Use of Immune Globulin Intravenous (Human), 10% (IVIG), plus Rituximab as Agents to Reduce Donor Specific Antibodies, Improve Transplant Rates and Outcomes in Highly-HLA Sensitized Patients Awaiting Deceased Donor Kidney Transplantation.

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A. **RITUXIMAB + IVIG FOR DESENSITIZATION PROTOCOL SYNOPSIS**

PROTOCOL ID:	CSMC: Rituximab + IVIG 2013
TITLE:	"Use of Immune Globulin Intravenous (Human), 10% (IVIG), plus Rituximab as Agents to Reduce Donor Specific Antibodies, Improve Transplant Rates and Outcomes in Highly-HLA Sensitized Patients Awaiting Deceased Donor Kidney Transplantation."
SHORT TITLE:	Rituximab + IVIG for Desensitization
IND SPONSOR:	Genentech, Inc. South San Francisco, CA.
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CLINICAL TRIAL MATERIAL:	Immune Globulin Intravenous (Human), 10% Rituximab (Chimeric anti-CD20, anti-B-cell)
MANUFACTURERS:	Genentech Inc. South San Francisco, CA.
STATISTICAL COORDINATING CENTER:	Cedars-Sinai Medical Center
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HYPOTHESIS

Rates of transplantation for highly-HLA sensitized patients (HS) are exceedingly low (<10% of patients with PRAs > 30% receive DD kidneys each year). IVIG 10% has been demonstrated as an effective desensitization agent in placebo controlled trials (1). However, in this study, only 35% of treated patients vs 17% placebo were able to receive a working kidney transplant, most from DD. Recent uncontrolled data from our group demonstrated a robust effect on improvement of transplant rates for HS patients (80% treated) using a combination of IVIG + rituximab (2,3). This was also associated with improved reductions in donor-specific antibody levels (DSA) and flow-cytometry crossmatches at the time of transplant (4-8). This protocol appeared to be particularly effective in improving rates of transplantation for HS patients awaiting DD transplantation. Our group has also developed novel methodologies to predict the risk of antibody-mediated rejection (ABMR) post-transplant based on DSA and FCMX results after desensitization. This allows us to improve the prediction of acceptable donor-specific antibody (DSA) levels that are permissive for allowing transplantation to go forward (3-8).

With these considerations and observations in mind, we have formulated the following hypothesis. We believe that the addition of the novel B-cell depleting agent (Rituximab) to IVIG treatment will offer significant benefits in reducing anti-HLA (DSA) thus improving rates and outcomes of DD transplantation above that seen for highly-HLA sensitized patients awaiting DD transplant on the UNOS list. In addition, we will assess the ability of Rituximab to lower DSA levels and reduce allograft injury post-transplantation. Protocol biopsies will be performed at time of transplant and at 12M post-transplant to determine the incidence of chronic antibody mediated rejection (Transplant Glomerulopathy {TG}). These biopsies will be read by the pathologist at participating institutions and graded according to Banff Criteria (2005) (9).

TARGET POPULATION:

Patients with end-stage renal disease on dialysis (ESRD, CKD Stage V), ages 18-65 years who are awaiting deceased donor (DD) kidney transplantation on the UNOS wait list have a Panel Reactive Antibody (PRA) >30% and a significant sensitization history (previous pregnancies, blood transfusions and/or previous transplants) with sufficient wait time to allow frequent DD offers with positive crossmatches (CMXs) that prohibit transplantation.

STUDY OBJECTIVES:

The primary objective of this revised protocol will be to examine the safety and efficacy of IVIG 2gm/kg (maximum dose 140g) given on day#0 & day #30 plus Rituximab 1gm given on day#15.Transplanted patients will receive additional doses of Rituximab 1gm at 3 months post-transplant if DSA levels remain or become +. OR at 6M if de novo DSA occur. All transplanted patients who remain DSA-, will not receive additional Rituximab. All transplanted patients will have a protocol biopsy at 12 months. .

This trial is designed to determine if Rituximab + IVIG is an effective and safe desensitization protocol and if DSAs persisting or arising at 3M and 6M post-transplant can be effectively reduced by additional doses of Rituximab. This should be reflected in reduced rates of TG for Rituximab-treated patients. This is the PRIMARY END POINT. Secondary end points include and will be evaluated based on time line in appendix A:

- Renal allograft survival at 12M

- Reduction in anti-HLA antibodies (class I/II)
- Recipient survival at 12M
- The number of acute rejection episodes
- Rates of serious infectious complications (i.e., serious infections requiring hospitalizations and/or i.v. antibiotics)
- Adverse events, toxicity assessments
- Abnormal laboratory studies: Hematology, Chemistry, Liver Function, and research tests on study
- Clinical significant abnormalities in physical examination, vital signs, weight, ECG or Chest X-ray on study
- Serial donor-specific antibody assessments
- Pre & Post-transplant determinations of CD4+/CD25+FoxP3+CD127+ & Th17 cell populations (Cedars-Sinai Patients Only).
- Pre & Post-Transplant determinations of CD19+, CD38+, CD27+ cells (Cedars-Sinai Patients Only).
- Post-Transplant biopsy at T0 and T12M assessed by Banff Criteria.
- HACA determinations at serial intervals

PRELIMINARY DATA

Our group recently reported on the use of a combination of intravenous immune globulin and rituximab to reduce levels of anti-HLA antibodies and improve transplantation rates in highly-HLA sensitized patients (HS). This was an open label, single center, phase I/II study that aimed to evaluate the effectiveness of IVIG + rituximab in reducing PRA levels and T-cell CMXs to improve transplant rates. This study enrolled 20 HS adults who were awaiting kidney transplantation. The results of this study showed that the combination reduced PRA values, improved crossmatch (CMX) results that allowed for transplantation of 80% of entered patients with patient and graft survival (100%/94%) at 1 year without significant complications (2). It is important to note that the sensitization level of these patients would have prevented them from ever receiving a kidney transplant without this therapy. The protocol required two 1gm doses of rituximab, however during routine monitoring we noted that excellent depletion of B-cells was achieved after a single 1gm dose.

