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BRIEF COMMUNICATION

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Preemptive treatment of early donor-specific antibodies with IgA- and IgM-enriched intravenous human immunoglobulins in lung transplantation

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This retrospective study presents our 4-year experience of preemptive treatment of early anti-HLA donor specific antibodies with IgA- and IgM-enriched immunoglobulins. We compared outcomes between patients with antibodies and treatment (case patients) and patients without antibodies (control patients). Records of patients transplanted at our institution between March 2013 and November 2017 were reviewed. The treatment protocol included one single 2 g/kg immunoglobulin infusion followed by successive 0.5 g/kg infusions for a maximum of 6 months, usually combined with a single dose of anti-CD20 antibody and, in case of clinical rejection or positive crossmatch, with plasmapheresis or immunoabsorption. Among the 598 transplanted patients, 128 (21%) patients formed the case group and 452 (76%) the control group. In 116 (91%) patients who completed treatment, 106 (91%) showed no antibodies at treatment end. Fourteen (13%) patients showed antibody recurrence thereafter. In case versus control patients and at 4-year follow-up, respectively, graft survival (%) was 79 versus 81 (P = .59), freedom (%) from biopsy-confirmed rejection 57 versus 53 (P = .34), and from chronic lung allograft dysfunction 82 versus 78 (P = .83). After lung transplantation, patients with early donor-specific antibodies and treated with IgA- and IgM-enriched immunoglobulins had 4-year graft survival similar to patients without antibodies and showed high antibody clearance.

Abbreviations: AMR, antibody-mediated rejection; CI, confidence interval; CLAD, chronic lung allograft dysfunction; CMV, Cytomegalovirus; ECMO, extracorporeal membrane oxygenation; eDSA, early anti-HLA donor specific antibodies; EVLP, ex-vivo lung perfusion; FEV_1, forced expiratory volume in 1 second; FFP, fresh frozen plasma; HLA, human leucocyte antigen; ICU, intensive care unit; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins; IQR, interquartile range; ISHLT, International Society for Heart and Lung Transplantation; MFI, mean fluorescence index; PC, platelets concentrate; PGD, primary graft dysfunction; PRBCs, peripheral red blood cells; SD, standard deviation; tPE, therapeutic plasmapheresis.

Igor Tudorache and Gregor Warnecke share senior authorship.

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KEYWORDS
clinical research/practice, graft survival, immunosuppression/immune modulation, intravenous immunoglobulin/IVIG, lung (allograft) function/dysfunction, lung transplantation/pulmonology, major histocompatibility complex (MHC), rejection: antibody-mediated (ABMR)

1 | INTRODUCTION

The development of antibodies against donor human leukocyte antigens (donor specific antibodies, DSA) after lung transplantation has been associated with antibody-mediated rejection (AMR), chronic lung allograft dysfunction (CLAD) and patient mortality.[1-9]

However, there are many open questions concerning DSA and AMR treatment.[10] Different protocols have been used, making any conclusion about treatment efficacy difficult.[11-17] Treatment of clinical AMR has shown suboptimal efficacy, since the graft dysfunction may not be reversible anymore.[12,17]

Since March 2013, at our institution, patients who developed DSA early after transplantation (eDSA) have been treated with a protocol based on successive infusion of IgA- and IgM-enriched intravenous human immunoglobulins (IgGAM, Pentaglobin, Biotest AG, Dreieich, Germany). In our experience, treated patients showed good eDSA clearance and short-term graft survival that was comparable to survival of patients without eDSA.[14]

This retrospective study presented our 4-year experience of early DSA treatment with IgGAM in lung transplantation. We compared outcomes between patients with eDSA treated with IgGAM and patients without eDSA.

2 | METHODS

2.1 | Patients

The in-hospital and follow-up records of patients who underwent lung transplantation at our institution between March 2013 and November 2017 were retrospectively reviewed.

Patients who showed eDSA after transplantation and were treated with IgGAM formed the $eDSA^+/IgGAM^+$ group (case group). The outcomes of $eDSA^+/IgGAM^+$ patients were compared to the outcomes of patients who did not show eDSA after transplantation ($eDSA^-$ patients, control group).

Patients, who showed eDSA and were treated without IgGAM ($eDSA^+/IgGAM^-$ patients), and the few patients who showed eDSA but were not treated at all ($eDSA^+$/no-treatment patients), were excluded from the study. However, their results were reported in the supporting information section.

Follow-up ended on November 1, 2017 and was 100% completed.

The hospital ethical review board waived the need of patient consent to the study, since all patients had given their consent to handle their personal data for research purposes at the time of listing to lung transplantation. In addition, in $eDSA^+/IgGAM^+$ patients, a patient consent was obtained to perform the additional DSA controls at follow-up.

2.2 | Variable definition

The present study focused on the treatment of early DSA, which were defined as DSA, which were detected during initial hospitalization after lung transplantation, before hospital discharge.

eDSA clearance was defined as absence of DSA in two consecutive Luminex-based SPA (LIFECODES, Immucor Transplant Diagnostics, Inc., Stamfort, CT) controls. DSA recurrence was defined as a renewed positivity of previously cleared DSA at Luminex-based SPA control.

