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A Phase I/II Trial to Evaluate the Safety and Tolerability of Ides® (IgG endopeptidase) to Eliminate Donor Specific HLA Antibodies (DSAs) and Prevent Antibody-Mediated Rejection Post-Transplant in Highly-HLA Sensitized Patients.

IgG endopeptidase

PROTOCOL

STUDY TITLE:

A Phase I/II Trial to Evaluate the Safety and Tolerability of IdeS® (IgG endopeptidase) to Eliminate Donor Specific HLA Antibodies (DSAs) and Prevent Antibody-Mediated Rejection Post-Transplant in Highly-HLA Sensitized Patients.

Study Drug

IdeS® (IgG endopeptidase)

Support Provided By

Hansa Medical AB
Lund, Sweden
hansamedical.com
NCT: 02426684
FDA IND: 124, 301

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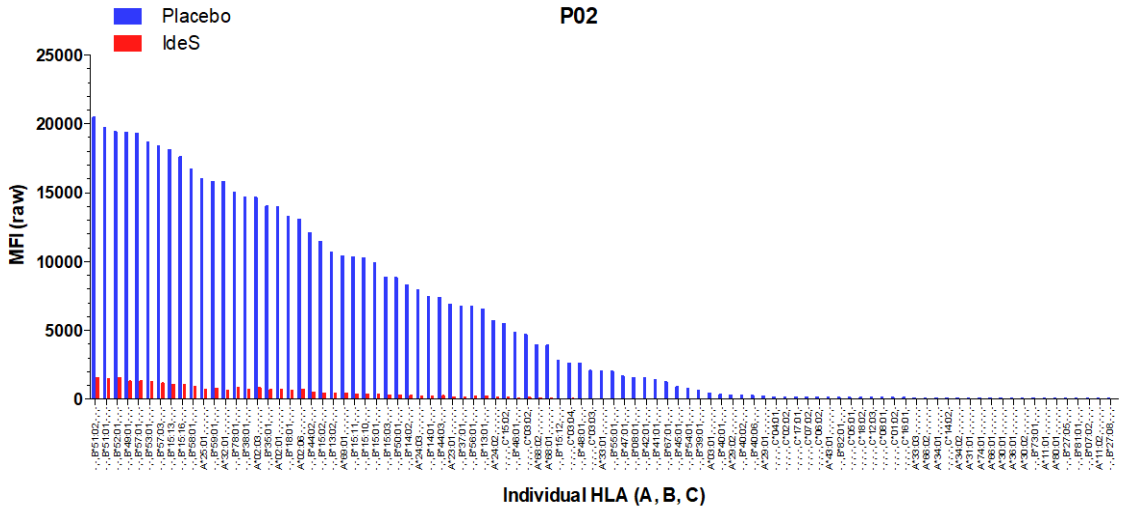
1.1. IdeS®: An Important New Therapeutic Option for Prevention & Treatment of ABMR

IdeS® is an IgG-degrading enzyme of *S.pyogenes* that cleaves all four human subclasses of IgG with strict specificity. IgG degrading activity is a common strategy employed by pathogenic bacteria. IdeS hydrolyzes human IgG in positions 236 and 237 of the lower hinge region of IgG heavy chains. This activity is important since the lower hinge region of IgG is critical for interaction with Fc receptors and complement binding. Thus the proteolytic activity on IgG molecules at this site prevents IgG mediated antibody-dependent cellular cytotoxicity and complement mediated injury to the *S. pyogenes*.

1.2. Therapeutic Use of IdeS® for Prevention of Antibody- Mediated Injury

As mentioned above, alloantibodies are a major deterrent to access to and success of life-saving organ transplants. Despite advancements in desensitization, designing efficient and effective means of removal of pathogenic HLA antibodies remains a significant medical challenge. Preliminary data suggests that IdeS® exhibits fast and efficient enzymatic digestion of pathogenic antibodies in animal models of autoimmunity and on human autoantibodies[23, 26]. Data from patients with autoimmune neuromyelitis optica (NMO) shows that the complement activating and ADCC activity of the autoantibody, anti-aquaporin-4, is completely inhibited *in vitro* by IdeS® treatment. In addition, *in vitro* treatment of DSA positive serum from highly-HLA sensitized patients awaiting transplantation show complete inhibition of DSA activity in the solid phase luminex assay (see example in figure 1). The collective data from 12 highly sensitized patients demonstrated that IdeS treatment significantly reduced the reactivity in all tested patient sera to individual MHC class I and II antigens, as well as the reactivity in complement dependent cytotoxicity tests.

Figure 1



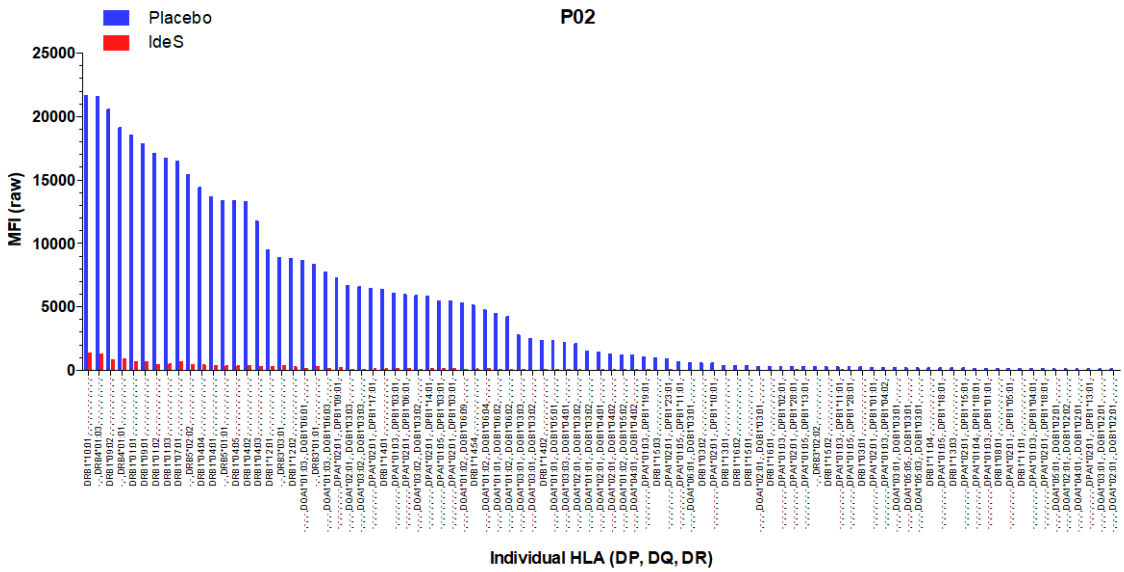


Figure 1. Efficacy of IdeS on anti-HLA IgG in serum from a sensitized patient. Graph shows the MFI (Raw) against individual antigens for (upper graph) MHC class-I (A, B and C) and (lower graph) MHC class-II (DP, DQ and DR) after mock (blue) and IdeS (red) treatment. MFI: Mean fluorescent intensity.

1.3. Study Hypothesis

Since alloantibodies are the major mechanism by which allografts are lost and are also exclusionary for transplantation of highly-HLA sensitized patients and that current approaches to desensitization and treatment of ABMR are not sufficient to completely overcome these barriers, an unmet medical need exist to improve the access to and success of renal allografts in sensitized patients. To this end, we hypothesize that the use of IdeS® pre-transplant in HS patients will represent a more robust and complete technique to eliminate DSA from the sera of HS patients. A single dose administration of IdeS® in the pre-operative period to HS patients with positive DSAs and flow cytometry crossmatches will durably eliminate circulating DSAs, allow transplantation to occur without ABMR and, in conjunction with standard desensitization therapy, result in a durable suppression of DSA levels thus eliminating the risk for ABMR.

1.4. Rationale for Study

Antibody to HLA antigens has a strong impact on mediation of allograft injury and loss. As depicted in Figure 2 below, pre-formed or *de novo* DSAs activate complement, induce endothelial cell proliferation antigens and mediate ADCC resulting in a progression of allograft dysfunction and loss. More than 5000 renal allografts are lost each year in the U.S., approximately 75-80% to antibody mediated injury[13]. Thus, understanding the pathophysiology of ABMR and B-cell activation are critical to improving the longevity of existing allografts and development of successful strategies for desensitization to prevent ABMR.

Figure 2

Course of Acute and Chronic Antibody-Mediated Allograft Injury

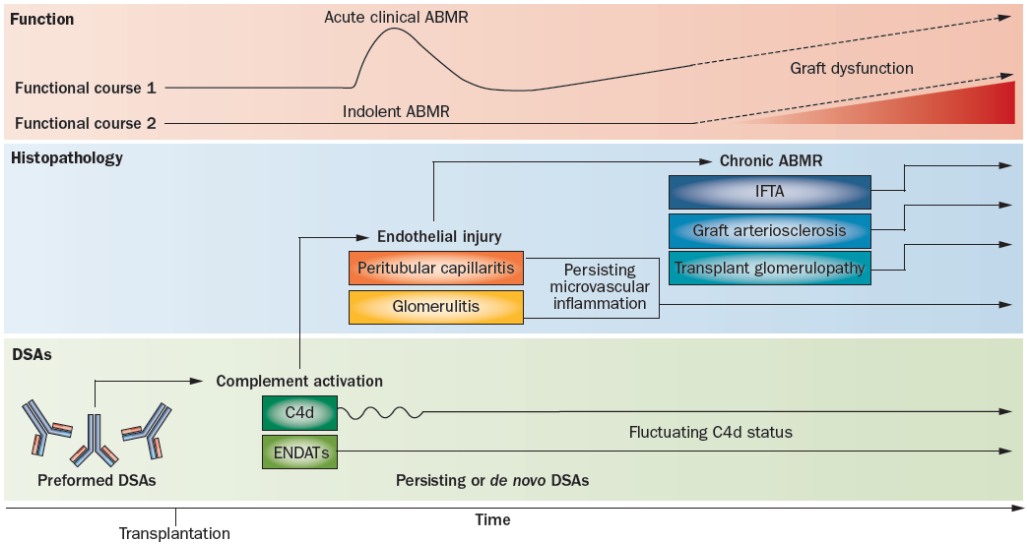


Figure 2 shows the time course of DSAs , their impact on allograft histology and the eventual progrssion to allograft failure, leaving the recipient highly HLA sensitized. It is important to point out the multi-faceted ability of DSAs to mediate allograft injury. Many of the pathologic features were once thought to be consequencs of CNI toxicity leading to reduced dosing of these critical medications which further accelerated allograft loss. Emerging knowledge in this area is critical for development of newer techiniqes for suppression of DSA responses.

