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CD40-CD40L Blockade: Update on Novel Investigational Therapeutics for Transplantation

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Abstract

Effective immune responses require antigen presentation by major histocompatibility complexes (MHC) with cognate TCR and antigen-independent costimulatory signaling for T cell activation, proliferation, and differentiation. Among several costimulatory signals, CD40-CD40L is of special interest to the transplantation community as it plays a vital role in controlling or regulating humoral and cellular immunity. Blockade of this pathway has demonstrated inhibition of donor-reactive T cell responses and prolonged the survival of transplanted organs. Several anti-CD154 and anti-CD40 antibodies have been used in the transplantation model and demonstrated the potential of extending allograft and xenograft rejection-free survival. The wide use of anti-CD154 antibodies was hampered due to thromboembolic (TE) complications in transplant recipients. These antibodies have been modified to overcome the TE complications by altering the antibody binding fragment (Fab) and Fc (Fragment, crystallizable) receptor region for therapeutic purposes. Here, we review recent preclinical advances to target the CD40-CD40L pair in transplantation.

Introduction:

Co-stimulation blockade has been shown as an effective way to suppress the immune response in transplantation against donor antigens and prolong graft survival. Several costimulatory (e.g., CD28:CD80/CD86, CTLA: CD80/CD86 and CD40: CD40L (CD154)) pathways play a pivotal role in transplantation. The CD40/CD40L co-stimulation pathway is among the most promising therapeutic targets in transplantation. Blockade of this pathway by anti-CD154 (MR-1) and anti-CD154 (5-C8) antibodies has prolonged graft survival and induced tolerance in mice and nonhuman primates (NHPs).[1–7] Anti-CD154 antibodies have demonstrated therapeutic potential, but thromboembolic (TE) complications have been seen during preclinical and clinical testing; therefore, further use of unmodified antibodies has stopped.[8,9] Lately, anti-CD40 antibodies, due to lack of TE potential, have been preferred in experimental transplantation as they inhibit the same costimulation pathway. Prolonged graft survival in NHP models has been reported.[10–14] Since the potential of CD40/CD40L

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costimulatory blockade by anti-CD40 and CD154 antibodies has been recognized, many clinically viable antibodies targeting this pathway have been produced for transplantation over the last 20 years. In this review, we discuss newer agents undergoing clinical trials to interrupt CD40/CD40L interactions in transplantation to prolong graft survival. Despite excellent results of significantly prolonging graft survival, none of these antibodies have reached clinical approval yet.

CD40/CD40L Pathway

CD40/CD40L is a member of the TNFR/TNF (tumor necrosis factor receptor/tumor necrosis factor) superfamily and is crucial for the functional activation of B cells and dendritic cells (DCs). CD154 (CD40L) is expressed mainly on activated T and B cells and platelets; its expression can be induced on monocytic cells, natural killer cells, mast cells, and basophils[15]. CD154 is involved in selecting T-cell clones during the negative selection process in the thymus and thereby shapes the peripheral TCR repertoire. However, CD40 is expressed on B cells along with other cells, e.g., dendritic cells (DCs), monocytes, platelets, and macrophages, as well as by nonhematopoietic cells such as myofibroblasts, fibroblasts, epithelial, and endothelial cells.[16,17] Mechanistically, the interaction of CD40 of B cells with its ligand CD154 on T cells promotes B cell proliferation, immunoglobulin (Ig) production, isotype switching, and somatic hypermutation (SHM) of the immunoglobulin. This enhances Ig affinity for antigens and the formation of long-lived plasma cells and memory B cells.[18] Likewise, the engagement of CD40 on antigen-presenting cells stimulates the release of pro-inflammatory cytokines and chemokines,[19] which influence the immune response downstream. CD40/CD40L costimulatory pathway plays an important role in T-cell-mediated dendritic cells (DCs) activation/maturation and macrophage activation (Figure 1). Blocking of this interaction significantly affects humoral response[20] and has also been shown to prolong graft survival[1,3,7] in experimental transplantation models. Whereas CD154 interaction with APCs enhance donor reactive CD8+ T cell infiltration and accelerate allograft rejection, blockade of this CD40/CD40L pathway with anti-CD154 antibody can delay donor-reactive CD4+ and CD8+ T-cell expansion and differentiation and also help to convert peripheral donor reactive regulatory T-cells to antigen-specific induced regulatory T (iTreg) cells[21] and potentially induce tolerance. Several studies have reported preventing allograft rejection by inhibiting donor-reactive T-cell responses and promoting long-term graft survival by anti-CD154 antibodies in mice[22,23] and nonhuman primates (NHPs).[3,4,7] Recently, Wolf et al reported that CD154 is also ligand for integrin molecule (Mac-1, i.e., αMβ2, CD11b/CD18) expressed on innate immune cells (e.g., myeloid cells, including monocytes, macrophages, neutrophils and lesser extent to activated lymphocytes except NK cells).[24] Therapeutic inhibition of Mac-1 (CD11b/CD18) effectively inhibits inflammation and thrombosis by reducing leukocyte adhesion and migration.[25] Liu et al. have demonstrated that blocking of CD11b along with anti-CD40 antibody prolongs skin allograft survival in murine model.[26] Leo Buhler and his colleagues have also demonstrated the importance of CD40/CD40L co-stimulation pathway-blockade in xenotransplantation[27] and later, several investigators, including us reported a prolonged xenograft survival in NHPs.[5,12,14,28–31]

