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Comparative Evaluation of α CD40 (2C10R4) and α CD154 (5C8H1 and IDEC-131) in a Nonhuman Primate Cardiac Allotransplant Model

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Abstract

Background—Specific blockade of T cell costimulation pathway is a promising immunomodulatory approach being developed to replace our current clinical immunosuppression therapies. The goal of this study is to compare results associated with 3 monoclonal antibodies directed against the CD40/CD154 T cell costimulation pathway.

Methods—Cynomolgus monkey heterotopic cardiac allograft recipients were treated with either IDEC-131 (humanized α CD154, n=9), 5C8H1 (mouse-human chimeric α CD154, n=5), or 2C10R4 (mouse-rhesus chimeric α CD40, n=6) monotherapy using a consistent, comparable dosing regimen for 3 months after transplant.

Results—Relative to the previously reported IDEC-131 treated allografts, median survival time (MST 35 $\pm$ 31 days) was significantly prolonged in both 5C8H1 (142 $\pm$ 26, p<0.002) and 2C10R4 (124 $\pm$ 37, p<0.020) treated allografts. IDEC-131 treated grafts had higher CAV severity scores during treatment relative to either 5C8H1 (p=0.008) or 2C10R4 (p=0.0002). Both 5C8H1 (5 of 5 animals, p=0.02) and 2C10R4 (6/6, p=0.007), but not IDEC-131 (2/9), completely attenuated IgM antidonor alloAb production during treatment; 5C8H1 (5/5) more consistently attenuated IgG alloAb production compared to 2C10R4 (4/6) and IDEC-131 (0/9). All evaluable explanted grafts experienced antibody-mediated rejection. Only 2C10R4 treated animals exhibited a modest, transient drop in CD20⁺ lymphocytes from baseline at d14 after transplant (-457 $\pm$ 152 cells/ μ L) compared to 5C8H1 treated animals (16 $\pm$ 25, p=0.037), and the resurgent B cells were primarily of a naïve phenotype.

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Conclusion—In this model, CD154/CD40 axis blockade using IDEC-131 is an inferior immunomodulatory treatment than 5C8H1 or 2C10R4, which have similar efficacy to prolong graft survival and to delay CAV development and antidonor alloAb production during treatment.

Introduction

Current clinical immunosuppressive regimens are incompletely effective at preventing chronic rejection and are associated with a myriad of adverse effects. Considerable attention has thus been given to exploring alternative immunomodulatory approaches. The 2 major T cell costimulation pathways that provide second signals required for T cell activation, CD28/B7 (CD80/CD86) and CD40/CD154 (CD40L), have been identified as logical targets to improve clinical transplant outcomes. The CD40/CD154 pathway plays a pivotal role in T cell-mediated dendritic cells (DC) and macrophage activation, development of Th1-type immune responses, and cytotoxic T cell priming.¹ As such, considerable effort has been directed toward development of clinically useful antibodies to both CD40 and CD154 for the protection of graft rejection in transplantation and for the treatment of autoimmune disease.

CD154 is a member of the TNF family and is rapidly induced on T cells following T cell receptor (TCR) activation. By targeting activated T cells, α CD154 demonstrated prolonged acute rejection-free periods following allotransplantation in NHP renal,²⁻⁵ skin,⁶ heart,⁷ and islet cells allografts.⁸ However, preclinical and human clinical trials of α CD154 were thwarted by thromboembolic (TE) complications,⁹⁻¹¹ which is likely due to expression of CD154 on activated platelets that stabilizes arterial thrombi in a CD40-independent manner.^{12, 13} Hence, α CD40 agents were developed in anticipation of an improved safety profile.

CD40 is a cell surface glycoprotein in the TNF receptor family that is widely expressed, both constitutively on antigen presenting cells (APCs), including B cells, DCs, and macrophages, and induced after inflammatory stimulation on endothelial cells and fibroblasts.¹ Despite the ubiquitous nature of CD40, overt harmful downstream effects of CD40 blockade have not been demonstrated in other preclinical models, and clinical success has been demonstrated with several different α CD40 mAb.¹⁴⁻²¹

Here we compare the efficacy of 3 contemporary monoclonal antibodies (mAb) that block the CD40/CD154 costimulation pathway, tested in a preclinical nonhuman primate (NHP) model: IDEC-131 and 5C8H1, a mouse-human chimeric α CD154 mAbs, and 2C10R4, a mouse-rhesus chimeric α CD40 mAb.