Figure 3A

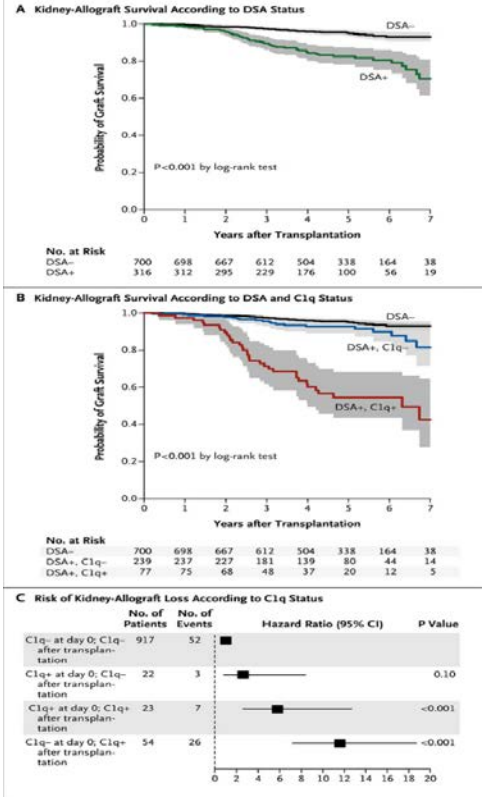


Figure 3B

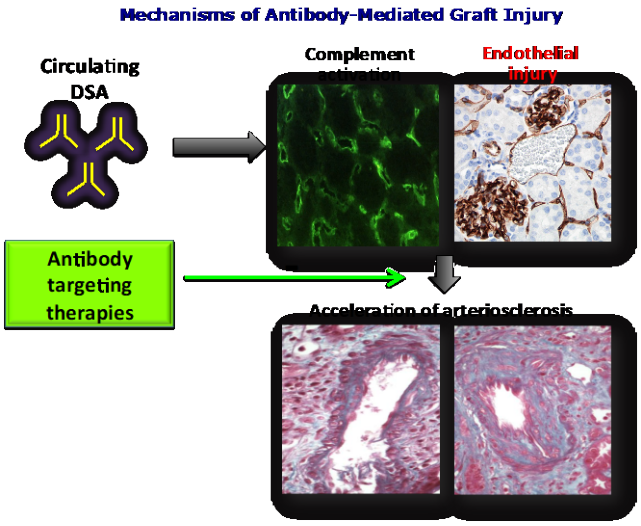


Figure 3A & 3B: Figure 3A shows the impact of DSAs on long term allograft survival in patients transplanted in France (Loupy et al, NEJM, 2013)[14]. These investigators showed that DSAs have a negative impact on long-term graft survival, but when segregated by ability to activate complement (C1q+ DSAs), the impact was significantly magnified. In fact, those who developed C1q+ DSAs post-transplant has a 12 fold greater risk for allograft loss than C1q- DSA patients. Figure 3B shows the mechanisms of alloantibody injury to grafts which include complement activation endothelial injury through direct actions of alloantibody on ENDATs and ADCC as well as induction of accellerated atherosclerosis in allografts[13].

Clearly, the most important way to approach antibody mediated allograft injury is through the development of antibody-targeted therapies. This would address all pathogenic mechansism associated with alloantibody and allow grafts to contine functioning for much longer periods of time. This would improve the length and quality of life of allograft recipients and reduce costs to the health care system.

Figure 4A & 4B below show data from our center on the long-term outcomes in patients who experienced ABMR after desensitization v. those who did not.

Figure 4A

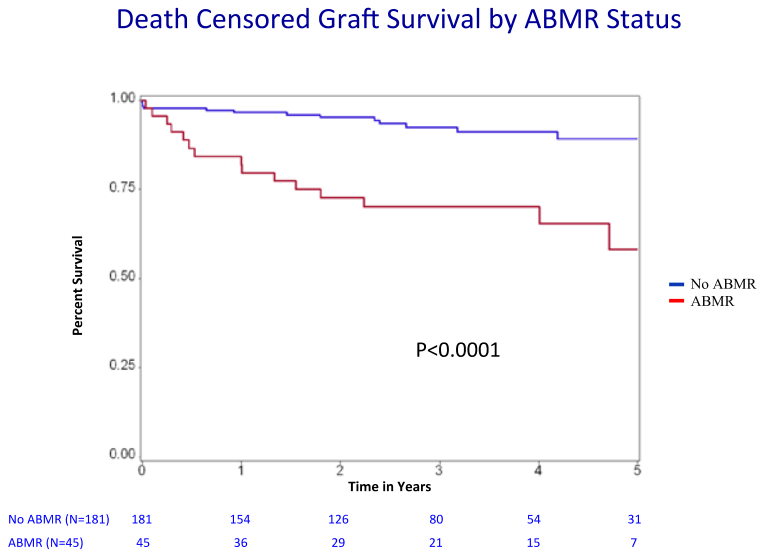
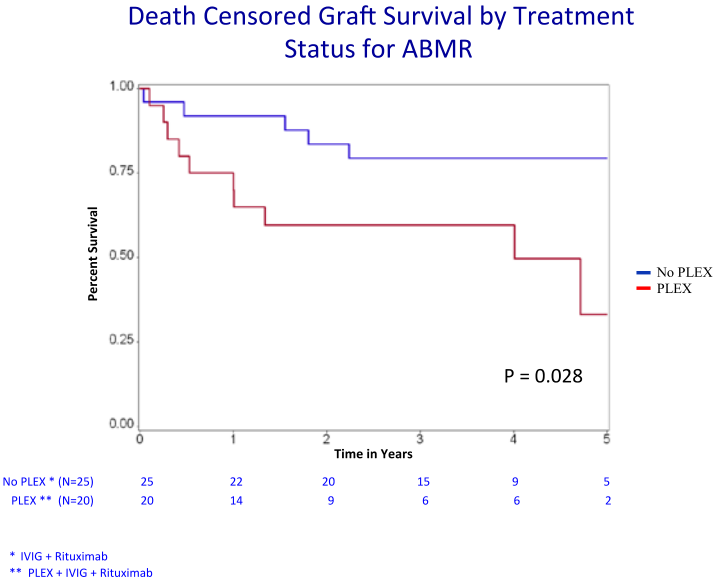


Figure 4B



* IVIG + Rituximab
** PLEX + IVIG + Rituximab

Figure 4A & 4B. We retrospectively reviewed the outcomes of 226 patients who underwent desensitization with IVIG + Rituximab +/- PLEX at Cedars-Sinai Medical Center. Patients were divided into two groups. Group 1 had no ABMR(80%) while Group 2 experienced ABMR(20%). As can be seen in Figure 3A, the long-term outcomes in HS patients who are ABMR+ are significantly worse than those who were ABMR-. In fact, data for the 5 year outcomes of Group 1 are similar to non-sensitized patients in the UNOS data base. This suggest prevention of ABMR is critical for an excellent long-term outcome of allografts. Assessment of factors associated with risk for ABMR showed that re-transplantation, female gender, ESRD caused by glomerulonephritis and presence of stronger class I and class II DSAs at time of transplant were most important. (Table 1 below). Figure 3B shows the difficulty in treating ABMR after transplant in HS Patients. Patients were treated with IVIG + rituximab or IVIG + rituximab + PLEX. Those treated with IVIG + rituximab did better, but this likely reflects a selection bias as more severe cases were treated with PLEX. Clearly, the most important thing we can accomplish is to develop new strategies to prevent ABMR alltogether to allow the long term benefits of transplantation to be manifest in these patients.

Table 1

Characteristics Predisposing to ABMR after Desensitization

Characteristic	No ABMR (n=181)	ABMR (n=45)	P
Age, years	49.2±13.0	41.0±11.4	0.0001
Male sex	54 (29.8)	22 (48.9)	0.015
Deceased donor	121 (66.9)	32 (71.1)	0.584
Race			
Caucasian	78 (43.1)	20 (44.4)	0.870
African American	38 (21.0)	15 (33.3)	0.080
Hispanic	45 (24.9)	6 (13.3)	0.097
Asian	15 (8.3)	2 (4.4)	0.381
Other	5 (2.8)	2 (4.4)	0.559
Primary disease			
Hypertension/diabetes mellitus	84 (30.3)	20 (44.4)	0.812
Glomerulonephritis/vasculitis	53 (27.2)	24 (53.2)	0.002
Congenital cystic/dysplastic	10 (5.5)	2 (4.4)	0.772
Others	43 (23.8)	13 (28.9)	0.475
Unknown	23 (12.7)	3 (6.7)	0.255
Previous transplants			
0	90 (50.0)	9 (20.5)	0.0003
1	68 (37.8)	20 (45.5)	0.397
≥2	22 (12.2)	15 (34.1)	0.0005
Other sensitizing events			
Pregnancy	80 (47.6)	12 (27.9)	0.032
Blood transfusions	92 (54.8)	19 (44.2)	0.301
Time on waitlist (mo)	92.9±49.6	131.3±66.3	0.0004
Time to transplant (mo)	4.0±6.9	3.0±3.7	0.449
PRA % at transplant			
Class I	70.3±44.3	83.11±23.92	0.151
Class II	60.9±34.3	59.5±40.6	0.903
Class I >80%	109 (61.6)	32 (71.1)	0.236
Class II >80%	68 (41.2)	24 (57.1)	0.064
T-cell FCMX (MCS) at transplant	60.2±71.3	130.4±103.9	<0.001
Pronase	31.5±76.1	71.2±90.0	0.019
B-cell FCMX (MCS) at transplant	212.8±143.1	249.4±135.8	0.136
Pronase	145.6±123.6	216.0±109.8	0.007
Positive DSA at transplant, any			
Class I only	44 (24.3)	7 (15.6)	0.208
Class II only	26 (14.4)	5 (11.1)	0.570
Both Class I & Class II	43 (23.7)	28 (62.2)	<0.001
None	68 (37.6)	5 (11.1)	0.0007
HLA Matches			
Zero match	36 (19.9)	14 (31.1)	0.105
1-2 Ag Matches	104 (57.5)	24 (53.3)	0.617
≥3 Ag Matches	41 (22.7)	7 (15.6)	0.297
Induction			
Alemtuzumab	143 (79.0)	41 (91.1)	0.061
Daclizumab	28 (15.5)	3 (6.7)	0.124
Thymoglobulin	6 (3.3)	1 (2.2)	0.704
Simulect/basiliximab	4 (2.2)	0 (0.0)	0.314

2. Experience with IdeS® in Humans

In January 2014, Hansa Medical, Lund, Sweden announced the successful completion of a Phase I study of IdeS® use in humans. The phase I study was performed between March 2013 and January 2014 and included 29 healthy subjects. The study was a double blind, placebo-controlled, randomized study who received single ascending intravenous doses of IdeS® or placebo. Five groups were dosed with the highest dose group receiving IdeS doses of 0.24 mg/kg BW (table 2). Study objectives were to evaluate safety, tolerability, pharmacokinetics and IgG-cleaving efficacy of the drug candidate IdeS®. Details of the study are presented below.