CD40/CD40L Blocking antibodies

Anti-CD154 (CD40L) and anti-CD40 antibodies, like all antibodies, have antigen binding (Fab) and Fc (Fragment, crystallizable) fragments which contain heavy and light chains. Several monoclonal antibodies have been engineered (Figure 2) to target CD40/CD40L and have demonstrated the potential of prolonging allograft and xenograft rejection-free survival in transplantation preclinical models (Table 1 and 2). These blocking antibodies have significant therapeutic potential in transplantation by their effect on inhibiting donor reactive T cell responses and inducing long-term survival in both murine and nonhuman primate models.

Anti-CD154 Antibodies

The CD40/CD40L blockade pathway using an anti-CD40L, non-B cell depleting antibody has shown great promise in rodent and nonhuman primate models for inhibiting rejection and prolonging graft survival.[4–7,32] Treatment with anti-CD154 antibody has effectively reduced donor-specific antibody (DSA) production in these models. Co-stimulation blockade by anti-CD154 antibody prolongs graft survival, but its wide use in the experimental and clinical setting is limited initially due to thromboembolic (TE) complications.[8,9] CD154 is expressed on platelets, and an anti-CD154 antibody is thought to potentially activate them, which then augments the thrombin-generating coagulation cascade.[33] In contrast, thromboembolic complications were not observed with newer modified anti-CD154 reagents. Currently, several anti-CD154 antibodies are being used in transplantation with variable results.

Ruplizumab (hu5C8)—Ruplizumab (hu5C8) is a mouse-human IgG1κ chimeric recombinant monoclonal antibody (mAb) against CD40L. Treatment with hu5C8 antibody has been shown to prevent acute renal and primary skin allograft rejection and prolonged cardiac xenograft survival in NHPs.[3,4,7] Kenyon et al. and others have demonstrated long-term survival and function of intrahepatic islet allografts in NHPs with hu5C8 as well.[7,34–36] Despite prolonged survival, Kawai et al. have reported a high incidence of thromboembolic complications in monkeys who have received renal allografts.[8] Heparin (100 units/kg) administration before the antibody treatment prevented thrombotic complications significantly. We and others have also used 5C8 antibodies in pig to baboon xenotransplantation model and demonstrated the prevention of early graft rejection and prolonged cardiac xenograft survival.[5,28,37,38] Cardiac xenograft survival was prolonged significantly in NHPs, but thromboembolic complications were encountered after anti-CD154 antibody treatment.[5,38]

Toralizumab (IDEC-131)—IDEC-131 is a humanized anti-CD154 mAb with the variable regions of the murine antibody and human γ1/κ constant regions and binds to CD154 on T cells with high specificity and avidity. Clinically, IDEC-131 has been used to treat systemic lupus erythematosus (SLE) autoimmune disease, and John Davis and their colleagues conducted a phase I clinical trial.[39] Xu et al. treated rhesus monkeys with a low (i.e., 15 mg/kg/dose) and high (i.e., 20 mg/kg/dose) dose of IDEC131 along with rapamycin and donor-specific transfusion and reported prolonged skin allograft survival up to 246 days[32] and suggested that it may have led to operational tolerance (i.e., no immunosuppression and

normal allograft function).[40] Pfeiffer et al. reported that the treatment with IDEC-31mAb modestly extended cardiac allograft survival (up to 56 days) in cynomolgus monkeys.[6] Wu et al. tested the IDEC-131 mAb in the pig-to-baboon heterotopic cardiac xenotransplantation model. They reported 7 days of median xenograft survival (range 6 to 11), and all the animals had induced anti-pig antibodies to either Gal or non-Gal antigens.[41] O'Neill and their colleagues have reported that IDEC-131 is inferior to huC58 mAb in a nonhuman primate cardiac allotransplant model.[42]

ABI793—ABI793 is a novel human anti-human CD154 mAb generated by Schuler et al. using human immunoglobulin (Ig) transgenic mice (HuMAb-Mouse, Medarex Inc., Annandale, NJ).[43] The CD40L epitope used in this antibody differed from hu5c8 (CD154) mAb. ABI793 belongs to the human immunoglobulin-G1:kappa family, and it has been shown to inhibit in vitro and human mixed lymphocyte reactions in a dose-dependent manner. Schuler et al. reported that ABI793 has also been efficacious in cynomolgus and rhesus monkey renal transplantation models, and treatment with ABI793 (20 mg/kg) prolonged the renal allograft survival (median graft survival of 108 days) in cynomolgus monkeys.[43] Thrombocytopenia and severe acute tubular necrosis (ATN) episodes were observed unrelated to rejection and responded to fluid and diuretic treatment.[43,44] A low dose of ABI793 was ineffective and could not prevent allograft cellular rejection.[43] Some recipients underwent rejection and showed signs of chronic and acute thromboembolic vascular lesions with hemorrhages in the lung and brain of allograft recipients. Kanmaz et al. have reported that recipients with chronic active rejection showed relatively strong alloantibody responses.[44] However, Knosalla et al. have used ABI793 mAb in pig-to-baboon xenotransplantation and reported that baboon recipients could be well-tolerated and prevent an elicited antibody response to pig antigens.[45] Although, this study was too small to conclude whether any other complications (e.g., ATN, thrombotic complications, thrombocytopenia, etc.) which were seen before will occur in this nonhuman primate species. Hering et al. tested its efficacy in the xenotransplantation model and reported prolonged porcine islets xenograft survival up to 158 days in immunosuppressed nonhuman primates.[46]