Materials and Methods

Animal Model

All protocols were approved by the Institutional Animal Care and Use Committee at the University of Maryland School of Medicine and were conducted in compliance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals*. Captive-bred and wild-caught cynomolgus monkeys (*Macaca fascicularis*) of Chinese and Indonesian origin were used. Males and females weighing 2.9-9.7 kg were selected as organ

recipients of ABO blood type-compatible donors. Major histocompatibility complex (MHC) class II mismatch was ensured by maximizing mixed lymphocyte reaction response and achieving a stimulation index >3 for each donor-recipient pair. The development of antidonor class I (T cell) alloAb demonstrated class I mismatch. Animals that did not develop alloAb had genomic DNA Illumina sequencing spanning exon 2 for all class I loci (Madison, WI) to confirm a high degree of Mafa-A and Mafa-B class I mismatch.

Surgical Procedures

All recipients underwent heterotopic intra-abdominal cardiac transplantation, as described previously.²² Open cardiac biopsies were performed by protocol on postoperative days (d) 14, 42, 90, and 150, if applicable. Biopsies were occasionally omitted or delayed due to anemia or weight loss. Core temperature and graft heart rate, systolic blood pressure, and diastolic blood pressure were assessed at least once daily using intra-abdominal telemetry (D70-PCTP or L11, Data Sciences International, St. Paul, MN) until time of graft explant.

Immunosuppression Treatment Groups and Dosing

5C8H1 is a mouse-human IgG1 κ chimeric anti-CD154 antibody, and 2C10R4 is a mouse-rhesus IgG4 κ anti-CD40 antibody.²¹ Both were obtained from the NIH Nonhuman Primate Reagent Resource (Boston, MA). IDEC-131, a humanized IgG1 anti-CD154 mAb, was a gift from Biogen Idec Inc. (San Diego, CA). Three IDEC-131 recipients received IDEC-131 intravenously (IV) at 30mg/kg on d0, d3, d7, and d14; 20 mg/kg on d28, d56, and d84 (total 180 mg/kg). This regimen was intensified, as described previously,²³ and subsequent recipients received either IDEC-131 (n=6), 5C8H1 (n=5), or 2C10R4 (n=6) IV at 30 mg/kg on d0, d3, d7, and d14; 10 mg/kg on d21, d28, d35, and d42; 20 mg/kg on d56 and d84 (total 200 mg/kg).

In the IDEC-131 treatment group only, 3 monkeys with suspected or biopsy-confirmed rejection were treated with steroid boluses (Solu-Medrol, Pharmacia, Kalamazoo, MI; 10 mg/kg IV daily for 3 doses, n=3), antithymocyte globulin (ATG; NR-ATG, DCI Inc., Nashville, TN or Thymoglobulin, Sangstad, Menlo Park, CA; 10 mg/kg IV daily for 3 doses, n=1), or with anti-CD20 rituximab (Rituxan, Genetech, South San Francisco, CA; 20 mg/kg IV once, n=1; Table 1).

In the 2C10R4 treatment group, coagulated blood in ethylenediaminetetraacetic acid (EDTA) tubes on routine blood draws on d7-11 raised suspicion for hypercoagulability in 1 animal, and on d7 heparin (50 units/kg/hr IV for 2 days, 15 units/kg/hr IV for the following 19 days) and aspirin (20 mg PO daily until day of rejection) were given (FB6E, Table 1). Routine monitoring for CMV was not performed, and antiviral medications were not used.

Allograft Rejection and Failure Criteria

Primary graft survival was defined as time to first rejection episode or graft failure and served as the primary study endpoint for all survival analyses to minimize the bias of IDEC-131 receiving treatment for acute rejection episodes. Acute rejection episode criteria included 1 or more of the following: intra-abdominal body temperature >38.5°C, decline in heart rate >20% below baseline or <120 beats per minute, pulse pressure (=systolic –

diastolic in mmHg) <30mmHg, or weak palpable graft contraction lasting >12 hours. Graft failure was defined by 1 or more of the following criteria for 2 consecutive days: decline in heart rate >20% below baseline or <120 beats per minute, pulse pressure <30mmHg, or nonpalpable graft contraction.

