Kidney allocation based on proven acceptable antigens results in superior graft survival in highly sensitized patients

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Highly sensitized renal transplant candidates accumulate on transplant waiting lists since they produce antibodies to many HLA antigens, which in this way become unacceptable. Organ allocation to these patients is usually based on avoiding transplantation of organs bearing these unacceptable antigens. In contrast, allocation through the Eurotransplant Acceptable Mismatch (AM) program is based on extension of the patient's own HLA type with so-called acceptable HLA antigens to which strictly no antibodies are formed, as shown by extensive laboratory testing. We questioned which type of allocation results in the best long-term graft survival. Therefore, we selected 58,727 cadaveric single renal transplant recipients transplanted within Eurotransplant between 1996 and 2015 and determined factors influencing graft survival for patients transplanted through the AM program. Next, we compared ten-year graft survival of patients with various sensitization grades who received a renal transplant through regular allocation to that of highly sensitized patients transplanted through the AM program. Unlike regular allocation, no effect for HLA mismatches existed for AM patients, while factors that did affect graft survival were similar to those of the general kidney transplant population. AM patients had significantly superior ten-year graft survival compared to highly sensitized patients transplanted on the basis of avoidance of unacceptable mismatches. Strikingly, graft survival of AM patients receiving a repeat transplant was similar to that of nonsensitized repeat transplant recipients. Thus, allocation of kidneys to highly sensitized patients based on proven acceptable antigens results in a significantly better graft survival compared to mere avoidance of unacceptable mismatches.

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Exposure to foreign human leucocyte antigen (HLA) molecules by pregnancy, blood transfusion, or transplantation can lead to sensitization in the form of alloantibodies.[1] HLA-specific antibodies are included in the immunological profile of a patient, and the transplantation-relevant antibody specificities are regarded as unacceptable antigens for the patient in question. Consequently, this results in the avoidance of organ offers that harbor ≥1 of these "unacceptable" antigens. Highly sensitized patients are difficult to transplant, and thus they accumulate on the transplant waiting list.[2] Several strategies are being followed to transplant highly sensitized patients, such as desensitization, paired donor exchange, as well as the avoidance of unacceptable mismatches by virtual crossmatching with priority for highly sensitized patients, such as in the new US kidney allocation system introduced by the Organ Procurement and Transplantation Network (OPTN).[3–5] While desensitization can create a window of opportunity to perform the transplant with a negative crossmatch, antibody-producing plasma cells remain present, which often leads to recurrence of the donor-specific antibodies that can contribute to (chronic) allograft rejection.[6–9] The probability of a highly sensitized patient receiving an organ through paired donor exchange is slim due to relatively small pools of donors.[2] Alternatively, allocation based on the avoidance of unacceptable mismatches with priority for highly sensitized patients could potentially be beneficial to highly sensitized patients by means of shorter waiting times,[5,10,11] but as we will show here, has limited benefit for long-term graft survival.

The Eurotransplant Acceptable Mismatch (AM) program was initiated more than 25 years ago to enhance transplantation of highly sensitized renal transplant candidates. Instead of avoiding transplantation of organs harboring unacceptable antigens, this program makes use of proven "acceptable" antigens, defined as antigens to which the patient has never formed antibodies, as proven by extensive laboratory tests.[12] Acceptable antigens are defined by the lack of antibody reactivity in complement-dependent cytotoxicity assays using target cells mismatched for a single HLA antigen, or single antigen-expressing cell lines. Additionally, since the early 2000s, B-cell epitope analysis using HLAMatchmaker (Rene Duquesnoy, Pittsburgh, PA) for HLA class I is used to aid in defining acceptable antigens. The increased chance of receiving an organ is achieved by allocation based on the

patient's own HLA with the addition of acceptable antigens. Through this addition of acceptable antigens to the patient's own HLA phenotype, and mandatory shipment of a compatible organ to AM patients, increased rates of transplantation of highly sensitized patients have been achieved.[13]

Here, we aimed to determine which factors influence long-term graft survival of AM patients. Furthermore, we compared long-term graft survival rates of patients transplanted on the basis of acceptable mismatches and those transplanted on the basis of avoidance of unacceptable mismatches to determine the true benefit of utilizing acceptable mismatches for allocation.

RESULTS

Factors influencing 10-year graft survival within the AM program

The study design is depicted in a flow diagram (Figure 1), whereas clinical characteristics of patients who received an organ through the AM program are listed in Supplementary Table S1. We performed univariate Cox regression analysis to determine which factors affected 10-year graft survival for AM patients (Table 1). Male donor sex was associated with a decreased risk of graft loss (hazard ratio [HR]: 0.63; 95% confidence interval [CI]: 0.468 to 0.851; $P = 0.003$). Additionally, recipient age >50 years decreased (HR: 0.67; 95%

