heart transplant patients, tacrolimus clearance after continuous IV infusion was 2.4 L/h.[103] After tacrolimus oral administration, CL/F was 0.23±0.08 $L.h^{-1}/kg$ after a single dose,[102] 0.19±0.08 $L.h^{-1}/kg$ ten days and 0.23±0.15 $L.h^{-1}/kg$ two months after transplantation,[115] with high coefficients of variation (42-65%). In a cohort of 12 lung and heart-lung transplant patients, CL/F = was 68.2±29.8 L/h in CF patients and 36.49±18.98 L/h in non-CF patients ($p < 0.05$),[112] which is higher than the above mentioned values (considering a mean bodyweight of 70 kg) and than those reported by Aumente-Rubio et al[109] in heart transplant patients (11.6±5.5 L/h); this may be explained by the difference in patients (heart-lung transplant vs heart recipients) and by the difference in the PK approach (one-compartment PK model vs non-compartmental PK analysis).

$T_{1/2}$ was determined mainly using non-compartmental PK analysis and was also found to be highly variable (CV = 30-64%).[100,102,103,105,114] When tacrolimus was assayed using a HPLC-MS/MS method, $T_{1/2}$ at steady-state was 10.7±5.3 h in heart transplant patients[100] and 7.9±2.3 h in lung transplant recipients.[114] The elimination half-life determined from tacrolimus concentrations measured by immunoassays may be overestimated due to the lack of specificity and the cross-reactions reported between the assay antibody and tacrolimus

metabolites. Indeed, in a cohort of 8 heart transplant patients, $T_{1/2}$ reached 33.3 h (mean: 13.8 h, 95%CI = 7.0-20.7 h). [111]

1.2.1.4 Drug-drug interactions

We did not find any formal PK studies investigating tacrolimus drug interactions in thoracic transplant recipients. Shitrit et al reported an interaction between itraconazole and tacrolimus in 40 patients with lung transplantation followed for more than one year.[116] All patients were on tacrolimus and received prophylactic itraconazole for the first 6 months post-transplantation. In those patients, the mean dose of tacrolimus required rose by 76% after itraconazole withdrawal (3.26±2.1 mg/day during the first 6 months compared to 5.74±2.9 mg/day during the following 6 months, $p < 10^{-4}$).

In other solid organs transplantation, various lists of drugs that may increase or decrease whole blood concentrations have been compiled.[101] Since tacrolimus is a substrate for CYP3A and P-gp like cyclosporine, the expected drug interactions are essentially the same[95] and any drug known to induce or inhibit these systems may increase or decrease its blood concentrations (erythromycin, calcium-channel blockers, imidazole anti-fungal agents).

1.2.1.5 Special populations

The PK of tacrolimus changes in time after transplantation and is affected by many factors, including liver function, patient age (pediatric transplant recipients require 2- to 4-fold higher doses of tacrolimus than adults to maintain similar C_0[101]), time of administration (diurnal variations, co-administration of food), associated drugs, and modifications in the GI tract motility and secretions observed in CF patients.[101,109]

Lower bioavailability of tacrolimus has been reported in CF patients, probably partly because of fat malabsorption due to pancreatic insufficiency.[110,112]

Walker et al reported that heart-lung and double lung transplant recipients with CF required a 39% higher dose of tacrolimus than patients without CF in order to reach similar C_0.[117] In a study performed in 22 heart-lung transplant patients (11 CF), tacrolimus CL/F after oral administration was higher in CF patients (68.22±29.80 vs 36.49±18.98 L/h, $p < 0.05$),[112] who required significantly higher doses to reach comparable exposure. In those patients, AUC_{0-12}/dose and AUC_{0-4}/dose were approx. half of those of non-CF patients ($p < 0.004$ and $p < 0.001$, respectively).[110] Comparatively to non-CF patients, exposure indices in CF patients (C_0, C_3, C_{max}, AUC_{0-12}, AUC_{0-4}) were characterized by higher inter-individual variability (CV% 24 to 46% vs 19 to 30%) and comparable intra-individual variability (CV% 16 to 40% vs 17 to 37%).[110]

1.2.2 Therapeutic drug monitoring

Issues relating to the TDM of tacrolimus have been addressed by numerous authors and recently reviewed by Staatz & Tett.[101] Like cyclosporine, tacrolimus is characterized by a narrow therapeutic index and significant systemic side effects.[8,52,115] As stressed above, tacrolimus absorption is erratic and incomplete with a variable metabolism mediated by intestinal and hepatic CYP3A isoenzymes, resulting in high intrapatient and interpatient PK variability. As a result, the correlation between dose and blood concentrations is poor,[52,115] confirming that the administered dose cannot reliably predict systemic exposure to tacrolimus, rendering it difficult to propose a suitable *a priori* dosing regimen for heart and lung transplant patients.[100] This was the rationale for monitoring tacrolimus blood concentrations to optimize immunosuppression.[52,94] However, the poor sensitivity and specificity of the first commercial assays used was an obstacle in the exploration of tacrolimus PK for optimizing TDM.[52]

1.2.2.1 Single concentrations monitoring

Most results of clinical studies in kidney and liver transplantation suggest that C_0 levels of tacrolimus are, to some extent, predictive of efficacy and toxicity.[52] Accordingly, tacrolimus dose after thoracic transplantation is mainly adapted to C_0 levels. The following target C_0 were proposed: in lung transplantation, 10-25 µg/L for the first 2 weeks, 10-20 µg/L for the next 6 to 10 weeks, and 10-15 µg/L thereafter;[98] after heart transplantation, 15-20 µg/L for the first 2 months, 10-15 µg/L from the third to the sixth month, and 8-10 µg/L after 6 months.[95] However, the authors did not mention on which assay these target levels were based, making it difficult to correctly interpret them, and to implement them in routine clinical practice.

Studies of the correlation between tacrolimus C_0 and AUC_{0-12} have reported a wide range of results, from $r^2 = 0.20$[111] to around 0.80 (Table IX).[103,104,109] Some studies have shown that the correlation between blood concentration and AUC can be improved by using different time points: a good correlation was found between AUC_{0-12} and C_3[110] or C_4.[104,105,109]

In other transplant types (mainly kidney), the reliance on C_0 monitoring has been questioned due to the observations that stable function, rejection or toxicity may be associated with the same range of tacrolimus concentrations.[101,109] Unfortunately, no study in thoracic transplantation has been performed with the intent of exploring the relationship between single concentrations (C_0 or other concentrations) and efficacy or toxicity.

1.2.2.2 AUC monitoring

As with cyclosporine, tacrolimus AUC rather than single concentrations may be used for TDM, as it is a better marker of patient's exposure to tacrolimus.

In studies in thoracic transplantation, tacrolimus AUC_{0-12} was mainly calculated using the linear trapezoidal rule.[100,102-105,109-111] As with cyclosporine, AUC_{0-4} was considered as a surrogate exposure index and AUC_{0-4} values reported in a few studies in heart[109] and in heart-lung[110,112] transplant recipients. However, AUC_{0-4} still has to be validated as a reliable estimate of AUC_{0-12} and as a reliable marker of clinical outcomes in heart or lung transplant patients.

As another way to avoid the numerous blood samples needed for the determination of AUC_{0-12}, some authors investigated sparse sampling strategies. In heart transplantation, Aumente Rubio et al proposed two algorithms, one with 3 sampling times (0, 2 hours and 4 hours, r^2 = 0.97 with trapezoidal AUC_{0-12}) and one with only 2 sampling times (0 and 2 hours, r^2 = 0.95).[109] In lung transplantation, Morton et al failed to find an efficient sparse sampling strategy,[114] but Saint-Marcoux et al developed and validated Bayesian estimators for the determination of tacrolimus AUC_{0-12} in CF and non-CF patients, and proposed the following sampling strategy: 0, 1 hour and 3 hours for non-CF patients and 0, 1.5 hours and 4 hours for CF patients.[112]

In a study of 25 primary heart transplant recipients, the authors found a significant difference in the AUC_{0-12} after the first oral dose between patients who experienced acute rejection and those who did not (71 vs 168 h.µg/L, p = 0.012).[103] However, this paper does not provide any detail about patient's follow-up, so there is no information on when acute rejection occurred.

So far, no prospective studies have been conducted in thoracic transplantation to investigate the relationships between tacrolimus inter-dose AUC and clinical outcome. *A fortiori*, the use of AUC as a monitoring tool still remains to be validated in prospective controlled studies, and optimal AUC targets to be defined in heart and lung transplantation.

2 Mycophenolate, purine synthesis antagonist

Two compounds containing mycophenolate are currently available, mycophenolate mofetil (MMF, Cellcept®) and mycophenolate sodium (EC-MPS, Myfortic®). As mycophenolate sodium has been released later and its use in thoracic transplantation more rarely reported, this review will focus on MMF only.

Following oral administration, MMF, the 2,4-morpholino-ester of the active moiety mycophenolic acid (MPA), is rapidly hydrolyzed to MPA by esterases in the stomach, small intestine, blood, liver and tissues.[118,119] MPA exerts its immunosuppressive effects by reversibly inhibiting inosine 5'-monophosphate deshydrogenase (IMPDH),[120] a key enzyme in the *de novo* pathway of purine biosynthesis,[121,122] essential for DNA replication when cells proliferate. Through IMPDH inhibition, MPA specifically blocks the proliferation and clonal expansion of T and B lymphocytes, providing effective immunosuppression in transplant patients.[121-124]

The use of MMF has dramatically increased in the past decade, MMF-based regimens representing now approx. 80% and 50% of maintenance strategies in adult heart and lung transplant recipients, respectively, and 50% in pediatric lung transplant recipients.[2,3,42,125]

MMF is available in oral (capsules, tablets and powder for oral suspension) and IV formulations.[122] It is generally combined with a calcineurin inhibitor and corticosteroids for rejection prophylaxis after solid organ transplantation.[124,126] Reduced rejection rates, improved graft and patient survival with MMF compared with AZA have lead it to progressively replace AZA as the antiproliferative agent of choice after thoracic transplantation, although it has not been approved by most of the national health agencies in lung transplantation.[2,5,127,128] There has been a growing interest in MMF-based as part of calcineurin inhibitor- or steroid-sparing strategies, as MMF is free of toxicities adversely affecting long-term patient and graft survival (nephrotoxicity, hypertension, metabolic perturbations).[120]

2.1 Pharmacokinetics

As opposed to other immunosuppressants, MMF dose in adults is not based on patient body size or weight. MMF is usually administered at a fixed oral dose of 0.75-1.5 g twice daily, depending on the associated calcineurin inhibitor.[119,120,124,129] Oral suspension can be administered via a nasogastric tube, if necessary.[118]

Little is known about the PK of MPA after thoracic transplantation.[119,124] Most PK studies have been conducted in renal transplant patients, in whom MMF exhibits time-dependant PK (concentrations increase with time following transplantation over the first 1-3 months).[124] However, data derived from renal transplant recipients cannot be readily extrapolated to thoracic organ transplant patients, as neither the heart nor the lungs are involved in the elimination of mycophenolate. Although blood pressure may have some impact on MPA glomerular filtration rate, cardiac and pulmonary functions do not impact so much MPA

plasma concentrations.[119,124] In a PK study of MMF in 50 stable thoracic (23 heart and 27 lung) transplant recipients (0.2-19.7 years post-transplantation), no significant variations in MPA exposure indices or PK parameters was observed with post-transplantation time (Tables XI & XII).[130] On the contrary, an increase of MPA C_0 throughout the follow-up period of 40.0±20.5 months was reported ($p = 0.0162$) in 57 "stable" lung transplant recipients (all non-CF patients), but the time elapsed between transplantation and enrolment was not reported and the authors did not give any hypothesis on the cause of this increase.[131] Consequently, currently available data on thoracic transplantation does not allow any reliable conclusion to be drawn on the evolution of MPA PK parameters with post-transplantation time, even in the early periods.

Like calcineurine inhibitors, the PK of MMF is complex and somewhat erratic,[118] with large intra- and inter-individual variability.[8,9,52,120,124,126,132,133] All studies we identified in thoracic transplant patients were performed using a model-independent PK approach, reporting mean and standard deviation, or range of exposure indices and PK parameters derived from individual data (Tables XI & XII).[119,121,122,124,130,132,134-137] We could not find any study using either the iterative two-stage (ITS) method or popPK analysis. Exposure indices of MPAG and AcMPAG were reported in 3 studies.[122,130,136] Finally, except for four studies in which MPA was assayed by EMIT[123,126,134,135] and one in which the analytical method was not reported,[137] all the PK studies we identified measured MPA, MPAG and AcMPAG concentrations with HPLV-UV.

2.1.1 Absorption, bioavailability

The PK of MPA after oral and IV MMF administration is characterized by an important variability. In a study involving 9 heart transplant patients who received 1.5 g BID MMF by IV infusion over three hours, immediately after transplantation and for 5 days, C_{max} displayed large inter-individual variations, occurring between 1 and 3 hours and ranging from 2.2 to 18.4 mg/L.[122] Following oral administration, MMF is rapidly absorbed (within 5 minutes of ingestion) in the upper gastro-intestinal tract because of its high solubility at a low pH.[8] Hydrolysis to MPA is responsible for the rapid disparition of MMF from the plasma.[118] MPA C_{max} after oral dosing is also highly variable, with a coefficient of variation ranging from approximately 30 to 80% depending on the studies,[119,121,124] and usually occurs within 1 to 2 hours following oral MMF administration (range 20 minutes-12 hours).[119,121,122,130,134,135,137] Delayed absorption has been reported when the drug is taken with food.[131]

In a review by Shaw et al, the bioavailability of MPA was reported to be incomplete, suggesting metabolism in the gastro-intestinal tract. This pre-systemic metabolism may take a part in the high overall PK variability of MPA.[133] On the contrary, the above mentioned study performed in 9 heart transplant patients reported excellent, high and consistent MPA bioavailability after MMF oral dosing compared with the IV formulation (oral F = 71 to 125%).[122]

Secondary peaks of MPA plasma concentration have been observed between 6 and 12 hours after drug administration and have been attributed to enterohepatic circulation.[8,124,131,138,139] This phenomenon is believed to contribute 10-60% to MPA exposure.[118,130,140] However, these secondary peaks are sometimes higher than the first peaks and in such cases, cannot be accounted for by enterohepatic cycling.[141] Further exploration of the absorption of MPA would thus be of major interest.

2.1.2 Distribution

In whole blood, MPA is poorly distributed into cellular fractions, with over 95% found in plasma.[118] In patients with normal renal and liver function, MPA is extensively and tightly bound to human serum albumin (97-99%).[118-120,133,142] The variations in MPA PK and pharmacodynamics (PD) may be partly explained by this affinity for serum albumin. Indeed, as only unbound MPA is pharmacologically active, variations in the free fraction may be responsible for variations of IMPDH inhibition and thus of the therapeutic response.[118,119,133,142] Moreover, in clinical situations with decreased serum albumin levels, such as severe hepatic impairment, or decreased serum albumin binding capacity, such as severe renal impairment, the increased proportion of free MPA may result in a higher clearance.[119,133] It has been suggested that in such situations with variations of plasma serum albumin, free MPA concentration might be worth monitoring,[119,120] though no paper reported that free MPA was better correlated to effects than total MPA plasma levels. Moreover, measuring free MPA is out of reach of traditional immunoassays.

Data on MPA protein binding in heart and lung transplantation was reported in only a few papers. In a cohort of 38 heart transplant patients (time post-transplantation: 310±278 days), the free MPA fraction (f) was 1.9±0.4%, with a free MPA AUC_{0-12} ($fAUC_{0-12}$) = 0.83±0.30 h.mg/L,[132] which is consistent with those reported in 7 lung transplant patients (time post-transplantation: 4.4±3.9 years), in whom f = 2.9±0.6%, and $fAUC_{0-\tau}$ = 1.29±0.50 h.mg/L;[119] as well as with the free fraction reported in 7 heart transplant recipients (time post-transplantation: 6-23 days), in whom f = 3.6±3.9%.[143] In another group of 7 heart (n = 3) and

lung (n = 4) transplant patients, f was higher, decreasing from 6.1±2.8% immediately after transplantation (15±13 days) to 4.4±3.0% later on (125±73 days), with corresponding $fAUC_{0-\tau}$ of 2.22±2.32 h.mg/L and 1.50±2.16 h.mg/L, respectively.[124] However, these results are to be interpreted cautiously, as f was determined in each patient after pooling all the plasma samples of a given profile. Indeed, averaging fractions is mathematically different from dividing the mean free by the mean total concentration and is simply not coherent. Finally, a recent study performed in a cohort of 23 heart (and heart-kidney) and 27 lung transplant recipients (time post-transplantation: 0.2-19.7 years) in whom MMF was associated to cyclosporine or tacrolimus, reported f values ranging between 0.2 and 15%, with $fAUC_{0-12}$ normalized to a 1 g-MMF dose of 0.05 to 19.0 h.mg/L.[130] Although they were discussed, the factors potentially linked with these high variations in free fraction were not investigated (nor reported).

Once absorbed, MPA is rapidly distributed in the tissues, within approx. 2 hours after t_{max}.[133] Values for MPA V_d/F were reported in only one study, conducted in 27 lung and 23 heart (and heart-kidney) transplant recipients who received MMF associated with either cyclosporine or tacrolimus.[130] In the lung transplant group, mean V_d/F = 248 L (range: 54-645) and 125 L (range: 30-607) in the patients on cyclosporine (n = 11) and on tacrolimus (n = 16), respectively. In the heart transplant group, mean V_d/F = 102 L (range: 37-1,141) and 111 L (range: 53-261) in the patients on cyclosporine (n = 14) and on tacrolimus (n = 9), respectively. A statistically significant difference on V_d/F was found neither between heart and lung transplant recipients, nor between patients on cyclosporine and those on tacrolimus.

2.1.3 Metabolism and elimination

MPA is primarily metabolized by uridine diphosphate glucuronyl transferases (UGT, mainly UGT1A9, 1A7 and 1A8) into its pharmacologically inactive metabolite, 7-O-mycophenolic acid glucuronide (MPAG).[144] MPAG accounts for more than 90% of all MPA metabolites, but at least 4 other metabolites have been identified in plasma from renal, liver and cardiac transplant recipients,[144-147] including the acyl glucuronide of MPA (AcMPAG), which inhibits IMPDH *in vitro*.[122,148] The primary site of conversion is the liver, but MPA may also be glucuronidated in the gastro-intestinal tract mucosa and the kidney.[8,94,118] As for MPA, concentrations of MPAG and AcMPAG are highly variable throughout the dose interval. MPAG plasma concentrations are said to be usually 20 to 100 times higher than MPA concentrations,[118] but this seems to be variable across sampling times and studies. In 9 heart transplant patients, MPAG C_0 was on average 65-106 times higher than MPA C_0 after IV or

oral MMF administration, whereas MPAG C_{max} was only 10 to 16 times higher than MPA C_{max}.[122] This study was the only one we identified reporting MPAG C_{max} (51 to 187 mg/L) and t_{max} (40 minutes to 8 hours). In another set of 26 heart transplant recipients, MPAG C_0 was only 22-30 times higher than MPA C_0.[136] The overall difference in exposure between MPAG and MPA may be more precisely evaluated by comparing their AUC. The AUC of MPAG was reported in two studies in thoracic transplantation, and was on average 9 to 32 times higher than that of MPA.[122,130] In a study conducted in 7 heart transplant recipients,[143] MPAG free fraction was estimated to be 26±8%, which is consistent with literature reports in renal transplant recipients (MPAG free fraction of 18-37% in 27 "stable" patients; time post-transplantation not reported).[149] MPAG is excreted into the bile by a mechanism probably involving the multidrug resistance protein 2 (MRP2). Upon delivery to the GI tact, through the action of β-glucuronidases shed by gastro-intestinal tract bacteria, part of MPAG is deconjugated back to MPA, which then undergoes enterohepatic recirculation.[8,94,118,133]

As opposed to MPAG, AcMPAG was once thought to display a pharmacological activity similar to that of MPA as it did *in vitro* with the recombinant enzyme,[146] but a more recent *in vitro* study suggested that, due to a lower intrinsic inhibitory activity, a lower ability to cross cell membranes and lower plasma concentrations, AcMPAG probably exhibits a negligible IMPDH inhibition activity *in vivo*. (Gensburger O et al, unpublished observations) AcMPAG t_{max} varies between 40 minutes and 6 hours, AcMPAG C_0 and C_{max} are 2 to 4 times lower and 8 to 11 times lower than MPA C_0 and C_{max}, respectively.[122] In two studies, AcMPAG AUC was 2.5 to 6 times lower than MPA AUC [122,130], which seems to be higher than the average of 10.3% reported in renal transplantation – though in this study AcMPAG was determined as MPAG equivalent due to the absence of a standard compound for quantification.[150]

We found two studies reporting MPA CL/F, with diverging results, showing the difficulty of estimating this PK parameter using a non-compartmental approach. The first study, performed in 14 heart transplant recipients who were all on MMF-cyclosporine, reported CL/F = 1,397±389 L/h.[137] The second study included 27 lung and 23 heart transplant recipients who received MMF associated with either cyclosporine (n = 25) or tacrolimus (n = 25).[130] In the lung transplant group, mean CL/F was 54 L/h (range: 29-295 L/h) and 21 L/h (9-121) in patients on cyclosporine and tacrolimus, respectively (p = 0.026). In the heart transplant group, mean CL/F was 21 L/h (5-59) and 11 L/h (6-24) in patients on cyclosporine and tacrolimus, respectively (ns). CL/F in the lung transplant group was significantly higher than in the heart transplant group, regardless of the associated calcineurin inhibitor (36 vs 13 L, p = 5.10^{-4}). One of the hypotheses made by the authors to explain this difference between heart

and lung transplant recipients is that the higher albumin levels observed in the heart transplant group may have contributed to an increase in MPA protein binding and thus a decrease in total CL/F of MPA.

Apparent half-life of MPA estimated from apparent distribution volume and clearance reported in the abovementioned study[130] was comprised between 3.2 and 7.0 hours, which is shorter compared to 18 hours reported in a review published in 1997.[94]

2.1.4 Drug-drug interactions

The variability in MPA concentrations may be partly explained by drug-drug interactions affecting the MPA concentrations achieved for a given MMF dose. Indeed, the PK of MPA is known to be altered by concomitant medications.[142] First, it is strongly affected by the nature of the associated calcineurin inhibitor. Such observations were initially made in renal transplant patients on tacrolimus, who displayed significantly higher MPA C_0 and AUC than patients on cyclosporine.[151] In heart transplantation, a randomized study in 60 patients found significantly lower MMF dose requirements for patients on tacrolimus (n = 30) than on cyclosporine (n = 30) to achieve MPA C_0 targets of 0.45 to 4.0 mg/L (1.6±0.8 vs 2.8±1.0 g/day at one year post-transplantation, $p = 4.10^{-5}$).[152] A prospective, randomized controlled study in 50 heart transplant patients compared the MMF – tacrolimus (n = 25) and the MMF – cyclosporine (n = 25) combinations in terms of efficacy, toxicity and PK.[153] Both groups displayed similar MPA C_0 (2.3±0.5 µg/L vs 2.7±0.6 µg/L), although the patients on cyclosporine received higher doses of MMF (3.3±0.5 g/day vs 2.1±0.7 g/day, p = 0.04). In 30 adult stable lung transplant recipients (7 CF) over the first 2 years post-transplantation, MPA C_0 were 50% lower in patients on cyclosporine (n = 16, C_0 estimated at approx. 1.3 mg/L) than in patients on tacrolimus (n = 14, C_0 estimated at approx. 2.7 mg/L, $p < 10^{-5}$) despite substantially higher MMF doses (estimated at approx. 25 vs 22 mg/kg/day).[142] In a study in 26 heart transplant patients aged 1 month to 33 years, lower MPA concentrations and higher MPAG/MPA C_0 ratios were reported in a subset of 8 children on cyclosporine compared to 8 others on tacrolimus (C_0 = 1.6±1.5 vs 3.0±2.2 mg/L, p = 0.04 and average MPAG/MPA C_0 ratio = 25.2 vs 11.9, p = 0.005).[136] Unfortunately, the MMF dose was only reported in children on cyclosporine, allowing no dose comparison.

Two other studies compared MPA C_0 (assayed by EMIT) in patients on cyclosporine vs tacrolimus,[123,126] but both pooled data from kidney and lung transplant recipients. The first one included 120 kidney and 20 lung transplant patients and reported higher MPA C_0 in patients on tacrolimus than in patients on cyclosporine (3.63±2.63 vs 2.14±1.22 mg/L, p <

0.01) despite similar MMF doses (2 g/day).[126] The second one included 49 kidney and 11 lung transplant recipients and reported higher median dose-to-concentration (D/C) ratios in patients on cyclosporine than in patients on tacrolimus ($p < 10^{-4}$),[123] which is an atypical presentation of results similar to those of the previous studies.

This was confirmed by a later study where 27 lung transplant patients were included together with 23 heart (heart-kidney) transplant recipients,[130] where the difference between dose-normalized AUC (DN-AUC) of lung transplant recipients on cyclosporine (n = 11) or tacrolimus (n = 16) was significant (DN-AUC = 18.6 h.mg/L, range: 3.4-35.1, vs 41.4 h.mg/L, range: 8.3-115.3, p = 0.022). Consistently, MPAG/MPA AUC ratios were higher in patients on cyclosporine (28.5, range: 9.8-55.2) compared to patients on tacrolimus (12.6, range: 2.4-25.8, p = 0.002). On the contrary, no significant difference on DN-AUC was found between the 14 heart transplant recipients on cyclosporine (50.9 h.mg/L, range: 16.9-218.7) and the 9 on tacrolimus (106.8 h.mg/L, range: 40.9-180.5, p = 0.14). Neither was the difference between MPAG/MPA AUC ratios of these 2 groups of heart transplant recipients significant (11.9, range: 0.9-28.6 in patients on cyclosporine vs 8.7, range: 2.3-13.4 in patients on tacrolimus, p = 0.10).

