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**This is a pre print version of the following article:**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1562237> since 2016-05-23T18:11:14Z

*Published version:*

DOI:10.1093/ndt/gfv437

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(Article begins on next page)

# The DESCARTES-Nantes survey of kidney transplant recipients displaying clinical operational tolerance identifies 35 new tolerant patients and 34 almost tolerant patients

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## ABSTRACT

**Background.** Kidney recipients maintaining a prolonged allograft survival in the absence of immunosuppressive drugs and without evidence of rejection are supposed to be exceptional. The ERAEDTA-DESCARTES working group together with Nantes University launched a European-wide survey to identify new patients, describe them and estimate their frequency for the first time.

**Methods.** Seventeen coordinators distributed a questionnaire in 256 transplant centres and 28 countries in order to report as many 'operationally tolerant' patients (TOL; defined as having a serum creatinine <1.7 mg/dL and proteinuria <1 g/day or g/g creatinine despite at least 1 year without any immunosuppressive drug) and 'almost tolerant' patients (minimally immunosuppressed patients (MIS) receiving low-dose steroids) as possible. We reported their number and the total number of kidney transplants performed at each centre to calculate their frequency.

**Results.** One hundred and forty-seven questionnaires were returned and we identified 66 TOL (61 with complete data) and 34 MIS patients. Of the 61 TOL patients, 26 were previously described by the Nantes group and 35 new patients are presented here. Most of them were noncompliant patients. At data collection, 31/35 patients were alive and 22/31 still operationally tolerant. For the remaining 9/31, 2 were restarted on immunosuppressive drugs and 7 had rising creatinine of whom 3 resumed dialysis. Considering all patients, 10-year death-censored graft survival post-immunosuppression weaning reached 85% in TOL patients and

100% in MIS patients. With 218 913 kidney recipients surveyed, cumulative incidences of operational tolerance and almost tolerance were estimated at 3 and 1.5 per 10 000 kidney recipients, respectively.

Conclusions. In kidney transplantation, operational tolerance and almost tolerance are infrequent findings associated with excellent long-term death-censored graft survival.

Keywords: frequency, graft survival, kidney transplantation, minimally immunosuppressed patients, operational tolerance

## INTRODUCTION

The natural history of an untreated allograft in humans is graft rejection. Immunosuppressive drugs made organ transplantation possible but even the latest generation of these drugs carry the risk of major infectious, malignant or metabolic complications. Together with acute and chronic rejections, immunosuppression side effects heavily affect the long-term survival of both allografts and patients.

The induction of a tolerant state, intended as a selective acceptance of the allograft by the host immune system, was always a highly desirable goal in transplantation.

Despite outstanding successes, induction of tolerance protocols remain risky and are not ready for clinical routine.

Interestingly, a very limited number of organ recipients have been described as maintaining a prolonged allograft survival despite the accidental discontinuation of any immunosuppressive drugs. Kidney recipients displaying operational tolerance

may have withdrawn their immunosuppressive regimen of their own accord—by noncompliance—or may have been advised to do so by their nephrologist on the grounds of serious infections or malignancies. This condition was termed as ‘clinical operational tolerance’ in reference to its spontaneous apparition.

The recently accepted definition of clinical operational tolerance is that of a good and stable graft function for at least 1 year after complete immunosuppression withdrawal.

This apparently unambitious definition contrasts with that of experimental tolerance. Aside from this criteria, laboratory animals must fulfil very stringent conditions to be declared tolerant including in vitro evidence that donor-specific responses are absent or suppressed and a normal histologic appearance of the transplanted allograft. Often, the demonstration that the donor recipient accepts a second graft from the same donor but rejects a third party graft is required. In practice, physicians and patients—either compliant or not—are often reluctant to perform a biopsy of a well-functioning transplant. In practice, in vitro tests or validated surrogate biomarkers are not yet available and challenge transplantations would obviously be non-realistic and futile. As a consequence, the concept of operational tolerance permits a broader range of situations than the strict concept of experimental tolerance does.