The definitions of other variables and outcomes are reported elsewhere.[3,13,14,18-20] Details on patient management after transplantation at our institution are reported in the supporting information section of this manuscript.[3,13,14]

2.3 | eDSA detection protocol

All patients were screened for anti-HLA antibodies at the time of listing to lung transplantation, and for eDSA, immediately before lung transplantation, on day 14 and before hospital discharge or upon indication. In the Luminex analysis, a low threshold of 1000 mean fluorescence index (MFI) was used to detect eDSA.

At follow-up, in $eDSA^+/IgGAM^+$ patients, Luminex-based DSA controls were performed at the beginning of each IgGAM treatment session and, after treatment end, every 6 months. In $eDSA^-$ as well as excluded $eDSA^+/IgGAM^-$ and $eDSA^+$/no-treatment patients, DSA were not regularly assessed, but only upon indication.

2.4 | eDSA treatment protocols

In March 2013, an IgGAM-based treatment protocol replaced the previous rather ineffective eDSA treatment protocol which had been based only on therapeutic plasmapheresis (tPE) and a single dose of anti-CD 20 antibody (Rituximab).[13,14] Pentaglobin was used, since it has been demonstrated that its IgA and IgM components conferred additional immunomodulatory and antimicrobial effects.[21] eDSA treatment with IgGAM represents an off-label use of IVIG.

Treatment was usually performed preemptively, since most of the patients showed only serologic evidence of eDSA (possible subclinical AMR[9]). In those patients with graft dysfunction, dysfunction was defined as worsening of blood oxygenation and/or lung function tests, unexplained by concomitant infection. In this case, diagnosis of definite clinical AMR was not made, since transbronchial biopsies

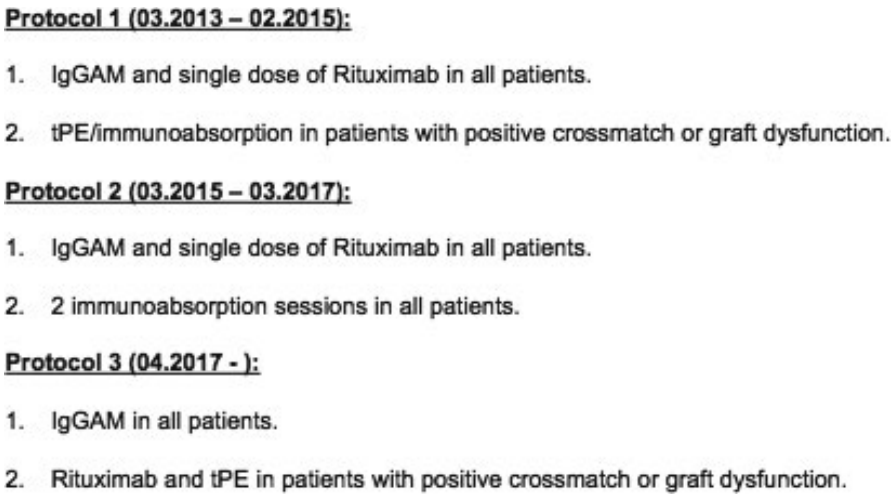

FIGURE 1 During the study period, three IgGAM-based treatment protocol were employed at our institution. In the first protocol, 3 or 5 sessions of tPE preceded the first IgGAM dose in those patients with graft dysfunction or positive crossmatch. In the second protocol, 2 sessions of immunoabsorption using tryptophan columns preceded the first IgGAM dose in all patients, in an effort to shorten treatment time. In both protocols, a single dose of Rituximab (375 mg/m^2) was administered following the first IgGAM dose. Since April 2017, immunoabsorption has been eliminated, and tPE and Rituximab were given only in case of presence of positive crossmatch or graft dysfunction. IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins; tPE, therapeutic plasmapheresis

were usually not performed early after transplantation for safety reasons (possible clinical AMR[9]).

IgGAM therapy consisted of a first infusion of 2 g/kg of IgGAM followed by additional infusions of 0.5 g/kg of IgGAM every 4 weeks until eDSA clearance or for a maximum of 6 months. Other procedures and drugs, comprising 3 distinct successive treatment protocols, were added to the first IgGAM infusion (Figure 1).

More treatment details are reported in the supporting information section.

2.5 | Statistics

IBM SPSS 24.0 (IBM, NY) was used for the data analysis. Primary endpoints were graft survival and eDSA clearance at treatment end. Secondary endpoints were patient survival, freedom from pulsed-steroid therapy, biopsy-confirmed acute rejection, CLAD, retransplant and infection requiring hospitalization.

Categorical and continuous variables were summarized as percentages and median with interquartile range (IQR), respectively. The non-parametric Mann-Whitney test and the Chi-squared test or the Fisher's exact test were used for group comparisons of continuous and categorical variables, respectively.

Survival estimates along with freedom from endpoints were calculated by the product-limit method of Kaplan-Meier. Differences between groups were quantified using the log-rank test.

