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This is the overview 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

INTRODUCTION AND AIMS: Rarely, kidney recipients have been reported as maintaining a prolonged allograft survival without evidence of rejection in the complete absence of immunosuppressive drugs. ERA-EDTA-DESCARTES working group together with Nantes University launched a European-wide survey to identify them and estimate their cumulative incidence.

METHODS: 17 coordinators accounting for 28 European countries distributed a standardized questionnaire to 256 transplant centres in order to identify any active or past operationally tolerant patients (defined as having a serum creatinine <1.7 mg/dL and proteinuria <1 g/day or /g creatinine despite at least one year without any immunosuppressive drug) but also “almost tolerant” patients (similar criteria but on low-dose steroid regimen, <10 mg/day). The total number of kidney recipients ever followed at each centre was also recorded.

RESULTS: 147 questionnaires covering 218,913 transplants were returned, which allowed to identify 66 operationally tolerant (61 with complete data) and 34 almost tolerant patients, consistent with cumulative incidences of 3 and 1.5 patients reported per 10,000 kidney recipients, respectively. Of the 61 operationally tolerant patients, twenty-six were previously described by Nantes group and 35 are presented here. Most of them were noncompliant patients. After a median follow-up of 191 [IQR: 147-252] months, 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 who 3 resumed dialysis. Over 85% of both tolerant and almost tolerant surviving patients displayed a functioning graft 10 years after immunosuppression withdrawal.

CONCLUSION: In kidney transplantation, operational tolerance and almost tolerance are infrequent, variable in duration and robustness and associated with excellent death-censored graft-survival.

KEY WORDS: operational tolerance, kidney transplantation, almost tolerance, minimally immunosuppressed patients, graft survival.

SHORT SUMMARY:

This is a survey assessing for the first time the cumulative incidences of tolerance and almost tolerance in European-wide cohorts of kidney recipients, defining their characteristics as well as patient and graft survival rates. This survey should be the basis for setting up or completing biocollection and database in order to perform mechanistic studies.

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 (1), malignant (2) or metabolic (3) complications, including the latest generation of immunosuppressants. Together with acute and chronic rejections, immunosuppression side effects heavily affect the long-term survival of both allografts and patients (4).

The induction of a tolerance 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 (5, 6), induction of tolerance protocols remain risky and not ready for entering the 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 (7-9). This last condition was termed as “operational tolerance” in reference to its spontaneous apparition. The recently accepted definition of operational tolerance is that of a good and stable graft function for at least one year after complete immunosuppression withdrawal (7, 8, 10). Operational tolerance is distinctly rare in kidney transplantation (11, 12). Indeed, less than 200 cases of tolerant kidney transplant recipients have been reported to date (7, 9-11, 13, 14) among more than half a million kidney transplants performed worldwide (15). Kidney recipients displaying operational tolerance may have withdrawn their immunosuppressive regimen by their own – by noncompliance - or may have been advised to do so by their nephrologist on the grounds of serious infections or malignancies (9). So far, many predictive biomarkers have been proposed (10, 14, 16-19) but they still lack validation in immunosuppression minimization trials. This fact, along with the serious consequences of acute kidney rejection refrained care providers to test for tolerance by simply discontinuing immunosuppression, even in a stepwise manner (20). 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 find out 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.

METHODS

Survey

Seventeen national or regional coordinators from 28 European countries (Supplementary material, Table 1) sent a standardized questionnaire to 256 transplantation centres or centres offering transplantation consultations between 10 Sept 2013 and 12 Nov 2014. Centre investigators were asked to report anonymized data on operationally tolerant and almost tolerant 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 centre own resources: through computerized database or physician interrogations. At last, the total number of kidney recipients ever performed at each centre was recorded. This survey included updated data from the 27 patients previously described by the Nantes group (9). They were used for the calculation of cumulative incidence and for survival analysis.

Patients and controls

Operationally tolerant patients (TOL) were defined as allogeneic kidney recipients maintaining a good graft function - i.e. a serum creatinine below 1.7 mg/dL and a proteinuria below 1 g/day or /g creatinine - for at least one year after complete immunosuppression withdrawal (7). We identified 66 TOL from whom 61 provided sufficient data to enter all analysis while 5 only participated to the calculation of cumulative incidence.