Table 2. Dose groups and number of subjects dosed in clinical study 11-HMedIdeS-01

Dose group	**Dose**	**No of healthy subjects**	
		IdeS	Placebo
1	0.010 mg/kg BW	4	2
2	0.010 mg/kg BW	4	2
3	0.040 mg/kg BW	4	2
4	0.12 mg/kg BW	4	1
5	0.24 mg/kg BW	4	2

2.1 IdeS® Treatment of Highly-HLA Sensitized Patient with Incompatible Transplantation

One patient in the ongoing phase II study in Uppsala, Sweden has been transplanted with a graft from a deceased donor. Patient received two doses of 0.12 mg/kg BW IdeS 11 and 12 August (20 hours between doses). Patient was offered a graft 14 August. Cytotoxic and FACS crossmatch tests were positive with historic serum, i.e. patient had DSA. After two doses of IdeS, both crossmatch tests were negative and the patient was transplanted. The effects of IdeS® on DSA levels as detected by luminex are shown in Figure 5A below. As can be seen, all DSAs were reduced to normal range after IdeS® therapy. The patient's course post-transplant is shown in Figure 5B below. Briefly, the patient did well post-transplant without infections or rejection episodes. The patient returned to work on 10/30/14.

Figure 5A

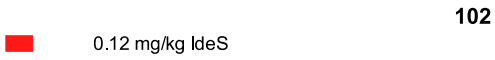


Figure 5A. Graph shows the MFI (Raw) against individual antigens for (upper graph) MHC class-I (A, B and C) and (lower graph) MHC class-II (DP, DQ and DR) after mock (blue) and IdeS (red) treatment. MFI: Mean fluorescent intensity.

Figure 5B

Figure 5B. Graph shows the course of serum Cr post-transplant. The patient did well without rejection or proteinuria and has returned to work today 10/30/14.

2.2 Safety

2.2.1 Adverse Events

A total of 77 AEs were observed in 24 (82.8%) of the subjects and 39 related AEs were observed in 14 (48.3%) of the subjects. In the 0.01 mg/kg dose group there were 24 AEs (12 related) in seven of the eight subjects, in the 0.04 mg/kg dose group there were 11 AEs (6 related) in the four subjects and in the 0.12 mg/kg dose group there were 7 AEs (2 related) in three of the four subjects. One subject who experienced a probable infusion reaction reported 12 of these AEs. In the Placebo group 14 AEs (7 related) was observed in six of the nine subjects. None of the AEs were reported as serious, met any dose limiting toxicity criteria or lead to withdrawal of study drug. The most commonly reported AE was nasopharyngitis, reported for 50 % (10 out of 20) of the subjects on IdeS and for 67% (6 out of 9) of the subjects on placebo. Headache was reported at nine occasions by seven subjects, all on IdeS. Seven incidences of fatigue were reported by six subjects, five on IdeS and one on placebo.

One subject in the highest dose group experienced a possible infusion reaction 2 minutes before completion of the infusion. Symptoms were sinus tachycardia, pharyngeal oedema, chest discomfort, nasal congestion and flushing. The subject was given 2 mL tavegyl (antihistamine)1 mg/mL i.v. and 2 mL betapred (glucocorticoid) 4 mg/mL i.v. and symptoms resolved within 15 minutes. The IdeS infusion was not interrupted and the subject received a full dose. A similar reaction was experienced in one subject dosed with placebo. This subject had three episodes of tacycardia in connection with dosing and he also had elevated IL-6 levels 6 hours after dosing.

2.2.2 Infections

Since IdeS degrades IgG antibodies there was an initial concern that study subjects would have an increased risk of infection. Subjects were screened for inherited immunoglobulin disorders, e.g. IgA deficit, before inclusion in the study. Furthermore, concerns were raised that study subjects could be carriers of subclinical bacterial agents (for example pneumococci) with an increased risk of infection due to reduction of plasma IgG. Therefore, subjects received antibiotic prophylaxis (Spektramox (amoxicillin/clavulanate) tablets (1x1, 500 mg/125 mg) until serum IgG levels had returned to $\geq$4.5 g/L. All study subjects compiled to the antibiotic treatment and there were no signs of an increasing rate of infections within the study group.

2.2.3 Pharmacokinetics (PK)

IdeS concentrations in serum were detected by a qualified but not validated LC-MS/MS method based on four peptides derived from IdeS. The analysis was performed at Lund University and serum concentration versus time data was analysed by non-compartmental analysis (NCA). The pharmacokinetic parameters were calculated up to 24 hours post dosing, as the remaining concentrations were around or below the estimated quantitative range of the method and thus had a high degree of uncertainty.

None of the analysed peptides could be detected in the pre-dose samples or in samples from the nine placebo subjects. However, IdeS could be detected in samples from the 20

subjects given IdeS, thereby confirming dosing. The IdeS concentration increased with dose in the dose interval given, 0.01-0.24 mg/kg IdeS and the increase in the serum concentration one minute before end of infusion was dose proportional (figure 6).

Figure 6

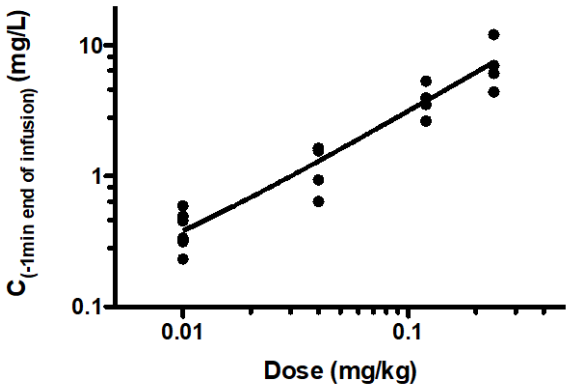


Figure 6. Comparison of serum IdeS concentration one minute before end of infusion versus dose levels of IdeS (0.01, 0.04, 0.12, and 0.24 mg/kg) (logarithmic scale: circles individual concentrations). Analyte: peptide LFEYFK

For subjects dosed with 0.12 and 0.24 mg/kg IdeS, in total 10 blood samples were collected up to 1 week post dose. The serum concentration versus time curve could be described by a multi-phase elimination curve (figure 7) and the main fraction of IdeS was eliminated during the first 24 hours post dosing. During the first 6 hours after dosing, the mean half-life was 4.1 (±2.6) hours at 0.12 mg/kg and 4.9 (±2.8) hours at 0.24 mg/kg. The C_{max} was 5.0 (±2.5) mg/L at 0.12 mg/kg and 8.3 (±3.7) mg/L at 0.24 mg/kg. The C_{max} and AUC increased with dose in humans.

Figure 7

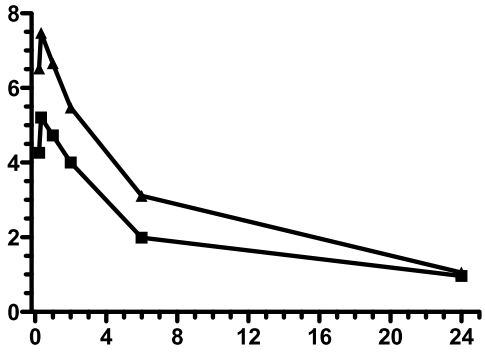


Figure 7 Comparison of serum concentration of mean values of four peptides (peptide A: AFPYLSTK, peptide B: AIYVTDSDSNASIGMK, peptide C: GGIFDAVFTR and

peptide D: LFEYFK) versus time profiles up to 24 hr post dose of IdeS after 15 infusion of 0.12 mg/kg or 0.24 mg/kg IdeS.

2.2.4 Pharmacodynamics (PD)

SDS-PAGE analysis revealed that complete conversion of the IgG pool into single cleaved IgG (scIgG) was observed in three out of eight subjects dosed with 0.01 mg/kg BW IdeS. ScIgG represents partially cleaved IgG where one of the two IgG heavy chains is cut by IdeS. A minor fraction of scIgG was further converted into $F(ab')_2$- and Fc-fragments in these subjects and a weak reduction in plasma IgG was seen. At the next dose level, 0.04 mg/kg BW, the SDS-PAGE analysis showed partial effect in one subject, intermediate effect in one subject and close to full effect in one subject.

The SDS-PAGE analysis of subjects dosed with 0.12 mg/kg BW of IdeS revealed that full or close to full effect was accomplished within 24 hours in all four subjects, i.e. IgG was fully cleaved into $F(ab')_2$- and Fc-fragments (figure 8A and 8B). Furthermore, maximum conversion into $F(ab')_2$- and Fc-fragments was reached six hours after dosing and this was confirmed using the PD-assay which showed that the level of intact IgG (plus scIgG) reached minimum levels six hours after dosing (figure 8). The PD-assay is a validated quantitative sandwich-ELISA based on a Fab-specific capture and an Fcγ-specific detector that measure intact IgG and scIgG, but not the F(ab')2/Fcγ-fragments generated through IdeS cleavage.

In subjects dosed with 0.24 mg/kg BW of IdeS the IgG pool was converted into scIgG already during dosing and maximal effect was accomplished 2-6 hours after dosing in all four subjects (see SDS-PAGE in figure 8A and B). Thus, IgG cleavage was more rapid at the highest dose level (i.e. 0.24 mg/kg BW), compared to the lower dose (i.e. 0.12 mg/kg BW). The rapid cleavage of human IgG into $F(ab')_2$ and Fc seen with SDS-PAGE analyses was confirmed with the PD-assay which showed that 2-6 hours after dosing low plateau level was reached at less than 5% remaining IgG and it was concluded that this signal mainly originated from scIgG (figure 8). Newly synthesized intact IgG was again detectable in serum two weeks after dosing in all subjects and after three weeks the level of intact IgG had further increased and constituted the main IgG fraction in serum (figure 9). See figure 10 for a summary of the SDS-PAGE analysis results.