In order to account for the influence on outcomes of the variables which showed a statistical significant difference ($P \leq .05$) among included eDSA$^+$/IgGAM$^+$ and eDSA$^-$ patients, propensity scores were developed based on 4 covariates in a logistic regression model with IgGAM treatment for eDSA as the dependent variable. The variables were age at transplantation under 18 years old, pulmonary artery hypertension as indication to transplantation, lung retrieval with portable ex-vivo lung perfusion (EVLP), and evidence of antibodies against HLA class II before transplantation (Tables 1-4).

Study endpoints were thus evaluated using propensity scores as balancing scores in two ways[22]: first, 123 eDSA$^+$/IgGAM$^+$ patients were 1:1 matched to 123 eDSA$^-$ patients. Second, all included patients were stratified into quintiles on the basis of having similar propensity scores. Each endpoint was then evaluated within each quintile.

P-values ≤ .05 were considered significant.

3 | RESULTS

3.1 | Patient groups

Between March 2013 and November 2017, among the 598 patients who underwent lung transplantation at our institution, 146 (24%) patients showed a positive crossmatch or eDSA, and the remaining 452 (76%) patients did not (control group). Percentage of eDSA$^+$/crossmatch$^+$ patients for each study year is reported in Figure S1. Among the 146 patients, 128 (88%) patients underwent treatment with IgGAM (eDSA$^+$/IgGAM$^+$ group, case group). Among the remaining 18 (12%) patients, 8 (5%) patients were treated only with tPE and a single dose of Rituximab (eDSA$^+$/IgGAM$^-$ group), and 10 (7%) patients were not treated at all (eDSA$^+$/no-treatment group). Patient groups are reported in Figure 2. Pretransplant, intraoperative, and posttransplant recipient and donor characteristics in eDSA$^+$/IgGAM$^+$ vs. eDSA$^-$ patients are reported in Tables 1 to 4 and in Tables S1 and S2.

3.2 | eDSA

In case group, 21 (16%) patients showed pre-formed eDSA. The remaining 107 (84%) patients developed de-novo eDSA. eDSA were

TABLE 1 Preoperative recipient data

Variable	eDSA$^+$/IgGAM$^+$ (n = 128)	eDSA$^-$ (n = 452)	*P* value
Female sex	61 (48)	213 (47)	.84
Age (y)	49 (31-58)	52 (38-59)	.25
Age < 18 y	18 (14)	26 (6)	.002
Age > 60 y	19 (15)	66 (15)	.95
BSA (m^2)	1.70 (1.54-1.90)	1.74 (1.56-1.94)	.76
Transplant indication			
COPD	38 (30)	116 (26)	.33
Pulmonary fibrosis	36 (28)	160 (35)	.12
Cystic fibrosis	24 (19)	99 (22)	.44
Pulmonary hypertension	15 (12)	17 (4)	<.001
Re-transplant	11 (9)	32 (7)	.56
Other	5 (4)	28 (6)	.32
Associated pulmonary artery hypertension	47 (37)	182 (40)	.47
LAS score	36.1 (32.6-42.4)	36.1 (33.2-41.6)	.99
Preoperative mechanical ventilation	3 (2)	15 (3)	.57
Preoperative intensive care unit	14 (11)	40 (9)	.47
Preoperative ECMO/iLA	13 (10)	25 (6)	.062

Values are expressed as median (IQR, interquartile range) or N of patients (%). BSA, body surface area; COPD, chronic obstructive pulmonary disease; ECMO, extracorporeal membrane oxygenation; eDSA, early donor-specific antibodies; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins; iLA, interventional Lung Assist Novalung; LAS, lung allocating score.

TABLE 2 Donor and intraoperative recipient characteristics

Variable	eDSA$^+$/IgGAM$^+$ (n = 128)	eDSA$^-$ (n = 452)	*P* value
Donor characteristics			
Female sex	71 (56)	212 (47)	.091
Age (y)	51 (38-59)	50 (37-59)	.49
Age > 70 y	7 (6)	30 (7)	.63
BSA (m^2)	1.90 (1.77-2.05)	1.91 (1.77-2.08)	.83
Ventilation time (d)	4 (2-8)	4 (2-7)	.87
pO_2 (100%, mmHg)	397 (329-453)	377 (312-441)	.48
Smoking history	55 (43)	183 (41)	.63
Contusion	13 (10)	37 (8)	.49
Aspiration	7 (6)	26 (6)	.90
Lung preservation			
Celsior	113 (88)	367 (83)	.15
Portable EVLP	3 (2)	32 (7)	.047
Intraoperative recipient characteristics			
Single lung	3 (2)	12 (3)	.86
Double lung	125 (98)	440 (97)	.84
Cardiopulmonary bypass	2 (2)	9 (2)	1.00
Intraoperative ECMO	34 (27)	118 (26)	.95
Postoperative extended ECMO	16 (13)	39 (9)	.19
Ischemic time (min)			
First lung	400 (315-477)	401 (319-495)	.96
Second lung	507 (429-590)	507 (414-604)	.97

Values are expressed as median (IQR, interquartile range) or N of patients (%). BSA, body surface area; ECMO, extracorporeal membrane oxygenation; eDSA, early donor-specific antibodies; EVLP, ex-vivo lung perfusion; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins.

more often against donor HLA class II than I antigens (81% vs. 25%, Table 3). Twelve (9%) patients showed eDSA against both HLA class antigens. Median time to eDSA positivity was 14 (11-20) days. Before treatment start, median MFI value was 4279 (2264-9983). Median cumulative MFI value was 4961 (2290-11 197).

