INVITED REVIEW

Advances in targeting co-inhibitory and co-stimulatory pathways in transplantation settings: the Yin to the Yang of cancer immunotherapy

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Summary

In the past decade, the power of harnessing T-cell co-signaling pathways has become increasingly understood to have significant clinical importance. In cancer immunotherapy, the field has concentrated on two related modalities: First, targeting cancer antigens through highly activated chimeric antigen T cells (CAR-Ts) and second, re-animating endogenous quiescent T cells through checkpoint blockade. In each of these strategies, the therapeutic goal is to re-ignite T-cell immunity, in order to eradicate tumors. In transplantation, there is also great interest in targeting T-cell co-signaling, but with the opposite goal: in this field, we seek the Yin to cancer immunotherapy's Yang, and focus on manipulating T-cell co-signaling to induce tolerance rather than activation. In this review, we discuss the major T-cell signaling pathways that are being investigated for tolerance induction, detailing preclinical studies and the path to the clinic for many of these molecules. These include blockade of co-stimulation pathways and agonism of coinhibitory pathways, in order to achieve the delicate state of balance that is transplant tolerance: a state which guarantees lifelong transplant acceptance without ongoing immunosuppression, and with preservation of protective immune responses. In the context of the clinical translation of immune tolerance strategies, we discuss the significant challenge that is embodied by the fact that targeted pathway modulators may have opposing effects on tolerance based on their impact on effector vs regulatory T-cell biology. Achieving this delicate balance holds the key to the major challenge of transplantation: lifelong control of alloreactivity while maintaining an otherwise intact immune system.

KEYWORDS

co-inhibitory blockade, co-stimulation blockade, transplantation

1 | INTRODUCTION

The last 5 years have witnessed tremendous progress in cancer immunotherapy. This has included advances in both antibody- and cell-based therapies, with the success of each of these modalities significantly reliant on their ability to regulate co-stimulatory and co-inhibitory signaling to optimize T-cell anti-tumor responses. The prevailing strategies for controlling and enhancing T-cell signaling for cancer eradication have focused on modulating both sides of the signaling equation, to enhance T-cell activation. Thus, they have harnessed co-stimulatory signaling to enable antigen-driven activation of chimeric antigen receptor (CAR)-T cells, and blocked coinhibitory signaling to re-engage hypofunctional T cells in the tumor microenvironment. In stark contrast to the typically suppressive tumor microenvironment, transplanted allogeneic or xenogeneic tissues or cells

can create a pro-inflammatory environment resulting in graft injury or loss for tissue transplants, and graft-versus-host disease (GVHD) or donor cell rejection for donor hematopoietic lineage cell infusions. If the strategy surrounding cancer immunotherapy is focused on harnessing the Yang—the optimized activation and 'heat' in T cells, then in transplantation, we seek the 'cooling' Yin, in focusing on controlling these same co-stimulatory and co-inhibitory pathways, toward the goal of inducing antigen-specific tolerance in T cells rather than T-cell assault.

In what follows we will review the work in the field of transplant tolerance, highlighting the many successes that have been achieved by targeting T-cell co-stimulatory and co-inhibitory pathways, and discuss the challenges that we still face on the road to the ultimate goal of tolerance-on-demand for all transplant patients. In discussing successes and challenges, we will examine the complexity of manipulating T-cell co-signaling, given that both conventional, effector T cells (Teffs) are targeted by these approaches, along with regulatory T-cell (Treg) populations, for which these manipulations may have opposing biologic effects and hence, transplantation tolerance outcomes. The search for the ideal pathway, which, when manipulated, could simultaneously tilt the balance toward tolerance in both Teffs and Tregs, remains one of the holy grails of our field of inquiry.

Transplantation tolerance is defined as the lifelong acceptance of transplanted organs or cells, in the absence of any immunosuppressive medications, and with preservation of protective immunity. Immune tolerance thus has three major components, all which must co-exist in transplant patients. Each is critical, and all are inter-related, thus raising the bar needed for achieving transplantation tolerance, especially in large animal models and patients. Efforts to blunt co-stimulation pathways in order to accomplish this goal began in earnest over two decades ago, when CTLA4Ig was first synthesized for the purpose of precluding a productive immune response in antigen-activated T cells.[1–4] These seminal studies demonstrated that this new way of interfering with T-cell co-signaling could potently downregulate T cell and antibody responses to xenogeneic (xeno) and allogeneic (allo) tissues and were heralded in the lay press as having reliably achieved the holy grail of tissue transplantation tolerance. Individual co-stimulatory pathway blockade reports were closely followed by a series of important publications with combination therapy. These included a 1996 study by Dr. Christopher Larsen and colleagues that demonstrated that the coordinated blockade of B7 signaling (through CTLA4Ig) and CD40/CD154 signaling (through an anti-CD154 monoclonal antibody) could produce long-term tolerance to concurrently transplanted skin and cardiac allografts, while preserving the recipient's ability to reject non-donor allografts transplanted after co-stimulatory pathway blockade had been discontinued.[5] Such reports resulted in the birth of combinatorial co-stimulation blockade for generating antigen-specific graft tolerance, a paradigm that continues to be vigorously pursued in the present day. In what follows we provide an overview of this field, as it relates to immune tolerance induction, and discuss the relevant pathways, cells and clinical applications, highlighting where we have been, and where we need to go to make immune tolerance a reality for the patients we treat.

2 | SIMULTANEOUS BLOCKADE OF B7/CD28 AND B7/CTLA-4: CONFRONTING THE DOUBLE-EDGED SWORD OF CO-STIMULATION AND CO-INHIBITION

The field of co-stimulation blockade began with the search for molecules that paired with antigen-specific TCR stimulation to optimize T-cell activation after cognate antigen recognition. To accomplish this, researchers used agonistic monoclonal antibodies directed at newly identified T-cell surface molecules, and identified the immunoglobulin (Ig)-superfamily member CD28 as a critical T-cell co-signaling molecule which was required to optimally initiate, amplify, and sustain T-cell responses.[6] Subsequent studies demonstrated that there were two distinct CD28 ligands, CD80 (B7-1) and CD86 (B7-2), that were expressed as dual coreceptors on antigen-presenting cells (APC).[7–11] While this was a central discovery, the complexities of signaling through this pathway and its surrogates is still incompletely understood, yet key to ongoing efforts to target the CD28 pathway to control T-cell activation.

The first reagent used to inhibit CD28 signaling was developed by Linsley et al.[12], who developed a soluble CD28Ig, reasoning that it could block CD28 signaling by providing an alternative binding site for CD28 ligands. While the data supported this molecule as the first 'costmulatory blockade' reagent, its affinity for the ligand(s) of CD28 was relatively low such that in vivo experiments (and clinical applications) with this reagent were not sufficiently encouraging for further testing. At this time, a molecule with molecular similarity to CD28, named cytotoxic T lymphocyte antigen-4 (CTLA-4), now also known as CD152, was discovered Dr. Pierre Goldstein at the Pasteur Institute.[13,14] CTLA-4 was first presumed to act as stimulator of T-cell activation. However, further studies by several laboratories subsequently showed that, while CTLA-4 was upregulated during T-cell activation, the signal delivered by CTLA-4 engagement functioned as a negative, rather than positive regulator of T-cell function.[15,16]

Given the shared structure between CTLA-4 and CD28 as well as B7 ligand binding (and prior to its unequivocal identification as a negative regulator of T-cell function),[14] a CTLA4Ig fusion protein, consisting of the extracellular domain of CTLA-4 fused to an IgG tail (to prolong its half-life), was developed, with the hopes that CTLA4Ig-mediated blockade of positive T-cell co-signaling would dominate over blocking the CTLA-4 inhibitory pathway. Dr. Peter Linsley and colleagues indeed showed that CTLA4-Ig was capable of binding B7 at clinically relevant concentrations and in doing so, inhibited T-cell alloproliferation and T-dependent B-cell antibody production.[4] This breakthrough discovery ushered in the era of T-cell modulation for clinical control of undesired, aggressive donor and host T- and B-cell immune responses that precluded transplantation tolerance. As briefly mentioned above, in vitro characterization studies with CTLA4Ig were quickly followed with demonstrations of the ability of CTLA4Ig to modulate allo- and xenoimmunity in vivo, including producing striking prolongation of xeno-islet graft survival in mice.[2,3,17] While further studies in mice, non-human primate (NHP), and patients have indicated that this agent

is not capable of producing tolerance, these first in vivo studies were striking in their demonstration of the impact of this first targeted co-stimulation blockade approach in small animal model systems.

Given the ability of CTLA4Ig to impact both T- and B-cell function, it was seen as a potentially important new therapeutic for auto- as well as alloimmune indications. Murine studies demonstrated striking activity in models of lupus-like disease[18] and collagen-induced arthritis,[19] and more variable results against murine experimental allergic encephalitis (EAE), a preclinical model of a multiple sclerosis (MS)-like disease.[20] The result in EAE is noteworthy in that it foreshadowed future clinical observations with CTLA4Ig in renal transplant (and may be related to the impact that this molecule has on Tregs, discussed in detail below) in that it found that higher doses of CTLA4Ig worsened, rather than improved results with this agent against EAE.[20] The results in murine models of lupus, and especially arthritis, spurred the initial clinical trials of CTLA4Ig, which focused on patients with psoriasis[21] and rheumatoid arthritis (RA). In a series of Phase II and clinical trials and now with over a decade of follow-up, CTLA4Ig (known as abatacept and marketed as Orencia™) has shown significant clinical activity for patients with RA,[22–26] and was the first FDA-approved co-stimulation blockade agent, approved for use in RA in 2005. Given its significant activity in RA, it is somewhat surprising that CTLA4Ig has not developed a larger sphere of clinical indications, with trials in MS, asthma, Type I diabetes, ulcerative colitis, and lupus not yielding significant enough clinical improvement to lead to efforts to obtain FDA approval for those diseases[27–31] especially in the context of other therapeutic options available for treatment of these diseases. Whether the suboptimal clinical results are due to an unwanted impact of abatacept on CTLA4-mediated co-inhibitory signaling, untoward effects on Tregs in these diseases, or other causes has not been determined. Despite the lack of widespread clinical applications in autoimmunity, the success of abatacept in RA patients supported continuing clinical investigation of B7 blockade with CTLA4Ig for other indications, especially solid organ and hematopoietic stem cell transplantation (HCT). In HCT, our work and that of others in murine, canine and NHP models suggested that abatacept has biologic activity in preventing GVHD.[32–37] This led to a promising first-in-disease feasibility trial[38] which demonstrated the pharmacodynamic impact of abatacept in controlling T-cell proliferation early post-transplant, providing the foundational data for our ongoing Phase II study designed to evaluate the efficacy of abatacept in preventing severe GVHD after either mismatched or matched unrelated donor HCT (Clinicaltrials.gov # NCT01743131).

In solid organ transplant, although the abatacept formulation of CTLA4Ig showed significant promise in murine models,[39–51] including evidence for a critical role in immune tolerance when combined with CD154-based co-stimulation blockade (discussed in detail below), initial studies in NHP renal transplant models were underwhelming.[52,53] These results may have been mechanistically linked to the fact that CD28 and CTLA-4 compete for B7 ligands (CD80 and CD86) that are required for CD28 activation, and that the interaction of CD80 with both CD28 and CTLA-4 appears substantially better than that of CD86. Because abatacept has decreased affinity for CD86 compared to CD80, CD80/CD86 interactions with CD28 and CTLA-4 may be incompletely

blocked with the abatacept formulation of CTLA4Ig.[12] Therefore, the group at Bristol-Myers Squibb, in collaboration with Larsen's group at the Emory Transplant Center decided to create directed mutations in the CTLA4Ig molecule designed to increase its affinity for CD86.[52] The resulting formulation, differing in two amino acids from abatacept (known first as LEA29Y and subsequently as belatacept, and now marketed as Nulojix™), increased both affinity and avidity of CTLA4Ig toward both B7 molecules. In doing so, belatacept resulted in increased blockade of both CD28- and CTLA-4 mediated T-cell activation in vitro and prolonged allograft survival in NHP models when compared to abatacept.[48,52] A subsequent series of Phase II and Phase III trials in renal transplant patients demonstrated that belatacept led to improved long-term renal functional outcomes after transplant and fewer off-target toxicities.[54] These results have been verified in long-term follow-up studies.[55–57]

Two important observations from the belatacept trials point to important biological complexities of targeting T-cell co-stimulation using CTLA4Ig: First was the observation that patients treated with a low-intensity regimen of belatacept actually had superior graft outcomes than those treated with a higher intensity regimen.[54] Second was the observation that while overall results were improved with belatacept, patients treated with this drug experienced higher rates of early (reversible) rejection events.[54–57] There are two potential explanations for these observations: First, is the fact that belatacept, like abatacept, not only targets CD28 positive co-signaling but also targets CTLA-4-based co-inhibition. CD28 is a highly expressed but low-affinity receptor, in contrast to CTLA-4, which is in low abundance but is a higher affinity receptor. At high in vivo concentrations, both CD28 and CTLA-4 would be blocked, whereas at lower in vivo concentrations, CTLA4Ig would preferentially block the high abundant CD28 receptor and restricting available ligand for the low abundance CTLA-4 receptor. CTLA-4 has a clear regulatory function as evidenced by CTLA-4 knockout mice.[58] These mice develop fatal lymphoproliferative disease at 3-4 weeks of age, suggesting a key role for CTLA-4 in maintaining self-tolerance. By restricting available ligand for the low abundance CTLA-4 receptor at low CTLA4Ig concentrations, tolerance induction induced by CTLA-4 signaling remains more intact than the co-stimulatory CD28 pathway.[59] This points to the knifes-edge that many of these studies may be on, in terms of the balance of CTLA4Ig being a co-stimulation *blocker* or *enhancer*, dependent upon the relative effects on co-stimulatory CD28 and co-inhibitory CTLA-4 pathways, which could lead to disease-specific impacts on efficacy given differential reliance on CD28 vs CTLA-4 signaling in different auto- and alloimmune entities.

A second important consideration also exists, as mentioned at the beginning of this review: this is the impact of CTLA4Ig on CD4$^+$ CD25$^+$ FOXP3$^+$ Tregs. As one of the key regulatory mechanisms to combat Teffs, Tregs represent a major force for tolerance induction in normal immune homeostasis. These cells have been well documented to express both CD28 and CTLA-4. Their activation augments Treg CTLA-4 expression,[60,61] critical for the ability of Tregs to prevent autoimmunity, in part by competing for B7 ligands that would otherwise be engaged by CD28 on conventional T cells (Tconv).[58,62] In addition to the role that Treg-expression of CTLA-4 may have on

restraining immune activation, Tregs also rely on CD28 expression in order to maintain their peripheral homeostasis.[63,64] Thus, an agent like belatacept might well produce an unexpected negative impact on tolerance induction through the impact of CTLA4Ig on CD28 signaling in Tregs. In fact, these data are consistent with results from our group and others that show that CTLA4Ig can lead to decreased levels of Tregs after both solid organ[65,66] and HCT,[67] which raise the possibility (although this has not been directly proven yet) that one of the potential relative contraindications of the use CTLA4Ig may be an undesired negative effect on Treg homeostasis and function.

3 | DIRECT TARGETING OF CD28: SUPERIOR TO CTLA4-IG?

Given the fact that CTLA4Ig can target both co-stimulation through CD28 and co-inhibition through CTLA-4, there has been deep interest for many years in directly targeting CD28 alone rather than targeting B7 ligands (that would affect CD28 along with CTLA-4). However, progress in this field was negatively affected by the tragic Phase I trial of TGN1412 in healthy controls, in which super-agonist activation by the cross-linked anti-CD28-specific intact mAb produced life-threatening cytokine storm in all six participants.[68,69] Recently, at least two independent efforts have led to second-generation CD28-targeting molecules that are incapable of cross-linking and have demonstrated broad safety in a number of preclinical settings and in clinical trials. Bristol-Myers Squibb is developing anti-CD28 'domain-specific' antibodies that are pegylated to prolong their half-life.[70] Preclinical work has demonstrated significant in vitro and in vivo activity of these molecules.[71,72] The clinical formulation, lilizumab, has now completed Phase I studies,[73,74] and is being studied in a Phase II trial in lupus as well as in a proof-of-concept study in Sjögren's syndrome.

Led by Dr. Bernard Vanhove, Effimune independently developed a pegylated anti-CD28 Fab, 'FR104', which has now been licensed to Janssen Biotech. FR104 has been shown to have biologic activity in a number of autoimmune and alloimmune settings, including in prolonging renal allograft survival in NHP and in preventing xenoGVHD in a NHP-→→ mouse model recently developed by our group.[75–81] Importantly, FR104 performed superiorly to belatacept in NHP models of renal transplant[80] and human xeno-GVHD.[76] The mechanisms that were critical for this improved activity have not been determined, although experiments in both systems suggest that both CTLA-4- and Treg-mediated mechanisms may be operative.[76,77,80] FR104 is also now in clinical trials, being studied in patients with RA.