Despite this minimalist definition, operational tolerance is distinctly rare in kidney transplantation. Indeed, <200 cases of tolerant kidney transplant recipients have been reported to date among more than half a million kidney transplants performed worldwide. Several predictive biomarkers have been proposed to detect or to

predict operational tolerance after drug withdrawal, but none of them has been shown in prospective, controlled trials to allow safe immunosuppression withdrawal. This fact, along with the serious consequences of acute kidney rejection, refrained care providers from testing for tolerance by simply discontinuing immunosuppression, even in a stepwise manner. Also, the exact frequency of operational tolerance among kidney recipients is unknown.

The ERA-EDTA-DESCARTES transplantation working group together with Nantes University (France) set up a European-wide survey to discover and describe new operationally tolerant kidney recipients and to evaluate the cumulative incidence of this phenotype. We aimed to identify new cohorts of operationally tolerant patients for further immunological and molecular studies.

## MATERIALS AND METHODS

### Survey

Seventeen national or regional coordinators from 28 European countries (Supplementary data, Table S1) sent a standardized questionnaire to 256 transplantation centres or centres offering transplantation consultations between 10 September 2013 and 12 November 2014. Centre investigators were asked to report anonymized data on operationally tolerant (TOL) and almost tolerant (minimally immunosuppressed, MIS) patients.

Considering rare and, sometimes, transient conditions, we encouraged the report of every patient with a history of operational tolerance either active or past, alive or dead. Patient screening was performed according to each centre's own resources through a computerized database or physician recollections.

In parallel with the inventory of TOL patients, investigators were asked to report on the total number of kidney transplants ever performed at each centre. Finally, this survey included updated data from the 27 patients previously described by the Nantes group. They were used for the calculation of cumulative incidence and for survival analysis.

### Patients and controls

TOL patients were defined as allogeneic kidney recipients maintaining a good graft function—for working purposes, we chose the clear definition of a serum creatinine below  $<1.7$  mg/dL and a proteinuria  $<1$  g/day or g/g creatinine—for at least 1 year after complete immunosuppression withdrawal. We identified 66 TOL patients of which 61 provided sufficient data to enter the complete analysis while 5 only contributed to the calculation of cumulative incidence.

MIS patients ( $n = 34$ ) fulfilled the same criteria but were still receiving prednisone (or steroid equivalent) at a dose lower than 10 mg/day. Patients with higher creatinine and/or proteinuria but who maintained a stable graft function during at least 1 year without immunosuppression were also considered for analysis ( $n = 4/61$  for TOL and  $1/34$  for MIS). Since we know from previous studies that tolerant states could be transient only

but also that those patients were probably rare and precious, we chose to report TOL and MIS patients whether prevalent or not, i.e. inclusive of patients no longer tolerant at the time of the report because of death, resumption of immunosuppressive drugs or declining graft function. Consequently, we also asked our collaborators to report on the full number of transplantations performed at their centre. Finally, we excluded patients in whom operational tolerance resulted from an intervention (e.g. allogeneic stem cell transplantation) or from a transplantation between monozygotic twins.

For comparison purposes, the TOL cohort (n = 61) was subdivided into 'new' (n = 35) and 'historical' TOL (n = 26) patients, for those previously described by the Nantes group.

### Data collection

Data were collected using a standardized data form or updated (for Nantes historical TOL patients). The questionnaire included enquiries about recipient's demographics (sex, date of birth, past medical and renal history), donor characteristics (age, sex, living or deceased donors), immunological data [number of human leucocyte antigen (HLA) system mismatches, anti-HLA antibodies, Epstein–Barr virus (EBV) and cytomegalovirus (CMV) serological status], immunosuppression and tolerance periods (durations, outcomes and graft function).