In summary, dose-adjusted MPA concentrations are significantly higher and MPAG/MPA concentrations ratios lower when MMF is associated with tacrolimus than cyclosporine.[136,142]

The mechanisms underlying the interactions between MPA and calcineurin inhibitors are still being investigated. At first, some authors postulated that the increase of MPA concentrations in patients on tacrolimus was caused by the inhibition of UGTs and thus of MPA glucuronidation by tacrolimus.[154] However, most authors now agree that these differences in MPA concentrations are due to a diminution in exposure caused by cyclosporine, rather than an increase caused by tacrolimus.[133] Indeed, it has been suggested that cyclosporine decreases MPA exposure by inhibiting the biliary excretion of MPAG into the gut, reducing back-transformation of MPAG into MPA through enterohepatic circulation.[126,133,142,155,156] It has also been suggested that cyclosporine might inhibit the deglucuronidation of MPAG to MPA by inactivating the β-glucuronidases in the gastro-intestinal tract.[157]

Consistently, in a recent study in 62 heart transplant recipients, exposure to MPA was shown to be higher in patients on sirolimus compared to those on cyclosporine for similar doses of MMF, as evidenced by significantly higher dose-adjusted AUC_{0-12} in patients on sirolimus (31.9±16.1 vs 61.0±27.4 h.mg/L/1000 mg MMF, $p < 0.001$),[134] which is in favour of cyclosporine being the interacting drug rather than tacrolimus and sirolimus.

In conclusion, the associated immunosuppressive drugs (cyclosporine, tacrolimus, sirolimus) need to be taken into account when defining MMF dose, as the under-immunosuppression potentially resulting from decreased MPA exposure in patients on cyclosporine may strongly influence clinical outcome.[142] Also, the influence of corticoids, which are known to induce the expression of the UGTs and have been shown to induce MPA metabolism in renal transplant patients,[158] might be worth investigating in thoracic transplantation.

2.1.5 Special populations

The PK of mycophenolate can be affected by several other factors such as pre-existing conditions (age, pathology, activity of UGTs and drug transporter MRP-2), and clinical status (time post-transplantation, gastro-intestinal motility, hypoalbuminemia, liver disease, renal dysfunction). These factors can alter plasma MPA, MPAG and AcMPAG total and free concentrations and global exposure, hence the degree of immunosuppression.
Pediatric thoracic transplantation will be briefly presented before focussing on CF.

2.1.5.1 Paediatrics

Children display different PK profiles from those of adults',[44,45] and different MMF disposition rates are expected in children vs adults, based on the ontogeny of UGTs.[159] Very little information is available on appropriate dosing or on PK of MMF in children. Similarly to adults, MMF in children is rapidly absorbed and hydrolyzed to MPA, with a first peak occurring approximately 45 minutes after intake and a potential second peak at a variable time. However, there is no information on the correlation between MPA exposure and clinical outcome in this population.[160] The recommended MMF starting dose is 1800 mg/m^2/day in two divided doses in children < 2 years and 1200 mg/m^2/day in older children and adolescents.[120,159] We identified two studies performed in heart transplant children. The first one was a retrospective review of MPA C_0 in 44 children (aged 7 days to 18 years; associated calcineurin inhibitor not reported).[160] MMF doses (in mg/kg and mg/m²) required to reach the target C_0 < 3 mg/L tended to decrease with increasing age (2.2±0.7 g/m² between 0 and 1 year, 2.2±0.9 g/m² between 1 and 5 year, 1.8±0.9 g/m² between 5 and 10 years, 1.6±0.8 between 10 and 16 years, and 1.6±0.6 beyond 16 years). However, the authors emphasized the limitations of their study, which included the retrospective design and more importantly the diversity of the population in terms of indication for MMF treatment (acute rejection prophylaxis or treatment) and time post-transplantation (7 days to 10 years). In the second study, a comparison was made between 8 children (with no more precision) and 10 adults

who received MMF in association with cyclosporine.[136] Adults and children received comparable MMF daily doses (1,280±360 vs 1,180±260 mg/m², ns) and displayed comparable MPA C_0 (2.3±2.2 vs 2.2±2.0 mg/L, ns), but MPAG C_0 and MPAG/MPA C_0 ratios were significantly higher in adults (98±47 vs 49±38, $p < 10^{-4}$ and 37.7±40.2 vs 25.2±21.7, $p = 0.016$). The authors hypothesized that this might be explained by the existence of an alternate pathway in children with enhanced MPAG clearance compared to adults, or by higher glomerular filtration and tubular secretion rates in children, or even that cyclosporine-mediated inhibition of MPAG excretion may also be a pathway specific to adults, not active (or to a lesser extent) in younger patients. In our opinion, not all these hypotheses have the same credibility, and we would rather favour the higher renal excretion of MPAG in children, which can also be put forward for CF patients.

2.1.5.2 Cystic fibrosis

In CF patients, chronic hepatic and gastro-intestinal disorders affect drug absorption, and lower serum albumin levels may result in an increased plasma clearance of protein-bound drugs, resulting in potentially higher risks for under-immunosuppression and therapeutic failure.[142] Unfortunately, little data is available on the PK of MPA in this population.[124] In a study of lung transplant recipients, patients with CF received on average 30% higher MMF doses than weight-matched patients without CF, all being given tacrolimus (doses estimated at approx. 32 vs 22 mg/kg/day, $p < 10^{-5}$), to reach MPA C_0 of approx. 2.5 vs 1.5 mg/L (ns).[142] Among the 21 patients recruited in another study, 5 had CF and received similar MMF doses compared to the remaining 16 patients (data not shown).[161] In CF patients, the metabolic AUC ratio of MPAG/MPA was significantly lower than in non-CF patients (14.3 vs 25.1, $p < 0.01$), which according to us could result from increased renal MPAG excretion, decreased hepatic metabolism or increased biliary excretion in CF patients, in order of decreasing probability. However, due to the small number of patients, these results would need to be confirmed and further investigations on the metabolism of MPA in CF patients would be of major interest.

2.2 Therapeutic drug monitoring

The advantages and limitations of TDM of MMF in transplantation were discussed recently in two reviews.[120,162] While TDM is mandatory for calcineurin inhibitors, it is not for MPA and MMF is usually administered at a fixed oral dose (adults: 2-3 g/day in combination with cyclosporine and 1.5-2 g/day in combination with tacrolimus; children, at least in renal

transplantation: 600 mg/kg body weight twice daily, up to a maximum daily dose of 2 g). As a consequence, MPA levels have not been routinely monitored despite PK and PD (variations of IMPDH inhibition) variability comparable to that of calcineurin inhibitors.[8,124,163] However, a significant relationship between MPA AUC and clinical outcomes and the clinical benefits of MMF monitoring for dose individualization have been demonstrated in a growing number of studies, including prospective concentration-controlled clinical trials, notably in renal transplantation and, to some extent, in heart transplantation.[16,124,132,164-166] Controlled exposure to MPA is essential to maximize its immunosuppressive effects and minimize its toxicity.[120] Despite this, MPA monitoring is still debated and no consensus has been reported on the best TDM tool for the optimal management of graft rejection and adverse effects (diarrhea, leucopenia, infections).[118,120,135] TDM guidelines for MMF in heart and lung transplant patients are even more limited.[120]

2.2.1 Single concentrations monitoring

When performed, MMF monitoring is sometimes based on MPA C_0 concentrations. In heart transplantation, the current recommendation is an MPA C_0 target of 1.2-3.5 mg/L when MPA is assayed by HPLC ($C_0 \geq 2$ mg/L when MPA is assayed by EMIT).[120]

Studies of the relationship between MPA C_0 and AUC_{0-12} in heart transplantation have reported poor correlation, with coefficients of determination (r^2) ranging from 0.36 to 0.69.[132,134,135,137] A better correlation was found with different time points, such as C_{40min} ($r^2 = 0.82$),[134] C_4 ($r^2 = 0.86$), C_6 ($r^2 = 0.85$) or C_8 ($r^2 = 0.93$) (Table XI).[135] However, AUC_{0-12} was not always measured and was sometimes estimated using multiple linear regression equations developed in other populations or transplant types.[134]

It is noteworthy that C_0 may not be the minimal concentration during the dosing interval, because of secondary peaks that occur between 6 and 12 hours after drug administration and of more or less delayed absorption, which translates into decreasing blood levels in the 10-60 min post-dose.[141] These secondary peaks may also explain the poor correlation of MPA C_0 with AUC_{0-12}.[124,163]

2.2.2 AUC monitoring

2.2.2.1 Full AUC

Mycophenolate is characterized by a poor correlation between blood concentrations and dose. In a study performed in 120 kidney and 20 lung transplant recipients on MMF and

cyclosporine (n = 107) or tacrolimus (n = 33), no correlation was found between MMF dose and MPA C_0 assayed by EMIT.[126]

As with calcineurin inhibitors, AUC_{0-12} is likely to be the most useful TDM tool rather than single concentrations. The recommended therapeutic range for MPA AUC_{0-12} after renal transplantation is 30-60 h.mg/L.[120] In thoracic transplantation, MPA AUC_{0-12} was calculated using the linear trapezoidal rule in almost all studies we identified. In two studies in heart transplant patients co-administered cyclosporine or sirolimus,[132,134] AUC_{0-12} was estimated using the EMIT assay and sparse sampling algorithms previously developed using HPLC in adult kidney transplant recipients treated with MMF-cyclosporine. The estimation precision is thus questionable because, as previously discussed sparse sampling strategies are usually specific of the transplant population, analytical method and associated immunosuppressant, unless validated in another population of interest. Fortunately, in these two studies the AUC estimates were not used for dose adjustment.

2.2.2.2 Sparse sampling strategies

As discussed previously, the determination of full AUC_{0-12} is impractical compared to other strategies such as C_0 or abbreviated sampling estimation of the drug's exposure. No abbreviated AUC has been proposed as a surrogate exposure index in thoracic transplantation. Experience of sparse sampling strategies is limited to three studies, two in heart and one in lung transplantation (Table XI). In heart transplant patients, the proposed sampling strategies included three or four MPA concentrations: at 1.25h, 2h, 6h and possibly 4h after MMF administration in the first study[121]; and at 0.5h, 1h, 2h and possibly the trough in the second.[167] In lung transplantation, an algorithm was proposed for the estimation of $\log(AUC_{0-12})$, based on the log(concentration) measured at pre-dose and either 1.5h or 2h post-dose.[138] Of note, both algorithms were developed using concentration-time profiles from a small number of patients. Clinical acceptability of sparse sampling strategies is better when the samples are drawn within 4 hours post-dose, which is not the case of the former. On the other hand, samples drawn before 6 to 8 hours post-dose are likely to miss MPA enterohepatic recycling,[118] unless Bayesian estimators, able to compensate for this, are used.[168]

2.2.2.3 Bayesian forecasting

To our knowledge, MAP-Bayesian procedures using popPK models have been developed for the estimation of AUC_{0-12} in renal transplant patients,[168,169] but not in thoracic

transplantation. Much still has to be done in order to refine TDM strategies for MMF after thoracic transplantation.

2.2.3 Impact of MMF TDM on patient outcome

Only a few studies have evaluated the relationship between exposure to MPA and clinical outcomes in heart transplantation,[129,132,136,139,163,170] and one in lung transplantation,[142] most of which were retrospective and dealt only with C_0 (Table XIII).

2.2.3.1 C_0 monitoring

In a retrospective study conducted in 215 heart transplant patients who received MMF associated with either cyclosporine (n = 191) or tacrolimus (n = 24), the incidence of acute rejection was higher when patients had MPA C_0 < 2 mg/L (EMIT) compared to patients with MPA C_0 > 2 mg/L (14.9 vs 8.8% between 0 and 6 months post-transplant and 11.3 vs 4.2% between 6 and 12 months post-transplant, p = 0.05 for both).[139] However, this difference was not further observed in patients beyond the first year post-transplantation. More importantly, patients who were within the therapeutic range for the associated calcineurin inhibitor in addition to MPA C_0 > 2 mg/L had lower rejection rates compared to patients who had only MPA C_0 > 2 mg/L (3.6 vs 15.5%, p = 0.002).

Forty-eight EMB were performed at the time of blood sampling in 26 heart transplant patients on MMF associated with cyclosporine or tacrolimus.[163] In the cyclosporine group, the overall acute rejection incidence was approximately 20% with MPA C_0 values of 1.65±0.97 mg/L (cyclosporine C_0 < 175 µg/L in all samples), whereas in the tacrolimus group, the overall acute rejection incidence was approximately 60% (p = 0.02) with MPA C_0 values of 2.86±2.07 mg/L, (tacrolimus C_0 < 10 µg/L in 52% of samples). These results are obviously very surprising and might be due to the dispersion of the individual exposure to both MPA and calcineurin inhibitor, even though the difference in MPA levels between rejectors and non-rejectors was not statistically significant.

In another retrospective study of 147 EMB from 20 patients in the first year after transplantation, the mean MPA C_0 (assayed by EMIT) was significantly lower in patients with acute rejection (n = 11, C_0 = 1.36 mg/L) than in those without (n = 9, C_0 = 1.76 mg/L, p = 0.015).[170]

In 26 heart transplant adults and children aged 1 month to 33 years who received MMF associated with either cyclosporine (n = 8 children + 10 adults) or tacrolimus (n = 8 children), lower MPA C_0 were associated with EMB grade ≥ 2 (ISHLT classification) compared to

grade < 2 (1.2±0.9 vs 2.5±2.3 mg/L, p = 0.02).[136] and no patient experienced grade 2 or 3A rejection when MPA C_0 was > 2.5 mg/L. Of note, issues regarding the heterogeneity of the population in this study have already been addressed in the PK section.

A statistically lower MPA C_0 (assayed by EMIT) was also reported in lung transplant patients with (n = 14, 28 acute rejection episodes) compared to patients without (n = 16) acute rejection (2.11±0.08 vs 2.71±0.11 mg/L, $p < 10^{-5}$).[142]

A prospective study conducted in 45 patients was organized in 2 phases:[129] during the first phase, 15 patients received fixed-dose of MMF (2 g/day, resulting in 5/15 patients without acute rejection; during the second phase, 30 patients received MMF at doses adjusted on C_0 with a target of 2.5 to 4.5 mg/L, resulting in 27/30 rejection-free patients, which, even if not statistically significant and despite the sequential design of the study, advocates for further trials.

2.2.3.2 AUC monitoring

The relationship between MPA AUC_{0-12} and acute rejection was demonstrated in renal transplant recipients[120] and confirmed by a recent study which demonstrated that TDM of MMF based on MPA AUC_{0-12} (estimated using Bayesian forecasting) compared to fixed-dose MMF resulted in a significant decrease of the acute rejection incidence:[16] patients who benefited from MPA TDM had fewer acute rejection episodes compared to patients of the fixed dose group (12% vs 30%, p = 0.01). Interestingly, a more than 10-fold inter-individual variability of MPA AUC values was reported in heart transplant patients receiving a fixed dose of MMF.[133]

In a cohort study, thirty-eight cardiac transplant recipients were divided into 3 groups based on the rejection severity on their biopsy (grade 0, n = 22; grade 1, n = 13; grade 2/3, n = 3).[132] Patients with grade 2 or 3 rejection had a lower mean total MPA AUC_{0-12} (26.1±6.6 vs 42.8±14.0 h.mg/L, p < 0.08) and unbound MPA AUC_{0-12} (0.49±0.11 vs 0.81±0.25 h.mg/L, p < 0.05) compared with patients without rejection (EMIT assay). However, due to the very limited number of patients with grade 2/3 rejection in this study and the already mentioned methodological limitations (estimation algorithm developed for renal transplantation and HPLC assay), this finding is to be used with caution.

Some authors hypothesized that high concentrations of MPA and AcMPAG may account for adverse effects such as gastro-intestinal disturbances and bone marrow suppression.[150,164,171-173] According to other authors, the occurrence of GI toxicity is probably not related to systemic exposure to MPA or MPAG, but rather to high concentrations of MPA and/or its

metabolites in the gut.[94,133] No study except for one[129] addressed the relationship between exposure to MPA and toxicity, more particularly gastro-intestinal toxicity, in heart or lung transplantation. In this study performed in 45 heart transplant recipients, the incidence of gastro-intestinal toxicity was 40% in patients who received fixed dose of MMF (2 g/day), and 30% in patients who benefited from MMF dose adjustment on C_0 (target: 2.5-4.5 mg/L). Unfortunately, no formal statistical comparison was made between the 2 groups, preventing us from drawing any conclusion from this observation.

To conclude, though prospective clinical validation is still missing, there is much indirect evidence that optimized exposure to MPA is crucial for successful thoracic transplantation and that MMF therapy can be improved by TDM.

3 mTOR inhibitors

Sirolimus and everolimus display a distinctive mechanism of action from that of calcineurin inhibitors. They form a complex with FKBP12, which inhibits the mTOR (mammalian target of rapamycine) involved in the proliferation of lymphocytes, blocking DNA and protein synthesis and consequently the proliferation of IL2 activated B and T cells. mTOR inhibitors reduce T-cell activation in cell cycle stages later than calcineurin inhibitors: their mechanism of action is downstream of and complementary to that of calcineurin inhibitors. [1,9,174,175]

3.1 Sirolimus

Sirolimus (rapamycin, Rapamune®) has been used mainly in renal transplantation, usually in combination with a calcineurin inhibitor or MMF and a corticosteroid, or as a calcineurin-sparing agent (because it allows the associated calcineurin inhibitor to be discontinued or its dose to be decreased). In thoracic transplantation, although used off-label, sirolimus has been of great interest as a rescue therapy when other immunosuppressants are inefficient or contra-indicated and in patients with impaired renal function.[9,175] Sirolimus was demonstrated to display a protective effect against the development of bronchiolitis obliterans in stable lung transplant patients.[1] However, it has been associated with interstitial pneumonia[176] and bronchial anastomotic dehiscence.[177] Consequently, in lung transplantation, it has been recommended not to introduce sirolimus before the third month post-transplantation. In heart transplantation, the safety of early CNI withdrawal has been questioned because of an associated increase in acute rejection rate, an observation that led to the discontinuation of a randomized trial of early CNI replacement by sirolimus. According to Zuckermann et al, CNI withdrawal should not be attempted until after the first year post-transplantation.[178]

These limitations and side-effects may explain why the use of sirolimus in thoracic transplantation has so far been limited.

3.1.1 Pharmacokinetics

The majority of PK studies of sirolimus were performed in healthy volunteers and kidney transplant recipients,[94] and we identified only one in heart transplantation.[179] This brief review will thus be mainly based on the PK and TDM of sirolimus in renal transplantation, as already reviewed in more detail in two articles.[175,180]

The standard sirolimus dosing strategy in renal transplantation consists of a loading dose of 6 mg/day, followed by a maintenance dose of 2 mg/day, with dose adjustment based on target C_0 values. In patients with high rejection risks and in heart transplant recipients, the loading dose is 15 mg/day and the maintenance dose 5 mg/day.[175,181-183]

In kidney transplant recipients, sirolimus exhibits wide inter- and intra-individual PK variability, like calcineurin inhibitors do.[52,180]

3.1.1.1 Absorption

Sirolimus is rapidly absorbed (t_{max} ranging between 0.7 and 3 hours), with an apparent oral bioavailability in association with cyclosporine of approx. 15% and a variable C_{max} ranging between 14 and 355 µg/L.[52,94,175,180] Sirolimus displays an intensive intestinal and hepatic metabolism by CYP3A4/CYP3A5 and is a substrate of P-gp, accounting for its low bioavailability and high PK variability.[94,144,175]

In a study conducted in 22 healthy adult volunteers, high fat meal decreased sirolimus AUC by 35%.[184] The drug should thus be administered consistently with or without food in order to reduce fluctuations in drug exposure.[180]

3.1.1.2 Distribution

In whole blood, sirolimus is extensively distributed in cellular elements (much more so than calcineurin inhibitors), mainly in erythrocytes (95%), lymphocytes (1%) and granulocytes (1%). The remaining 3% are found in plasma, where only 4% are bound to soluble plasma proteins, and 40% to lipoproteins, although the latter ratio goes up with increasing sirolimus concentrations.[94,180] Its high hydrophobic properties result in a wide distribution in lipid membranes of body tissues and confer sirolimus a large V_d/F (5.6-16.7 L/kg).[180]

In a popPK study performed in 31 *de novo* heart transplant recipients using a one-compartment PK model with first-order absorption and elimination, the estimated V_d/F ranged

approximately between 1,200 and 1,600 L, which is slightly higher than values reported in renal transplant recipients.[179]

3.1.1.3 Metabolism

Sirolimus, like cyclosporine and tacrolimus, is metabolized in the liver by the CYP3A4, and to a lesser extent CYP3A5 isoenzyme.[52,94] The large inter-individual variability of sirolimus PK may be related to the variability in its metabolism,[180] which consists mainly of O-demethylation and/or hydroxylation.[175] Over 16 metabolites have been identified in the bile of rats receiving sirolimus.[180] In patients' whole blood, concentrations of metabolites exceed those of the parent drug,[94] but there is no evidence that they display significant biological activity (< 10% of that of sirolimus at most).[180] An *in vitro* study with human liver microsomes showed that CYP3A5 exhibited a lower activity than CYP3A4 for sirolimus metabolism, but as CYP3A4 was more inhibited by cyclosporine, cyclosporine could unveil the influence of CYP3A5 and of its **1/*3* polymorphism on sirolimus disposition.[144] However, the *CYP3A5*1/*3* polymorphism was shown to have a dramatic impact on the sirolimus CL/F and C_0 actually achieved over the first 1-3 months post-transplantation despite TDM in renal transplantation, probably due to its higher impact on sirolimus intestinal metabolism and first pass effect.[185]

3.1.1.4 Elimination

Sirolimus is characterized by a long elimination half-life, varying between 44 and 87 hours, which allows once daily administration.[94,180] Steady-state is thus normally reached on average 6 days after the initiation of therapy, which justifies the use of a loading dose to achieve steady-state concentrations rapidly. The primary route for the clearance of sirolimus is biliary, over 90% of the metabolites being recovered in the faeces.[180] A large intra-patient (CV = 86%) and inter-patient variability (CV = 90%) on CL/F has been reported in stable renal transplant recipients, with 4- to 8-fold differences.[175,180]

The only published study on the PK of sirolimus in heart transplantation aimed to build a popPK model to estimate sirolimus CL/F and its intra- and inter-patient variability.[179] Thirty-one *de novo*, adult heart transplant recipients between 4 days and 5 years post-transplantation, on sirolimus, cyclosporine and oral prednisone were included. Sirolimus dose was adjusted on C_0 (target: 8-18 µg/L, assay: LC-MS/MS), and PK modelling was performed using NONMEM. As the majority of blood samples were collected towards the end of the dosing interval with a median of 20 hours, no data was available during the absorption phase.

Consequently, a one compartment PK model with first-order absorption and elimination was used, and the rate constant of absorption k_a was fixed at a value of 0.752 L/h. In this population, the maximum *a posteriori* Bayesian estimate of CL/F was 7.1 L/h (95%CI = 6.75-7.41 L/h), which is similar to values reported in renal transplantation. A 21% increase in sirolimus CL/F was observed for every 100 mg increase in the cyclosporine daily dose. Patients with primary diagnosis of ischemic heart disease had a 62% higher CL/F compared to others, and patients with hypertriglyceridemia (TG ≥ 2 mmol/L) had a 38% lower CL/F compared to patients with TG < 2 mmol/L, though the meaning of these findings is not quite clear.

3.1.1.5 Drug-drug interactions

Concomitant administration of sirolimus with cyclosporine microemulsion (but not the oil-based formulation) results in a 50-80% increase in sirolimus AUC because of the inhibition of CYP3A and P-gp by cyclosporine, with no modification of cyclosporine AUC. Moreover, a synergistic PD interaction between cyclosporine and sirolimus has been widely reported.[52,180] These are the reasons why sirolimus has been largely used as a cyclosporine-sparing agent.

As a substrate of CYP3A4 and P-gp, sirolimus has a potential for drug interactions similar to that of cyclosporine.[52] In renal transplantation, the association of sirolimus with diltiazem and ketoconazole resulted in a 60 and 990% increase of sirolimus AUC respectively, whereas the association with rifampicin resulted in an 82% decrease of sirolimus AUC.[180,186] On the contrary, in a study in 31 *de novo* heart transplant patients, sirolimus C_0 was lower in patients during diltiazem administration (14.1 µg/L, range: 9.7-21.9) than at other times (18.9 µg/L, range: 12.6-26.0; $p < 10^{-5}$),[179] which is not consistent with the known inhibitory effect of diltiazem on P-gp and hepatic metabolism, nor with the calculated sirolimus clearance in the same paper, which was lower under diltiazem. This might be due to an error in C_0 reporting in this paper.

Combination of cerivastatin with cyclosporine + sirolimus may result in a significant increase in cerivastatin exposure, with possible HMG-CoA reductase inhibitor-associated toxicity.[175]

3.1.2 Therapeutic drug monitoring

A wide intra-individual variability of sirolimus PK has been reported in renal transplant patients, with CV = 42-82% on observed and dose-adjusted C_{max}, C_0 and AUC values.[180,185] High variability was similarly observed in 31 *de novo* heart transplant patients, with the C_0 ranging between 0.2 and over 70 µg/L, despite targeting 8-18 µg/L,[179] and a poor correlation

between the dose and C_0 ($r^2 = 0.162$, $n = 524$, $p < 0.001$). The wide range of C_0 in patients receiving the same dose is due to the variability in drug absorption and clearance.[180]

On the other hand, better though variable correlation was reported in renal transplant patients between steady-state C_0 and AUC (assay: LC-MS/MS), with $r^2 = 0.85$,[52,180] or $r^2 = 0.43$[185].

