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Update on CD40 and CD154 blockade in transplant models

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Abstract

Generation of an effective immune response against foreign antigens requires two distinct molecular signals: a primary signal provided by the binding of antigen-specific T-cell receptor to peptide-MHC on antigen-presenting cells and a secondary signal delivered via the engagement of costimulatory molecules. Among various costimulatory signaling pathways, the interactions between CD40 and its ligand CD154 have been extensively investigated given their essential roles in the modulation of adaptive immunity. Here, we review current understanding of the role CD40/CD154 costimulation pathway has in alloimmunity, and summarize recent mechanistic and preclinical advances in the evaluation of candidate therapeutic approaches to target this receptor-ligand pair in transplantation.

Keywords

alloimmunity; CD40/CD154; costimulation; Tcells; tolerance; transplantation

Transplantation is firmly established as the best available treatment for acute or chronic end-stage failure of vital organs, including heart, lung, liver and kidney. However, current success in controlling alloimmune injury to the graft relies on an array of potent immunosuppressive agents, which are nondonor-specific in their action and associated with many undesirable side effects. Consequently, transplant recipients are relatively vulnerable to infections, drug toxicities, malignancy and drug costs, while regimen complexity and the inconvenience of regular drug monitoring contribute to noncompliance and related complications. In recent years, novel immunomodulatory strategies based on selectively targeting T cell costimulatory pathways have gained considerable attention as a potential means to control pathogenic alloreactive responses against transplanted organs and tissues, and perhaps promote emergence of protective, alloantigen-specific ‘regulatory’ immune mechanisms.

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T cells govern the adaptive immune responses after transplantation. T cells require both high-affinity antigen-specific T-cell receptor (TCR) binding (signal 1) and simultaneous engagement of costimulatory molecules (signal 2) to become 'optimally' activated. Among the many various costimulatory pathways that have been discovered to play a pivotal role in transplantation, the best characterized are CD28/CD152 (CTLA-4)/B7 (CD80/86) and CD40/CD154 (CD40L) receptor/ligand interactions [1–6].

Therapeutic manipulation of CD28/B7 costimulatory pathway has shown great promise for controlling pathogenic alloimmunity in both rodent [7–12] and nonhuman primate (NHP) models [13,14]. Based on clinical trials that demonstrated safety and efficacy in renal allograft recipients, belatacept, an inhibitor of B7/CD28 costimulation, was approved by the US FDA in a calcineurin inhibitor free regimen. Thus, costimulation blockade has already emerged as a novel and promising class of immunosuppression, and a landmark example of successfully translating from bench to bedside.

Based on striking efficacy in preclinical models, clinical trials using humanized or chimeric anti-CD154 monoclonal antibodies (mAb) blocking CD40/CD154 interactions were undertaken in the early 2000s. Unfortunately, progress halted due to the incidence of thromboembolic events. Nevertheless, because CD40/CD154 signaling plays such a critical role in the regulation of immune responses during allograft rejection, developing alternative clinically viable agents to target both CD40 and CD154 remains very attractive, and several candidate biologic agents based on this pathway are under current investigation for treatment of autoimmune disease or for prevention of transplant rejection. In this review, we focus on the current knowledge of the CD40/CD154 costimulatory pathway. Concentrating on recent progress, we summarize a large body of experimental work from initial landmark studies to the more recent evaluation of new therapeutic reagents to block this pathway in transplantation.

Structure & expression of CD40 & CD154

CD40 is a cell surface glycoprotein in the TNF receptor (TNFR) family [15]. CD40 is constitutively expressed, and its expression further increased upon activation, on antigen-presenting cells (APCs), including B cells, dendritic cells (DCs) and macrophages; expression of CD40 is also inducible on parenchymal cells such as endothelial cells and fibroblasts after inflammatory stimulation [16]. Its known ligand, CD154, belongs to the TNF family and is rapidly induced on T cells (CD4⁺ and some CD8⁺ cells) following TCR activation. First identified on activated T cells, CD154 is also expressed on platelets [17], as well as monocytes [18] and B cells [19,20]. CD154 can also be shed from activated platelets and lymphocytes in a soluble form (sCD154).