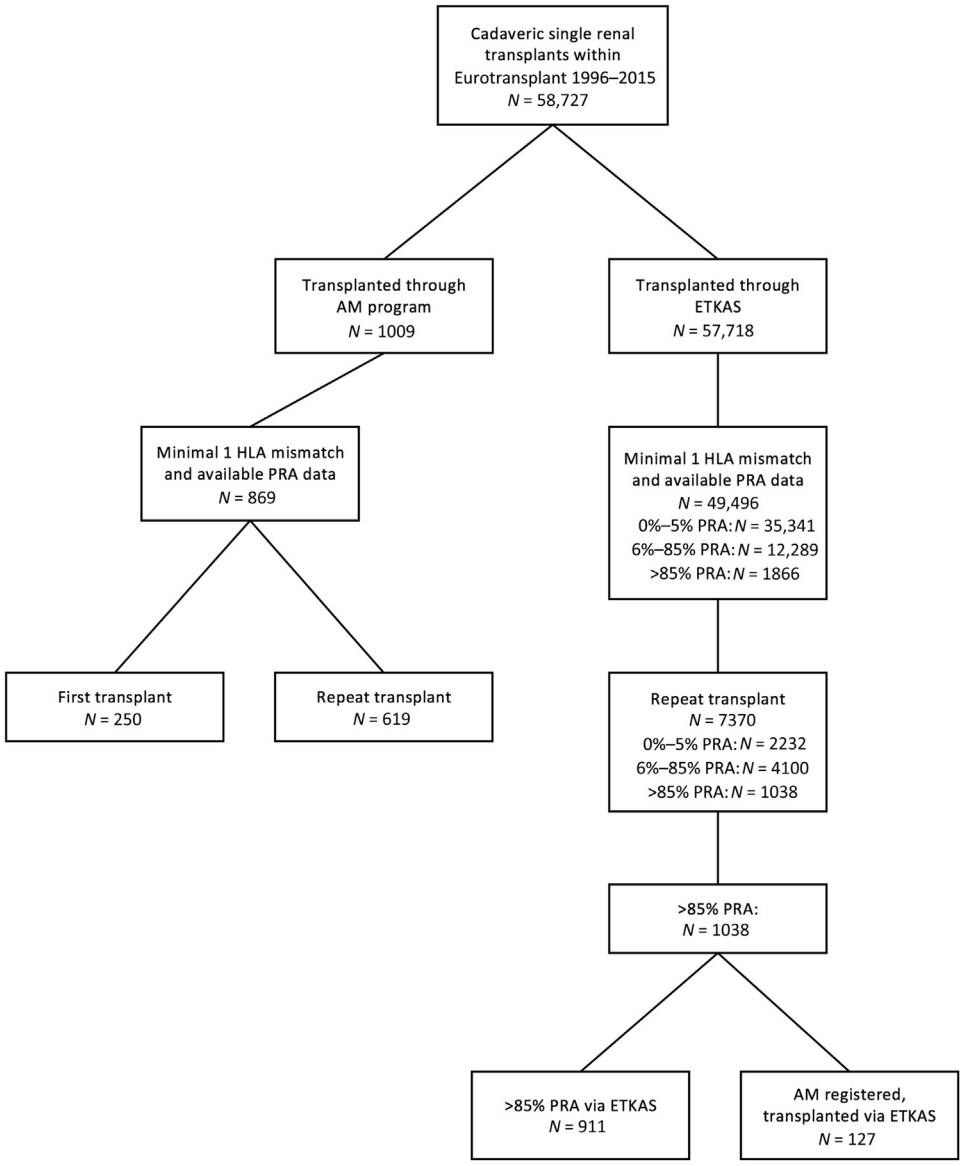


Figure 1 | Flow diagram of the study design. All cadaveric single renal transplants carried out in the Eurotransplant area between 1996 and 2015 were included in this study ($N = 58,727$). For death-censored graft survival comparison between patients transplanted through the Eurotransplant Kidney Allocation System (ETKAS) and the acceptable mismatch (AM) program, all patients receiving a renal transplant with a minimum of 1 HLA-A, HLA-B, or human leukocyte antigen (HLA)–DR broad antigen mismatch and available panel reactive antibody (PRA) data were selected ($n = 50,365$), from which 869 transplants were through the AM program and 49,496 through ETKAS.

Table 1 | Factors affecting 10-year graft survival of AM patients

	Cox regression					
	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
A-B mismatch (split antigen level)[a]						
0, 1, 2, 3, 4	0.99	0.853–1.142	0.859			
DR mismatch (split antigen level)[a]						
0, 1, 2	0.96	0.725–1.283	0.805			
Tx period						
1996–2005 (ref)						
2006–2015	1.05	0.753–1.457	0.784			
Sex of recipient						
Female (ref)						
Male	1.26	0.934–1.697	0.130			
Sex of donor						
Female (ref)						
Male	0.63	0.468–0.851	0.003	0.65	0.483–0.879	0.005
Blood group O donor						
No (ref)						
Yes	1.14	0.832–1.571	0.409			
Age of recipient (yr)						
≤50 (ref)						
>50	0.67	0.484–0.915	0.012	0.67	0.486–0.919	0.013
Age of donor (yr)						
≤50 (ref)						
>50	1.48	1.095–1.989	0.010	1.47	1.088–1.981	0.012
Current PRA (%)						
0–5 (ref)						
>5	1.86	1.0575–3.275	0.031	1.82	1.033–3.204	0.038
Donor type						
HB (ref)						
NHB	1.31	0.616–2.799	0.481			
CIP (h)[a]						
<18 (ref)						
≥18	1.06	0.764–1.472	0.724			
Waiting time (yr)[b]						
≤3 (ref)						
>3–6	0.73	0.516–1.057	0.098			
>6	1.01	0.682–1.501	0.956			
Repeat transplant						
No (ref)						
Yes	1.15	0.821–1.597	0.425			

CI, confidence interval; CIP, cold ischemic period; HB, heart beating; HR, hazard ratio; NHB, non–heart beating; PRA, panel reactive antibody; ref, reference value; Tx, transplantation.
[a]Lower number due to missing data.
[b]Based on Eurotransplant Network Information System data.

CI: 0.484 to 0.915; $P = 0.012$), and donor age >50 years increased the risk of graft loss (HR: 1.48; 95% CI: 1.095 to 1.989; $P = 0.011$). Within Eurotransplant, the status of highly sensitized is based on peak antibody levels, because both historical and current immunization is deemed important.[14,15] Patients who remain immunized based on their current serum show a higher risk of graft loss than patients who have a panel reactive antibody (PRA) of 0% to 5% (nonimmunized) in their current serum (HR: 1.86; 95% CI: 1.058 to 3.275; $P = 0.031$). Factors not affecting the risk of graft loss were HLA mismatches (either HLA-A and HLA-B, or HLA-DR, all at the split level), period of transplantation, recipient sex, donor blood group O, donor type (heart beating vs. non–heart beating), cold ischemic period (CIP), waiting time, and receiving a first or repeat transplant.