Drug Trough Levels and Receptor Coverage

Serum trough α CD154 and α CD40 levels were checked retrospectively by enzyme-linked Immunosorbent Assay (ELISA; SDC Materials and Methods). The peripheral blood CD40 receptor saturation was measured by flow cytometry prior to each 2C10R4 administration and biweekly after completion of the drug treatment. Peripheral blood was stained for CD20 (L27, BD Biosciences, San Jose, CA) and CD40 (5C8, Biolegend San Diego, CA), and the CD40 saturation was calculated as the percentage of CD20⁺CD40⁺ among CD20⁺ cells subtracted from 100%. A tube with excess 2C10R4 served as positive control. The samples were processed on a Verse flow cytometer (BD Biosciences) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Blood Collection and Lymphocyte Detection

Routine blood collection was performed, lymphocytes stained for flow cytometry and analyzed for CD20⁺ B memory cell phenotypes (SDC Materials and Methods).^{24, 25}

Antidonor Alloantibody Detection

Antidonor alloAb was measured retrospectively by flow cytometry using archived frozen donor splenocytes and recipient sera, as described previously.^{26, 27} Antidonor alloAb elaboration was defined as consistently detected IgM-positive or IgG-positive donor CD3⁺CD20⁻ T cells of >10% relative to donor serum prior to transplant.²⁸

Histology and Cardiac Allograft Vasculopathy Grading

Biopsy and explant cardiac tissue specimens were fixed with 10% formalin, processed for paraffin embedding, and stained with hematoxylin and eosin (H&E). Cellular myocardial infiltrates were classified by the 2005 International Society for Heart and Lung Transplantation (ISHLT) revised criteria for cardiac allograft rejection.²⁹ Cardiac allograft vasculopathy (CAV) severity with H&E staining was scored on an average of 3 ± 0.3 and 28 ± 2.5 vessels in each biopsy and explanted graft specimen, respectively, by 3 independent evaluators (NAO or LB, TZ, and RNP), who were blinded with respect to treatment group.²⁶ Biopsies with poor tissue preservation or absence of identifiable myocardial tissue were classified as nondiagnostic (ND). Pericardial granulation tissue was excluded from rejection and CAV assessments.

Immunofluorescence Staining and Antibody-Mediated Rejection Score

Evaluable cardiac tissue specimens were stained for CD68⁺ and C4d and scored using the 2013 ISHLT guidelines for antibody-mediated rejection (AMR) scoring (SDC Materials and Methods).³⁰

Cellular infiltration of explanted allografts by CD3⁺ and CD20⁺ lymphocytes was evaluated in a similar manner. Each explant had 8-9 representative images per staining and was

independently scored by 3 blinded evaluators (NAO, TZ, and CTL). The mean score of the representative images per graft was reported (SDC Table 1).

Statistical Analysis

Survival analysis was examined by the Kaplan-Meier method and compared by log-rank test. Continuous variables were expressed as the mean±standard error of the mean (SEM) or median and [interquartile ranges (IQR)], and these were compared using either the 2-tailed unpaired t test to compare 2 groups or as analysis of variance (ANOVA) to compare 3 or more groups. Nominal variables were compared using a contingency table and the Fisher's exact test. P values less than 0.05 were considered statistically significant. All statistical analyses were performed on a personal computer with GraphPad InStat (version 3.01; GraphPad Software, San Diego, CA).

Results

Pharmacokinetics

IDEC-131, 5C8H1, and 2C10R4 serum trough levels were generally >300 µg/mL for the first month and >100 µg/mL for the 90 days following transplantation (Figure 1A). Trough levels during the 90-day treatment for 2C10R4 treated allografts (mean±SEM; 376±22 µg/mL) were significantly higher than IDEC-131 treated allografts (289±20; p=0.005) but not different than the 5C8H1 group. CD40 receptor coverage was nearly 100% in 2C10R4 treated animals through d118 (Figure 1B).