CD40/CD154 pathway in humoral & cellular immunity

CD40/CD154 interaction is essential for the development of thymus-dependent humoral immune responses. Ligation of CD40 on B cells by CD154 on T cells promotes B cell proliferation, immunoglobulin (Ig) production, isotype switching and memory B-cell generation. Deficiency in CD40 or CD154 results in hyper IgM syndrome, a genetic disorder characterized by low levels of serum IgG, IgA, IgE with normal to high IgM and a high risk

for opportunistic infections [21]. Similarly, genetic ablation of CD40 or CD154 in mice results in defective humoral immunity [22–24].

It has been firmly established that CD40/CD154 costimulatory pathway plays a central role in T-cell-mediated DC activation/maturation and macrophage activation. Engagement of CD40 on these APCs stimulates the release of pro-inflammatory cytokines and chemokines, as well as CD28-mediated costimulation by upregulating B7–1 (CD80) and B7–2 (CD86) (Figure 1), enhances expression of major histocompatibility complex (MHC) molecules by DCs and induces macrophage effector functions. CD40-activated DCs and macrophages produce IL-12 [25–29], which plays an important role in the polarization of Th1-type immune responses and cytotoxic T lymphocyte priming [30].

CD154 blockade in rodent models of transplantation

Given its critical role in mediating many aspects of immune responses, the CD40/CD154 pathway represents a promising potential therapeutic target for the prevention of transplantation rejection. Early studies by Larsen *et al.* demonstrated that interrupting the CD40/CD154 signal pathway with anti-CD154 antibody (MR1) is effective in preventing acute cardiac allograft rejection and alloantibody responses in mice [31]. Subsequent studies have demonstrated the beneficial effect of anti-CD154 on the prolongation of graft survival in a number of rodent models (islet, limb, corneal and marrow). However, on its own, CD154 blockade is not sufficient to prevent chronic rejection of fully MHC mismatched cardiac allografts, suggesting that adjunct treatment will be required to fully control T-cell recognition/activation.

When used in combination with donor-specific transfusion (DST) or transient CD28 blockade with CTLA4-Ig (B7-blocker), anti-CD154 prevents cardiac allograft vasculopathy (CAV) and leads to long-term donor-specific tolerance in murine cardiac and islet allografts [31–33]. Although the mechanisms by which combination strategies induce peripheral tolerance has not been fully elucidated, many factors have been implicated in this process, including clonal deletion of alloreactive cells (apoptosis), anergy and the induction of antigen-specific T regulatory cells (Tregs). Interestingly, the administration of CTLA4-Ig impedes the beneficial effects of DST + anti-CD154 [34], underscoring the critical importance of CTLA-4 in the establishment of allograft tolerance induced with the DST + anti-CD154 regimen.

Blockade of the CD40/CD154 pathway induces the expansion of antigen-specific Tregs [35–37], a mechanism requiring expression of CD40 on CD8⁺ T cells [38]. In addition, anti-CD154-induced tolerance can be transferred to naive recipients by the adoptive transfer of CD4⁺ Tregs from tolerized recipients [39,40]. However, in skin transplantation, CD154 blockade fails to induce tolerance in naive mice. Unlike heart and islet allograft rejection, which is primarily mediated by CD4⁺ T cells, destructive immune responses against allogeneic skin grafts can be elicited by either CD4⁺ or CD8⁺ T cells. The combination of DST and anti-CD154 substantially prolonged survival of MHC-mismatched skin allografts, however, only 20% of the recipient mice exhibited indefinite graft survival [41]. By contrast, the addition of thymectomy to the same treatment resulted in permanent skin graft

survival in most recipients [42]. DST in combination with anti-CD154 leads to early deletion of peripheral alloreactive CD8⁺ T cells and the induction of allospecific CD4⁺ Tregs. The failure to maintain skin tolerance with this treatment regimen in euthymic mice was attributed to the emergence of new thymic emigrants (presumably CD8⁺ T cells), which overwhelm the capacity of immunoregulatory mechanisms [43,44].