Subsequent multivariate analysis confirmed that donor sex, recipient and donor age, as well as a current PRA >5% remained significantly associated with the risk of graft loss (Table 1).

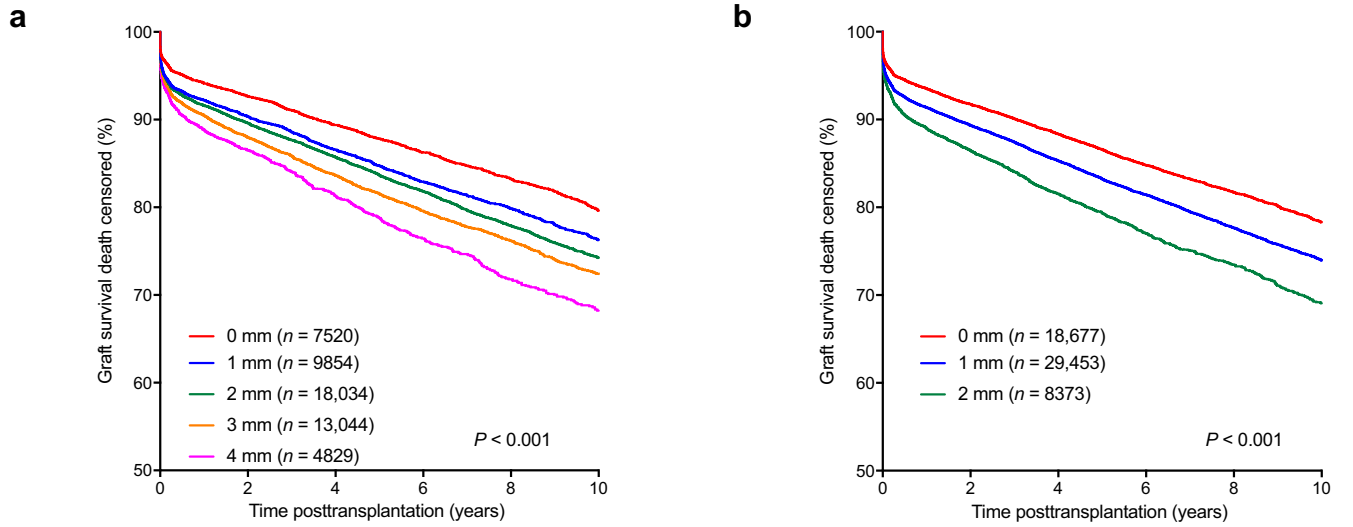
No effect of HLA-A and HLA-B, or HLA-DR mismatches on graft survival of AM patients

Previously, we have shown that there was no match effect of HLA-A, HLA-B, and HLA-DR at the broad antigen level for patients transplanted through the AM program.[12] We here extend these findings by analyzing HLA class I and HLA class II separately, both at the split antigen level. Patients transplanted through regular allocation within Eurotransplant (Eurotransplant Kidney Allocation System [ETKAS]) showed a dose-dependent decrease in 10-year graft survival for both HLA-A and HLA-B ($P < 0.001$) (Figure 2a), and HLA-DR mismatches ($P < 0.001$) (Figure 2b). In contrast, AM patients showed no match effect for either HLA class I ($P = 0.400$) (Figure 2c) or HLA class II ($P = 0.871$) (Figure 2d). It is noteworthy that in the latter analysis 2 HLA-DR mismatches hardly occur, due to the minimal match criteria of either 2 HLA-DR, or one HLA-DR with one HLA-B antigen match, that are adhered to for the large majority of AM patients.

Ten-year graft survival of patients transplanted through the AM program

To determine whether a beneficial effect of matching based on proven acceptable antigens exists, we next analyzed the 10-year death censored graft survival of patients transplanted through the AM program or ETKAS. For these and all further analyses, patients with a minimum of 1 HLA mismatch were selected. Furthermore, ETKAS patients were subdivided according to the level of sensitization (0%–5% peak PRA: nonsensitized; 6%–85% peak PRA: intermediately sensitized; and >85% peak PRA: highly sensitized), as defined by complement-dependent cytotoxicity (CDC) assays. Our analysis showed that AM patients ($n = 869$) had superior 10-year graft survival (72.8%) compared with their highly sensitized counterparts transplanted through ETKAS (62.4%, $P < 0.001$), whereas no statistically significant difference between AM patients and intermediately sensitized ETKAS patients (71.3%, $P = 1.000$) was observed. Nonsensitized patients had the highest 10-year graft survival rate of 74.8% ($P = 0.030$) (Figure 3a).