In addition, whenever necessary, the physicians who sent back the questionnaires were contacted by e-mail to complete all the required information. In cases of uncertainty regarding the exact month when operational tolerance started in the context of noncompliance, we arbitrarily chose a start date of 15 June of the first year of complete immunosuppression withdrawal.

### Statistical analysis

Results from continuous variables with and without normal distribution were expressed as mean  $\pm$  standard deviation (SD) and median and interquartile range (IQR), respectively, and categorical data were expressed as percentages. TOL and MIS patients were compared by using Student's t-test for normally distributed data, Mann–Whitney U-test for non-normally distributed data and Fisher's exact test or  $\chi^2$  for categorical variables. Death-censored graft survival and patient survival analysis were performed for the whole TOL and MIS cohorts according to the Kaplan–Meier method. Statistical analyses were performed using STATA software, version 12 (StataCorp LP). Statistical significance was taken below the 5% level.

P-values were calculated with full non-normalized data.

## RESULTS

Cumulative incidence of operational tolerance and almost tolerance among kidney recipients

One hundred and forty-seven out of 256 questionnaires were returned reporting on a total of 218 913 transplants that were performed over a cumulative period of 3635 years. Sixty-six eligible TOL and 34 MIS patients were identified (Figure 1).

Overall, tolerance and almost tolerance were reported in 3 [95% confidence interval (CI): 2.64–3.37] and 1.5 (95% CI: 1.53–1.58) patients out of 10 000 kidney recipients, respectively.

Considering the higher frequency of TOL patients in France, we compared France with the remaining European countries: TOL patients were reported in 3.9 out of 10 000 kidney recipients in France versus 1.7 out of 10 000 outside France ( $P = 0.07$ ).

#### Characteristics of the 35 new operationally tolerant patients

Important medical or administrative data were missing in 5 out of 66 patients entering the survey. Among the 61 patients with complete data, 26 have been described previously.

Thirty-five new TOL subjects are detailed here (see flow chart in Figure 2A and Table 1), of which 31 fulfilled the definition of good graft function as described above. The remaining four have displayed suboptimal (either serum creatinine or proteinuria above the limits) but stable function for at least 1 year without immunosuppressive therapy and also entered the study as TOL (patients referred to as ‘T6, T9, T22 and T31’ in Figure 3A). These 35 newly described patients were mainly males of European ancestry. Of note, glomerulonephritis/sclerosis or pyelonephritis was reported as primary renal disease in 51%, while diabetes or hypertension was reported in 6%. Patients ( $n = 35$ ) were transplanted at a mean age of  $29 \pm 13$  years. They received their first graft in 88% of cases after spending 17 (8–26) ( $n = 26$ ) months on dialysis. Four patients out of 35 were pre-emptively transplanted. Donors were deceased in 60% of cases ( $n = 21/35$ ), males in 70% ( $n = 23/33$ ) and had a mean age of  $33.5 \pm 11$  years. The cohort was composed of 25% ( $n = 8/31$ ) full HLA-matched donor–recipient pairs. The remaining 23 patients had a mean number of HLA-A, -B and -DR mismatches of  $2.8 \pm 1$ . One-quarter had a history of alloimmunization prior to transplantation, detected either by a complement-dependent cytotoxicity assay or by Luminex. Three out of 35 patients experienced an episode of biopsy-proven rejection before the period of immunosuppression discontinuation. Several patients ( $n = 4/25$ ) developed CMV ( $n = 2$ ) or EBV ( $n = 2$ ) seroconversion under immunosuppression and four were diagnosed with malignancy (lymphoproliferative disease in three and multiple skin cancers in one). The median time passed off immunosuppression was 108 (58–156) months. The majority (90%) of the patients discontinued their immunosuppressive medications because of noncompliance, mental illness or social considerations. At the latest observation of tolerance, median creatininemia was 1.35 (1.1–1.48) mg/dL. Proteinuria exceeding 300 mg/day (but below 1 g/day) was noted in 9 out of 29 patients (31%).