Minimally immunosuppressed patients (MIS or almost tolerant 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 one year without immunosuppression were also considered for analysis (n=4/61 for TOL and 1/34 for MIS). TOL and MIS patients were also reported when tolerance was no longer ongoing at the time of the report because of death, resumption of

immunosuppressive drugs or declining graft function. We excluded patients in whom operational tolerance resulted from an intervention (e.g. allogeneic stem cell transplantation). For comparison purpose, 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 (9).

Data collection

All 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 HLA mismatches, anti-HLA antibodies, EBV and CMV serological status); immunosuppression and tolerance periods (durations, outcomes, 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 on the 15th June of the first year of complete immunosuppression withdrawal.

Statistical analysis

Results from continuous variables with and without normal distribution were expressed as mean ± 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-square 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. A log-rank test was used to compare TOL with MIS. In addition, crude and adjusted hazard ratios (HRs) derived from the Cox model were used to assess the HR of patient survival among MIS in comparison to TOL. We adjusted HRs for history of cancer, age at immunosuppression withdrawal and period of time with a functioning transplant. The proportional hazard assumption in the final Cox model was fulfilled. 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

147 out of 256 questionnaires were returned reporting on a total of 218,913 transplants that were performed over a cumulative period of 3,635 years. 66 eligible TOL tolerant and 34 MIS were identified (**Figures 1a and 1b**). 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 number of TOL patients identifed in France, we also looked at these frequencies after excluding French patients in order to avoid biasing. 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 previously described (9). 35 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 4 have displayed suboptimal (either serum creatinine or proteinuria above the limits) but stable function for at least one 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% of them, while diabetes or hypertension was reported in 6%. Patients (n=35) were transplanted at a mean age of 29±13 years 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 31±13 year-old. The cohort was composed of 25% (n=8/31) of full HLA-matched donor-recipient pairs.

The remaining 23 patients had a mean number of HLA A, B and DR mismatches of 2.8±1. One quarter had a history of allo-immunization 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 4 were diagnosed with malignancy (lymphoproliferative disease in 3 and multiple skin cancers in 1). 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 patient's median creatinine was 1.35 [1.1-1.48] mg/dL. Proteinuria exceeding 300 mg/day (but below 1g/day) was noted in 9 out of 29 patients (31%).

Characteristics of MIS patients

We identified 34 MIS patients (**Table 1**). 33 fulfilled the definition of a good kidney function while the remaining one presented a suboptimal (either serum creatinine or proteinuria above the limits) but stable graft function for at least one year with 7.5 mg prednisone per day (patient referred to as "M10" in figure 3b). End-stage kidney disease resulted from either glomerulonephritis/sclerosis or pyelonephritis in 59% and from diabetes or hypertension in less than 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±14 years. Sixteen percent of the donor-recipient pairs were HLA complete matches (n=5/32) while the others displayed an overall mean number of 3.2±1 mismatches with 0.9±0.7 mismatches at HLA-DR. Seven patients out of 28 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 creatinine 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 1g/day).

New operational 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 4 patients displayed suboptimal graft function (either serum creatinine or proteinuria above the limits) however were stable for at least one 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 2 patients are still free of dialysis. At last, a third group of 8 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 (1 haematuria and 1 glomerulopathy of unknown significance at biopsy) and the 6 others had a rising creatinine leading to dialysis in 3. 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 had persistent almost tolerance 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 only for 47 [32-99] months, after which, one was restarted on a second immunosuppressive drug for a creeping increase in creatinine and 5 others had a declining graft function exceeding the limits described in the methods section. Overall this cohort had functioning grafts for already 62 [44-146] months, whilst 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, graft and tolerance 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 (P=0.0002). In univariate Cox model, MIS patients had a relative hazard ratio of death of 4.95 [95% CI: 1.95-12.45] in comparison to TOL (P=0.001). After adjustment for history of cancer during the immunosuppression period (in 4 TOL out of 35 and 27 MIS out of 32), age at immunosuppression withdrawal and period of time with a functioning transplant (Supplementary material, **Table 2**), we still observe an excess risk of death in MIS patients compared to TOL however with borderline significance (HR: 3.08 [95% CI: 1.02-9.3, P=0.05]. 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.

DISCUSSION

In this large European-wide survey, operationally tolerant and almost tolerant patients were reported in 3 and 1.5 patients per 10,000 kidney recipients, respectively. This is the first attempt to identify and characterise these patients methodically on a vast territory. Rare diseases are both challenges and opportunities and their study requires specific strategies (21). 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. Furthermore, the survey response rate (nearly 60%) can be considered high. The personal contacts between the national coordinators and the local investigators were critical to this success.