H106—H106 is humanized anti-CD154 mAb and it has shown to be efficacious in xenotransplantation model. Cardona and colleagues demonstrated engraftment of adult porcine islet xenografts in diabetic nonhuman primates after Anti-CD154 (H106) antibody treatment to rhesus macaque.[30] These results suggest that a costimulation blockade-based immunosuppressive regimen by H106 prevents hyperacute rejection.

Fc receptor-modified CD40L Antibodies

Recent evidence suggests that the Fc region of anti-CD154 antibodies interacting with Fc receptors plays an important role in platelet activation and aggregation of platelets.[9] Ferrant et al. and others reported that the Fc effector function of anti-CD154 antibody was a necessary component for therapeutic benefit.[47,48] Therefore, an Fc-disabled, glycosylated anti-CD154 heavy chain variant was created, which was found to be effective in models of autoimmune diseases and transplantation.[49] A novel blocking domain Ab construct (dAb) was developed that targets CD40L but has disrupted the FcγR binding and complement-

fixing function. Pinelli et al. demonstrated that the Fc-silent anti-CD154 domain antibody inhibits alloreactive T cell expansion and attenuates cytokine production of antigen-specific T cells in an allogeneic mouse model. They have also suggested that it can promote the conversion of Foxp3+-induced Treg cells like other conventional anti-CD154 antibodies.[50] Because of their ability to convert effector T cells into induced Treg in the presence of CTLA4-Ig, suggests that Fc-silent anti-CD154 antibodies have the potential for their translation to clinical use. Several monoclonal antibodies were engineered with various Fc fragments (altered or modified) and have been tested in multiple experimental and preclinical transplant models, summarized below.

CDP7657 (Dapirolizumab Pegol)—CDP7657 is a high-affinity polyethylene glycosylated monovalent Fab fragment without the functional Fc portion of the anti-CD154 antibody. Shock et al. demonstrated that a high dose (60 mg/kg i.v.) of CDP7657 could inhibit CD154-dependent immune responses without activating platelets and thrombotic complications in rhesus monkeys.[51] Phase 2 clinical trial of dapirolizumab pegol was conducted in patients with moderate-to-severe active systemic lupus erythematosus.[52]

Tegoprubart (AT1501)—Tegoprubart is a humanized IgG1 with a high affinity for CD154 and inhibits both CD40/CD40L and CD11 costimulatory signaling pathways. It is a synthetic antibody developed by Eledon Pharmaceuticals. AT-1501 is being tested to treat autoimmune diseases, i.e., amyotrophic lateral sclerosis (ALS), IgA nephropathy, and an autoimmune kidney disorder. AT-1501 has been shown to promote islet allograft survival in Nonhuman Primates.[52] It is also being tested in Phase-I/II clinical trials for the prevention of renal transplant rejection in Canada.[53] A single dose of Tegoprubart was found to be safe and well-tolerated in healthy volunteers and ALS patients in a Phase 1 trial and initiated a Phase 2a trial to test multiple ascending doses of the therapy.[54]

Letolizumab (BMS-986004)—Letolizumab is an Fc-silent anti-CD154 domain humanized mAb designed to treat inflammatory diseases and developed by Bristol-Myers Squibb. It inhibits T-cell mediated proliferation and differentiation of B cells like others. It also inhibits the production of platelet-derived anti-glycoprotein (GP) IIb/IIIa antibodies by B cells and GPIIb/IIIa-dependent T-cell proliferation. This mAb prevents platelet destruction and helps increase platelet counts and its direct binding with CD40L and Fcγ-RIIA of platelets, which prevent anti-CD154-induced thromboembolism.[55] Phase I/II clinical trial studies' for BMS-98600 and sirolimus along with tacrolimus are undergoing in patients with hematologic cancer or blood disorder to avoid graft versus host disease after donor stem cell transplant.[56]

Dazodalibep (HZN-4920/VIB4920)—Dazodalibep is an Fc-deficient CD154 antagonist fusion human mAb developed by Horizon Therapeutics to prevent autoimmune active Rheumatoid Arthritis. It binds to CD154 on activated T cells and blocks the interaction with CD40-expressing B cells. It is currently in Phase 2 clinical trial for Sjögren's syndrome and rheumatoid arthritis autoimmune disease. A Phase 2a clinical trial is also active to Evaluate the Safety and Efficacy of VIB4920 along with Belatacept for Prophylaxis of Allograft Rejection in Adults Receiving a Kidney Transplant.