4 | CD40:CD154-DIRECTED CO-STIMULATION BLOCKADE: THE STORY OF ON-TARGET POTENCY BEING SIDELINED BY AN UNEXPECTED TOXICITY

In preclinical studies, there has been no class of molecules that has seen more success in transplant tolerance induction than those that target CD40: CD154 signaling. CD40 (a member of the tumor necrosis factor receptor (TNFR) superfamily, expressed on most APCs), and its ligand, CD154 (expressed primarily on activated T cells, and also found on other hematopoietic cells, discussed below) are critical for T-cell function, as well as for the B- and T-cell interactions that result in the generation of high affinity antibodies that can be pathological in solid organ transplantation and HCT.[82] Consistent with its TNFR structure, CD40 signaling depends on its interactions with TNFR-associated factors (TRAFs),[83] which when engaged, lead to T-cell activation through a variety of secondary signals, including NF-kB, Jnk, and AKT pathways.[82]

The importance of CD40:CD154 engagement likely is due to its critical impact both on T-cell activation and on the initiation and perpetuation of the germinal center response in lymph nodes, thus being a critical hub for both T- and B-cell immunity.[84,85] The potential translational importance of targeting this pathway has been demonstrated numerous times, in murine, canine, and NHP models. The early studies concentrated on blocking CD154, and led to the striking result that a single dose of the MR1 anti-CD154 mAb permitted long-term cardiac transplant acceptance when combined with donor splenocyte infusion, and that anti-CD154 mAb infusion led to skin acceptance in mice.[86,87] In addition, striking synergy was observed when blockade of CD154 was combined with B7 blockade (using CTLA4Ig), which was demonstrated in numerous publications, including the seminal observation of its ability to lead to tolerance to both heart and skin allografts in the allogeneic Balb/c→→ C3H model.[86] Additionally, a series of studies by the Sykes, Larsen, and Blazar laboratories demonstrated the potent effects of anti-CD154 mAb on facilitating donor chimerism in suboptimally conditioned recipients, attributed to either peripheral host anti-donor Teff deletion followed by central tolerance or alternatively, by peripheral immune regulatory mechanisms.[42,44,49,87–94] Moreover, when anti-CD154 mAb was combined with donor bone marrow, immune tolerance to skin allografts was achieved even in the stringent B6→→ Balb/c model system.[46,49,50]

Several mechanistic studies have provided important insights into the mechanisms of CD154 blockade and tolerance induction. For example, anti-CD154 mAb added to an in vitro mixed lymphocyte reaction induced antigen-specific tolerance, measured both in vitro and in vivo in a GVHD reaction, with evidence that this effect was dependent upon Tregs.[46,88,95–98] Notably, activated Tregs express high levels of CD154, although a direct signaling mechanism for CD154 in T cells has not been described. In other studies, investigators demonstrated that anti-CD154-induced tolerance resulted in the abortive expansion of the alloreactive, Teff pool with a loss of cytokine production, activation marker expression, and absence of memory T-cell markers. Additionally, the tolerant alloreactive T-cell phenotype induced by CD154 was long-lived but could be reversed by in vivo Treg depletion.[46,88,95–98] Dr. Mandy Ford and colleagues further showed that blockade of this pathway not only controlled donor-reactive CD8^{+} T-cell responses but also was associated with the expansion of peripherally generated antigen-specific CD4^{+} FoxP3^{+} Tregs.[99–101] These observations suggest that blockade of CD154 may create the balance that was missing with CTLA4Ig, with pro-tolerogenic effects on both Tconv and Treg populations.

The impressive results in mice led to a large number of studies in NHP. In those studies, anti-CD154 antibodies were a critical component for the prolonged acceptance of both bone marrow and renal allografts.[67,102–105] While the induction of true immune tolerance in mice was not able to be directly extrapolated to NHP, CD154 blockade results were sufficiently impressive that they led to widespread interest in the clinical translation of CD154-directed therapies, as potential adjuncts to both conventional immunosuppression and CTLA4Ig-based therapeutics.

Unfortunately, the clinical translation of the first anti-CD154 antibodies was halted quickly, when thromboembolic complications were noted. This was found to be due to the expression of CD154 on activated platelets, leading to anti-CD154-mediated platelet activation and clotting in treated patients.[106–109] This unfortunate turn of events led the field to explore CD40 blockade as a potential alternative to CD154. While numerous studies have shown that CD40 blockade can also serve to prolong allograft survival in both mouse models and NHP,[110–120] the combined impact on both Tconv and Tregs noted for anti-CD154 antibodies has not yet been demonstrated for those blocking CD40.

Nonetheless, the large body of preclinical evidence supporting a central role of CD40:CD154 pathway blockade in prolonging allograft survival has sustained significant interest in CD40 for clinical translation, with a Phase II trial of the anti-CD40 antibody ASKP1240 currently underway in renal transplantation (Clinicaltrials.gov # NCT01780844). As with CD28, the advent of single-chain domain-specific antibodies has also presented an important solution to the clinical toxicity observed with anti-CD154 antibodies that were dependent on cross-linking. A domain-specific antibody for CD154 has now been developed, and has shown significant activity in murine models of alloimmunity.[100,121] These reagents are now being tested in NHP models of solid organ transplant and early-phase clinical trials are being planned.

5 | ICOS/L, A SUPPORTING PLAYER IN GERMINAL CENTER TARGETING AND CO-STIMULATION BLOCKADE

Structurally similar to CD28, inducible T-cell co-stimulator (ICOS, also known as CD278) is also an Ig superfamily member, expressed as a homodimer in activated (but not resting) T cells, as well as NK and NKT cells.[122–124] As with CD28, binding of ICOS to its ligand, CD275, results in T-cell activation, including expanded T-cell proliferation, survival, cytokine production, and effector differentiation.[125–127]

Consistent with these properties, ICOS blockade inhibited murine acute GVHD induced by CD4^{+} and/or CD8^{+} T cells and also was shown to promote alloengraftment of donor bone marrow in suboptimally conditioned recipients. Murine experiments also have documented that ICOS is required for T-mediated B-cell function, including a critical role in T-follicular helper (Tfh) generation and CD40-mediated Ig isotype switching.[127–129] While the impact of ICOS signaling on T-cell function is striking[130] and positive effects have been seen in acute GVHD models, monotherapy mAb-mediated ICOS blockade in a solid organ cardiac allograft model only demonstrated a modest impact, although synergistic activity was seen when ICOS blockade was combined with cyclosporine to produce >100 day survival in a cardiac transplant model.[131] ICOS/L blockade has also been shown to effectively partner with anti-CD40L in prolonging islet allograft survival[132,133] and blockade of ICOS; ICOSL reduced alloreactivity in an acute GVHD.[134] Moreover, ICOS has been demonstrated to be up-regulated on activated T cells in a canine model of chronic GVHD,[135] and in a murine model of chronic GVHD model induced by injecting parental splenocytes into unirradiated F1 recipients, antiICOS mAb inhibited B-cell activation, autoantibody production and glomerulonephritis, the primary manifestation of chronic GVHD in that model system.[136] In addition, using a murine multi-organ system model of chronic GVHD with a hallmark of lung and bronchiolitis obliterans that is due to antibody production and depostion, blockade of ICOS in mice with active chronic GVHD restored pulmonary function to the level seen in no-GVHD controls.[137] In that setting, ICOS/L blockade resulted in a significant reduction in germinal center formation, essential for chronic GVHD generation in that model system.

Whether these encouraging results in mice and canine models will be translatable to the clinic is unclear. Indeed, a recent study showed that in a NHP renal transplant model, ICOS blockade did not prolong allograft survival, either as a monotherapy or when combined with belatacept.[138] However, combination blockade of this pathway with other potential partners was not tested in this study, nor was a comprehensive examination of its impact on diseases that are more reliant on GC formation. The mixed results notwithstanding, an anti-ICOSL antibody (AMG-557) is currently in clinical trials for lupus (Clinicaltrials.gov # NCT01683695 and NCT00774943), and as such, a direct test of the impact of ICOS pathway blockade in patients is being undertaken.

6 | TARGETING OX40:OX40L: AN ADDITIONAL AVENUE FOR THE 'RIGHT' TARGETING OF TCONV AND TREGS?

Given the complexities of the impact that co-signaling pathway blockade makes on both conventional and regulatory T-cell populations, the search for the ideal therapeutic continues in the fields of both autoimmunity and transplantation. New studies are identifying a role for OX40 (CD134):OX40L (CD252) blockade in transplant immunomodulation, given that, like CD154 pathway blockade, it may be able to simultaneously control Tconv effector function while potentially permitting Treg homeostasis and function. It has been well documented that OX40 is upregulated on CD4»CD8 cells during T-cell activation.[139–142] In several murine acute GVHD models, OX40:OX40L blockade augmented survival associated with anti-host hyporesponsiveness with the major effects being seen on CD4^{+} T cell-mediated acute GVHD.[143–147] OX40 is also upregulated on human alloreactive T cells, and depletion of CD134^{+} T cells during an allogeneic mixed lymphocyte culture reaction with HLA mismatched stimulator cells dramatically reduced anti-host but not anti-third party alloresponses.[148]

In the context of solid organ transplantation, blockade of OX40:OX40L signals was shown to prevent allograft rejection in murine models, in a manner that is non-redundant with blockade of CD28 or CD154.[149] Of note, several of the murine solid organ allograft studies showed that OX40:OX40L blockade did not impact T-cell proliferation, but rather, inhibited T-cell survival, and thus resulted in fewer cells available to infiltrate and reject allografts.[141,142] Of significant interest is the fact that in contrast to the positive impact that OX40 ligation makes on Tconvs, Vu et al.[150] have shown that its ligation on Tregs results in the opposite effect, inhibiting Treg survival[142] and resulting in inhibition of their ability to suppress Tconv activation. These results have led to significant interest in the field, and experiments in NHP investigating the impact of OX40:OX40L are underway in models of both solid organ and HCT, which are documenting that targeting this pathway may be an important avenue for tolerance induction (V. Tkachev, D. Casson, P. Bland-Ward and L. S. Kean, unpublished results). If these effects in NHP are robust, OX40:OX40L blockade agents are poised to be one of the next agents in the clinical pipeline for controlling auto- and alloimmune activation.

7 | 4-1BB/4-1BBL BLOCKADE: MORE CENTRAL TO TUMOR IMMUNOLOGY THAN TRANSPLANT IMMUNOLOGY?

4-1BB has emerged as a major component of CAR-T cell engineering,[151,152] as a key intracellular signaling domain that can lead to long-term T-cell persistence in vivo after CAR-T cell adoptive transfer. However, the impact of blockade of this pathway on solid organ transplant outcomes is, in general, less striking. A member of the TNF family of receptors, 4-1BB (also known as CD137 and tnfrsf9), is expressed on activated Tconv (but not their naïve counterparts), Tregs,[153] as well as being expressed on B cells and dendritic cells.[154-157] An agonistic anti-4-1BB mAb amplified donor anti-host alloreactive T-cell responses, accelerating GVHD as well as cardiac and skin allograft rejection, associated with heightened CD8^{+} T-cell proliferation and IFNg response.[158] In other studies, both CD4^{+} and CD8^{+} T cell-mediated alloresponses were regulated by 4-1BB:4-1BBL interactions to approximately the same extent as assessed in acute GVHD and host bone marrow allograft rejection models,[159] and 4-1BB receptor-facilitated CD4^{+} T cell-mediated alloresponses were partly CD28 independent. Additionally, agonistic 4-1BB antibodies increased the graft-versus-leukemia effect of a suboptimal number of donor splenocytes given in a delayed fashion after bone marrow transplantation by bolstering allogeneic responses resulting in leukemia elimination. In other studies, host 4-1BBL deficiency reduced the capacity of the host to resist the GVHD lethality effect of delayed donor splenocyte infusion, which was used to simulate the clinical settings in which donor lymphocytes are given later post-transplant to treat or prevent leukemia relapse.[160] Consistent with these murine data, selective depletion of human alloreactive T cells after a mixed lymphocyte reaction was highly effective in reducing alloresponses.[161] In parent-into-F1 unirradiated GVHD models, blocking anti-CD137 mAb ameliorated acute GVHD, while unfortunately

enhancing chronic GVHD associated with reduced CD8^{+} T cells expansion and IFNg expression along with enhanced antibody production.[162]

In solid organ allograft models, using 4-1BBL-deficient recipients, skin allograft survival was not prolonged, and the combination of 4-1BB and CD28 deficiency only slightly delayed rejection kinetics.[163] These data suggest that 4-1BB was not the main pathway of escape in the absence of CD28 signaling. Despite the lack of striking effect of the genetic absence of 4-1BBL, blockade of the pathway has had positive impact on allograft survival in mice, prolonging corneal, intestinal, and cardiac allotransplants.[164-167] Signaling through 4-1BB and its ligand therefore clearly play a role in alloimmunity; however, its supporting role in this process has thus far hindered its pathway toward the clinic for control of T-cell activation, quite distinct from the central role it has played in CAR-mediated T-cell activation and subsequent tumor clearance.

8 | BLOCKADE OF T-CELL TRAFFICKING TO SECONDARY LYMPH NODES AND TARGET ORGANS AND TISSUES: IMPACT ON TRANSPLANT OUTCOMES AND INTERACTIONS WITH CO-SIGNALING BLOCKADE

One of the most critical steps in both organ transplant rejection and GVHD pathogenesis is the recruitment of activated T cells to target organs. As such, there is high interest in the impact that adhesion and chemokine blockade may have on both processes. Much of the work on adhesion blockade has been pioneered in autoimmune disease, where several targeted therapies have been investigated.[168-173] These include blockade of the lymphocyte function antigen-1 (LFA-1; CD11a) pathway, the alpha4 (CD49d), beta 1 (CD29) integrin very late antigen-4 (VLA-4) pathway, and the sphingosine-1-phosphate receptor (S1P1R) pathway that controls lymphocyte egress from lymph nodes and can be blocked by the S1P1R antagonist, FTY720.[174-196] While the predominance of the published data suggest important roles for both LFA-1 and VLA-4 pathway blockade in improving GVHD and graft acceptance,[176,177,197-199] the latter analyzed in murine, NHP, and patient trials, the significant risk of infectious disease with both these agents has limited their use in transplant settings.[184] Thus, while LFA-1 blockade has shown some promise in both murine and some, but not all NHP studies, and was also included in several islet transplant clinical protocols, the identification of the risk of progressive multi-focal leukoencephalopathy (PML) in patients treated with the anti-LFA-1 antibody efilizumab for autoimmune disease prompted it to be withdrawn from the market. Similarly, while preclinical efficacy was observed with the anti-VLA-4 antibody natalizumab in both autoimmune and transplant settings, it has also been associated with a significant risk of PML. Patient advocacy has retained its approval for MS, but, given this risk, there has been limited adoption for other indications, especially in settings where there are viable alternatives.

As with anti-LFA-1 and anti-VLA4, there is also evidence to suggest that FTY720 can prevent acute GVHD, and at least temporarily

prevent rejection as assessed in preclinical murine models of transplantation.[200–214] In murine acute GVHD systems, FTY720 has been shown to prevent GVHD lethality without impairing graft-versus-lymphoma or -leukemia effects. Mechanisms in these studies have been attributed to impairing lymphocyte migration, inducing donor anti-host alloreactive T-cell apoptosis, and reducing splenic CD11c^{+} cells along with T-cell responder frequencies.[205,208,212,214,215] In a chronic GVHD scleroderma model, FTY720 suppressed the immune response by promoting the expansion of Tregs and reducing vascular damage and infiltration of immune cells into the skin. In contrast to these positive results in GVHD in mice, in a large animal DLA non-identical acute GVHD model, FTY720 was ineffective in reducing acute GVHD, though the number of recipients studied was somewhat limited.[213] In the context of non-myeloablative transplants, FTY720 treatment in mice markedly but only transiently inhibited graft rejection due to the emigration of mature graft rejecting T cells from the host thymus upon drug withdrawal. However, when combined with tolerigenic anti-CD154 mAb, graft rejection did not occur upon drug withdrawal.[205] In the canine model, graft rejection rates did not differ from non-treated historical controls.[216] Although clinical studies with FTY720 have been completed and have resulted in drug approval for MS, and there was initially significant enthusiasm for it as a potential novel therapy to prevent GVHD and organ graft rejection, trials in renal transplant patients failed to show an advantage of this drug compared to standard therapy, despite encouraging early results. Thus, FTY720 is no longer being evaluated in this setting.