The relationship between sirolimus C_0 and efficacy has been evaluated in renal transplantation.[175] Current TDM strategies are based on C_0, but appropriate C_0 for the prevention of acute rejection depends on the concomitant immunosuppressive regimen and would thus probably need to be refined. In renal transplant patients treated with sirolimus, cyclosporine and prednisone, $C_0 < 5$ µg/L (assays: LC/MS, LC/UV) was associated with an increased risk for acute rejection episodes, whereas $C_0 > 15$ µg/L was associated with a higher risk of adverse events (anemia, leukopenia, decreased platelet count, hypertriglyceridemia).[180] In association with cyclosporine, the following target values were proposed for sirolimus C_0: 5 to 10 µg/L for 30% reduction in usual cyclosporine exposure, 10 to 15 µg/L for 60% reduction in usual cyclosporine exposure, and ≥ 20 µg/L for no cyclosporine exposure.[52] In CNI-free studies in renal transplantation, where the C_0 target was 30 µg/L before M2 and 15 µg/L after M2, 41% and 27.5% acute rejection episodes were reported in patients on sirolimus associated with azathioprine and prednisone and in those on sirolimus associated with MMF and prednisone, respectively.[180]

As sirolimus has a long elimination half-life, the following schedule for C_0 monitoring was proposed: initially not more than once every 4 days, then once a week during the first month and once every two weeks during the second month. Moreover, sirolimus dose adjustments should be based on C_0 obtained more than 5-7 days after a dosage change or initiation of therapy.[175]

Sirolimus monitoring is a regulatory requirement in Europe. However, in a recent review, routine TDM of sirolimus was not deemed necessary in all patients after the first 2 months of dose titration, but only in case of suspected toxicity or non-compliance, and in some high-risk categories, identified as: young children (5-11 years), patients with hepatic impairment, patients on enzyme inhibitors or inducers, patients at high risk of rejection, patients on a CNI-sparing strategy.[175]

In conclusion, sirolimus pharmacogenetic-PK studies are required in heart or lung transplantation, in order for this drug to be used safely in these populations. Moreover, although there appears to be a relationship between sirolimus C_0 and clinical outcomes, the value of sirolimus TDM needs to be tested in the clinical setting,[52] and the demonstration of the superiority of sirolimus TDM over fixed dose regimens requires controlled trials.[175]

3.2 Everolimus

Everolimus (SDZ RAD, RAD, Certican®) was derived from sirolimus in order to improve its PK, and in particular to increase its oral bioavailability,[174,187] and decrease its half-life. It is currently the only mTOR inhibitor with regulatory approval in heart transplant recipients, as it has been shown to reduce significantly the incidence of acute rejection, CAV, graft loss, and death.[9,178,187,188] To date, there is limited evidence in favour of the use of everolimus in lung transplantation. Given the fibroproliferative process underlying the BOS and everolimus general antiproliferative effect, everolimus may prevent the onset of BOS after lung transplantation.[174] Significant clinical benefit of everolimus vs AZA on pulmonary function and BOS was evidenced in stable lung transplant recipients.[189]

3.2.1 Pharmacokinetics

The few PK studies of everolimus performed to date have been conducted in healthy volunteers and kidney and liver transplant recipients. Only exposure indices have been reported in heart and heart-lung transplantation.

3.2.1.1 Absorption

Everolimus is characterized by a low oral bioavailability of around 16%, with t_{max} ranging between 0.5 and 4 h depending on the type of patients (healthy volunteers, solid organ transplant patients).[187,190] Everolimus, like sirolimus, is a P-gp substrate, which probably affects its absorption.[175,190] As high-fat meals seem to influence its PK, it was recommended to take everolimus consistently with or without food, in order to reduce fluctuations in drug exposure.[190]

The dispersible tablets tested during a pediatric development program in 19 renal transplant children (aged 2-16 years), showed a 10% lower bioavailability relative to the conventional ones.[191]

3.2.1.2 Distribution

In whole blood, over 75% of everolimus is partitioned into erythrocytes.[187,190] In a popPK analysis of everolimus (one-compartment model) in 673 kidney transplant recipients, V_d/F was estimated to be 110±5 L.[192]

3.2.1.3 Metabolism

Everolimus is metabolized extensively in the gastro-intestinal tract and liver by CYP3A4 (mainly), 3A5 and 2C8, so the large inter-individual variability in everolimus bioavailability

and biotransformation is related to the variations in activity of these enzymes and P-gp.[175,187,190] The major metabolic pathways are thought to be hydroxylation and demethylation, leading to the production of at least 11 metabolites, whose immunosuppressive or toxic activities are unknown.[190] Patients with hepatic impairment were shown to have significantly lower everolimus CL/F, with longer $T_{1/2}$ (by 84%) and higher AUC (by 115% higher) compared to healthy subjects. As a consequence, it was recommended to reduce the everolimus dose by half in such patients.[190]

3.2.1.4 Elimination

About 98% of everolimus is excreted in bile as metabolites and only 2% is eliminated in urine.[190] Everolimus displays a shorter half-life than sirolimus, ranging between 18 and 35 hours in kidney and liver transplant patients, which allows twice daily administration and a steady-state to be achieved more quickly.[187,190] In healthy volunteers, CL/F and $T_{1/2}$ were 19.7±5.4 L/h and 32.2±6.1 h, respectively.[190] As already mentioned, no clearance or half-life values have been reported so far in heart or lung transplant recipients.

3.2.1.5 Drug-drug interactions

Everolimus and cyclosporine are given as a combination therapy because of their synergistic interaction.[9,190] As they are both substrates for CYP3A and P-gp (cyclosporine inhibits whereas everolimus induces P-gp), they may interfere with each other's absorption or elimination.[193] Any alteration in P-gp and inhibition of CYP3A4 metabolism may result in the accumulation of everolimus and/or cyclosporine.[187] In a PK study conducted in 634 heart transplant patients on everolimus (n = 420) or AZA (n = 214) associated with cyclosporine and corticosteroids, similar cyclosporine C_0 were achieved with lower doses of cyclosporine in patients on everolimus vs those on AZA ($p = 10^{-4}$), resulting in higher dose-normalized cyclosporine C_0 in patients on everolimus vs those on AZA ($p = 10^{-4}$).[193]

Similar results were reported in a PK study conducted in 213 patients on everolimus (n = 101) or AZA (n = 112) associated with cyclosporine and corticosteroids, with cyclosporine C_0 during the first month of 220±102 µg/L in patients on everolimus vs 176±76 µg/L in patients on AZA ($p < 0.02$).[194]

A PK conversion study (switch from AZA to everolimus or from cyclosporine to tacrolimus) was recently performed in 12 adult heart transplant patients (2 groups of 6 patients, aged 18-65 years) over 3 months post-transplantation.[195] Two full PK profiles per patient were collected, before and 6-8 weeks after conversion. Everolimus, cyclosporine and tacrolimus

were assayed by LC-MS/MS. The first group received tacrolimus adjusted on C_0 (target: 6-10 µg/L), and was converted from AZA to everolimus adjusted on C_0 (target: 3-8 µg/L), showing no significant difference in the PK of tacrolimus before and after the conversion. The second group received everolimus adjusted on C_0 (target: 3-8 µg/L) and was switched from low-dose cyclosporine (target C_0: 60-80 µg/L) to tacrolimus (target C_0: 6-10 µg/L). A significant decrease ($p < 0.05$) of everolimus exposure before and after conversion from cyclosporine to tacrolimus was found, on C_0 (4.2±1.3 vs 2.3±1.2 µg/L), C_{max} (9.1±1.9 vs 5.9±1.1 µg/L) and AUC_{0-12} calculated using the linear trapezoidal rule (64.2±6.8 vs 33.7±9.7 h.µg/L). In conclusion, no significant effect of everolimus on the PK of tacrolimus was evidenced, but the PK interaction between everolimus and cyclosporine led to 1.9-fold and 8-fold higher everolimus AUC_{0-12} and C_0 on concomitant cyclosporine compared with tacrolimus.

In healthy volunteers, rifampicin was reported to increase significantly everolimus CL/F (+172%), and shorten $T_{1/2}$ from 32 to 24 hours ($p = 10^{-4}$), with a significant decrease of its AUC (-63%, $p = 10^{-4}$).[196] In *de novo* renal transplant patients, erythromycin and azithromycin resulted in a significant decrease in everolimus CL/F (-22 and -18%, respectively). Fluconazole had no significant influence, but itraconazole induced a 74% reduction in everolimus CL/F.[192]

Analysis of PK profiles of everolimus in 13 lung and heart-lung transplant recipients found 2.3-fold higher everolimus C_0 in patients on azole antifungal drugs compared to others ($p < 10^{-4}$).[194] Fluconazole appeared to have minimal influence on everolimus exposure whereas itraconazole and ketoconazole had a stronger effect.

3.2.1.6 Special populations

3.2.1.6.1 Paediatric transplantation

We found no published study in heart or lung pediatric patients. A review published in 2004 analyzed two studies performed in pediatric renal transplant patients on everolimus associated with cyclosporine and prednisone.[190]

In the first study (n = 19 stable transplant recipients), V_d/F and CL/F were positively correlated with age, weight and body surface area (BSA) ($p < 0.001$) and were significantly lower to those of adults, whereas elimination half-life was similar, regardless of age, and comparable to that of adults. The authors recommended adjustment of everolimus doses to the bodyweight in children.[197]

In the second study (n = 19 *de novo* transplant recipients followed for 6 months after transplantation), a positive correlation was also found between CL/F and age, weight and

BSA. Intra- and inter-patient variability on AUC was 29 and 35%, respectively. The authors recommended adjustment of everolimus doses to the body surface area in children.[198]

3.2.1.6.2 Cystic fibrosis

One study reported the PK of single-dose everolimus in patients with CF, using a non-compartmental approach.[199] This was a phase I multicenter, randomized, double-blind, cross-over study in 19 lung and heart-lung transplant recipients (7 CF, 12 non-CF), beyond 3 months post-transplantation treated with cyclosporine, azathioprine and prednisone. Each patient received everolimus at a low-dose (0.035 mg/kg without exceeding 2.5 mg) and at a high-dose (0.10 mg/kg without exceeding 7.5 mg), in random order and separated by a wash-out period. Everolimus was assayed using LC-MS/MS, and $AUC_{0-\tau}$ was calculated using the trapezoidal rule. Dose-normalized C_{max} was significantly lower in CF patients vs non-CF patients (low-dose everolimus: 12.4±3.9 vs 13.8±3.1 $\mu g.L^{-1}$; high-dose everolimus group: 10.2±2.3 vs 12.8±2.5 $\mu.L^{-1}$; p = 0.03 for patients pooled across everolimus dose levels), whereas everolimus AUC/dose (which is the inverse of CL/F) did not differ significantly between the patients with and those without CF. $T_{1/2}$ was similar in all groups, ranging from 25 to 29 hours.

Similarly, the analysis of everolimus PK profiles in 13 lung and heart-lung transplant recipients showed no significant difference in exposure between CF and non-CF patients.[194]

3.2.2 Therapeutic drug monitoring

Everolimus is characterized by a narrow therapeutic index.[187,190] Moreover, data from various solid organ transplant populations (adult renal, cardiac and liver transplantation and pediatric renal transplantation) suggests that during the first year post-transplantation, there is a good relationship between everolimus concentrations and the clinical outcomes (efficacy, thrombocytopenia).[190,200] Consequently, TDM has been recommended for everolimus.[187]

In heart transplantation, two PK studies were conducted in the patients of study B253,[188] a prospective randomized double-blind efficacy trial in which 634 heart transplant patients (aged 52±11 years) on cyclosporine and corticosteroids were randomized to receive either 0.75 or 1.5 mg of everolimus BID (n = 209 and 211), or azathioprine (n = 214). Based on exposure-efficacy and exposure-safety analyses, everolimus C_0 of 3 to 8 µg/L was shown to ensure an optimal benefit/risk ratio.[178]

The first study focused on the PK of everolimus (assay: ELISA) during the first 6 months post-transplantation.[193] A measurement of C_0 was performed in all patients and AUC_{0-12}

estimated in 55 of them, using the trapezoidal rule based on 5 samples drawn over 8 hours (C_{12} was extrapolated from C_5 and C_8 by log-linear regression). No statistically significant difference in dose-normalized C_{max} or AUC was found between the 0.75-mg BID and the 1.5-mg BID dose groups, showing dose proportionality. A good correlation was found between C_0 and AUC ($r^2 = 0.81$, $p < 0.001$). Intra-individual variability on C_{min}, C_{max} and AUC was 38%, 30% and 30%, and inter-individual variability 40%, 33% and 37%, respectively. Sixty-nine and 80% of patients with C_0 comprised between 3.6 and 5.3 µg/L and between 5.4 and 7.3 µg/L respectively, remained rejection-free vs 65% of patients with $C_0 \leq 3.5$ µg/L, and cox proportional hazard analysis showed that the relative risk for biopsy-proven acute rejection was 2.4 for $C_0 < 3$ µg/L vs C_0 in the 3-7 µg/L range ($p < 10^{-5}$). However, ROC analysis showed that $C_0 = 3.4$ µg/L was the best one, able to distinguish rejectors from non-rejectors, yielding 75% sensitivity and 47% specificity. These variable thresholds and targets, apparently chosen for statistical reasons are puzzling and probably weaken the conclusions of this study. No significant relationship was evidenced between exposure and the level of triglycerides or serum creatinine, but there was a significant correlation between C_0 and thrombocytopenia ($r^2 = 0.85$, $p = 0.03$).

The second study evaluated, throughout the first year post-transplantation, the relationship between everolimus C_0 and cyclosporine C_0 on the one hand and efficacy (biopsy-proven acute rejection, BPAR) and safety (renal function, serum lipids, platelet counts) on the other.[201] Everolimus C_0 were stable in the first year post-transplantation and averaged 5.2±3.8 and 9.4±6.3 µg/L in patients treated with 1.5 and 3 mg/day, respectively. The same correlation as in the previous study was found between C_0 and AUC ($r^2 = 0.81$). The incidence of biopsy-proven acute rejection (BPAR ≥ grade 3A based on ISHT classification) was 44% in patients with $C_0 < 3$ µg/L, 24% in patients with $C_0 = 3$-8 µg/L, and 17% in patients with $C_0 \geq 8$ µg/L, leading to a relative risk of BPAR of 2.5 ($p = 10^{-4}$) when $C_0 < 3$ µg/L vs $C_0 = 3$-8 µg/L. The relative risk of BPAR when $C_0 = 3$-8 µg/L vs $C_0 \geq 8$ µg/L was not significant. No effect of everolimus exposure on renal events (serum creatinine > 200 µmol/L) was found, but hypercholesterolemia, hypertriglyceridemia and thrombocytopenia tended to increase with higher everolimus C_0.

A PK study of everolimus was performed in lung and heart-lung transplant recipients (aged 46±14 years, 3-36 months after transplantation at enrollment, 23% CF) enrolled in a 3-year randomized, multi-center, double-blind, parallel group study comparing everolimus (n = 89) and azathioprine (n = 100) as part of a cyclosporine-based immunosuppressive regimen.[194] Patients received an initial everolimus dose of 1.5 mg BID, stabilized at 1.2±0.4 mg BID

between M3 and M12. Everolimus concentration time profiles were obtained in 13 patients (assay: LC-MS). AUC_{0-12} was calculated (method: N/R) using 5 blood samples drawn between 0 and 8 hours and considering C_{12} equal to C_0 at steady-state. T_{max} ranged between 1 and 5 hours, and dose-normalized C_0 were regarded as stable throughout the observation period, with intra- and inter-individual CV of 35 and 37%, respectively (C_0: 7.3±2.6 µg/L at M2, 9.2±4.7 µg/L at M6, 7.6±3.1 µg/L at M12). Everolimus AUC was 130±31 h.µg/L at M2 (n = 13), 144±52 h.µg/L at M6 (n = 9) and 138±32 h.µg/L at M12 (n = 7). Over the range of everolimus C_0 measured throughout the study (2-36 µg/L), the exposure-efficacy relationship (efficacy criteria: FEV1, BOS and BPAR) appeared to be in the near-maximal plateau region. A significant relationship was found between everolimus C_0 over the first 2 months and the incidence of cholesterol > 6.5 mmol/L (r^2 = 0.95, p = 0.025) as well as the incidence of triglycerides > 2.9 mmol/L (r^2 = 0.96, p = 0.022). A significant relationship was also found between everolimus C_0 during the first month and platelet count < 100 G/L (r^2 = 0.93, p = 0.038). A tendency was found between renal impairment and the increase in everolimus C_0 (p = 0.051), which might be attributed to the synergistic nephrotoxic effect of cyclosporine associated with everolimus, as already described for sirolimus *in vitro*.[202,203] From data obtained in this population, the everolimus C_0 range of 3 to 12 µg/L was considered as safe and well tolerated. Unfortunately, the number of AUC values available in this study was too small to be able to study the AUC – effect relationships.

In conclusion, clinical evidence suggests that TDM of everolimus is probably beneficial in thoracic transplantation, C_0 as measured by LC-MS/MS being a good predictor of clinical efficacy. The recommended minimum effective concentration for everolimus when used in conjunction with a cyclosporine-corticosteroid immunosuppressive regimen in heart and lung transplant recipients is 3 µg/L. The upper bond has not been formally established because patients have tolerated levels up to 15 µg/L without damage. These levels are usually achieved within 1 to 2 weeks, and everolimus dose adjustment should always be based on blood trough levels obtained at least 4-5 days after the previous dose change. It is noticeable that correlation coefficients between C_0 and AUC are suboptimal, which suggests that AUC monitoring might represent further improvement in everolimus dose personalisation, but almost everything remains to be done, particularly AUC-effects observational studies and C_0/AUC comparative trials.

In heart transplant patients, cyclosporine dose reduction coupled with everolimus TDM could optimize immunosuppressive efficacy while reducing treatment-related toxicity.[178,187,194,200]

The use of everolimus in CNI-free regimens, maybe with higher C_0 (or AUC) targets to maintain immunosuppressive efficacy has not been studied extensively.[178]

4 Discussion

The increasing success of thoracic transplantation is largely attributable to the development of effective immunosuppressive strategies. However, there is still a high rate of mortality on the mid- and long-term, as the percentage of 5-year survival is still only 70% after heart transplantation and 50% after lung transplantation.[2,3] Substantial improvement of the long-term survival after thoracic transplantation may be obtained by adapting to some extent the immunosuppression to the recipient's rejection risk factors. In other solid organ transplantations, TDM derived from PK studies has been demonstrated to be crucial to improve patients' outcome, by targeting individualized doses of different immunosuppressants.[11] An overview of the PK and the clinical evidence in favour of the TDM of immunosuppressants in thoracic transplantation has been lacking.

4.1 Pharmacokinetics

Like in other solid organ transplant populations, the pharmacokinetics of the immunosuppressive agents in thoracic transplant recipients is characterized by wide inter- and intra-individual variability. A number of studies have been performed in order to compare the PK of Neoral® to that of Sandimmune® in heart and, to a lesser extent, in lung transplantation. Indeed, the old oil-based cyclosporine formulation Sandimmune®, which was characterized by erratic PK and consequently random efficacy was replaced in the mid 90's by the microemulsion Neoral®, characterized by less variability and consequently a more constant efficacy. Little is known about the pharmacokinetics of MPA and tacrolimus in heart and lung transplant recipients, even though both have been available for around 15 years. Their introduction into the immunosuppressive armamentarium occurred progressively in both heart and lung transplantation, based on clinical experience, though in most countries tacrolimus and MMF have still not been approved by health authorities for lung transplantation. Given their increased use in thoracic transplant recipients, their PK deserves to be explored more in depth. Moreover, with the recent approval of another formulation of tacrolimus (Advagraf®) by the EMEA, further investigation of its PK will also be necessary. Finally, there are hardly any studies on the pharmacokinetics of the mTOR inhibitors sirolimus and everolimus after thoracic transplantation, probably because they have been developed and released more recently. Sirolimus use in heart and lung transplantation has been questioned due to a poor

benefit/risk ratio with the prescription schemes proposed. To our knowledge, the vast majority of studies on the PK of sirolimus were performed in healthy volunteers and in kidney transplant recipients, while its PK in thoracic transplantation was studied by only one team.[179] There are only few studies of the PK of everolimus in thoracic transplantation, as it is the drug most recently developed and introduced in the maintenance immunosuppressive strategy. Its anti-proliferative activity slowing down the process of cardiac vessels intima thickening, a major mechanism of cardiac allograft vasculopathy, makes it a very promising molecule.[204-207] Moreover, its synergistic immunosuppressive activity with cyclosporine allows the use of low doses of cyclosporine and makes it a key drug in calcineurin inhibitor-sparing strategies, which is of major interest in patients with renal dysfunction, but its potential synergistic effect on cyclosporine nephrotoxicity reinforce the need for combined pharmacokinetic studies in heart and lung transplant recipients.

4.2 Therapeutic drug monitoring

4.2.1 Evidence-based TDM

TDM comprises the measurement and interpretation of drug exposure and aims at assisting physicians in the choice of drug dose for an individual patient. The state of the art of evidence-based TDM should be consistent comparative, randomized clinical trials showing the superiority of TDM *vs* fixed dose, on hard clinical outcomes (morbidity, mortality, graft function). Despite the paucity of such trials (althought there might be a publication bias by which small studies with negative outcome are less likely to be published than studies of similar size but with a positive outcome), the clinical benefits of TDM have been validated by usage and are now well recognized in most situations. In clinical practice, TDM is even mandatory for calcineurin inhibitors and mTOR inhibitors due to the poor relationship between the dose given and the systemic concentrations achieved. Consequently, one may question the actual necessity of imposing upon the medical staff and patients similar clinical trials with different graft types, when a clear effect has already been demonstrated, for instance in renal transplantation. Indeed, once the clinical usefulness of TDM has been demonstrated in a given graft type, it might be acceptable to extrapolate the results to the other graft types, provided a case-control or a cohort study (regarded as of a lower evidence level) confirms a good exposure-outcome relationship. A good example for that would be MMF, for which no clear correlation has been established between dose and MPA plasma

levels, much like with calcineurin inhibitors and mTOR inhibitors, and which probably deserves to be monitored as much as the other molecules.

The gold-standard exposure index is in most cases the full AUC, but when it is not easy to measure, a surrogate marker (abbreviated AUC, AUC calculated using sparse sampling algorithms or Bayesian estimators, single concentrations) can be proposed based on tight correlation with the former. These correlation studies are indeed of the lowest evidence level but can be useful to evaluate the pertinence of a simplified TDM strategy.

4.2.2 Is there clinical evidence for TDM of the immunosuppressants after thoracic transplantation?

TDM of cyclosporine has been recognized as an essential tool in the management of allograft transplant recipients and has been performed routinely in transplantation for almost 20 years.[52,53] However, much of the information available has been obtained in renal and liver transplantation, where there is still no clear consensus about the optimal monitoring method. We found very little data on the practices and outcomes of cyclosporine monitoring after thoracic transplantation. Various observational studies sought for a relationship between C_0 and clinical outcome, with sometimes positive[28,74-79] and sometimes negative[20,54,80] results. Similarly, a number of authors found a relationship between C_2 and clinical outcomes and thus advocated for C_2 monitoring,[10,56,84-88] while such a relationship could not be evidenced in a number of other studies.[20,22,28,51,89-92] The superiority of C_2 over C_0 monitoring in terms of clinical outcome has been demonstrated in few studies, either in terms of efficacy, or in terms of toxicity (nephrotoxicity, infections).[10,55,79,86] There is no published consensus to date for C_2 target levels after heart or lung transplantation, although tentative targets have been suggested based upon retrospective or single-center experience on small numbers of adult patients.[10,23,56,89] Our literature search allowed us to identify only two studies evaluating the potential relationship between AUC and clinical outcomes in heart transplant recipients, with diverging results,[54,55] and none in lung transplant recipients. A few methods have been proposed to estimate the AUC using a limited number of samples, three of which seem clinically applicable, one based on blood samples taken at 1 and 4 hours and multilinear regression,[21] the other two requiring 3 samples (0, 1 and 3 hours) and Bayesian estimation.[31,32] However, to date, the cyclosporine AUC target values have not been established and no studies in heart or lung transplantation have demonstrated the superiority of AUC management over C_0 or C_2 monitoring in terms of clinical outcomes.

Tacrolimus dosage after thoracic transplantation is generally adapted to C_0 levels, mainly based on the results of clinical studies in kidney or liver transplantation. Studies of the relationship between C_0 and AUC_{0-12} in thoracic transplant recipients have reported a wide range of correlation coefficient values.[103,104,109,111] Some authors proposed to monitor tacrolimus C_3[110] or C_4[104,105,109] levels because of their good (and sometimes better) correlation with AUC_{0-12}. AUC_{0-4} was proposed as a surrogate exposure index for heart[109] and heart-lung[110,112] transplant patients. Sparse sampling strategies have been proposed for the determination of the tacrolimus inter-dose AUC, based either on multilinear regression and 2 blood samples (C_0 and C_2) or 3 blood samples (C_0, C_2 and C_4),[109] or on Bayesian estimators and 3 blood samples taken at 0, 1 and 3 hours and 0, 1.5 and 4 hours for non-CF and CF patients, respectively.[112] Unfortunately, up until now, no prospective study has been conducted in thoracic transplantation to investigate the relationships between tacrolimus AUC_{0-12}, AUC_{0-4} or single concentration-time points and clinical outcomes (acute rejection or toxicity), compare them and propose target levels.