The aim of this report was to evaluate the efficacy of IVIG + rituximab on reduction of anti-HLA antibodies to a level that was permissive for LD or DD transplantation without incurring the risk of ABMR and immediate graft loss. Here we report on a modified approach to desensitization using IVIG (2gm/kg X2) + rituximab (1 gram) in HS patients awaiting kidney transplantation.

PATIENTS AND METHODS

Between July 2006 and February 2009, 76 HS patients awaiting LD or DD transplantation who met the following criteria for desensitization were evaluated. All patients had flow cytometry PRAs >30% (75% of patients were ≥80%). All patients had significant sensitizing risks such as multiple blood transfusions, pregnancies and/or previous transplants. All had positive pre-transplant T-cell flow cytometry crossmatches with prospective donors and/or had donor specific antibodies (DSA). Patients who had positive pre-transplant B-cell flow cytometry CMXs only, were eliminated from this analysis. These patients received IVIG and rituximab therapy as described below (2, 4-8).

IVIG/Rituximab Desensitization Protocol

All HS patients received non-sucrose containing IVIG 10% [2.0 grams /kilogram (maximum 140gm per dose) on day 1 & day 30], plus rituximab [1gram administered on day 15]. A donor-specific flow cytometry crossmatch (FCMX) was performed pre-transplant. An acceptable CMX is defined as a negative CDC, at least at a 1:2 dilution of sera. A positive T and B cell FCMX with a shift of <250 CS were also acceptable (Negative: < 100 MCS for B-cell & <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 previously reported (4-8). B-cell FCMX data obtained prior to June 2008 was not valid due to the presence of rituximab in test samples. However, pronase treatment of crossmatches was performed after that time. Insufficient numbers of pronase treated B cell crossmatches have been obtained to date and therefore, these data are not included in this study. Thus, a heavier reliance on T-cell FCMX data and DSA values (<100,000 SFI units) were used as the primary determinants of crossmatch acceptability as previously described. (4-8).

This strategy was also used in HS DD transplant candidates who were on the United Network of Organ Sharing (UNOS) list for >5 years who received frequent DD offers with positive cross matches. After completion of the desensitization protocol, patients usually waited 4-6 months for acceptable cross match offers. Because the half-life of IVIG is only 30 days, an additional IVIG dose was given at the time of transplant. All transplanted patients received an additional dose of IVIG (2gm/kg, maximum dose 140g) 10-14 days post-transplant.

Data were gathered before and after each IVIG infusion, at time of transplant, and months 1, 6, 12, and 24 post-transplant. Safety data included monitoring patients for infusion related side effects (such as fever, headache, shortness of breath during and immediately after infusion), viral infections and CNS related side effects. The patients were followed to determine the proportion with reductions in anti-HLA antibodies that subsequently obtained and retained a viable and functioning kidney allograft. The monitoring protocols were similar to those previously reported (2).

All IVIG doses were infused over a 4 hour hemodialysis session as previously described (9). Rituximab infusions were administered in an outpatient infusion center over a 6 hour period with frequent monitoring of vital signs. To reduce the frequency of infusion-related side effects, all patients were pre-treated with intravenous methylprednisolone (40mg - 125mg), acetaminophen (650 milligrams by mouth) and diphenhydramine (50 mg by mouth) 30-60 minutes prior to scheduled IVIG and rituximab infusions.

Donor-Specific Crossmatching and Anti-HLA Antibody Analysis

The FCMX and CDC were performed as previously described (4-8). Three-color FCMXs were performed according to the method of Bray et al (10), using FACScan cytometer (Becton-Dickinson, San Jose, CA). T-cell FCMXs were considered positive at more than 50 mean channel shifts (MCS) and B-cell FCMX were considered positive at more than 100 MCS. B-cell cross matches were always positive after rituximab therapy for up to 6 months or longer. These B cell cross match results were considered invalid. To address this issue, pronase treated B & T-cell cross matches have been performed since June 2008 (4-8). The negative cut off values for pronase treated cells is 70 MCS for T cells and 130 MCS for B cells.

The binding level of DSA was determined by the multianalyte bead assay performed on the Luminex platform. The single antigen Luminex bead assay was standardized with Quantiplex beads (One Lambda, Inc., Canoga Park, CA) and results were expressed as standard fluorescence intensity (SFI) as previously reported (4-8). These data were obtained on sera from patients treated after June 2007. Flow PRA Screening was performed per manufacturer's instructions as previously described. Briefly, 25 µl patient serum was mixed with 5µl of FlowPRA® screening beads (One Lambda Canoga Park, CA). Any significant shift of the bead population to the right of the negative control marker >50% is considered as positive. The percentage of Flow PRA Screening was calculated from the positive reactivity bead population.

RESULTS

Success of Desensitization

76 HS patients who met criteria for desensitization were successfully transplanted

(31LD/45DD). The mean follow-up time was 12.2 ± 8.4 months and 37% of patients had 12-24 months follow-up (table 1). All patients in this group were deemed to have high immunologic risk (54% had more than one previous transplant, 25% had PRA 30-79% and 75% had PRA≥80% {table 2}).