The majority of AM patients are repeat transplant candidates (71.2% vs. 14.9% in all other recipients of a renal transplant, $P < 0.001$). To determine the true benefit of defining acceptable antigens, we therefore compared 10-year death-censored graft survival of patients who received a repeat transplant, both within ($n = 619$) and outside the AM program ($n = 7370$) (Figure 3b). In this analysis, we confirmed that AM patients have superior long-term graft

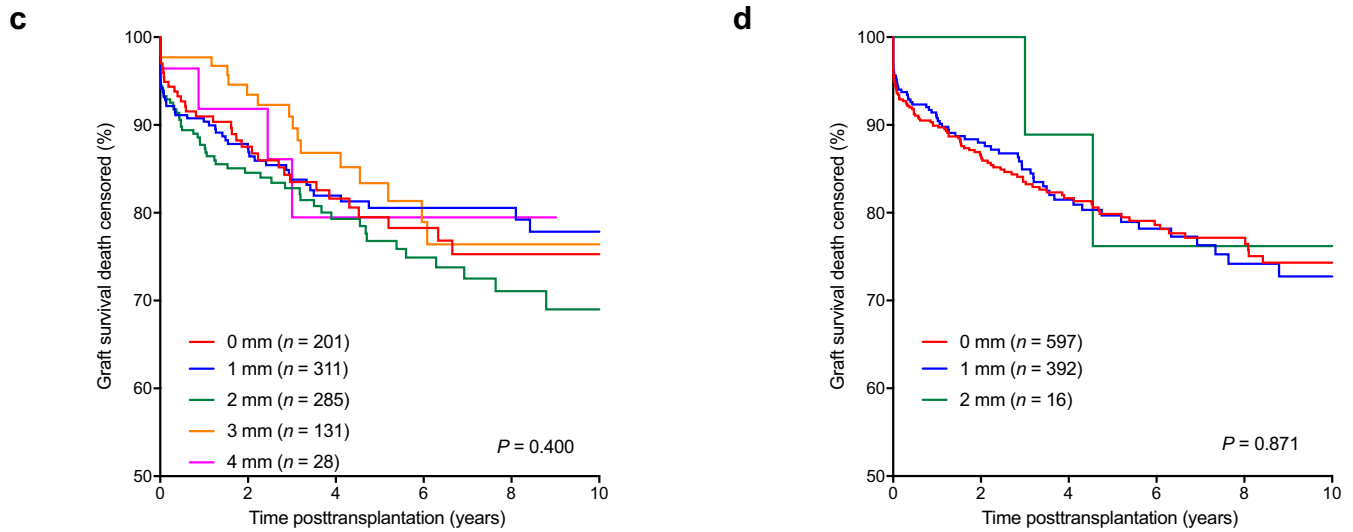


Number of transplants

0 mm	7520	5278	4032	3281	2577	1870
1 mm	9854	6609	5018	4003	3114	2195
2 mm	18,034	11,687	8537	6688	5036	3416
3 mm	13,044	8029	5584	4228	3117	2031
4 mm	4829	2785	1876	1331	907	540

Number of transplants

0 mm	18,677	12,690	9472	7564	5808	4068
1 mm	29,453	18,861	13,693	10,666	7080	5391
2 mm	8373	4920	3366	2433	1734	1043



Number of transplants

0 mm	201	115	83	56	40	30
1 mm	311	186	123	92	61	41
2 mm	285	162	102	68	41	22
3 mm	131	80	55	32	17	12
4 mm	28	18	11	4	1	0

Number of transplants

0 mm	597	355	243	169	111	70
1 mm	392	224	145	92	59	40
2 mm	16	10	8	5	2	1

Figure 2 | Match effect of human leukocyte antigen (HLA) mismatches at split antigen level. (**a**) Ten-year death-censored graft survival of patients transplanted through the Eurotransplant Kidney Allocation System stratified by HLA class I mismatches or (**b**) HLA class II mismatches. (**c**) Ten-year death-censored graft survival of patients transplanted through the acceptable mismatch program stratified by HLA class I mismatches or (**d**) HLA class II mismatches. Patients with HLA data at the split level were selected. HLA-A28, HLA-B14, HLA-B70, and HLA-DR3 were analyzed at the broad level due to the lack of split data in the Eurotransplant database, whereas other antigens lacking split information were not considered in this analysis. *P* values calculated with log-rank test. mm, mismatch.

survival (72.6%) compared with highly sensitized ETKAS patients (55.0%, $P < 0.001$). Graft survival of patients who received a repeat transplant through the AM program to intermediately sensitized (63.1%) and nonsensitized (69.3%) patients who received a repeat transplant through regular allocation was comparable ($P = 0.053$ and $P = 1.000$,