The cumulative incidence of operational tolerance described in this study is an approximation only as it is difficult to accurately determine medication compliance/adherence. Detection of non-compliance relies on patient's acknowledgement and, when available, on undetectable drug blood levels, prescription assistant software, pharmacy repertories or national security system records. Finally, completing the required data for TOL or MIS patients is time-consuming, and some of our closest colleagues confessed us not having found the time reporting them. It is likely therefore that the cumulative incidence of tolerant patients reported here is an underestimate. I addition, a lot's of the centres were

probably not able to capture reliably the patients who may have temporarily fulfilled the criteria.

This study highlights that viral seroconversions, anti-HLA-immunization, history of auto-immune disease, episodes of graft rejection were all conditions that were compatible with the later installation of operational tolerance. These are additional evidences that operational tolerance is acquired and specific and not the consequence of a generalized immune deficiency (7, 22).

TOL and MIS patients also demonstrated prolonged death-censored graft survival and, for TOL patients only, a remarkable patient survival. European-wide data from the Collaborative Transplant Study report patient and death-censored graft survival for kidney patients below 75% at ten years (23). Although the cohorts are not comparable, it is striking that more than 85% of surviving TOL and MIS patients had a functioning graft 10 years after the beginning of the tolerance or almost tolerance period. For most of them, this represented more than 20 years of functioning graft. In line with this finding, a previous report (11) highlighted that, in 2004, 8 out of 9 kidney recipients with the world longest graft survival were actually clinically tolerant. Seven of them still had good renal function after 39 to 40.5 years. Importantly, it is not clear whether the excellent patient and graft survivals we observed were consequences of immunosuppression minimization or whether some condition 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 (9), 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 IS.

The duration of operational tolerance and almost tolerance were however extremely variable. They represent an unstable phenotype, which may be interrupted at anytime, 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 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 (24). All these patients share the same designation of operational tolerance. Here is thus a pressing need for reliable, and clinically available biomarkers going beyond binary criteria based on creatinine and/or proteinuria levels. Several biomarkers have been proposed (10, 14, 16-18) but we still lack knowledge on their predictive and discriminative values based on prospective studies (19, 25)

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. This study confirms that operational tolerance is not unlimited over time. Operational tolerance is metastable in nature, not black or white and “every degree is represented”. This was previously described a long time ago, when the first definition of tolerance was formulated in animal models (26). This study, descriptive in nature, should help setting up or continuing networks (Indices of tolerance, RISET, ITN: see summary at http://www.kcl.ac.uk/lsm/research/divisions/timb/research/tolerance/index.aspx) that aim at ongoing prospective data collection with long-term follow-up, in addition to collection of biological material in order to support further clinical, immunological and molecular studies.

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See separate document.

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Table 1. Demographic data of tolerant and minimally immunosuppressed patients.

	New TOL patients N=35	Historic TOL patients N=26	All TOL N=61	MIS cohort N=34	*P* – value for the comparison of All TOL vs MIS
RECIPIENT FEATURES					
Age at transplantation date – years*	29.1 ± 13	34.6 ± 16	31.5 ± 14	37.6 ± 14	*0.05*
Male gender	26 (74)	19 (73)	45 (74)	17 (90)	***0.02***
European origin	29 (83)	22 (88)	51 (85)	28 (90)	*NS*
Primary nephropathy					*NS*
According to ERA-EDTA classification					
Glomerulonephritis/sclerosis	11 (31)	13 (50)	24 (39)	14 (41)	
Pyelonephritis	7 (20)	4 (15)	11(18)	6 (18)	
Polycystic kidney disease	3 (9)	0	3 (5)	3 (9)	
Hypertension	1 (3)	0	1 (2)	0	
Renal vascular disease	0	0	0	1 (3)	
Diabetes	1 (3)	3 (11)	4 (7)	1 (3)	
Miscellaneous	7 (20)	4 (15)	7 (11)	5 (145)	
Unknown	5 (14)	2 (8)	7 (11)	4 (12)	
History of dialysis	28 (85)	20 (95)	48 (90)	32 (94)	*NS*
Dialysis vintage – months (excluding zero)[&]	(n=26) 17 [8-26]	(n=19) 36 [17-63]	(n=45) 24 [10-36]	(n=32) 25 [12-36]	*NS*
History of auto-immune disease	3 (9)	5 (23)	8 (14)	4 (12)	*NS*
History of cancer before transplantation period	2 (6)	2 (8)	4 (7)	1 (3)	*NS*
TRANSPLANTATION FEATURES					
Donor age - years*	(n=31) 33.5 ± 11	(n=23) 26.9 ± 15.4	(n=54) 30.7 ± 13	(n=33) 32 ± 14	*NS*
Male gender	23 (70)	16 (70)	39 (70)	21 (64)	*NS*
Deceased donor	21 (60)	21 (81)	42 (70)	24[§] (71)	*NS*
First kidney transplant	31(88)	22 (85)	53 (87)	31 (91)	*NS*
Cold ischemia time - minutes[&]	(n=26) 907 [70-1385]	(n=25) 1245 [720-1860]	(n=51) 1110 [84-1440]	(n=29) 1020 [600-1320]	*NS*