#### Characteristics of minimally immunosuppressed patients



We identified 34 MIS patients (Table 1). Thirty-three fulfilled the definition of a good kidney function, while the remaining one presented a suboptimal (serum creatinine above the limit) but stable graft function for at least 1 year with 7.5 mg prednisone per day (patient referred to as 'M10' in Figure 3B). Endstage kidney disease resulted from either glomerulonephritis/sclerosis or pyelonephritis in 59% and from diabetes or hypertension in <3%. Dialysis duration was 25 (12–36) months for 27 patients while 2 were pre-emptively transplanted. One patient received a combined kidney–pancreas transplant. Mean donor age was  $32 \pm 14$  years. Sixteen per cent of the donor–recipient pairs were HLA complete matches ( $n = 5/32$ ), while the others displayed an overall mean number of  $3.2 \pm 1$  mismatches with  $0.9 \pm 0.7$  mismatches at HLA-DR. Eleven patients out of 31 had evidence of HLA immunization prior to transplantation.

Twenty-seven patients experienced 32 malignancies under immunosuppression, mainly lymphoproliferative diseases ( $n = 20$ ). This was the major reason for the physician-driven decision of immunosuppression weaning. At the latest observation of almost tolerance, mean creatinemia was 1.23 (0.96–1.5) mg/dL and 10 patients out of 32 (31.2%) displayed proteinuria above 300 mg/day (but below 1 g/day).

#### New operationally tolerant patients follow-up

Data on newly described TOL patients ( $n = 35$ ) covered a median period of 191 (145–255) months post-transplantation. At the time of data capture (see flow chart in Figure 2A), a first group of 23 patients were still operationally tolerant after a median time of 79 (39–120) months without immunosuppression.

One out of 23 died with good graft function. A second group of four patients displayed suboptimal graft function (either serum creatinine or proteinuria above the limits), however, were stable for at least 1-year period without immunosuppressive medications.

Their grafts survived for 60 (35–120) months. One out of four died with a functioning graft and another one required dialysis.

The remaining two patients are still dialysis free. Lastly, a third group of eight patients lost their tolerant state after a period of 53 (36–77) months. Two of them were restarted on immunosuppressive medications for undefined graft injury (one haematuria and one glomerulopathy of unknown significance at biopsy) and the six others had a rising creatinine leading to dialysis in three. Their grafts had functioned with no treatment for 85 (45–127) months. Individual trajectories of TOL patients are depicted in Figure 3A.

#### New minimally immunosuppressed patients follow-up

Data on MIS patients covered a median period of 219 (160–287) months post-transplantation ( $n = 34$ ). Among these 34 MIS patients, 27 were persistently almost tolerant at the time of data capture (see flow chart in Figure 2B). Almost tolerance status lasted 88 (32–99) months. Six other MIS patients displayed good graft function on low-dose steroids for only 47 (32–99) months, after which one was restarted on a second immunosuppressive drug for a creeping increase in creatinine and five others had a declining graft function

exceeding the limits described in the Materials and methods section. Overall, this cohort had functioning grafts for already 62 (44–146) months, while maintained on small doses of corticosteroids only. Finally, a single patient continued to maintain a functioning graft with a suboptimal serum creatinine (1.9 mg/dL at latest observation) 66 months after drug minimization. Individual trajectories of MIS patients are depicted in Figure 3B.

#### Patient and graft survival

As illustrated in Figure 4, 10-year patient survival after the establishment of operational tolerance and almost tolerance was 90% (95% CI: 75–96) and 59% (95% CI: 41–74), respectively.

Death-censored graft survival at 10 years after the establishment of operational tolerance or almost tolerance was 87.1% (95% CI: 71.2–95.6) and 100% respectively. We did not directly compare TOL with MIS patients because those populations were different with regard to cancer incidence rates and other confounding factors.