Allograft Survival

The median survival time (MST) of 5C8H1 (142, range 90 – 173 days, n=5, p<0.001) or 2C10R4 (124, 25 – 178, n=6, p=0.002) was significantly longer than IDEC-131 treated allograft recipients (35, 9 – 112, n=9, Figure 2). One of the six 2C10R4 treated animals (FC9J, 89d, serum trough level 239 µg/mL, 88% receptor coverage) experienced rejection within the 90-day treatment period, and another animal (FA53, 2C10R4, d117) experienced graft rejection and subsequent failure with 100% receptor coverage and low (41µg/mL) serum trough levels. Two animals in the 2C10R4 treatment group (FB9N and DV36) and 1 animal (MDL5X) in the 5C8H1 group had functional, beating allografts removed per protocol or for health reasons (Table 1). All allografts in the IDEC-131 treatment group were explanted due to graft failure from rejection.

Three IDEC-131 treated allografts that demonstrated evidence of clinically overt acute rejection (M51, M327, and M348) received steroids (Table 1). While 1 animal additionally received ATG (M51) and recovered graft function from this early acute rejection episode, both M327 and M348 failed to recover function and were explanted shortly thereafter.

Acute Cellular Infiltration Histology

Acute cellular rejection, as graded by ISHLT score, increased significantly within each treatment group as time after transplantation increased (IDEC-131 p=0.01; 5C8H1 p=0.02; 2C10R4 p=0.02; Figure 3, SDC Figure 1), but there was not a significant difference between

treatment groups and median ISHLT scores graded on biopsies or explanted grafts at <30, 30-89, 90-119, or 120d.

Myocardial cellular infiltration of rejected explants in each treatment group was predominately CD68⁺ macrophages and CD3⁺ T cells with scattered foci of CD20⁺ B cells (Figure 4). While the CD3⁺ infiltration scores were similar across the 3 treatment groups, 5C8H1 treated grafts had significantly more CD20⁺ cells (mean score 0.3±0.2) than IDEC-131 treated grafts (0.1±0.0; p=0.04, Figure 4C).

Cardiac Allograft Vasculopathy Histology

CAV severity scores increased significantly from time of transplant to explant in both the IDEC-131 (p<0.0001) and 2C10R4 treatment groups (p=0.01; Figure 5, SDC Figure 2). In 5C8H1 treated allografts, CAV biopsy scores were heterogeneous following transplantation. Biopsies and explanted grafts from 30 – 89d, which are under treatment, had significantly higher CAV scores in the IDEC-131 treated grafts (median [IQR]: 2.0 [1.8-2.4]) compared to either 5C8H1 treated (0.8 [0.4-0.8]; p=0.008) or 2C10R4 treated grafts (0.4 [0.3-1.1]; p=0.0002), which is consistent with the earlier graft failure in the IDEC-131 group. A representative myocardial vessel with severe CAV is shown in SDC Figure 3.

Antidonor Alloantibody Production

Overall, incidence of antidonor alloAb production during treatment was less with 5C8H1 (IgM: 0/5 animals, p=0.02; IgG: 0/5, p=0.0005, Figure 6, middle panel) and 2C10R4 (IgM: 0/6, p=0.007; IgG: 2/6, p=0.01, Figure 6, bottom panel) than IDEC-131 treated allografts (IgM: 7/9; IgG: 9/9, Figure 6, top panel). In the IDEC-131 group, IgM alloAb elaboration preceded the explant of 7 allografts by 26±13 days (Figure 6, left top panel), and IgG alloAb elaboration preceded all allograft explants by 27±12 days (Figure 6, right top panel).

In 5C8H1 treated allografts, IgM and IgG alloAb were detected prior to rejection in only 1 animal (MFA6L) 149 days after transplant, which was 65d and 24d, respectively, after treatment cessation at 84d and prior to rejection and explant of the allograft at 173d. At the time of explant, IgM alloAb was detected in 3 of 4 5C8H1 treated recipients (Figure 6, left middle panel), and IgG alloAb was detected in 2 of 4 recipients (Figure 6, right middle panel).

In the 2C10R4 treated allografts, IgM alloAb elaboration was detected in 1 animal (FB6E) 154d after transplant, which was 24d prior to rejection and explant (Figure 6, left bottom panel), and coincided with explant in a second animal (DV2R). IgG alloAb elaboration preceded explant in 3 animals by 19±5 days and coincided with explant in the 4th animal (DV2R). No statistically significant difference in alloAb elaboration was detected at 90d or graft failure between the 5C8H1 and 2C10R4 treated allograft recipients.