IMMUNOLOGICAL FEATURES					
Full matched transplants	8 (26)	5 (22)	13 (24)	5 (16)	*NS*
HLA A - B - DR mismatching (excluding full matched pairs)*	(n=23) 2.8 ± 1.1	(n=18) 2.8 ± 1.1	(n=41) 2.8 ± 1.1	(n=27) 3.2 ± 1	*NS*
HLA DR mismatching (excluding full matched pairs)*	(n=23) 1.2 ± 0.7	(n=18) 0.6 ± 0.6	(n=41) 0.7 ± 0.6	(n=27) 0.9 ± 0.7	*NS*
Anti-HLA immunization prior transplantation (either positive PRA or anti-HLA antibodies)	7 (25)	11 (42)	18 (33)	11 (32)	*NS*
Anti-HLA immunization after transplantation (either positive PRA or anti-HLA antibodies; donor-specific, non specific or undetermined)	1(5)	12 (50)	13 (30)	3 (20)	*NS*
De novo donor specific antibodies after transplantation	1 (5)	6 (28.6)	7 (17.1)	3 (20)	*NS*
IMMUNOSUPPRESSION PERIOD					
IL-2 receptor antagonists	8 (23)	2 (8)	10 (17)	3 (9)	*NS*
Antilymphocyte globulins	9 (26)	9 (36)	18 (30)	9 (27)	*NS*
Other induction agents	0 (0)	3 (11)	3 (5)	4 (12)	*NS*
Steroids	34 (100)	25 (96)	60 (100)	34 (100)	
Tacrolimus	11 (32)	1 (4)	12 (20)	5 (15)	*NS*
Cyclosporine	22 (63)	15 (58)	37 (61)	23 (67)	*NS*
Mycophenolate acid derivatives	14 (41)	5 (19)	19 (32)	13 (39)	*NS*
Azathioprine	17 (50)	20 (77)	37 (62)	14 (42)	*0.07*
mTOR inhibitors	2 (6)	0	2 (3)	(n=33) 0	*NS*
Cytomegalovirus seroconversion	2 (8)	(n=18) 0	2 (5)	3 (13)	*NS*
Epstein-Barr virus seroconversion	2 (9)	4 (25)	6 (15.8)	3 (15)	*NS*
Patient with a history of cancer occurring during immunosuppression	4 (11)	10 (40)	14 (23)	27(79)	***< 0.0001***
Post-transplant lymphoma disease	3	6	9	20	
Kaposi sarcoma	0	0	0	3	
Adenocarcinoma	0	3	3	4	
Skin cancer	1	5	6	5	
History of anticancer chemotherapy	2 (6)	4 (15)	6 (10)	19 (56)	***< 0.0001***