Unlike calcineurin inhibitors, MPA TDM is not mandatory and MMF is usually administered at a fixed oral dose. Consequently, MPA levels have not been routinely monitored, despite variability in its pharmacokinetics and its pharmacodynamics comparable to that of other immunosuppressants,[8,124,163] and TDM guidelines for MMF in heart and lung transplantation are very limited. When performed, despite poor correlation between MPA C_0 and AUC_{0-12}, the TDM of MMF is often based on C_0,[132,134,135,137] with a recommended target range in heart transplantation of 1.2-3.5 mg/L (MPA assayed by HPLC).[120] The relationship between MPA C_0 and efficacy was evaluated in a few studies in heart transplantation,[129,132,136,139,163,170] and in a single one in lung transplantation,[142] showing that a higher rejection incidence was related to lower C_0. No abbreviated AUC has been proposed as a surrogate for inter-dose AUC in thoracic transplantation, and the experience of sparse sampling strategies using multilinear regression equations is limited to two studies with small numbers of patients.[121,138] Even though it was clearly demonstrated in renal transplantation,[120] the relationship between MPA AUC_{0-12} and rejection, or between any exposure index and toxicity (such as gastro-intestinal side-effects, the mechanism of which is still debated) has not been demonstrated in thoracic transplantation yet.[94,133,161] To date, no study comparing the different exposure indices in thoracic transplant recipients has been performed and no AUC target established and MMF dose individualization has not been validated *vs.* fixed-dose regimens. However, there is increasing evidence in favour of the clinical benefit of MPA AUC_{0-12} monitoring, particularly in renal transplantation.[16,120]

The current TDM strategies for both sirolimus and everolimus are based on C_0. There appears to be a good relationship between C_0 and clinical outcomes for both molecules. Most studies on sirolimus were performed in renal transplantation, and we found none evaluating the clinical benefits of sirolimus C_0 monitoring in thoracic transplantation. Everolimus was evaluated in various solid organ transplant populations, including heart transplantation. A target everolimus C_0 range of 3 to 8 µg/L was proposed for heart transplant recipients, based on the results of only 2 studies where a limited number of patients were enrolled.[193,201] The only study performed in lung transplantation failed to show a relationship between everolimus C_0 and efficacy, but a significant relationship was found between C_0 and toxicity.[194] Based on these results, an everolimus C_0 target range of 3 to 12 µg/L was proposed for lung transplant recipients. We found no study in thoracic transplant patients evaluating the relationship between sirolimus or everolimus AUC and effect or looking for the best TDM tools in this population.

Because appropriate exposure for the prevention of acute rejection depends on the concomitant immunosuppressive regimen, the use of sirolimus or everolimus in CNI-free regimens in stable patients, maybe with higher C_0 (or AUC) targets to maintain immunosuppressive efficacy, also needs to be studied extensively.

Induction therapy with a polyclonal anti-lymphocyte or anti-thymocyte globulin is now used in around 50% of thoracic transplant recipients, with a significant decrease of the prevalence of acute graft rejection episodes.[2,3] Induction therapy allows a quicker and stronger immunosuppression peri-operatively, and one could hypothesize that the level of maintenance immunosuppression should be driven by the presence or not of induction. The therapeutic benefit of a higher level of immunosuppression, in terms of rejection, might be however counterbalanced by a higher risk of toxicity, in particular infections and lymphoproliferative disorders. Anyway, the TDM of immunosuppressants used as maintenance therapy may be as relevant in patients with induction as in those without, but specific targets for CNI, MPA or mTOR inhibitors levels should then be defined in these patients in order to avoid overimmunosuppression and optimize the benefit/risk ratio.

4.3 What are the needs to improve the therapeutic management of thoracic transplant patients?

4.3.1 Development of sophisticated TDM tools

Heart and lung transplantations are not comparable with kidney transplantation. Firstly, the incidence of acute rejection is higher. Secondly, where graft failure in kidney transplant recipients can be handled by dialysis, graft loss in heart or lung transplant patients generally leads to death. Thirdly, patients with thoracic transplantation are more often administered drugs that intensively interact with the PK of the immunosuppressants (azole antifungals, calcium channel blockers, HMG-CoA reductase inhibitors), implying that the immunosuppressive treatment has to be closely monitored to avoid under- or over-immunosuppression. Therefore, it is legitimate to expect the development of sophisticated TDM tools dedicated to thoracic transplantation, in order to evaluate accurately and precisely patients' exposure to the drugs at large, and to immunosuppressants in particular. Bayesian forecasting presents two major advantages compared to AUC monitoring based on the measurement of full concentration profiles or AUC estimation with multiple linear regression algorithms or from a single concentration: (i) it is characterized by a high flexibility in sampling times, as long as the true sampling times are known; (ii) in addition to the AUC, it can estimate several PK parameters and exposure indices simultaneously. Therefore, it can help identify absorption and elimination problems in specific populations such as for instance, diabetic or CF patients.

The use of Bayesian estimators for cyclosporine and MMF monitoring has been validated in renal transplantation by clinical studies.[16,208] In thoracic transplantation, although Bayesian estimators have been set up for the calculation of the AUC of cyclosporine[30-32] and of tacrolimus,[112] they remain to be validated as TDM tools. Indeed, the implementation in routine clinical practice of efficient Bayesian estimators requires prior validation in large, collaborative, prospective clinical trials. Bayesian estimators should also be developed for the TDM of MMF, sirolimus and everolimus in thoracic transplant patients. Furthermore, as multiple immunosuppressive strategies now arise and as these patients often receive interacting drugs, Bayesian estimators should be built, and ideally validated, for the different immunosuppressive associations, and for the association of the immunosuppressant of interest with other types of interacting drugs.

4.3.2 Clinical trials dedicated to TDM and dose individualization

Once relationships have been established between clinical outcome and different markers of drug exposure, the steps necessary towards optimal TDM and dose adjustment are: (i) to identify the most predictive exposure index, either retrospectively from the exposure-effects relationships, or prospectively (which is seldom the case) by comparing the clinical outcome of different groups monitored using different exposure indices and their appropriate targets; (ii) to determine the target values for this exposure index, *i.e.* those associated with the best benefit/risk ratio, which again can be done retrospectively (such as with ROC curve analyses) or prospectively (concentration-controlled studies); and (iii) to validate the clinical usefulness of TDM using this marker and these target values in comparative trials *vs.* the standard of care at the time of the trial. In conclusion, large cohort TDM studies definitely need to be conducted in thoracic transplant patients.

One difficulty with the immunosuppressants is that they are always used in combination regimens, making the establishment of therapeutic ranges for individual drugs complex, as the exposure – effect relationship for a given drug may not be the same depending on the nature or even the dose of the molecules it is associated with.

Another difficulty is that patients' follow-up in clinical trials is most of the time limited to the first one to three years post-transplantation, focusing on the incidence of acute rejection as the primary efficacy endpoint and on primary toxicity endpoints related with over-immunosuppression (infections, cancer, post-transplant lymphoproliferative disorders) or on molecule-specific side effects (nephrotoxicity, hypertension, hypertrichosis, gingivitis, diabetes for calcineurin inhibitors; gastro-intestinal and haematological toxicity for MMF; hyperlipidemia for sirolimus; nephrotoxicity, hyperlipidemia, thrombocytopenia for everolimus). Such short follow-up durations consequently limit the conclusions to the short-term outcomes and lead to underestimate the incidence and/or severity of cumulative toxicities (which can be particularly pertinent in lung transplantation where the doses given are higher than in other types of transplantation). If one wants to validate the benefits of therapeutic or TDM strategies in these populations and improve the evaluation of their risk, longer follow-up periods using hard clinical outcomes (morbidity, mortality, graft function) and other long-term outcomes (cardiac allograft vasculopathy, bronchiolitis obliterans syndrome, long-term cumulative toxicity such as renal insufficiency, metabolic disorders, etc.) should be considered. Long-term clinical trials are often expensive and complicated to conduct. One option could thus be to switch from clinical trials to large epidemiologic or cohort studies collecting all the information on therapeutic and monitoring strategies,

compliance, side effects and quality of life, together with clinical outcomes. This would allow comparing the different therapeutic strategies and exposure levels, thus helping delineate the best therapeutic regimen and define precise exposure targets for each clinical condition and in each situation in the different transplant (sub)populations.

4.3.3 Perspectives

Increased knowledge and a better use of older drugs would also be of great interest. Even though they probably are the oldest and most widely used immunosuppressive drugs in medicine, there is almost no published experience regarding the application of TDM to corticosteroid therapy. Despite the emergence of steroid-sparing strategies, prednisolone therapy is still largely used, sometimes at high doses, in paediatric and adult thoracic transplant patients.[3,42] High doses of corticosteroids are believed to induce the expression of a number of enzymes, including UGTs, which may interfere with the disposition of MPA.[157] Moreover, inter-individual variability of the pharmacokinetics of prednisolone has been reported in lung[209] and in heart transplant recipients,[210] suggesting that optimization of corticosteroid therapy using TDM might also improve the management of thoracic transplant patients.

It has been suggested that pharmacodynamic monitoring of the cellular targets of immunosuppressant drugs may better reflect clinical outcomes than drug level monitoring. For instance, IMPDH pretransplant activity has been demonstrated to be associated with clinical outcome after renal transplantation.[211] Thus, pharmacodynamic monitoring of calcineurin activity, IMPDH activity or IL2 levels may address some of the limitations of PK monitoring, either alone or associated with PK monitoring. However, the assays available for the pharamcodynamic monitoring are either non-existant or technically challenging, expensive and time-consuming, which could limit their use in routine monitoring and dose adjustment. Moreover, pharmacodynamic monitoring has not yet reached the level of evidence PK monitoring has. Although promising, its complementarity with, or superiority over PK monitoring still has to be proven.[162]

Finally, in the last decade, single nucleotide polymorphisms (SNPs) have been intensively investigated and found on the genes encoding P-glycoprotein (P-gp), the cytochromes P450 (CYP450) and the uridine-diphosphate glucuronyl-transferases (UGT), all of which are involved in the disposition of most immunosuppressants. Some of the SNPs were found to be associated with altered protein expression or function, and with drug PK variability. A few SNPs have also been reported for IMPDH and IL2,[212-214] involved in the immunosuppressive

response, some of which are potentially associated with pharmacodynamic variability. The implications of these findings are important for transplant patients' care, as the efficacy and toxicity of a given drug or association of drugs may be different depending on the genotype. Moreover, the combination of multiple substrates for P-gp, CYP450's and UGTs can cause competitive inhibition of the proteins or up-regulate their function. Therefore, the addition of such agents to a transplant patient's drug regimen may be accompanied by modifications in drug disposition or effect, which may be different depending on the genotype of the patient. Therefore, pharmacogenetic characterization of transplant patients (such as MDR1 and CYP3A5 genotypes for calcineurin inhibitors and mTOR inhibitors, and UGT1A9 and MRP2 for mycophenolate) may have the potential to optimize immunosuppressive therapy, in addition or, less likely as a replacement to the TDM approach. However, the level of evidence is low once again. Further investigations are thus needed in thoracic transplant patients in order to verify the clinical significance of these SNPs, before being able to conduct prospective, comparative trials investigating the impact of treatment individualisation based on this approach. If confirmed, *a priori* genotyping may become a new useful tool to help select the appropriate drugs and optimal starting doses for an individual patient, and thus improve clinical outcomes and participate in thoracic transplantation progress.

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Table I. Cyclosporine exposure indices in cohort studies
Results are expressed as mean±SD, unless otherwise specified

Ref	Number of patients	Post-transplant period	Cyclosporine dose	Co-administered immunosup-pressant	Assay	Measured AUC (h.µg/L)	C_0 (µg/L)	C_2 (µg/L)	C_{max} (µg/L)	T_{max} (h)
HEART TRANSPLANTATION										
[23]	16 adults (out of 35 in the global trial)		NEO	N/R	*FPIA TDx*	Non-compartmental PK analysis; AUC_{0-12} Trap				
						AUC/D: (h.µg/L)/mg cyclosporine	*C_0/D: (µg/L)/mg cyclosporine*		*C_{max}/D: (µg/L)/mg cyclosporine*	
		Week 1	200±58 mg			6,521±1,940 AUC_{0-12}/D = 34.2±12.6	329±119 C_0/D = 1.8±0.8	--	1,136±315 C_{max}/D = 6.0±2.1	1.9±0.6
						r^2 C_0-AUC_{0-4} = 0.16; r^2 C_2-$AUC0_{-4}$ = 0.6				
		Week 12	159±40 mg			6,214±1,760 AUC_{0-12}/D = 39.4±7.3	287±99 C_0/D = 1.9±0.6	--	1,368±369 C_{max}/D = 8.8±1.8	1.8±0.4
						r^2 C_0-AUC_{0-4} = 0.4; r^2 C_2-$AUC0_{-4}$ = 0.4				
		Week 52	162±37 mg			6,111±1,627 AUC_{0-12}/D = 38.4±10.3	284±74 C_0/D = 1.8±0.5	--	1,256±379 C_{max}/D = 7.9±2.2	2.2±0.6
						r^2 C_2-AUC_{0-4} = 0.7				
[29]	20 male adults 58.8±10.2 years	Stable 36.8±27.1 months	NEO and SAND 1.14±0.4 mg/kg TID	N/R No corticosteroids	*FPIA TDx*	Non-compartmental PK analysis; AUC_{0-8} Trap				
			SAND			3,278±682	297±62	--	732±178	2.63±1.21
			NEO			3,798±757 ($p < 0.001$)	312±49 (ns)	--	935±250 ($p < 0.001$)	1.36±0.49 ($p < 0.001$)
[19]	47 adults 54±12 years	Stable 49±19 months	NEO + SAND[(1)] None	D (mg/kg/day) 3.90±1.03	N/R	*EMIT* SAND	Non-compartmental PK analysis; AUC_{0-12} Trap 3,655±1,120	167±77	--	827±204	2.27±0.47

			n = 11			NEO	4,911±935 (p < 0.05)	179±64 (p < 0.02)		1,147±307 (p < 0.01)	1.55±0.52 (p < 0.001)
	52±8 years	61±15 months	DILT n = 11	2.98±0.64		SAND NEO	3,605±1,079 4,747±923 (p < 0.05)	147±79 171±61 (p < 0.02)	--	S: 816±183 1,288±415 (p < 0.01)	2.09±0.54 1.45±0.69 (p < 0.05)
	52±9 years	32±15 months	KETO n = 13	0.79±0.27		SAND NEO	3,601±933 3,703±772 (ns)	194±72 189±54 (p < 0.02)	--	480±164 532±93 (ns)	2.61±0.96 2.00±0.57 (p < 0.05)
	47±12 years	28±5 months	DILT + KETO n = 12	0.90±0.54		SAND NEO	4,070±1,090 4,369±993 (ns)	201±68 181±62 (p < 0.02)	--	574±192 652±204 (p < 0.01)	2.58±0.90 2.33±0.49 (ns)
[58]	47 adults	Stable	NEO(1)		N/R	*EMIT*	Non-compartmental PK analysis; AUC_{0-12} and AUC_{0-5} Trap				
				D (mg/day)							
			None n = 11	302±68			AUC_{0-12} = 4,912±935 AUC_{0-5} = 3,356±641	179±64	1,088±165	1,147±307 (CV = 27%)	1.5±0.5 (CV = 33%)
							r^2 C_0-AUC_{0-5} = 0.710 (p = 0.001); r^2 C_2-AUC_{0-5} = 0.197 (p = 0.17)				
			DILT n = 11	243±55			AUC_{0-12} = 4748±924 AUC_{0-5} = 3,304±655	171±61	1,022±237	1,222±501 (CV: 41%)	1.4±0.7 (CV: 50%)
							r^2 C_0-AUC_{0-5} = 0.650 (p = 0.005); r^2 C_2-AUC_{0-5} = 0.650 (p = 0.005)				
			KETO n = 14	66±35			AUC_{0-12} = 3,703±824 AUC_{0-5} = 1,980±414 (vs "none", p < 0.05)	201±48	503±110	503±110 (CV: 22%)	2.2±0.5 (CV: 23%)
							r^2 C_0-AUC_{0-5} = 0.176 (p: N/R); r^2 C_2-AUC_{0-5} = 0.870 (p = 10^{-4})				
			DILT + KETO n = 12	87±60			AUC_{0-12} = 4,386±1,082 AUC_{0-5} = 2,406±675 (vs "none", p < 0.05)	179±68	627±209	659±224 (CV: 34%)	2.4±0.5 (CV: 21%)
							r^2 C_0-AUC_{0-5} = 0.022 (p: N/R); r^2 C_2-AUC_{0-5} = 0.898 (p = 10^{-4})				
[21]	15 adults 48±10 years	Clinically stable 5.2±4.4 years	NEO 2.7±0.7 mg/kg/day D adjusted on C_{av} = 200-300 µg/L		ATG AZA Prednisolone	*FPIA* *TDx*	Non-compartmental PK analysis; AUC_{0-12} Trap				
							1,285±367 C_{av} = 371±123	162±55	--	1,101±326	1.1±0.4
							r^2 C_0-AUC_{0-12} = 0.60; r^2 C_2-AUC_{0-12} = 0.94; r^2 C_4-AUC_{0-12} = 0.95				

[22]	22 adults	Until Year 4	NEO(2)	ATG Prednisone	*EMIT*	Non-compartmental PK analysis; 2 consecutive periods				
		Month 1	Group I D adjusted on C_0	MMF D = 1g BID		--	197±98	1199±476	--	--
		Month 3				--	222±60	1202±587	--	--
		Month 6				--	218±83	999±467	--	--
		Month 12				--	--	664±203	--	--
		Month 24				--	--	593±208	--	--
		Month 36				--	--	561±147	--	--
		Month 1	Group II D adjusted on C_2	MMF D = 1.5 g BID		--	--	809±160 (p = 0.02)	--	--
		Month 3				--	--	644±178 (p = 0.003)	--	--
		Month 6				--	--	665±169 (p = 0.02)	--	--
		Month 12				--	--	616±221 (ns)	--	--
		Month 24				--	--	464±234 (ns)	--	--
		Month 36				--	--	451±164 (ns)	--	--
[31]	14 patients *de novo*	3 PK profiles/patient	NEO D adjusted on C_0	N/R	*FPIA* *TDx*	Exposure indices and PK parameters estimated using ITS method, Ciclo 1.3 software Absorption model based on a gamma distribution				
						AUC_{0-12}/D: (h.µg/L)/mg cyclosporine	*C_0/(100-mg D): (µg/L)/100 mg cyclosporine*	*C_2/D: (µg/L)/mg cyclosporine*	*C_{max}/D: (µg/L)/mg cyclosporine*	
		Week 1 (W1)	200±61.2 mg BID			6,395±1,921 AUC_{0-12}/D = 34±13	310±134.6 C_0/(100-mg D) = 156±70	C_2/D = 5.47±2.33	1,128±299 C_{max}/D = 6±2	1.78±0.46

		Month 3 (M3)	152±36.0 mg BID (vs W1, p<0.05)			5,780±1,365 AUC_{0-12}/D = 37±8	270±66.7 C_0/(100-mg D) = 163±34	C_2/D = 7.78±1.05 (vs W1, p < 0.05)	1,380±371 C_{max}/D = 9±2 (vs W1, p < 0.05)	1.63±0.40
		Year 1 (Y1)	164±40.1 mg BID			5,967±1,600 AUC_{0-12}/D = 38±10	284±74.2 C_0 (100-mg D) = 163±48	C_2/D = 6.98±2.17 (vs W1, p < 0.05)	1,216±410 C_{max}/D = 8±2	1.22±0.41
						Bayesian estimator (AUC_{0-12}), best sampling times: C_0, C_1, C_3 for the three periods BE vs Trap: bias% = -0.20 to +3.06, ns; RMSE% = 1.61 to 18.59%				
LUNG TRANSPLANTATION										
[24]	11 adults All CF SL	> 6 months Medically stable		N/R	*SRIA*	Non-compartmental PK analysis; AUC_{0-12} Trap – Crossover study				
			Capsules (1 week)			4,164±1,467	--	--	613±242	3.64±2.16
			NEO (1 week)			5,318±1,670	--	--	931±458	2.27±1.2 (p = 0.23)
			Ratio NEO/Capsules			*1.48 (p = 0.047)*	--	--	*1.91 (p=0.045)*	--
[4]	20 adults 1 CF 8 SL, 12 DL	Stable, > 3 months	NEO, D (mean, range) = 330 mg/day (150-50) D adjusted on C_0:	ATG (n = 6) MMF Steroids	*FPIA Mc*	Non-compartmental PK analysis; AUC_{0-4} Trap; data are presented as mean (range)				
		< 1 year n = 10	M1-M3 350-400 M4-M12 300-350			3,700 (2,400-5,700)	322 (248-492)	1,180 (890-1,700)	1,057 (303-2,337)	--
		1-3 years n = 8	M13-M24 N/A M25-M36 250-300			2,440 (1,030-3,600)	222 (68-403)	780 (380-1,500)	929 (401-1,360)	--
		> 3 years n = 2	> M36 150-200			N/R (990-2,050)	N/R (134-143)	N/R (270-670)	N/R (333-804)	--
						r^2 C_0-AUC_{0-4} = 0,64; r^2 C_1-AUC_{0-4} = 0,44; r^2 C_2-AUC_{0-4} = 0,85; r^2 C_3-AUC_{0-4} = 0,67; r^2 C_4-AUC_{0-4} = 0,64				
[66]	14 adults 8 SL, 6 DL	Stable	NEO Steady-state	N/R	*FPIA TDx*	Non-compartmental PK analysis; AUC_{0-12} Trap				
						5,183±1,835	--	--	--	--
[6]	12 adults 53.2±7.4 years	Weeks 1 & 2	NEO D adjusted on C_0 = 300-	Daclizumab Prednisolone	*EMIT*	Non-compartmental PK analysis; AUC_{0-6} Trap (interpolation of C_5 values)				

			400 µg/L	MMF		3,443±1,451	361±118	C_1 = 481±231 C_2 = 682±314 C_3 = 715±347 C_4 = 658±271 C_6 = 571±260	--	--
						r^2 C_0-AUC_{0-6} = 0.31 (p = 0.001); r^2 C_1-AUC_{0-6} = 0.73 (p < 0.001); r^2 C_2-AUC_{0-6} = 0.88 (p < 0.001) r^2 C3-AUC_{0-6} = 0.89 (p < 0.001); r^2 C4-AUC_{0-6} = 0,84 (p < 0.001) ; r^2 C6-AUC_{0-6} = 0.54 (p = 0.001)				
HEART AND LUNG TRANSPLANTATION										
[27]	22 adults(3)	Month 1	D adjusted on C and clinical data	AZA Prednisolone	*Pc* *NSRIA*	Non-compartmental PK analysis				
	11 CF 11 non-CF		16.7±7.2 mg/kg/day 8.2±1.9 mg/kg/day (p<0.01)			--	892±385 CF vs non-CF: p = 0.58	--	--	--
[26]	9 patients(4) All CF 12-32 years	Stable 10 months – 7.5 years	Switch from SAND to NEO D adjusted on C_0 mean±SD median (range)	N/R	*SRIA*	Non-compartmental PK analysis; AUC_{0-12} Trap; data are presented as median (range) Crossover study; 9 PK profiles for each cyclosporine formulation				
						AUC/D: (h.µg/L)/mg cyclosporine	*C_{min}/D: (µg/L)/mg cyclosporine*		*C_{max}/D: (µg/L)/mg cyclosporine*	
			NEO 12.6±6.7 mg/kg/day 450 mg/day (125-500)			6,032 (3,931-11,939) AUC_{0-12}/D = 19,76 (8,73-47,97) (CV = 51%)	C_{min} = 224 (131-393) C_{min}/D = 0.60 (0.29-2.37) CV on C_0: Intra-ind. 36% Inter-ind. 79%	--	1,360 (729-2,360) C_{max}/D = 4.53 (1.62-7.69)	1.0 (1.0-4.0)
			SAND 8.5±3.6 mg/kg/day 450 mg/day (125-1000)			4,179 (3,377-7,833) AUC_{0-12}/D = 9,29 (4,92-29,61) (CV = 63%)	C_{min} = 172 (85-443) C_{min}/D = 0.43 (0.17-3.22) CV on C_0:	--	595 (402-1,065) C_{max}/D = 1.32 (0.65-3.22)	3.0 (0.0-6.0)

							Intra-ind.45% Inter-ind.130%			
			NEO/SAND			*1.77 (0.56-4.35)*	*1.58 (0.41-9.71)*	--	*2.17 (0.78-8.51)*	--
[28]	50 adults 9 CF 19 SL, 9 DL 22 HL	f/up: 12 months	D adjusted on C_0 (µg/L) = M1-M2: 300-400 M3-M12: 200-300	RATG AZA Prednisolone	*EMIT*	Non-compartmental PK analysis ; 289 PK profiles ; AUC_{0-6} Trap AUC_{0-6} CF vs non-CF: ns AUC_{0-6}/D: CF < non-CF (p<0.001)				
		Week 2	NEO n = 28			3,582-6,822	--	--	--	--
		Week 4				4,601-9,536	--	--	--	--
		Month 6				2,955-6,478	--	--	--	--
		Month 9				3,698-6,365	--	--	--	--
						r^2 C_0-AUC_{0-6} = 0.671; r^2 C_2-AUC_{0-6} = 0.962; r^2 C_6-AUC_{0-6} = 0.651				
		Week 2	SAND n = 22			735-5,526, p < 0.001	C_0/D, NEO vs SAND: ns	C_2/D: NEO > SAND (significant)	--	--
		Week 4				1,256-8,199, p < 0.001				--
		Month 6				550-7,839, p < 0.001		C_6/D, NEO vs SAND: ns	--	--
		Month 9				586-9,400, p < 0.001			--	--
						r^2 C_0-AUC_{0-6} = 0.574; r^2 C_2-AUC_{0-6} = 0.890; r^2 C_6-AUC_{0-6} = 0.608				
[25]	20 patients 10 CF 6 DL, 4 HL	Clinically stable Median: 21 months	NEO D adjusted on C_0 = 250-350 µg/L	N/R	*EMIT*	Non-compartmental PK analysis; AUC_{0-12} Trap AUC_{0-12} = 7,639±3,353 to 8,388±2,859 AUC_{0-4} = 3,876±2,876 to 4,481±1,311 r^2 C_2-AUC_{0-12} = 0.76; r^2 C_3-AUC_{0-12} = 0.87 r^2 C_2-AUC_{0-4} = 0.90; r^2 AUC_{0-4} – C_3 = 0.81	267±96 to 269±85	1,219±802 to 1,603±614	1,664±824 to 1,874±539	1.40±0.39 to 2.11±1.39 *(CF & non-CF)*