It should be noted that each of these three modifiers of T-cell trafficking have demonstrated more efficacy in autoimmune disorders than in transplant settings. It is still not clear what the mechanistic underpinnings of this observation are; however, one of the important observations in large animal models, similar to the murine model of suboptimal conditioning and bone marrow graft rejection discussed above, has been the rebound effect that occurs when drug was withdrawn. Thus, in both NHP renal[185,190] and bone marrow transplant (A. Page and L.S. Kean, unpublished), rejection occurred rapidly after withdrawal of anti-LFA1, ostensibly from alloreactive cells that were restrained from reaching their targets but not deleted or tolerized by the anti-integrin treatment. While patients treated long-term with these agents might benefit from the chronic inhibition of T-cell trafficking, this strategy would be expected to increase the cumulative risk of infectious toxicities.

As with the adhesion and egress inhibitors, there has been significant interest in blocking chemokine-chemokine receptor interactions to improve transplant outcomes. Of the many chemokines that could be targeted, CCR5 has been investigated most thoroughly, after having been shown to be a central molecular mediator of T-cell trafficking to GVHD target organs, including the GI tract.[217,218] In preclinical studies, CCR5 blockade has shown protection against GVHD in sublethally irradiated recipients, associated with reduced homing to the intestinal Peyer's patches in some models using knockout donor T cells,[217,219] and, somewhat paradoxically, acceleration of GVHD in heavily but not lightly irradiated recipients.[219,220] Based upon the positive data in murine non-myeloablated recipients, the CCR5 inhibitor

maraviroc (NCT00948753), an FDA-approved drug designed to limit CCR5-mediated HIV entry into CD4 T cells, was given for 1-month post-transplant, along with standard-of-care pharmacological agents, to high-risk patients receiving matched unrelated or one antigen mismatched unrelated donor grafts. Although acute GVHD of the skin was not eliminated, patients given maraviroc had a low incidence of GI and liver GVHD which constituted one of the first demonstrations of a target organ-specific effect of a biologic anti-GVHD prophylaxis regimen.[221] The durability of the impact of maroviroc on GVHD control is still undetermined, however, and there may be conditioning-regimen-specific effects in patients as seen in rodent models.[219] This agent is now being investigated as part of a large multicenter study sponsored by the BMT-CTN (Clinicaltrials.gov # NCT02208037), where issues of conditioning and durability of the GVHD protective effect will be investigated more thoroughly.

9 | LFA-3 (CD58): CD2 BLOCKADE: A MISSED OPPORTUNITY?

In the last several years, there have been multiple demonstrations of the potential importance of targeting LFA3:CD2 with an LFA-3/Fc fusion protein to control T-cell (and NK cell) activation and effector function, with several important lines of evidence suggesting that it might preferentially target memory rather than naïve T cells. These have included in vivo studies of antiCD2 mAb in rodent xenogenic islet and allogeneic cardiac transplant models,[222] as well as in vitro studies of the human alloresponse,[223] and translational studies in NHP demonstrating a role for CD2 blockade in renal transplant.[224] The clinical benefits of anti-CD2 mAb could be seen in studies in both bone marrow transplant[225] and Type I diabetes[226,227] indicating the potential importance of this pathway in controlling T cell-mediated disease. However, although alefacept is currently FDA approved for the treatment of psoriasis, the parent company for the marketed version of the drug decided to discontinue marketing this drug in 2011 for financial reasons, making future clinical applications uncertain. Given the wide array of indications in which this agent may have significant activity, and its potentially unique predisposition for memory, rather than naïve T cells, this likely represents a missed opportunity, and one that the transplant and autoimmune communities would welcome revisiting.

10 | THE CENTRAL ROLE OF TREGS IN TRANSPLANT TOLERANCE

In solid organ transplant models, it is clear that achieving long-term allograft survival and/or tolerance through the manipulation of co-signaling pathways is associated with an expansion of Tregs.[228] What is less clear, however, is whether this is a specific result of blocking co-stimulatory pathways per se, or rather, a secondary effect of antigen presentation in an environment where inflammation has been deliberately suppressed, promoting the expansion of pre-existing Tregs

(tTregs) and the induction of de novo induced pTregs. Obviously, these are not mutually exclusive hypotheses; however, there is some evidence in favor of the latter. For example, recent studies indicate that CD28 is an important homeostatic survival signal for Tregs[229] and thus CTLA4Ig would interfere with this survival signal. We suggest that promotion of Tregs in the setting of co-stimulatory blockade likely is an indirect effect of inhibition of inflammation, which promotes TGF-b. In addition, inflammatory cytokines are able to activate the PI-3 kinase pathway in T cells, which, when unopposed, inhibits Treg differentiation and promotes destabilization of pre-existing Tregs.[230] Thus, dampening inflammation may promote Tregs via multiple mechanisms. In BMT, it is increasingly clear that Tregs can play a major role in controlling unwanted alloreactivity, and can function both to prevent acute GVHD and treat chronic GVHD. Thus, our group and others have shown that Treg adoptive transfer early after transplant is safe, feasible, and has efficacy in reducing acute GVHD, both in murine models[231–233] and in patient trials.[234–243] Important studies have also shown that adoptive transfer of these cells can also function to promote engraftment[244] and do not impair GVL.[245–248] These data underscore the central role that Tregs play in both establishing and maintaining successful engraftment, and in restraining alloreactivity. Thus, in an era of manipulation of T-cell co-signaling with targeted therapies, choosing those that 'walk the fine line' of restraining effector T-cell activation while supporting Treg reconstitution and function will likely be necessary in order to achieve immune tolerance after both solid organ and HCT.

11 | THE KEY ROLE OF MEMORY T CELLS IN ESCAPING CO-STIMULATORY PATHWAY BLOCKADE AND APPROACHES TO PREVENT TMEMORY CELL ESCAPE

As noted above, CD28 is required for optimal activation, function, and survival of naïve T cells, with a particularly important role in CD4 T-cell function. However in a number of studies (albeit not all), memory T cells have been shown to be relatively independent of CD28 co-stimulation.[249] Indeed, mouse models of solid or tissue transplantation have clearly demonstrated that co-stimulatory blockade is relatively ineffective in animals harboring pre-existing anti-donor-specific T cells.[250] This presents a particularly problematic clinical situation as it can be anticipated that a large number of patients, even without evidence of prior antigenic exposure or preexisting anti-donor humoral immunity, will have memory T cells capable of recognizing donor antigen. This is likely due to a variety of factors, most prominently heterologous immunity[251,252] and the frequent expression of more than one TCRab heterodimer on human T cells[253] (the latter implying that a T cell with anti-donor alloantigen specificity based on one TCR might have memory phenotype/function acquired due to prior exposure to the antigen recognized by its second TCR).

Studies to overcome pre-existing memory have focused on non-CD28-mediated pathways. Preclinical studies in mice suggest that OX-40 may be a useful target,[254] and data in non-human primates points

to CD2 as a potential useful target for memory T cells.[224] As noted above, this has been supported by an early-phase clinical study in type I diabetes suggesting that using LFA3Ig (a soluble CD2-ligand) may deplete Teffector/memory cells and/or promote T-cell exhaustion.[226]

12 | THE CO-INHIBITORY PATHWAYS: TURN THEM OFF TO FIGHT CANCER, TURN THEM ON TO FIGHT REJECTION AND GVHD

The significant success in re-awakening anti-tumor T-cell responses through checkpoint blockade has opened up new treatment possibilities for patients for whom conventional chemotherapeutic approaches are inadequate. In the clinic, these approaches have focused on blockade of two prominent inhibitory pathways: CTLA-4 and PD-1 (programmed death-1). Given the success of blockade of both of these pathways in activating T-cell immunity, there has been much interest in activating these pathways for tolerance induction. We have discussed the CTLA4 pathway at length above, which plays a central role in the function of both Teffs and Tregs. Unfortunately, an agonistic reagent that specifically activates CTLA-4 is not available in the clinic; therefore, agonism of this pathway for immune tolerance induction is not currently a viable clinical goal.

The other behemoth of the checkpoint blockade pathways for cancer immunotherapy is the PD-1 pathway. PD-1 was described as a novel Ig supergene family member by Professor Tatsuku Honjo in 1992.[255] PD-1 is expressed on dendritic cells, NK, NKT cells, and on activated T cells. In BALB/c mice, PD-1 deficiency can result in autoimmune cardiomyopathy, consistent with its negative regulatory function.[256] The first identification of the critical importance of this pathway in antigen-responsive T cells in vivo was made by Dr. Rafi Ahmed's group, which showed that PD-1 is upregulated on exhausted T cells that accumulate during chronic infection.[257] Further studies showed that working through both immunoreceptor tyrosine-based inhibitory and switch motifs, PD-1 recruits SHP phosphatases leading to the inactivation of multiple T-cell signaling pathways.[258]

Experiments with knockout mice for PD-1 and one of its ligands, PD-L1, as well as with PD-1 and PD-L1 blocking antibodies, demonstrated the necessity of this pathway for co-stimulation blockade-mediated allograft acceptance.[259–261] Additionally, acute GVHD studies indicate a major role for PD-1 in downregulating lethality, as shown with PD-1 KO donor T cells and anti-PD-1 blocking mAbs, which were demonstrated to function via an IFNg-dependent mechanism.[262–264] In addition, in a sublethally irradiated minor antigen mismatched model of sclerodermatous chronic GVHD, blockade of the PD-1 pathway worsened disease.[265] These data reinforce the central role that PD-1 pathways play in T-cell activation. While this has yielded several important new drugs to augment T-cell responses by blocking this pathway, unfortunately, no agonists of PD-1 have yet been developed to inhibit T-cell auto- or alloactivation, and thus it is not possible to manipulate this pathway clinically to improve solid organ or bone marrow transplant outcomes.

13 | PD-L1 VS PD-L2: PREDOMINANCE OF PD-L1 DUE TO MORE PROMISCUOUS TISSUE EXPRESSION?

In its negative regulatory functionality, PD-1 is known to interact with both PD-L1 and PD-L2, and the relative importance of these ligands has been a matter of significant investigation. PD-L1 (B7-H1, CD274) is expressed constitutively on hematopoietic cells and non-hematopoietic cells and upregulates after activation. PD-L2 (B7-DC, CD273) expression is restricted primarily to DCs, macrophages, and cultured bone marrow-derived mast cells. The broad PD-L1 expression suggests an important role in inhibiting immune responses in lymphoid and non-lymphoid organs.

Using a TCR transgenic system, we demonstrated that only blockade of PD-L1, and neither PD-1 nor PD-L2 abrogated skin graft tolerance in a transplant model with MHC Class II disparity.[266] In the same study, we found that in an adoptive transfer model, both PD-1 and PD-L1 were critical early after transfer, with PD-L1 becoming the predominant inhibitor of T-cell alloproliferation as time progressed. This effect was dependent on the presence of CD4$^+$ Tregs, suggesting that a multicellular mechanism of transplant acceptance may be in play, with both Teffs and Tregs necessary for the ultimate control of alloreactivity. The prominence of PD-L1 signaling in a model of Class II-mediated alloreactivity was also demonstrated by Yang et al., who found that in Class II-disparate cardiac allograft model, signaling through PD-L1 was critical for allograft acceptance, but blockade of neither PD-1 nor PD-L2 had a negative impact on engraftment.[267,268] Moreover, PD-L1 was found to be critical for the induction of Tregs.[269] In acute GVHD studies, PD-L1 has been shown to be the dominant host PD-L1 ligand that controls apoptosis of donor T cells, Teff expansion, function, and metabolism.[270] This is also true when delayed lymphocyte infusions are given. The expression on non-hematopoietic cells likely substantially contributes to this dominance, resulting in a markedly diminished GVL effect after delayed lymphocyte infusion.[264,271,272] Recent studies also indicate that PD-L1 expression on donor T cells regulates acute GVHD lethality, further complicating interpretation of the use of anti-PD-L1 mAbs.[273] Indeed, in an autoimmune-like minor histocompatibility antigen mismatched GVHD model, host APC PD-L1 reacting with CD80, was shown to augment donor Treg survival and expansion, suppressing GVHD.[274,275]

Another potential explanation for the predominance of PD-L1 signaling in controlling allograft acceptance came from the discovery that PDL1 (and not PD-L2) can interact with CD80 in addition to PD1.[276] In acute GVHD, PD-L1/CD80 interactions induce apoptosis of donor T cells in a PD-1-dependent manner, resulting in amelioration of GVHD. Similarly, PD-L1/CD80 interactions can control diabetogenic Teffs. Thus, PDL1 may provide a parallel track for the control of alloreactivity that renders it as a central player in allograft survival. Evidence for this role was further strengthened by the identification of a monoclonal antibody that specifically targets the PDL1:CD80 binding motif, providing a structural basis for the previously identified biologic activity.[268]

14 | THE DOWNSIDE OF BLOCKING CO-INHIBITORY RECEPTORS: UNWANTED T-CELL ACTIVATION, GVHD, AUTOIMMUNITY

While this review is focused on manipulation of co-stimulatory and co-inhibitory molecules to control rather than stimulate T-cell function, it is important to consider the impact of these agents on both auto- and alloimmunity, as patients at risk for both these processes are now being treated with checkpoint blockade agents. There have now been several reports of immune activation syndrome in patients treated with both anti-CTLA-4 (ipilimumab) and anti-PD-1 (novolumab). These include evidence for pulmonary, skin, cardiac, hepatic, GI, and bone marrow disease.[277–282] The range of clinical severity of these diseases is large, and unfortunately includes some patients who have required extensive hospitalization and treatment with broad-acting immunosuppressive drugs, with patient death occurring in some instances. However, there are also numerous reports of patients with de novo inflammation or flare of known autoimmune disease being successfully treated with conventional immunosuppression while continuing to receive checkpoint blockade. Indeed, early-phase data showed that administration of ipilimumab was feasible in patients with recurrent hematologic cancers after allogeneic HCT, and resulted in durable responses, especially of extramedullary acute myeloid leukemia, although immune-mediated toxic effects and GVHD occurred.[283] In other studies, PD-1 pathway blocking reagents have been used to treat relapse post-allo-HCT, although early data do point to higher acute GVHD incidence and severity in treated patients.[284] It is still not clear what the impact of combining these two opposing therapies will be on tumor biology, as a large enough study has not yet been undertaken, but, at least in melanoma and small cell lung cancer, pre-existing autoimmunity is not considered an absolute contraindication for treatment with checkpoint blockade.

The issue of intercurrent alloactivation during checkpoint blockade or CAR-T therapy is also critical to the design of relapse-prevention strategies for patients that have undergone allogeneic BMT. In these patients, there is concern about the risk of induction or exacerbation of GVHD with checkpoint blockade, and it is not currently offered clinically. Although murine studies have suggested that GVHD can occur with allogeneic CAR therapy in the setting of transplant,[285] this has not been a major problem in patients, and many post-allo-BMT patients are treated with CAR-T cells.[286] However, there is concern with giving CAR-T cells in post-transplant patients who are being treated for GVHD, and this is not currently standard practice, given the concern of exacerbating GVHD with these therapies. There is also a concern for negatively impacting the efficacy of CAR-T cells when patients are receiving treatment with steroids and other agents to control GVHD. Engineering CAR-T cells to be resistant to these agents may increase the application of this therapy post-transplant. However, given the potential hazard of having a T-cell population that cannot be controlled by conventional immunosuppression,[287] having a suicide trigger engineered into these T cells is considered a necessary safety criteria.[288,289]

15 | CO-INHIBITORY LIGANDS OTHER THAN CTLA-4 AND PD-1

In addition to CTLA-4 and PD1⁻, another of the major co-inhibitory molecules, LAG-3, has also become a major target for checkpoint blockade, with trials of anti-LAG-3 antibodies in glioblastoma, other solid tumors as well as hematologic malignancies.[290–292] In immune tolerance studies, its expression on induced Tregs is upregulated by exposure to IL-27 and is critical for the optimal function of these cells in controlling autoimmune diseases.[292,293] In acute GVHD, studies have shown that LAG-3 KO Tconv induce significantly more severe GVHD than wildtype Tconv and that the absence of LAG-3 on CD4 but not CD8 T cells is responsible for worsening GVHD.[294] Notably, Tregs from LAG-3 KO mice were functionally intact. While there is significant interest in modulating LAG-3 to enhance control of both auto- and alloimmunity, no targeted agents to upregulate this molecule yet exist.