History of rituximab use	1 (3)	3 (11)	4 (7)	7 (21)	*0.05*
Biopsy-proven acute rejection	3 (9)	5 (19)	8 (13)	5 (15)	*NS*
Immunosuppression exposure - months[&]	(n=35) 108 [58-156]	(n=26) 128 [88-163]	(n=61) 111 [65-161]	(n=34) 141 [80-164]	*NS*
Off immunosuppression period					
Age at immunosuppression arrest - years[&]	36 [29-47]	46 [31-57]	40 [31-52]	49 [35-62]	***0.04***
Cause of immunosuppressive withdrawal					***< 0.0001***
Doctor driven for cancer	2 (6)	6 (23)	8 (13)	22 (81)	
Doctor driven for other reasons	1 (3)	1 (4)	2 (3)	3 (11)	
Patient driven	32 (91)	19 (73)	51 (84)	1 (4)	
Last good serum creatinine (last available before eventual degradation) – mg/dL*	(n=31) 1.35 [1.1-1.48]	(n=26) 1.46 [1.12-1.63]	(57) 1.39 [1.11-1.6]	(n=33) 1.23 [0.96-1.5]	*0.08*
> median (1.34 mg/dL)			33 (58)	12 (36)	*0.1*
Proteinuria > 300 mg and < 1 g/day or /L	9 (31)	15 (60)	24 (44)	10 (31)	*NS*
Period of optimal graft function without immunosuppressant - months[&]	(n=31) 70 [39-114]	(n=26) 130 [69-172]	(n=57) 92 [49-136]	(n=33) 72 [30-123]	*NS*
Period of functioning transplant without immunosuppressant, irrespective of creatinine - months[&]	77 [39-120]	153 [80-173]	107 [63-155]	78 [31-132]	*0.09*
> median (93 months)			34 (56)	13 (38)	*0.05*

Note: Results from continuous variables were expressed as mean ± standard deviation (SD) (*) or median (interquartile range [IQR]) ([&]); any missing data are indicated. Categorical data were expressed as number (percentage); any missing data can be deduced from numbers with their percentages.

NS designates non significant P values (< 0.05) however all P values ≤ 0.1 are mentioned.

[§] One combined kidney-pancreas transplantation.

SUPPLEMENTARY MATERIAL

SM, Table 1. Participating countries or regions and coordinating investigators

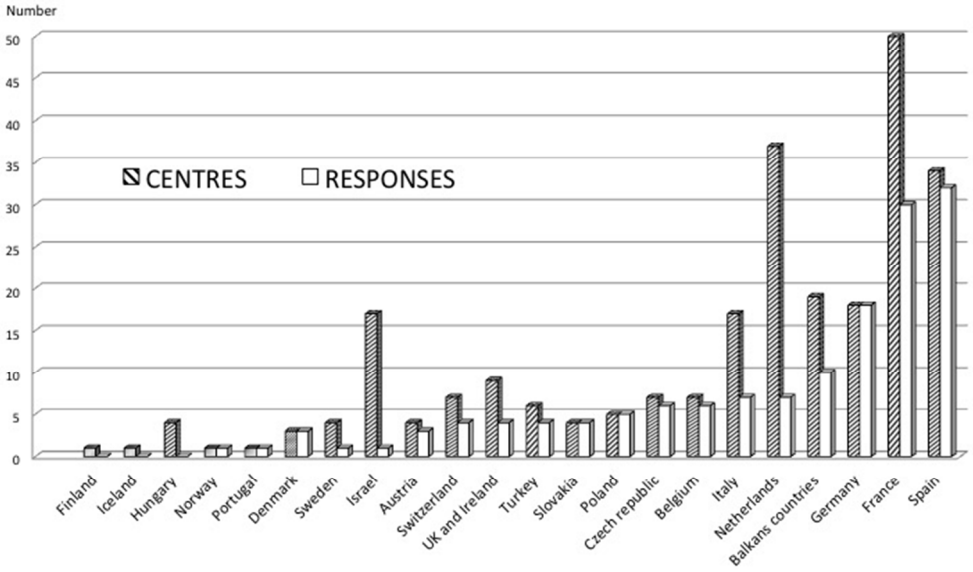
Country of region	Coordinating investigator(s)
Austria	R. Oberbauer
Balkan countries – Albania, Bosnia, Croatia, Greece, Macedonia, Serbia, Slovenia	G. Spasovski
Belgium	D. Abramowicz, A. Massart
Czech Republic and Slovakia	O. Viklicky
France	S. Brouard, M. Hazzan
Germany	K. Budde
Iberic countries – Spain and Portugal	J. Pascual
Nordic countries – Denmark, Finland, Iceland Norway, Sweden	S.S. Sorensen
Ireland and United Kingdom	C. Dudley
Israël	A. Yussim
Italy	U. Maggiore
Netherlands	JW. De Fijter
Poland	M. Klinger
Switzerland	K Hadaya
Turkey	M. Sever

SM, Table 2. Cox model describing the excessive risk of death in MIS patients compared to TOL.