Figure 8A

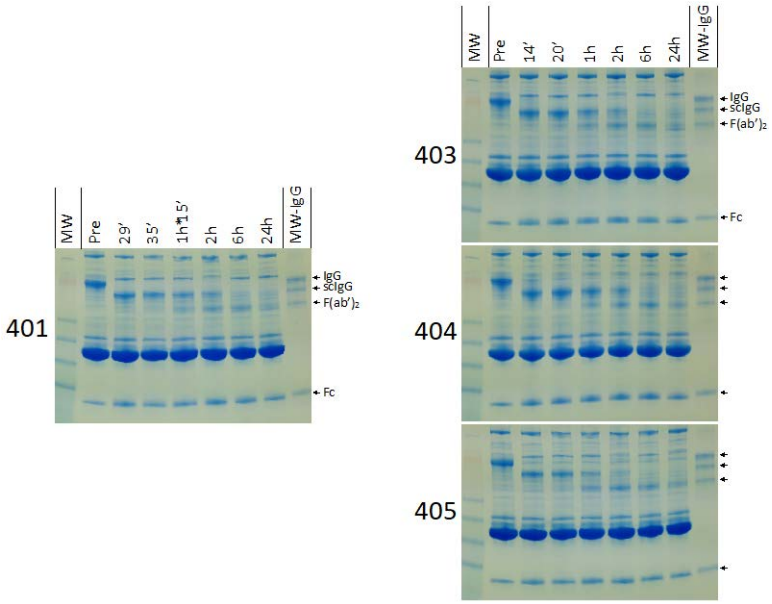


Figure 8B

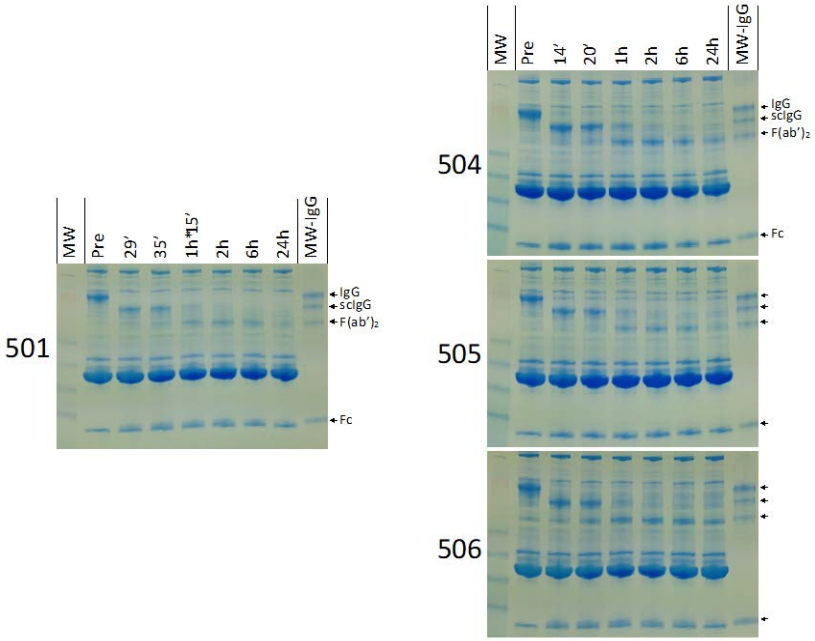


Figure 8A&B. SDS-PAGE analysis of serum from subjects dosed with A) 0.12 mg/kg and B) 0.24 mg/kg IdeS showing protein banding pattern from pre-dosing and up to 24 h after dosing. Intact IgG and single-cleaved IgG (scIgG), Fc and F(ab')$_2$ are indicated in the figure. A marker containing a mix of human IgG, scIgG, F(ab')$_2$ and Fc was also loaded on the gels (last lane). A comparison of the two dose groups shows that IgG cleavage is more rapid in the higher dose group.

Figure 9

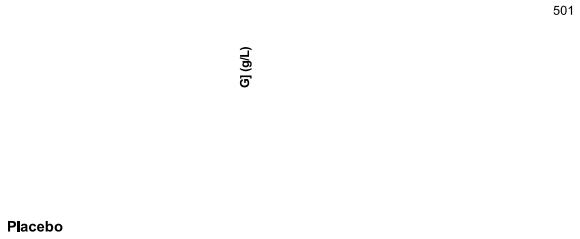


Figure 9. Serum IgG levels in serum from subjects in the two highest dose groups determined using the ELISA PD method performed by Covance laboratories. A, C & E) 0-64 days. B & D) 0-72 hours.

Figure 10A

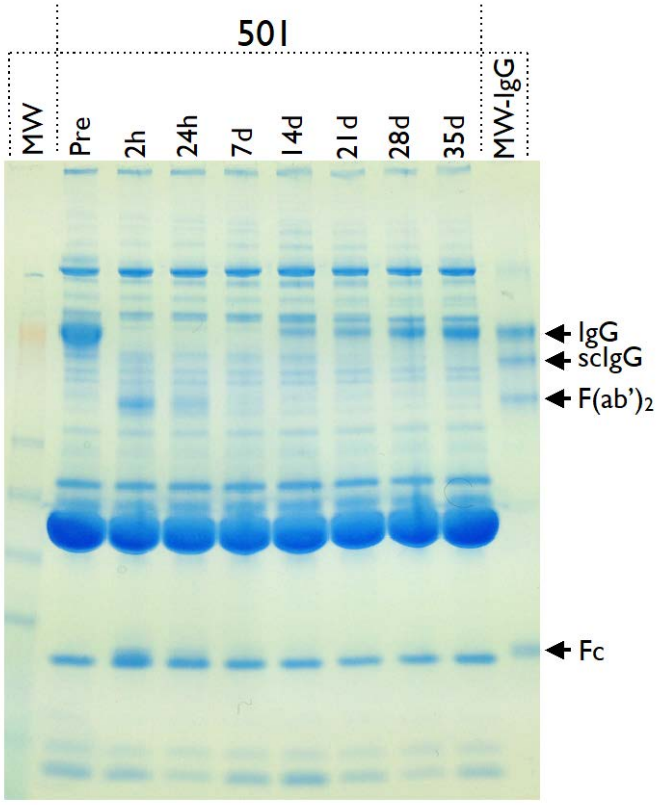
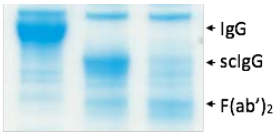
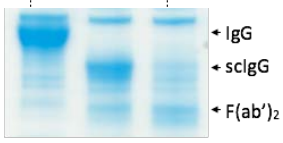


Figure 10A. SDS-PAGE analysis of serum from one subject in the 0.24 mg/kg BW illustrating that newly synthesized IgG can be detected after 14 days and has further increased after three weeks to constitute the main IgG fraction in serum.

Figure 10B



Dose (mg/kg)	No effect	Partial	Full effect	N
Placebo	7			7
0.01*	2	2		4
0.04	1	3		4
0.12		3	1	4
0.24			4	4



Dose (mg/kg)	No effect	Partial	Full effect	N
Placebo	9			9
0.01	5	3		8
0.04	1	2	1	4
0.12			4	4
0.24			4	4

*Figure 10B. Scoring of the effect A) 2 hours and B) 24 hours after administration of IdeS or placebo. The effect was scored as "No effect": intact IgG represents the dominating IgG-band, "Partial": scIgG represents the dominating IgG-band or "Full effect": scIgG is close to non-detectable as the result of conversion to F(ab')$_2$ and Fc. A representative SDS-PAGE showing the different categories is incorporated in the figures and the different cleavage products are marked. *Serum (2 hours) was not available from group 1.*

2.2.5 Immunogenicity

In the first human IdeS study special precautions were taken to prevent hypersensitivity reactions. IdeS originates from Streptococcus pyogenes and it is previously known that a significant proportion of the population has preformed IgG against IdeS (HMed Doc. No. 2012-041). Anti-IdeS IgE (>0.10 kU/L) has not been detected in any individual tested so far. However, it is presumed that individuals with preformed IdeS antibodies of both IgE and IgG type have an increased risk of allergic reactions against IdeS. Therefore, specific in vitro test systems (IdeS-ImmunoCap) for the quantitative measurement of IdeS-specific IgE and IgG antibodies has been developed by Thermo Fisher Scientific, Uppsala, Sweden. The IdeS-ImmunoCap was used to screen study subjects before

inclusion and subjects positive for IgE antibodies were not included in the study. Subjects with elevated IgG antibody titers (>15 mg/L) were also excluded from the first in man study.

A total of 78 subjects were screened in the 11-HMedIdeS-01 study. None of the tested individuals had detectable IgE against IdeS but all tested individuals had detectable IgG. The median level of anti-IdeS IgG was 10.6 mg/L (range 2.1-90.8 mg/L) and 28% of the tested individuals had anti-IdeS IgG titers over 15 mg/L and were excluded from the study. Serum samples from included subjects were analysed for anti-IdeS IgG at the following time points; Day 1 (pre-dose), Day 2 (24 h post dosing), Day 3 (48 h), Day 4 (72 h), Day 7 (1 week), Day 14, (2 weeks), Day 21 (3 weeks), Day 28 (4 weeks) and Day 64 (2 months). The majority of the study subjects (dosed with a single dose of IdeS) responded with an increase of serum anti-IdeS IgG (figure 11). The response was non-detectable one week after dosing but had reached close to peak levels two weeks after dosing. The median pre-dose level (all subjects) of anti-IdeS IgG was 5.3 mg/L (range: 2.0-10.6 mg/L) and at day 14 the median level of all subjects dosed with IdeS was 104 mg/L (range: 3.1-1744 mg/L). Two months after dosing the levels of anti-IdeS IgG had started to decrease in the majority of individuals and the median anti-IdeS IgG level of all subjects dosed with IdeS was 87.8 mg/L (range: 10.5-764 mg/L). Although the individual variation in the magnitude of the anti-IdeS IgG response was large there was clearly a stronger response among the subjects receiving 0.12 or 0.24 mg/kg IdeS compared to subjects receiving 0.01 or 0.04 mg/kg. It can be concluded that the anti-IdeS IgG response is very similar in kinetics and magnitude to the response reported for other drugs of bacterial origin, such as streptokinase and staphylokinase (Collen et al., 1997; Declerck et al., 1994; Mainet et al., 1998).

Figure 11

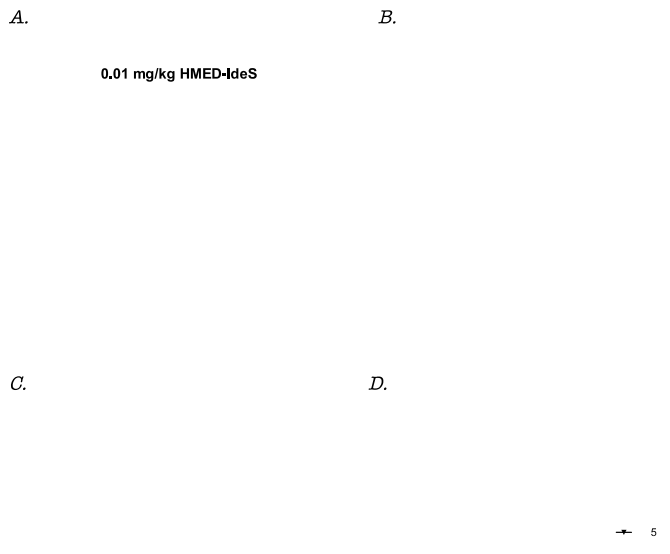


Figure 11. Anti-IdeS IgG levels in human serum from subjects receiving a single dose, A) 0.01 mg/kg, B) 0.04 mg/kg, C) 0.12 mg/kg and D) 0.24 mg/kg, of IdeS. Day 1 represents the pre-dose

sample. Note the different y-axis scales. The samples were analysed using the IdeS-ImmunoCAP (Thermo Fisher Scientific/Phadia) on a Phadia® 250 instrument. The cut-off (LLOQ) for IgG was 2 mg/L.

3 Primary Objectives

This study will be an open label design to assess the efficacy of IdeS® in eliminating DSAs in HS patients who are DSA+ and flow cytometry (FCMX) crossmatch + at time of transplant. Safety determinations will be aimed at assessments of any side effects associated with IdeS® administration and risk for infectious complications associated with IdeS® therapy for prevention of ABMR. Limited efficacy determinations will include incidence of allograft rejection, DSA levels at multiple times post-transplant (Day 0, 30, 90 and 180), determinations of renal function, proteinuria (day 0 through day 30), and pathologic assessments of allograft biopsies at 6M after completion of IdeS® therapy. Patients will be followed and assessed for infectious risk after transplantation as well.