More limited data exist for B7-H3 (CD276), a B7 family member overexpressed in a variety of solid tumors, which can be associated with poor prognosis. In allograft rejection, different studies have demonstrated either the promotion of rejection or prolongation of allograft survival by B7-H3 suppression of Th1 responses.[295,296] In acute GVHD, B7-H3 expression by donor and host cells negatively regulates GVHD lethality.[297] Similar to LAG3-3, clinical agonistic reagents are not available. Notably, both resting and activated murine Tregs were found to lack B7-H3 messenger RNA.[297,298]

Similar to B7-H3, B7-H4 is another B7 family member that is a negative regulator of T-cell responses. This molecule is expressed on tumors and can be associated with a poor prognosis. Although GVHD studies have not been reported to date, B7-H4 expression has been shown in multiple studies to prolong islet allografts and in CD28-deficient recipients, B7-H4 blockade resulted in accelerated cardiac allograft rejection associated with increased frequencies of IFNg, IL4, and granzyme B-producing splenocytes.[299] Moreover, the efficacy of CTLA4Ig in promoting allograft survival was diminished in mice that did not have an intact B7-H4 pathway. B7-H4 agonistic reagents are not clinically available at this time.

2B4 (CD244, SLAMf4) is also a central co-inhibitory molecule. Although it was originally identified on NK cells, it is clear that 2B4 is upregulated on activated T cells.[300–303] While delineation of the major function of 2B4 has proven complex (and has included its associated with both T-cell activation and inhibition), studies from Dr. Arlene Sharpe's laboratory,[304] and from Dr. Mandy Ford and others provide strong evidence for its role as a co-inhibitor of CD8⁺ T-cell alloresponses in vivo.[72,305] Although the 2B4 pathway has not yet been harnessed for the promotion of tumor immunity, these studies suggest that it may be a viable candidate, especially in combination with either PD-1 or CTLA-4 blockade.

T-cell immunoglobulin mucin-3 (TIM-3) was also found to be a critical player in transplantation tolerance when the Kuchroo laboratory demonstrated that anti-TIM3 blocking antibodies prevented transplant tolerance by interfering with Treg function.[306,307] TIM-3 is expressed on pathogenic T cells in acute GVHD and its ligand, galectin-9 is upregulated in inflamed tissues.[308] Tim-3/galectin-9 blockade accelerates acute GVHD, while conversely, galectin-9 transgenic recipients have demonstrated reduced acute GVHD. Pardoxically, TIM-3/galectin-9 blockade in recipients of Treg-depleted grafts reduced GVHD due to activation-induced Teff cell death as a result of increased IFNg production. In skin allograft recipients, activation of the TIM-3/galectin-9 pathway negatively regulates CD8⁺ alloreactive T cells and prolongs allograft survival,[309,310] a finding also observed with cardiac allografts.[311,312] TIM3⁺ Tregs, which are frequently PD-1⁺, were found to be a short-lived Treg subset that can restrain skin allograft rejection responses.[313] Unlike CTLA-4 and PD-1, an agonistic reagent does exist for TIM3, with its ligand, Galectin-9 recently being shown to promote acceptance of solid organ allografts.[314–316] Thus, TIM3 represents a molecule of considerable interest for both the cancer immunotherapy and transplant tolerance fields, with promise as both immune modulator and immune stimulator.

16 | THE SPECIAL ROLE OF LARGE ANIMAL MODELS IN UNDERSTANDING CO-SIGNALING IN TRANSPLANTATION

Most of the pathways and molecules involved in T-cell co-signaling have been first identified in murine models, using both genetic knockout technologies and antibody- and small molecule-based blockade. While these murine studies have been absolutely critical to the progress in the field, canine models[135,213,317–322] and NHP models[323] have been central to the translation of co-stimulation blockade to the clinic, in both solid organ and bone marrow transplant. The best example of this is with the FDA approval of belatacept for clinical use in renal transplantation. In the path to the clinic, Bristol-Myers Squibb closely collaborated with academic investigators to first design the higher avidity version of CTLA4Ig that belatacept represents,[52] and then performed the key IND-enabling studies in NHP prior to launching pivotal clinical trials. These NHP studies closely predicted both the success of belatacept in being a true calcineurin-sparing agent (with all of the attendant advantages in terms of renal and cardiovascular function that accompanies a calcineurin-free regimen) and also predicted the belatacept-resistant rejection that has been observed clinically. For belatacept, the cycle of murine→→ NHP→→ patient and back to murine and NHP studies has been realized, with the detailed investigation of belatacept-resistant rejection being vigorously pursued in both murine and NHP models. While a clear solution to this issue has not yet been identified, ongoing studies in mice and NHP focused on identifying the causative mechanisms, and using a range of agents designed to overcome these early rejection responses is underway.

The utility of NHP models in moving co-stimulation blockade to the clinic has also been demonstrated in the field of BMT. While the success of both abatacept in RA and belatacept in renal transplant should have predicted the utility of these approaches in bone marrow transplant (along with the murine data that supported a role for this pathway in the pathogenesis of acute GVHD),[32–37] until the

demonstration of the activity of CTLA4-Ig in preventing alloreactivity in a canine[318] an NHP model,[33] there was hesitation in the field to use in vivo co-stimulation blockade as a novel approach for GVHD prevention (There had been one trial of ex vivo exposure of bone marrow products to either CTLA4Ig or anti-B7 antibodies,[324] but this was not followed with significant adoption by the BMT field.) The demonstration in NHP that combination co-stimulation blockade could significantly protect recipients from GVHD provided the necessary rationale for clinical translation of this strategy to patients.[38] We are now completing a large Phase II study (Clinicaltrials.gov# NCT01743131) designed to determine both the benefits and risks of the addition of abatacept to standard-of-care GVHD prophylaxis in both adult and pediatric transplant patients.

Importantly, while NHP studies proved critical in identifying both the efficacy and the breakthrough rejection of belatacept in renal transplantation, not all clinical toxicities have been correctly identified in NHP studies. A major example of this is the fact that studies in cynomolgus macaques, one of the most prevalent of the NHP models used, did not identify the risk of uncontrolled T-cell activation and cytokine storm that accompanied clinical use of the cross-linking anti-CD28 agonist targeting the C''D loop of CD28, TGN1412,[68] likely due to the lack of expression of CD28 on CD4$^+$ effector cells in macaques, and its increased expression on the human counterpart.[325–327] Vanhove et al.[328] have subsequently shown that baboons represent a much more predictive NHP model for testing the toxicity of reagents targeting CD28, and used this model to provide compelling data for the safety of their single-chain anti-CD28 reagent, FR104. They have gone on to demonstrate the potential efficacy of this therapeutic (originally developed by Effimune, now licensed to Janssen) in a number of NHP models of transplantation,[76–81,328] and it has now completed Phase I trials in RA (Clinialtrials.gov # NCT02800811). BMS has also developed a similar single-chain anti-CD28 antibody, 'Lulizumab', which has also been moved into early-phase clinical trials for Sjogrens syndrome and lupus (Clinicaltrials.gov # NCT02843659 and NCT02265744).

The importance (and also the limitations of NHP models) in facilitating the translation of new therapeutics to the clinic have also been demonstrated with antiCD154 and anti-CD40 antibodies. In murine tolerance models, strategies targeting CD154 have long been demonstrated to be among the most potently tolerogenic, for both solid organ and BMT applications. These striking results in mice were recapitulated in NHP models,[104,329–331] where anti-CD154 antibodies were found to be critical components of calcineurin-sparing regimens for many solid organ transplant models as well as for bone marrow chimerism-induction. Given the many positive attributes of targeting this pathway, both in terms of its impact on effector cells as well as on Tregs, there was much enthusiasm for translation to the clinic, and two trials were opened, one in renal transplant (Clinicaltrials.gov # NCT00001857) and one for patients with lupus neprhritis.[332] However, both clinical trials were halted shortly after their initiation, given an unusually high rate of thromboembolic complications in treated patients.

Subsequently, Kawai et al.[109] reported a new regimen that incorporated antiCD154 antibodies in NHP that also demonstrated the thromboembolic events; however, many previous studies had failed to identify this complication. The combined lessons with anti-CD28 and anti-CD154 have pushed the field of NHP transplantation research to evolve more stringent practices for evaluating the potential toxicity and efficacy of therapeutics prior to clinical translation, and the road to the clinic through primate investigation has continued to be robust.

Following the identification of the thrombosis risk with anti-CD154, there was a significant push to develop and evaluate anti-CD40 reagents in NHP for use in transplantation, and these studies suggested that targeting CD40 had significant efficacy, on par with that observed for CD154-directed therapies. These NHP results are now being translated to the clinic, with the first anti-CD40 antibodies being tested for psoriasis and renal transplantation (clinicaltrials.gov # NCT01585233, NCT01780844 and NCT02217410). In addition, the thromboembolic complications with the conventional anti-CD154 antibodies have now been overcome, with domain-specific, single-chain antibodies (rigorously determined to not induce thrombosis), having been developed. These are being re-introduced into the fields of both autoimmunity and transplantation with high hopes for significant efficacy without toxicities.

The experience with both anti-CD28 and anti-CD154 antibodies have reinforced the contention that no animal model is a perfect surrogate for carefully conducted clinical trials. However, the strengths of NHP models have been critical for the streamlining of translation of co-stimulation blockade to the clinic for autoimmunity and transplantation. These studies permit combination therapies that are more difficult to interrogate in murine models, given significant differences in pharmacokinetics and in receptor:ligand expression patterns in mice compared to humans. They also permit the direct evaluation of reagents that are developed for human use, which usually cross-react with NHP targets but rarely do so with their murine counterparts. Finally they permit intensive monitoring of both the blood and transplant target organs (eg, kidney, heart, skin, GI tract) such that both the peripheral and tissue-specific immunologic consequences of transplant and novel therapeutic strategies can be fully assessed. This is essential for both the evaluation of safety and efficacy, with the proviso that careful attention be paid to any species-specific heterogeneity of target expression, as occurred with TGN1412.

In order to maximize their utility for clinical transplantation, the field of NHP experimentation has greatly benefited from recent advances that help assure that the immune milieu of these transplants is understood in the greatest detail possible. One significant example is the establishment of a new 'gold standard' in transplant studies, where the degree of MHC disparity between donors and recipients is now expected to be defined, and equivalent among different treatments in any transplant series.[102,333] Until 2010, this degree of information about the MHC was not included in transplant planning, which opened up these studies to the potential confounder of serendipitously close MHC matching leading to transplant outcomes that were more successful than they otherwise would have been (or vice versa with highly disparate transplant pairs).

Our group and others created a new paradigm in NHP transplant planning by applying first microsatellite-[102,333] and now

MHC-allele-level typing to multiple NHP colonies,[334] with the NIAID providing critical support for this effort, by funding the detailed MHC typing of their sponsored macaque colony. In addition to the rigorous MHC matching that is now possible in NHP transplant experiments, the detailed annotation of the rhesus transcriptome[335,336] has allowed a systems approach to understanding the impact that new therapies make on the T-cell transcriptional networks controlling transplant outcomes. This has led to new insights about the breakthrough allo-reactivity[336] that occurs with both standard and CTLA4Ig-based immunomodulation for GVHD prevention, as well as the identification of new potential targeted therapies to control the T-cell activation that leads to GVHD.[337] These advances have helped solidify the depth of analysis that is possible with NHP models of T-cell co-signaling, thereby helping to optimize the ability of these models to most efficiently advance novel targeted therapeutics to the clinic.

17 | CONCLUSIONS AND PERSPECTIVES

In this review, we have discussed the co-signaling pathways that lead to T-cell activation. However, our focus has not been on controlling these pathways in order to activate T cells, or awaken them from senescence (what one might call harnessing the 'Yang' in T cells) but rather, we have concentrated on the field of immune tolerance, a field that is focused on the Yin, or the specific 'cooling' of T cells, to control T-cell activation and to lead to lifelong acceptance of allografts, without the need for ongoing immunosuppression. This work has its philosophical underpinnings in the landmark work of Billingham, Brent, and Medawar, over 60 years ago, which introduced the concept that under some conditions, lifelong acceptance of foreign cells and tissues could occur.[338] As we have learned more about the complex molecular networks that control T-cell activation, and T-cell tolerance, two things have happened:

First, we are more and more able to manipulate T-cell programming to achieve our goals (whether they be T-cell activation or T-cell tolerance) and second, we are increasingly aware of the extremely delicate balance that exists, that makes these manipulations extremely difficult to fully control. This has been shown in both the fields of cancer immunotherapy and in immune tolerance work. In cancer immunotherapy, we have seen toxicities directly attributable to our unleashing of T-cell activation in a manner that has many fewer brakes than exist in the natural setting. In immune tolerance, we have seen the consequences of 'over-cooling' of T-cell immunity, which can lead to defects in protective immunity against both infectious pathogens and tumor cells. Moreover, our increasing awareness of the impact of manipulating T-cell co-signaling pharmacologically, affecting both effector and regulatory T cells, has led to an understanding that we must induce immune tolerance on a 'knifes-edge': We must seek to understand in exquisite detail the complex systems that control T-cell programming such that we can simultaneously induce tolerance in all T-cell subpopulations, both effector and regulatory. The ongoing work in murine NHP and patient trials are geared toward just this goal: understanding the programming that naturally induces tolerance, and recapitulating this with targeted therapies such that we induce just the right amount of Yin for our patients undergoing transplantation.

CONFLICTS OF INTEREST

B. R. B. has the following conflicts to declare: He is a founder of Tmunity Therapeutics, Inc. He receives funding from Tmunity Therapeutics Inc, Kadmon Corporation and Nektar Therapeutics. L. S. K. receives research funding from Bristol-Myers Squibb and Kymab Ltd. L. A. T. has financial interests in Third Rock Ventures and Neon Therapeutics.

REFERENCES

1. Lin H, Bolling SF, Linsley PS, et al. Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4Ig plus donor-specific transfusion. *J Exp Med.* 1993;178:1801–1806.
2. Linsley PS, Wallace PM, Johnson J, et al. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. *Science.* 1992;257:792–795.
3. Lenschow DJ, Zeng Y, Thistlethwaite JR, et al. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science.* 1992;257:789–792.
4. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. *J Exp Med.* 1991;174:561–569.
5. Larsen CP, Elwood ET, Alexander DZ, et al. Long-term acceptance of skin and cardiac allografts after blocking CD40 and CD28 pathways. *Nature.* 1996;381:434–438.
6. June CH, Ledbetter JA, Linsley PS, Thompson CB. Role of the CD28 receptor in T-cell activation. *Immunol Today.* 1990;11:211–216.
7. Linsley PS, Clark EA, Ledbetter JA. T-cell antigen CD28 mediates adhesion with B cells by interacting with activation antigen B7/BB-1. *Proc Natl Acad Sci USA.* 1990;87:5031–5035.
8. Freeman GJ, Freedman AS, Segil JM, Lee G, Whitman JF, Nadler LM. B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J Immunol.* 1989;143:2714–2722.
9. Gimmi CD, Freeman GJ, Gribben JG, et al. B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc Natl Acad Sci USA.* 1991;88:6575–6579.
10. Freeman GJ, Gribben JG, Boussiotis VA, et al. Cloning of B7-2: A CTLA-4 counter-receptor that costimulates human T cell proliferation. *Science.* 1993;262:909–911.
11. Lenschow DJ, Su GH, Zuckerman LA, et al. Expression and functional significance of an additional ligand for CTLA-4. *Proc Natl Acad Sci USA.* 1993;90:11054–11058.
12. Peach RJ, Bajorath J, Brady W, et al. Complementarity determining region 1 (CDR1)- and CDR3-analogous regions in CTLA-4 and CD28 determine the binding to B7-1. *J Exp Med.* 1994;180:2049–2058.
13. Brunet JF, Denizot F, Luciani MF, et al. A new member of the immunoglobulin superfamily–CTLA-4. *Nature.* 1987;328:267–270.
14. Harper K, Balzano C, Rouvier E, Mattei MG, Luciani MF, Golstein P. CTLA-4 and CD28 activated lymphocyte molecules are closely related in both mouse and human as to sequence, message

expression, gene structure, and chromosomal location. *J Immunol*. 1991;147:1037–1044.

15. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity*. 1994;1:405–413.

16. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*. 1995;182:459–465.

17. Turka LA, Linsley PS, Lin H, et al. T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection in vivo. *Proc Natl Acad Sci USA*. 1992;89:11102–11105.

18. Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. *Science*. 1994;265:1225–1227.

19. Knoerzer DB, Karr RW, Schwartz BD, Mengle-Gaw LJ. Collagen-induced arthritis in the BB rat. Prevention of disease by treatment with CTLA-4-Ig. *J Clin Invest*. 1995;96:987–993.