Effects of Desensitization on cPRA, Crossmatch and DSA Results

The effect of desensitization on flow cytometry PRA determinations were performed on a subset of 39 HS patients. This data show that the mean pre-treatment class I PRA was 79.7±25.6 % vs post-treatment 67.1±28.6%, (p=0.0001). The mean pre-treatment class II PRA was 59.7±29.2 vs. post-treatment 49.7±27.8, (p=0.01). This reduction is less than reported previously (2). Reasons for this difference include a change in technique from cytotoxicity-based PRA determinations to solid phase antibody testing using flow cytometry and luminex bead technologies. Both cytotoxicity and solid phase assays are susceptible to interference from IVIG but only for a period of less than a week after IVIG administration. Twenty five percent of treated patients experienced a reduction in flow cytometry PRA of 25% or greater. Analyses of transplantation and outcomes by flow cytometry PRA (Class I or II) and donor type are shown in figure 1. Seventy five percent of patients had flow cytometry PRA ≥80% (19LD/38DD) before desensitization. These patients also experienced more ABMR episodes. A significant reduction was seen in FCMX positivity against donor T-cells for patients receiving LD and DD transplants after desensitization (Fig 2A & 2B). Figure 2A shows the MCS results for T-cell FCMXs for all 31 individual LD recipients were available. Figure 2A shows the mean values for MCS pre-treatment and at transplant. Briefly, T-cell FCMX were significantly reduced from 183±98 MCS prior to therapy to 68±58 MCS at time of transplant (p<0.000006). T-cell FCMX data for DD recipients pre-treatment and at transplant are shown in figure 2B. Briefly, a significant reduction in mean T-cell FCMX was seen after desensitization in DD recipients (162±41 MCS pre-treatment vs. 125±49 MCS at transplant {p=0.05}). Table 3 shows rejection type and graft loss by DSA.

An analysis of AR (CMR and ABMR) over the 24 month observation period is shown in table 3. Briefly, 37% of patients had AR episodes (8%CMR and 29% ABMR). There was a very significant association of ABMR risk with the presence of DSA >100,000 SFI Units at time of transplant (17/29 with DSA >100,000 had ABMR while only 5/42 with DSA<100,000 experienced ABMR). There is no significant association between DSA >100,000 or <100,000 SFI units with graft loss to ABMR. The data also include 2 patients who developed late ABMR (>12M) due to non-compliance, both with graft losses.

Outcomes of Highly-HLA Sensitized Patients Transplanted After Desensitization

Patient and graft survival, acute rejection episodes, infectious complications, and renal function were monitored after transplantation. A summary of the patient demographics, immunologic profiles and infectious complications is shown in (tables 1 and 2). Patients who received DD transplants waited 95±46 months on the transplant waitlist before receiving desensitization with IVIG + rituximab, but waited only 4.2±4.5 months after treatment for transplantation. Patients who received DD transplants did not receive additional points toward transplantation for participation in this protocol.

Patient and Graft Survival

Patient and graft survival (non-death censored) up to 24 months were 95% and 84% {LD (100%/90%) vs. DD (91%/80%)} (Figure 3A&B). Graft losses occurred in nine patients: ABMR (6), non-adherence (2), and surgical complications (1). Four deaths occurred at 1, 6 and 10 months post-transplant due to donor-transmitted fungal sepsis (#1), bacterial sepsis (1), and myocardial infarction (#2). Data are summarized in table 2. No significant differences in outcomes were seen among the three induction regimens used.

Acute Rejection Episodes

Acute rejection episodes occurred in 37% of transplanted patients. Twenty nine percent (22/76) of AR episodes were C4d+ antibody mediated rejections (ABMR) (table 3). Most rejection episodes occurred within the first month post-transplant and were reversible with treatment. Seven graft losses were due to ABMR. All but 1 patient who lost their allograft to ABMR showed progressive elevations in DSAs.

Renal Function
Mean serum creatinine values at 1 month, 6 months, 12 months, and 24 months were 1.9±1.5, 1.3±0.4, 1.5±1.1 and 1.3±0.3 milligrams per deciliter, respectively.

Adverse Events, Serious Adverse Events and Infections
No patients to date have developed neurological symptoms suggestive of PML. Viral infections were seen post-transplant in 6 patients (8%) who were treated with IVIG and rituximab for desensitization (table 2). Two CMV, 3 PBK viremia, and 1 CMV/PVB19. No patient developed BK nephropathy. One patient died of donor transmitted fungal infection at 1 month post-transplant, another patient died of bacterial sepsis after prolonged bowel obstruction at one year post transplant. Minimal infusion-related side effects were noted (shortness of breath in several patients requiring additional methylprednisolone 125mg x1). Lack of significant infusion-related side-effects is likely due to the pre-medication regimen used prior to infusion and the long infusion times (2). Tables and figures are shown below:

Table 1: Demographics for highly sensitized patients desensitized with IVIG and rituximab (N=76)

Total Transplants	76
Male/Female (N=76)	27/49 (64% F)
Type (DD/LD) (N=76)	45/31 (59% DD)
Age Range (Years)	17-82yrs.
Mean Follow-Up Post Tx (N=76)	
Percent w. 12-24M Follow-up	28 (37%)
Percent w. 6-12M Follow-up	15 (20%)
Percent w. <6M Follow-up	33 (43%)
Race: (N=76)	
Caucasian	31 (41%)
African American	13 (17%)
Hispanic	22 (29%)
Other	10 (13%)
Etiology of ESRD: (N=76)	
Diabetes/HTN	26 (34%)

FSGS	3 (4%)
SLE	5 (7%)
Other (GN, Alport's, OU, CIN)	42 (55%)

DD denotes deceased donor. LD denotes living donor, M denotes month. AA denotes African-American. ESRD denotes end-stage renal disease. HTN denotes hypertension. FSGS denotes focal and segmental glomerulosclerosis. SLE denotes systemic lupus erythematosus. GN denotes glomerulonephritis. OU denotes obstructive uropathy. CIN denotes chronic interstitial nephritis.