3.3 | eDSA treatment and IgGAM side effects

Treatment was performed preemptively in 110 (86%) patients. The remaining 18 (14%) patients had evidence of graft dysfunction.

Before the first IgGAM infusion, 18 (14%) patients underwent tPE (3 sessions in 13 patients and 5 sessions in 5 patients), and 37 (29%) patients 2 sessions of immunoabsorption. A single dose of Rituximab was given in 112 (88%) patients after the first IgGAM infusion. A hundred and eight (84%) patients underwent at least one consecutive 0.5 g/kg IgGAM infusion (median 3, [2-5] infusions) at follow-up (median treatment time 3 [2-5] months). Figure S2 shows eDSA treatment. There was no difference between protocols 1 and 2 regarding the number of additional 0.5 g/kg IgGAM infusions (median 4 vs. 3, *P* = .35) or treatment time (median 4. vs. 3 months, *P* = .16).

Overall, 493 IgGAM infusions (2 g/kg, n = 128, and 0.5 g/kg, n = 365) were performed. During IgGAM infusions, anemia, defined as a drop of the haemoglobin value below 8 g/dl or of at least 2 g/dl after IgGAM infusion, was detected 26 (5%) times; allergic reaction, 6 (1.2%) times; nausea and abdominal pain, 22 (4.5%) times. In one (0.7%) patient, IgGAM treatment was withdrawn earlier as intended per protocol due to recurrent abdominal pain at each IgGAM infusion.

3.4 | eDSA clearance

eDSA clearance is reported in Figure 3 and Table 5. Among the 128 eDSA$^+$/IgGAM$^+$ patients, 116 (91%) patients completed

TABLE 3 Anti-HLA antibodies

Variable	eDSA$^+$/IgGAM$^+$ (n = 128)	eDSA$^-$ (n = 452)	*P* value
Preoperative anti-HLA antibodies			
Anti-HLA I	26 (20)	83 (18)	.62
Anti-HLA II	40 (31)	86 (19)	.003
Anti-HLA I + anti-HLA II	9 (7)	24 (5)	.46
Cumulative mismatches			
HLA A + B	3 (2-4)	3 (3-4)	.04
HLA A + B + DR	5 (4-6)	5 (4-5)	<.001
Postoperative anti-HLA antibodies[a]			
Anti-HLA I	56 (44)	98 (22)	<.001
Anti-HLA II	111 (87)	116 (26)	<.001
Anti-HLA I + anti-HLA II	43 (34)	45 (10)	<.001
Postoperative anti-HLA eDSA			
HLA A	15 (12)		
HLA B	21 (16)		
HLA C	2 (2)		
HLA DR	12 (9)		
HLADQ	103 (81)		
Positive crossmatch	10 (8)		

Values are expressed as median (IQR) or N of patients (%). eDSA, early donor specific antibodies; HLA, human leukocyte antigen; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins.All patients who developed anti-HLA antibodies after lung transplantation were considered, independently of DSA positivity.

TABLE 4 Postoperative data

Variable	eDSA$^+$/IgGAM$^+$ (n = 128)	eDSA$^-$ (n = 452)	*P* value
PGD score grade 2 or 3			
24 h	20 (16)	51 (11)	.18
48 h	21 (17)	57 (13)	.27
72 h	17 (13)	44 (10)	.24
Rethoracotomy for bleeding	8 (6)	36 (8)	.51
New dialysis	6 (5)	38 (8)	.16
Postoperative pulsed steroid therapy	49 (38)	133 (30)	.061
Secondary ECMO	2 (2)	9 (2)	1.00
Tracheostomy	12 (9)	35 (8)	.55
Ventilation time, h	11 (8-14)	11 (8-17)	.84
ICU stay, d	2 (1-5)	2 (1-4)	.23
Hospital stay, d	25 (22-34)	22 (21-27)	<.001
In-hospital mortality	4 (3)	21 (5)	.45
Immunosuppressive therapy at discharge after transplantation[a]			
Cyclosporine	0	3 (1)	1.00
Tacrolimus	124 (100)	428 (99)	1.00
Mycofenolate mofetil	123 (99)	431 (100)	.22
Immunosuppressive therapy at last outpatient control[a]			
Cyclosporine	4 (3)	54 (13)	.003
Tacrolimus	117 (95)	375 (87)	.012
Mycofenolate mofetil	112 (92)	401 (93)	.76
Everolimus	11 (9)	21 (5)	.088

Values are expressed as median (IQR, interquartile range) or N of patients (%). ECMO, extracorporeal membrane oxygenation; eDSA, early donor-specific antibodies; ICU, intensive care unit; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins' PGD, primary graft dysfunction.
[a]In-hospital deaths (n = 25) are excluded.

treatment as intended per protocol at follow-up end. Among the remaining 12 (9%) patients, 4 (3%) patients had died in-hospital, 4 (3%) patients were still on treatment, and 4 (3%) patients terminated treatment earlier as intended per protocol (due to evidence of carcinoma, n = 1; IgGAM side effects, n = 1; early retransplant, n = 1; recurrent hospital stays due to infection, n = 1). At treatment end, eDSA were cleared in 106 (91%) out of 116 patients. Among these 106 patients, the same eDSA recurred in 14 (13%) patients at a median of 9 (6-18) months after treatment end. No new DSA was detected. At the last DSA control, performed at a median of 23 (7-36) months after transplantation, 98 (92%) out of 106 patients did not show any DSA. eDSA clearance was worse in patients with preformed than de novo eDSA and in patients with graft dysfunction (Table 5).