Simultaneous blockade of the CD28/B7 and CD40/CD154 pathways is a promising regimen to delay or prevent graft rejection. Aside from targeting CD28/B7 on the ligand side using the widespread B7-directed blocking reagent CTLA4-Ig, selective targeting of the CD28 receptor using anti-CD28 monoclonal antibody (JJ319) [45] or monovalent single chain variable antagonist antibody fragment (α 28scFv) [46] both synergized with CD40/CD154 blockade in promoting long-term allograft survival in rodents. By directly targeting CD28 molecules on T cells, selective CD28 blockade might offer advantage over B7 blockade by favoring B7-mediated coinhibitory signals delivered through CTLA-4 and/or PD-L1, suppressing IL-21 elaboration by follicular Th cells [47], and thereby facilitating the induction of peripheral allograft tolerance [4,14]. Further supporting this emerging paradigm, ligation of CTLA-4 dramatically abrogated cardiac allograft acceptance and intragraft tolerogenic gene expression induced by CD28 blockade [46].

In addition, a wide variety of biological agents have been used in combination with CD154 blockade, and many have yielded promising results. These include biologics targeting other costimulatory/coinhibitory molecules, such as ICOS [48,49] and PD-L1 [50,51], as well as antibodies targeting adhesion/costimulatory molecule and cytokines, such as LFA-1 [52–54], CD45RB [55], IL-2 [56], IL-7 [57] and IL-15 [58].

CD28/CD154 costimulation blockade-independent rejection

In a murine skin allograft model, Trambley *et al.* showed that CD8⁺ T cells are able to reject allografts in the absence of CD4⁺ T cells in the context of costimulation blockade of both CD40 and CD28 pathways. Accordingly, depletion of CD8⁺ T cells reversed CD4-independent, costimulatory blockade-resistant rejection of murine skin grafts [59]. The ability of CD8⁺ T cells to mount effective alloresponses and cause rejection is significantly influenced by the recipient's immunogenetic background, since C3H mice show a marked prolongation of BALB/c skin graft survival following CD28 and CD154 blockade, whereas C57BL/6 mice with the same grafts are refractory to this treatment [60]. A population of CD8⁺ asialo GM1⁺ T cells was identified as a pivotal mediator of this type of rejection [59].

A critical role for OX40 (CD134, another member of TNFR costimulation pathway family) in mediating CD28/CD154-independent rejection has been reported. Interruption of the OX40/OX40L, but not ICOS/ICOSL, 4-1BB/4-1BBL or CD27/CD70 pathways, markedly improved skin graft survival in the absence of CD28/CD154 costimulation [61]. In addition, under the cover of CD28/CD154 coblockade, CD4⁺ and CD8⁺ T-cell-mediated rejection is supported by OX40 costimulation [62]. These findings reflect the fact that families of costimulatory receptors exhibit considerable functional redundancy or overlap, although CD28 and CD40 pathways dominate.

Alloreactive memory T cells constitute a potent barrier to the successful induction of allograft tolerance through classical costimulation blockade [63–67]. In humans, pre-existing ‘naive’ effector memory T cells, possibly elicited by ‘cross’-priming by environmental or infectious antigens, comprise a significant proportion of circulating T cells, coincidentally ‘cross’-react with donor alloantigens and thus mediate rejection responses [68]. It has been generally accepted that several populations of memory cells are less dependent on the costimulatory signals (signal 2) provided by CD28/B7 and/or CD40/CD154 for function [69–71]. However, other recent studies demonstrated that memory T cells may require CD28 costimulation for recall responses under certain circumstances [72–76]. Moreover, a critical threshold of memory T cell is necessary to precipitate costimulation blockade-resistant rejection [64]. In addition, a current working hypothesis is that the increased precursor frequency of ‘primed’ donor-reactive memory T cells contributes to their resistance to costimulation blockade [77]. In murine models, the ability of memory T cells to mediate CD28/CD154 blockade-resistant rejection can be abrogated by NF-κB inhibitor (deoxyspergualin) [64], anti-OX40L [78], anti-LFA-1 or anti-VLA-4 [79], implicating each of these pathways in costimulation pathway-independent allograft injury mechanisms.