20. Racke MK, Scott DE, Quigley L, et al. Distinct roles for B7-1 (CD-80) and B7-2 (CD-86) in the initiation of experimental allergic encephalomyelitis. *J Clin Invest*. 1995;96:2195–2203.

21. Abrams JR, Lebwohl MG, Guzzo CA, et al. CTLA4Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest*. 1999;103:1243–1252.

22. Moreland LW, Alten R, Van den Bosch F, et al. Costimulatory blockade in patients with rheumatoid arthritis: A pilot, dose-finding, double-blind, placebo-controlled clinical trial evaluating CTLA-4Ig and LEA29Y eighty-five days after the first infusion. *Arthritis Rheum*. 2002;46:1470–1479.

23. Kremer JM, Westhovens R, Leon M, et al. Treatment of rheumatoid arthritis by selective inhibition of T-cell activation with fusion protein CTLA4Ig. *N Engl J Med*. 2003;349:1907–1915.

24. Genovese MC, Becker JC, Schiff M, et al. Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N Engl J Med*. 2005;353:1114–1123.

25. Kremer JM, Dougados M, Emery P, et al. Treatment of rheumatoid arthritis with the selective costimulation modulator abatacept: Twelve-month results of a phase iib, double-blind, randomized, placebo-controlled trial. *Arthritis Rheum*. 2005;52:2263–2271.

26. Kremer JM, Genant HK, Moreland LW, et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: A randomized trial. *Ann Intern Med*. 2006;144:865–876.

27. Viglietta V, Bourcier K, Buckle GJ, et al. CTLA4Ig treatment in patients with multiple sclerosis: An open-label, phase 1 clinical trial. *Neurology*. 2008;71:917–924.

28. Parulekar AD, Boomer JS, Patterson BM, et al. A randomized controlled trial to evaluate inhibition of T-cell costimulation in allergen-induced airway inflammation. *Am J Respir Crit Care Med*. 2013;187:494–501.

29. Orban T, Bundy B, Becker DJ, et al. Type 1 Diabetes TrialNet Abatacept Study Group. Co-stimulation modulation with abatacept in patients with recentonset type 1 diabetes: A randomised, double-blind, placebo-controlled trial. *Lancet*. 2011;378:412–419.

30. Sandborn WJ, Colombel JF, Sands BE, et al. Abatacept for Crohn's disease and ulcerative colitis. *Gastroenterology*. 2012;143(62–9):e4.

31. Merrill JT, Burgos-Vargas R, Westhovens R, et al. The efficacy and safety of abatacept in patients with non-life-threatening manifestations of systemic lupus erythematosus: Results of a twelve-month, multicenter, exploratory, phase IIb, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum*. 2010;62:3077–3087.

32. Blazar BR, Taylor PA, Linsley PS, Vallera DA. In vivo blockade of CD28/CTLA4: B7/BB1 interaction with CTLA4-Ig reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice. *Blood*. 1994;83:3815–3825.

33. Miller WP, Srinivasan S, Panoskaltsis-Mortari A, et al. GVHD after haploidentical transplantation: A novel, MHC-defined rhesus macaque model identifies CD28⁻ CD8⁺ T cells as a reservoir of breakthrough T-cell proliferation during costimulation blockade and sirolimus-based immunosuppression. *Blood*. 2010;116:5403–5418.

34. Saito K, Sakurai J, Ohata J, et al. Involvement of CD40 ligand-CD40 and CTLA4-B7 pathways in murine acute graft-versus-host disease induced by allogeneic T cells lacking CD28. *J Immunol*. 1998;160:4225–4231.

35. Hakim FT, Cepeda R, Gray GS, June CH, Abe R. Acute graft-versus-host reaction can be aborted by blockade of costimulatory molecules. *J Immunol*. 1995;155:1757–1766.

36. Wallace PM, Johnson JS, MacMaster JF, Kennedy KA, Gladstone P, Linsley PS. CTLA4Ig treatment ameliorates the lethality of murine graft-versus-host disease across major histocompatibility complex barriers. *Transplantation*. 1994;58:602–610.

37. Via CS, Rus V, Nguyen P, Linsley P, Gause WC. Differential effect of CTLA4Ig on murine graft-versus-host disease (GVHD) development: CTLA4Ig prevents both acute and chronic GVHD development but reverses only chronic GVHD. *J Immunol*. 1996;157:4258–4267.

38. Koura DT, Horan JT, Langston AA, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: A first-in-disease trial. *Biol Blood Marrow Transplant*. 2013;19:1638–1649.

39. Pree I, Bigenzahn S, Fuchs D, et al. CTLA4Ig promotes the induction of hematopoietic chimerism and tolerance independently of Indoleamine-2,3-dioxygenase. *Transplantation*. 2007;83:663–667.

40. Blaha P, Bigenzahn S, Koporc Z, Sykes M, Muehlbacher F, Wekerle T. Shortterm immunosuppression facilitates induction of mixed chimerism and tolerance after bone marrow transplantation without cytoreductive conditioning. *Transplantation*. 2005;80:237–243.

41. Wekerle T, Kurtz J, Bigenzahn S, Takeuchi Y, Sykes M. Mechanisms of transplant tolerance induction using costimulatory blockade. *Curr Opin Immunol*. 2002;14:592–600.

42. Wekerle T, Kurtz J, Sayegh M, et al. Peripheral deletion after bone marrow transplantation with costimulatory blockade has features of both activation-induced cell death and passive cell death. *J Immunol*. 2001;166:2311–2316.

43. Wekerle T, Kurtz J, Ito H, et al. Allogeneic bone marrow transplantation with co-stimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nat Med*. 2000;6:464–469.

44. Wekerle T, Sayegh MH, Ito H, et al. Anti-CD154 or CTLA4Ig obviates the need for thymic irradiation in a non-myeloablative conditioning regimen for the induction of mixed hematopoietic chimerism and tolerance. *Transplantation*. 1999;68:1348–1355.

45. Wekerle T, Sayegh MH, Hill J, et al. Extrathymic T cell deletion and allogeneic stem cell engraftment induced with costimulatory blockade is followed by central T cell tolerance. *J Exp Med*. 1998;187:2037–2044.

46. Adams AB, Durham MM, Kean L, et al. Costimulation blockade, busulfan, and bone marrow promote titratable macrochimerism, induce transplantation tolerance, and correct genetic hemoglobinopathies with minimal myelosuppression. *J Immunol*. 2001;167: 1103–1111.

47. Adams AB, Pearson TC, Larsen CP. Conventional immunosuppression and costimulation blockade. *Philos Trans R Soc Lond B Biol Sci*. 2001;356:703–705.

48. Adams AB, Shirasugi N, Durham MM, et al. Calcineurin inhibitor-free CD28 blockade-based protocol protects allogeneic islets in nonhuman primates. *Diabetes*. 2002;51:265–270.

49. Durham MM, Bingaman AW, Adams AB, et al. Cutting edge: Administration of anti-CD40 ligand and donor bone marrow leads to hemopoietic chimerism and donor-specific tolerance without cytoreductive conditioning. *J Immunol*. 2000;165:1–4.

50. Kean LS, Durham MM, Adams AB, et al. A cure for murine sickle cell disease through stable mixed chimerism and tolerance induction after nonmyeloablative conditioning and major histocompatibility complex-mismatched bone marrow transplantation. *Blood*. 2002;99:1840–1849.

51. Kean LS, Manci EA, Perry J, et al. Chimerism and cure: Hematologic and pathologic correction of murine sickle cell disease. *Blood*. 2003;102:4582–4593.

52. Larsen CP, Pearson TC, Adams AB, et al. Rational development of LEA29Y (belatacept), a highaffinity variant of CTLA4-Ig with potent immunosuppressive properties. *Am J Transplant*. 2005;5:443–453.

53. Levisetti MG, Padrid PA, Szot GL, et al. Immunosuppressive effects of human CTLA4Ig in a non-human primate model of allogeneic pancreatic islet transplantation. *J Immunol*. 1997;159:5187–5191.

54. Vincenti F, Larsen C, Durrbach A, et al. Belatacept Study Group. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med*. 2005;353:770–781.

55. Vincenti F, Rostaing L, Grinyo J, et al. Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med*. 2016;374:333–343.

56. Rostaing L, Vincenti F, Grinyo J, et al. Long-term belatacept exposure maintains efficacy and safety at 5 years: Results from the long-term extension of the BENEFIT study. *Am J Transplant*. 2013;13:2875–2883.

57. Vanrenterghem Y, Bresnahan B, Campistol J, et al. Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies). *Transplantation*. 2011;91:976–983.

58. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity*. 1995;3:541–547.

59. Sansom DM. CD28, CTLA-4 and their ligands: Who does what and to whom? *Immunology*. 2000;101:169–177.

60. Schmidt EM, Wang CJ, Ryan GA, et al. Ctla-4 controls regulatory T cell peripheral homeostasis and is required for suppression of pancreatic islet autoimmunity. *J Immunol*. 2009;182:274–282.

61. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science*. 2008;322:271–275.

62. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science*. 1995;270:985–988.

63. Tang Q, Henriksen KJ, Boden EK, et al. Cutting edge: CD28 controls peripheral homeostasis of CD4$^+$CD25$^+$ regulatory T cells. *J Immunol*. 2003;171:3348–3352.

64. Zhang R, Huynh A, Whitcher G, Chang J, Maltzman JS, Turka LA. An obligate cell-intrinsic function for CD28 in Tregs. *J Clin Invest*. 2013;123:580–593.

65. Bluestone JA, Liu W, Yabu JM, et al. The effect of costimulatory and interleukin 2 receptor blockade on regulatory T cells in renal transplantation. *Am J Transplant*. 2008;8:2086–2096.

66. Riella LV, Liu T, Yang J, et al. Deleterious effect of CTLA4-Ig on a Treg-dependent transplant model. *Am J Transplant*. 2012;12:846–855.

67. Zheng HB, Watkins B, Tkachev V, et al. The knife's edge of tolerance: Inducing stable multilineage mixed chimerism but with a significant risk of CMV reactivation and disease in rhesus macaques. *Am J Transplant*. 2016; doi:10.1111/ajt.14006.

68. Suntharalingam G, Perry MR, Ward S, et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med*. 2006;355:1018–1028.

69. Hanke T. Lessons from TGN1412. *Lancet*. 2006;368:1569–1570; author reply 70.

70. Suchard SJ, Davis PM, Kansal S, et al. A monovalent anti-human CD28 domain antibody antagonist: Preclinical efficacy and safety. *J Immunol*. 2013;191:4599–4610.

71. Liu D, Suchard SJ, Nadler SG, Ford ML. Inhibition of donor-reactive CD8$^+$ T cell responses by selective CD28 blockade is independent of reduced ICOS expression. *PLoS ONE*. 2015;10:e0130490.

72. Liu D, Krummey SM, Badell IR, et al. Ford ML 2B4 (CD244) induced by selective CD28 blockade functionally regulates allograft-specific CD8$^+$ T cell responses. *J Exp Med*. 2014;211:297–311.

73. Shi R, Honczarenko M, Zhang S, et al. Pharmacokinetic, pharmacodynamic, and safety profile of a novel anti-CD28 domain antibody antagonist in healthy subjects. *J Clin Pharmacol*. 2016; doi:10.1002/jcph.791.

74. Yang Z, Wang H, Salcedo TW, et al. Integrated pharmacokinetic/pharmacodynamic analysis for determining the minimal anticipated biological effect level of a novel anti-CD28 receptor antagonist BMS-931699. *J Pharmacol Exp Ther*. 2015;355:506–515.

75. Mary C, Coulon F, Poirier N, et al. Antagonist properties of monoclonal antibodies targeting human CD28: Role of valency and the heavy-chain constant domain. *mAbs*. 2013;5:47–55.

76. Poirier N, Mary C, Dilek N, et al. Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody. *Am J Transplant*. 2012;12:2630–2640.

77. Poirier N, Dilek N, Mary C, et al. FR104, an antagonist anti-CD28 monovalent fab' antibody, prevents alloimmunization and allows calcineurin inhibitor minimization in nonhuman primate renal allograft. *Am J Transplant*. 2015;15:88–100.

78. Vierboom MP, Breedveld E, Kap YS, et al. Clinical efficacy of a new CD28-targeting antagonist of T cell co-stimulation in a nonhuman primate model of collagen-induced arthritis. *Clin Exp Immunol*. 2016;183:405–418.

79. Haanstra KG, Dijkman K, Bashir N, et al. Selective blockade of CD28-mediated T cell costimulation protects rhesus monkeys against acute fatal experimental autoimmune encephalomyelitis. *J Immunol*. 2015;194:1454–1466.

80. Ville S, Poirier N, Branchereau J, et al. Anti-CD28 antibody and belatacept exert differential effects on mechanisms of renal allograft rejection. *J Am Soc Nephrol*. 2016; doi:10.1681/ASN.2015070774.

81. Hippen KL, Watkins B, Tkachev V, et al. Preclinical testing of anti-human CD28 Fab' antibody in a novel nonhuman primate (NHP) small animal rodent model of xenogenic graft-versus-host disease (GVHD). *Transplantation*. 2016; doi:10.1097/TP.0000000000001465.

82. Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. *Immunol Rev*. 2009;229:152–172.

83. Pullen SS, Labadia ME, Ingraham RH, et al. High-affinity interactions of tumor necrosis factor receptorassociated factors (TRAFs) and CD40 require TRAF trimerization and CD40 multimerization. *Biochemistry*. 1999;38:10168–10177.

84. Foy TM, Shepherd DM, Durie FH, Aruffo A, Ledbetter JA, Noelle RJ. In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39. *J Exp Med*. 1993;178:1567–1575.

85. Van den Eertwegh AJ, Noelle RJ, Roy M, et al. In vivo CD40-gp39 interactions are essential for thymusdependent humoral immunity. I. In vivo expression of CD40 ligand, cytokines, and antibody production delineates sites of cognate T-B cell interactions. *J Exp Med*. 1993;178:1555–1565.

86. Hancock WW, Sayegh MH, Zheng XG, Peach R, Linsley PS, Turka LA. Costimulatory function and expression of CD40 ligand, CD80, and CD86 in vascularized murine cardiac allograft rejection. *Proc Natl Acad Sci USA*. 1996;93:13967–13972.

87. Larsen CP, Alexander DZ, Hollenbaugh D, et al. CD40-gp39 interactions play a critical role during allograft rejection. Suppression of allograft rejection by blockade of the CD40-gp39 pathway. *Transplantation*. 1996;61:4–9.

88. Fehr T, Takeuchi Y, Kurtz J, Wekerle T, Sykes M. Early regulation of CD8 T cell alloreactivity by CD4$^+$CD25$^-$ T cells in recipients of anti-CD154 antibody and allogeneic BMT is followed by rapid peripheral deletion of donor-reactive CD8$^+$ T cells, precluding a role for sustained regulation. *Eur J Immunol*. 2005;35:2679–2690.

89. Kawai T, Sogawa H, Boskovic S, et al. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. *Am J Transplant*. 2004;4:1391–1398.

90. Kurtz J, Shaffer J, Lie A, Anosova N, Benichou G, Sykes M. Mechanisms of early peripheral CD4 T-cell tolerance induction by anti-CD154 monoclonal antibody and allogeneic bone marrow transplantation: Evidence for anergy and deletion but not regulatory cells. *Blood*. 2004;103:4336–4343.

91. Niimi M, Pearson TC, Larsen CP, et al. The role of the CD40 pathway in alloantigen-induced hyporesponsiveness in vivo. *J Immunol*. 1998;161:5331–5337.

92. Taylor PA, Lees CJ, Waldmann H, Noelle RJ, Blazar BR. Requirements for the promotion of allogeneic engraftment by anti-CD154 (anti-CD40L) monoclonal antibody under nonmyeloablative conditions. *Blood*. 2001;98:467–474.

93. Blazar BR, Taylor PA, Noelle RJ, Vallera DA. CD4(+) T cells tolerized ex vivo to host alloantigen by anti-CD40 ligand (CD40L:CD154) antibody lose their graft-versushost disease lethality capacity but retain nominal antigen responses. *J Clin Invest*. 1998;102: 473–482.

94. Blazar BR, Taylor PA, Panoskaltsis-Mortari A, et al. Blockade of CD40 ligand-CD40 interaction impairs CD4$^+$ T cell-mediated alloreactivity by inhibiting mature donor T cell expansion and function after bone marrow transplantation. *J Immunol*. 1997;158:29–39.

95. Smook ML, Heeringa P, Damoiseaux JG, et al. Leukocyte CD40L deficiency affects the CD25(+) CD4 T cell population but does not affect atherosclerosis. *Atherosclerosis*. 2005;183:275–282.