Antibody-Mediated Rejection (AMR) Score

Given this striking pattern of alloAb development, AMR was scored on rejected explanted allografts. All evaluable explants demonstrated evidence of AMR, and cumulative pathologic AMR (pAMR) scores were similar between the 3 groups (SDC Table 1). The

C4d capillary distribution scores were significantly higher in the 5C8H1 treated (1.5 ± 0.2 ; $p=0.03$) and 2C10R4 treated (1.6 ± 0.2 ; $p=0.01$) than IDEC-131 treated allografts (0.5 ± 0.2 ; Figure 7A-B). Distribution of intravascular CD68⁺ macrophages was similar across treatment groups (Figure 7C-D).

Blood Lymphocytes

Peripheral blood CD20⁺ lymphocytes in 5C8H1 treated animals increased significantly from d0 (568 ± 69 cells/ μ L) to d90 (934 ± 101 , $p=0.024$), whereas the number of CD20⁺ cells remained similar from d0 (913 ± 220 cells/ μ L) to d90 (1477 ± 370) in 2C10R4 treated animals. On d14 after transplant, there was a modest, though significant, drop in CD20⁺ lymphocytes from baseline in the 2C10R4 treated animals (-457 ± 152 cells/ μ L) compared to 5C8H1 treated animals (16 ± 25 , $p=0.037$; Figure 8A, SDC Figure 4). After recovery of this B cell reduction in 2C10R4 treated animals, a naïve B cell phenotype predominated (Figure 8D and F). Memory B cell phenotype in the 1 evaluable 5C8H1 treated animal (MFA6L) remained generally stable after transplantation (Figure 8C and E).

Peripheral blood CD3⁺ lymphocytes after transplantation did not differ significantly from d0 in either the 5C8H1 or 2C10R4 treated animals or between treatment groups at any point (Figure 8B).

Anecdotally, 1 animal (FB6E) was noted to have coagulated blood in EDTA tubes on 2 routine blood draws on d7-11. This observation led to a clinical decision to deploy a 3-week heparin drip and oral aspirin. No clinically significant TE event was noted in any animal in this treatment group, and hypercoagulability was not observed in association with either of the 2 unexpected, unexplained deaths that occurred in the group treated with 2C10R4.

Discussion

Selective inhibition of 1 or more of the T cell costimulation pathways has been shown to increase allograft survival, delay or prevent elaboration of antidonor alloantibody (alloAb), and attenuate chronic rejection.^{23, 31-32} As predicted by extensive work in murine models, our prior work in NHP has shown that CD40/CD154 blockade is tolerogenic to influenza vaccination,³⁴ which prompted our initial evaluation of this treatment in a cardiac transplant model. As a predicate for exploring the combination of novel immunomodulatory strategies³² with optimized blockade of the CD40/CD154 axis to promote peripheral regulatory tolerance, here we report a preclinical comparison of 3 monoclonal antibody drug candidates that block CD40 (2C10R4) or CD154 (IDEC-131, 5C8H1).

As previously reported, blockade of α CD154 by IDEC-131 significantly, albeit modestly, increased median allograft survival from 6 to 39 days.^{7, 23, 35} By using a directly comparable treatment regimen, here we show that both 5C8H1 and 2C10R4 significantly prolong cardiac allograft survival compared to historical IDEC-131 treated allografts. Additionally, 5C8H1 and 2C10R4 are each associated with improved control of pathogenic immunity, as demonstrated by reduced CAV severity scores during the second and third months of treatment and attenuation of alloAb development during treatment relative to IDEC-131, which is concordant with findings in several different NHP models using 5C8H1 or

2C10R4.^{2-8, 20, 36} Although the trend toward lower concentrations of IDEC-131 may also have played a role, the stark increase in allograft survival with 5C8H1 compared to IDEC-131 is likely attributable to the differences in recognized CD154 epitopes, reduced binding affinity of IDEC-131 to CD154, Fc-related functionality, or some combination of these factors.