Based on these considerations, targeting of T cell memory might potentiate the clinical efficacy of costimulation blockade and thus facilitate transplantation tolerance. The finding that LFA-1 and VLA-4 participate in memory T-cell-mediated costimulation blockade-resistant rejection may have significant implication for clinical translation [79], as both anti-LFA-1 mAb (efalizumab) and anti-VLA-4 mAb (natalizumab) were FDA-approved to treat autoimmune diseases such as psoriasis, multiple sclerosis and Crohn’s disease. In a preclinical study of islet transplantation, increased levels of LFA-1 were detected on donor-specific memory T cells and anti-LFA-1 (TS-1/22) in combination with either belatacept or basiliximab and sirolimus facilitated allograft survival [80]. Moreover, in pilot clinical transplant trials, LFA-1 blockade with efalizumab has shown significant promise in preventing renal and islet allograft rejection [81–83]. However, chronic administration of efalizumab and natalizumab has been associated with increased risk of progressive multifocal leukoencephalopathy (PML). Although relatively rare (<0.5%), PML is a devastating disorder with no known treatment, and consequently efalizumab was voluntarily withdrawn from the market [84], while natalizumab is currently available for specific autoimmune indications where the risk/benefit ratio is deemed favorable. As costimulation blockade has emerged as a viable transplant immunosuppressive strategy, the efficacy of targeting LFA-1 or VLA-4 as useful adjuvant to abrogate costimulation-resistant memory alloresponses remains to be fully explored clinically, and will presumably require identification of a strategy to assure improved safety, particularly with respect to PML.

Recent studies have also shown that the precursor frequency of ‘naive’ yet alloantigen-responsive T cells has an influence on the effects of costimulation blockade. The presence of high initial CD4⁺ or CD8⁺ donor-reactive T-cell precursor frequency might render the transplant recipients resistant to costimulation blockade [85–87]. These discoveries might have important clinical implication as the influence of alloreactive T-cell precursors could be minimized or eliminated through the selection of donor-recipient combinations with low pretransplant donor-reactive CD4⁺ and CD8⁺ T cell frequencies [77]. These results also

suggest that some T cell-depleting agents such as anti-CD52 or anti-CD3 mAb might be useful for potentiating the efficacy of costimulation blockade by reducing the initial frequency of graft-specific T cells [77,85]. On the other hand, pan-T- (and B-, for anti-CD52) depletion also perturbs regulatory cell populations that may be essential to facilitate graft immunoprotection as donor-reactive cell populations recover.

CD154 blockade in NHP models of transplantation

hu5C8 (ruplizumab)

CD154 blockade has been investigated extensively in NHP models of transplantation (Table 1). hu5C8 is a recombinant humanized anti-CD154 monoclonal antibody that incorporates the complementarity determining region of the anti-human CD154 mAb, 5C8, in a human IgG1 framework. Kirk *et al.* initially reported that treatment with hu5C8, alone or in conjunction with CTLA4-Ig, effectively prevented renal graft rejection in juvenile rhesus monkeys [88]. Remarkably, when used as an induction regimen followed by a 5-month maintenance therapy, hu5C8 monotherapy induced long-term rejection-free survival of renal allografts, in some cases for more than 1 year after the cessation of hu5C8 treatment [89]. In addition, hu5C8 promoted islet [90,91] and skin [92] allograft survival in rhesus monkeys and baboons. Taken together, these findings clearly demonstrate that hu5C8 can induce extended and robust immunosuppression to prevent allograft rejection. However, uniform transplant tolerance was not achieved by CD154 blockade alone.