3.1 Major Secondary Objectives

To determine if IdeS® treatment, can prevent or significantly reduce ABMR episodes and C4d deposition in incompatible allografts transplanted to highly-HLA sensitized patients from ~25% to <5% in CMX+ donor-specific antibody (DSA)+ patients. Assess allograft function up to 6 M post-transplant, determine renal function using SCr, MDRD GFR calculations and DSA levels. We will also record any late ABMR episodes after IdeS® therapy. This study protocol is shown in Figure 4.

3.2 Inclusion Criteria

- ✓ End-stage renal disease awaiting transplantation on the UNOS list.
- ✓ No known contraindications for therapy with IVIG10%/Rituximab, plasmapheresis (PLEX) or IdeS®.
- ✓ Age 18-70 years at the time of screening.
- ✓ Calculated PRA (CPRA)≥ 50% demonstrated on 3 consecutive samples, Patient highly-HLA sensitized and a candidate for DD transplantation after desensitization at CSMC.
- ✓ At transplant, patient must have donor-specific antibody/ crossmatch positive (DSA/CMX+) non-HLA identical donor.
- ✓ Pre-transplant vaccination with Streptococcus pneumoniae and Nisseria meningitides
- ✓ Subject/Parent/Guardian must be able to understand and provide informed consent.

3.3 Exclusion Criteria

- ✓ Use of IVIG 7 days prior to planned IdeS® administration
- ✓ Recipients of Extended Criteria Donors (ECD) or Living Donors (LD)
- ✓ Lactating or pregnant females.
- ✓ Women of child-bearing age who are not willing or able to practice FDA-approved forms of contraception.
- ✓ HIV-positive subjects.
- ✓ Subjects who test positive for HBV and HCV DNA and/or RNA PCRs.

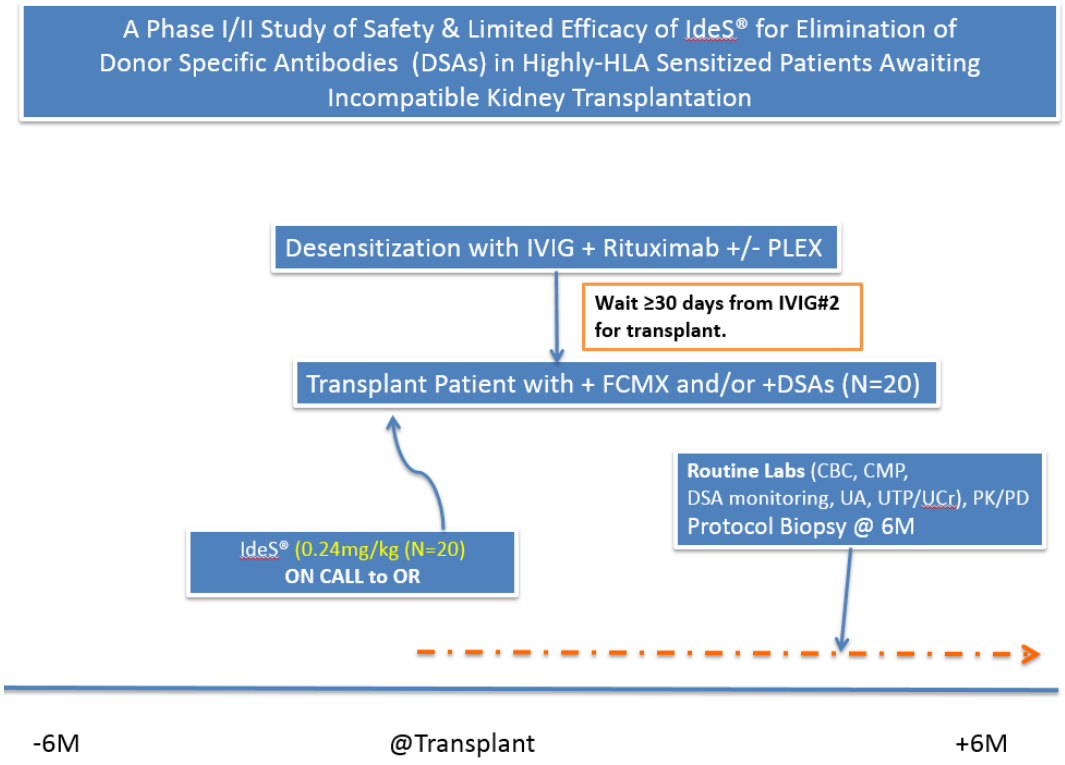
- ✓ Subjects with active TB.
- ✓ Subjects with selective IgA deficiency, those who have known anti-IgA antibodies, and those with a history of anaphylaxis or severe systemic responses to any part of the clinical trial material.
- ✓ Subjects who have received or for whom multiple organ transplants are planned.
- ✓ Recent recipients of any licensed or investigational live attenuated vaccine(s) within two months of the screening visit (including but not limited to any of the following:
    - Adenovirus [Adenovirus vaccine live oral type 7]
    - Varicella [Varivax]
    - Hepatitis A [VAQTA]
    - Rotavirus [Rotashield]
    - Yellow fever [Y-F-Vax]
    - Measles and mumps [Measles and mumps virus vaccine live]
    - Measles, mumps, and rubella vaccine [M-M-R-II]
    - Sabin oral polio vaccine
    - Rabies vaccines [IMOVAX Rabies I.D., RabAvert])
- ✓ A significantly abnormal general serum screening lab result defined as a WBC < 3.0 X 10^3/ml, a Hgb < 8.0 g/dL, a platelet count < 100 X 10^3/ml, , an SGOT > 3X upper limit .
- ✓ Individuals deemed unable to comply with the protocol.
- ✓ Subjects with active CMV or EBV infection as defined by CMV-specific serology (IgG or IgM) and confirmed by quantitative PCR with or without a compatible illness.
- ✓ Subjects with a known history of previous myocardial infarction within one year of screening.
- ✓ Subjects with a history of clinically significant thrombotic episodes, and subjects with active peripheral vascular disease.
- ✓ Subjects with Protein C and Protein S deficiency
- ✓ Use of investigational agents within 4 weeks of participation.
- ✓ Known allergy/sensitivity to IdeS® infusions

4 Study Design & Methods

Background: This is a single center, Phase I/II, open label exploratory study. The trial will primarily examine the safety and tolerability of IdeS® (Hansa Medical, Lund Sweden) given 4-6 hours prior to kidney transplantation to reduce or prevent complement-dependent, antibody-mediated rejection (ABMR) in 20 subjects (adult) who are highly-HLA sensitized (HS) as determined by the Calculated Panel Reactive Antibody Test (CPRA), have undergone desensitization with IVIG + rituximab and/or plasmapheresis and are awaiting LD/DD kidney transplant at Cedars-Sinai Medical Center. Patients will be screened for study eligibility once transplant offers are entertained. If the patient agrees to participate in the study, a donor-specific crossmatch will be performed to detect anti-HLA antibodies and donor-specific anti-HLA antibodies (DSA) which are associated with ABMR and/or graft loss. DSA will be detected using solid phase assay systems currently utilized at the Cedars-Sinai Medical Center HLA Laboratory (Dr. Nancy Reinsmoen, Director, Phone: 310-423-4979)[10]. These anti-HLA antibodies may result naturally or from previous pregnancy, transfusions, or prior transplants. If acceptable crossmatches and DSA levels are seen after desensitization, twenty patients will proceed to DD transplantation as previously described[23-25]. Patients receiving

transplants will have pre-transplant labs for screen ADA, DSAs, FCMX, C3 and C4 obtained as outlined (See Study Protocol in Appendix A). In addition to the standard post-transplant immunosuppressive protocol, all patients will receive 0.24mg/kg IdeS® on day 0 on call to the OR prior to transplant procedure. Pre-medications will be given to patients as a precaution with Solumedrol 40mg IVP, Tylenol 650mg PO and Benadryl 50mg PO to minimize the risk of infusion reactions.Crossmatch and DSA tests will also be assessed on day 1 post-transplant to determine the effect of IdeS®. A protocol biopsy will be performed at 6M to assess the allograft for evidence of ABMR, including C4d staining using Banff 2013 criteria[8, 16]. Since ~25% of HS patients experience ABMR post-transplant and 80% of these ABMR episodes occur in the 1st post-transplant month, we feel the assessment of the potential impact of IdeS® therapy is best assessed in this time period. Patients who have evidence of allograft dysfunction will have non-protocol biopsies for cause. After completion of the IdeS® therapy, patients will be followed up to 6M to assess allograft function and ABMR episodes as well as DSAs. A protocol biopsy will be performed at 6M. The protocol is summarized in Fig. 12.

Figure 12



The entered subjects will be followed to determine if the use of IdeS® for prevention of ABMR in this high risk transplant population is safe and without infectious risks. In addition, we will determine the proportion of who developed evidence of ABMR within 6M of completion of the study. We will assess the transplanted patients to determine the number who sustain a viable and functioning kidney allograft for 6 months. All subjects will be evaluated on an intent-to-treat basis. The subject accrual rate will be limited to no more than five subjects per month in the initial three months to assure safety to all subjects. Repeat laboratories will be performed at the completion of IdeS® therapy to determine effect on levels and correlation with any potential events. A detailed analysis of the study is discussed below.

4.1 Study Analysis

This single-center, Phase I/II, trial is designed to examine the safety, tolerability and limited efficacy of human IdeS® (0.24mg/kg) given as per protocol (Appendix A) in 20 subjects (adults only) who are highly-HLA sensitized and are awaiting DD kidney transplant. Patients considered for this study will be desensitized using high-dose IVIG + rituximab and/or plasmapheresis[8, 23-25]. Patients who have received IVIG within 7 days from transplant offer will be excluded from study in order to minimize interactions with IdeS® infusion and circulation of IgG from IVIG infusion[27]. Once transplant offers are entertained, a donor-specific crossmatch will be performed to detect anti-HLA antibodies which are associated with risk for ABMR. The specifics of our patient selection for desensitization, desensitization protocol, CMX testing and post-transplant protocols are outlined below:

4.2 Defining the Sensitized Patient

For the purposes of this study, we define HLA sensitization as a patient awaiting kidney transplantation on the UNOS waitlist who has a CPRA of $\geq$50% who also has demonstrable DSA using luminex bead technology and a history of sensitizing events (previous transplants, blood transfusions and/or pregnancies). These individuals must also have sufficient wait time on the UNOS list to allow for frequent offers with a history of positive crossmatches (DD) or an incompatible (LD) with a positive flow cytometry (FCMX), complement-dependent cytotoxicity (CDC+) crossmatch[23-25].