Among the 10 eDSA$^+$/no-treatment patients, 9 (90%) did not show DSA at last control, performed at a median of 17 (6-28) months after transplantation. eDSA$^+$/no-treatment patients showed no pre-formed eDSA and had a lower prevalence of eDSA against donor HLA class II antigens (60% vs. 81%, *P* = .094). The median MFI value at first positive DSA control was lower in eDSA$^+$/no-treatment than eDSA$^+$/IgGAM$^+$ patients (2037, IQR 1506-3191, *P* = .012).

3.5 | Outcomes

Median follow-up was 24 (11-40) months and did not differ between eDSA$^+$/IgGAM$^+$ and eDSA$^-$ patients (*P* = .76). Outcomes of eDSA$^+$/IgGAM$^+$ versus eDSA$^-$ did not show significant statistical differences between groups (Table 6 and Figure 4A-D). However, freedoms from biopsy confirmed rejection (Figure 4B) and from pulsed steroid therapy (Figure 4C) at 6 months after transplantation were higher in eDSA$^+$/IgGAM$^+$ than eDSA$^-$ patients. These results were

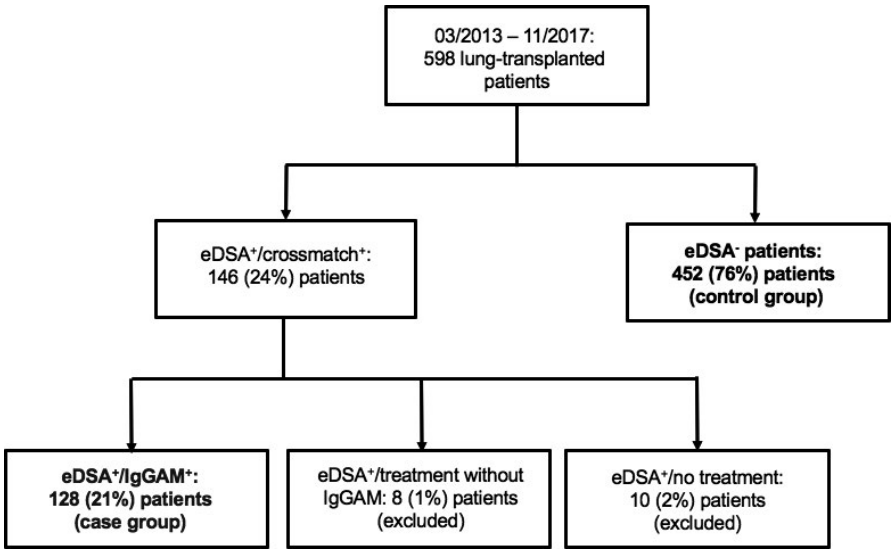

FIGURE 2 Figure 2 shows patient groups. Patients who developed eDSA and were treated with IgGAM (n = 128) formed the case group. Patients without eDSA (n = 452) formed the control group. Both groups are marked in bold. eDSA, early donor-specific antibodies; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins

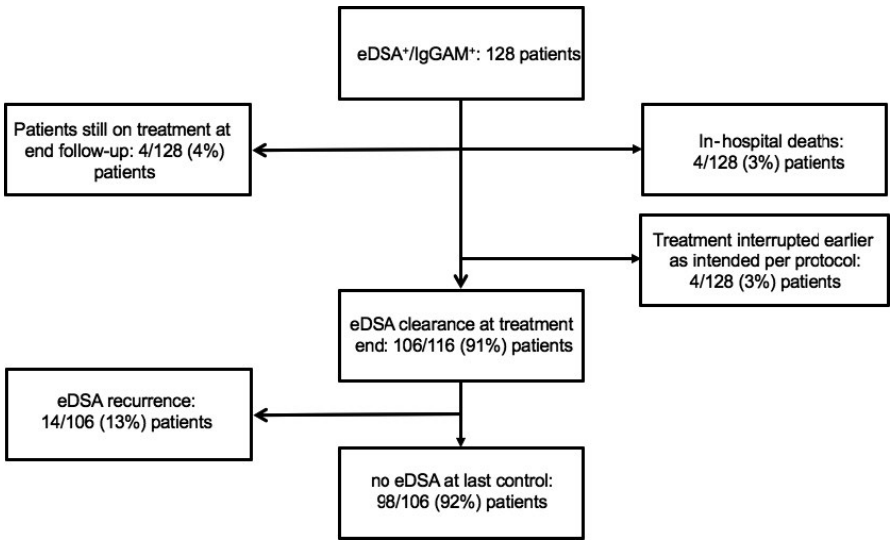

FIGURE 3 Figure 3 shows eDSA clearance, at treatment end and at last DSA control performed at a median of 23 (7-36) months after transplantation. eDSA, early donor-specific antibodies

confirmed after propensity score matching and stratification according to quintiles of propensity scores (Tables S3-S6).