Using a cynomolgus monkey heterotopic heart allograft model, we demonstrated that CD154 blockade with hu5C8 significantly prolonged survival, but was not sufficient to prevent CAV [93]. More recently, we observed that intensive hu5C8 therapy (30 mg/kg on days 0, 3, 7 and 14; 10 mg/kg on days 21, 28, 35 and 42; 20 mg/kg on days 56 and 84) potentially increased cardiac allograft survival (median survival time [MST] = 133 days); all recipients retained excellent allograft function during treatment [111]. Moreover, a short peritransplant course of sc28AT, an anti-CD28 scFv fragment conjugated to α -1-antitrypsin (selective CD28 blockade, 3-week induction regimen) [14], when added to hu5C8, resulted in remarkably reduced cellular infiltration and significant CAV prevention in graft biopsies/explanted functional grafts within the first 3 months after transplantation (Zhang *et al.*, manuscript in preparation). These results demonstrated that perioperative addition of selective CD28 blockade to anti-CD154 therapy favorably modulates primate cardiac alloimmunity and prevents chronic allograft vasculopathy in NHP.

IDEC-131 (toralizumab)

IDEC-131 is a humanized anti-human CD154 mAb that incorporates the variable regions of the murine antibody clone 24-31 onto a human framework and uses human γ 1/ κ constant regions [112]. In a rhesus monkey skin transplant model, IDEC-131 prolonged allograft survival when combined with rapamycin (with or without DST) [95]. IDEC-131 was also found to prevent acute renal graft rejection in rhesus monkeys, and was particularly effective when combined with rapamycin and DST, resulting in long-term rejection-free renal graft survival without ongoing treatment (>500 to >1000 days, three of five recipients) and donor-specific skin graft acceptance (two long-term survivors tested) [94]. Our group has shown

that IDEC-131 extended cardiac allograft survival modestly in cynomolgus monkeys [96], and early graft failure with IDEC-131 could be overcome by antihuman thymocyte globulin (ATG) induction therapy [97]. We have also demonstrated that IDEC-131 suppressed both primary and secondary antibody responses to influenza antigens, even when revaccination was given after IDEC-131 treatment withdrawal [113,114]. These findings suggest that CD154 inhibition has a tolerizing effect on immunity to viral antigens in primates.

ABI793 & H106

In addition to hu5C8 and IDEC-131, two other clones of anti-CD154 mAb, ABI793 and H106, have been investigated and shown to be efficacious in promoting renal allograft survival [98–100].

hu5C8 is superior to IDEC-131 in promoting cardiac allograft survival in cynomolgus monkeys

To date, four mAbs to CD154 (hu5C8, IDEC-131, ABI793 and H106) have been evaluated in preclinical transplant models, but we are not aware of reports comparing efficacy between different reagents. In our preclinical NHP heterotopic heart transplant model, we compared hu5C8 and IDEC-131, using the same treatment dose and schedule (30 mg/kg on days 0, 3, 7 and 14; 10 mg/kg on days 21, 28, 35 and 42; 20 mg/kg on days 56 and 84). We found that although intensive blockade of CD154 with either IDEC-131 [97] or hu5C8 (aforementioned observation) effectively attenuated acute rejection of cardiac allografts, hu5C8 monotherapy was superior to IDEC-131 in promoting extended graft survival (MST 133 days vs 35 days). Moreover, almost all of the animals treated with IDEC-131 elaborated reproducibly detectable IgM and/or IgG donor-reactive antibodies despite continued treatment [97], whereas hu5C8-treated animals lacked detectable alloantibody reactivity during therapy [111]. It is notable that in published reports, IDEC-131 appeared to be generally less effective than hu5C8 in promoting skin allograft survival in rhesus monkeys [92,95] and in patients with systemic lupus erythematosus (SLE) [115,116], although other direct comparisons were not performed. Our study provides evidence that hu5C8 is probably more efficacious than IDEC-131 in promoting cardiac allograft survival in NHPs, and we speculate that biologic differences between the two agents in binding affinity for CD154 and/or epitope specificity may account for observed differences in efficacy.