Neither transient CD40 nor CD154 blockade alone could induce a state of tolerance. As trough levels, and receptor coverage in the 2C10R4 group, declined after cessation of treatment, allografts were invariably rejected. The single 2C10R4 treated animal with 100% receptor coverage at the time of rejection (d117) and the animal with rejection within the 90-day treatment period (FC9J; d89, serum trough level 239 µg/mL, 88% receptor coverage on date of explant) suggest a pathway for αCD40-independent rejection. Several plausible mechanisms may explain this finding. We hypothesize that the broad distribution and constitutive expression of CD40 on cells of hematopoietic origin may decrease the effective concentration of 2C10R4 in the secondary lymphoid organs and in the graft where “professional” antigen presentation occurs. In addition, binding of 2C10R4 to soluble CD40 in plasma may artificially increase reported plasma levels of 2C10R4 to the extent that 2C10R4-CD40 complexes are detected in our assay. In contrast, CD154 is conditionally expressed after signal 1-mediated T cell activation. In the absence of a reservoir of antigen in plasma, effective concentrations of free 5C8H1 may be higher and, thus, available to more efficiently inhibit T-DC and T-B cell interactions. Unfortunately, due to this induced expression of CD154, we do not have an assay developed to reliably measure receptor coverage in these animals. Alternatively, T cells may receive a costimulation signal via another pathway, such as CD28/B7 or another costimulatory pathway. We are currently evaluating the combination of different agents that block the CD40/CD154 and B7/CD28 T cell costimulation pathways³⁷ to determine if these agents provide any synergy.

Treatment with αCD40 and αCD154 is thought to inhibit B cell sensitization or maturation,^{1, 20} and the consequential inhibition of, or delay in, production of antidonor alloantibody is thought to be an important mechanism by which these treatments inhibit or delay acute and chronic rejection. Here we show delayed elaboration of both IgM and IgG antidonor alloAb during ongoing treatment and for variable intervals after the 90-day treatment in all evaluable 5C8H1 treated animals and 2 of the 4 evaluable 2C10R4 treated animals. In the two 2C10R4 animals with potential αCD40-independent rejection, alloAb was detected prior to clinical evidence of rejection and graft failure. When evaluating explant AMR scores, 5C8H1 and 2C10R4 groups had similar capillary C4d and intravascular CD68⁺ macrophages distribution patterns, which were both higher than IDEC-131 treated animals likely because of prolonged graft survival and exposures to subclinical episodes of rejection. Overall, αCD40/CD154 could delay, though not completely prevent, development of AMR.

While preclinical and clinical trials using αCD154 were complicated by thromboembolic (TE) complications,⁹⁻¹¹ likely due to CD154 expression on activated platelets, subsequent studies found reduced TE rates with perioperative heparin or ketorolac administration.³⁸ Although our study was not designed to evaluate the frequency of subclinical thromboembolic events, we did not observe any clinical events associated with 5C8H1 (or

the other agents used here), even without routine heparin or NSAID use. Since CD154 can also circulate in a soluble form, sCD154 could potentially complex with α CD154 antibodies in the circulation, and such immune complexes were shown to potently activate platelets and cause thrombosis via the Fc γ RIIA IgG receptor.³⁹ A novel α CD154 blocking reagent that lacks the Fc domain, CDP7657, reportedly does not induce TE events in NHP⁴⁰ and is currently under evaluation for safety in patients with systemic lupus erythematosus (Clinicaltrials.gov Identifier NCT01764594). In our estimation, this promising development coupled with our current observations justify future studies to reevaluate α CD154 treatment in preclinical transplantation models, and in the clinic, based on the favorable immunomodulatory effects shown in this and other studies.

Nevertheless, the TE complications in 5C8H1 clinical trials motivated us, and others, to evaluate CD40 blockade as an alternative. Initially, a chimeric mouse antihuman CD40 with human IgG1 monoclonal antibody, Chi220, was developed and studied in NHP renal⁴¹ and islet models³⁷; however, this treatment was found to profoundly deplete and activate B cells, was highly immunogenic due to the chimeric nature of the mAb, and was associated with multiple morbidities attributable to over-immunosuppression.^{37, 41-42} In part, this may be due to IgG1 engineered heavy chain epitopes that can bind Fc-receptors and activate complement.^{16,21, 37, 41} Importantly, the Fc region of the 2C10R4 antibody we used in this study is of the IgG4 subclass to reduce complement and Fc γ receptor binding.⁴³ Whether the antibody could initiate activation of CD40 expressing cells including endothelial cells has not been formally verified, but it is unlikely based on the absence of in vitro B cell activation by 2C10R4.²¹