eDSA$^+$/IgGAM$^+$ patients showed better graft survival (P = .005) and freedom from retransplant (P = .02) than excluded eDSA$^+$ patients (Table S7), and particularly better freedom from retransplant (P = .003) than eDSA$^+$/no-treatment patients (Table S8). However, this could have been confounded by the small number of excluded patients.

In eDSA$^+$/IgGAM$^+$ patients, outcomes did not differ after stratification according to presence of preformed versus de novo eDSA, use of tPE or immunoabsorption, use of treatment protocol 1 versus 2, and eDSA clearance at treatment end (Tables S9, S10, S11, S12, respectively). However, eDSA$^+$/IgGAM$^+$ patients who had a negative crossmatch, did not have graft dysfunction at treatment time, and received Rituximab, had better graft survival (Tables S13, S14, S15, respectively).

Finally, outcomes were similar between a small number of eDSA$^+$/no-treatment patients and eDSA$^-$ patients, except for a higher incidence of retransplant in eDSA$^+$/no-treatment patients (Table S16).

Median forced respiratory volume in 1 second (FEV_1) values (% predicted) did not differ between eDSA$^+$/IgGAM$^+$ versus eDSA$^-$ patients at discharge (68 vs. 64, P = .88), at 1-year follow-up (87 vs. 88, P = .23), and at last outpatient assessment (80 vs. 84, P = .29), performed at 24 (12-37) months after transplantation.

TABLE 5 eDSA clearance at treatment end

Stratification	Clearance at treatment end (n = 106/116[a], 91%)
HLA class	
I (n = 27)	24 (83%)
II (n = 96)	88 (92%)
P value	.60
Pre-formed vs. de novo DSA	
De novo (n = 101)	98 (97%)
Preformed (n = 15)	8 (53%)
P value	<.001
MFI values before treatment	
Cleared (n = 106)	3654 (2084-9164)
Not cleared (n = 10)	8360 (4428-12 089)
P value	.082
Cumulative[b] MFI values before treatment	
Cleared (n = 106)	4729 (2186-9898)
Not cleared (n = 10)	7716 (3940-15 351)
P value	.13
Crossmatch	
Positive (n = 8)	7 (88)
Negative (n = 108)	99 (92)
P value	.52
tPE/immunoabsorption	
Yes (n = 48)	42 (88)
No (n = 68)	64 (94)
P value	.31
Rituximab	
Yes (n = 106)	97 (92)
No (n = 10)	9 (90)
P value	.61
Treatment protocol	
Protocol 1 (n = 81)	74 (91)
Protocol 2 (n = 32)	29 (91)
P value	.90

Values are expressed as median (IQR) or N of patients (%). DSA, donor specific antibodies; MFI, mean fluorescence index, tPE, therapeutic plasmapheresis.

[a]12 patients were not considered in this analysis (4 patients still on IgGAM treatment; 4 patients died in-hospital; in the remaining 4 patients, treatment was interrupted earlier as per protocol).

[b]Sum of the single MFI, in case a patient showed eDSA against more than one antigen.

TABLE 6 Outcomes at follow-up

Variable	eDSA$^+$/IgGAM$^+$ (n = 128)	eDSA$^-$ (n = 452)	*P* value
Patient survival (%)			
1 y	94 ± 2	92 ± 1	
4 y	82 ± 4	83 ± 3	.59
Graft survival (%)			
1 y	93 ± 2	91 ± 1	
4 y	79 ± 5	81 ± 3	.58
Causes of death after hospital discharge[a]			
CLAD	4 (3)	8 (2)	.35
Infection	4 (3)	5 (1)	.11
Malignancy	4 (3)	6 (1)	.18
Cardiac	0	1 (0.2)	1.00
Other	1 (1)	8 (2)	.69
Freedom from biopsy-confirmed rejection (%)			
6 mo	74 ± 4	63 ± 3	
1 y	67 ± 5	61 ± 3	
4 y	57 ± 5	53 ± 3	.34
ISHLT biopsy grade			
A1	34 (32)	128 (34)	.62
A2	10 (9)	41 (11)	.63
A3	0	3 (1)	1.00
Freedom from pulsed steroid therapy (%)			
6 mo	73 ± 4	64 ± 2	
1 y	58 ± 5	60 ± 3	
4 y	43 ± 5	47 ± 3	.82
Freedom from CLAD (%)			
1 y	99 ± 1	99 ± 1	
4 y	82 ± 5	78 ± 4	.83
Freedom from re-transplant (%)			
1 y	98 ± 1	99 ± 1	
4 y	95 ± 3	97 ± 1	.28
Freedom from infection (%)			
1 y	74 ± 4	78 ± 2	
4 y	48 ± 8	63 ± 3	.15

Values are expressed as mean ± SD (%) or N of patients (%). CLAD, chronic lung allograft dysfunction; ISHLT, International Society of Heart and Lung Transplantation.