Thromboembolic side effects associated with anti-CD154 mAb

Treatment with several different anti-CD154 mAbs was associated with thromboembolic complications in initial clinical trials and subsequently reported in pre-clinical studies. Phase II trials using hu5C8 in lupus patients [117] and in kidney transplantation [118] were suspended after the occurrence of thromboembolic complications. In a Phase II study, although IDEC-131 was found to be well-tolerated in SLE patients, (although there were no beneficial effects compared with placebo [116]), but further clinical development of IDEC-131 was halted after thromboembolism was observed in a Crohn's disease patient. In NHP studies, both hu5C8 and ABI793 have been associated with thromboembolic events [99,119–121]. Although thromboembolism was not observed with all humanized anti-CD154 mAbs, it is generally accepted that thromboembolic complications may be a class effect of anti-CD154 mAbs independent of epitope specificity.

The undesirable thrombotic effects associated with anti-CD154 mAbs may result from binding to CD154 elaborated by activated platelets, either expressed on the platelet surface or released in soluble form (sCD154) [122]. Importantly, in NHP studies, the use of clinically applicable perioperative anticoagulation (prophylactic dose heparin) and aspirin during anti-CD154 mAb treatment abrogated thrombotic events [94,120,123]. In addition, a relatively short treatment course of anti-CD154 mAb may diminish the occurrence of thromboembolic complications in experimental models [94].

Improved reagents targeting CD154

The finding that the Fc domain of anti-CD154 mAbs binding to platelet Fc receptor (CD32A) contributes to platelet activation and aggregation [124,125] suggests that an anti-CD154 Ab without Fc-mediated functions may avoid the risk for platelet aggregation and thromboembolism that have been associated with anti-CD154 mAbs. For example, an Fc-disabled aglycosylated anti-CD154 mAb [126] and a clinical translatable Fc-silent anti-CD154 domain antibody [127] have shown promise in mouse transplant models.

Another way to circumvent adverse thromboembolic events might be to use a noncross-linking monovalent antibody specific for CD154, similar to the development of nonactivating antibody fragments (scFv or Fab) recently used to block the CD28/B7 pathway [14,46,128–129]. Accordingly, a novel monovalent pegylated anti-CD154 Fab antibody fragment (CDP7657) is currently under evaluation in Phase I trials for the therapy of SLE (ClinicalTrials.gov Identifier: NCT01764594). The efficacy of such anti-CD154 antibody fragments in the setting of transplantation has yet to be described.

CD40 blockade

An alternative approach to block CD40/CD154 interaction, one that might not evoke undesirable platelet-associated prothrombotic activity, is the use of mAb directed to CD40 (Table 1), the receptor for CD154 that is expressed constitutively on multiple immune, vascular and other cell types. In murine models of transplantation, a CD40-specific mAb 7E1-G2b was shown to effectively synergize with CTLA4-Ig to promote both bone marrow chimerism and skin graft survival [130]. In addition, CD40-Ig gene transfer, alone or combined with CD28/B7 targeting, was successfully used to prolong heart and liver allograft survival by several groups [45,131].