4.3 Defining ABMR

For purposes of this investigation, antibody-mediated rejection (ABMR) is defined as follows:

- ✓ Deterioration of allograft function in a high-risk transplant recipient (i.e. sensitized patient with history of DSAs) measured by serum Cr and eGFR (defined as a decline >20% from baseline).
- ✓ Association with the presence of DSA (usually increasing in strength) measured by luminex techniques.
- ✓ Biopsy evidence of capillaritis, inflammation and C4d deposition.

Figure 13 below shows the course of a highly-sensitized (HS) patient who developed ABMR after receiving desensitization for incompatible kidney transplantation. Note,

DSAs increase with evidence of allograft dysfunction. This is associated with significant biopsy findings including evidence of complement activation and inflammation.

Figure 13

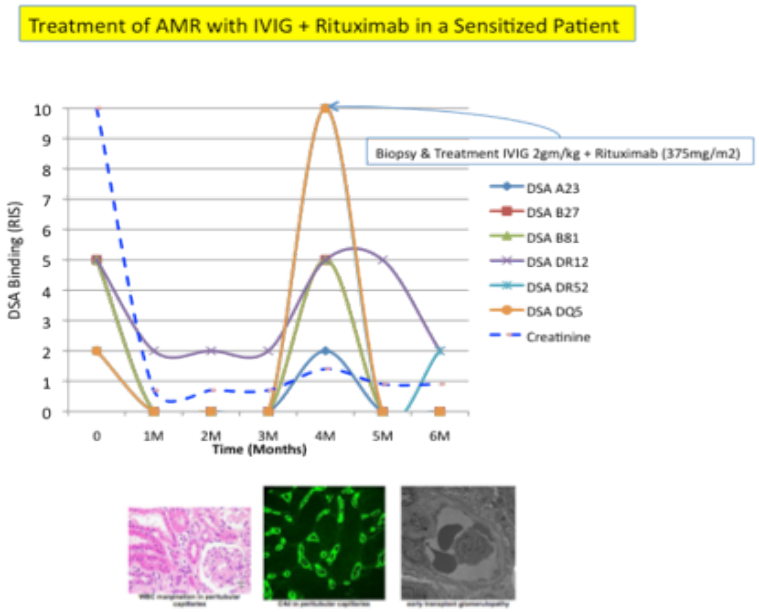


Figure 13 shows the course of DSAs after transplantation in this HS patient who received desensitization with IVIG + rituximab prior to transplant. Three DSAs were present at transplant (two moderate strength and one low strength) as determined by a Relative Intensity Scale (RIS) where 0 points is given for a negative DSA, 2 for low strength (1000-2000 MFI), 5 for moderate (2000-5000 MFI) and 10 for high (>5000 MFI). The course of SCr, DSAs and biopsy are shown as well as responses to treatment with IVIG + rituximab.

The presence of DSAs has a strong correlation with C4d deposition and biopsy evidence of ABMR[3, 9, 12-14]. Thus, this study will assess the ability of IdeS® to alter or prevent ABMR in DSA + patients. Important observations will be the assessment of efficacy of IdeS® in enzymatic cleavage of DSAs, the duration of this effect and the potential for rebound DSA responses after this unique therapy. We will also assess development of anti-IdeS® antibodies and determine if these responses are altered by desensitization.

4.3.1 Treatment of Antibody Mediated Rejection (ABMR)

Biopsy-proven rejection episodes are treated with “pulse” methylprednisolone (10mg/kg/day, max 1000mg for >100kg for 3 days) and anti-thymocyte globulin (1.5mg/kg daily X 4) for cell-mediated rejection episodes that are unresponsive to pulse steroids. For patients experiencing ABMR, patients will initially receive pulse methylprednisolone (10mg/kg/day, max 1000mg for >100kg) IV daily x 3 doses then, depending on severity, IVIG 10% solution 2gm/kg (max 140g for >70kg) IV X1 dose followed by rituximab (375mg/m2) IV X1 dose. In cases where rapid deterioration of allograft function is seen and/or thrombotic microangiopathy is seen on biopsy, the patient will receive plasma exchange X3-5 sessions followed by anti-C5 (Eculizumab®) IV weekly X4 weeks (1200mg week #1 followed by 900mg/ weekly for 3 additional

weeks). Efficacy of therapy will be assessed by determining renal functional improvement, monitoring DSA responses and repeat allograft biopsies, if needed.

4.4 IVIG/Rituximab Desensitization Protocol

All HS who meet criteria for desensitization will receive IVIG 10% solution (2.0 g/kg [maximum 140 g per dose] on days 1 and 30) and rituximab (1 g administered on day 15). When a DD kidney offer becomes available, a donor-specific FCMX is performed pre-transplant. An acceptable CMX is defined as a negative complement-dependent cytotoxicity (CDC), at least at a 1:2 dilution of sera. A positive T-and B-cell FCMX with a shift of less than 225 channel shifts (CS) is also acceptable (negative: <100 mean channel shifts [MCS] for B cell and <50 MCS for T cell). Solid-phase antibody analysis was also used to define the specificity of the antibodies detected, to follow the effect of desensitization, and the strength of DSA as reported previously[23-25]. Since the B-cell cytotoxicity and FCMX crossmatches are falsely elevated by rituximab, a heavier reliance on T-cell FCMX data and DSA values (<100,000 standard fluorescence intensity [SFI] units) is used as the primary determinants of CMX acceptability as described previously and illustrated below.

An analysis of the effects of the IVIG + rituximab desensitization protocol on DSA levels is shown in Figure 14A. Figure 14B shows the relationship of DSA levels and FCMX results. The data confirm that a cutoff of ~225 channel shifts (CS) for FCMX and DSAs <100,000 SFI units is associated with lower risk for ABMR compared with higher DSA levels and FCMX >225 CS.

Figure 14A

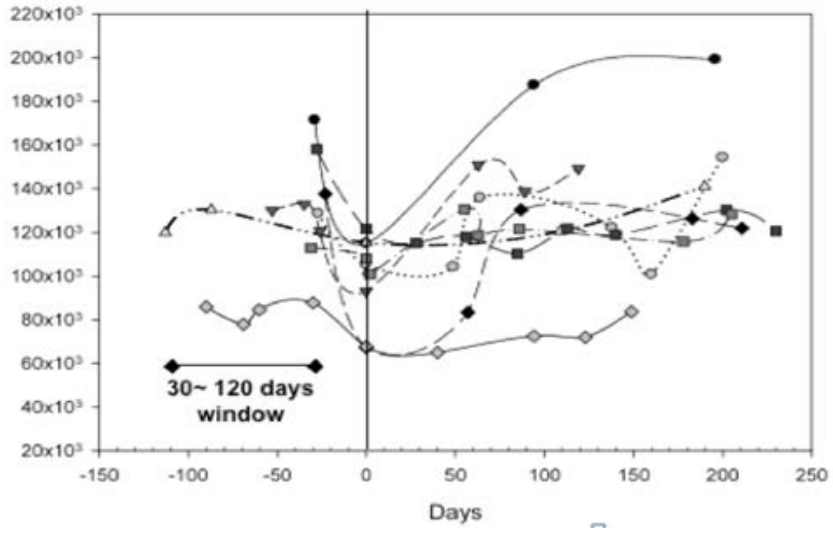


Figure 14A: Sequential antibody profiles subsequent to one round of immunomodulatory therapy (IVIG + rituximab).The value of each data point is represented as the average of all single antigen beads equal or greater than 20,000 SFI. Patient 4853 serum contained anti-HLA class I antibodies only. Sera from patients 901, 301, 15622, 17075, 19211, 19521, and 25059 contained both class I and class II anti-HLA antibodies.

Figure 14B

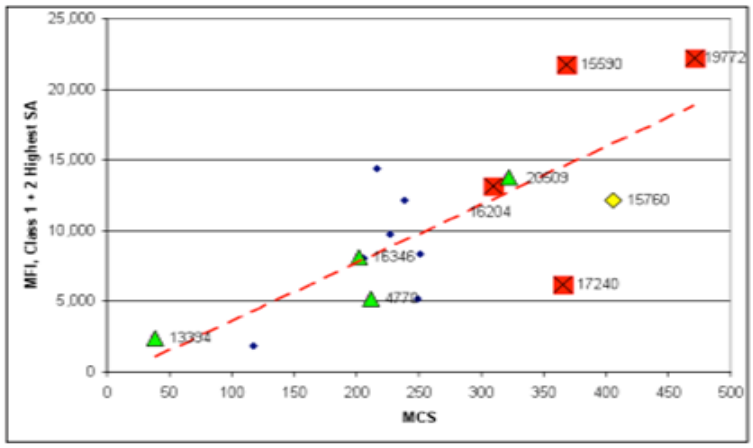


Figure 14B: Determining acceptable single antigen bead strength by using the sum of the highest DSA values for class I and class II beads versus BPFXM. ◆ *Patients with no ABMR episodes,* ⊠ *patients with an ABMR episode within 42 day.* ◇ *A patient with ABMR within 50 to 200 days and patients with* △ *ABMR episodes after 200 days.*

By using the criteria outlined, 74% (80/108) HS (C PRA>80%) DD recipients treated with desensitization (1/06-12/09) were transplanted. A total of 26% (28/108) patients remained on the waitlist during this period with mCPRA 95. Four of these patients were transplanted in 2010. Of the 80 patients transplanted, 42 (53%) were transplanted with a positive donor specific T and/or B cell flow cytometry XM. A total of 19 (24%) patients experienced ABMR +/- CMR ranging from 3 to 535 days post-transplant with a median of 109 days. Seven of these patients were diagnosed with ABMR +/- CMR within the first 42 days. Two patients were diagnosed with late ABMR +/- CMR at post-transplant days 144 and 249. One of these patients had CMV detected by PCR before the diagnosis of ABMR and one patient had CMV detected by PCR at the time of ABMR diagnosis.. Another patient had Parvovirus B19 positive PCR reaction prior to the diagnosis of ABMR (post-transplant day 535). One patient was noncompliant (post-transplant day 139) and lost the graft. Twenty-eight patients (mCPRA 91) were transplanted with a negative donor specific flow cytometry XM. Pretreatment samples were available for 25 of these 28 patients and showed all pretreatment XMs were negative with the donor samples. Among these 28 patients, none had ABMR and 8 had CMR. The CMR occurred between 15 and 773 days post-transplant with the median time at 58 days. A total of 10 patients received zero HLA ABDR mismatched grafts (mCPRA 90), one had ABMR after 101 days post-transplant and one had CMR at 404 days post-transplant.