Table 2: *Immunologic & infectious profiles for highly sensitized patients receiving transplants after desensitization with IVIG and rituximab. (N=76)*

Transplant by Flow PRA (Class I or II) & Donor Type (N=76)		
Type	DD (N=45)	LD (N=31)
30-79%	7 (16%)	12 (39%)
≥80%	38 (84%)	19 (61%)
Previous Transplants (N=76)		
0	35 (46%)	
1	28 (37%)	
≥2	13 (17%)	
Infectious Complications (N=8 {11%})		
Viral (N=6{8%})		
CMV/PVB19	1 (1%)	
CMV	2 (3%)	
Polyoma BK	3 (4%)	
Fungal (N=1 {1%})		
Coccidiomycosis from donor	1(1%)†	
Bacterial (N=1 {1%})		
Sepsis post-bowel obstruction	1(1%)†	
Death post transplant (N=4 {5%})	DD (N=4)	LD(N=0)
Infections (N=2)		
Fungal	1 (1%)	
Bacterial	1 (1%)	
Myocardial infarction (N=2)	2 (3%)	
HLA Matches (N=76)		
0 Ag match	12 (16%)	

1 Ag match	34 (44%)
2 Ag match	12 (16%)
≥3 Ag match	18 (24%)

Flow PRA denotes panel reactive antibody as determined by flow cytometry assays. DD denotes deceased donor. LD denotes living donor. CMV denotes cytomegalovirus. PVB 19 denotes Parvovirus B19. HLA denotes human leukocyte antigen. †= death.

Figure 1: This figure shows an analysis of transplantation and outcomes by PRA and donor type. 75% of patients had PRA >80%. There were also more ABMR episodes and graft losses to ABMR in this group.

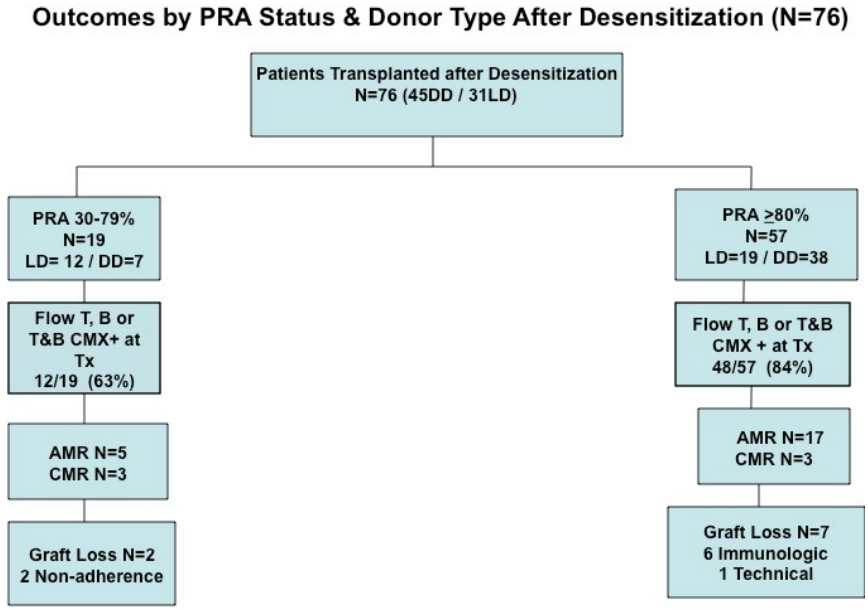


Table 3: Shows AR episodes and graft losses in the patients receiving transplants after desensitization. Relationship of ABMR to DSA levels at time of transplant is also shown.

Rejection Types & Graft Loss by DSA	Outcomes	Significance
Total AR Episodes	28/76 (37%)	
Total CMR Episodes (3DD / 3LD)	6/76 (8%)	
Total Graft Loss to CMR	0/76 (0%)	
Total AMR Episodes (11DD / 11LD)	22/76 (29%)	
AMR+ (DSA<100,000 SFI Units)* AMR- (DSA<100,000 SFI Units)	5 42	
AMR+ (DSA >100,000 SFI Units)* AMR- (DSA >100,000 SFI Units)	17 12	**P<0.0000004
Graft Loss to AMR by DSA <100,000 SFI Units	2/5*** (40%)	
Graft Loss to AMR by DSA >100,000 SFI Units	5/17*** (29%)	P=NS

*DSA values obtained at time of transplant

**Statistics is by χ^2 analysis

***This data includes 2 patients with late AMR due to non-compliance 12 months post-transplant.

Figure 2A & B: This figure shows T-cell FCMX values for pre-treatment and at time of transplant for 31 HS LD (2A) and 45 HS DD (2B) recipients who were desensitized with IVIG+ rituximab. A negative T-cell FCMX is <50 MCS.

Figure 2A

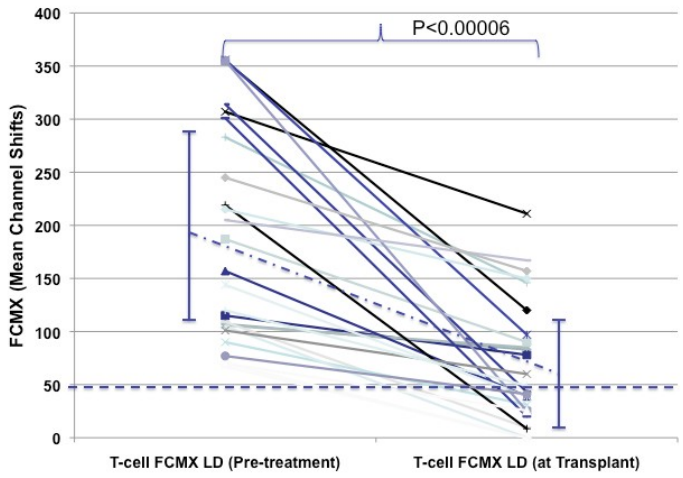


Figure 2B

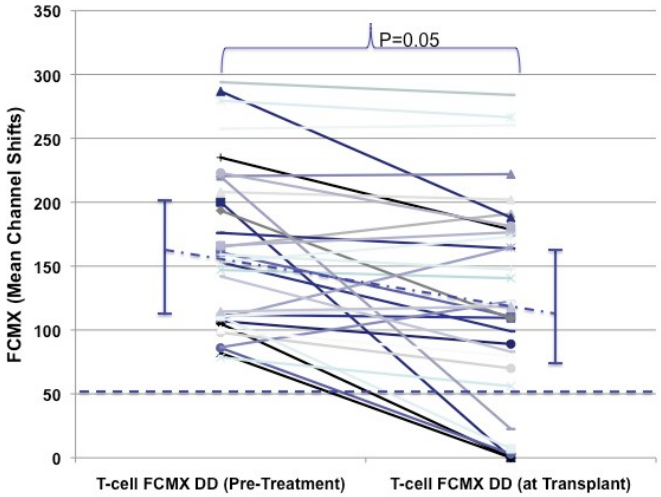


Figure 3A & B: This figure shows non-death censored patient and graft survival values at 0M, 1M, 6M, 12M and 24M for 76 HS patients who were desensitized with IVIG + rituximab. (3A) Patient and Graft Survival for all patients up to 24M post transplant. (3B) Patient and Graft Survival by donor type LD (N=31) and DD (N=45) for the same period.

