The immunobiology of CD27 and OX40 and their potential as targets for cancer immunotherapy

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Abstract

In recent years monoclonal antibodies (mAbs) able to reinvigorate anti-tumor T cell immunity have heralded a paradigm shift in cancer treatment. The most high profile of these mAbs block the inhibitory checkpoint receptors PD-1 and CTLA-4 and have improved life expectancy for patients across a range of tumor types. However, it is becoming increasingly clear that failure of some patients to respond to checkpoint inhibition is due to inadequate T-cell priming. For full T-cell activation two signals must be received and ligands providing the second of these signals, termed costimulation, are often lacking in tumors. Members of the TNF receptor superfamily (TNFRSF) are key co-stimulators of T cells during infection and there has been an increasing interest in harnessing these receptors to augment tumor immunity. We here review the immunobiology of two particularly promising TNFRSF target receptors, CD27 and OX40, and their respective ligands CD70 and OX40L, focusing on their role within a tumor setting. We describe the influence of CD27 and OX40 on human T cells based on in vitro studies and on the phenotypes of several recently described individuals exhibiting natural deficiencies in CD27/CD70 and OX40. Finally we review key literature describing progress in elucidating the efficacy and mode of action of OX40- and CD27-targeting mAbs in pre-clinical models and provide an overview of current clinical trials targeting these promising receptor/ligand pairings in cancer.

Introduction

The concept of a two-signal model of T-cell activation was initially proposed by Lafferty and colleagues based on earlier work on the regulation of B-cell activation by Bretscher & Cohn[1]. Subsequently, Jenkins, Schwartz and others demonstrated that full T-cell activation required not only T-cell receptor (TCR) occupancy by MHC-anchored peptide, but also short-range costimulatory signals provided by non-T accessory cells[2]. The identity of the first costimulatory receptor, CD28, was revealed using anti-CD28 monoclonal antibodies (mAbs) which when added to T-cell cultures provided the second signal necessary for T-cell proliferation and IL-2 secretion[3]. Identification of additional costimulatory receptors came about in the 1980s with the discovery of T-cell determinants recognized by newly isolated mAbs. A number of these mAbs were found to enhance T-cell proliferation, but only when combined with reagents that induced TCR triggering, thus fulfilling the true definition of a costimulatory signal. With the advent of expression cloning in the late 1980s and early 1990s many costimulatory receptors and their natural ligands were molecularly cloned[4,5], thus paving the way to detailed analysis of their roles in immunity. Based on structural features, costimulatory receptors can be grouped primarily into those that belong to either the immunoglobulin superfamily (IgSF; eg. CD28, ICOS and CD226) or the tumor necrosis factor receptor superfamily (TNFRSF), including CD27, OX40, 4-1BB, GITR, death receptor 3 and CD30[3,6-8]. In addition T cells express inhibitory receptors (eg. CTLA-4, PD-1, TIGIT and BTLA, all of which are also members of the IgSF) that negatively regulate T-cell responses through cell-intrinsic as well as cell-extrinsic mechanisms with CTLA-4 and PD-1 partly mediating their effects by countering the costimulatory activity of CD28[9-13]. The discovery that costimulatory signals can boost the magnitude of the T-cell response and the generation of effector and memory T cells, led to the notion that targeting these pathways may promote cellular immune responses against tumors or those elicited by vaccination. Conversely, blockade of costimulatory signaling has the potential to ameliorate overt T-cell responses in autoimmune and graft-versus-host disease as well as prevent rejection after allogeneic transplantation. Currently two drugs (Abatacept and Belatacept) based on a CTLA-4-Ig fusion protein, a CD28 antagonist, are in clinical use. Although not broadly efficacious, CTLA-4-Ig is effective in ameliorating symptoms of rheumatoid arthritis, juvenile idiopathic arthritis, common variable immunodeficiency syndrome when caused by

haploinsufficiency in *CTLA-4* or dysregulated CTLA-4 trafficking, and in preventing rejection of renal transplants[3,14,15]. Despite impressive results in these conditions, treatment with CTLA-4-Ig is not fully protective and therefore current efforts are focused on combining CTLA-4-Ig with blockade of additional costimulatory receptors[16]. The choice of the targeted costimulatory receptor will be influenced by a number of factors, including the dynamics of receptor and ligand expression, the presence of the receptor on a disease-relevant cell population and the extent of functional diversity and redundancy between the various receptors. Therefore, a thorough understanding of the biology of costimulatory receptors and knowledge of their relevance to human disease are critical for selecting appropriate receptors for therapeutic intervention.

In this review we summarize recent advances in the understanding of the immunobiology of OX40 and CD27 focusing on their role in human immune responses and their potential as targets for promoting anti-tumor immunity.

Structure of OX40, CD27 and their ligands

In common with other members of the TNFRSF, OX40 (also known as TNFRSF4 or CD134) and CD27 (TNFRSF7) are type I transmembrane glycoproteins characterized by the presence of cysteine-rich domains (CRDs) which typically incorporate 3 disulfide bridges[17]. While human OX40 and CD27 are similar in length (277 and 260 residues respectively), and exhibit some homology at the protein level, OX40 contains 4 CRDs, whereas CD27 contains only 3[17] (see Figure 1). Both mature proteins are larger than predicted (approximately 30kDa) at 50-55kDa, due to N-linked and O-linked glycosylation[18-22].

Unlike OX40, and indeed all other members of the TNFRSF, membrane-bound CD27 is a disulfide-linked homodimer due to interactions in cis between cysteine residues at position 165 of the mature protein[18,22], although monomeric CD27 can be detected in serum under inflammatory conditions[23]. Soluble CD27, at 32kDa, is smaller than the membrane-bound form from which it is processed in activated T cells[24-26]. Interestingly the 32kDa form can

also be detected on the surface of activated T cells likely as part of a heterodimeric complex between processed 32kDa and mature 50kDa protomers[24,27]. While soluble OX40 has also been detected in human serum, levels are at least 30-fold lower than reported concentrations of serum CD27[28-30].