#### DISCUSSION

Here, we described 61 operationally tolerant and 34 almost tolerant kidney recipients at the same time. This unique, and likely the world's largest, data collection on tolerance in kidney transplantation should help draw more stable conclusions on those traits.

First, this study highlights that viral seroconversions, anti-HLA immunization, episodes of graft rejection and young age but also, history of autoimmune diseases were all conditions that were compatible with the later installation of operational tolerance. This is accumulating evidence that operational tolerance is acquired and specific and not the consequence of a generalized immune deficiency process and, notably, immunosenescence. The abundance of MIS patients displaying a good graft function years after cancer diagnosis and subsequent immunosuppression weaning also argues against a global immunodeficiency state. Second, TOL and MIS patients demonstrated a prolonged death-censored graft survival and, for TOL patients only, a surprisingly long patient survival. European-wide data from the Collaborative Transplant Study report patient and death-censored graft survival for kidney patients below 75% at 10 years. Although the cohorts are not comparable, it is striking that 85 and 100% of surviving TOL and MIS patients, respectively, had a functioning graft 10 years after immunosuppression weaning. For most of them, this represented >20 years of functioning graft. In line with this finding, a previous report highlighted that, in 2004, eight out of nine kidney recipients with the world's longest graft survival were actually clinically tolerant. Seven of them still had good renal function after 39–40.5 years. Importantly, it is not clear whether the excellent patient and graft survival we observed were a consequence of immunosuppression minimization or whether some conditions associated with the development of operational tolerance (such as graft quality, recipient health or HLA matches) also confer a survival benefit, leading to a selection bias. In this regard, in the previous report on the 27 historical cases of operational tolerance, no clinical differences were found between TOL patients and the two matched groups of patients with stable graft function and those who rejected their graft after arrest of immunosuppression.

Third, the duration of operational tolerance and almost tolerance was, however, extremely variable. They are unstable phenotypes, which may be interrupted at any time, even after several years. Regarding historical cases of operational tolerance, we have previously stressed the wide disparities among operationally tolerant patients. Whereas some will virtually never develop any measurable immunological response towards the graft, others will mount immunological responses such as donor-specific antibodies, yet compatible with a prolonged allograft survival; finally, a third non-stable group will surreptitiously develop a damaging process that will end in graft loss in just a few years. All these patients share the same designation of operational tolerance. There is thus a pressing need for reliable and clinically available biomarkers that go beyond binary criteria based on creatinine and/or proteinuria levels. Several biomarkers have been proposed, but we still lack knowledge on their predictive and discriminative values based on prospective studies.

We believe that the data presented here will be useful to confront phenotypic assumptions about those patients but also to support the development or the continuation of large networks dedicated to operational tolerance mechanistic studies (indices of tolerance, RISET, ITN: see summary at <http://www.kcl.ac.uk/lsm/research/divisions/timb/research/tolerance/index.aspx>).

This study should also constitute an appeal to new clinicians to join our research efforts.

Patient dispersion is indeed a major obstacle for knowledge and research into operational tolerance. Our strategy directly tackled this issue. The existence of a working group within the ERA-EDTA dedicated to transplantation (DESCARTES) and the interest of the Nantes hospital group for such patients for more than a decade provided a very helpful platform to access a high number of nephrologists across Europe. In particular, the personal contacts between the national coordinators and the local investigators were critical to this success. Unfortunately, rare conditions also challenge the realization of prospective controlled studies. Most of the time, retrospective uncontrolled case analyses—typically registries—remain the most valuable research options. As a consequence, systematic numbering of patients or prevalence could be difficult to assess, recruitment strategies could be biased and patient-related information could suffer lack of uniformity.