TNX-1500—TNX-1500 is a humanized mAb against CD154. It has an antibody binding fragment (Fab) region of hu5C8, and a modified Fc region (IgG4) engineered to reduce FcγRIIa-binding associated with the risk of thrombosis. Efficacy of TNX-1500 has been tested in heterotopic kidney[57] and heart[58] allotransplant model in NHPs. Lassiter et al. demonstrate that monotherapy of TNX-1500 prolongs the renal allograft survival, whereas treatment with MMF resulted in increased allograft failure.[57] Miura et al. also demonstrated that standard treatment of TNX-1500 mAb to NHPs prolongs allograft survival without thromboembolic complications. And TNX-1500 mAb also inhibits alloimmunity, alloantibody production, and class switching of immunoglobulin subtypes.[58]

Anti-CD40 antibodies

Blockade of CD40-CD40L interaction with an anti-CD40 antibody is a potent immunosuppressive agent to prolong graft survival in an experimental transplantation model. It affects T cell-dependent immunoglobulin class switching, germinal center formation, CD8 T cell priming, dendritic cell longevity, cytokine production, and endothelial activation.[17,18,59,60] Anti-CD40 mAb has been shown to augment immune responses to boost anti-tumor and anti-virus activity.[17,59,61] Anti-CD40 antibody has been shown to deplete antigen-presenting cells, but several nondepleting antibodies are available, which have shown high efficacy in bone marrow, islet, and renal transplantation in NHPs.[10,12–14,47,62] Its treatment successfully suppresses the immune response and dramatically prolongs graft survival in mice and NHPs.[12–14,63,64] Humanized anti-CD40 antibody has also demonstrated a rejection-free 2-month survival of first pig to human heart transplantation.[65]

7E1-G1 and 7E1-G2b—These are rat IgG1 and IgG2b antibodies specific for mouse CD40 antigens. 7E1-G1 is a potent agonist, but 7E1-G2b (anti-CD40 Ab) demonstrated anti-CD40 properties similar to Anti-CD40L agents in promoting allogeneic bone marrow chimerism and skin graft survival in mouse model.[66] Therefore, 7E1-G2b is an attractive agent for co-stimulation blockade-based tolerance regimens in mouse models.

ch5D12—ch5D12 is a molecularly engineered chimeric mAb (IgG(4), which has the variable domains of the heavy and light chains of the murine version of 5D12 (mu5D12). This chimeric antibody was tested in an experimental autoimmune encephalomyelitis model[67] and Crohn's disease.[68] ch5D12 binds to B cells, monocytes, and dendritic cells but not to peripheral CD4+ or CD8+ T cells. It can also weekly bind with activated endothelial cells and inhibit T-cell proliferation, antigen-presenting cell (APC) activation, and GC formation. Treatment of ch5D12 in cynomolgus monkeys inhibits the IL-8 and IL-12 cytokine production from pro-inflammatory T helper-1 cells. Haanstra et al. reported that a high dose of ch5D12 extended kidney allograft survival in a rhesus monkey model. ch5D12 treatment and anti-CD86 antibody prevented kidney allograft rejection, and an additional 12-week course of low-dose cyclosporine A (CsA) treatment resulted in long-term drug-free survival without donor-specific antibodies.[69]

3A8—3A8 is mouse anti-human CD40 mAb which has been shown to prolong the engraftment of hematopoietic stem cells (HSCs) in rhesus macaque along with CTLA4-Ig

and Sirolimus. Treatment with 3A8 alone developed a rhesus anti-mouse antibody (RAMA) response, which suggests that 3A8 is immunogenic and negatively affects the potency of CD40 blockade. Badell et al. demonstrated that the efficacy of 3A8 in an alloislet transplantation model in NHPs results in similar immunomodulation.[10] The addition of CTLA4-Ig treatment along with 3A8, basiliximab, and sirolimus significantly prolonged graft survival without depleting B cells and donor-specific antibodies (DSA) were also not produced.[10,70] We have also used 3A8 mAb along with the previously described immunosuppression (B and T-cell induction, MMF, and a tapered dose of steroids, i.e., the Mohiuddin IS regimen) in a pig-to-baboon heterotopic cardiac xenotransplantation model and demonstrated that 3A8 binds and activates baboon B cells in vitro, but only modestly suppressed antibody production in vivo.[64] Cardiac xenograft survival in baboons was also not extended (the most prolonged survival was 28 Days).

Chi220 (BMS-224819)—Chimeric mouse anti-human CD40 (IgG) monoclonal antibody has been used in the treatment of arthritis and transplantation. Treatment with Chi220 has modestly extended kidney and islet allograft survival[13,47] in rhesus macaques, but most recipients developed donor-specific antibodies (DSA) despite peripheral B cell depletion. At the same time, both Chi220 and CTLA-4-Ig could suppress the DSA without having an augmented effect on allograft Survival.[13] Adams et al. demonstrated that Chi220-treated animals alone or in combination with CTLA-Ig deplete peripheral CD20+ B cells. This depletion is rapid but transient,[14,47] suggesting that Chi220 is a weak agonist. Thompson et al. have used Chi220 in pig to NHPs islet xenotransplantation model and reported effective engraftment and prolonged survival of porcine islet in NHPs.[14]