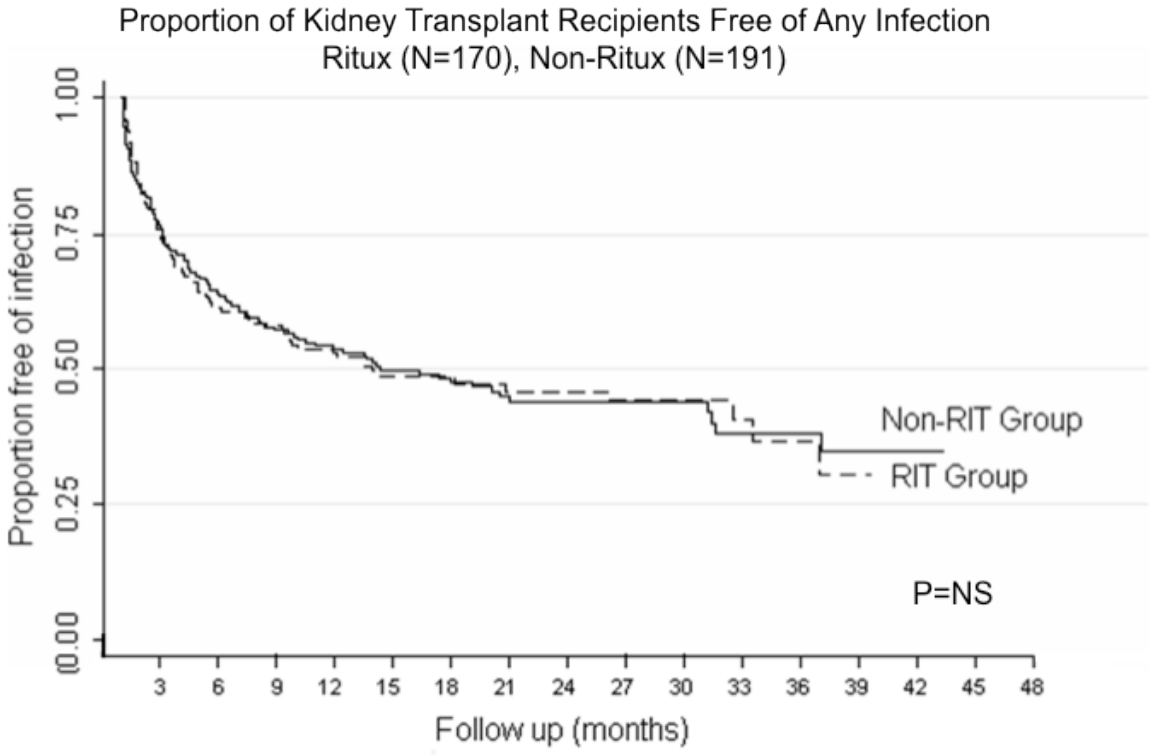
In this data set of 108 patients, we saw an ABMR rate of 24% using careful selection criteria based on FCMX and DSA data at transplant. When compared a concomitant group of non-sensitized patients transplanted during the same time period (N=190), we saw an ABMR rate of <1% in this non-sensitized, low risk group. Thus ABMR is very rare in non-sensitized patients and in patients who are DSA + but FCMX negative who have received desensitization prior to transplantation.

4.5 Monitoring for AE/SAEs Post-Transplant in HS Patients

Adverse events (AEs) and serious adverse events will be monitored post-transplant. These include careful attention to infectious complications potentially associated with IdeS® therapy.

Infectious complications associated with IVIG + rituximab desensitization and alemtuzumab induction therapy followed by maintenance therapy with tacrolimus, MMF and prednisone have been assessed by our group. Briefly, we evaluated 170 patients who were desensitized with IVIG + rituximab followed by alemtuzumab induction and maintenance therapy with tacrolimus, MMF and steroids. This was compared to a concomitant group of non-sensitized, low-risk transplants (N=191) who did not receive IVIG, rituximab or alemtuzumab (induction with IL-2R blockers). A careful analysis of all infections and serious infections that occurred over the next 4 years was compiled and is shown in Figure 15A &15B. Briefly, these data show that the use of this desensitization

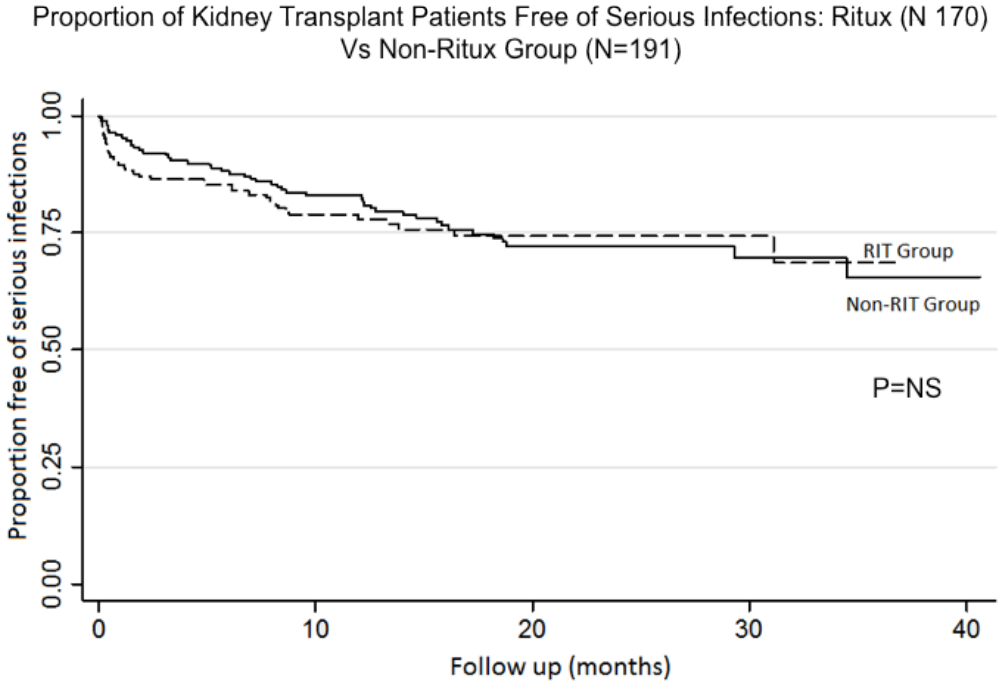
Figure 15A



protocol followed with alemtuzumab induction does not increase the risk for common or serious infections post-transplant compared to a low risk group of patients. Serious infections were defined as any viral infection and fungal or bacterial infections requiring i.v. antibiotics or hospitalizations[19]. Thus risk for infections in the study group (IdeS®) after desensitization will likely be similar and comparable to non-sensitized patients. All patients entered into this study will receive vaccinations for *Streptococcus pneumoniae* and *Neisseria meningitides* pre-transplant.

In addition, all patients treated with IdeS® will also receive antibiotic prophylaxis with ciprofloxacin for 4 weeks post-transplant. It is also noted that our protocol includes a post-transplant dose of IVIG 2 gm/kg (maximum 140 grams) administered at 7 days post-transplant. This will provide additional anti-infective support during the IgG reconstitution after IdeS® therapy.

Figure 15B



15A & B: This figure shows the proportion of kidney transplant recipients who developed infections (all infections 15A, or serious infections 15B) post-transplant. Patients were in the low-risk group (non-Ritux #191) who did not receive IVIG/ritux or alemtuzumab or (Ritux #171) who received desensitization with rituximab, IVIG followed by transplantation. Follow up for up to 45 months shows that infection rates are similar. The commonest infection was urinary tract infection in both groups. The incidence of CMV was greater in the non-Ritux group while there were more BK infections in the Ritux group. No patients in the Ritux group developed PTLD or PML while 2 patients in the non-Ritux group developed PTLD.

4.6 Infection Prophylaxis Protocols and Viral Monitoring Post-Transplant

All study patients, regardless of their cytomegalovirus (CMV) status, receive IV ganciclovir while inpatients and valganciclovir as outpatients for 6 months, with dose adjustments for renal function. Fungal prophylaxis was accomplished with fluconazole 100 mg daily for 1 month. *Pneumocystis jirovecii* pneumonia and bacterial prophylaxis is accomplished with trimethoprim 80 mg and sulfamethoxazole 400 mg daily for 12 months.

Viral polymerase chain reaction assays for CMV, Epstein Barr virus, Parvovirus B-19, Polyoma virus BK and JC will be performed on study patients monthly for 6 months post-

transplantation. Methodologies used for monitoring viral replication have been described previously [25].

4.7 Outcomes of HS Patients vs. Non-Sensitized Patient Cohort

As part of the suggested revisions of this protocol, we have evaluated a group (N=170) of patients who underwent desensitization with IVIG + rituximab and were transplanted (2006-2010). This was compared to a group of non-sensitized patients who were transplanted during the same time period who did not receive IVIG, rituximab or alemtuzumab induction. The patient characteristics are shown in Table 1 below. Briefly, the only significant findings were the higher PRA in the Ritux group, more White (non-Hispanics) in the non-Ritux group, more females in the Ritux group, more lymphocyte depleting agents in the Ritux group and significantly longer dialysis times for the Ritux group. In Table 3, we see the outcomes up to 45 months post-transplant in the two groups. Briefly, there are no significant differences between the two groups in regards to patient and graft survival and infection rates however, there were more rejections in the Ritux group. As previously mentioned, the ABMR rate for sensitized patients (CPRA >80%) was 24% vs. <1% for the non-Ritux group.

Table 3

	RIT Group (N=170)	Non-RIT Group (N=191)	P
Age (years), mean±SD	46.6±13.6	51.9±14.3	<0.001
Male gender, n (%)	69 (41.0)	141 (73.8)	<0.001
Cause of ESRD, n (%)			<0.001
DM	31 (18.2)	71 (37.2)	
HTN	21 (12.4)	28 (14.7)	
Glomerulonephritis	64 (37.7)	36 (18.9)	
Cystic Disease	6 (3.5)	18 (9.4)	
Other	28 (16.5)	36 (18.9)	
Unknown	20 (11.8)	2 (1.1)	
PRA, n (%)			
<10%	26 (15.3)	174 (91.1)	<0.001
10-80%	51 (30.0)	17 (8.9)	
>80%	93 (54.7)	0 (0)	
Type of donor, n (%)			
Living	80 (47.1)	68 (35.6)	0.027
Deceased	90 (52.9)	123 (64.4)	
Lymphocyte depletion, n (%)			
No	47 (27.6)	110 (57.6)	<0.001
Yes	123 (72.4)	81 (42.4)	
Race/Ethnicity, n (%)			
White (non-Hispanic)	73 (42.9)	92 (48.1)	0.015
Hispanic	44 (25.9)	64 (33.5)	
African American	29 (17.1)	22 (11.5)	
Asian	15 (8.8)	12 (6.3)	
Other	9 (5.3)	1 (<1)	
Length of follow-up (days), mean±SD	479.0±272.9	608.4±301.6	<0.001
Dialysis Vintage (days), mean±SD	1694.8± 1662.5	1059.0±982.5	<0.001

Table 3 shows patient survival, graft survival, and number of patients who developed Infections in the Ritux vs. non-Ritux group.