Interactions	Crude hazard ratios			Adjusted hazard ratios		
	cHR	95% CI	*p-value*	aHR	95% CI	*p-value*
Tolerance group						
MIS	4.93	1.95-12.45	***0.001***	3.08	1.02-9.3	***0.05***
TOL	1			1		
History of cancer under immunosuppression						
Yes	4.6	1.68-12.57	***0.003***	1.25	0.40-3.95	*NS*
No	1			1		
Age at transplantation	1.07	1.03-1.10	***<0.0001***			
Age at immunosuppression withdrawal	1.06	1.03-1.09	***<0.0001***	1.05	1.02-1.09	***0.002***
Rituximab use						
Yes	1.38	0.40-4.72	*NS*			
No	1					
Recipient gender						
Male	0.84	0.33-2.14	*NS*			
Female	1					
Azathioprine use						
Yes	1.02	0.42-2.45	*NS*			
No	1					
Last good serum creatinine						
≤ median (1.345 mg/dL)	1.35	0.57-3.15	*NS*			
> median (1.345 mg/dL)	1					
Period with a functioning transplant						
≤ median (93 months)	13.5	4.06-44.7	***<0.0001***	18.6	3.6-96.4	***0.001***
> median (93 months)	1			1		

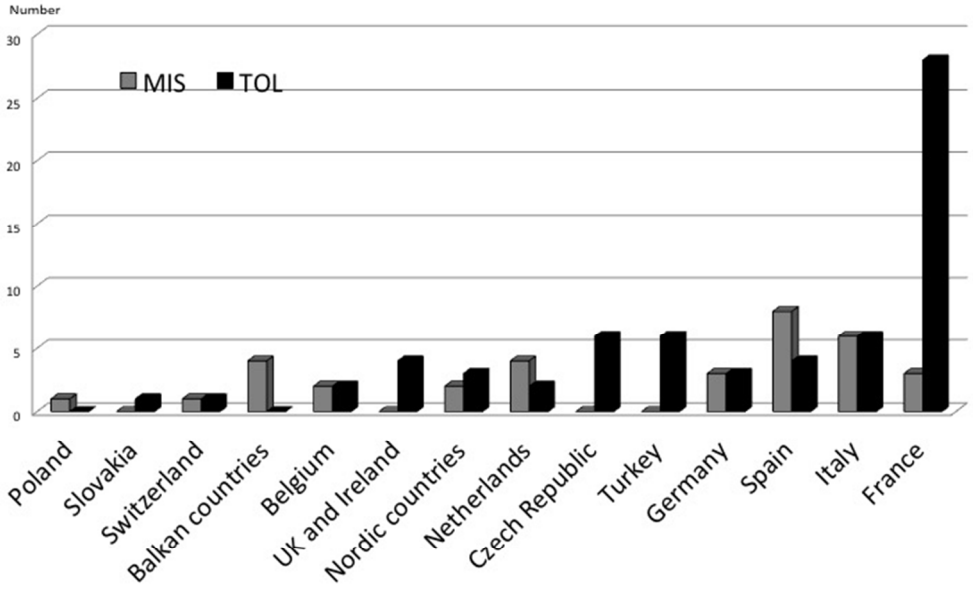
Note: aHR: adjusted hazard ratio for tolerance group, history of cancer under immunosuppression, age at immunosuppression withdrawal and period with a functioning transplant, CI: confidence interval, cHR: crude hazard ratios were calculated for each factor found unbalanced between TOL and MIS in table 1. P values are only detailed if below 0.1. *NS: not significant.*

FIGURE 1a



254x190mm (72 x 72 DPI)