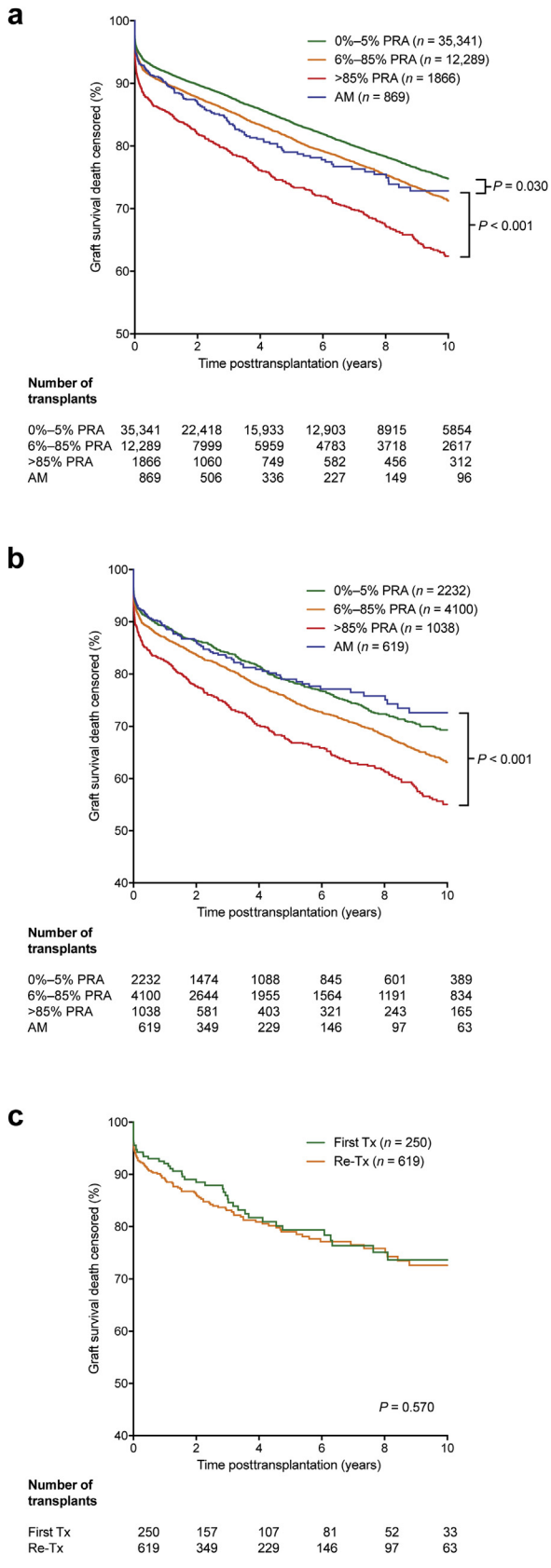


Figure 3 | Comparison of 10-year death-censored graft survival between patients transplanted through acceptable mismatch (AM) or through the Eurotransplant Kidney Allocation System. (**a**) Selection of patients with a minimum of 1 human leukocyte antigen mismatch. (**b**) Additional selection on repeat transplant recipients. (**c**) Comparison of 10-year death-censored graft survival between first and repeat transplant recipients within the AM program. *P* values calculated with log-rank test. PRA, panel reactive antibody; Tx, transplantation.

respectively). Additionally, we observed no difference in graft survival between first and repeat transplants within the AM program (73.7% vs. 72.6%, $P = 0.570$) (Figure 3c).

Factors affecting 10-year graft survival of highly sensitized patients

When comparing highly sensitized patients that received a repeat transplant through the AM program ($n = 619$) to highly sensitized patients receiving a repeat transplant through ETKAS ($n = 1038$), it was clear that the former are better matched for both HLA class I and HLA class II (both $P < 0.001$), most probably due to the aforementioned minimal match criteria (Table 2). Furthermore, the era of transplantation was different, with the majority of AM patients being transplanted between 2006 and 2015 (78.5% vs. 44.6%, $P < 0.001$). Blood group B was slightly overrepresented in the AM group ($P = 0.049$), whereas donor blood group was enriched for blood group O in the AM cohort ($P < 0.001$), most likely due to blood group compatibility for AM patients versus blood group identity in ETKAS. The percentage of donors age ≤50 years was higher for AM patients than for ETKAS patients ($P = 0.020$), whereas the percentage of patients with a current PRA of 0% to 5% was higher in patients in the ETKAS cohort ($P < 0.001$). Lastly, waiting time to transplant shifted toward a shorter waiting time for patients in the AM program as compared to ETKAS patients (waiting time since failure of previous graft, $P < 0.001$).

We next performed univariate Cox regression analysis on all highly sensitized patients who received a repeat transplant with a minimum of 1 HLA mismatch ($n = 1657$) to determine the variables that affect 10-year graft survival (Table 3). Four or more HLA-A, HLA-B, or HLA-DR mismatches increased the risk of graft loss (HR: 1.42; 95% CI: 1.122 to 1.791; $P = 0.004$), due to the match effect for ETKAS patients (Supplementary Figure S1). Receiving a transplant in a later period (2006–2015 vs. 1996–2005) was associated with a reduced risk of graft loss (HR: 0.58; 95% CI: 0.472 to 0.706; $P < 0.001$), as well as male donor sex (HR: 0.80; 95% CI: 0.660 to 0.960; $P = 0.002$). Both recipient and donor age affected the risk of graft loss, with recipient age >50 years decreasing (HR: 0.73; 95% CI: 0.593 to 0.902; $P = 0.004$), and donor age >50 years increasing the risk of graft loss (HR: 1.75; 95% CI: 1.447 to 2.106; $P < 0.001$). Likewise, a CIP of ≥18 hours increased the risk of graft loss (HR: 1.28; 95% CI: 1.042 to 1.569; $P = 0.018$). Finally, receiving a transplant through the AM program decreased the risk of graft loss (HR: 0.58; 95% CI: 0.470 to 0.723; $P < 0.001$). Factors not

Table 2 | Comparison of highly sensitized AM and ETKAS patients

	Tx AM program				
	No		Yes		
	$n = 1038$		$n = 619$		
	No.	Percent	No.	Percent	Chi-square *P* value
A-B mismatch (broad antigen level)					
0	68	6.6%	64	10.3%	<0.001
1	312	30.1%	274	44.3%	
2	418	40.3%	204	33.0%	
3	215	20.7%	65	10.5%	
4	25	2.4%	12	1.9%	
DR mismatch (broad antigen level)					
0	228	22.0%	312	50.4%	<0.001
1	640	61.7%	295	47.7%	
2	170	16.4%	12	1.9%	
A-B-DR mismatch (broad antigen level)					
1	129	12.4%	212	34.3%	<0.001
2	278	26.8%	231	37.3%	
3	394	38.0%	140	22.6%	
4	185	17.8%	30	4.9%	
5	46	4.4%	6	1.0%	
6	6	0.6%	0	0.0%	
Tx period					
1996–2005	575	55.4%	133	21.5%	<0.001
2006–2015	463	44.6%	486	78.5%	
Sex of recipient					
Female	479	46.2%	294	47.5%	0.594
Male	559	53.9%	325	52.5%	
Sex of donor					
Female	447	43.1%	271	43.8%	0.776
Male	591	56.9%	348	56.2%	
ABO recipient					
O	401	38.6%	219	35.4%	0.049
A	452	43.6%	266	43.0%	
B	121	11.7%	101	16.3%	
AB	64	6.2%	33	5.3%	
ABO donor					
O	452	43.6%	431	69.6%	<0.001
A	446	43.0%	157	25.4%	
B	99	9.5%	29	4.7%	
AB	41	4.0%	2	0.3%	
Age of recipient (yr)					
≤50	666	64.2%	393	63.5%	0.783
>50	372	35.8%	226	36.5%	
Age of donor (yr)					
≤50	611	58.9%	400	64.6%	0.020
>50	427	41.1%	219	35.4%	
Donor type					
HB	1009	97.2%	595	96.1%	0.225
NHB	29	2.8%	24	3.9%	
Current PRA (%)[a]					
0–5	207	20.0%	72	11.6%	<0.001
6–85	537	51.8%	384	62.0%	
>85	293	28.3%	163	26.3%	
CIP (h)[a]					
<18	453	53.6%	242	52.8%	0.807
≥18	393	46.5%	216	47.2%	
Waiting time (yr)[a,b]					
≤3	239	25.5%	219	36.6%	<0.001
>3–6	279	29.8%	188	31.4%	
>6	419	44.7%	191	31.9%	