Figure 3A

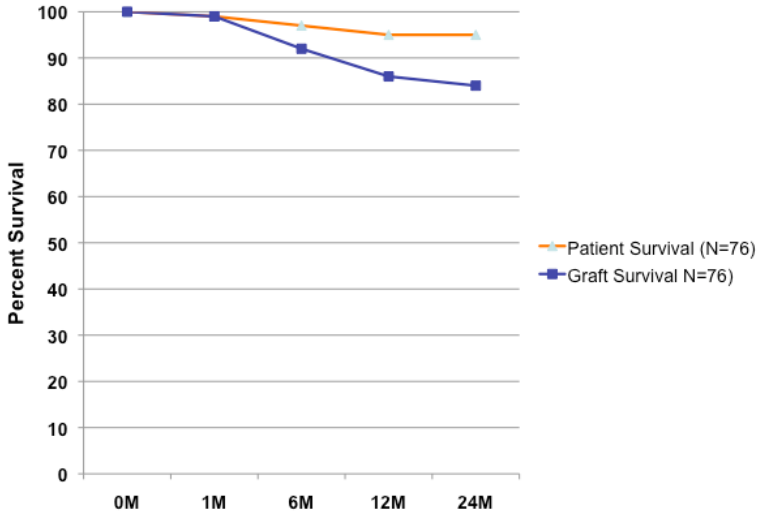
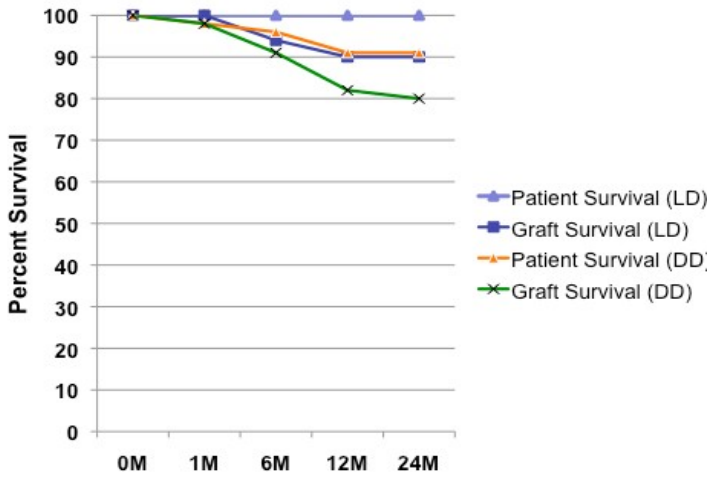


Figure 3B



STUDY DESIGN:

This single (or multiple)-center, Phase I/II, exploratory study will use an open label design. The revision of this protocol is attributed to the failure of the placebo controlled protocol which showed a significant increase in SAEs in the IVIG + placebo group (3 antibody-mediated rejection episodes of 5 transplanted compared with 0 ABMR episodes in the IVIG + Rituximab group, p=0.038). The original study was put on hold and this iteration represents a redesign of the protocol to investigate the optimal dosing of Rituximab for desensitization and for modification of DSA levels post-transplant. The trial will examine the safety and efficacy of human polyclonal IGIV 10%, when given at [2.0 gm/kgx2], + Rituximab 1gm to reduce DSA to a level that is permissive for DD transplantation in 75 subjects (adults only ages ≥18 yrs) who are highly-HLA sensitized and are awaiting DD kidney transplant. Once transplant offers are entertained, a donor-specific crossmatch will be performed to detect anti-HLA antibodies which are associated with acute rejection or graft loss. (These anti-HLA antibodies may result naturally or from some previous pregnancy, transfusions, or prior transplants). If acceptable crossmatches and DSA levels are seen, the patients will proceed to DD transplantation. Patients receiving transplants will receive an additional dose of IVIG at transplantation (within 10 days) and will receive additional doses of Rituximab 1g) at 3M post-transplant if they remain or develop DSAs. OR at 6M if de novo DSAs occur (Fig 5 & 6). Patients who are desensitized and not transplanted within 9 M after desensitization will have completed the study and can be treated as best judged by the participating center. The protocol is shown in Figure 4 below.

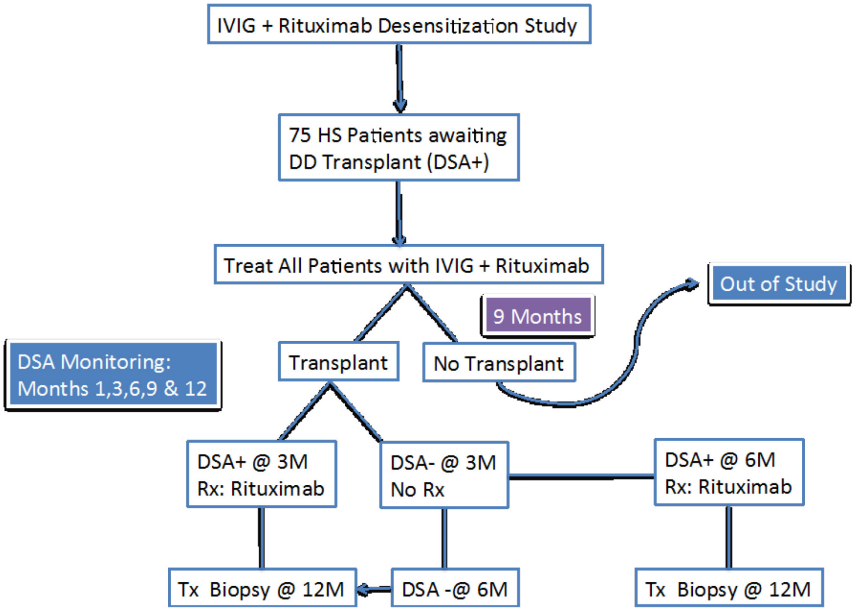


Figure 4: Study Protocol for revised IVIG + Rituximab Study.