96. Bour-Jordan H, Salomon BL, Thompson HL, Szot GL, Bernhard MR, Bluestone JA. Costimulation controls diabetes by altering the balance of pathogenic and regulatory T cells. *J Clin Invest*. 2004;114:979–987.

97. Ochando JC, Yopp AC, Yang Y, et al. Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3$^+$ regulatory T cells. *J Immunol*. 2005;174:6993–7005.

98. Zhai Y, Meng L, Gao F, Wang Y, Busuttil RW, Kupiec-Weglinski JW. CD4$^+$ T regulatory cell induction and function in transplant recipients after CD154 blockade is TLR4 independent. *J Immunol*. 2006;176:5988–5994.

99. Ferrer IR, Wagener ME, Song M, Kirk AD, Larsen CP, Ford ML. Antigen-specific induced Foxp3$^+$ regulatory T cells are generated following CD40/CD154 blockade. *Proc Natl Acad Sci USA*. 2011;108:20701–20706.

100. Pinelli DF, Wagener ME, Liu D, et al. An anti-CD154 domain antibody prolongs graft survival and induces Foxp3(+) iTreg in the absence and presence of CTLA-4 Ig. *Am J Transplant*. 2013;13:3021–3030.

101. Zhang N, Schroppel B, Lal G, et al. Regulatory T cells sequentially migrate from inflamed tissues to draining lymph nodes to suppress the alloimmune response. *Immunity*. 2009;30:458–469.

102. Larsen CP, Page A, Linzie KH, et al. An MHC-defined primate model reveals significant rejection of bone marrow after mixed chimerism induction despite full MHC matching. *Am J Transplant*. 2010;10:2396–2409.

103. Kim EJ, Kwun J, Gibby AC, et al. Costimulation blockade alters germinal center responses and prevents antibody-mediated rejection. *Am J Transplant*. 2014;14:59–69.

104. Kirk AD, Burkly LC, Batty DS, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med*. 1999;5:686–693.

105. Kirk AD, Harlan DM, Armstrong NN, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci USA*. 1997;94:8789–8794.

106. May AE, Kalsch T, Massberg S, Herouy Y, Schmidt R, Gawaz M. Engagement of glycoprotein IIb/IIIa (alpha(IIb)beta3) on platelets upregulates CD40L and triggers CD40L-dependent matrix degradation by endothelial cells. *Circulation*. 2002;106:2111–2117.

107. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature*. 1998;391:591–594.

108. Charafeddine AH, Kim EJ, Maynard DM, et al. Platelet-derived CD154: Ultrastructural localization and clinical correlation in organ transplantation. *Am J Transplant*. 2012;12:3143–3151.

109. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med*. 2000;6:114.

110. Badell IR, Thompson PW, Turner AP, et al. Nondepleting anti-CD40-based therapy prolongs allograft survival in nonhuman primates. *Am J Transplant*. 2012;12:126–135.

111. Gilson CR, Milas Z, Gangappa S, et al. Anti-CD40 monoclonal antibody synergizes with CTLA4-Ig in promoting long-term graft survival in murine models of transplantation. *J Immunol*. 2009;183: 1625–1635.

112. Lowe M, Badell IR, Thompson P, et al. A novel monoclonal antibody to CD40 prolongs islet allograft survival. *Am J Transplant*. 2012;12:2079–2087.

113. Rothstein DM, Livak MF, Kishimoto K, et al. Targeting signal 1 through CD45RB synergizes with CD40 ligand blockade and promotes long term engraftment and tolerance in stringent transplant models. *J Immunol*. 2001;166:322–329.

114. Thompson P, Cardona K, Russell M, et al. CD40specific costimulation blockade enhances neonatal porcine islet survival in nonhuman primates. *Am J Transplant*. 2011;11:947–957.

115. Page A, Srinivasan S, Singh K, et al. CD40 blockade combines with CTLA4Ig and sirolimus to produce mixed chimerism in an MHCdefined rhesus macaque transplant model. *Am J Transplant*. 2012;12:115–125.

116. Adams AB, Shirasugi N, Jones TR, et al. Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol*. 2005;174:54250.

117. Haanstra KG, Ringers J, Sick EA, et al. Prevention of kidney allograft rejection using anti-CD40 and anti-CD86 in primates. *Transplantation*. 2003;75:637–643.

118. Pearson TC, Trambley J, Odom K, et al. Anti-CD40 therapy extends renal allograft survival in rhesus macaques. *Transplantation*. 2002;74:933–940.

119. Aoyagi T, Yamashita K, Suzuki T, et al. A human anti-CD40 monoclonal antibody, 4D11, for kidney transplantation in cynomolgus monkeys: Induction and maintenance therapy. *Am J Transplant*. 2009;9:1732–1741.

120. Imai A, Suzuki T, Sugitani A, et al. A novel fully human antiCD40 monoclonal antibody, 4D11, for kidney transplantation in cynomolgus monkeys. *Transplantation*. 2007;84:1020–1028.

121. Xie JH, Yamniuk AP, Borowski V, et al. Engineering of a novel anti-CD40L domain antibody for treatment of autoimmune diseases. *J Immunol*. 2014;192:4083–4092.

122. Hutloff A, Dittrich AM, Beier KC, et al. ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. *Nature*. 1999;397:263–266.

123. Simpson TR, Quezada SA, Allison JP. Regulation of CD4 T cell activation and effector function by inducible costimulator (ICOS). *Curr Opin Immunol*. 2010;22:326–332.

124. Dong C, Juedes AE, Temann UA, et al. ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature*. 2001;409:97–101.

125. Gonzalo JA, Tian J, Delaney T, et al. ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses. *Nat Immunol*. 2001;2:597–604.

126. Rottman JB, Smith T, Tonra JR, et al. The costimulatory molecule ICOS plays an important role in the immunopathogenesis of EAE. *Nat Immunol*. 2001;2:605–611.

127. Bauquet AT, Jin H, Paterson AM, et al. The costimulatory molecule ICOS regulates the expression of c-Maf and IL-21 in the development of follicular T helper cells and TH-17 cells. *Nat Immunol*. 2009;10:167–175.

128. Xu H, Li X, Liu D, et al. Follicular T-helper cell recruitment governed by bystander B cells and ICOS-driven motility. *Nature*. 2013;496:523–527.

129. McAdam AJ, Greenwald RJ, Levin MA, et al. ICOS is critical for CD40-mediated antibody class switching. *Nature*. 2001;409:102–105.

130. Schenk AD, Gorbacheva V, Rabant M, Fairchild RL, Valujskikh A. Effector functions of donor-reactive CD8 memory T cells are dependent on ICOS induced during division in cardiac grafts. *Am J Transplant*. 2009;9:64–73.

131. Ozkaynak E, Gao W, Shemmeri N, et al. Importance of ICOS-B7RP-1 costimulation in acute and chronic allograft rejection. *Nat Immunol*. 2001;2:591–596.

132. Nanji SA, Hancock WW, Luo B, et al. Costimulation blockade of both inducible costimulator and CD40 ligand induces dominant tolerance to islet allografts and prevents spontaneous autoimmune diabetes in the NOD mouse. *Diabetes*. 2006;55:27–33.

133. Nanji SA, Hancock WW, Anderson CC, et al. Multiple combination therapies involving blockade of ICOS/B7RP-1 costimulation facilitate long-term islet allograft survival. *Am J Transplant*. 2004;4:526–536.

134. Taylor PA, Panoskaltsis-Mortari A, Freeman GJ, et al. Targeting of inducible costimulator (ICOS) expressed on alloreactive T cells down-regulates graft-versus-host disease (GVHD) and facilitates engraftment of allogeneic bone marrow (BM). *Blood*. 2005;105:3372–3380.

135. Sato M, Storb R, Loretz C, et al. Inducible costimulator (ICOS) up-regulation on activated T cells in chronic graftversus-host disease after dog leukocyte antigen-nonidentical hematopoietic cell transplantation: A potential therapeutic target. *Transplantation*. 2013;96:34–41.

136. Ogawa S, Nagamatsu G, Watanabe M, et al. Opposing effects of anti-activation-inducible lymphocyteimmunomodulatory molecule/inducible costimulator antibody on the development of acute versus chronic graft-versus-host disease. *J Immunol*. 2001;167:5741–5748.

137. Flynn R, Du J, Veenstra RG, et al. Increased T follicular helper cells and germinal center B cells are required for cGVHD and bronchiolitis obliterans. *Blood*. 2014;123:3988–3998.

138. Lo DJ, Anderson DJ, Song M, et al. A pilot trial targeting the ICOS-ICOS-L pathway in nonhuman primate kidney transplantation. *Am J Transplant*. 2015;15:984–992.

139. Chen AI, McAdam AJ, Buhlmann JE, et al. Ox40-ligand has a critical costimulatory role in dendritic cell: T cell interactions. *Immunity*. 1999;11:68998.

140. Kopf M, Ruedl C, Schmitz N, et al. OX40-deficient mice are defective in Th cell proliferation but are competent in generating B cell and CTL Responses after virus infection. *Immunity*. 1999;11:699–708.

141. Kinnear G, Wood KJ, Marshall D, Jones ND. Anti-OX40 prevents effector T-cell accumulation and CD8$^+$ T-cell mediated skin allograft rejection. *Transplantation*. 2010;90:1265–1271.

142. Kinnear G, Wood KJ, Fallah-Arani F, Jones ND. A diametric role for OX40 in the response of effector/memory CD4$^+$ T cells and regulatory T cells to alloantigen. *J Immunol*. 2013;191:1465–1475.

143. Tsukada N, Akiba H, Kobata T, Aizawa Y, Yagita H, Okumura K. Blockade of CD134 (OX40)-CD134L interaction ameliorates lethal acute graft-versus-host disease in a murine model of allogeneic bone marrow transplantation. *Blood*. 2000;95:2434–2439.

144. Blazar BR, Sharpe AH, Chen AI, et al. Ligation of OX40 (CD134) regulates graft-versus-host disease (GVHD) and graft rejection in allogeneic bone marrow transplant recipients. *Blood*. 2003;101:3741–3748.

145. Sanchez J, Casano J, Alvarez MA, et al. Kinetic of regulatory CD25high and activated CD134$^+$ (OX40) T lymphocytes during acute and chronic graft-versus-host disease after allogeneic bone marrow transplantation. *Br J Haematol*. 2004;126:697–703.

146. Miura Y, Thoburn CJ, Bright EC, Arai S, Hess AD. Regulation of OX40 gene expression in graft-versus-host disease. *Transplant Proc*. 2005;37:57–61.

147. Moncrieffe H, Coles M, Stockinger B. The influence of CD4 T-cell subsets on control of CD4 T-cell-mediated graft-versus-host disease. *Immunology*. 2008;125:459–468.

148. Ge X, Brown J, Sykes M, Boussiotis VA. CD134-allodepletion allows selective elimination of alloreactive human T cells without loss of virus-specific and leukemiaspecific effectors. *Biol Blood Marrow Transplant*. 2008;14:518–530.

149. Demirci G, Amanullah F, Kewalaramani R, et al. Critical role of OX40 in CD28 and CD154-independent rejection. *J Immunol*. 2004;172:1691–1698.

150. Vu MD, Xiao X, Gao W, et al. OX40 costimulation turns off Foxp3$^+$ Tregs. *Blood*. 2007;110:2501–2510.

151. Jensen MC, Riddell SR. Designing chimeric antigen receptors to effectively and safely target tumors. *Curr Opin Immunol*. 2015;33:9–15.

152. Ruella M, June CH. Chimeric antigen receptor T cells for B cell neoplasms: Choose the right CAR for you. *Curr Hematol Malig Rep*. 2016;11:368–384.

153. Schoenbrunn A, Frentsch M, Kohler S, et al. A converse 41BB and CD40 ligand expression pattern delineates activated regulatory T cells (Treg) and conventional T cells enabling direct isolation of alloantigen-reactive natural Foxp3$^+$ Treg. *J Immunol*. 2012;189:5985–5994.

154. Pollok KE, Kim YJ, Hurtado J, Zhou Z, Kim KK, Kwon BS. 4-1BB T-cell antigen binds to mature B cells and macrophages, and costimulates anti-mu-primed splenic B cells. *Eur J Immunol*. 1994;24:367–374.

155. Pollok KE, Kim YJ, Zhou Z, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol*. 1993;150:771–781.

156. Dawicki W, Watts TH. Expression and function of 4-1BB during CD4 versus CD8 T cell responses in vivo. *Eur J Immunol*. 2004;34:743–751.

157. Lee SW, Vella AT, Kwon BS, Croft M. Enhanced CD4 T cell responsiveness in the absence of 4-1BB. *J Immunol*. 2005;174:6803–6808.

158. Shuford WW, Klussman K, Tritchler DD, et al. 4-1BB costimulatory signals preferentially induce CD8$^+$ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. *J Exp Med*. 1997;186:47–55.

159. Blazar BR, Kwon BS, Panoskaltsis-Mortari A, Kwak KB, Peschon JJ, Taylor PA. Ligation of 4-1BB (CDw137) regulates graft-versus-host disease, graft-versus-leukemia, and graft rejection in allogeneic bone marrow transplant recipients. *J Immunol*. 2001;166:3174–3183.

160. Blazar BR, Lees CJ, Martin PJ, et al. Host T cells resist graft-versus-host disease mediated by donor leukocyte infusions. *J Immunol*. 2000;165:4901–4909.

161. Martins SL, St John LS, Champlin RE, et al. Functional assessment and specific depletion of alloreactive human T cells using flow cytometry. *Blood*. 2004;104:3429–3436.

162. Kim J, Choi WS, La S, et al. Stimulation with 4-1BB (CD137) inhibits chronic graft-versus-host disease by inducing activationinduced cell death of donor CD4$^+$ T cells. *Blood*. 2005;105:2206–2213.

163. DeBenedette MA, Wen T, Bachmann MF, et al. Analysis of 4-1BB ligand (4-1BBL)-deficient mice and of mice lacking both 4-1BBL and CD28 reveals a role for 4-1BBL in skin allograft rejection and in the cytotoxic T cell response to influenza virus. *J Immunol*. 1999;163:4833–4841.

164. Asai T, Choi BK, Kwon PM, et al. Blockade of the 4-1BB (CD137)/4-1BBL and/or CD28/CD80/CD86 costimulatory pathways promotes corneal allograft survival in mice. *Immunology*. 2007;121:349–358.

165. Wang J, Guo Z, Dong Y, et al. Role of 4-1BB in allograft rejection mediated by CD8$^+$ T cells. *Am J Transplant*. 2003;3:543–551.

166. Saiki H, Suzuki J, Kosuge H, et al. Blockade of the 4-1BB pathway attenuates graft arterial disease in cardiac allografts. *Int Heart J*. 2008;49:105–118.

167. Cho HR, Kwon B, Yagita H, et al. Blockade of 4-1BB (CD137)/4-1BB ligand interactions increases allograft survival. *Transpl Int*. 2004;17:351–361.

168. Chalkley JJ, Berger JR. Progressive multifocal leukoencephalopathy in multiple sclerosis. *Curr Neurol Neurosci Rep*. 2013;13:408.

169. Bamias G, Clark DJ, Rivera-Nieves J. Leukocyte traffic blockade as a therapeutic strategy in inflammatory bowel disease. *Curr Drug Targets*. 2013;14:1490–1500.

170. Joshi A, Bauer R, Kuebler P, et al. An overview of the pharmacokinetics and pharmacodynamics of efalizumab: A monoclonal antibody approved for use in psoriasis. *J Clin Pharmacol*. 2006;46:10–20.

171. Calabrese LH, Molloy E, Berger J. Sorting out the risks in progressive multifocal leukoencephalopathy. *Nat Rev Rheumatol*. 2015;11:119–123.

172. Kothary N, Diak IL, Brinker A, Bezabeh S, Avigan M, Dal Pan G. Progressive multifocal leukoencephalopathy associated with efalizumab use in psoriasis patients. *J Am Acad Dermatol*. 2011;65:546–551.

173. Carson KR, Focosi D, Major EO, et al. Monoclonal antibody-associated progressive multifocal leucoencephalopathy in patients treated with rituximab, natalizumab, and efalizumab: A Review from the Research on Adverse Drug Events and Reports (RADAR) Project. *Lancet Oncol*. 2009;10:816–824.

174. Stoppa AM, Maraninchi D, Blaise D, et al. Anti-LFA1 monoclonal antibody (25.3) for treatment of steroid-resistant grade III-IV acute graft-versus-host disease. *Transpl Int*. 1991;4:3–7.

175. Le Mauff B, Hourmant M, Rougier JP, et al. Effect of anti-LFA1 (CD11a) monoclonal antibodies in acute rejection in human kidney transplantation. *Transplantation*. 1991;52:291–296.