In NHP models, initial studies using chimeric anti-human CD40 mAbs (Ch5D12 and Chi220) [100–102] demonstrated that these CD40-depleting agents prolong renal and islet allograft survival, although arguably less effectively than anti-CD154 mAbs. As expected, these antibodies significantly deplete B cells, which prevalently express CD40; the importance of this finding remains incompletely explored. 3A8 and 4D11 (ASKP1240), antihuman CD40 mAbs that are associated with some degree of B activation *in vitro* and/or B cell depletion *in vivo*, prolong islet, kidney and liver allograft survival [103–106] and synergize with CTLA4-Ig [107,108]. Recently, a novel chimeric mouse-rhesus mAb against rhesus macaque CD40 (2C10) was developed that lacks agonistic properties and does not deplete B cells *in vivo* [109]. Treatment with 2C10 inhibited T cell-dependent antibody responses to keyhole limpet hemocyanin (KLH), prolonged islet allograft survival in rhesus

monkeys compared with recipients treated with basiliximab and sirolimus alone [109], and consistently promotes cynomolgus cardiac allograft survival in our hands as well [110]. Although the number of observations to date is small, efficacy appears to be comparable to that achieved by anti-CD154 treatment with hu5C8 [110].

Taken together, these preclinical data demonstrate that targeting CD40, especially with nonagonistic, nondepleting reagents, appears to be an attractive candidate approach to promote tolerogenic immunomodulation in transplantation. Defining efficacy relative to selective CD154 blockade remains important to efforts to identify a suitable alternative to CD154 inhibition, particularly if the prothrombotic features of selective CD154 targeting prove not to be dissociable from therapeutic efficacy. As such, clinical trials of α CD40 (ASKP1240) are in progress (ClinicalTrials.gov Identifier: NCT01780844).

In addition, CD40 gene silencing, in the graft, recipient, or both, by the use of small interfering RNA (siRNA) is another innovative approach to transiently abrogate CD40/CD154 signaling [132].

Conclusion & future perspective

The critical importance of CD40/CD154 costimulation interaction in regulating many pathogenic, and tolerogenic, aspects of adaptive immune responses suggests that interfering with this pathway is likely to yield one or more effective strategies for therapeutic immunomodulation in transplantation. Although translation of traditional anti-CD154 mAb preparations to the clinic has been delayed by thromboembolic complications, several different approaches to address this issue appear promising. Meanwhile, therapeutic efficacy with CD40-specific agents in rodent and NHP models has advanced targeting of this molecule into clinic evaluation. In conclusion, targeting the CD40/CD154 pathway is quite promising as a candidate therapeutic strategy to safely alleviate pathogenic alloimmune responses, may promote tolerogenic immune modulatory mechanisms, and thus appears poised to significantly improve long-term outcomes in human transplantation.

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Executive summary

CD154 blockade in rodent models of transplantation

- α CD154 mAb (MR1) monotherapy prevents acute allograft rejection in transplant models, but does not prevent chronic rejection.
- Combination therapies exhibit synergism with α CD154.
- Donor-specific transfusion and CTLA4-Ig enhance the efficacy of α CD154.
- Selective CD28 blockade synergizes with α CD154 in a CTLA-4-dependent manner.
- Biologics targeting ICOS, PD-L1, LFA-1, CD45RB, IL-2, IL-7 or IL-15 synergize with α CD154.
- Immune injury refractory to CD28/CD154 costimulation blockade is mediated by:
 - CD4-independent CD8⁺ T cells, especially CD8⁺ asialo GM1⁺ T cells.
 - OX40/OX40L costimulation.
 - Alloreactive/heterologous memory T cells.
 - High precursor frequency of antigen-specific T cells.

CD154 blockade in nonhuman primate models of transplantation

- α CD154 mAb monotherapy prevents rejection.
- Modulation of alloimmunity, but not alloimmune tolerance, was achieved.
- Tolerance was demonstrated to vaccine antigens administered during α CD154 treatment.
- hu5C8 appears to be more effective than IDEC-131 in a NHP cardiac transplant model.
- Selective CD28 blockade synergizes with hu5C8.

CD40 blockade in rodent transplant models

- α CD40 mAb synergizes with CTLA4-Ig to promote bone marrow chimerism and skin graft survival.
- CD40-Ig gene transfer alone, or combined with CD28/B7 targeting, prolongs heart and liver allograft survival.