2C10—2C10 is a mouse-rhesus IgG chimeric antibody. It is engineered to contain either rhesus IgG1 (2C10R1) or IgG4 (2C10R4) heavy and kappa light chain constant regions that can effectively block the interaction of T cell-bound CD154 with CD40 on B cells and antigen-presenting cells. This chimeric mouse-rhesus mAb binds to a unique epitope of CD40 different from several other anti-CD40 mAb and lacks agonistic properties. Treatment with recombinant 2C10 isotypes resulted in a modest change in peripheral B cell counts[71] and prevented antigen-specific antibody formation in a dose-dependent manner. Chimeric rhesus 2C10R4 (IgG4) mAb was tested in an allogenic islet transplant model in NHPs. Lowe et al. reported a significantly prolonged alloislet graft survival (median rejection-free allograft survival was 280 days compared to 8 days for control) in NHPs.[71] Kwun et al. have used 2C10 along with proteasome inhibitor (Bortezomib/ Belatacept) in presensitized rhesus macaques who received a kidney allograft.[72] They demonstrated that 2C10/Bortezomib/Belatacept treated recipients had significantly reduced bone marrow plasma cells and prolonged allograft survival. We and others have also used 2C10 chimeric antibody in a pig-to-baboon xenotransplantation model and reported prolonged survival of cornea, kidney, islet, and heart xenograft.[11,12,14,28,73–76] We have reported the most extensive cardiac xenograft survival and demonstrated that withdrawal of 2C10 antibody in cardiac xenograft recipients triggers donor-specific antibodies and rejection of heterotopic cardiac xenograft.[12] 2C10 treatment has also been found to be very effective for prolonging the survival of life-supporting cardiac xenografts.[77,78]

Bleselumab 4D11(ASKP1240)—4D11 is type 4 immunoglobulin G (IgG4) human antagonistic anti-CD40 mAb. It inhibits human CD154-induced proliferation and suppresses both delayed-type hypersensitivities. 4D11 does not induce antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) and prevents renal allografts rejection in cynomolgus monkeys[79] and suppresses anti-donor antibodies.[80] B cell counts decrease in the periphery of 4D11 treated animals and recover after cessation of mAb. None of the renal allograft recipients treated with 4D11 did have anti-drug antibodies and thromboembolic complications. Watanabe et al. evaluated the effect of 4D11 on islet transplantation (ITx) in cynomolgus monkeys. They observed a prolonged allograft survival without adverse events in single donor-derived allogeneic ITx.[81] These encouraging results suggest that it could be a promising immunomodulating agent in clinical organ transplantation.

A phase I/II clinical trial for 4D11 (ASKP1240), i.e., Bleselumab, was initiated in de novo kidney transplant recipients.[82] Harland et al. reported that Bleselumab treatment with tacrolimus was not worse than standard care for renal transplant recipients. Whereas Bleselumab and MMF-receiving recipients had higher biopsy-proven acute rejection episodes (6 and 36 months) after transplantation.[83]

Clinical Trials:

Iscalimab (CFZ533): Phase 1 Clinical trial—CFZ533 is humanized IGg1 mAb against anti-CD40 mAb developed by Novartis and XOMA for treating autoimmune diseases. CFZ533 was also tested in a clinical trial for the treatment of allograft rejection in renal (phase 1)[84] and liver transplant recipients (phase 2)[85] to replace calcineurin inhibitors (CNI). Early phase 1 clinical trial results from renal transplant recipients looked promising. Farkash E et al. demonstrated that recipients receiving CFZ333 had normal allograft histology as compared to standard care and suggested that it has the potential to improve long-term outcomes.[86] However, a review of interim data from the clinical trial found that CFZ333 treatment was inferior to tacrolimus-based therapy in preventing organ rejection. Therefore, the phase 1 Clinical trial was halted for kidney transplant patients[87]; however, the phase 2 trial in liver transplant recipients is continuing and Novartis exploring its potential treatment for other conditions.[88]

KPL-404: Phase 1 Clinical Trial—KPL-404 is a humanized anti-CD40 mAb designed by Kiniksa Pharmaceuticals to block the CD40/CD40L pathway and suppress T-cell Dependent Antibody Response (TDAR). Phase 1 clinical trial was performed and demonstrated safety and tolerability in healthy volunteers.[89] A high dose of KPL404 (10mg/Kg) suppresses the TDAR after KLH antigen challenge.[90] KPL-404 can potentially treat conditions or diseases where dysregulation of the CD40-CD154 pathway is involved. A phase 2 Clinical trial is underway to evaluate the safety and pharmacokinetics of KPL-404 for 12 weeks in rheumatoid arthritis patients.[91] Most recently, KPL404 was used in first pig-to-human cardiac xenotransplantation after getting a FDA approval in the “expanded access (EA)” program to avoid rejection of a genetically modified cardiac xenograft.[92]

NJA-730a: Phase 1 Clinical Trial—Besides mAb against anti-CD40, NapaJen Pharma has developed an oligo, a short single strand of synthetic RNA (NJA-730). It is an anti-CD40 oligonucleotide combined with a beta-glucan delivery vehicle with the potential to treat acute graft-versus-host disease (GVHD). The half-life of NJA-730 is approximately 30 minutes and can be rapidly eliminated from circulation. A single dose of an ortholog of NJA-730 in a mouse model of bone marrow transplantation found that it significantly prolongs the engraftment of bone marrow in murine recipients (up to 45 days after the transplantation) and can effectively prevent GVHD. A Phase 1, first-in-human clinical trial of NJA-730 in Australia reported that NJA-730 treatment is safe and tolerable in approximately 80 healthy adult male volunteers.[93]