While 2C10R4 is a nondepleting IgG4 isotype, in our study CD20⁺ B cells mildly, but consistently, dropped by about 500 cells/ μ L in 2C10R4-treated allograft recipients 2 weeks after transplantation, similar to previous studies.^{20, 21} The relative decrease in B cells was self-limiting, with B cells generally returning to baseline by d35, and was unlike the large degree of depletion seen with previously developed IgG1 isotype α CD40 mAb.^{16, 21, 37, 40} Here we found a nonspecific decrease across all peripheral B cell phenotypes and a naïve resurgent B cell phenotype. This transient reduction may be due to Fc-mediated homing out of the peripheral blood or sequestration events. Alternatively, B cell homeostasis may be impaired in absence of CD40 signals during intense and systemic CD40 blockade.⁴⁴ Severe weight loss (>25%) with 2C10R4 has been reported,³⁶ but we did not observe any clinically significant weight loss with our 2C10R4 monotherapy group (Table 1).

Several other “nondepleting” α CD40 mAb have since been developed, including 4D11,¹⁴⁻¹⁵ 3A8,¹⁶ and ASKP1240,¹⁷⁻¹⁹ in addition to 2C10R4. Overall, each of these reagents has demonstrated clinical success in NHP solid organ allotransplantation models. Based on this encouraging data, “nondepleting” α CD40 antibodies are moving forward in Phase 2 clinical trials in kidney transplant recipients (Clinicaltrials.gov Identifier NCT01780844).

In summary, our report is the first direct comparative evaluation of 3 monotherapy mAb that block the CD154/CD40 pathway of T cell activation in a stringent NHP cardiac allograft model. We report that CD154 blockade using IDEC-131 is inferior to 5C8H1 or CD40 blockade with 2C10R4 when administered according to a comparable dosing regimen.

5C8H1 and 2C10R4 have similar safety and efficacy profiles, and thus are both promising reagents for clinical translation and preclinical development of tolerance induction strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

alloAb	alloantibody
ANOVA	analysis of variance
AMR	antibody-mediated rejection
APC	antigen presenting cell
ATG	antithymocyte globulin
CAV	cardiac allograft vasculopathy
CD	cluster of differentiation
CMV	cytomegalovirus

d	days
DC	dendritic cells
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
H&E	hematoxylin and eosin
ISHLT	International Society for Heart and Lung Transplantation
IV	intravenous
mAb	monoclonal antibody
MHC	major histocompatibility complex
MST	median survival time
NHP	Nonhuman primate
SD	standard deviation
SEM	standard error of the mean
TCR	T cell receptor
TE	thromboembolism
TNF	tumor necrosis factor

Figure 1.

A) Mean serum trough levels measured by ELISA for IDEC-131 (green), 5C8H1 (blue), and 2C10R4 (red) with SEM in negative error bars. Open circles represent drug dosing: 30 mg/kg on d0, d3, d7, d14; 10 mg/kg on d21, d28, d35, d42; 20 mg/kg on d56 and d84. **B)** Mean peripheral blood CD40 receptor coverage (%) measured by flow cytometry for 2C10R4 (red) with SEM demonstrated in error bars. Open circles represent drug dosing, as above.

Receptor coverage was nearly 100% through d118, which coincided with 2C10R4 trough levels falling below 100 µg/mL.

Figure 2.

Allograft Survival. Primary graft survival was defined as the time to first rejection episode or graft failure and served as the primary study endpoint for all survival. Graft failure was defined by 1 or more of the following criteria for 2 consecutive days: decline in heart rate >20% below baseline or <120 beats per minute, pulse pressure <30mmHg, or nonpalpable graft contraction. Allograft MST is significantly longer in the 5C8H1 (142 ± 26 days, $p < 0.001$) and 2C10R4 (124 ± 37 , $p = 0.002$) treatment groups than IDEC-131 (35 ± 31). (+) represent allografts removed without evidence of rejection, and are censored from survival analyses.

Figure 3. Median ISHLT scores represented in box and whisker plot from all protocol biopsies and explanted allografts. Median ISHLT scores within each treatment group increased as time after transplantation increased. There was not a significant difference between treatment groups and median ISHLT scores at any time after transplantation.