Table 4

	RIT Group N=170	Non-RIT Group N=191	P
Overall Patient Survival, n (%)	165 (97.1)	183 (96.3)	NS
Overall Graft Survival, n (%)	154 (90.6)	170 (89.0)	NS
Rejection, n (%)	47 (27.7)	36 (18.9)	0.047
Any Infection, n (%)	88 (51.8)	103 (53.9)	NS
Bacterial Infections, n (%)	59 (34.7)	75 (39.1)	NS
Viral Infections, n (%) CMV viremia BKV viremia	37 (21.8) 17 (10.0) 18 (10.6)	48 (25.1) 29 (15.2) 11 (5.8)	NS NS 0.092
Fungal Infections, n (%)	10 (5.9)	10 (5.2)	NS
Serious Infections, n (%)	39 (22.9)	49 (25.5)	NS

Table 4 shows the overall survival by treatment group and risk for common viral injections post-transplant. Briefly, no differences were seen between the two groups for any type of infection or serious infections.

5 Dosing of IdeS®

IdeS is a clear colorless liquid. It is formulated at 10 g/L in PBS and intended for intravenous administration after dilution. Refer to table 5 for composition.

Table 5. Study drug

Ingredient	**Quantity (g/L)**	**Function**
IdeS drug product	10	Active ingredient
Phosphate buffered saline:		
Potassium Chloride (KCl)	0.20	Buffer
Monopotassium Phosphate (KH_2PO_4)	0.20	Buffer
Sodium Chloride (NaCl)	8.0	Buffer
Disodium Phosphate (Na_2HPO_4)	1.43	Buffer

5.1 Storage and Handling

All patients will receive treatment with IdeS. IdeS will be supplied to the hospital pharmacy by Biovian in 7 mL vials packed into cartons containing 10 vials each. Vials should be kept dark at – 20°C. IdeS infusion solution will be prepared at the study unit. Administration will be performed using an infusion bag with a filter containing infusion set and an infusion pump. Details on preparation, labeling and administration of IdeS are described in the pharmacy manual. (see Appendix B).

5.1.1 Collection, Transport & Shipping to the Laboratories

Samples for Anti-IdesS Antibody Levels will be collected according to the protocol outlined in Appendix C. These samples will be pooled and mailed to the

laboratories (Addresses listed in Appendix C). Thus, results will be available at the end of the study.

5.2 Patient Monitoring

Patients will be closely monitored for hypersensitivity reactions such as hives, urticaria, tightness of chest, wheezing, hypotension, and/or anaphylaxis during infusion of IdeS®, which are all common side effects of biological drugs. Patients will also be monitored for any signs of infections, although there were no signs of increased infection rates in healthy subjects who received IdeS. Patients with ongoing infections will not be included in the study.

5.3 Adverse Drug Reactions

A first in man single ascending dose study in 29 healthy subjects has previously been performed with doses up to 0.24 mg/kg BW with a favorable safety profile. Since IdeS degrades IgG there was concern that study subjects would have an increased risk of infection and that subclinical infections (e.g. pneumococci) would pose a problem. Therefore, subjects received antibiotic prophylaxis until plasma IgG levels had returned (>4.5 g/L) and there were no signs of an increased rate of infections within the study group. The adverse events that were reported, none of which were reported as serious, were as anticipated from to the biological nature of the drug. Hence, headache and fatigue were more common in the IdeS treated subjects compared to placebo. One possible infusion reaction was reported among the placebo treated subjects and one among the IdeS treated, the latter resolved within 15 minutes after treatment with antihistamine and glucocorticoids and the infusion was completed. All adverse events were mild or moderate.

5.3.1 Ides and Proteinuria

A phase I, first in man, double blind and randomized study with single ascending doses of IdeS has been conducted and the objective was to assess the safety, efficacy, pharmacokinetics, and immunogenicity of IdeS in healthy human subjects following intravenous administration.

A total of 29 healthy subjects were included and randomized into four dose groups. The subjects in each dose group were randomized to either IdeS or placebo. The starting dose was 0.01 mg/kg body weight (BW) (n_{IdeS} = 8 and $n_{Placebo}$ = 4) and after evaluation by the data monitoring committee the dose was stepwise increased to 0.04 mg/kg BW (n_{IdeS} = 4 and $n_{Placebo}$ = 2), 0.12 mg/kg BW (n_{IdeS} = 4 and $n_{Placebo}$ = 1) and 0.24 mg/kg BW (n_{IdeS} = 4 and $n_{Placebo}$ = 2). The subjects were followed until day 64 after dosing with more intensive monitoring during the first week. All subjects were male Caucasians with a median age of 23 years (range: 20-41), weight 76 kg (range: 59-100) with a body mass index of 23 kg/m2 (range: 20-30) and there were no statistical significant differences in demographics between the groups.

As part of the safety assessment urinalysis (U-glucose, U-hemoglobin and U-protein) was performed using Multistix (Siemens, Germany). The proteinuria was scored as negative, trace, +1 (0.3 g/L), +2 (1.0 g/L), +3 (3.0 g/L) and +4 (≥20 g/L) according to the manufacturer's instructions. A transient and mild proteinuria was observed 24-48 hours after dosing in subjects administered an IdeS dose that resulted in significant cleavage of IgG (Fig. 16). In the placebo treated group the proteinuria varied between negative and +1 during the 64 day period. In the highest dose group, where IgG was fully cleaved into $F(ab')_2$ and Fc within 2-6 hours, peak levels of proteinuria at +2 was observed in three of four subjects. This peak probably reflected the clearance of IgG cleavage products from the circulation. None of the treated subjects had a strong elevation in proteinuria and in all subjects the values were rapidly normalized. No subjects had any signs of hematuria correlating to IdeS treatment.

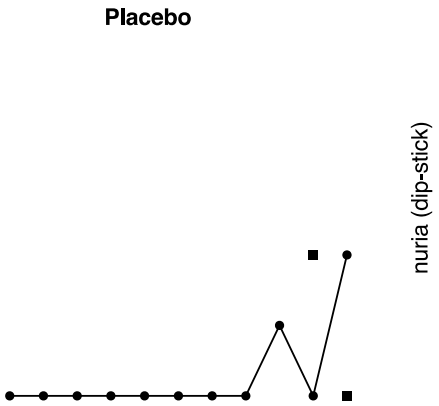


Figure 16. Proteinuria was monitored as a safety assessment throughout the study. Multistix (Siemens) were routinely used at the hospital and transient proteinuria was detected in several subjects which correlated to IgG cleavage. A) Subjects given placebo (n = 9). B) Subjects given a single dose of 0.24 mg/kg BW IdeS (n = 4). Data represents proteinuria in individual subjects at the indicated time-points after treatment.

To minimize adverse outcomes, patients with extended criteria donors (ECD) will be excluded from the study. The level of proteinuria post Ides is not clinically significant since proteinuria rapidly decreases to baseline day 3-4 after IdeS® administration. Proteinuric effects of IdeS will be monitored closely post IdeS for the first 4 weeks after transplant (see appendix A) to determine if there is a sustained effect.

6 Therapy Stopping Points

As indicated previously, the study will be halted and re-evaluated by the Data and Safety Monitoring Board (DSMB) if any patient in the study group develops SAEs or evidence of severe infusion related or infectious complications. Reassessment of the study goals and complications will be done and discussed with Hansa Medical, the DSMB and FDA prior to proceeding (see appendix D).

7 Statistical Analysis

Due to the exploratory nature of this study that involves safety endpoints only and the small sample size that is not powered for efficacy end points, our primary objective will be to assess safety end points and limited efficacy. Most important will be tolerability in the ESRD population and determination of the effects of IdeS® treatment on circulating DSAs as well as the durability of the effect and efficacy in reducing the number and severity of ABMR episodes. We will however plan to assess biopsies at 6M to determine if there are durable effects of IdeS® in prevention of ABMR both acute and chronic.

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Appendix A

Study/Protocol Title: "A Phase I/II Trial to Evaluate the Safety & Tolerability of the Human IgG endopeptidase (IdeS®) for Elimination of DSAs & Prevention of Antibody-Mediated Rejection Post-Transplant in Highly-HLA Sensitized Patients"

Study visit	Screening	Transplant Day 0	1 h ± 15 min	2h ± 15 min	6h ± 30min	Day 1 ± 2h	Day 2 ± 2h	Day 3 ± 6h	Day 4 ± 1d	Day 5 ± 1d	Day 7 ± 3d	Day 14 ± 3d	Day 21 ± 3d	Day 30 ± 3d	Day 90 ± 7d	Day 180 ± 30d
Prophylaxis: Ganciclovir for inpatients and Valganciclovir for outpatients	X															
Serum Igg		X													X	X
PPD/Quantiferon Test	X															
Informed Consent	X															
Inclusion/Exclusion criteria review	X															
Medical History	X															
Complete Physical Exam	X	X				X	X	X	X	X	X	X	X	X	X	X
Vital signs/weight	X	X				X	X	X	X	X	X	X	X	X	X	X
12-lead ECG (SOC)- w/in 6M	X															
Chest X-Ray (SOC) --w/in 6M	X															
Safety laboratory tests (CBC, CMP, UA)	X	X				X	X	X	X	X	X	X	X	X	X	X
Urine Total Protein, Urine Creatinine (UTP/UCr) (if urine available)		X				X	X	X	X	X	X	X				
Review Historical Serologies for HIV, HBV, HCV, CMV, & EBV	X															

EBV, CMV, PBK, PJC DNA PCR	X										X	X	X		
Pregnancy test (for WOCP)	X	X													
Estimated GFR (using MDRD equation)	X	X				X	X	X	X	X	X	X	X	X		

Study visit	**Screening**	**Transplant Day 0**	**1 h ± 15 min**	**2h ± 15 min**	**6h ± 1h**	**Day 1 ± 6h**	**Day 2 ± 6h**	**Day 3 ± 6h**	**Day 4 ± 6h**	**Day 5 ± 1d**	**Day 7 ± 2d**	**Day 14 ± 3d**	**Day 21 ± 7d**	**Day 30 ± 7d**	**Day 90 ± 14d**	**Day 180 ± 30d**
Pneumococcal Vaccine & Meningcoccal Vaccine (Menactra® & Menomune®)	X															
Desensitization w IVIG + Rituximab*	X															
Screen ADA (Freeze and batch out/analyze at the end of the study)	X															
ADA (Sent Out)		X (pre-dose)				X					X	X		X	X	X
C3, C4 Levels								X						X		
PD analysis (Sent Out)		X (pre-dose)	X		X	X					X		X	X	X	
PK analysis (Sent Out)		X (pre-dose)	X	X	X	X	X	X	X	X	X	X				
Donor Specific Antibodies (DSA)		X			X	X								X	X	X
IdeS® Administration		X														
Alemtuzumab Administration**									X							
Ciprofloxacin Prophylaxis daily x 30d		X					X	X	X	X	X	X	X	X		
Repeat IVIG Post Tx											X					

CNI Levels						X	X	X	X	X	X	X	X	X	X	X
Allograft biopsy																X
Adverse Event Monitoring		X				X	X	X	X	X	X	X	X	X	X	X

Revised 10/15/15

* Or Plasmapheresis [PLEX]

** Give alemtuzumab 4 days +/- 6 hours after IdeS administration