We reported 3 and 1.5 TOL and MIS patients per 10 000 kidney recipients, respectively. Of note, the cumulative incidence of operational tolerance described in this study is an approximation as it is difficult to accurately determine medication compliance/adherence. Detection of noncompliance relies on the patient's acknowledgement and, when available, on undetectable drug blood levels, prescription assistant software, and pharmacy repertories or national security system records. Completing the required data for TOL or MIS patients is time-consuming, and some of our closest colleagues confessed to us not having found the time to report them. Finally, a lot of the centres were not able to reliably capture patients who may have temporarily fulfilled the criteria.

It is likely, therefore, that the cumulative incidence of tolerant patients reported here is a minimum evaluation. We acknowledge that unsystematic patient screening, absence of method uniformity in the biological tests reported and the lack of prospective biological and histological follow-up are limitations in this work. However, in the setting of a rare trait usually associated with noncompliance and patient concealment, this survey represents a valuable effort of not less than 145 kidney transplant practitioners across Europe. This

survey brings further evidence that some transplant patients may spend prolonged periods without immunosuppressive drugs. We showed that operational tolerance was associated with excellent patient and graft outcomes, but not necessarily unlimited over time. As already stressed in the setting of experimental tolerance a long time ago, operational tolerance is also metastable in nature, not black or white, and ‘every degree is represented’. This study, descriptive in nature, should help to set up or continue networks to support further clinical, immunological and molecular studies.

## ACKNOWLEDGEMENTS

This survey was funded by the ERA-EDTA-DESCARTES working group, the Fonds Erasme (research grant), the Fonds Carine Vyghen, the Fonds Horlait-Dapsens, the RTRS Fondation de Coopération Scientifique CENTAURE and the IHUCesti project, which received French government financial support managed by the National Research Agency via the ‘Investment Into The Future’ program ANR-10-IBHU-005. J.Pascual is supported by FIS-FEDER PI13/00598 and REDINREN RD12/0021/0024. We are also very indebted to the colleagues who replied to the survey: Albania: M. Barbullushi, A. Idrizi, University Hospital Center Mother Teresa, Tirana. Austria: G. Mayer, Medical University, Innsbruck; A. Rosenkranz, Medical University, Graz. Belgium: J. Sennesael, BBJTP, Brussels; J.-L. Bosmans, University Hospital, Antwerpen; M. De Meyer, Cliniques universitaires Saint Luc UCL, Brussels; S. Van Laecke, University Hospital, Ghent. Bosnia and Herzegovina: E. Mesic, University Hospital, Tuzla; H. Resic, University Hospital, Sarajevo.

Croatia: N. Basic Jukic, University Hospital, Zagreb; M. Knotek, University Hospital Merkur, Zagreb. Czech Republic: T. Seeman, Department of Pediatrics, University Hospital Motol, Prague; P. Navratil, University Hospital, Hradec Kralove; J. Dedochova, University Hospital, Ostrava; K. Krejci, University Hospital, Olomouc. Denmark: K. Skov, University Hospital, Aarhus; C. Bistrup, University Hospital, Odense.

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Guillaume; P. Grise, D. Guerrot, I. Etienne, M. Hanoy, M. Godin, and B. Legallicier, Hopital de Bois Guillaume CHU

Rouen, Bois Guillaume; P. Merville, CHU Pellegrin, Bordeaux; G. Robert, CHU de Bordeaux, Bordeaux; J.-P. Boiteux and P. Deteix, CHU Hopital G. Montpied, Clermont Ferrand; V. Audard, S. Homs, T. Kofman and P. Lang, Hopital Henri Mondor (Ap Hp), Creteil; B. Charpentier, Hopital de Bicetre (Ap Hp), Le Kremlin Bicetre; M. Dehennault, R. Novo and Ch. Samaille, Hop Jeanne de Flandre Chr Lille, Lille; J.-P. Rerolle, CHU Dupuytren Limoges, Limoges; Y. Berland, P. Brunet, N. Jourde Chiche, E. Lechevallier, Ch. Maurin, V. Moal, R. Purgus and H. Vacher Coponat, Hopital de la Conception, Marseille 5eme; L. Cabaniols, T.Murez and G. Poinas, Hopital Lapeyronie CHU Montpellier, Montpellier; G. Favre, A. Jeribi and O. Moranne, Hopital Pasteur, Nice; I. Jollet, Efs Centre Atlantique, Poitiers; E. Verdier, CHU la Miletrie, Poitiers; T. Frouget, P. Le Pogamp, J. Rivalan and C. Vigneau, C. H. R. Pontchaillou Rennes, Rennes; Y. Georg and P. Wolf, CHU de Strasbourg/Nouvel Hopital Civil, Strasbourg. Germany: M. Wiesener, University Hospital, Erlangen; W.