The decision to accept a DD transplant will rest entirely with the participating transplant center.

The subjects will be followed to determine the proportion who receive a transplant within 9M of completion of the infusions. In addition we will assess the transplanted patients to determine the number who sustain a viable and functioning kidney allograft for 12 months at which time a repeat study biopsy will be performed. 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.

Figure 5: Pre-Transplant Desensitization Protocol for deceased donor recipients:

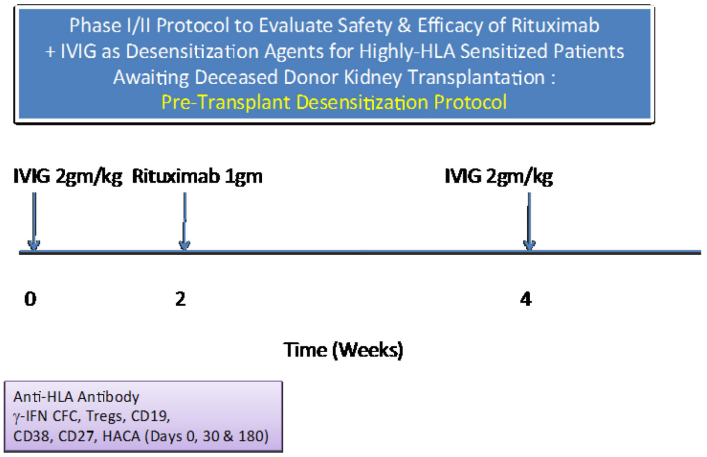
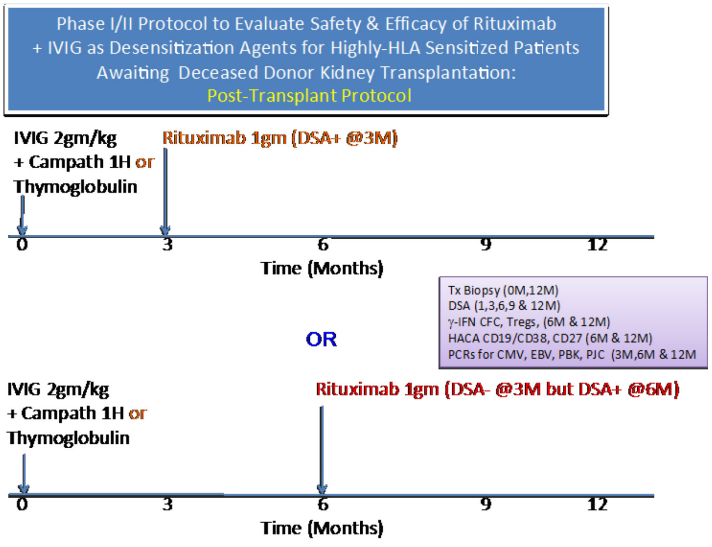


Figure 6 Shows the Post-Transplant Management Protocol for Patients Receiving DD Transplants.



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ACCRUAL GOAL: 75 subjects

DURATION OF ACCRUAL: 24 months

DURATION OF FOLLOW-UP:	9 months for all desensitized subjects, 12 months for all transplanted subjects
THERAPY STOPPING RULES:	Safety stopping rules for the study include: The study will be put on hold and re-evaluated for safety signals if significant adverse events (serious infusion reactions, serious infections or death and or graft loss from early ABMR) are seen in three of the first 5 patients. In addition, if biopsy proven ABMR is seen in 4 of the first 10 patients entered, the study will be stopped for re-evaluation. Efficacy Stopping Rules: The DSMB will review cumulative safety and efficacy data according to a pre-specified schedule, not less frequently than quarterly. Based on these data (after the pre-specified data reviews, the DSMB may recommend alterations or stopping of the protocol.
CONCOMITANT MEDICATIONS:	Concomitant therapy will include a standard immunosuppressive regimen of mycophenolate mofetil (CellCept®) at 1200 mg/m^2/day given in two divided doses, Tacrolimus (Prograf®) given at 0.05 to 0.15 mg/kg divided twice daily, and prednisone loading dose and taper as per center protocol. Induction therapy using Campath 1H 30mg SQ/IV at the time of transplant **OR** Thymoglobulin® (total dose not to exceed 6.0mg/kg) IV per center protocol will be given to subjects on study. The safety and efficacy of Campath 1H induction therapy after desensitization with IVIG + Rituximab has been previously described for adults (11,12). Therapy for recurrent biopsy proven rejection will be initiated as standard of care at participating institutions. The recommended initial rejection therapy will consist of pulse Solumedrol per center protocol. Alternatively, Thymoglobulin will typically be given for 5-10 days, depending on the time of return of creatinine levels to baseline. If resistant ABMR is seen, the use of IVIG +Rituximab (anti-CD20 monoclonal antibody) and/or plasmapheresis will be considered. In addition, the use of monoclonal antibodies to the complement component C5 (Eculizumab®, Alexion, Cheshire, CT) may be used at the discretion of the investigators.
DATA SAFETY MONITORING	A major component of this study will be to determine the safety of this combination of drugs for desensitization and subsequent transplantation. In order to insure this is done, a Data Safety Monitoring Board (Drs. Jordan, Dr. Lill (Bone Marrow Transplantation) & Vo) will be created to monitor AEs and SAEs that occur during the study. Cedars-Sinai will maintain the database for all patients entered into the study and will be responsible for accumulation of safety data and reporting to participating centers and Genentech with each occurrence.
RECIPIENT INCLUSION CRITERIA:	1. End-stage renal disease. 2. No known contraindications for therapy with IGIV10%/Rituximab. 3. Age 18-70 years at the time of screening. 4. CPRA≥ 30% demonstrated on 3 consecutive samples, UNOS wait time sufficient to allow DD offers, history of sensitizing events, positive crossmatch with the intended donor. 5. Subject/Parent/Guardian must be able to understand and provide informed consent.
RECIPIENT EXCLUSION CRITERIA:	1. Lactating or pregnant females. 2. Pediatric patients <18 years of age 3. Women of child-bearing age who are not willing or able to practice FDA-approved forms of contraception. 4. HIV-positive subjects. 5. Subjects who test positive for HBV infection [positive HBVsAg, HBVcAg, or