While there are many anti-CD40/CD40L monoclonal antibodies, only some of them have made initial clinical trials. Unfortunately, it is impossible to compare these reagents side by side, even in the preclinical setting, because of the manufacturer's intellectual property licensing. Also, these antibodies have variable affinities for their targets and are epitope specific, sometimes with differing effects in vivo, even after promising in vitro development. Novel pathways have also discovered that augment CD40/CD40L signaling. Recently, Liu and Ford have reported that blockade of CD40/CD40L by the anti-CD154 antibody (MR-1) was more effective than the anti-CD40 antibody (7E1G2b) in prolonging graft survival in a murine transplant model as CD154 binds with the CD11b receptors and inhibits donor-reactive CD8+ T cell infiltration. Blocking both CD154 and CD11b (M1/70) enhances the efficacy of anti-CD154 antibodies by inhibiting cellular trafficking.[26]

Conclusion

There are many novel therapeutics targeting the CD40/CD40L pathway for prolonging allograft and xenograft survival in preclinical transplantation models. Anti-CD154 antibody blocks this interaction and deplete of CD4 T cells. It also promotes production of induced regulatory T (iTreg) cells and dampens the cell mediated immune response in transplant recipient. Whereas anti-CD40 antibody inhibit the clonal expansion of B cells and plasma cells to produce antibody and suppress humoral immune response. Anti CD40 (2C10R4) is also a nondepleting antibody. Some of these agents have progressed to clinical trials in autoimmune diseases and allotransplantation with mixed results, but others show promise as adjunctive immunomodulators. Xenogeneic immune response is primarily antibody mediated; therefore, anti-CD40 antibody treatment that inhibit B cell activation and antibody production is beneficial for preventing xenograft rejection. Studies have also demonstrated the dependence of xenograft survival on blockade of this pathway and antibody mediated rejection occurring after the anti-CD40 treatment is stopped. It also appears that the blocking CD40/CD40L pathway improves xenograft rejection-free survival by curbing xenoantigen-specific elicited antibody-mediated rejection and perhaps blocking the T cell help. Other studies in NHP allotransplant models have shown the effectiveness of monotherapy with these agents. It may even be that blocking CD40/CD40L pathway with anti-CD154 and anti-CD11b antibodies could be superior to anti-CD40 or anti-CD154 antibodies alone, as blocking both CD11b and CD154 can inhibit alloreactive CD8+ cells and help in converting and producing iTreg cells.[26] All these studies demonstrate CD40/CD40L agents' therapeutic potential, but it is still unclear whether a single anti-CD154 /anti-CD40 antibody or a

combination of 2 or more is better at preventing rejection. We believe that it is also possible that anti-CD154 treatment alone or in combination may benefit more to allotransplantation and anti-CD40 antibody can control antibody mediated xenorejection. However, this finding still needs to be validated. Whether CD40/CD40L agents with alternative approaches will supplant current immunosuppression regimens has not been proven yet. However, preclinical data suggests their role in preventing antibody mediated processes from co-stimulation signaling provided by antigen presenting cells to B-cells could be a sustainable alternative to traditional immunosuppression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATN	Acute Tubular Necrosis
ADCC	Antibody-dependent Cellular cytotoxicity
CSA	Cyclosporin A
DCs	Dendritic cells
Fab	Fragment antigen-binding
Fc	Fragment, crystallizable
mAb	Monoclonal antibody
NHP	nonhuman primates
TE	Thromboembolic
TNFR/TNF	Tumor necrosis factor receptor/tumor necrosis factor