	10 non-CF 5 DL, 5 HL	36.5 months				AUC_{0-12} = 7,202±1,610 to 7,447±1,870 AUC_{0-4} = 4,081±1,135 to 4,348±1,262 AUC/D: CF < non-CF r^2 C_2-AUC_{0-12} = 0.66; r^2 C_3-AUC_{0-12} = 0.82 r^2 C_2-AUC_{0-4} = 0.78; r^2 C_3-AUC_{0-4} = 0.63	300±392 to 346±112	1,372±337 to 1,545±530	1,710±482 to 1,862±572	1.40±0.39 to 2.11±1.39 *(CF & non-CF)*
[32]	19 adults	Stable (no evidence of rejection within previous 3 months)	NEO D adjusted on C_0 = 250-350 µg/L	N/R	*EMIT*	PK parameters estimated using ITS method, CICLO 1.3; absorption described by a gamma distribution 3 consecutive PK profiles (P)/patient within 5 days Parameters standardized to a 100-mg dose *AUC/D: (h.µg/L)/mg cyclosporine*				
	9 CF 6 L, 3 HL		250±76 mg BID (175-425)			AUC_{0-12} P1: 7.97±2.46 P2: 8.33±3.25 P3: 8.23±3.25 CV%: Inter: 31.2; Intra: 10.6 AUC_{0-12}/D P1: 34.6±1.30 P2: 35.6±13.0 P3: 34.6±16.9 CV%: Inter: 41.3; Intra: 10.6 AUC_{0-4} P1: 4.54±1.50 P2: 4.63±1.31 P3: 4.43±1.51 CV%: Inter: 29.5; Intra: 9.5 AUC_{0-4}/D P1: 20.1±8.7 P2: 20.1±7.0 P3: 19.8±9.7 CV%: Inter: 39.3; Intra: 9.7	P1: 254±92 P2: 258±87 P3: 254±102 CV%: Inter: 32.2 Intra: 13.3	P1: 1641±638 P2: 1544±621 P3: 1492±678 CV%: Inter: 34.7 Intra: 14.7	P1: 1,952±732 P2: 1,977±479 P3: 1,966±708 CV%: Inter: 29.0 Intra: 11.1	P1: 1.47±0.24 P2: 1.33±0.34 P3: 1.33±0.34 CV%: Inter: 13.2 Intra: 15.9

10 non-CF 5 L, 5 HL	175±52 mg BID (125-275) p<0.025	AUC_{0-12} (ns) P1: 7.44±1.79 P2: 7.29±1.98 P3: 7.11±1.66 CV%: Inter: 23.8; Intra: 7.4 AUC_{0-12}/D (p < 0.005) P1: 41.6±7.7 P2: 41.2±9.5 P3: 40.6±8.5 CV%: Inter: 19.7; Intra: 7.5 AUC_{0-4} (ns) P1: 4.19±1.19 P2: 4.30±1.27 P3: 4.05±1.15 CF vs non-CF : CV%: Inter: 27.3; Intra: 10.3 AUC_{0-4}/D (p < 0.005) P1: 23.4±3.9 P2: 24.2±5.4 P3: 22.7±4.9 CV%: Inter: 18.2; Intra: 10.4 Bayesian estimator (AUC_{0-12}), best sampling times: C_0, C_1, C_3 BE vs Trap on AUC_{0-12}, AUC_{0-4}, C_{max}, t_{max}, C_0: bias% < 5.3; RMSE% < 15	P1: 346±112 P2: 309±90 P3: 300±92 CF < non-CF CV% Inter: 28.9; Intra: 11.9 C_0/D: CF < non-CF (p<0.01)	P1: 1545±530 P2: 1500±426 P3: 1372±337 (ns) CV% Inter: 26.5 Intra: 12.9 C_2/D: CF < non-CF (p<0.01)	P1: 1,752±427 P2: 1,959±576 P3: 1,802±587 CV%: Inter: 26.9 Intra: 12.6	P1: 1.62±0.31 P2: 1.34±0.19 P3: 1.55±0.46 CV%: Inter: 17.6; Intra: 15.1

(1) Co-administration of enzyme inhibitors for minimum 2 years
(2) In absence of ARE, dose decreased if SCr increased > 20% from baseline
(3) Only adults except for 4 patients ≤ 16 years
(4) 10 patients planned in the study: 9 L and 1 HL

Legend:
ARE: Acute rejection episode – ATG: Anti-thymocyte globulin – AZA: Azathioprine – BE: Bayesian estimator – C: concentration – CF: Cystic fibrosis – D: Dose – DILT: Diltiazem – DL: Double lung transplantation – f/up: follow-up – HL: Heart-lung transplantation – Intra-ind.: Intra-individual – Inter-ind.: Inter-individual – KETO: Ketoconazole – MMF: Mycophenolate mofetil – NEO: Neoral – N/R: not reported – SAND: Sandimmune – SCr: Serum creatinine – SL: Single lung transplantation – Trap: Trapezes.

Table II. Cyclosporine pharmacokinetic parameters in cohort studies
Results are expressed as mean±SD, unless otherwise specified

Ref	Number of patients	Post-transplant period	Cyclosporine dose	Co-administered immunosup-pressant	Assay	F, transfer k	V_d/F *(unless otherwise specified)*	CL/F *(unless otherwise specified)*	$t_{1/2}$ (h) *(unless otherwise specified)*
HEART TRANSPLANTATION									
[23]	16 adults (out of 35 in the global trial)		NEO	N/R	*FPIA TDx*	Non-compartmental PK analysis			
		Week 1	200±58 mg			--	--	--	--
		Week 2	159±40 mg			F = 57±9%	--	--	--
		Week 52	162±37 mg			--	--	--	--
[29]	20 male adults 58.8±10.2 years	Stable 36.8±27.1 months	NEO and SAND 1.14±0.4 mg/kg TID	N/R No corticosteroids	*FPIA TDx*	2-compartment open model: 1-order absorption, 1-order elimination			
						k: h^{-1}	*V_1, V_2: L/kg*	*CL: $L.h^{-1}/kg$*	
			SAND			F = 66±16% k_a = 1.99±1.42 k_{10} = 0.23±0.07 k_{12} = 0.37±0.14 k_{21} = 0.12±0.04	V_1 = 1.16±0.77 V_2 = 5.38±1.37	CL = 0.22±0.04	17.27±3.23
			NEO			F = 75±19% (p<0.001) k_a = 2.71±1.47 (p<0.05) k_{10} = 0.24±0.07 (ns) k_{12} = 0.41±0.16 (ns) k_{21} = 0.12±0.05 (ns)	V_1 = 1.00±0.43 (ns) V_2 = 5.84±1.37 (ns)	CL = 0.21±0.04 (ns)	18.83±3.58 (ns)
			After IV infusion				V_1 = 1.86±0.72 (ns) V_2 = 5.58±1.57 (ns)	CL = 0.20±0.04 (ns)	17.68±4.29 (ns)
[19]	47 adults	Stable	NEO + SAND(1)	N/R	*EMIT*	Non-compartmental PK analysis			
			D (mg/kg/day)			*$t_{1/2\ abs}$: h*		*CL/F: L/h*	
	54±12 years	49±19 months	None n = 11 3.90±1.03		SAND	$t_{1/2\ abs}$ = 2.72±1.45	--	46.7±21.1	--
					NEO	$t_{1/2\ abs}$ = 0.51±0.28 (p < 0.001)	--	31.8±9.5 (p<0.05)	--

	52±8 years	61±15 months	DILT n = 11	2.98±0.64		SAND NEO	$t_{1/2\ abs}$ = 1.44±1.16 $t_{1/2\ abs}$ = 0.58±0.62 ($p < 0.05$)	-- --	36.7±14.1 26.8±9.0 ($p<0.05$)	--
	52±9 years	32±15 months	KETO n = 13	0.79±0.27		SAND NEO	$t_{1/2\ abs}$ = 1.65±1.22 $t_{1/2\ abs}$ = 0.67±0.34 ($p < 0.01$)	-- --	9.7±4.3 9.1±3.8 (ns)	-- --
	47±12 years	28±5 months	DILT + KETO n = 12	0.90±0.54		SAND NEO	$t_{1/2\ abs}$ = 1.81±1.09 $t_{1/2\ abs}$ = 1.07±0.57 ($p < 0.05$)	-- --	10.1±6.4 9.1±4.9 (ns)	-- --
[30]	47 adults	Stable (months)	NEO + SAND(1)		N/R	*EMIT*	2-compartment PK analysis using ABBOTTBASE pharmacokinetic system (PKS); Structural model and PK parameters selected from a study in stable heart transplant recipients by Baraldo et al[29]			
				D (mg/kg/day)			*k: h^{-1}*	*V_1: L/kg*	*CL: $L.h^{-1}/kg$*	
		49±19	None n = 11	3.90±1.03			F = 75% (fixed) k_a = 2.7 h^{-1} (fixed) k_{12} = 0.41 h^{-1} (CV = 39%) k_{21} = 0.12 h^{-1} (CV = 42%)	V_1 = 1 (CV = 43%)	CL = 0.21 (CV = 19%)	--
		61±15	DILT n = 11	2.98±0.64						
		32±15	KETO n = 14	0.79±0.27			With no covariate: • r^2 = 0.871 ($p<0.001$); bias% = 11.7; RMSE% = 13.4 • Underestimation of AUC			
		28±5	DILT + KETO n = 11	0.90±0.54			With "disposition factor": (0.5 for CL if K or K+D; 0.76 for V_d if K+D) • r^2 = 0.698 ($p<0.001$); bias% = 2.2; RMSE% = 16.6 • Overestimation of AUC in patients on diltiazem Bayesian model: best sampling strategy: C_0, C_1, C_2			
[21]	15 adults 48±10 years	Clinically stable 5.2±4.4 years	NEO 2.7±0.7 mg/kg/day D adjusted on C_{av} = 200-300 µg/L		ATG AZA Prednisolone	*FPIA* *TDx*	Non-compartmental PK analysis using non linear regression program WinNonLin			
								V_d/F: mL/kg	*CL/F: $mL.min^{-1}/kg$*	
							--	2,421±945	5.7±1.7	5.0±1.3
[31]	14 patients *de novo*	3 PK profiles/patient	NEO D adjusted on C_0		N/R	*FPIA* *TDx*	Population exposure indices and PK parameters estimated using ITS method, CICLO 1.3 Absorption model based on a gamma distribution			
							MAT, SDAT: h	*A, B: L^{-1}*		*λ_1, λ_2: h^{-1}* *$t_{1/2}(\lambda_1)$, $t_{1/2}(\lambda_2)$: h*

		Week 1	200±61.2 mg BID			MAT = 1.25±0.40 SDAT = 0.48±0.20	A = 0.307±0.167 B = 0.637±0.267	--	λ_1 = 5.78±3.32 λ_2 = 0.47±0.17 $t_{1/2}(\lambda_1)$ = 0.26±0.40 $t_{1/2}(\lambda_2)$ = 1.90±1.09
		Mont 3	152±36.0 mg BID (vs W1, p<0.05)			MAT = 1.27±0.40 SDAT = 0.45±0.19	A = 0.771±0.630 B = 0.799±0.299	--	λ_1 = 3.18±2.19 λ_2 = 0.52±0.13 $t_{1/2}(\lambda_1)$ = 0.28±0.13 $t_{1/2}(\lambda_2)$ = 1.47±0.63
		Year 1	164±40.1 mg BID			MAT = 1.59±0.47 SDAT = 0.66±0.32	A = 0.690±0.445 B = 0.783±0.229	--	λ_1 = 2.595±1.368 (vs W1, p<0.05) λ_2 = 0.529±0.097 $t_{1/2}(\lambda_1)$ = 0.35±0.22 $t_{1/2}(\lambda_2)$ = 1.36±0.27
LUNG TRANSPLANTATION									
[66]	14 adults 8 SL, 6 DL	Stable	NEO Steady-state	N/R	*FPIA* *TDx*	--	--	--	9.52±5.71
HEART AND LUNG TRANSPLANTATION									
[27]	22 adults(2)	Month 1	D adjusted on C and clinical data	AZA + prednisolone	*Pc* *NSRIA*	PK parameters estimated using a Bayesian program for parameter estimation[48]			
							V_d/F: L/kg	*CL/F: mL.min^{-1}/kg*	
	11 CF		16.7±7.2 mg/kg/day			--	7.2±5.3	3.5±1.3	--
	11 non-CF		8.2±1.9 mg/kg/day (p<0.01)			--	4.3±2.2 (ns)	1.7±0.5 (p=0.003)	--
[32]	19 adults	Stable (no evidence of rejection within previous 3 months)	NEO D adjusted on C_0 = 250-350 µg/L	N/R	*EMIT*	PK parameters estimated using ITS method, CICLO 1.3; absorption described by a gamma distribution 3 consecutive PK profiles (P)/patient within 5 days Parameters standardized to a 100-mg dose			
						MAT, SDAT: h	*V_1/F: L* *F.A, F.B: L^{-1}*	*CL/F: L/h*	*λ_1, λ_2: h^{-1}*
	9 CF 6 L, 3 HL		250±76 mg BID (175-425)			F set to 1 MAT = 1.00±0.21 SDAT = 0.30±0.09	V_1/F = 88±41 F.A = 0.72±0.70 F.B = 0.68±0.34	50±20	λ_1 = 4.14±3.01 λ_2 = 0.36±0.11

10 non-CF 5 L, 5 HL	175±52 mg BID (125-275) p<0.025		F set to 1 MAT = 1.13±0.28 (ns) SDAT = 0.37±0.15 (ns)	V_1/F = 74±44 (ns) F.A = 1.13±1.36 (ns) F.B = 0.77±0.30 (ns)	50±14 (ns)	λ_1 = 2.16±1.75 ($p < 0.01$) λ_2 = 0.49±0.12 ($p < 0.01$)

(1) Co-administration of enzyme inhibitors for minimum 2 years
(2) Only adults except for 4 patients ≤ 16 years

Legend:
ATG: Anti-thymocyte globulin – AZA: Azathioprine – C: concentration – CF: Cystic fibrosis – D: Dose – DILT: Diltiazem – DL: Double lung transplantation – F.A and F.B: estimated intravenous coefficients – f/up: follow-up – HL: Heart-lung transplantation – KETO: Ketoconazole – λ1, λ2: disposition rate constants – MAT: Mean absorption time – MMF: Mycophenolate mofetil – NEO: Neoral – N/R: not reported – SAND: Sandimmune – SDAT: Standard deviation of absorption time – SL: Single lung transplantation – V1: volume of central compartment – V2: volume of peripheral compartment.

Table III. Cyclosporine population pharmacokinetic studies

Ref	Study	Subjects	PK parameters, exposure indices	ISV (%)	RRV	Covariates	Comments
HEART TRANSPLANTATION							
[33]	Retrospective data from patients who underwent routine cyclosporine PK monitoring (HPLC-UV) NONMEM ADVAN2 TRANS2 One-compartment PK model First order absorption and elimination	2 groups of patients: • Index n = 36; 51 years (18-63) • Predictive performance n = 33; 50 years (25-65) Oral SAND for at least 3 months post-transplantation + AZA + prednisone	k = CL/V Fixed k_a = 0.3 h^{-1} Fixed V = 4 x WT Initial analyses, F = 0.3 – then adjusted: F = 0.2 + 10 x POD-7 | /[(POD + 10) x 60] Cl = 0.281 x WT x (0.629 x DIL + 1 – DIL)	CL: 20.2	Add. 77.4 µg/L	POD Diltiazem	Over-prediction of concentrations (from 150 to 400 µg/L), especially within the first two weeks post-transplantation Predictive performance on concentrations: • When F = 0.3: ME = 42.1 µg/L [35.6-48.6] RMSE = 102.2 µg/L [61.0-143.4] • When F adjusted: ME = 21.3 µg/L [15.0-27.6] RMSE = 92.5 µg/L [54.0-131.0]
HEART AND LUNG TRANSPLANTATION							
[34]	Retrospective PK analysis of study performed by Trull et al. TDM 1999 (NEO vs SAND) – EMIT NONMEM ADVAN2 TRANS2 One-compartment PK model First order absorption and elimination	48 adults (8 CF) 18 SL, 9 DL, 21 HL NEO, n = 27 – SAND, n = 21 (D = 417±218 mg/day) + ATG, AZA, prednisolone Follow-up: Week 1 to week 52	1,004 samples collected CL/F (L/h) = 22.1 (19.5-24.7) V_d/F (L) = 147 (130-164)	CL/F: 17.1	Prop. 44.0% Add. 76.4 µg/L	On CL/F: Itraconazole, CF, WT On F: SAND, time post-transplantation	Comments of Saint-Marcoux[37] • Underestimation of C_2 values (95%CI = +5 to +115 µg/L) • Overestimation of C_6 values (95%CI = -127 to -69 µg/L) • Final model not validated, robustness not tested • ISV of V_d/F not estimated • Influence of individual factors tested only against CL/F

Legend
Add.: Additive – AZA: Azathioprine – CF: Cystic fibrosis – ISV: Inter-subject variability – ME: Mean error – NEO: Neoral – POD: post-operative day – Prop.: Proportional – RMSE: Root mean square error – RRV: Residual random variability – SAND: Sandimmune – WT: Weight.

Table IV. Pharmacokinetic methods for therapeutic drug monitoring of cyclosporine after thoracic transplantation (derived from Fernandez de Gatta et al. Clin Pharmacokinet 2002 and Dumont RJ et al. Clin Pharmacokinet 2000).[43,53]

Method	Advantages	Limitations
Single concentration		
C_0	• Standard method: simple and practical (inpatients and outpatients)	• Does not reflect absorption, drug exposure, or elimination • Does not give information on other PK parameters • Prediction of clinical outcome: conflicting data
C_2	• Surrogate marker of absorption (Neoral®) • Higher sensitivity as indicator of AUC and clinical effect than C0	• Rigid collection time • No distinction between poor absorbers and slow absorbers • Prediction of clinical outcome: conflicting data
Other time points (C_3, C_4, C_6)	• C_0 better correlated with AUC	• Rigid collection time • No distinction between poor absorbers and slow absorbers • Hardly any data on their relationship with clinical outcomes
AUC		
Full	• The best indicator of drug exposure, absorption profile and clinical outcome • Characterization of abnormal absorption patterns on concentration-time profile • Allows the calculation of oral pharmacokinetic parameters • Reduces analytical variability	• Need for multiple blood samples: impractical for routine clinical use • Expensive • Inconvenient for patients (outpatients ++) • Optimal targets to be defined for most immunosuppressants
Abbreviated (ex: 4 h post-dose)	• Good predictor of absorption profile and full AUC	• Further studies required • Optimal targets to be defined
Sparse sampling strategies	• Balance between precision and practicality • Acceptable predictor of AUC	• Multi-linear regression not based on a PK model. Equations result from correlations and cross-correlations between sampling times and AUC • Rigid collection times • Analytical method- and centre-specific, often not validated in independent populations
Bayesian forecasting		
	• Flexibility in sampling times • Limited number of samples needed • Can easily be integrated in clinical practice • Simultaneous estimation of individual PK parameters and exposure indices • Identification of absorption or elimination problems (*e.g.*, gastroparesis)	• Only a few studies in small populations • Predictive performance not tested • Requires sophisticated PK models and software

Table V. Summary of selected sparse sampling strategies for cyclosporine monitoring after Neoral® administration

Ref	Patient group	Number of patients	AUC interval (h)	Equation	r^2	%PE
[21]	HTx Stable	15	12	$AUC_{0-12} = 531 + 0.64*C_1 + 8.12*C4$	0.973	N/R
[215]	HTx Week 1 Month 3 Year 1	20 Validation: 17[215] then 14[31]	12	$AUC_{0-12} = 330.841 + 2.235*C_2 + 7.970*C_6$	0.951 0.918 0.949	-8.68 to +6.24 -7.3 to +8.58 -5.33 to +6.49
[6]	LTx < Week 2	12	N/R	$AUC_{0-6} = 0.496*C_2 + 0.517*C_3 + 776.9$	0.94	NR
[66]	LTx Stable	8 Validation: 6	τ, N/R	$AUC_{0-\tau} = 185.62 + 1.75*C_1 + 4.91*C_3$ $AUC_{0-\tau} = 53.54 – 5.47*C_0 + 2.11*C_1 + 6.44*C_3$	0.904 0.908	-6.96 to +13.05 -10.31 to 22.43

Legend
HTx: Heart transplantation – LTx: Lung transplantation – N/R: not reported – PE: Prediction error.

Table VI. Cyclosporine exposure – efficacy studies
Results are presented as mean±SD, unless otherwise specified

Ref	Number of patients	Immonsuppressants	Sampling periods	*Analytical method*, exposure	Event (number of episodes)	Comments
HEART TRANSPLANTATION						
[54]	31 adults OHT 52±10 years	Cyclosporine D adjusted on C_0: < M6 200-275 µg/L M6-M12 150-250 µg/L > M12 100-200 µg/L D = 194±57 mg BID (4.8±1.4 mg/kg BID)	244±178 days Median (range): 223 (22-582)	*HPLC-UV* Calculation of AUC: AUC_{0-12}: MAP-BE[1] or sparse-sample algorithms[2-4] AUC_{0-4}: sparse-sample algorithm[5] C_0 215±68 µg/L C_2 949±204 µg/L AUC_{0-12} 4,875±956 to 5,897±1,457 h.µg/L AUC_{0-4} 2958±579 h.µg/L C_0 242±62 µg/L (ns) C_2 1,359±474 µg/L (ns) AUC_{0-12} 6,723±2,119 to 7,445±1,871 h.µg/L (ns) AUC_{0-4} 3970±1150 h.µg/L vs. 2958±579 (ns)	Routine surveillance EMB (ISHLT) Rejection (≥ grade 2) n = 3 patients; all had C_2 < 1250 µg/L No rejection (< grade 2)	Trend for a significant relationship between C_2 values and the incidence of rejection. Number of patients experiencing rejection too small: • To reach statistical significance based on C_0 or C_2 thresholds; • To perform ROC curve.
[93]	60 adults	Cyclosporine D adjusted on C_0: Y1 300-400 µg/L Y2 200-250 µg/L Y3 150-180 µg/L > Y3 > 150 µg/L ATG Corticosteroids until M12 AZA or MMF (n = 10)	3-60 months	*FPIA-AxSYM* 4 AUC/patient = 240 AUC, calculated from C_0, C_2, C_4, C_6 Constant absorbers, AUC CV < 15% (n = 21) AUC = 6,521 h.µg/L Inconstant absorbers, AUC CV > 15% (n = 39) AUC = 6,751 h.µg/L (ns)	At least one ARE grade 3A (ISHLT) in 20/60 patients Acute rejection n = 4 patients (19%) Acute rejection n = 16 patients (41%), $p < 0.05$	No difference between "rejection" and "no rejection" groups on mean C_2. Variability on absorption = risk factor for rejection.
[55]	10 children out of 50 in the global trial	SAND or NEO, D adjusted on C_0 (and blood cell count): ≤ M1 200-300 µg/L > M1 150-200 µg/L (D: median, range) ATG	*De novo* ≥ 2 months	*HPLC/MS*	Rejection: IMEG, echocardiography, TDE, BPAR (≥ grade 2 – ISHLT) ROC analysis: best sensitivity and specificity for C_2 < 600 µg/L – Se = 100%, Sp = 83% • C_2 < 600: rejection 14, no rejection 0 • C_2 > 600: rejection 6, no rejection 30	Prospective blinded analysis Small number of patients with rejection: study not powerful enough? Impossible to define different C_2 target levels in relation to adjunctive

		AZA or MMF					immunosuppressants
	8.1±6.3 years	D = 8.6 mg/kg (4-21)	2.2±1.6 years	C_2 345±163 µg/L C_{min} 213±29 µg/L C_{max} 467±38 µg/L AUC_{0-12} 3,615±508 h.µg/L AUC_{0-4} 1,498±132 h.µg/L	< Y1: 456±164 µg/L Y1-Y2: 428±196 µg/L Y2-Y5: 244±147 µg/L	Rejection (BPAR: n = 14) n = 5 patients	Impossible to assess chronic rejection
	8.9±5.6 years	D = 7.9 mg/kg (3.7-24), ns	2.4±1.8 years	C_0: ns C_2 = 952±310 µg/L (p<0.001) C_{min} 219±32 µg/L (ns) C_{max} 966±231 µg/L (p<0.001) AUC_{0-12} 5,530±889 h.µg/L (p < 0.001) AUC_{0-4} 2,713±536 h.µg/L (p<0.001)	< Y1: 1,134±356µg/L Y1-Y2: 996±266 µg/L Y2-Y5: 859±274 µg/L	No rejection n = 5 patients	
HEART AND LUNG TRANSPLANTATION							
[80]	31 adults (12 CF) HL	Cyclosporine median D (mg/kg/day) • starting: 2.7 • maintenance: 10.3 (CF patients: TID) ATG, AZA, Prednisone	< 3 months	*SRIA* Observed C_0 (median):	< W3: 347.5 µg/L W3-M3: 445.5 µg/L	Treated ARE (66% BPAR) If C_0 CV > 40%: RR = 1.51 (95%CI = 1.01-2.27)	Variability on C_0: risk factor for subsequent rejection; no such relationship for C_0 itself
[28]	50 patients (9 CF) 19 SL, 9 DL, 22 HL	NEO (n = 28) vs SAND (n = 22), D adjusted on C_0: M1-M2 300-400 µg/L M3-M12 200-300 µg/L ATG, AZA, prednisolone	12 months	*EMIT* 289 PK profiles C_0 = 394 µg/L C_0 = 449 µg/L (p = 0.042)		 Patients with at least one treated ARE Patients with no ARE	Study designed to compare the PK of NEO vs SAND, not to establish a relationship between drug exposure and efficacy Relationship C_2, C_6 or AUC_{0-6} – incidence of treated AR: ns
[20]	48 patients SL, DL, HL	NEO (n = 27) vs SAND (n = 21), D adjusted on C_0: M1-M2 300-400 µg/L M3-M12 200-300 µg/L (CF patients: TID)	Weeks 1, 2, 3, 4, 13, 26, 39, 52	*EMIT* 341 AUC_{0-6} 3 groups based on C_2 (µg/L) at M1: low, < 1000 (18); intermediate, 1000-1500 (16); high >1500 (14)		ARE frequency similar in the 3 C_2 groups AR-free periods: Intermediate C_2 (226 days) >> low C_2 (197 days) and high C_2 (191 days) (p<0.0001)	

	ATG, AZA, prednisolone		C_2 875±546 µg/L AUC_{0-6} 4,036±1,904 h.µg/L	Median number of ARE > 2	
			C_2 1,114±633 (p = 0.01) AUC_{0-6} 4,870±2,182 (p = 0.01) C_0 and C_6: ns	Median number of ARE ≤ 2	

Legend
ARE: Acute rejection episode – ATG: Antithymocyte globulin – AZA: Azathioprine – BPAR: Biopsy-proven acute rejection – CF: Cystic fibrosis – D: Dose – DL: Double lung transplantation – EMB: Endomyocardial biopsy – HL: Heart-lung transplantation – IMEG: Intramyocardial electrocardiogram – MAP-BE: Maximum *a posteriori* Bayesian estimation – MMF: mycophenolate mofetil – NEO: Neoral – OHT: Orthotopic heart transplantation – SAND: Sandimmune – SL: Single lung transplantation – TDE: Tissue Doppler echocardiography.