AM, acceptable mismatch; CIP, cold ischemic period; ETKAS, Eurotransplant Kidney Allocation System; HB, heart beating; NHB, non–heart beating; PRA, panel reactive antibody; Tx, transplantation.
[a]Lower number due to missing data.
[b]Waiting time since failure of previous transplant.

affecting the risk of graft loss were recipient sex, donor blood group O, donor type (heart beating vs. non–heart beating), current PRA, and waiting time.

Subsequent multivariate analysis on variables significant in the univariate analysis confirmed that besides HLA mismatch, transplant period, donor sex, recipient and donor age, receiving a transplant through the AM program remained significantly associated with the risk of graft loss (Table 3). CIP did not independently affect the risk of graft loss in the multivariate model.

The use of proven acceptable antigens is superior to the use of unacceptable antigens only

A proportion of highly sensitized patients that received a transplant through ETKAS were actually registered on the AM waiting list. For this subset of patients, acceptable antigens had been defined, but the ETKAS allocation was based on avoidance of unacceptable antigens only. To validate the benefit of using acceptable antigens for allocation to highly sensitized patients, we performed graft survival analysis on AM patients transplanted through the AM program ($n = 619$), AM patients transplanted through ETKAS ($n = 127$) and all other highly sensitized patients transplanted through ETKAS ($n = 911$). When acceptable antigens were defined, but not used for allocation, 10-year graft survival was comparable to that of highly sensitized patients allocated based on avoidance of unacceptable antigens (57.4% vs. 54.8%, $P = 1.000$) (Figure 4). AM patients allocated through the AM program had significantly better 10-year graft survival (72.6%) compared with that of both patient groups ($P = 0.041$ and $P < 0.001$, respectively).

DISCUSSION

It is of great importance to transplant highly sensitized patients with optimal long-term graft survival, as well as minimizing the chance of additional sensitization. In our initial analysis, we showed that patients transplanted through the AM program have a substantially superior graft survival compared with that of highly sensitized patients transplanted on the basis of avoidance of unacceptable mismatches and a slightly inferior graft survival compared with nonsensitized

Table 3 | Factors affecting 10-year graft survival of all highly sensitized repeat transplant recipients

	Cox regression					
	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
A-B-DR mismatch (broad antigen level)						
1, 2, 3 (ref)						
4, 5, 6	1.42	1.122–1.791	0.003	1.27	1.001–1.618	0.049
Tx period						
1996–2005 (ref)						
2006–2015	0.58	0.472–0.706	<0.001	0.62	0.506–0.771	<0.001
Sex of recipient						
Female (ref)						
Male	1.04	0.859–1.250	0.711			
Sex of donor						
Female (ref)						
Male	0.80	0.660–0.960	0.017	0.82	0.677–0.985	0.034
Blood group O donor						
No (ref)						
Yes	1.16	0.963–1.400	0.118			
Age of recipient (yr)						
≤50 (ref)						
>50	0.73	0.593–0.902	0.004	0.77	0.620–0.949	0.014
Age of donor (yr)						
≤50 (ref)						
>50	1.75	1.447–2.106	<0.001	1.80	1.489–2.174	<0.001
Donor type						
HB (ref)						
NHB	1.09	0.614–1.936	0.769			
Current PRA (%)[a]						
0–5 (ref)						
>5	1.16	0.895–1.505	0.263			
CIP (h)[a]						
<18 (ref)						
≥18	1.28	1.042–1.569	0.018			
Waiting time (yr)[a,b]						
≤3 (ref)						
>3–6	1.02	0.800–1.313	0.847			
>6	0.99	0.778–1.257	0.930			
Tx via AM program						
No (ref)						
Yes	0.58	0.470–0.723	<0.001	0.71	0.564–0.891	0.003