[a]Patients who died before hospital discharge (n = 25) were not considered.

4 | DISCUSSION

This study represents the largest single-centre case series on treatment of early DSA in lung transplantation published so far.[11-13,15-17]

IVIG are a consolidated component of AMR treatment protocols in renal transplantation.[23,24] In lung transplantation, conversely, there is no consensus on when and how AMR must be treated.[11-17]

In the first published case series on preemptive DSA treatment with IVIG after transplantation, Hachem et al showed a DSA clearance of 65% at treatment end. Outcomes were worse in patients who did not clear DSA than in patients who did.[11] Witt et al reported that treatment with IVIG and Rituximab cleared DSA in 9 out of 21 (43%) patients with acute AMR. Six (29%) patients died in-hospital of refractory AMR. Among survivors, 14 (93%) patients developed CLAD.[12] Vacha et al treated 16 patients with acute AMR using a combination

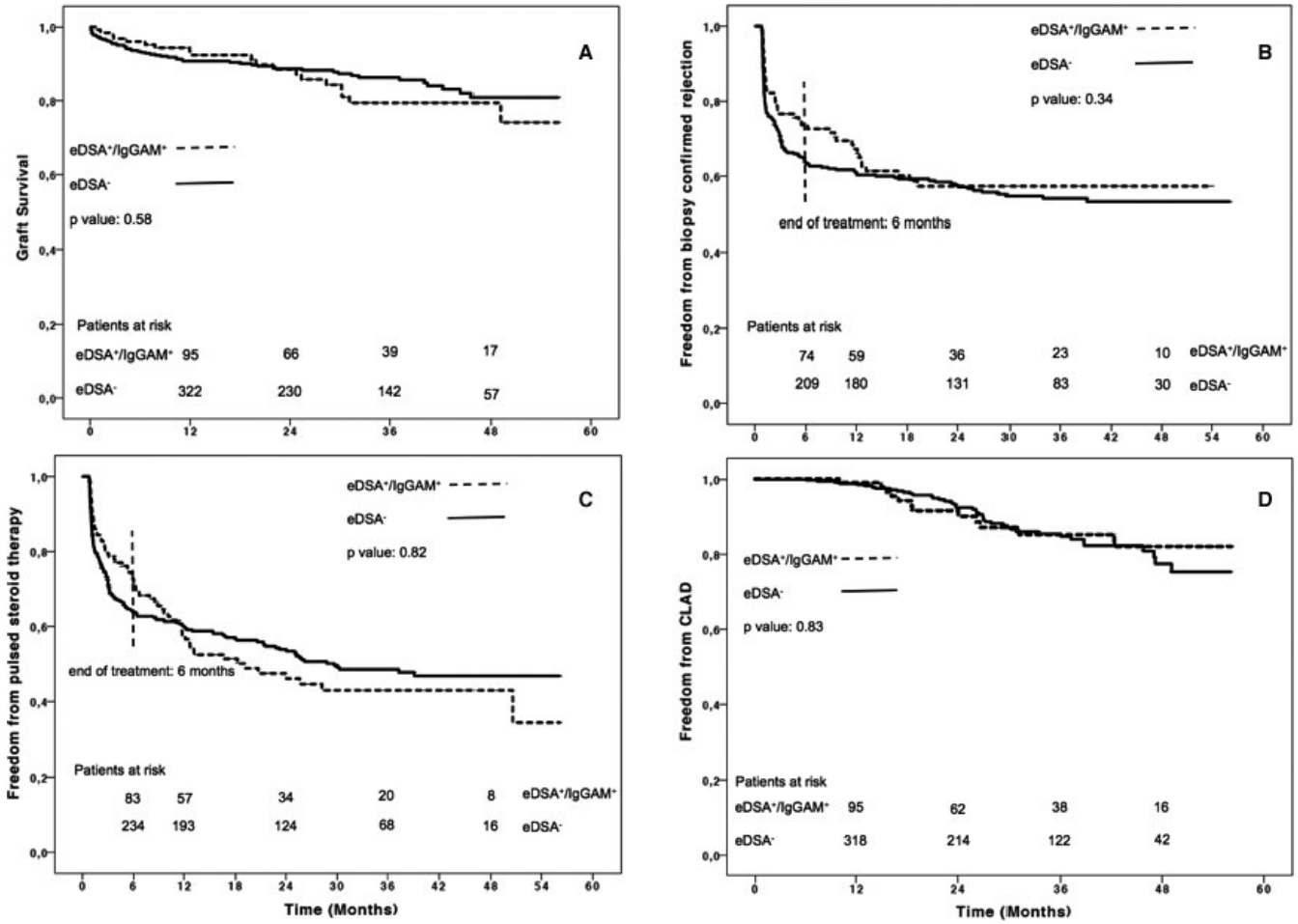

FIGURE 4 Figure 4 shows graft survival (A), freedom from biopsy confirmed rejection (B), freedom from pulsed steroid therapy (C), and freedom from CLAD (D), between $eDSA^{+}/IgGAM^{+}$ vs. $eDSA^{-}$ patients. Patients at risk are reported above the X axis. In B and C a dotted line at 6-month follow-up marks the treatment end. CLAD, chronic lung allograft dysfunction; eDSA, early donor-specific antibodies; IgGAM, IgA- and IgM-enriched intravenous human immunoglobulins