Table VII. Cyclosporine exposure – toxicity studies
Results are presented as mean±SD, unless otherwise specified

Ref	Number of patients	Immunosuppressants	Sampling periods	*Analytical method*, exposure	Event (number of episodes)	Comments
HEART TRANSPLANTATION						
[54]	31 adults OHT 52±10 years	Cyclosporine D adjusted on C_0: < M6 200-275 µg/L M6-M12 150-250 µg/L > M12 100-200 µg/L D = 194±57 mg BID (4.8±1.4 mg/kg BID)	244±178 days Median (range): 223 (22-582)	*HPLC-UV* Calculation of AUC: AUC_{0-12}: MAP-BE[1] or sparse-sample algorithms[2-4] AUC_{0-4}: sparse-sample algorithm[5] Rejection vs no rejection: C_0 215±68 µg/L C_2 949±204 µg/L AUC_{0-12} 4,875±956 to 5,897±1,457 h.µg/L AUC_{0-4} 2958±579 h.µg/L C_0 242±62 µg/L (ns) C_2 1,359±474 µg/L (ns) AUC_{0-12} 6,723±2,119 to 7,445±1,871 h.µg/L (ns) AUC_{0-4} 3970±1150 h.µg/L vs. 2958±579 (ns)	Impairment of renal function: • If SCr > 2.5 mg/dL • n = 5 patients No relationship between cyclosporine C_0 or C_2 and impairment of renal function.	
[93]	60 adults	Cyclosporine D adjusted on C_0: Y1 300-400 µg/L Y2 200-250 µg/L Y3 150-180 µg/L > Y3 > 150 µg/L ATG Corticosteroids until M12 AZA or MMF (n = 10)	3-60 months	*FPIA-AxSYM* 4 AUC/patient = 240 AUC, calculated from C_0, C_2, C_4, C_6 Constant absorbers, AUC CV < 15% (n = 21) AUC = 6,521 h.µg/L Inconstant absorbers, AUC CV > 15% (n = 39) AUC = 6,751 h.µg/L (ns)	Mean SCr rise of 50% from baseline in both groups (ns) % patients: Hypertension 48% Neurological events 24% Gum hyperplasia 14% New onset hyperlipidemia treated with statins 48% Hypertension 56% Neurological events 41% Gum hyperplasia 18% New onset hyperlipidemia treated with statins 51%	
LUNG TRANSPLANTATION						
[79]	15 adults (0 CF) 3 SL, 12 DL	Cyclosporine D adjusted on C_0 = 200-300 µg/L	42-359 days	*EMIT* C_0 = 289±93 µg/L	CMV infection or disease	• C_0 and C_2: same sensitivity (84.6%) • Better specificity of C_0

		AZA, methylprednisolone		C_2 = 1,583±421 µg/L C_0 = 222±82 µg/L (p<0.05) C_2 = 1,074±423 µg/L (p<0.05)	n = 9 patients (13 episodes) No CMV infection or disease	(66.7%) vs C_2 (57.1%)
HEART AND LUNG TRANSPLANTATION						
[77]	32 patients 24 HTx, 28 HL	Cyclosporine D adjusted on C_0 (target: N/R) ATG, AZA, steroids	< 3 months	*SRIA* First 5 postoperative days, 88% C_0 < 200 µg/L	Renal function (624 complete sets of dose, C_0 and SCr) No correlation between dose and change in renal function Correlation between log C_0 and 1/SCr: • Simultaneous measurements: r^2 = -0.33 (95%CI = -0.52;-0.10) • In the subsequent 5-day period: r^2=-0.69 (95%CI = -0.69;-0.15)	Exclusion of all data up to the first nadir in plasma creatinine following transplantation (mean time: 6.7±3.6 days)
[28]	50 patients (9 CF) 19 SL, 9 DL, 22 HL	NEO (n = 28) vs SAND (n = 22), D adjusted on C_0: M1-M2 300-400 µg/L M3-M12 200-300 µg/L ATG, AZA, prednisolone	12 months	*EMIT* 289 PK profiles Patients with at least one treated ARE C_0 = 394 µg/L Patients with no ARE C_0 = 449 µg/L (p = 0.042)	Renal function: None of C_0, C_2, C_6 or AUC_{0-6} correlated with any of the markers of cyclosporine nephrotoxicity	Study designed to compare the PK of NEO vs SAND, not to establish a relationship between drug exposure and efficacy
[20]	48 patients SL, DL, HL	NEO (n = 27) vs SAND (n = 21), D adjusted on C_0: M1-M2 300-400 µg/L M3-M12 200-300 µg/L (CF patients: TID) ATG, AZA, prednisolone	Weeks 1, 2, 3, 4, 13, 26, 39, 52	*EMIT* 341 AUC_{0-6} 3 groups based on C_2 (µg/L) at M1: low, < 1000 (18); intermediate, 1000-1500 (16); high >1500 (14) Median number of ARE > 2 C_2 = 875±546 µg/L AUC_{0-6} = 4,036±1,904 h.µg/L Median number of ARE ≤ 2 C_2 = 1,114±633 (p = 0.01) AUC_{0-6} = 4,870±2,182 (p = 0.01) C_0 and C_6: ns	Infections (71 episodes): • Patients with infection episodes > 2 vs ≤ 2: C_0, C_2, C_6, AUC ns • Number of infection episodes ns among patients stratified on C_2 Renal function (1,693 observations): • Difference between groups: ns Blood pressure (1,891 measurements): • Linear trend ↗ BP – C_2 strata (p < 0.001) • No correlation between BP and exposure indices	

Legend:
ARE: Acute rejection episode – ATG: Antithymocyte globulin – AZA: Azathioprine – BP: Blood pressure – CF: Cystic fibrosis – D: Dose – DL: Double lung transplantation – HL: Heart-lung transplantation – HTx: Heart transplantation – MAP-BE: Maximum *a posteriori* Bayesian estimation – MMF: Mycophenolate mofetil – NEO: Neoral – N/R: not reported – OHT: Orthotopic heart transplantation – SAND: Sandimmune – SCr: Serum creatinine – SL: Single lung transplantation.

Table VIII. Cyclosporine TDM or concentration-controlled studies

Ref	Study	Patients	Time post-transplant	Immunosuppressants	*Analytical method*, exposure index and target	Evaluated endpoints and results	Comments
HEART TRANSPLANTATION							
[59]	Prospective study Consecutive patients, randomized in 2 groups	20 adults	< 1 year	SAND	*EMIT*	BPAR: ISHLT classification	
		Group I 53±8 years n = 10	11±2 months	Thymoglobuline AZA Prednisone	D adjusted on C_0 = 150-250 µg/L	Efficacy: • BPAR: 50% (grade I: 70/172 biopsies: 40.7%) Others: • Cyclosporine D at end of f/up : 3.5±1 mg/kg/day • Mean cyclosporine D adjustment/patient: 1/month • Total cyclosporine cost: 5,106±1,045 CDN $	
		Group II 58±4 years n = 10	10±3 months		D adjusted on C_6 = 150-250 µg/L	Efficacy: • BPAR: 50% (grade I: 47/111 biopsies: 29.7%, p=0.04) • Left VEF and graft atherosclerosis similar in both groups Toxicity: • Renal function similar in both groups (SCr, GFR) Others: • Cyclosporine D at end of f/up : 2.6±0.6 mg/kg/day (p=0.002) • Mean cyclosporine D adjustment/patient: 1/month • Total cyclosporine cost: 3,589±1,116 CDN $ (p=0.005)	
[56]	Prospective study Randomization to C_0 or C_2 monitoring Follow-up: 6 months	30 adults	Stable, ≥ 1 year No BPAR in previous 6 months SCr < 250 µmol/L	NEO (Week 0: conversion from SAND to NEO)	*EMIT*	Routine biopsies and after each dose reduction No instance of BPAR ≥ grade 2 (ISHLT classification)	No comparison of outcome between two groups based on monitored exposure index: this study rather looked for a correlation between a single time-point concentration and AUC_{0-4} and AUC_{0-12}
		Group I 59±7 years n = 15		Thymoglobuline AZA Prednisone	• Week 2: D adjusted on C_0 = 100-200 µg/L • ≥ Week 6: D adjusted on C_2 = 300-600 µg/L	D increase in 8 patients after the first visit D reduction in all patients after the second visit • C_0 = 147±39 µg/L • C_1 = 762±385 µg/L • C_2 = 835±320 µg/L • C_4 = 379±138 µg/L • AUC_{0-4} (Trap) = 2,467±804 h.µg/L	

		Group II 56±9 years n = 15			• Week 2: D adjusted on C_2 = 200-400 µg/L • ≥ Week 6: D adjusted on C_2 = 300-600 µg/L	D reduction after the first visit • C_0 = 102±35 µg/L (p = 0.0001) • C_1 = 621±402 µg/L (ns) • C_2 = 555±271 µg/L (p = 0.01) • C_4 = 218±83 µg/L (p = 0.001) • AUC_{0-4} (Trap) = 1,723±808 h.µg/L (p = 0.02)	
[10]	Longitudinal follow-up of all patients followed at heart transplantation clinic 2-phase study	114 adults 57±9 years	Stable, ≥ 1 year	NEO ATG (most patients) Cyclosporine monotherapy (7.9%) or + AZA + prednisone (40.3%) or + AZA (36%) or + prednisone (15.8%)	*EMIT*	Primary endpoint: clinical benefit = composite measure • Positive cardiac outcomes (no mortality, no acute rejection, no decrease in LVEF > 10%), and • Positive renal outcomes (absence of increase in SCr > 10%)	Limitation: sequential longitudinal assessment of two strategies for NEO D adjustment in the same patients
				Phase 1 (10±4 months)	D adjusted on C_2 = 300-600 µg/L	Primary endpoint: • Clinical benefit: 69.3%	
				Initial D = 2.6±0.8 mg/kg/day	C_0 = 136±42 µg/L C_2 = 776±316 µg/L	Secondary endpoints: • Incidence of acute rejection: 0.87% • Incidence of mortality: 7.9% • Incidence of increase in SCr > 10%: 18.1%	
				Final D = 1.9±0.6 mg/kg/day	C_0 = 87±36 µg/L C_2 = 422±153 µg/L		
				Phase 2 (10±2 months)	Dose adjusted on C_0 = 100-200 µg/L	Primary endpoint: • Clinical benefit: 43.3% RR = 1.6 (p = 10^{-5})	
				Initial D = 1.9±0.6 mg/kg/day	C_0 = 86±35 µg/L C_2 = 428±159 µg/L	Secondary endpoints: • Incidence of acute rejection: 0.96% (ns) • Incidence of mortality: 9.6% (ns) • Incidence of increase in SCr > 10%: 48.9% RR = 0.37 (p<10^{-4}) • LVEF: stable across both time periods	
				Final D = 2.4±0.6 mg/kg/day	C_0 = 134±46 µg/L C_2 = 592±156 µg/L		

[22]	Prospective analysis of 2 consecutive periods		*De novo* f/up: 4 years	NEO In absence of ARE, D decreased if SCr increased > 20% from baseline ATG, MMF, prednisone	*EMIT*	Efficacy • Routine EMB: ARE ≥ grade 3A (ISHLT classification) • Echocardiography • Coronary angiography Toxicity • Renal function: SCr, ClCr (Cockroft-Gault), GFR	MMF D: group 1 ≠ 2 D/kg different at M1
		13 adults 54±9 years		Group I (MMF D: 1 g BID)	• < M9: D adjusted on C_0 < M3: 200-300 µg/L M4-M6: 150-250 µg/L M6-M9: 100-200 µg/L • > M9: D adjusted on C_2 = 400-600 µg/L	Efficacy • Incidence of ARE ≥ grade 3A ○ Month 6: 38.5% ○ Month 12: 38.5% ○ Month 24: 46% ○ Month 36: 54% • Time to acute rejection: 363±462 days • 3 deaths • LVEF: 58±5 (M3) to 60±5% (M12) • Incidence of graft atherosclerosis at M12: 11%	
		9 adults 53±12 years		Group II (MMF D: 1.5 g BID)	• D adjusted on C_2 < M3: 600-800 µg/L M4-M6: 500-700 µg/L > M6: 400-600 µg/L	Efficacy • Incidence of ARE ≥ grade 3A (ns) ○ Month 6: 11% ○ Month 12: 44% ○ Month 24: 56% ○ Month 36: 56% • Time to acute rejection: 344±237 days (ns) • No death • LVEF: 59±2 (M36) to 61±2% (M24) (ns) • Incidence of graft atherosclerosis at M12: 0% (ns) Toxicity: • Renal function, incidence of infections: ns	
[86]	2-phase prospective study Paired determination of C_0 and C_2 Investigators blinded	58 adults 56±11 years	2.03±1.28 years (0.3-3.6)	Cyclosporine ATG or basiliximab Prednisone AZA or MMF	*FPIA-TDx*	Toxicity: • Incidence of life-threatening infections • Renal dysfunction (SCr, ClCr) Efficacy • BPAR ≥ grade 2 (ISHLT)	Short follow-up in both phases. C_0 or C_2 values in the whole population for

	to exposure index				Phase I (6 months): D adjusted on C_0 • M3-M6: 300-350 µg/L • M6-M12: 250-300 µg/L • M12-M24: 200-250 µg/L • >M24: 150-200 µg/L	Toxicity • 8 infection episodes • Renal function deterioration in 2 patients Efficacy: • ARE ≥ grade 3, 7/58 patients • Rejection vs no rejection: C_0 = 195±121 vs 197±100, ns C_2 = 777±326 vs 1,015±422, p=0.022	each phase: N/R. No monitoring of MPA levels.
					Phase II (6 months): D adjusted on C_2 associated with no rejection during phase I • M3-M6: 1,403±285 • M6-M12: 1,175±215 • M12-M24: 947±170 • >M24: 824±120	Toxicity • 6 infection episodes (ns) • Renal function deterioration in 1 patient; no difference in SCr and calculated ClCr Efficacy: • ARE ≥ grade 3, 6/56 patients (ns) • Rejection vs no rejection: C_0 = 204±85 vs 209±138, ns C_2 = 765±297 vs 967±470, p=0.03	
[51]	Prospective randomized controlled study f/up: 6 months	125 adults	Stable, > 1 year	Cyclosporine	*CEDIA*		Short follow-up duration.
		Group I n = 62 55±10 years	6.4±2.8 years	± Prednisone ± MMF or AZA	D adjusted on C_0 = 80-120 µg/L	Primary endpoint • Decrease of cyclosporine D: 11 mg/day Secondary endpoints • Deaths: 1 patient • ARE: no suspicion • Infections: 9.7% • ClCr: + 0.54 mL/min	Low incidence of AR after the first year post-transplant. Study not powered to detect a small possible effect of C_2 vs C_0 monitoring on AR.
		Group II n = 63 55±9 years	6.4±2.5 years		D adjusted on C_2 = 300-600 µg/L	Primary endpoint • Decrease of cyclosporine D: 26 mg/day (p = 0.0025) – C_0 in target range Secondary enpoints • Deaths: 1 patient • ARE: no suspicion • Infections: 15.9% (p = 0.14) • ClCr: -0.16 mL/min (p = 0.61)	

HEART AND LUNG TRANSPLANTATION

[88]	Single center study using historic controls Intent-to-treat analysis		*De novo*	Cyclosporine	*EMIT*			Larger proportion of DL ($p < 0.01$) and of CF patients ($p < 0.01$) in C_2 vs C_0 group.
	Transplantation between 1989 and 2000	338 patients 124 SL, 150 DL, 64 HL		ATG[(1)] AZA Prednisolone	C_0 group Target: N/R		Primary endpoint : efficacy (ISHLT classification) • Freedom from AR: M3: 51% – M6: 45% – M12: 41% • Freedom from BOS: Y1: 87% – Y3: 53% – Y5: 36% • Actuarial survival: 30-days: 93% – Y1: 83% – Y3: 66% – Y5 : 55% Secondary endpoint: toxicity • Chronic renal failure within 5 years: 18 patients	
	Transplantation between 2001 and 2002	50 patients 3 SL, 44 DL, 3 HL	f/up: 16-1,790 days (1,185±426)	AZA (n = 15) or MMF (n = 35) Prednisolone	C_2 group Day 0-2 Day 2-7 Day 8-30 Day 30-60 Day 61-90 Day 91-180 Day 181-365 Day >365	 > 800 > 1,200 1,200-1,700 1,200-1,500 800-1,200 700-1,000 600-900 600-800	Primary endpoint : efficacy (ISHLT classification) • 15 patients with ARE (23 episodes/171 TBB) 18/23 episodes within 2 weeks of subtherapeutic level • 12 patients with ARE/25 with C_2 subtherapeutic levels • Freedom from AR ($p = 0.001$) M3: 74% – M6: 69% – M12: 69% • Freedom from BOS ($p = 0.002$) Y1: 98% – Y3: 79% – Y5: 59% • Actuarial survival ($p = 0.029$) 30-days: 98% – Y1: 94% – Y3: 82% – Y5 : 77% Secondary endpoint: toxicity • Chronic renal failure in one patient, with SCr > 250 mg/L (needed dialysis and renal transplantation); ($p > 0.10$) • SCr = 122±64 mg/L	
LUNG TRANSPLANTATION								
[92]	Sequential groups	36 adults (17 CF) DL	*De novo* f/up: 3 months	Cyclosporine Prednisolone	*EMIT*			C_0 group older than C_2 group
		n = 18		AZA (n = 14) or MMF (n = 4)	D adjusted on C_0 Week 1: 450 µg/L Month 3: 250 µg/L		Efficacy • FEV1 at M3: no difference • AR (A and B): similar rates in both groups (numbers: N/R) • Survival at M3: 100%	Baseline SCr lower in C_0 group Associated immunosuppressants different between the
		n = 18		AZA (n = 2)	D adjusted on C_2			

				or MMF (n = 16)	Week 1: 1,200 µg/L Month 3: 800 µg/L	Toxicity • SCr: greater increase from baseline in C_0 group • Infections: similar rates in both groups (1.85 vs. 1.79 events per 100 patients-days)	2 groups
[87]	Uncontrolled single-center pilot study	15 adults with renal dysfunction (5 CF) 5 SL, 10 DL	3.5±2.7 years (0.2-9.0)	Cyclosporine AZA (n = 10) or MMF (n = 5) Prednisolone	*EMIT* Switch from C_0 to C_2 monitoring C_2 = 300-600 µg/L	Primary endpoint • Renal function improvement at 3 and 12 months: SCr = 0.20±0.07 mmol/L at baseline to 0.15±0.05 mmol/L at M3 ($p < 0.001$) Secondary endpoint • Stable lung function except for 1 ARE Cyclosporine D (mg/kg/day) divided by two within 3 months: 6.4±7.3 to 3.9±3.7 at M3 ($p = 0.031$) to 3.1±2.7 at M12 ($p = 0.041$)	

(1) Patients transplanted before 1995

Legend
AR: Acute rejection – ARE: Acute rejection episode – ATG: Antithymocyte globulin – AZA: Azathioprine – BOS: Bronchiolitis obliterans syndrome – BPAR: Biopsy proven acute rejection – ClCr: Creatinine clearance – D: dose – EMB: Endomyocardial biopsy – f/up: Follow-up – GFR: Glomerular filtration rate – LVEF: Left ventricular ejection fraction – M: Month – MMF: Mycophenolate mofetil – NEO: Neoral – N/R: not reported – SAND: Sandimmune – SCr: Serum creatinine – TBB: Transbronchial biopsy – Y: Year.