HBVeAg/DNA] or HCV infection [positive Anti-HCV (EIA) and confirmatory HCV RIBA].

6. Subjects with active TB.
7. 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.
8. Subjects for whom multiple organ transplants are planned.
9. 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])*
10. 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 > 5X upper limit of normal, and an SGPT >5X upper limit of normal range.
11. Individuals deemed unable to comply with the protocol.
12. Subjects with active CMV or EBV infection as defined by CMV-specific serology (IgG or IgM) and EBV-specific serology (IgG or IgM) and confirmed by quantitative PCR with or without a compatible illness.
13. Subjects with a known history of previous myocardial infarction within one year of screening.
14. Subjects with a history of clinically significant thrombotic episodes, and subjects with active peripheral vascular disease.
15. Use of investigational agents within 4 weeks of participation.

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1.0 STATISTICAL CONSIDERATIONS

1.1 IVIG + Rituximab for Desensitization

Primary Objective:

This trial is designed to determine if Rituximab + IVIG is effective in improving rates of transplantation for highly-HLA sensitized DD candidates on the UNOS waiting list over a 9M period of time after completion of treatment. Results will be compared to transplant rates in a group of age and anti-HLA antibody matched patients who remain on the UNOS wait list during the study period. This is the PRIMARY END POINT. Secondary end points include and will be evaluated based on time line in appendix A:

Transplantation rate assumptions:

- 60-65% in the IVIG + Rituximab group
- ~15% in the non-desensitized patients on UNOS wait list

1.2 Target Sample Size

The target sample size is based on the statistical estimates of adequate sample size to power the study at 95% confidence. 95% Confidence Intervals for the 12-month Transplant Rate Assumes a 50% transplant rate (Expected proportion = 0.50) at 12 months for IVIG + Rituximab

Confidence interval for proportion using normal approximation (n large)

	1	2	3
Confidence level, 1-□	0.95	0.95	0.95
1 or 2 sided interval?	2	2	2
Expected proportion, □	0.50	0.50	0.50
Distance from proportion to limit, □	0.127	0.117	0.113
n	60	70	75

We do not have a good estimate of the IVIG + Rituximab transplant rate at 12 months in this patient population. We expect the rate to be at least 50%, hopefully as high as 60 to 65%. This is based on our experience over the past 7 years where 67% of deceased donor recipients were transplanted after desensitization (Vo et al. Transplantation 2013;65:1-7). This article is included. Thus these estimates are reliable in our hands.
For estimation using a 95% confidence interval, the assumed 50% transplant rate is conservative in that the width of the 95% confidence interval will be maximum when the assumed rate is 50%, i.e. the estimate will be least precise when the assumed rate is 50%.

Table 3 shows the upper limit of the 95% confidence interval estimates of the historical transplant rate at 3 years to be about 32% (highest at 38% in 2006).
Column 3 (Distance from proportion to limit) in the above power and sample size table shows that the lower limit of the 95% confidence interval for the IVIG + Rituximab transplant rate at 12 months (assuming the rate = 50%) is about 39% [50% – 11.3% = 38.7%], which is higher than the upper limit of the 95% confidence interval for the historical rate at 3 years (even in 2006).
If the observed IVIG + Rituximab transplant rate is > 50%, then the lower level of the 95% confidence interval will be > 39% and the evidence for the effectiveness of IVIG + Rituximab will be stronger.
For example, if the observed transplant rate is 60%, then the 95% confidence interval (based on n = 75) is approximately 49% to 71%.
[As I wrote in the document: "If the observed 12-month IVIG + Rituximab transplant rate is > 50%, then the effectiveness evidence is even stronger (the lower limit of the CI will be higher)."]

If the 9-month transplant rate is 50% (or 60 to 65%) then the 12-month rate should be even higher. During the placebo controlled phase of this study, the 13/15 patients were transplanted at a mean of 7 months post treatment. (7 IVIG + Placebo v. 6 IVIG + Rituximab). The mean wait time on dialysis for this group was > 11 years prior to being entered into the study and transplanted. Our original time of assessment of transplantation

was 6M. However, with this preliminary data, we felt it better to look at 9M since this would likely be more reasonable and would give a fair assessment of the efficacy of the therapy. We feel we are being conservative with these estimates. Hopefully, this clarifies the sample size calculation issues.

Column 1: the lower limit of the CI is 0.373 or about 37% when the sample size is 60.

Column 2: the lower limit of the CI is 0.383 or about 38% when the sample size is 70.

Column 3: the lower limit of the CI is 0.387 or about 39% when the sample size is 75.

If the upper limit of the UNOS CI (historical data) is below the lower limit of the above CI, then the CI do not overlap and there is evidence that IVIG + Rituximab is more effective re the 12-month transplant rate.