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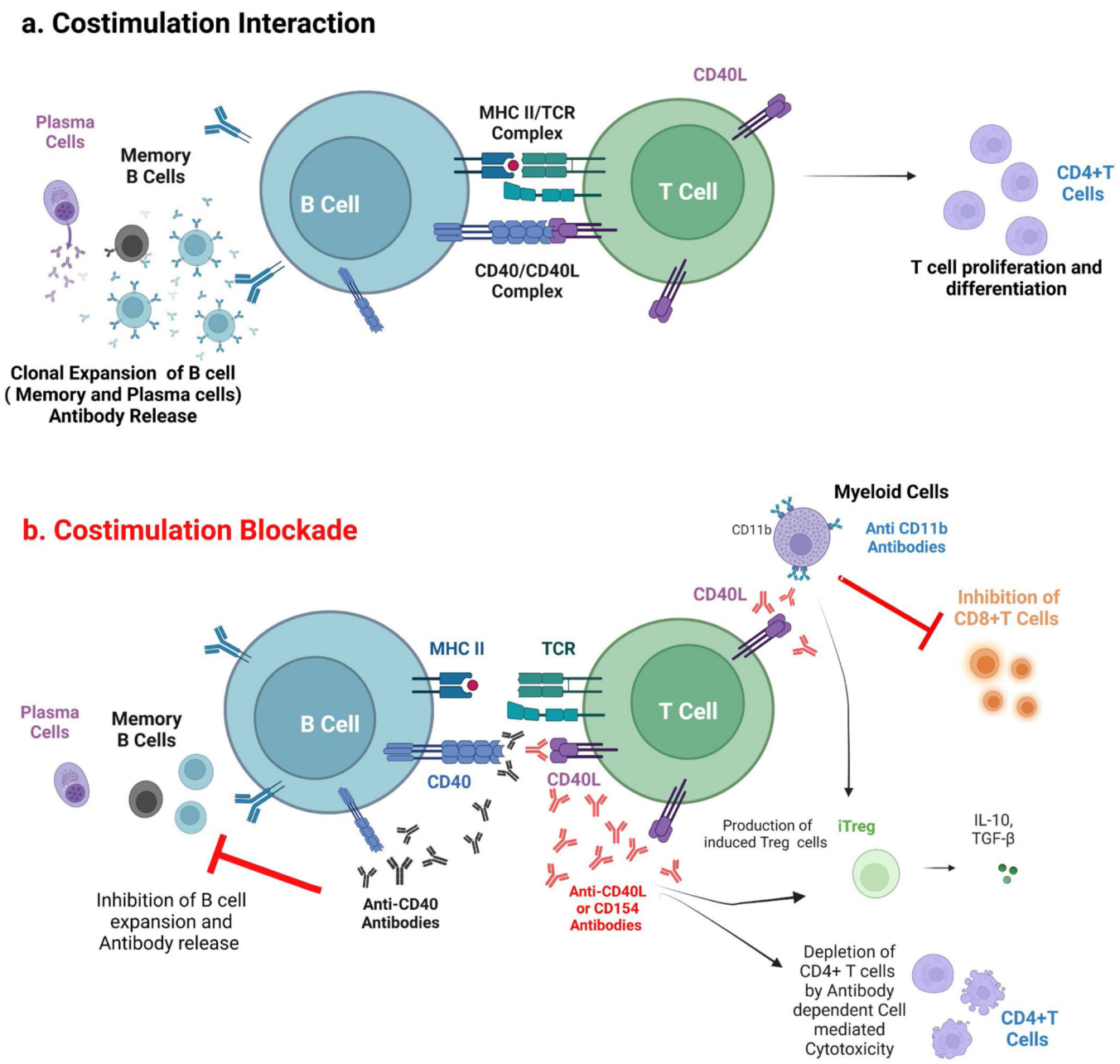

Figure 1. Mechanism of CD40/CD154(CD40L) pathway:

a. Costimulation interaction: Ligation of CD40 on B cells with CD154 (CD40L) on T cells activates B cells differentiation and clonal expansion that enhances the formation of long-lived plasma cells and memory B cells which release antibodies and produce cytokines which activate other immune cells, and likewise CD40L interaction with CD40 on B cells stimulated T cells and help in proliferation and differentiation of T cells. b. Costimulation Blockade: Anti-CD154 antibody (in red) blocks the interaction and depletes alloreactive T cells (CD4+) cells by ADCC and helps produce induced regulatory T (iTreg) cells. Anti-CD40 antibody (in black) blocks the interaction and inhibits clonal expansion of B

cells, plasma, and memory B cells, reducing antibody production. Blocking CD154 (in red) and CD11b (in blue) inhibits alloreactive CD8+ T cells and helps produce iTreg cells.