Table IX. Tacrolimus exposure indices in cohort studies
Results are expressed as mean±SD (range), unless otherwise specified

Ref	Number of patients	Post-transplant period	Tacrolimus dose	Co-administered Immunosup-pressant	Assay	Measured AUC	C_0 (µg/L)	C_{max} (µg/L)	t_{max} (h)	Others
HEART TRANSPLANTATION										
[102]	14 adults 55.5 years (23-61)	< 6 months	First D = 0.03-0.40 mg/kg (median: 0.052 mg/kg)	ATG AZA Prednisone	*MEIA – IMx*	Non-compartmental PK analysis using Pharm-NCA computer program; AUC Trap; median (range) PK profiles after the first dose of tacrolimus				
						AUC_{0-12} = 155.6 (103.5-728.7)	--	23.0 (10.7-84.4)	2 (1-4)	C_{min} = 8.3 (< 5-54.1)
						$AUC_{0-\infty}$ = 285.3 (121.8-1540.0)				
						Best correlation: C_{12}-AUC: C_{12} = 0.0371 x AUC0-∞ – 2.1; r^2 = 0.98 ($p<0.001$)				
[100]	11 adults OHT		First D up to 0.15 mg/kg BID D adjusted on C_0 < 15 µg/L	N/R		Non-compartmental PK analysis using TOPFIT v2.0; t_{max}: median (range)				
		Profile 1 (day 1) NG tube	D = 0.053±0.031 mg/kg (0.026-0.143) CV = 59%		*ELISA*	$AUC_{0-\infty}$ = 191.8±137.1 (32.9-422.2) CV = 72% – n = 7	--	23.6±22.4 (1.3-76.6) CV = 95% – n = 10	2.84 (2-9.5) n = 10	--
					HPLC-MS/MS	$AUC_{0-\infty}$ = 141.9±93.6 (41.6-254.9) CV = 66% – n = 5	--	20.8±15.9 (2.1-49.4) CV = 77% – n = 10	2.75 (1.5-9.5) n = 10	--
		Profile 2 (last day) Hard capsules	D = 0.076±0.069 mg/kg (0.013-0.250) CV = 91%		*ELISA*	$AUC_{0-\infty}$ = 257.4±76.3 (144.8-442.7) CV = 30% – n = 10	--	34.2±18.2 (19.0-84.7) CV = 53% – n = 10	1.96 (0-4) n = 10	--
					HPLC-MS/MS	$AUC_{0-\infty}$ = 211.1±77.2 (104.2-362.9) CV = 37% – n = 10	--	33.1±14.8 (13.3-66.6) CV = 45% – n = 10	1.5 (1-8) n = 10	--
[103]	25 adults 47±10 years	Immediate post-transplantation		Induction: ATG/OKT3	*HPLC-MS/MS*	Non-compartmental analysis using computer program PC Modfit v/6; AUC_{0-12} Trap				

		period								
	n: N/R		D = 0.075 mg/kg/day							
			First dose			78.1±59.0	--	14.7±7.8	2.1±1.7	--
			Steady-state			186±68.7		26.6±9.5	1.9±1.2	
	n: N/R		D = 0.15 mg/kg/day							
			First dose			142±116	--	24.5±13.7	1.9±1.2	--
			Steady-state			198±73.1		29.9±14.5	1.8±0.6	
						r^2 C_{min} – AUC_{0-12}: first D = 0.86; steady-state = 0.79				
[115]	19 adults 46 years (28-48) OHT		D = 0.06 mg/kg/day (0.04-0.08) D adjusted on C_0 = 5-20 µg/L	ATG AZA Prednisone	*MEIA – IMx*	2-compartment open model using P-Pharm popPK software				
		PK1 (day 10)				--	12.5	--	--	--
		PK2 (month 2)				--	15.0	--	--	--
[111]	8 adults 45-60 years	4 to 16 months (mean: 10.6)	D = 0.02-0.1 mg/kg/day D adjusted on C_0 = 5-12 µg/L	MMF Prednisone	*MEIA II – IMx*	Non-compartmental PK analysis using PK Calc software; AUC Trap; Mean (range)				
						AUC_{0-12} = 151.7 (129.2-174.2)	7.9 (6.0-9.7)	22.9 (18.3-27.5)	2.2 (1.5-2.9)	C_{12} = 7.6 (6.2-9.0) (vs C_0: ns)
						$AUC_{0-\infty}$ = 317.5 (189.2-445.7)				
						r^2 AUC_{0-12}: • C_0, C_1, C_2, C_3 = 0.09 to 0.26 (ns) • C_4, C_5, C_6, C_7, C_8 = 0.53 to 0.64 ($p < 0.05$) • C_9, C_{10}, C_{11}, C_{12} = 0.70 to 0.80 ($p < 0.01$)				
[109]	22 adults 55±8 years (36-64) OHT	< Year 1	D = 0.3 mg/kg/day D adjusted on C_0 = 10-20 µg/L Steady-state	MMF or AZA Steroids	*MEIA – IMx*	Non-compartmental PK analysis using MOMENT software (n = 25 PK profiles); AUC Trap				
						AUC_{0-12} = 236.3±88.6 (81.5-419.8)	14.8±5.7 (3.9-25.9)	30.5±13.8 (10.3-57.8)	2.3±1.5 (0.5-6.0)	C_{av} = 19.7±7.4 (6.8-35.0)
						AUC_{0-4} = 93.5±40.8 (35.9-180.9)				
						r^2 AUC_{0-12}: • C_0 = 0.80 • C_1 = 0.40 • C_2 = 0.81 • C_4 = 0.90 • C_{12} = 0.89	r^2 AUC_{0-4}: • C_0, C_1 = 0.70 • C_2 = 0.96 • C_4 = 0.73	Sparse sampling algorithms for AUC_{0-12}: • $3.75 + 5.52*C_0 + 6.97*C_4$ – r^2 = 0.95 • $0.98 + 4.17*C_0 + 2.29*C_2 + 5.3*C_4$ – r^2 = 0.97		
[104]	13 patients		D adjusted on C_0 = 10-15 µg/L	ATG	*N/R*	Non-compartmental PK analysis performed using WinNonlin; AUC Trap				

						AUC/D: (µg.h/L)/mg tacrolimus					
		First dose	D = 0.077±0.011 mg/kg BID			$AUC_{0-\infty}$ = 186.7±96.6 CV = 52% $AUC_{0-\infty}$/D = 46.0±24.3 r^2 $AUC_{0-\infty}$: • $C_{0.5}$ = 0.020; p = 0.66 • C_1 = 0.143; p = 0.226 • C_2, C_3, C_4, C_6, C_8, C_{10} = 0.764 to 0.88; p < 0.05 • C_{12} = 0.895; p < 0.05	--	--	--	--
		First month Steady-state	D = 0.068±0.047 mg/kg BID			AUC_{0-12} = 202.6±61.3 CV = 30% AUC_{0-12}/D = 69.0±43.9 r^2 AUC_{0-12}: • C_0 = 0.841; p < 0.05 • C_1 = 0.469; p = 0.014 • $C_{0.5}$, C_2, C_3, C_{10}, C_{12} = 0.640 to 0.822; p < 0.05 • C_4, C_6, C_8 = 0.902 to 0.976; p < 0.05	--	--	--	--
[105]	23 patients 38 years (18-56)		D = 0.10 mg/kg/day D adjusted on C_0 = 10-20 µg/L	N/R	*MEIA – IMx*	Non-compartmental PK analysis					
		First dose				AUC_{0-12} = 116.3±58.9 Best r^2 C_4-AUC = 0.93	--	18.8±7.7	2.1±1.4	C_{12} = 5.8±2.7
		Day 3				AUC_{0-12} = 149.7±63.2 Best r^2 C_4-AUC = 0.92	--	23.7±10.4	2.2±1.3	C_{12} = 10.6±3.8
		Day 7				AUC_{0-12} = 216.6±89. 4 Best r^2 C_4-AUC = 0.95	--	32.4±11.5	1.9±0.8	C_{12} = 17.7±6.4
LUNG TRANSPLANTATION											
[117]	20 patients		D adjusted on C_0 = 7-12 µg/L	Patients converted	*N/R*	Non-compartmental PK analysis					
	8 CF	326 days	7.9±2.8 mg/day	from		--	10.3±2.6	--	--	--

		(120-689)	(0.16±0.06 mg/kg/day)	cyclospsorine to tacrolimus (rescue therapy)						
	12 non-CF	436 days (155-714)	6.4±3.7 mg/day (p = 0.14) 0.11±0.06 mg/kg/day (p = 0.08)			--	10.7±1.6 (ns)	--	--	--
[114]	16 adults (5 CF) 45.5±3.4 years	98±19 wks	D = 3.6±2.5 mg BID (0.25-7.0) D adjusted on C_0 = 5-15 µg/L	N/R	*HPLC-MS/MS*	Non-compartmental PK analysis (n = 22 profiles)				
						AUC_{0-8} = 115.8±45.2 (41.0-212.3)	10.5±5.7 (3.4-30.4)	21.6±11.8 (5.9-50.0)	2.3±1.3 (0.0-5.0)	C_2 = 19.1±11.7 (4.6-50.0)
						AUC_{0-4} = 67.9±31.1 (20.7-146.9)				
						r^2 C_2-AUC_{0-8} = 0.50; r^2 C_4-AUC_{0-8} = 0.53				
HEART AND LUNG TRANSPLANTATION										
[110]	22 adults		D adjusted on C_0 = 8-12 µg/L Steady-state	Low-dose corticoids	*MEIA – IMx*	Non-compartmental PK analysis – 3 full PK profiles/patient within 1 week; AUC trap; Median (range)				
						AUC/D: (h.µg/L)/mg tacrolimus	*C_0/D: (µg/L)/mg tacrolimus*	*C_{max}/D: (µg/L)/mg tacrolimus*		
	11 CF 30 years (21-43)	36.1 months	D = 12 mg/day (5-25)	AZA (n = 5) or MMF (n = 2)		AUC_{0-12} V1: 170 (140-260) V2: 180 (110-310) V3: 160 (100-270)	C_0 V1: 8.2 (6.0-14.0) V2: 9.2 (4.7-13.2) V3: 8.5 (5.2-13.2)	C_{max} V1: 23.4 (16.6-64.8) V2: 25.6 (15.6-65.7) V3: 23.4 (10.6-76.8)	t_{max} V1: 1.5 (0.5-6.0) V2: 1.5 (1.0-8.0) V3: 1.5 (0.5-5.0)	C_3 V1: 16.2 (11.4-30.5) V2: 21.6 (10.1-33.0) V3: 16.4 (6.5-26.0)
						AUC_{0-12}/D V1: 14 (8-34) V2: 17 (8-33) V3: 12 (9-32)	C_0/D V1: 0.7 (0.5-2.3) V2: 0.7 (0.4-1.7) V3: 0.7 (0.3-1.7)	C_{max}/D V1: 2.6 (1.2-4.7) V2: 3.2 (1.1-3.9) V3: 2.2 (1.2-3.4)		
						AUC_{0-4} V1: 80 (60-150) V2: 100 (50-170) V3: 70 (40-180)				C_3/D V1: 1.5 (0.7-3.3) V2: 1.7 (0.6-3.6) V3: 1.4 (0.9-3.3)
						AUC_{0-4}/D V1: 8 (4-6) V2: 10 (3-13) V3: 7 (4-14)				

						AUC/D smaller by about 50% in CF patients: AUC_{0-12}/D, $p < 0.004$; AUC_{0-4}/D, $p < 0.001$ r^2 AUC_{0-12}: • $C_{0.5} = 0.35$ • C_0, C_1, $C_{1.5} = 0.53$ to 0.58 • C_2, $C_{2.5}$, $C_4 = 0.71$ to 0.80 • $C_3 = 0.86$ r^2 AUC_{0-4}: • $C_0 = 0.34$ • $C_{0.5}$, $C_4 = 0.46$-0.53 • C_1, $C_{1.5}$, $C_3 = 0.79$ to 0.83 • C_2, $C_{2.5} = 0.89$-0.90				
	11 non-CF 51 years (31-56) $p < 0.01$	32.6 months $p = 0.97$	D = 6.1 mg/day (3-10) $p = 0.008$	AZA (n = 5)		AUC_{0-12} V1: 210 (120-270) V2: 180 (80-270) V3: 170 (80-240) AUC_{0-12}/D V1: 31 (17-71) V2: 33 (12-72) V3: 30 (12.69) AUC_{0-4} V1: 110 (60-140) V2: 80 (40-150) V3: 90 (40-130) AUC_{0-4}/D V1: 15 (6-43) V2: 15 (6-42) V3: 16 (6-39) r^2 AUC_{0-12}: • C_0, $C_{0.5} = 0.44$-0.58 • C_1, $C_{1.5}$, $C_2 = 0.72$ to 0.76 • $C_{2.5}$, $C_4 = 0.84$-0.87 • $C_3 = 0.92$	C_0 V1: 9.9 (6.4-14) V2: 10.9 (5.0-17) V3: 9.8 (5.4-13.7) C_0/D V1: 1.8 (1.0-2.4) V2: 1.9 (0.7-3.1) V3: 2.0 (0.8-3.4) r^2 AUC_{0-4}: • C_0, $C_{0.5} = 0.44$-0.48 • C_1, $C_{1.5}$, C_2, $C_{2.5}$, C_3, $C_4 = 0.83$ to 0.88	C_{max}: V1: 32.8 (15.5-57.8) V2: 29.1 (11.1-68.0) V3: 30.6 (11.6-43.5) C_{max}/D: V1: 5.5 (2.4-15.2) V2: 6.5 (1.4-14.1) V3: 5.6 (1.6-4.5)	t_{max} V1: 1.5 (1.0-4.0) V2: 1.0 (0.5-5.0) V3: 1.5 (1.0-3.0)	C_3 V1: 19.4 (12.5-25.0) V2: 16.3 (9.4-26.9) V3: 16.9 (11.0-26.4) C_3/D V1: 3.5 (1.9-8.3) V2: 3.6 (1.2-7.2) V3: 2.8 (1.6-6.3)
[112]	22 adults				*MEIA-IMx*	PK analysis using a one-compartment model with first-order elimination convoluted with a double gamma absorption phase – PK software Ciclo®2.3 3 full PK profiles/patient within 5 days (V1, V2, V3) *AUC/D: (h.µg/L)/mg tacrolimus*	*C_0/D: (µg/L)/mg tacrolimus*	*C_{mac}/D: (µg/L)/mg tacrolimus*		
	11 CF 30 years (21-43)	3-116 months	D = 0.23 mg/kg/day (0.09-0.47)	AZA (n = 5) or MMF (n = 2)		AUC_{0-12} V1: 182.3±48.2 V2: 187.4±66.0	C_0 V1: 9.05±2.29 V2: 8.91±3.20	C_{max} V1: 31.9±16.9 V2: 32.4±18.4	t_{max} V1: 1.5±0.6 V2 : 2.1±1.8	--

					V3: 169.7±60.5	V3: 8.52±3.10	V3: 26.2±17.7	V3: 1.7±1.2	
					AUC_{0-12}/D V1: 18.8±9.1 V2: 18.5±8.2 V3: 16.5±6.3	C_0/D V1 : 0.98±0.61 V2: 0.89±0.45 V3: 8.52±3.10	C_{max}/D V1: 3.0±1.3 V2: 2.9±1.2 V3: 2.2±0.7		
					AUC_{0-4} V1: 81.3±39.0 V2: 80.2±39.3 V3: 71.0±35.0				
					AUC_{0-4}/dose V1: 8.1±3.9 V2: 7.6±3.5 V3: 6.3±2.7				
					Bayesian estimator (AUC_{0-12}): best sampling times: C_0, $C_{1.5}$, C_4; $r^2 = 0.91$, mean bias -1.1% (-18.6;16.7%) BE vs Trap on AUC_{0-12}, AUC_{0-4}, C_{max}, t_{max}, C_0: bias% < 7.3%; RMSE% < 15%				
	11 non-CF 51 years (31-56) $p < 0.01$	9-86 months	D = 0.1 mg/kg/day (0.06-0.19)	AZA (n = 6)	AUC_{0-12} V1: 184.0±55.1 V2: 180.2±59.6 V3: 183.4±47.0	C_0 V1: 9.62±2.54 V2: 9.87±2.85 V3: 9.98±2.49	C_{max} V1: 33.1±15.0 V2: 31.1±17.4 V3: 30.1±10.8	t_{max} V1: 1.4±0.7 V2: 1.6±1.4 V3: 1.5±0.8	--
					AUC_{0-12}/D V1: 33.5±14.7 V2: 33.9±16.7 V3: 35.8±18.3	C_0/D V1: 1.71±0.49 V2: 1.82±0.72 V3: 1.90±0.81	C_{max}/Dose : V1: 5.9±3.4 V2: 6.0±3.8 V3: 5.7±3.5		
					AUC_{0-4} V1: 83.7±28.0 V2: 80.3±33.7 V3: 80.1±25.9				
					AUC_{0-4}/D V1: 15.3±7.8 V2: 15.1±8.5 V3: 15.9±9.3				
					Bayesian estimator (AUC_{0-12}): best sampling times: C_0, C_1, C_3; $r^2 = 0.96$, mean bias -3.6% (-17.4;6.8%) BE vs Trap on AUC_{0-12}, AUC_{0-4}, C_{max}, t_{max}, C_0: bias% < 4.6; RMSE% < 15.6				

Legend:

A_{IV}: intravenous coefficient – ATG: Anti-thymocyte globulin – AZA: Azathioprine – BE: Bayesian estimator – D: Dose – CF: Cystic fibrosis – MMF: Mycophenolate mofetil – NG: Nasogastric – N/R: not reported – Trap: Trapezes.

Table X. Tacrolimus pharmacokinetic parameters in cohort studies
Results are expressed as mean±SD (range), unless otherwise specified

Ref	Number of patients	Post-transplant period	Tacrolimus dose	Co-administered immunosuppres-sant	Assay	F, transfer k	V_d/F *(unless otherwise specified)*	CL/F *(unless otherwise specified)*	$T_{1/2}$ (h)
HEART TRANSPLANTATION									
[102]	14 adults 55.5 years (23-61)	< 6 months	First D = 0.03-0.40 mg/kg (median: 0.052 mg/kg)	ATG AZA Prednisone	*MEIA – IMx*	PK parameters after the first dose of tacrolimus *k_a, k_{el}: h^{-1}* *Lag-time: h*	*V_d/F: L/kg*	*CL/F: $L.h^{-1}$/kg*	
						Non-compartmental PK analysis using Pharm-NCA computer program			
						--	2.4±0.79	0.21±0.08	8.7±3.5 k_{el} = .091±0.040
						One-compartment open model with first-order elimination using P-Pharm computer program			
						k_a = 1.26±0.61 Lag-time = 0.22±0.13	2.0±0.4 (1.3-3.9) CV = 33%	0.23±0.08 (0.098-0.328) CV = 36.3%	6.5±2.4 k_{el} = 0.116±0.034
[100]	11 adults OHT		First D up to 0.15 mg/kg BID D adjusted on C_0 < 15 µg/L	N/R		Non-compartmental PK analysis using TOPFIT v2.0 software			
		Profile 1 (day 1) NG tube	D = 0.053±0.031 mg/kg (0.026-0.143) CV = 59%		*ELISA*	--	--	--	7.1±4.3 (2.4-15.9) CV = 60% – n = 7
					HPLC-MS/MS	--	--	--	4.0±1.0 (2.8-5.3) CV = 24% – n = 5
		Profile 2 (last day) Hard capsules	D = 0.076±0.069 mg/kg (0.013-0.250) CV = 91%		*ELISA*	--	--	--	14.1±6.3 (7.1-19.5) CV = 32% – n = 10
					HPLC-MS/MS	--	--	--	10.7±5.3 (1.8-18.5) CV = 50% – n = 10
[103]		Immediate post-transplantation period		N/R	HPLC-MS/MS	Non-compartmental PK analysis using PC Modfit v/6 software CL: L/h			

	10 adults 57±9 years		IV, continuous infusion D = 0.01 to 0.02 mg/kg/day	Induction: N/R		--	--	CL = 2.4	--
	25 adults 47±10 years		Oral	Induction: ATG/OKT3					After the first dose:
	n: N/R		D = 0.075 mg/kg/day			--	--	--	10.9±7.0
			D = 0.15 mg/kg/day			--	--	--	9.8±5.2
[115]	19 adults 46 years (28-48) OHT		D = 0.06 mg/kg/day (0.04-0.08) D adjusted on C_0 = 5-20 µg/L	ATG AZA Prednisone	*MEIA – IMx*	2-compartment open model – PK analysis using P-Pharm popPK software *k_a: h^{-1}* *Lag-time: h*	*V_1/F: L/kg*	*CL/F: $L.h^{-1}$/kg*	
		PK1 (day 10)				k_a = 1.1±0.5 (0.3-1.6) CV = 42% Lag-time = 0.22±0.09 (0.1-0.45) CV = 41%	V_1/F = 1.7±0.6 (0.7-3.5) CV = 36%	0.19±0.08 (0.09-0.38) CV = 42%	--
		PK2 (month 2)				k_a = 1.0±0.5 (0.1-1.8) CV = 48% Lag-time = 0.44±0.05 (0.29-0.50) – $p \leq 0.05$ CV = 11%	V_1/F = 1.1±0.4 (0.3-1.8) – $p < 0.01$ CV = 34%	0.23±0.15 (0.04-0.62) CV = 65%	--
[111]	8 adults 45-60 years	4 to 16 months (mean: 10.6)	D = 0.02-0.1 mg/kg/day D adjusted on C_0 = 5-12 µg/L	MMF Prednisone	*MEIA II – IMx*	Non-compartmental PK analysis using PK Calc software --	--	--	Mean = 13.8 (95% CI = 7.0-20.7)
[109]	22 adults 55±8 years (36-64) OHT	First year	D = 0.3 mg/kg/day D adjusted on C_0 = 10-20 µg/L Steady-state	MMF or AZA Steroids	*MEIA – IMx*	Non-compartmental PK analysis using MOMENT software (n = 25 PK profiles) CL/F: L/h --	--	11.6±5.5 (3.8-23.5)	--
[105]	23 adults 38 years (18-56)	First dose	D = 0.10 mg/kgd D adjusted on C_0 = 10-20 µg/L	N/R	*MEIA – IMx*	Non-compartmental PK analysis --	--	--	11.1±6.3
		Day 3				--	--	--	10.6±5.8

		Day 7				--	--	--	9.8±5.5
LUNG TRANSPLANTATION									
[114]	16 adults (5 CF) 45.5±3.4 years	98±19 wks	D = 3.6±2.5 mg BID (0.25-7.0) D adjusted on C_0 = 5-15 µg/L	N/R	*HPLC-MS/MS*	Non-compartmental PK analysis (n = 22 profiles) --	--	--	7.9±2.3 (4.0-12.0)
HEART-LUNG TRANSPLANTATION									
[112]	22 adults				*MEIA – IMx*	One-compartment model with first-order elimination convoluted with a double gamma absorption phase – PK analysis using Ciclo®2.3 software *MAT, SDAT: h* *A_{IV}: L^{-1}*	*V_1/F: L*	*CL/F: L/h; λ: h^{-1}*	
	11 CF 30 years (21-43)	3-116 months	D = 0.23 mg/kg/day (0.09-0.47)			MAT_1 = 1.10±0.68 $SDAT_1$ = 0.27±0.15 MAT_2 = 5.14±2.11 $SDAT_2$ = 1.96±0.68 A_{IV} = 4.03±1.67 r = 0.58±0.22	V_1/F = 2,011±1,740	68.22±29.80	λ = 0.64±0.33
	11 non-CF 51 years (31-56) p < 0.01	9-86 months	D = 0.1 mg/kg/day (0.06-0.19)	AZA (n = 6)		MAT_1 = 0.92±0.43 (ns) $SDAT_1$ = 0.30±0.10 (ns) MAT_2 = 5.47±2.30 (ns) $SDAT_2$ = 1.89±0.71 (ns) A_{IV} = 9.36±4.98 (p < 0.01) r = 0.62±0.12 (ns)	V_1/F = 444±326 (p < 0.01)	36.49±18.98 (p < 0.05)	λ = 0.80±0.31 (p < 0.01)

Legend
ATG: Antithymocyte globulin – AZA: Azathioprine – CF: Cystic fibrosis – D: Dose – NG: Nasogastric – N/R: not reported – OHT: orthotopic heart transplantation – r: fraction of the dose absorbed by the faster phase.

Table XI. Mycophenolate exposure indices in cohort studies
Results are expressed as mean±SD unless otherwise specified

Ref	Number of patients	Post-transplant period	MMF dose	Co-administered immunosuppressant	*Analytical method*, molecule	Measured AUC (h.mg/L)	C_0 (mg/L)	C_{max} (mg/L)	t_{max} (h)	Others
HEART TRANSPLANTATION										
[122]	9 patients 55±11 years (33-71)	First 10 days		Cyclosporine (same dose throughout the period)	*HPLC-UV*	Non-compartmental PK analysis; AUC Trap; data are presented as median (range)				
		Day 3	IV, D = 1.5 g BID Infusion over 3 hours (day 0 – day 5)	Prednisolone		AUC_{0-6}				
					MMF	7.5 (4.2-37.2)	--	4.7 (2.2-18.4)	2.0 (1.0-3.0)	--
						AUC_{0-12}				
					MPA	34.2 (22.3-52.1)	0.65 (0.24-1.35)	10.2 (7.6-16.7)	2.0 (1.0-3.0)	
					MPAG	1,030 (537-2,049)	69 (34-153)	110 (65-187)	3.0 (2.5-3.5)	
					AcMPAG	8.5 (5.6-11.9)	0.36 (0.09-0.69)	1.31 (0.99-1.68)	1.3 (1.0-1.7)	
		Day 5				AUC_{0-6}				
					MMF	6.5 (5.0-26.3)	--	3.7 (2.3-10.3)	2.0 (1.0-2.0)	--
						AUC_{0-12}				
					MPA	33.8 (23.7-43.1)	0.96 (0.26-1.11)	9.8 (6.1-11.2)	2.0 (2.0-3.0)	
					MPAG	881 (456-1,584)	63 (34-135)	109 (51-166)	3.2 (3.0-4.0)	
					AcMPAG	5.4 (1.8-9.3)	0.25 (<0.05-0.59)	0.86 (0.38-1.22)	3.0 (3.0-4.0)	
		Day 6	Oral, D = 1.5 g BID (day 5 – day 10)			AUC_{0-12}				
					MPA	29.7 (22.3-37.6)	0.56 (0.24-1.12)	6.0 (4.4-10.5)	2.0 (0.33-6.0)	--
					MPAG	959 (471-1,216)	56 (26-82)	96 (51-111)	4.0 (0.67-8.0)	
					AcMPAG	5.1 (3.2-5.9)	0.14 (<0.05-0.4)	0.77 (0.48-1.30)	2.0 (0.67-6.0)	
		Day 10				AUC_{0-12}				
					MPA	33.8 (26.6-40.3)	0.95 (0.48-1.39)	9.0 (3.6-10.8)	1.25 (0.67-1.25)	--
					MPAG	1,032 (573-1,498)	79 (28-122)	108 (59-179)	2.5 (1.25-4.0)	
					AcMPAG	6.4 (4.5-10.7)	0.32 (0.19-0.85)	0.90 (0.44-1.36)	2.0 (1.25-4.0)	
[121]	9 patients Mean: 59 years	Weeks 1, 2, 4, 8, 12	D = 1 g or 1.5 g BID	Cyclosporine Prednisone	*HPLC-UV*	Non-compartmental PK analysis (n = 44 full MPA profiles); AUC_{0-12} Trap				
						45.9±15.4 (13.4-91.7)	--	10.4±6.6	1.25	--

						r^2 C_2-AUC_{0-12} = 0.610; r^2 C_{12}-AUC_{0-12} = 0.003 Recommended sparse sampling algorithms: • AUC_{0-12} = 5.568 + 0.902*$C_{1.25}$ + 2.022*C_2 + 4.594*C_6; r^2 = 0.926 • AUC_{0-12} = 3.800 + 1.015*$C_{1.25}$ + 1.819*C_2 + 1.566*C_4 + 3.479*C_6; r^2 = 0.948				
[137]	14 patients 57±13 years	54±42 months	D = 35±7 mg/kg/day	Cyclosporine	*N/R*	Method for PK analysis: N/R				
					MPA	AUC_{0-12} = 63±11	--	--	1-2	C_{av} = 5.2±1.0
						r^2 AUC_{0-12} • C_0 = 0.48 • C_1, C_2 = 0.08-0.09 • C_3, C_4, C_6, C_8, C_{12} = 0.23 to 0.60				
[132]	38 patients OHT 53±10 years	310±278 days	Steady-state D = 1.1±0.4 g BID (13.6±4.9 mg/kg BID)	No induction Cyclosporine Prednisone	*HPLC-UV*	MPA AUC_{0-12} calculated from a 2-hour abbreviated AUC developed in renal transplant patients[216]				
					MPA Free MPA	AUC_{0-12} 44.5±16.1 0.83±0.30	1.2±0.6	--	--	f = 1.9±0.4%
						r^2 MPA C_0-AUC_{0-12} = 0.40 (p = 0.01) r^2 MPA C_0-MPA free fraction : ns				
[134]	62 patients Mean: 59 years	Stable, > 1 year	Steady-state		*EMIT*	Non-compartmental PK analysis; AUC_{0-12} estimated from C_0, C_{30}, C_{120} using a sparse sampling algorithm developed in renal transplant patients[120]				
						AUC/D: (h.mg/L)/g MMF	*C_0/D: (mg/L)/g MMF*	*C_{max}/D: (mg/L)/g MMF*		
	n = 47 57±9 years	3.6±4.0 years	D = 2.9±0.8 g/day D ≥ 3 g/d, n = 32	Cyclopsorine Corticosteroids (45% patients)	MPA	AUC_{0-12} 41.9±14.1 (19.7-81.8) < 40 in 50% pts with dose ≥ 3 g/day AUC_{0-12}/D = 31.9±16.1 (13.4-82.3)	C_0/D = 1.41±0.95	C_{max}/D = 18.9	Med.: 40 min Mean: 75 min	--
						r^2 AUC_{0-12} • C_0 = 0.36, p < 0.01 • C_{40}, C_{75}, C_{120} = 0.62-0.64, p < 0.01				

	n = 15 64±10 years	8.5±3.6 years ($p < 0.01$)	D = 1.9±0.7 g/day $p < 0.001$ D = 1-3 g/day, n = 15	Sirolimus Corticosteroids (13% patients; $p < 0.01$)	MPA	AUC_{0-12} 51.1±15.8 (34.4-87.6); $p < 0.03$ < 40 in 27% pts with dose = 1-3 g/day AUC_{0-12}/D = 61.0±27.4 (23.7-131.5); $p < 0.001$ r^2 AUC_{0-12} • C_0, C_{75}, C_{120} = 0.56-0.61, $p < 0.01$ • C_{40} = 0.82, $p < 0.01$	C_0/D = 5.10±3.41 $p < 0.001$	C_{max}/D = 21.8 (ns)	Med.: 40 min Mean: 75 min	--
	56 years	3.4 years	Mean D Before the switch: 2.9±1.0 g/day	Switch group (cyclosporine to sirolimus), n = 9	MPA	AUC_{0-12} 49.9±12.4 (34.3-56.9) AUC_{0-12}/D = 37.5±19.9 (20.6-73.6)	C_0/D = 4.85±4.39 (0.44-5.20)	--	--	--
			After the switch: 2.0±0.7 g/day ($p < 0.01$)			37.5±20.0 (34.6-70.1) ($p < 0.001$) AUC_{0-12}/D = 58.3±32.5 (27.7-131.5); $p < 0.001$	C_0/D = 2.00±1.68 (0.33-14.6) $p < 0.001$	--	--	--
[136]	26 patients 15±10 years (1 month – 33 years)	50% < 1 year 50% > 1 year	Steady-state D = 37.9±12.5 mg/kg = 1.207±0.302 g/m^2	Cyclosporine or tacrolimus Corticosteroids	*HPLC* MPA	Non-compartmental PK analysis; 120 samples --	2.2±2.0 $C_0 \geq 1$ mg/L in 50% of patients	--	--	--
					MPAG	--	48±4	--	--	--
	16 children		1.1±0.3 g/m^2/day	Cyclosporine, n = 8 40 samples	MPA	--	1.6±1.5	--	--	--
					MPAG	--	49±38	--	--	--
					MPAG/MPA	--	25.2±21.7	--	--	--