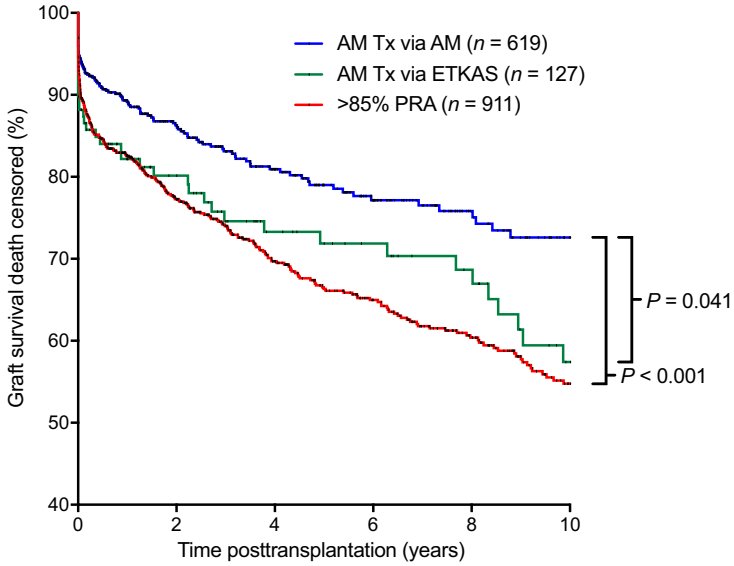
AM, acceptable mismatch; CI, confidence interval; CIP, cold ischemic period; HR, hazard ratio; PRA, panel reactive antibody; ref, reference value; Tx, transplantation.
[a]Lower number due to missing data.
[b]Waiting time since failure of previous transplant.

patients transplanted through regular allocation. However, when we analyzed repeat transplant candidates (the majority of AM patients), long-term graft survival was similar between AM patients and nonimmunized ETKAS patients. This indicates that clinically relevant antibodies in highly sensitized patients are mainly cytotoxic and can be detected by CDC.[16] However, due to the retrospective nature of this study, the effect of antibodies detected by solid phase assay only could not be determined[17] and will be subject to future studies. We additionally showed that long-term graft survival was significantly decreased when AM patients were transplanted based on allocation through the avoidance of unacceptable mismatches only. These data are of importance, because the OPTN has recently introduced a new kidney allocation system wherein highly sensitized patients are prioritized, but allocation remains based on the avoidance of unacceptable mismatches.[5,18] Our data suggest that despite prioritizing highly sensitized patients, continuation of allocation through the avoidance of unacceptable mismatches will lead to suboptimal graft survival. This is likely to result in more highly sensitized patients returning to the transplant waiting list after a failed transplant with a high chance of an even broader immunization status. Importantly, by using the AM program principle, besides excellent graft survival, there is also a shorter waiting time for highly sensitized patients compared with highly sensitized patients receiving an organ through regular allocation (Table 2 and Supplementary Figure S2). The current lack of a number of specific parameters in the Eurotransplant Network Information System (ENIS) led to some limitations of the current study. We could not analyze the occurrence of rejection episodes, the level of epitope mismatches (lack of high-resolution HLA typing data), or the effect of pretransplant solid phase assay–only detectable antibodies. The latter will be the subject of study in the Dutch multicenter Profiling Consortium of Antibody Repertoire and Effector functions (PROCARE) consortium study, in which the effect of pretransplant Luminex antibodies is studied for 6000 Dutch renal transplant recipients, including patients transplanted through the AM program.[19]

There are several, not mutually exclusive explanations as to why patients who receive a transplant based on proven acceptable mismatches have similar graft survival as nonsensitized patients do. First, for all AM patients, the absence of certain HLA antibodies in both historical and current sera is actively determined by using extended HLA antibody screening.[12] This is in contrast to normal allocation, in which only the presence of HLA antibodies is determined and the assumption is made that the other HLA antigens are thus acceptable.

The second explanation is that the acceptable antigens defined for a patient include the noninherited maternal antigens (NIMA) to which acquired neonatal tolerance has been established.[20] It has previously been shown that for many highly sensitized patients (58%), the proven acceptable HLA class I antigens included noninherited maternal antigens, in contrast to the noninherited paternal antigens of which only 8% were acceptable HLA class I antigens.[21] A third explanation is that the acceptable antigens harbor a high number of epitopes shared with the patient's own HLA repertoire. Indeed, for HLA class I epitope analysis by HLAMatchmaker is used in the AM program alongside antibody screening assays for identifying acceptable antigens and is likely to contribute to the beneficial outcomes.[22]

By defining acceptable antigens, and taking into consideration HLA epitope sharing,[12] the chance of additional sensitization on transplantation is reduced. Currently, acceptable antigens are defined at HLA-A, HLA-B, HLA-C, HLA-DRB, and HLA-DQB, but in the future, defining acceptable antigens based on additional HLA-DQA, HLA-DPA, and HLA-DPB information will probably lead to even



Number of transplants						
AM Tx AM	619	349	229	146	97	63
AM Tx ETKAS	127	75	55	47	40	27
>85% PRA	911	506	348	274	203	138

Figure 4| Comparison of 10-year death-censored graft survival between acceptable mismatch (AM) patients transplanted through the AM program, AM patients transplanted through Eurotransplant Kidney Allocation System (ETKAS), and highly sensitized patients outside the AM program transplanted through ETKAS. *P* values calculated with log-rank test. PRA, panel reactive antibody; Tx, transplantation.

better outcomes. Indeed, it has repeatedly been shown that the majority of antibodies formed after transplantation are directed at HLA-DQ.[23,24] With the advance in high resolution typing, a further step in the direction of epitope matching is impending.[25] Whereas acceptable antigens are already in part defined by epitope analysis for HLA class I, extension to HLA class II will be a major step forward. Indeed, it has already been shown that grafts with a low level of HLA class II epitope mismatches result in less antibody formation.[26,27] Finally, the use of solid phase assay to define acceptable antigens (even on the allele level) is likely to be beneficial, especially once high-resolution typing data can be obtained during deceased donor procedures. Of note, defining acceptability based on highly sensitive solid phase assay is a fine balance between excluding too many antigens thereby preventing transplantation, and the possibility to define acceptable antigens accurately to facilitate transplantation of highly sensitized patients.[28]

In conclusion, we show here that highly sensitized patients can be transplanted with a far superior long-term graft survival when allocation is based on proven acceptable mismatches instead of avoidance of unacceptable mismatches only. Improving long-term graft survival should be a priority in the allocation of highly sensitized patients and is feasible with the acceptable mismatch approach.