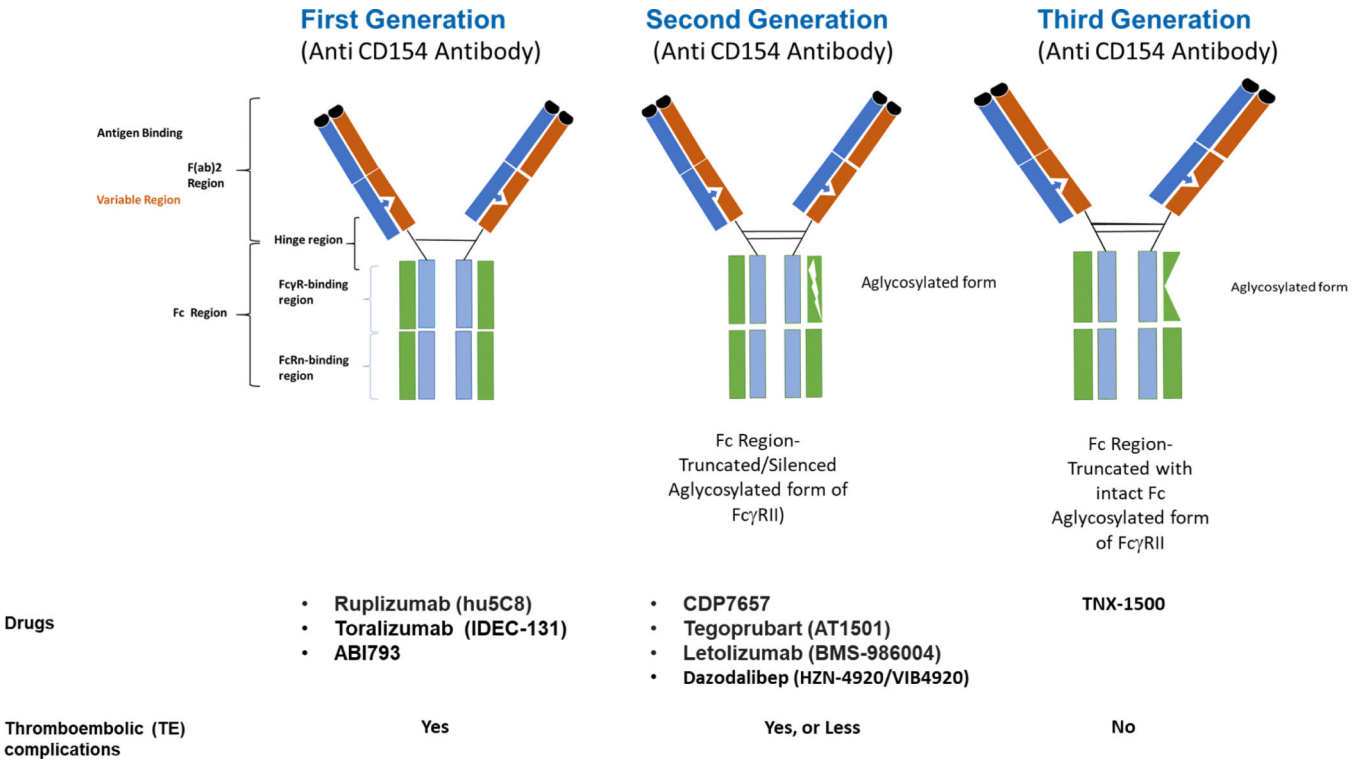

Figure 2. Anti-CD154 antibodies and their modified version:

First-generation anti-CD154 antibodies with intact F(ab)2 and FC region, Second and third-generation Anti CD154 antibodies with truncated /silenced Fc region

Table: 1.

Anti-CD154 antibodies targeting the CD40/CD40L pathway in the pipeline

Name of Agent	Experiment	Outcome	Clinical Trial Status
Anti-CD154			
Ruplizumab (hu5C8)	Cardiac Xenotransplantation[3–5,7,8,38]	Prolonged cardiac xenotransplantation up to 236 days in heterotopic model	
Toralizumab (IDEC-131)	Cardiac Xenotransplantation[6,32,40,41]	Prolonged cardiac Xenotransplantation	
ABI793	Cardiac Xenotransplantation[43–45] Islet Xenotransplantation[46]	Prolonged cardiac xenotransplantation. Prolonged wild type porcine islet transplantation in immunosuppressed nonhuman primates up to 154 days	
H106	Islet Xenotransplantation [30]	Engraftment of adult porcine Islet transplantation in rhesus macaque	
Fc receptor modified CD154 Antibody			
CDP7657 (Dapirolizumab Pegol)	SLE[51,94]	Inhibit immune responses without activating platelets and thrombotic complications	
Tegoprubart (AT1501)	Kidney Transplantation[52,53]	Promote islet allograft survival in Nonhuman Primates; Prevents renal transplant rejection	
Letolizumab (BMS-986004)	Hematopoietic stem cell Transplantation [55,56]	Inhibits the production of platelet-derived anti-glycoprotein (GP) IIb/IIIa antibodies and prevents thromboembolism	Phase 2
Dazodalibep (HZN-4920/VIB4920)	Kidney Transplantation[95]	Prevent renal Allograft Rejection	Phase 2a
TNX-1500	Kidney Transplantation[57] Cardiac Transplantation[58]	Monotherapy prolongs the renal allograft survival without thrombotic complications	

Table 2.

Anti-CD40 antibodies targeting the CD40/CD40L pathway in the pipeline

Name of Agent	Experiment	Outcome	Clinical Trial Status
Anti-CD40			
ch5D12	Kidney Allotransplantation[69]	Prevented rejection and extended kidney allograft survival up to 217 days in rhesus monkey	
3A8 (3A8R1)	Skin and Islet Transplantation[70] Cardiac Xenotransplantation[64]	Prolonged alloislet transplantation model up to 298 days in NHP; Demonstrated cardiac xenotransplantation up to 27 days in NHPs	
Chi220	Islet Transplantation[13,14,47]	Suppressed the primary immune response to cytomegalovirus and modestly prolonged renal and islet allograft survival; Prolonged survival of porcine neonatal Islet in Rhesus macaques	
2C10R4	Cardiac, Liver, Kidney Xenotransplantation[12,71,72,74,75,96]	Prolonged cardiac xenotransplantation up to 945 days in heterotopic and 264 days in life supporting orthotopic model; Prolonged liver Xenotransplantation up to 31 days	
Bleselumab 4D11 (ASKP1240)	Kidney Transplantation[79,80,82,83] Islet allotransplantation[81]	Prevents renal allografts rejection in cynomolgus monkeys and suppresses anti-donor antibodies	
Iscalimab (CFZ533)	Kidney Transplantation[84] Liver Transplantation[85]	Prevented allograft rejection in renal (phase 1) and liver transplant recipients	Phase 1
KPL-404	Rheumatoid Arthritis [90,91]	Suppresses the T-cell Dependent Antibody Response (TDAR); Has been used in first porcine to human cardiac xenotransplantation	Phase 1
Oligonucleotide (RNAi) for anti-CD40			
NJA-730a	Healthy Volunteer[93]	Prolongs the engraftment of bone marrow in murine recipients (up to 45 days)	Phase 1