Figure 4.

Rejected allograft cellular infiltration scores using immunofluorescence. **A)** Representative image of an explanted allograft (animal FA53) demonstrating cellular infiltration by T (CD3⁺, red) and B (CD20⁺, green) cells. The blue (DAPI) illustrates staining of cell nuclei. Original magnification $\times 200$. **B)** Mean CD3⁺ T cell cellular infiltration score in rejected explanted allografts (0: <10%; 1: 10 – 50%; 2 >50% infiltration) was similar in each treatment group. **C)** Mean CD20⁺ T cell cellular infiltration score in rejected explanted allografts using the same scoring system above. 5C8H1 treated animals had significantly higher CD20⁺ T cell infiltration than IDEC-131 treated animals. (*) represents $p=0.04$.

Figure 5.

Median CAV scores from all protocol biopsies and explanted allografts represented in box and whisker plot. CAV scoring system is as follows: 0: 0% intimal hyperplasia; 1: <10%; 2: 10-50%, 3: >50%. Biopsies and explanted grafts from 30 – 89 days following transplantation in the IDEC-131 treated allografts had significantly higher CAV than the 5C8H1 and 2C10R4 treated allografts. (*) represents $p=0.0005$.

Figure 6.

Antidonor alloantibody elaboration. Compared to IDEC-131 treated allografts, both 5C8H1 (0/5 animals, $p=0.021$) and 2C10R4 (0/6, $p=0.010$) completely attenuated IgM alloAb production during treatment (left panels), and IgG alloAb production was significantly attenuated in both 5C8H1 and 2C10R4 (right panels).

Figure 8.

Peripheral blood CD20⁺ (A) and CD3⁺ (B) lymphocytes were measured using flow cytometry for 5C8H1 (blue) and 2C10R4 (red) treated cardiac allograft recipients. Absolute cell numbers were calculated using CBC data, and data is shown here as a change in absolute cell number from baseline (group mean $\pm$ SEM). Proportion of memory B cell phenotype amongst CD20⁺ B cells using flow cytometry in 5C8H1 (n=1, C) and 2C10R4 (n=6, D). Absolute cell number of memory B cell calculated from CBC data is shown in 5C8H1 (n=1, E) and 2C10R4 (n=6, F). After the reduction of circulating peripheral blood B cells in 2C10R4, a naïve B cell phenotype (CD27⁻IgD⁺CD38⁺IgM^{intermediate}) predominated. NS, Nonclass-switched memory B cells (CD27⁺IgD⁺); IS, Isotype switched memory B cells (CD27⁺IgD⁻); DN, Double negative memory B cells (CD27⁻IgD⁻); Tr, Transitional B cells (CD27⁻CD38⁺IgM^{high}).

Table 1

Individual animal survival and clinical notes.

	Animal ID	Primary Survival (days)	Secondary Survival (days)	Rejection Treatment	Mean Body Weight	Clinical Notes
IDEC-131	M147	9				
	M51	10	53	Steroid ^a , ATG ^b (d10)		
	M350	28				
	M327	35	42	Steroid ^a (d35)		
	M643	35				
	M10682	43				
5C8H1	M18	58				
	M348	75	84	Steroid ^a , αCD20 ^c (d75)		
	M10670	112				
	MDL5X	>90				d90 explanted by protocol
	MC7-10057	113				
	MDM6A	133				
2C10R4	MDP28	152				
	MFA6L	173			0.7±0.7%	
	FB9N	>25			14.5±0%	Found dead; unknown etiology
	DV36	>69			-0.4±1.8%	Euthanized for mesenteric ischemia due to foreign body
	FC9J	89			4.1±0.8%	
	FA53	117			13.7±0.7%	
	DV2R	130			14.7±1.0%	
	FB6E	178			3.2±0.7%	Heparin (d7-28) ^d , ASA ^e (d7-179)

^a Steroid: Solumedrol 10 mg/kg IV daily for 3 doses^b ATG: Antithymocyte globulin 10 mg/kg IV for 3 doses^c αCD20: Rituxan 20 mg/kg IV once^d Heparin: 50 units/kg/hr IV continuous for 2 days, 15 units/kg/hr continuous for remaining 19 days^e ASA: Aspirin 20 mg PO daily