FIGURE 1b



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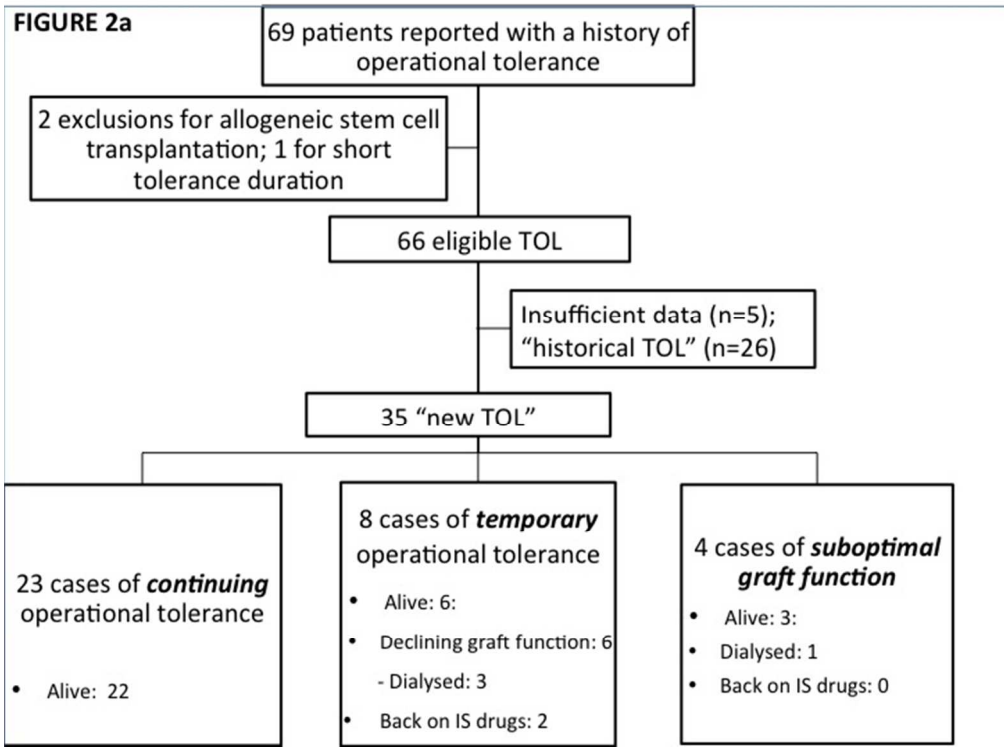

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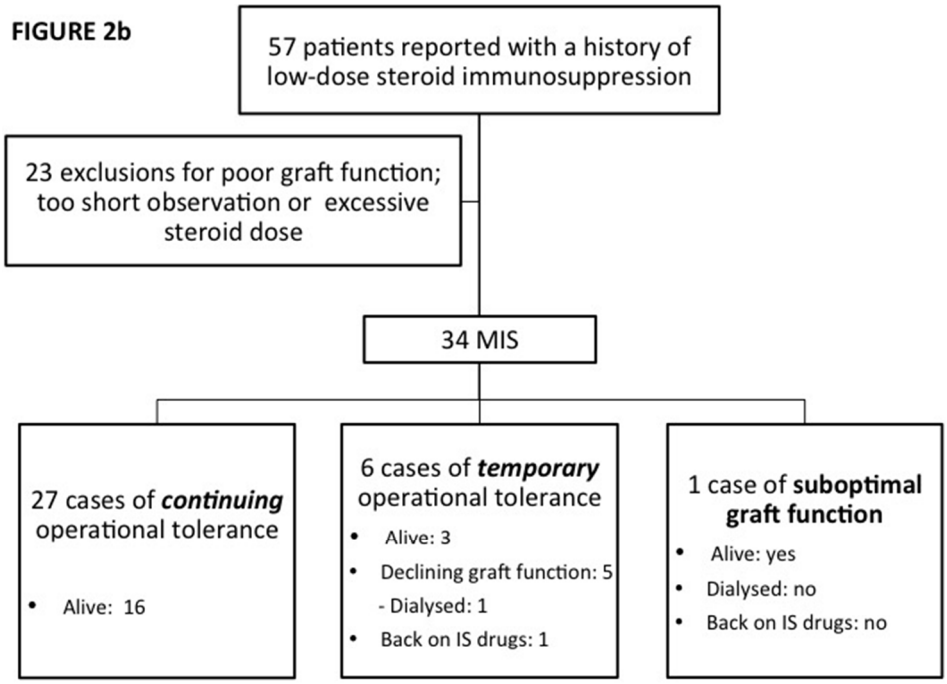
FIGURE 2b