176. Blazar BR, Carroll SF, Vallera DA. Prevention of murine graft-versus-host disease and bone marrow alloengraftment across the major histocompatibility barrier after donor graft preincubation with anti-LFA1 immunotoxin. *Blood*. 1991;78:3093102.

177. Blazar BR, Taylor PA, Panoskaltsis-Mortari A, Gray GS, Vallera DA. Coblockade of the LFA1:ICAM and CD28/CTLA4:B7 pathways is a highly effective means of preventing acute lethal graft-versus-host disease induced by fully major histocompatibility complex-disparate donor grafts. *Blood*. 1995;85:2607–2618.

178. Tchorsh-Yutsis D, Hecht G, Aronovich A, et al. Pig embryonic pancreatic tissue as a source for transplantation in diabetes: Transient treatment with anti-LFA1, anti-CD48, and FTY720 enables long-term graft maintenance in mice with only mild ongoing immunosuppression. *Diabetes*. 2009;58:1585–1594.

179. Kwun J, Farris AB, Song H, Mahle WT, Burlingham WJ, Knechtle SJ. Impact of leukocyte function-associated antigen-1 blockade on endogenous allospecific T cells to multiple minor histocompatibility antigen mismatched cardiac allograft. *Transplantation*. 2015;99:2485–2493.

180. Ramsey H, Pilat N, Hock K, et al. Anti-LFA-1 or rapamycin overcome costimulation blockade-resistant rejection in sensitized bone marrow recipients. *Transpl Int*. 2013;26:206–218.

181. Kitchens WH, Haridas D, Wagener ME, Song M, Ford ML. Combined costimulatory and leukocyte functional antigen-1 blockade prevents transplant rejection mediated by heterologous immune memory alloresponses. *Transplantation*. 2012;93:997–1005.

182. Reisman NM, Floyd TL, Wagener ME, Kirk AD, Larsen CP, Ford ML. LFA-1 blockade induces effector and regulatory T-cell enrichment in lymph nodes and synergizes with CTLA-4Ig to inhibit effector function. *Blood*. 2011;118:5851–5861.

183. Setoguchi K, Schenk AD, Ishii D, et al. LFA-1 antagonism inhibits early infiltration of endogenous memory CD8 T cells into cardiac allografts and donor-reactive T cell priming. *Am J Transplant*. 2011;11:923–935.

184. Kitchens WH, Larsen CP, Ford ML. Integrin antagonists for transplant immunosuppression: Panacea or peril? *Immunotherapy*. 2011;3:305–307.

185. Badell IR, Russell MC, Thompson PW, et al. LFA-1-specific therapy prolongs allograft survival in rhesus macaques. *J Clin Invest*. 2010;120:4520–4531.

186. Posselt AM, Bellin MD, Tavakol M, et al. Islet transplantation in type 1 diabetics using an immunosuppressive protocol based on the anti-LFA-1 antibody efalizumab. *Am J Transplant*. 2010;10:187080.

187. Wang Y, Li D, Jones D, et al. Blocking LFA-1 activation with lovastatin prevents graft-versus-host disease in mouse bone marrow transplantation. *Biol Blood Marrow Transplant*. 2009;15:1513–1522.

188. Vincenti F, Mendez R, Pescovitz M, et al. A phase I/II randomized openlabel multicenter trial of efalizumab, a humanized anti-CD11a, anti-LFA-1 in renal transplantation. *Am J Transplant*. 2007;7:1770–1777.

189. Beus JM, Hashmi SS, Selvaraj SA, et al. Heterologous immunity triggered by a single, latent virus in Mus musculus: Combined costimulation- and adhesion- blockade decrease rejection. *PLoS ONE*. 2013;8:e71221.

190. Anderson DJ, Lo DJ, Leopardi F, et al. Anti-leukocyte function-associated antigen therapy in a nonhuman primate renal transplant model of costimulation blockaderesistant rejection. *Am J Transplant*. 2016;16:1456–1464.

191. Kitchens WH, Haridas D, Wagener ME, et al. Integrin antagonists prevent costimulatory blockade-resistant transplant rejection by CD8(+) memory T cells. *Am J Transplant*. 2012;12:69–80.

192. Turgeon NA, Avila JG, Cano JA, et al. Experience with a novel efalizumab-based immunosuppressive regimen to facilitate single donor islet cell transplantation. *Am J Transplant*. 2010;10:2082–2091.

193. Paul LC, Davidoff A, Benediktsson H, Issekutz TB. The efficacy of LFA-1 and VLA-4 antibody treatment in rat vascularized cardiac allograft rejection. *Transplantation*. 1993;55:1196–1199.

194. Stegall MD, Ostrowska A, Haynes J, Karrer F, Kam I, Gill RG. Prolongation of islet allograft survival with an antibody to vascular cell adhesion molecule 1. *Surgery*. 1995;118:366–369; discussion 9–70.

195. Molossi S, Elices M, Arrhenius T, Diaz R, Coulber C, Rabinovitch M. Blockade of very late antigen-4 integrin binding to fibronectin with connecting segment-1 peptide reduces accelerated coronary arteriopathy in rabbit cardiac allografts. *J Clin Invest*. 1995;95:2601–2610.

196. Yang H, Issekutz TB, Wright JR Jr. Prolongation of rat islet allograft survival by treatment with monoclonal antibodies against VLA-4 and LFA-1. *Transplantation*. 1995;60:71–76.

197. Shiohara T, Moriya N, Gotoh C, Saizawa K, Nagashima M. Locally administered monoclonal antibodies to lymphocyte function-associated antigen 1 and to L3T4 prevent cutaneous graft-vs-host disease. *J Immunol*. 1988;141:2261–2267.

198. Harning R, Pelletier J, Lubbe K, Takei F, Merluzzi VJ. Reduction in the severity of graft-versus-host disease and increased survival in allogenic mice by treatment with monoclonal antibodies to cell adhesion antigens LFA-1 alpha and MALA-2. *Transplantation*. 1991;52:842–845.

199. Schlegel PG, Vaysburd M, Chen Y, Butcher EC, Chao NJ. Inhibition of T cell costimulation by VCAM-1 prevents murine graft-versus-host disease across minor histocompatibility barriers. *J Immunol*. 1995;155:3856–3865.

200. Khatri BO. Fingolimod in the treatment of relapsing-remitting multiple sclerosis: Long-term experience and an update on the clinical evidence. *Ther Adv Neurol Disord*. 2016;9:130–147.

201. Ziemssen T, Derfuss T, de Stefano N, et al. Optimizing treatment success in multiple sclerosis. *J Neurol*. 2016;263:1053–1065.

202. Ueda H, Takahara S, Azuma H, Kusaka M, Suzuki S, Katsuoka Y. Effect of a novel immunosuppressant, FTY720, on allograft survival after renal transplant in rats. *Eur Surg Res*. 2000;32:279–283.

203. Hoitsma AJ, Woodle ES, Abramowicz D, Proot P, Vanrenterghem Y. Group FTYPITS. FTY720 combined with tacrolimus in de novo renal transplantation: 1-year, multicenter, open-label randomized study. *Nephrol Dial Transplant*. 2011;26:3802–3805.

204. Huu DL, Matsushita T, Jin G, et al. FTY720 ameliorates murine sclerodermatous chronic graft-versus-host disease by promoting

expansion of splenic regulatory cells and inhibiting immune cell infiltration into skin. *Arthritis Rheum*. 2013;65:1624–1635.

205. Taylor PA, Kelly RM, Bade ND, Smith MJ, Stefanski HE, Blazar BR. FTY720 markedly increases alloengraftment but does not eliminate host anti-donor T cells that cause graft rejection on its withdrawal. *Biol Blood Marrow Transplant*. 2012;18:134152.

206. Weckbecker G, Pally C, Beerli C, et al. Effects of the novel protein kinase C inhibitor AEB071 (Sotrastaurin) on rat cardiac allograft survival using single agent treatment or combination therapy with cyclosporine, everolimus or FTY720. *Transpl Int*. 2010;23:543–552.

207. Lopes CT, Gallo AP, Palma PV, Cury PM, Bueno V. Skin allograft survival and analysis of renal parameters after FTY720 + tacrolimus treatment in mice. *Transplant Proc*. 2008;40:856–860.

208. Taylor PA, Ehrhardt MJ, Lees CJ, et al. Insights into the mechanism of FTY720 and compatibility with regulatory T cells for the inhibition of graft-versus-host disease (GVHD). *Blood*. 2007;110:3480–3488.

209. Suzuki S, Enosawa S, Kakefuda T, et al. A novel immunosuppressant, FTY720, with a unique mechanism of action, induces long-term graft acceptance in rat and dog allotransplantation. *Transplantation*. 1996;61:200–205.

210. Yanagawa Y, Sugahara K, Kataoka H, Kawaguchi T, Masubuchi Y, Chiba K. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II. FTY720 prolongs skin allograft survival by decreasing T cell infiltration into grafts but not cytokine production in vivo. *J Immunol*. 1998;160:5493–5499.

211. Chiba K, Yanagawa Y, Masubuchi Y, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. *J Immunol*. 1998;160:5037–5044.

212. Hashimoto D, Asakura S, Matsuoka K, et al. FTY720 enhances the activation-induced apoptosis of donor T cells and modulates graft-versus-host disease. *Eur J Immunol*. 2007;37:271–281.

213. Lee RS, Kuhr CS, Sale GE, et al. FTY720 does not abrogate acute graft-versus-host disease in the dog leukocyte antigennonidentical unrelated canine model. *Transplantation*. 2003;76:1155–1158.

214. Kim YM, Sachs T, Asavaroengchai W, Bronson R, Sykes M. Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720. *J Clin Invest*. 2003;111:659–669.

215. Blaha P, Bigenzahn S, Koporc Z, et al. The influence of immunosuppressive drugs on tolerance induction through bone marrow transplantation with costimulation blockade. *Blood*. 2003;101:2886–2893.

216. Xun CQ, Little MT, Zellmer E, et al. What role for FTY720, a novel immunosuppressive agent, in canine nonmyeloablative hematopoietic stem cell transplantation? *Transplantation*. 2002;73:310–313.

217. Murai M, Yoneyama H, Ezaki T, et al. Peyer's patch is the essential site in initiating murine acute and lethal graft-versus-host reaction. *Nat Immunol*. 2003;4:15460.

218. Murai M, Yoneyama H, Harada A, et al. Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J Clin Invest*. 1999;104:49–57.

219. Wysocki CA, Burkett SB, Panoskaltsis-Mortari A, et al. Differential roles for CCR5 expression on donor T cells during graft-versus-host disease based on pretransplant conditioning. *J Immunol*. 2004;173:845–854.

220. Welniak LA, Wang Z, Sun K, et al. An absence of CCR5 on donor cells results in acceleration of acute graft-vs-host disease. *Exp Hematol*. 2004;32:318–324.

221. Reshef R, Luger SM, Hexner EO, et al. Blockade of lymphocyte chemotaxis in visceral graftversus-host disease. *N Engl J Med*. 2012;367:135–145.

222. Chavin KD, Lau HT, Bromberg JS. Prolongation of allograft and xenograft survival in mice by anti-CD2 monoclonal antibodies. *Transplantation*. 1992;54:286–291.

223. Lo DJ, Weaver TA, Stempora L, et al. Selective targeting of human alloresponsive CD8^{+} effector memory T cells based on CD2 expression. *Am J Transplant*. 2011;11:22–33.

224. Weaver TA, Charafeddine AH, Agarwal A, et al. Alefacept promotes costimulation blockade based allograft survival in nonhuman primates. *Nat Med*. 2009;15:746–749.

225. Stenger EO, Chiang KY, Haight A, Qayed M, Kean L, Horan J. Use of alefacept for preconditioning in multiply transfused pediatric patients with nonmalignant diseases. *Biol Blood Marrow Transplant*. 2015;21:1845–1852.

226. Rigby MR, Harris KM, Pinckney A, et al. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J Clin Invest*. 2015;125:3285–3296.

227. Rigby MR, DiMeglio LA, Rendell MS, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol*. 2013;1:284–294.

228. Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. *Nat Rev Immunol*. 2012;12:417–430.

229. Daud AI, Loo K, Pauli ML, et al. Tumor immune profiling predicts response to anti-PD-1 therapy in human melanoma. *J Clin Invest*. 2016;126:3447–3452.

230. Huynh A, DuPage M, Priyadharshini B, et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat Immunol*. 2015;16:188–196.

231. Taylor PA, Panoskaltsis-Mortari A, Swedin JM, et al. L-Selectin(hi) but not the L-selectin(lo) CD4^{+}25^{+} Tregulatory cells are potent inhibitors of GVHD and BM graft rejection. *Blood*. 2004;104:3804–3812.

232. Steiner D, Brunicki N, Bachar-Lustig E, Taylor PA, Blazar BR, Reisner Y. Overcoming T cell-mediated rejection of bone marrow allografts by T-regulatory cells: Synergism with veto cells and rapamycin. *Exp Hematol*. 2006;34:802–808.

233. Steiner D, Brunicki N, Blazar BR, Bachar-Lustig E, Reisner Y. Tolerance induction by third-party "off-the-shelf" CD4^{+}CD25^{+} Treg cells. *Exp Hematol*. 2006;34:66–71.

234. Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: Kinetics, toxicity profile, and clinical effect. *Blood*. 2016;127:104451.

235. Hippen KL, Riley JL, June CH, Blazar BR. Clinical perspectives for regulatory T cells in transplantation tolerance. *Semin Immunol*. 2011;23:462–468.

236. Hippen KL, Merkel SC, Schirm DK, et al. Massive ex vivo expansion of human natural regulatory T cells (T(regs)) with minimal loss of in vivo functional activity. *Sci Transl Med*. 2011;3:83ra41.

237. Golovina TN, Mikheeva T, Brusko TM, Blazar BR, Bluestone JA, Riley JL. Retinoic acid and rapamycin differentially affect and synergistically promote the ex vivo expansion of natural human T regulatory cells. *PLoS ONE*. 2011;6:e15868.

238. Brunstein CG, Miller JS, Cao Q, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: Safety profile and detection kinetics. *Blood*. 2011;117:1061–1070.

239. June CH, Blazar BR, Riley JL. Engineering lymphocyte subsets: Tools, trials and tribulations. *Nat Rev Immunol*. 2009;9:704–716.

240. Riley JL, June CH, Blazar BR. Human T regulatory cell therapy: Take a billion or so and call me in the morning. *Immunity*. 2009;30:656–665.

241. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. *Blood*. 2014;124:638–644.

242. Martelli MF, Di Ianni M, Ruggeri L, et al. "Designed" grafts for HLA-haploidentical stem cell transplantation. *Blood*. 2014;123:967–973.

243. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood*. 2011;117:3921–3928.

244. Barao I, Hanash AM, Hallett W, et al. Suppression of natural killer cell-mediated bone marrow cell rejection by CD4$^+$CD25$^+$ regulatory T cells. *Proc Natl Acad Sci USA*. 2006;103:5460–5465.

245. Cohen JL, Trenado A, Vasey D, Klatzmann D, Salomon BL. CD4(+) CD25(+) immunoregulatory T Cells: New therapeutics for graft-versus-host disease. *J Exp Med*. 2002;196:401–406.

246. Edinger M, Hoffmann P, Ermann J, et al. CD4$^+$CD25$^+$ regulatory T cells preserve graft-versus-tumor activity while inhibiting graftversus-host disease after bone marrow transplantation. *Nat Med*. 2003;9:1144–1150.

247. Hoffmann P, Ermann J, Edinger M, Fathman CG, Strober S. Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. *J Exp Med*. 2002;196:389–399.

248. Taylor PA, Lees CJ, Blazar BR. The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood*. 2002;99:3493–3499.

249. Gudmundsdottir H, Turka LA. A closer look at homeostatic proliferation of CD4$^+$ T cells: Costimulatory requirements and role in memory formation. *J Immunol*. 2001;167:3699–3707.

250. Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am J Transplant*. 2002;2:501–509.

251. Adams AB, Pearson TC, Larsen CP. Heterologous immunity: An overlooked barrier to tolerance. *Immunol Rev*. 2003;196:147–160.

252. Adams AB, Williams MA, Jones TR, et al. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest*. 2003;111:1887–1895.

253. Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A. Expression of two T cell receptor alpha chains: Dual receptor T cells. *Science*. 1993;262:422–424.

254. Vu MD, Clarkson MR, Yagita H, Turka LA, Sayegh MH, Li XC. Critical, but conditional, role of OX40 in memory T cell-mediated rejection. *J Immunol*. 2006;176:1394–1401.

255. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J*. 1992;11:3887–3895.

256. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 2001;291:319–322.

257. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439:682–687.

258. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol*. 2005;25:9543–9553.

259. Wang L, Han R, Hancock WW. Programmed cell death 1 (PD-1) and its ligand PD-L1 are required for allograft tolerance. *Eur J Immunol*. 2007;37:2983–2990.

260. Ito T, Ueno T, Clarkson MR, et al. Analysis of the role of negative T cell costimulatory pathways in CD4 and CD8 T cell-mediated alloimmune responses in vivo. *J Immunol*. 2005;174:6648–6656.

261. Koehn BH, Ford ML, Ferrer IR, et al. PD1-dependent mechanisms maintain peripheral tolerance of donor-reactive CD8$^+$ T cells to transplanted tissue. *J Immunol*. 2008;181:5313–5322.

262. Blazar BR, Carreno BM, Panoskaltsis-Mortari A, et al. Blockade of programmed death-1 engagement accelerates graft-versus-host disease lethality by an IFN-gamma-dependent mechanism. *J Immunol*. 2003;171:1272–1277.

263. Schilbach K, Schick J, Wehrmann M, et al. PD-1-PD-L1 pathway is involved in suppressing alloreactivity of heart infiltrating t cells during murine gvhd across minor histocompatibility antigen barriers. *Transplantation*. 2007;84:214–222.

264. Koestner W, Hapke M, Herbst J, et al. PD-L1 blockade effectively restores strong graft-versus-leukemia effects without graft-versus-host

265. Fujiwara H, Maeda Y, Kobayashi K, et al. Programmed death-1 pathway in host tissues ameliorates Th17/Th1-mediated experimental chronic graft-versus-host disease. *J Immunol*. 2014;193:2565–2573.

266. Sandner SE, Clarkson MR, Salama AD, et al. Role of the programmed death-1 pathway in regulation of alloimmune responses in vivo. *J Immunol*. 2005;174:3408–3415.

267. Yang J, Popoola J, Khandwala S, et al. Critical role of donor tissue expression of programmed death ligand-1 in regulating cardiac allograft rejection and vasculopathy. *Circulation*. 2008;117:660–669.

268. Yang J, Riella LV, Chock S, et al. The novel costimulatory programmed death ligand 1/B7.1 pathway is functional in inhibiting alloimmune responses in vivo. *J Immunol*. 2011;187:1113–1119.

269. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*. 2009;206:3015–3029.

270. Saha A, Aoyama K, Taylor PA, et al. Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. *Blood*. 2013;122:3062–3073.

271. Asakura S, Hashimoto D, Takashima S, et al. Alloantigen expression on non-hematopoietic cells reduces graft-versus-leukemia effects in mice. *J Clin Invest*. 2010;120:2370–2378.

272. Matte-Martone C, Venkatesan S, Tan HS, et al. Graft-versus-leukemia (GVL) against mouse blast-crisis chronic myelogenous leukemia (BC-CML) and chronic-phase chronic myelogenous leukemia (CP-CML): Shared mechanisms of T cell killing, but programmed death ligands render CP-CML and not BC-CML GVL resistant. *J Immunol*. 2011;187:1653–1663.

273. Saha A, O'Connor RS, Thangavelu G, et al. Programmed death ligand-1 expression on donor T cells drives graft-versus-host disease lethality. *J Clin Invest*. 2016;126:2642–2660.

274. Yi T, Li X, Yao S, et al. Host APCs augment in vivo expansion of donor natural regulatory T cells via B7H1/B7.1 in allogeneic recipients. *J Immunol*. 2011;186:2739–2749.

275. Deng R, Cassady K, Li X, et al. B7H1/CD80 interaction augments PD-1-dependent T cell apoptosis and ameliorates graft-versus-host disease. *J Immunol*. 2015;194:560–574.

276. Butte MJ, Keir ME, Phamduy TB, Sharpe AH, Freeman GJ. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity*. 2007;27:111–122.

277. Menzies AM, Johnson DB, Ramanujam S, et al. AntiPD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab. *Ann Oncol*. 2016; doi:10.1093/annonc/mdw443.

278. Krecke N, Zimmer A, Friesenhahn-Ochs B, Muller CS, Vogt T, Pfohler C. Sneaky side effects and ineffectiveness of an immunotherapy with ipilimumab in a case of metastatic melanoma. *Dermatoendocrinol*. 2016;8:e1199307.

279. Koelzer VH, Buser T, Willi N, et al. Grover's-like drug eruption in a patient with metastatic melanoma under ipilimumab therapy. *J Immunother Cancer*. 2016;4:47.

280. Koelzer VH, Rothschild SI, Zihler D, et al. Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors—An autopsy study. *J Immunother Cancer*. 2016;4:13.

281. Verschuren EC, van den Eertwegh AJ, Wonders J, et al. Clinical, endoscopic, and histologic characteristics of ipilimumab-associated colitis. *Clin Gastroenterol Hepatol*. 2016;14:836–842.

282. Johnson DB, Sullivan RJ, Ott PA, et al. Ipilimumab therapy in patients with advanced melanoma and preexisting autoimmune disorders. *JAMA Oncol*. 2016;2:234–240.

283. Davids MS, Kim HT, Bachireddy P, et al. Leukemia and Lymphoma Society Blood Cancer Research Partnership. Ipilimumab for

disease after delayed adoptive transfer of T-cell receptor gene-engineered allogeneic CD8$^+$ T cells. *Blood*. 2011;117:1030–1041.

patients with relapse after allogeneic transplantation. *N Engl J Med.* 2016;375:143–153.

284. Singh AK, Porrata LF, Aljitawi O, et al. Fatal GvHD induced by PD-1 inhibitor pembrolizumab in a patient with Hodgkin's lymphoma. *Bone Marrow Transplant.* 2016;51:1268–1270.

285. Yang Y, Jacoby E, Fry TJ. Challenges and opportunities of allogeneic donorderived CAR T cells. *Curr Opin Hematol.* 2015;22:509–515.

286. Kochenderfer JN, Dudley ME, Carpenter RO, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood.* 2013;122:4129–4139.

287. Jacoby E, Yang Y, Qin H, Chien CD, Kochenderfer JN, Fry TJ. Murine allogeneic CD19 CAR T cells harbor potent antileukemic activity but have the potential to mediate lethal GVHD. *Blood.* 2016;127:1361–1370.

288. Jones BS, Lamb LS, Goldman F, Di Stasi A. Improving the safety of cell therapy products by suicide gene transfer. *Front Pharmacol.* 2014;5:254.

289. Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T cells. *Front Pharmacol.* 2014;5:235.

290. Okamura T, Fujio K, Sumitomo S, Yamamoto K. Roles of LAG3 and EGR2 in regulatory T cells. *Ann Rheum Dis.* 2012;71(Suppl 2):i96–i100.

291. Okamura T, Fujio K, Shibuya M, et al. CD4$^+$CD25$^-$LAG3$^+$ regulatory T cells controlled by the transcription factor Egr-2. *Proc Natl Acad Sci USA.* 2009;106:13974–13979.

292. Chen SY, Hsu WT, Chen YL, Chien CH, Chiang BL. Lymphocyte-activation gene 3(+) (LAG3(+)) forkhead box protein 3(-) (FOXP3(-)) regulatory T cells induced by B cells alleviates joint inflammation in collagen-induced arthritis. *J Autoimmun.* 2016;68:75–85.

293. Do JS, Visperas A, Sanogo YO, et al. An IL-27/Lag3 axis enhances Foxp3$^+$ regulatory T cell-suppressive function and therapeutic efficacy. *Mucosal Immunol.* 2016;9:137–145.

294. Sega EI, Leveson-Gower DB, Florek M, Schneidawind D, Luong RH, Negrin RS. Role of lymphocyte activation gene-3 (Lag-3) in conventional and regulatory T cell function in allogeneic transplantation. *PLoS ONE.* 2014;9:e86551.

295. Wang L, Fraser CC, Kikly K, et al. B7-H3 promotes acute and chronic allograft rejection. *Eur J Immunol.* 2005;35:428–438.

296. Ueno T, Yeung MY, McGrath M, et al. Intact B7-H3 signaling promotes allograft prolongation through preferential suppression of Th1 effector responses. *Eur J Immunol.* 2012;42:2343–2353.

297. Veenstra RG, Flynn R, Kreymborg K, et al. B7-H3 expression in donor T cells and host cells negatively regulates acute graft-versus-host disease lethality. *Blood.* 2015;125:3335–3346.

298. Mahnke K, Ring S, Johnson TS, et al. Induction of immunosuppressive functions of dendritic cells in vivo by CD4$^+$CD25$^+$ regulatory T cells: Role of B7-H3 expression and antigen presentation. *Eur J Immunol.* 2007;37:2117–2126.

299. Yamaura K, Watanabe T, Boenisch O, et al. In vivo function of immune inhibitory molecule B7-H4 in alloimmune responses. *Am J Transplant.* 2010;10:2355–2362.

300. Bengsch B, Seigel B, Ruhl M, et al. Coexpression of PD-1, 2B4, CD160 and KLRG1 on exhausted HCV-specific CD8$^+$ T cells is linked to antigen recognition and T cell differentiation. *PLoS Pathog.* 2010;6:e1000947.

301. Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8$^+$ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol.* 2009;10:29–37.

302. Raziorrouh B, Schraut W, Gerlach T, et al. The immunoregulatory role of CD244 in chronic hepatitis B infection and its inhibitory potential on virus-specific CD8$^+$ T-cell function. *Hepatology.* 2010;52:1934–1947.

303. Wherry EJ, Ha SJ, Kaech SM, et al. Molecular signature of CD8$^+$ T cell exhaustion during chronic viral infection. *Immunity.* 2007;27:670–684.

304. Brown DR, Calpe S, Keszei M, et al. Cutting edge: An NK cell-independent role for Slamf4 in controlling humoral autoimmunity. *J Immunol.* 2011;187:21–25.

305. Kim JR, Mathew SO, Patel RK, Pertusi RM, Mathew PA. Altered expression of signalling lymphocyte activation molecule (SLAM) family receptors CS1 (CD319) and 2B4 (CD244) in patients with systemic lupus erythematosus. *Clin Exp Immunol.* 2010;160:348–358.

306. Sabatos CA, Chakravarti S, Cha E, et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat Immunol.* 2003;4:1102–1110.

307. Sanchez-Fueyo A, Tian J, Picarella D, et al. Tim-3 inhibits T helper type 1-mediated auto- and alloimmune responses and promotes immunological tolerance. *Nat Immunol.* 2003;4:1093–1101.

308. Veenstra RG, Taylor PA, Zhou Q, et al. Contrasting acute graft-versushost disease effects of Tim-3/galectin-9 pathway blockade dependent upon the presence of donor regulatory T cells. *Blood.* 2012;120:682–690.

309. Wang F, He W, Yuan J, et al. Activation of Tim-3Galectin-9 pathway improves survival of fully allogeneic skin grafts. *Transpl Immunol.* 2008;19:12–19.

310. Wang F, He W, Zhou H, et al. The Tim-3 ligand galectin-9 negatively regulates CD8$^+$ alloreactive T cell and prolongs survival of skin graft. *Cell Immunol.* 2007;250:68–74.

311. He W, Fang Z, Wang F, et al. Galectin-9 significantly prolongs the survival of fully mismatched cardiac allografts in mice. *Transplantation.* 2009;88:782–790.

312. Boenisch O, D'Addio F, Watanabe T, et al. TIM-3: A novel regulatory molecule of alloimmune activation. *J Immunol.* 2010;185:5806–5819.

313. Gupta S, Thornley TB, Gao W, et al. Allograft rejection is restrained by short-lived TIM-3$^+$PD-1$^+$Foxp3$^+$ Tregs. *J Clin Invest.* 2012;122:2395–2404.

314. Tao YF, Lin F, Yan XY, et al. Galectin-9 in combination with EX-527 prolongs the survival of cardiac allografts in mice after cardiac transplantation. *Transplant Proc.* 2015;47:2003–2009.

315. Cai L, Zhou H, Fang Z, et al. Galectin-9 in combination with rapamycin induces cardiac allograft tolerance in mice. *Transplantation.* 2013;96:379–386.

316. Shimmura-Tomita M, Wang M, Taniguchi H, Akiba H, Yagita H, Hori J. Galectin9-mediated protection from allo-specific T cells as a mechanism of immune privilege of corneal allografts. *PLoS ONE.* 2013;8:e63620.

317. Chen Y, Fukuda T, Thakar MS, et al. Immunomodulatory effects induced by cytotoxic T lymphocyte antigen 4 immunoglobulin with donor peripheral blood mononuclear cell infusion in canine major histocompatibility complex-haplo-identical non-myeloablative hematopoietic cell transplantation. *Cytotherapy.* 2011;13:1269–1280.

318. Graves SS, Stone D, Loretz C, et al. Establishment of long-term tolerance to SRBC in dogs by recombinant canine CTLA4-Ig. *Transplantation.* 2009;88:317–322.

319. Graves SS, Stone DM, Loretz C, et al. Antagonistic and agonistic anti-canine CD28 monoclonal antibodies: Tools for allogeneic transplantation. *Transplantation.* 2011;91:833–840.

320. Mathes DW, Hwang B, Graves SS, et al. Tolerance to vascularized composite allografts in canine mixed hematopoietic chimeras. *Transplantation.* 2011;92:1301–1308.

321. Rosinski SL, Storb R, Strong RK, et al. Anti-CD28 antibody-initiated cytokine storm in canines. *Transplant Direct.* 2015;1. doi:10.1097/TXD.0000000000000516.

322. Jochum C, Beste M, Zellmer E, Graves SS, Storb R. CD154 blockade and donorspecific transfusions in DLA-identical marrow transplantation in dogs conditioned with 1 Gy total body irradiation. *Biol Blood Marrow Transplant.* 2007;13:164–171.

323. Kean LS, Singh K, Blazar BR, Larsen CP. Nonhuman primate transplant models finally evolve: Detailed immunogenetic analysis creates new models and strengthens the old. *Am J Transplant.* 2012;12:812–819.

324. Guinan EC, Boussiotis VA, Neuberg D, et al. Transplantation of anergic histoincompatible bone marrow allografts. *N Engl J Med*. 1999;340:1704–1714.

325. Eastwood D, Findlay L, Poole S, et al. Monoclonal antibody TGN1412 trial failure explained by species differences in CD28 expression on CD4+ effector memory T-cells. *Br J Pharmacol*. 2010;161: 512–526.

326. Pallardy M, Hunig T. Primate testing of TGN1412: Right target, wrong cell. *Br J Pharmacol*. 2010;161:509–511.

327. Pitcher CJ, Hagen SI, Walker JM, et al. Development and homeostasis of T cell memory in rhesus macaque. *J Immunol*. 2002;168: 29–43.

328. Poirier N, Mary C, Le Bas-Bernardet S, et al. Advantages of *Papio anubis* for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28. *mAbs*. 2014;6:697–707.

329. Kenyon NS, Chatzipetrou M, Masetti M, et al. Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci USA*. 1999;96:8132–8137.

330. Kawai T, Cosimi AB, Colvin RB, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. *Transplantation*. 1995;59:256–262.

331. Kimikawa M, Sachs DH, Colvin RB, Bartholomew A, Kawai T, Cosimi AB. Modifications of the conditioning regimen for achieving mixed chimerism and donorspecific tolerance in cynomolgus monkeys. *Transplantation*. 1997;64:709–716.

332. Boumpas DT, Furie R, Manzi S, et al. Group BGLNT. A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum*. 2003;48:719–727.

333. Johnson ZP, Eady RD, Ahmad SF, et al. Immunogenetic Management Software: A new tool for visualization and analysis of complex immunogenetic datasets. *Immunogenetics*. 2012;64:329–336.

334. Wiseman RW, Karl JA, Bimber BN, et al. Major histocompatibility complex genotyping with massively parallel pyrosequencing. *Nat Med*. 2009;15:1322–1326.

335. Zimin AV, Cornish AS, Maudhoo MD, et al. A new rhesus macaque assembly and annotation for next-generation sequencing analyses. *Biol Direct*. 2014;9:20.

336. Furlan SN, Watkins B, Tkachev V, et al. Systems analysis uncovers inflammatory Th/Tc17-driven modules during acute GVHD in monkey and man. *Blood*. 2016; doi:10.1182/blood-2016-07-726547.

337. Furlan SN, Watkins B, Tkachev V, et al. Transcriptome analysis of GVHD reveals aurora kinase A as a targetable pathway for disease prevention. *Sci Transl Med*. 2015;7:315ra191.

338. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature*. 1953;172:603–606.