Arns, Merheim Medical Center, Cologne; T. Rath, Westpfalz-Klinikum, Kaiserslautern; M. Nitschke, University Hospital Schleswig-Holstein, Lübeck; U. Kunzendorf, University Hospital Schleswig-Holstein, Kiel; B. Banas, University Hospital, Regensburg; M. van der Giet, Charité-Campus Benjamin Franklin, Berlin; C. Schinköthe, University Hospital, Jena; O. Hakenberg, Universitätsmedizin, Rostock; B. Krämer, Transplant Centre, Mannheim; R. Weimer, Internal Medicine/Nephrology, Gießen; M. Haubitz, Nephrologie, Klinikum Fulda; W. Gwinner, Medical School, Hannover.

Greece: J. Boletis, Laiko University Hospital, Athens; K. Siamopoulos, University Hospital, Ioannin. Ireland: P. J. Conlon, Beaumont Hospital, Dublin. Israel: Y. Frishberg, Shaare Zedek Medical Center, Jerusalem. Norway: A. Varberg Reisæter, Oslo University Hospital – Rikshospitalet. Poland: M. Krajewska, Medical University, Wroclaw; M. Nowicki, Medical University, Lodz; A. Dębska-Ślizień, Transplant Centre, Gdańsk; M. Durlík, Transplant Centre, Warsaw. Portugal: D. Machado, Hosp Santa Cruz, Carnaxide. Serbia: R. Naumovic, Clinic of Nephrology, Clinical Center of Serbia, Belgrade. Slovakia: Z. Zilinska, University Hospital, Bratislava; E. Lackova, University Hospital, Banska Bystrica; I. Dedinska, University Hospital, Martin.

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#### CONFLICT OF INTEREST STATEMENT

None declared.

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FIGURE 1 : (A) Number of centres contacted (dashed bars) and questionnaires returned (white bars) per country, ordered by ascending number of questionnaires returned. (B) Cumulative incidences of TOL (black bars) and MIS (grey bars) patients reported per 10 000 kidney recipients per country. Countries were ordered by ascending number of combined TOL + MIS incidences.

FIGURE 2 : (A) Flow chart and outcomes of TOL patients through the study. (B) Flow chart and outcomes of MIS patients through the study. IS, immunosuppressive.

FIGURE 3 : Individual trajectories of TOL (A) and MIS (B) patients. White bars account for the duration, in months, of the tolerance period with a good kidney function [serum creatinine (S creat) <1.7 mg/dL and proteinuria (U prot) <1 g/day or g/g creatinine]. Dotted white bars account for the duration of tolerance with a less good kidney function (not meeting the above criteria) but free of dialysis. Coloured bars represent the occurrence of either end of tolerance because of immunosuppression resumption (for TOL patients) or increased dose (for MIS patients) (blue), back on dialysis (red) or patient death (black).

FIGURE 4 : (A) TOL patient survival. (B) TOL patient death-censored graft survival. (C) MIS patient survival. (D) MIS patient death-censored graft survival. TOL and MIS patients who returned on higher immunosuppressive drug levels before reaching death or graft loss were excluded from death-censored graft survival analysis (patients T11, 28, 32, 48 and 60; M7 and 8).