of Bortezomib, Rituximab, tPE, and successive 0.5 g/kg IVIG infusions. DSA cleared in only 3 out of 11 patients (27.7%) at 6 months after treatment. Survival was 56.2% following treatment.[17] Finally, in the case series of Islam et al, 72 (22.2%) patients developed de novo DSA after lung transplantation and, in 25 (34.7%) patients, DSA cleared spontaneously. They treated only patients with graft dysfunction using tPE, Rituximab and IVIG, showing a DSA clearance of 53%.[16]

All these studies reconfirm that current treatment protocols are ineffective in cases of AMR with established graft dysfunction. Therefore, at our institution, we treat patients as soon as eDSA are detected, mainly preemptively (possible subclinical AMR). In our opinion, eDSA represent just the early measurable part of general allosensitization of host versus graft.[25] We observed that survival and outcomes were similar in treated patients versus patients without eDSA. In accordance with the previously reported literature, those patients with graft dysfunction (possible clinical AMR) showed worse survival and eDSA clearance than patients with only eDSA (possible subclinical AMR).

Freedom from biopsy confirmed rejection and from pulsed steroid therapy were higher during treatment time (Figure 4B,C) and decreased after treatment end, reconfirming that IgGAM may have a protective role against rejection. IgGAM are not per se immunosuppressive and have pleiotropic immunomodulatory effects, since they act on different points of the immunologic cascade.[21,24] IgGAM contain IgG (76%), IgM (12%), and IgA (12%), and can neutralize DSA in the periphery and scavenge activated complement through the IgM, IgG, and IgA components; inhibit the activation of antibody dependent cell mediated cytotoxicity through the IgG component; inhibit tissue migration of activated neutrophilic granulocytes and monocytes through the IgA component; and activate T regulatory cells through the IgG component.[21,26-29] Moreover, the IgM component also confers a protection against infections through pathogen opsonisation.[21] In our study, freedom from infection was similar among groups during treatment, but worsened thereafter in previously treated patients. This trend may be due to a late effect of Rituximab.

During the study period, we developed three different IgGAM-based protocols to treat eDSA, looking for the most appropriate therapy. In fact, therapies of AMR may also provoke side effects, and the benefit of treatment must be carefully evaluated against the risk of side effects, particularly in asymptomatic patients with eDSA. We usually combined

IgGAM with a single dose of Rituximab and, in some patients, with tPE or immunoabsorption. No difference was found in clearance and outcomes between protocol 1 and 2. The addition of 2 immunoabsorptions in all patients with eDSA did not add any benefit and did not reduce treatment time. Thus, since April 2017, we use a combination protocol with IgGAM, Rituximab and tPE for patients with a positive crossmatch or presence of graft dysfunction (possible clinical AMR), and only IgGAM in asymptomatic patients with eDSA (possible subclinical AMR, Figure 1).

Finally, 90% of untreated patients (n = 10) showed spontaneous eDSA clearance. Outcomes were mostly similar to treated patients, yet freedom from CLAD and re-transplant were worse in untreated patients. Moreover, in a recent publication, spontaneous DSA clearance was observed in 34.7% of patients and was associated with a lower risk of acute rejection.[16] Therefore, a randomized trial is required to demonstrate the real treatment efficacy by comparing outcomes between patients with DSA and treated versus patients with DSA without treatment.

5 | STUDY LIMITATIONS

A control group made of $eDSA^+$/no-treatment patients would have been more robust than a control group made of patients without eDSA, to demonstrate treatment effect. The choice of $eDSA^-$ patients instead of eDSA+/no-treatment patients was motivated by the fact that only few $eDSA^+$ patients were not treated, and that, according to the recent evidence in literature,[1-8] DSA^- patients have better graft function and survival than DSA^+ patients.

Moreover, in the present study, we investigated the efficacy of IgGAM therapy only in patients with early DSA. Therefore, the results of this study might not be necessarily extended to patients who develop late DSA. This aspect was not investigated, because, at follow-up, DSA were only controlled upon indication in patients without eDSA.

6 | CONCLUSIONS

After lung transplantation, outcomes of treated patients with eDSA were similar to the outcomes of patients without eDSA. These results were confirmed after matching and stratification into quintiles of propensity scores. Treated patients showed high antibody clearance, that persisted at follow-up end. However, further studies are required to demonstrate that IgAM therapy really improves outcomes and directly leads to eDSA clearance, since most of the $eDSA^+$/no-treatment patients cleared eDSA spontaneously.

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DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Dr. Fabio Ius and Dr. Gregor Warnecke report personal and congress fees paid from Biotest, outside the submitted work. Dr. Tobias Welte reports personal fees from Boehringer and from Roche outside the submitted work. The other authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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