CD40 blockade in primate transplant models

- Allograft survival prolonged by several α CD40 antibodies with variable degrees of agonistic and depletional activity (Ch5D12 and Chi220 and, to a lesser extent, 4D11/ASKP1240, 3A8).
- Nonactivating, nondepleting α CD40 antibody (2C10) inhibits antibody responses to KLH, and prolongs islet and cardiac allograft survival.

α CD40/CD154 mAbs being evaluated for/in clinic transplantation

- Initial translation of α CD154 mAbs to the clinic revealed thromboembolic complications.
- Several α CD154 Ab modifications appear to attenuate thromboembolic risk
 - Aglycosylated Fc-disabled α CD154 mAb.
 - Fc-silent α CD154 domain antibody (dAb).
 - Non cross-linking peggylated monovalent α CD154 Fab antibody fragment (CDP7657).
- α CD40 mAb (ASKP1240) in Phase II ‘efficacy and safety’ study in *de novo* kidney transplant recipients (NCT01780844).
- α CD154 (CDP7657) in Phase I ‘safety, dose-finding’ study in patients with systemic lupus erythematosus (NCT01764594).

Conclusion

- The CD40/CD154 costimulatory pathway regulates pathogenic alloimmunity, and its blockade consistently prevents transplantation rejection, and may promote alloimmune tolerance.
- Several approaches to CD40/CD154 blockade appear likely to prove safe and effective in clinical application, and to emerge as potentially attractive alternatives to ‘conventional immunosuppression’.

Figure 1. Role of CD40/CD154 in T-cell activation

Signaling following CD40/CD154 interactions promotes CD28-mediated costimulation by upregulating B7-1 and B7-2 expression on antigen-presenting cells and enhances T-cell activation by augmenting the potency of antigen-presenting cells to present antigen. In addition, CD28 engagement on T cells upregulates CD154 (CD40L) expression.
 +: positive (activating) costimulatory signal; -: negative (inhibitory) signal.

Table 1

CD40/CD154 blockade in nonhuman primate transplant models.

Drug name	Type of transplant	Main findings
CD154 blockade		
hu5C8	Kidney [88,89]	Prolonged graft survival (alone or combined with CTLA4-Ig)
	Islet [90,91]	Prolonged graft survival
	Skin [92]	Prolonged graft survival
	Heart [93] and unpublished data	Prolonged graft survival
		Attenuated CAV (combined with selective CD28 blockade)
IDEC-131	Kidney [94]	Prolonged graft survival
		Synergize with rapamycin/DST operational tolerance
	Skin [95]	Prolonged graft survival (combined with rapamycin)
	Heart [96,97]	Prolonged graft survival
		Less potent and efficacious than hu5C8
ABI793	Kidney [98,99]	Prolonged graft survival
H106	Kidney [100]	Prolonged graft survival (combined with CTLA4-Ig)
CD40 blockade		
Ch5D12 (chimeric)	Kidney [101]	Prolonged graft survival (alone or combined with anti-CD86 mAb)
Chi220 (chimeric)	Kidney [100]	Prolonged graft survival
		Suppress donor-specific Abs (combined with CTLA4-Ig)
	Islet [102]	Prolonged graft survival
		Synergize with belatacept
ASKP1240 (4D11)	Kidney [103]	Prolonged graft survival
	Islet [104]	Prolonged graft survival
	Liver [105]	Prolonged graft survival
3A8	Islet [106,107]	Prolonged graft survival (combined with basiliximab/sirolimus)
		Additional CTLA4-Ig suppress alloantibody
	Bone marrow [108]	Prolonged chimerism (combined with CTLA4-Ig/sirolimus)
2C10R1	Islet [109]	Prolonged graft survival (combined with basiliximab/sirolimus)
2C10R4	Islet [109]	Prolonged graft survival (combined with basiliximab/sirolimus)
	Heart [110]	Prolonged graft survival

CAV: Cardiac allograft vasculopathy; DST: Donor-specific transfusion.