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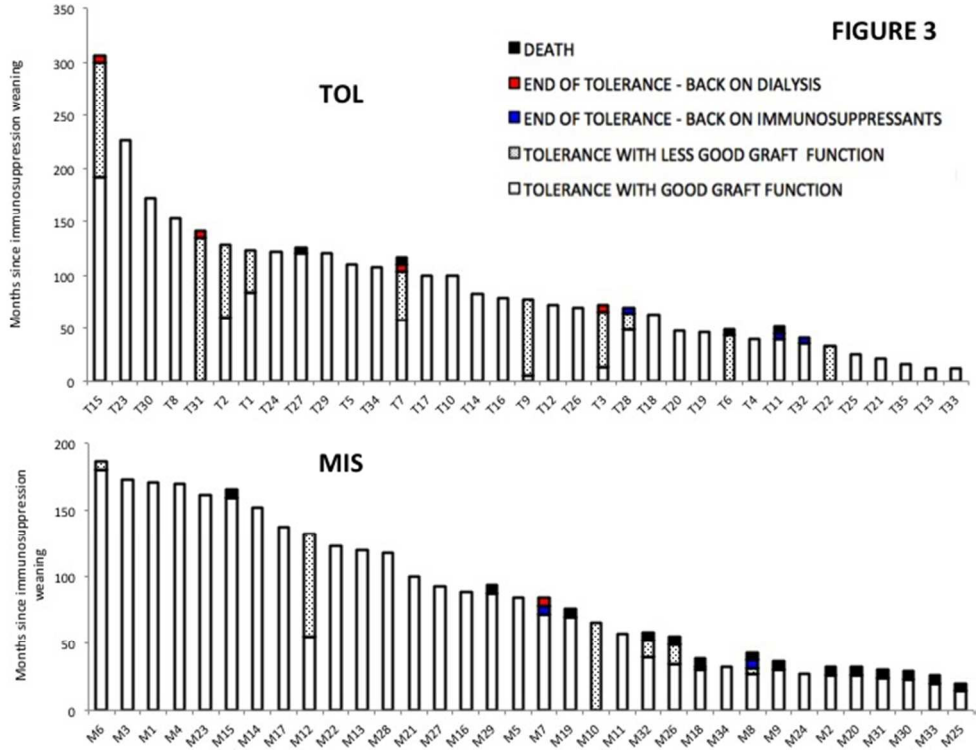

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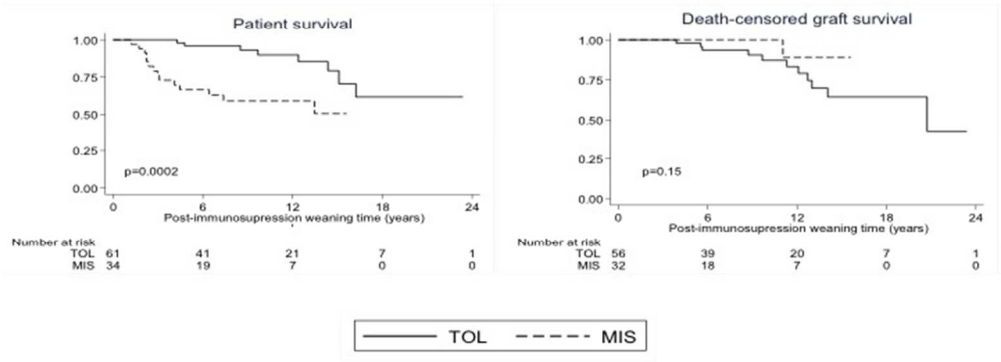
FIGURE 4



254x190mm (72 x 72 DPI)

TOL survey paper – legends of the figures

FIGURE 1a - Number of centres contacted (dashed rectangles) and questionnaires returned (white rectangles) per country, ordered by ascending number of questionnaires returned.

FIGURE 1b - Number of tolerant (TOL, black rectangles) and minimally immunosuppressed (MIS, grey rectangles) patients reported per country, ordered by ascending number of patients identified.

FIGURE 2a - Flow chart and outcomes of tolerant (TOL) patients through the study.

FIGURE 2b - Flow chart and outcomes of minimally immunosuppressed (MIS) patients through the study.

FIGURE 3 – Individual trajectories of tolerant (top panel) and minimally immunosuppressed (bottom panel) patients. White rectangles account for the duration, in months, of the tolerance period with a good kidney function (serum creatinine below 1.7 mg/dL and proteinuria below 1 g/day or /g creatinine). Dotted white rectangles account for the duration of tolerance with a less good kidney function (not meeting the above criteria) but free of dialysis. Coloured rectangles 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 - Patient (left panel) and death-censored graft survivals (right panel). Tolerant patients are represented by the plain black line and minimally immunosuppressed patients by the dashed black line. 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).

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