			D: N/R	Tacrolimus, n = 8 42 samples	MPA	--	3.0±2.2 (vs children on CsA, p = 0.04)	--	--	--
					MPAG	--	36±17 (vs children on CsA, p = 0.04)	--	--	--
	10 adults		1.3±0.4 g/m^2/day (vs. children on cyclosporine, ns)	Cyclosporine, n = 10 37 samples	MPA	--	2.3 ±2.2 (vs children on CsA, ns)	--	--	--
					MPAG	--	98±47 (vs children on CsA, p < 0.0001)	--	--	--
					MPAG/MPA	--	37.7±40.2 (vs children on CsA, p = 0.016)	--	--	--
[143]	7 patients 21-63 years	6-23 days	Steady-state D = 1 or 1.5 g BID	Cyclosporine Corticosteroids	*HPLC-UV*	Non-compartmental PK analysis --	--	--	--	fMPA = 3.6±3.9% fMPA – total MPA r^2 = 0.20 fMPAG = 26±8% fMPAG – total MPAG r^2 = 0.87 fMPA – fMPAG : r^2 = 0.83
[135]	23 adults n = 14 57±13 years	Stable, > 1 year	Steady-state 34.6±6.0 mg/kg/day	Group 1 Cyclosporine	*EMIT*	Non-compartmental analysis – AUC_{0-12} Trap 62.8±11.5	2.9±1.3	--	1-2	--

						r^2 AUC_{0-12} • C_{30}, C_1, C_2 = 0.06-0.09 • C_0, C_3, C_4, C_8, C_{12} = 0.23-0.69 • C_6 = 0.60				
	n = 9 54±12 years		24.3±11.6 mg/kg/day	Group 2 Tacrolimus		55±24	2.4±1.2	--	30 min-1 h	--
						r^2 AUC_{0-12} • C_{30} = 0.04 • C_0, C_1, C_2, C_3, C_{12} = 0.38-0.72 • C_4, C_6, C_8 = 0.85-0.93				
[167]	28 patients 54 years (19-67)	Stable, > 1 year 2.5±3 years	1.5 g/day (0.5-4.0) D adjusted on C_0 = 1.5-4 mg/L	Tacrolimus	*HPLC-UV*	Non-compartmental PK analysis –AUC_{0-12} Trap				
						42.0±18.3 (12.2-79.8)	2.0±1.2	--	--	C_{12h} = 2.0±1.7
						r^2 AUC_{0-12} • C_0, C_3 = 0.14-0.17 • C_2, C_{10}, C_{12} = 0.25-0.39 • $C_{0.5}$, C_1, C_6, C_8 = 0.48-0.57 Recommended sparse sampling algorithms: • $AUC_{0-12} = 1.11*C_{0.5} + 1.16*C_1 + 3.72*C_2$; $r^2 = 0.84$ • $AUC_{0-12} = 3.37*C_0 + 0.97*C_{0.5} + 1.20*C_1 + 2.70*C_2$; $r^2 = 0.87$				
HEART TRANSPLANT RECIPIENTS + LUNG TRANSPLANT RECPIENTS										
[124]	9 patients (5 HTx, 4 LTx) 53±11 years		Steady-state 2-3 g/day	ATG in HTx patients Cyclosporine (n = 7) or tacrolimus (n = 2) Prednisone	*HPLC-UV* Total and unbound MPA	Non-compartmental PK analysis using WinNonlin in the 7 patients on cyclosporine (4 HTx, 3 LTx) $AUC_{0-\tau}$, τ = N/R				
		Period 1 15±13 days (n = 10)	2.4±0.5 g/day			AUC = 30.1±18.2 DN-AUC = 26.0±15.0 fAUC = 2.22±2.32	3.79±4.04	C_{max} = 9.34±4.24 DN-C_{max} = 8.00±4.15	4.0±4.2	C_{min} = 0.33±0.58 f = 6.1±2.8 %
		Period 2 56±33 days (n = 46)	2.4±0.5 g/day			AUC = 19.8±11.0 DN-AUC = 18.2±12.0 fAUC = 1.01±0.81	2.21±2.63	C_{max} = 8.30±4.19 DN-C_{max} = 7.20±3.81	0.7±0.4	C_{min} = 0.28±0.50 f = 4.3±2.4 %

		Period 3 125±73 days (n = 114)	2.2±0.4 g/day			AUC = 36.8±29.7 DN-AUC = 32.5±23.7 fAUC = 1.50±2.16	2.20±2.29	C_{max} = 10.32±7.86 DN-C_{max} = 9.49±7.78	1.7±1.3	C_{min} = 1.21±1.78 f = 4.4±3.0 %
[130]	50 patients 56.5 years (20.7-77.6)		Steady-state		*HPLC-UV*	Non-compartmental PK analysis using WinNonlin				
	27 LTx (6 CF) 49.9 years (20.7-70.5)	2.1 years (0.2–14.0)	1.5 g BID (0.50–1.50) 0.034 g/kg/day (0.013–0.054)	Prednisone (n = 27) Cyclosporine (n = 11)		DN-AUC_{0-12}		DN-C_{max}		MPA DN-C_{min}
					MPA	18.6 (3.4-35.1)	--	5.66 (0.64-15.53)	1.0 (0.3-6.0)	0.44 (ND-1.05)
					MPAG	387 (152-909)	--	--	--	
					AcMPAG	8.7 (ND-147)	--	--	--	fMPA: N/R
					MPA	DN-AUC_{0-6}				
					MPAG	14.3 (2.5-28.2)				
					AcMPAG	226 (69-495)				
						5.8 (ND-76)				
					MPA	DN-AUC_{6-12}				
					MPAG	6.5 (0.9-16.8)				
					AcMPAG	162 (61-427)				
						3.0 (ND-70)				
					MPA	AUC_{6-12}/AUC_{0-12} ratio 0.27 (0.12-0.55) fAUC: N/R				
						MPAG/MPA = 28.5 (9.8-55.2) AcMPAG/MPA = 0.4 (ND-12.3)				
				Tacrolimus (n = 16)		DN-AUC_{0-12}		DN-C_{max}		MPA DN-C_{min}
					MPA	41.4 (8.3-115.31) (p = 0.022)	--	8.24 (1.81-37.11)	1.1 (0.3-10.0)	1.13 (0.17-3.64) (p = 0.002)
					MPAG	471 (72-928)	--	--	--	
					AcMPAG	12.7 (0.7-160)	--	--	--	fMPA = 1.9% (0.7-3.8)

					DN-AUC_{0-6}				
				MPA	25.4 (5.5-96.1)				
					(p = 0.046)				
				MPAG	257 (43-603)				
				AcMPAG	4.6 (0.5-101)				
					DN-AUC_{6-12}				
				MPA	14.8 (2.8-26.4)				
					(p = 0.007)				
				MPAG	166 (29-389)				
				AcMPAG	7.40 (ND-59)				
					AUC_{6-12}/AUC_{0-12} ratio				
				MPA	0.33 (0.17-0.56)				
					fAUC = 1.73				
					(0.51-2.43)				
					MPAG/MPA = 12.6				
					(2.4-25.8)				
					(p = 0.002)				
					AcMPAGMPA = 0.3				
					(0.05-2.4)				
21 HTx	4.0 years	0.75 g BID (0.25–1.50)	Prednisone (n = 1)						
(and heart +	(0.4–19.7)	(p < 0.05, lung vs heart)	Cyclosporine (n = 14)		DN-AUC_{0-12}		DN-C_{max}		MPA DN-C_{min}
kidney)				MPA	50.9 (16.9-218.7)	--	16.8 (3.6–47.3)	1.5 (0.3–12.0)	1.45 (0.34-3.02)
59.8 years		0.019 g/kg/day		MPAG	219 (50–1869)	--	--	--	
(23.3-77.6)		(0.008–0.038)		AcMPAG	17.9 (2.2-178)	--	--	--	fMPA = 2.2%
(p < 0.05)		(p < 10^{-4}, lung vs heart)							(0.2-15)
					DN-AUC_{0-6}				
				MPA	34.9 (10.7-134.0)				
				MPAG	408 (35-1142)				
				AcMPAG	7.7 (1.2-101)				
					DN-AUC_{6-12}				
				MPA	18.9 (5.3-102.2)				
				MPAG	287 (14-726)				
				AcMPAG	6.6 (0.8-77)				
					AUC_{6-12}/AUC_{0-12} ratio				
				MPA	0.33 (0.16-0.49)				
					fAUC =1.38				
					(0.05-12.26)				

						MPAG/MPA = 11.9 (0.9-28.6) AcMPAG/MPA = 0.2 (0.07-2.03)				
				Tacrolimus (n = 9)		DN-AUC_{0-12}		DN-C_{max}		MPA DN-C_{min}
					MPA	106.8 (40.9-180.5)	--	21.8 (9.9-44.5)	1.0 (0.4–10.0)	3.28 (1.29-8.40)
					MPAG	717 (113-1723)	--	--	--	(p = 0.009)
					AcMPAG	20.1 (2.6-109)	--	--	--	
										fMPA: 3.5% (0.5-14)
						DN-AUC_{0-6}				
					MPA	58.1 (23.9-90.1)				
					MPAG	419 (40-940)				
					AcMPAG	13.4 (1.7-80.3)				
						DN-AUC_{6-12}				
					MPA	29.2 (9.9-89.8)				
					MPAG	298 (60–829)				
					AcMPAG	9.4 (0.9-62.6)				
						AUC_{6-12}/AUC_{0-12} ratio				
					MPA	0.42 (0.23-0.61)				
						fAUC = 3.54 (0.85-18.89)				
						MPAG/MPA = 8.7 (2.3-13.4) AcMPAG/MPA = 0.3 (0.06-0.87)				
LUNG TRANSPLANTATION										
[119]	7 patients 50±10 years	4.4±3.9 years (0.3-11.5)	Steady-state, 1-3 g/day 2.4±0.9 g/day (35.5±14.1 mg/kg/day)	Cyclosporine Prednisone	*HPLC-UV*	Non-compartmental analysis using WinNonlin $AUC_{0-\tau}$, τ = N/R				
					Total Unbound	AUC = 45.8±18.4 DN-AUC = 23.6±15.8 fAUC = 1.29±0.50	--	C_{max} = 17.37±7.69 DN-C_{max} = 9.30±9.66	1.2±0.4	C_{min} = 3.12±1.41 f = 2.9±0.6%
[138]	19 adults (4 CF) 48±15 years	Stable 4.2±3.7 years	Steady-state 2.5±0.5 g/day	Prednisone Cyclosporine (n = 9) or tacrolimus (n = 10)	*HPLC-UV*	Non-compartmental analysis using WinNonlin 29.4±17.9	--	--	--	--

						r^2 C_0-$AUC0_{-12}$ = 0.714; r^2 C_2-AUC_{0-12} = 0.663; r^2 C_8-AUC_{0-12} = 0.884 ; otherwise, r^2 = 0.176-0.732 Recommended sparse sampling algorithms: • $\log(AUC_{0-12}) = 1.140 + 0.241*\log(C_0) + 0.406*\log(C_2)$; r^2 = 0.828, ME = -5.8%, RMSE = 6.0% • $\log(AUC_{0-12}) = 1.09 + 0.202*\log(C_0) + 0.411*\log(C_{1.5})$; r^2 = 0.791, ME = -5.7%, RMSE = 7.0%

Legend
D: Dose – DN: Normalized to a 1000 mg MMF dose – f: Free fraction – HTx: Heart transplantation – LTx: Lung transplantation – N/R: not reported – OHT: Orthotopic heart transplantation.

Table XII. Mycophenolate pharmacokinetic parameters in cohort studies
Results are expressed as mean±SD, unless otherwise specified

Ref	Number of patients	Post-transplant period	MMF dose	Co-administered immunosuppressant	*Analytical method*, molecule	F, transfer k	V_d/F *(unless otherwise specified)*	CL/F *(unless otherwise specified)*	$T_{1/2}$ (h)
HEART TRANSPLANTATION									
[122]	9 patients 55±11 years (33-71)	First 10 days		Cyclosporine (same dose throughout the period)	*HPLC-UV* MPA	Non-compartmental analysis – Data presented as medians (range) IV/PO AUC and C_{max} ratios were calculated using log-transformed data and are presented as mean [90%CI]			
		Day 3	IV (infusion over 3 hours) D = 1.5 g BID (days 0–5)	Prednisolone		--	--	--	--
		Day 5				--	--	--	--
		Day 6	Oral D = 1.5 g BID (days 5 – 10)			Day 6/Day 3: F = 82.9% [71.4-96.3] C_{max} PO/IV = 63% [48-82] Day 6/Day 5: F = 91.6% [79-106] C_{max} PO/IV = 74% [57-95]	--	--	--
		Day 10				Day 10/Day 3: F = 97.5% [83-114] C_{max} PO/IV = 77% [60-99] Day 10/Day 5: F = 108% [93-125] C_{max} PO/IV = 90% [71-115]	--	--	--
[137]	14 patients (57±13 yrs)	54±42 months	D = 35±7 mg/kg/day	Cyclosporine	N/R	Method for PK analysis: N/R			
						--	--	23.3±6.5 L/min	--
HEART TRANSPLANT RECIPIENTS + LUNG TRANSPLANT RECPIENTS									

[130]	50 patients 56.5 years (20.7–77.6)	2.7 years (0.2–19.7)	D = 1 g BID (0.25–1.50) 0.028 g/kg/d (0.008–0.054)		*HPLC-UV*	Non-compartmental PK analysis using WinNonlin			
			Steady-state		MPA MPAG AcMPAG		V_d/F: L	CL/F: L	
	LTx (n = 27) (6 CF) 49.9 years (20.7–70.5)	2.1 years (0.2–14.0)	1.5 g BID = (0.50–1.50) 0.034 g/kg/d (0.013–0.054)	Prednisone (27) Cyclosporine (11)		--	248.1 (54.1-644.6)	53.9 (28.5-294.7)	--
				Tacrolimus (16)		--	124.8 (29.8-607.1)	20.8 (8.7-120.5) (p = 0.026)	--
	HTx (n = 23) (5 HTx + KTx) 59.8 years (23.3–77.6) (p < 0.05)	4.0 years (0.4–19.7)	D = 0.75 g BID (0.25–1.50) (p < 0.05)	Prednisone (1) Cyclosporine (14)		--	101.5 (36.6-1141.1)	21.2 (4.6-59.2)	--
			0.019 g/kg/d (0.008–0.038) ($p < 10^{-4}$)	Tacrolimus (9)		--	111.0 (53.2-261.3)	11.0 (5.5-24.4)	--

Legend
CF: Cystic fibrosis – D: Dose – HTx: Heart transplantation – KTx: Kidney transplantation – LTx: Lung transplantation – N/R: not reported.

Table XIII. MMF exposure – effect studies in heart transplantation
Results are expressed as mean±SD, unless otherwise specified

Ref	Immunosuppressants	Number of patients	Sampling periods	Exposure (*analytical method*)	Event (Number of episodes)	Comments
[132]	MMF, steady-state 1.1±0.4 g BID (13.6±4.9 mg/kg BID)	38 patients OHT 53±10 years	310±278 days	MPA AUC, fAUC, C_0 *(HPLC-UV)* MPA AUC_{0-12} calculated from a 2-hour abbreviated AUC developed in renal transplant patients[216] AUC 42.8±14 fAUC 0.81±0.25 C_0 1.20±0.58 AUC 51.7±17.5 fAUC 0.95±0.34 C_0 1.24±0.72 AUC 26.1±6.6 ($p < 0.05$ vs grade 0 and 1) fAUC 0.49±011 ($p < 0.05$, vs grades 0 and 1) C_0 0.65±0.15 (ns vs grades 0 and 1)	3 groups (ISHLT classification) Grade 0 n = 22 patients Grade 1 n = 13 patients Grade 2/3 n = 3 patients	No significant differences in cyclosporine exposure Prednisone dose varying among the patients Small sample size (3 patients in the grade 2/3 rejection group) Patients sampled at varying times throughout their post-transplantation course
[163]	MMF, steady-state Increased to the maximal tolerated dose D = 2.1±0.9 g/day (1.0-4.0) Corticosteroids (50%) Cyclosporine (42%) MMF D = 2.4±0.9 g/day Tacrolimus (58%) MMF D = 1.8±0.8 g/day ($p = 0.01$)	26 patients 54±14 years (22-72)	3.0±1.7 years (1.0-7.8)	MPA and MPAG C_0 *(HPLC-UV)* "Therapeutic" C_0 levels: • MPA > 2 mg/L • Cyclosporine ≥ 175 µg/L • Tacrolimus ≥ 10 µg/L n = 19 samples MPA C_0 = 1.65±0.97 MPAG C_0 = 128.9±55.6 n = 29 samples MPA C_0 = 2.86±2.07 ($p = 0.02$) MPAG C_0 = 101.1±84.9 (ns)	48 routine EMB at time of blood sampling (ISHLT) Grade 0, 46%; grade 1A/1B, 33%; grade 2, 8%; grade 3A, 4% MPA C_0 vs overall incidence of rejection: ns Overall rejection ≈ 20% Cyclosporine C_0 < 175 µg/L in 100% of patients Overall rejection ≈ 60% (significantly higher, p: N/R) Tacrolimus C_0 < 10 µg/L in 52% of patients	
[136]	MMF, steady-state D = 37.9±12.5 mg/kg = 1.207±0.302 g/m^2 Cyclosporine or tacrolimus Corticosteroids	26 patients (16 children, 10 adults) 15±10 years (1 month – 33 years)	50% < 1 year 50% > 1 year	MPA and MPAG C_0 *(HPLC)* MPA C_0 = 2.5±2.3 MPA C_0 = 1.2±0.9 ($p = 0.02$)	78 EMB (ISHLT classification) Grade < 2 (n = N/R) Grade ≥ 2, (n = N/R) More frequent with MPA C_0 ≥ 2.5 mg/L ($p = 0.03$)	

[170]	MMF, 1.5 g BID (decreased based on clinical symptoms of toxicity) Cyclosporine (possibly converted to tacrolimus) Prednisone	20 patients	< 1 year (mean: 10.1 months)	MPA C_0 *(EMIT)* Median: 1.51 (DF50 = 0.96-2.23) Difference in cyclosporine and MPA C_0 between categories of biopsies: ns Median MPA C_0: 1.76 (range: 0.496-7.65) Median MPA C_0: 1.36 (range: 0.26-6.13); p = 0.015	147 EMB (ISHLT) Grade 0, n = 54; grade 1A, n = 61; grade 2, n = 16, grade 3A, n = 16 Patients without AR, n = 9 Patients with AR, n = 11	
[139]	MMF, 2 g/day D adjusted on C_0: 2-4 mg/L Cyclosporine (89%) or tacrolimus (11%) D adjusted on C_0 Prednisone (all)	215 patients 36±14 yrs		MPA C_0 *(EMIT)*; 892 plasma samples (3 groups based on C_0: < 2, 2-4, > 4, or 2 groups based on $C_0 < 2$ and $C_0 \geq 2$, to compare the incidence of rejection)	Scheduled EMB Acute rejection if grade ≥ 3A (ISHLT)	50 patients had samples taken on more than one occasion
	Rejectors: 2.8±0.8 g/day Non rejectors: 2.8±0.7 g/day (ns)	Period I n = 104	< 6 months	No difference in mean C_0 between patients with (C_0 = 2.2±2.0 mg/L) and without (C_0 = 2.3±1.7 mg/L) AR Higher proportion of $C_0 < 2$ in rejectors (n = 34/54) vs non rejectors (n = 194/401), p = 0.05	No difference in AR incidence between the 3 C_0 groups (p = 0.1): 7.8 to 14.9%	
				Difference in cyclosporine or tacrolimus C_0 between MPA C_0 groups: ns Mean daily MMF dose to reach MPA $C_0 > 2$, cyclosporine group vs tacrolimus group: ns • When MPA $C_0 < 2$, no difference on AR incidence in relation to therapeutic or subtherapeutic cyclosporine or tacrolimus C_0 (14.4 vs 13.9%, ns) • When MPA $C_0 > 2$, AR incidence is significantly reduced if cyclosporine or tacrolimus C_0 are therapeutic vs subtherapeutic (3.6 vs 15.4%, p = 0.002)		
	Rejectors: 2.5±1.0 g/day Non rejectors: 2.6±0.7 g/day (ns)	Period II n = 90	6-12 months	No difference in mean C_0 between patients with (C_0 = 2.0±1.5 mg/L) and without (C_0 = 2.7±2.0 mg/L) AR Higher proportion of $C_0 < 2$ in rejectors (n = 9/14) vs non rejectors (n = 71/188), p = 0.05	No difference in AR incidence between the 3 C_0 groups (p = 0.15): 4.0 to 11.3%	
	2.3±0.8 g/day 2.3±0.8 g/day (ns)	Period III n = 71	> 12 months	No difference in mean C_0 between patients with (C_0 = 2.4±2.0 mg/L) and without (C_0 = 2.6±2.2 mg/L) AR Comparable proportion of $C_0 < 2$ in rejectors (n =	No difference in AR incidence between the 3 C_0 groups (p = 0.13): 2.6 to 15.1%	

				13/27) vs non rejectors (n = 98/208), p = 0.92		
[129]	MMF Tacrolimus Prednisone (weaning at 6 months)	45 adults Primary OHT		MPA C_0 *(EMIT)* Tacrolimus C_0 *(IMx)*	EFFICACY Patient survival EMB (ARE, ISHLT classification)	TOXICITY GI toxicity Infections
	MMF D = 2 g/d (fixed) Tacrolimus D adjusted on C_0	Phase I n = 15 51±11 years	696±62 days (606-790)	Mean MPA levels (Months 0-6) 3.6±0.4 2.2±0.4 1.4±0.2	Patient survival: 100% Tacrolimus C_0, patients with vs without AR: ns 0 ARE/patient, n = 5 1-2 ARE/patient n = 7 patients 3 ARE/patient, n = 3	GI toxicity: n = 6 (40%) Infections: Bacterial, n = 10 (66.7%) Fungal, n = 8 (53.3%) Viral, n = 9 (60.0%)
	MMF and tacrolimus D adjusted on C_0	Phase II n = 30 54±9 yeras	436±88 days (175-562)	MPA C_0 target: 2.5-4.5	27 patients: rejection-free 3 patients with AR had confounding factors	GI toxicity: n = 9 (30%) Infections: Bacterial, n = 17 (56.7%) Fungal, n = 5 (16.7%) Viral, n = 7 (23.3%)

Legend:
AR: Acute rejection – ARE: Acute rejection episode – CF: Cystic fibrosis – D: Dose – f: Free fraction – EMB: Endomyocardial biopsy – GI: Gastro-intestinal – N/R: not reported.

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