MATERIALS AND METHODS

The AM program

Eligibility for inclusion into the AM program are a cumulative waiting time on the regular waiting list of at least 2 years, and a CDC PRA of >85% in either historic or current serum samples. Acceptable antigens are defined by using several assays as has been described elsewhere.[12] HLA matching on the patient's own HLA antigens and additional acceptable antigens is performed on the split antigen level. Minimal match criteria on identity of either 2 HLA-DR or 1 HLA-DR antigen with 1 HLA-B antigen at split level is adhered to. For patients with a chance of receiving a kidney through the AM program of <0.1% (based on immunological grounds), these minimal match criteria are abandoned. Furthermore, AM patients are transplanted based on blood group compatibility, whereas regular allocation through ETKAS is based on blood group identity.

Patients

We used a cohort study design to determine the benefit of receiving an organ based on acceptable mismatches, for which data were extracted from ENIS on December 5, 2015. Donor, transplant, recipient, and follow-up data were obtained from ENIS with informed consent of the Eurotransplant Tissue Typing Advisory Committee. All data were anonymous. We selected from this database all cadaveric single renal transplants carried out in the Eurotransplant area between 1996 and 2015, which determined the sample size ($N = 58{,}727$). From these data, patients transplanted through the AM program were selected ($n = 1009$). Patients included on the AM waiting list remain on the ETKAS waiting list as well. AM patients actually transplanted through ETKAS (and thus received an organ based on the absence of unacceptable antigens only) are included in the >85% PRA ETKAS group. For analysis of the effect of HLA antigen mismatches on 10-year graft survival, patients with HLA data at the split level were selected. HLA-A28, HLA-B14, HLA-B70, and HLA-DR3 were analyzed at the broad level due to the lack of split data in the Eurotransplant database,

whereas other broad antigens lacking split information were not considered in this analysis.

For death-censored graft survival comparison between patients transplanted through ETKAS and the AM program, all patients receiving a renal transplant with a minimum of 1 HLA-A, HLA-B, or HLA-DR broad antigen mismatch and available PRA data were selected because the effect of acceptable mismatches was studied. This led to a subset of 50,365 patients, from which 869 transplants were through the AM program. All transplants were performed on basis of a negative CDC crossmatch.

Data handling

Groupings of quantitative variables were based on the following strategies: transplant period was divided into 2 decades, recipient and donor age was set at 50 years based on previous studies,[29] percentage PRA was based on Eurotransplant definitions, CIP was based on previously published data,[30] waiting time was based on equal group size for AM patients (Table 2), and HLA mismatches were divided into equal categories. Missing HLA split typing data was due to technical limitations in HLA typing techniques (Supplementary Table S2), while missing data in current PRA, CIP, waiting time was due to incomplete records in ENIS (Supplementary Tables S2 and S3). In graft survival analyses, graft loss, death, date last seen, or date of data extraction were used as end points. Patients who died with a functioning graft were censored at time of death. Median follow-up times for all analyses are presented in Supplementary Table S4, and mortality rates for the patient groups compared in Figure 3b are presented in Supplementary Table S5.

Statistical analysis

The chi-square test was used to compare characteristics between 2 groups. Survival curves were calculated by the Kaplan-Meier method. Statistical significance was determined by using the log-rank test, corrected for multiple comparisons (Bonferroni method), where applicable. Inclusion criterion for inclusion in the multivariate analysis was a univariate P value of <0.1. Multivariate Cox regression analysis with backward elimination (Wald) was performed to determine independent effects on graft survival. P values were 2-tailed, and those <0.05 were considered statistically significant. SPSS version 23 (IBM, Armonk, NY) and GraphPad Prism, version 6.0h (GraphPad Software, La Jolla, CA) were used.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

GWH, MDW, JJvR, and SH analyzed the data analysis. SH and FHJC wrote the manuscript.

SUPPLEMENTARY MATERIAL

Figure S1. Human leukocyte antigen (HLA) mismatches are detrimental for graft survival of organs transplanted to highly sensitized patients through regular allocation and not for graft survival of those transplanted through the AM program. (**A**) Ten-year death-censored graft survival of highly sensitized patients transplanted through Eurotransplant Kidney Allocation System (ETKAS). (**B**) Ten-year death-censored graft survival of patients transplanted through the AM program. Mismatches are determined at the split antigen level. P values calculated with log-rank test. mm, mismatch.
Figure S2. Patients transplanted through the acceptable mismatch (AM) program ($n = 1009$) have a shorter waiting time compared with that of highly sensitized patients transplanted through Eurotransplant Kidney Allocation System (ETKAS). ETKAS patients are subdivided according to the level of sensitization (0%–5% peak panel reactive antibody [PRA]: nonsensitized $n = 41{,}483$; 6%–85% peak PRA: intermediately sensitized $n = 14{,}013$; and >85% peak PRA: highly sensitized $n = 2081$), as defined by complement-dependent cytotoxicity (CDC). Highly sensitized patients have to be on the regular waiting list for at least 2 years before they are allowed to enter the AM program, which is included in the AM program waiting time. Mean years with 95% confidence interval (CI) are shown.
Table S1. Clinical characteristics of patients who received an organ through the Eurotransplant Acceptable Mismatch (AM) program.
Table S2. Missing data for clinical characteristics of Eurotransplant Acceptable Mismatch (AM) cohort described in Table S1.
Table S3. Missing data for case-control study described in Table 2.
Table S4. Median follow-up time for all analyses.
Table S5. Mortality rates for the patient groups compared in Figure 3b.
Supplementary information is linked to the online version of the paper at www.kidney-international.org.

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