Given the UNOS data for transplant rates (Table 4 & 5), 60 should be sufficient as a sample size to show benefit of the IVIG + Rituximab treatment. However, 75 patients gives a better and more robust distinction. Since we will be looking at transplant rates at 9M post-treatment, and given the vagueries of organ allocation and patient readiness issues, we feel the choice of 75 patients will provide more security for an accurate and definitive study.

If the observed 12-month IVIG + Rituximab transplant rate is > 50%, then the effectiveness evidence is even stronger (the lower limit of the CI will be higher).

1.3 Planned Efficacy Evaluations

The efficacy end points will be evaluated after all 75 patients have been enrolled into the trial. We plan to evaluate rates of deceased donor transplantation as well as conversion rate of DSA and rates of ABMR in those transplanted. The efficacy of the proposed combination of IVIG + Rituximab will be determined by the statistical methods. These evaluations will be carried out in concert with our safety monitoring that is outlined in section 9.0. The appropriate stopping points and re-evaluation points are described there as well.

1.3.1 Primary Efficacy Variables

The primary efficacy variable is the number of highly-HLA sensitized patients receiving a deceased donor transplant within 9 months after completion of the treatment. From our previous experience, the anticipated transplant rates for patients receiving IVIG + Rituximab desensitization is ~70% (49,112, 131). The rates for DD transplantation in non-treated patients based on sensitization levels is shown in Figure 15 below. Briefly, the rates of transplantation for all sensitized patients are <12% per year for the past decade. This indicates that desensitization

with IVIG + Rituximab is likely to benefit this high risk group who are otherwise unlikely to receive a life-saving transplant.

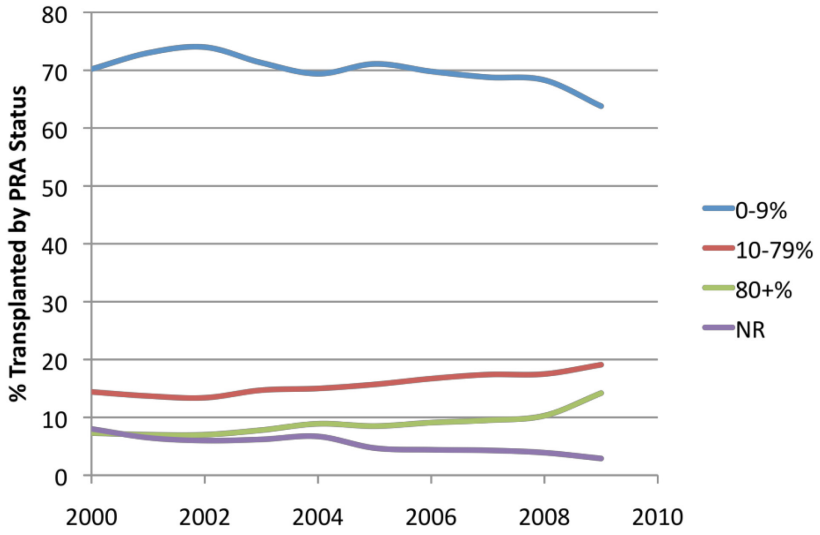

Figure 15: Data shown in figure 14 represent an analysis of 95,083 deceased donor transplants performed over the 10 year period 2000-2009. When analyzed by PRA status, one can see that <20% of all transplants performed are in highly-HLA sensitized patients. Data from the United States Renal Data Systems (usrds.gov) for 2009 also show that the wait time for a deceased donor kidney for patients with PRAs <10% is 4 years but increases to 8 years for patients with PRAs >10%. These trends have not changed over the past decade.

1.3.2 Secondary Efficacy Variables

In addition to the primary efficacy variables, we will evaluate a number of secondary efficacy variables. These include donor-specific antibody testing, patient and graft survival, infectious complications, immunologic testing, protocol biopsies and rates of allograft rejection. These end points are outlined in section 2.0 and Appendix A. Data will be analyzed in the treatment group.

1.3.3 Methods of Analysis

We aim to recruit 75 HS patients on the basis of a clinically relevant increase in the absolute rate of transplantation for highly-HLA sensitized deceased donor recipients of approximately 50 percentage points between the two groups during 9 months of follow-up, with an estimated event rate of 15% in the control UNOS study group. We determined that this number of patients would provide the study with a power of 95%, with a two-sided type I error of 5% and a nonadherence or loss to follow up rate of 5(or 10)%. A transplant rate of 60-70% in the intervention limb is based on our experience with desensitization in the recent years (49, 113, 131). All analyses shall be performed on the basis of the intention-to-treat principle. Patients that were transplanted outside of the study will be included in the analysis.

We shall compare the proportions of patients in the treatment group compared to the UNOS control group using time-to-event analysis with respect to outcomes such as the primary outcome (rate of transplantation) and certain secondary outcomes (such as recipient and graft survival, occurrence of rejection in the treatment group only). These outcomes shall be analyzed with the use of the log-rank test. Cox proportional-hazards regression shall be used to obtain unadjusted hazard ratios and to adjust for significant stratifying variables and to test for effect modification in all secondary analyses. Data from patients who are lost to follow-up shall be regarded as censored at the time of the last contact. Data from patients who do not receive transplantation shall be censored at end of 9 months post desensitization. We shall use Kaplan–Meier estimates of the proportion of patients with the above listed outcomes. We shall calculate the number of patients who would need to be desensitized to allow one deceased donor renal transplant from the hazard ratio and its 95% confidence interval.
For other outcomes, the Chi square or Fischer exact test shall be used if the variable is categorical, and the t-test or signed rank test if it is continuous variable.

To determine whether the treatment effect varies according to the patients' PRA status, a priori subgroup analysis shall be planned with patients stratified according to the titer of PRA (>80% or <80%), and a test of interaction shall be performed in a Cox model. Post hoc subgroup analyses shall be conducted with the use of other stratifying variables. Treatment effects shall be described in terms of hazard ratios and absolute risk differences with 95% confidence intervals. All P values reported shall be two-sided and adjudged significant if <0.05.