The only known ligands for OX40 and CD27 are OX40L (TNFSF4) and CD70 (TNFSF7) respectively. Both are type II transmembrane proteins and contain the conserved TNF-homology domain which enables trimerization[17]. Many TNFRSF ligands can be cleaved to generate a soluble form[17] and although the details of its generation are not clear, soluble OX40L, but not CD70 to our knowledge, can be detected in serum at ng/ml levels[28]. The crystal structure of trimeric human OX40L reveals a splayed pyramidal-type form with a relatively small area of interaction between protomers[20]. On activation three OX40 molecules bind to the OX40L trimer which is typical for ligand-receptor pairings in the TNFRSF[31]; in the case of OX40 CRDs 1, 2 and 3 all interact with OX40L[20]. While the crystal structure of the hCD27/CD70 complex has not been solved, modeling based on the crystal structures of a CD27 extracellular region/antibody complex and the homologous CD40/CD40L complex suggest that a similar 3:3 binding configuration occurs with CRD2 of CD27 interacting with CD70[21]. However, higher-order oligomers possibly dimers of trimers, are more effective ligands for both OX40 and CD27 activation[32,33].

Naturally-arising mutations which impair immunity have been identified in both human OX40 and CD27. For OX40, introduction of an additional cysteine residue within CRD1 (R65C; numbering includes the signal sequence) results in a substantial reduction in surface OX40 on activated human T cells and intracellular accumulation of a misfolded form of the protein[34]. Furthermore, the residual surface-expressed R65C OX40 protein is incapable of binding to OX40L[34]. Similarly for CD27, mutations C53Y, C96Y and R107C, which fall in CRDs 1, 2 and 3 respectively, all contribute to reduced expression of CD27[22,35] (see Figure 1). In contrast, while a patient with a mutation at R78W (in CRD2) showed defects in immunity consistent with impaired CD27 function, crystallographic studies suggest this is likely due to impaired interactions between CD27 and CD70 rather than a defect in CD27 expression[22,35].

Expression patterns

OX40 and CD27

Both OX40 and CD27 are expressed predominantly by T cells although expression patterns differ according to T-cell subset and differentiation/activation status. OX40 is transiently expressed by both human CD4+ and CD8+ T cells following TCR stimulation and is expressed more highly on CD4+ compared with CD8+ T cells in vitro and at tumor sites[36-39]. Thus, both CD4+ and CD8+ T cells are potential targets of OX40-directed immunotherapeutic agents in cancer. Furthermore, studies have shown Tregs to express more OX40 compared with conventional CD4+ T cells in multiple human tumors[38-41], raising the additional possibility of preferentially targeting the $OX40^{hi}$ population with depleting mAbs. Finally, OX40 has also been reported on human neutrophils (in which signaling supports survival) and murine, but not human to our knowledge, NK and NKT cells[42,43].

CD27 is expressed on resting $\alpha\beta$ CD4+ (Treg and Tconv) and CD8+ T cells and also a population of innate-like $V\gamma9V\delta2$-expressing T cells with anti-tumor function[44]. That CD27 is expressed constitutively on naïve T cells whereas OX40 requires TCR stimulation for expression suggests that CD27 may act earlier during priming than OX40. On human T cells CD27 is downregulated on effector cells but is expressed at reasonable levels by stem-cell memory cells and central-memory like cells[45]. In contrast, terminally-differentiated T_{EMRA} and effector-memory cells, comprise mixed populations of both CD27+ and CD27- subsets; while CD27+ cells predominate in the effector-memory population, the T_{EMRA} subset is largely CD27-[46,47]. For NK cells, loss of CD27 is similarly associated with the more effector-like $CD56^{dim}$ population, and even within the $CD56^{bright}$ subpopulation loss of CD27 is associated with greater lytic activity[48]. CD27 has also been described on human B cells, including germinal center, memory and plasma cells but not naive or transitional B cells[49,50]; the function of CD27 on human B cells is poorly understood although stimulation with CD70-transfected cells augments IgG production from human B cells in vitro[51]. Importantly, and similarly to OX40, CD27 is expressed by both effector and regulatory T cell populations at

sites of immune pathology and cancer making both populations potential targets for CD27-targeting treatments[52,53].

OX40L and CD70

Expression of OX40L and CD70 are tightly controlled and the influence of OX40 and CD27 in a given context is dependent on their availability. OX40L is induced on human DCs upon exposure to thymic stromal lymphopoietin, CD40L or TLR agonists although the ability of CD40L and TLR agonists alone to induce OX40L varies between studies[54-56]. In addition, human monocytes, neutrophils, mast cells, lymphoid tissue inducer cells, smooth muscle cells, endothelial cells and in vitro activated B cells can all express OX40L under appropriate conditions[34,57-62]. Furthermore, human CD4+ and CD8+ T cells treated with anti-CD3/CD28-coated beads in the presence of IL-2 and TGF-β1, or exposed to mature DCs, upregulate OX40L in vitro suggesting potential T-T co-stimulatory effects under inflammatory conditions[55,63]. With regards to autoimmunity, expression of OX40L by monocytes has been shown to promote T follicular-helper cell polarization and pathogenesis in human lupus[64] consistent with an association between *TNFSF4* polymorphism and susceptibility to SLE and other autoimmune diseases[65]. A similar mechanism may also contribute to the pathogenesis of sepsis given the enhanced OX40L expression by blood monocytes in septic patients and the requirement for macrophages for sepsis induction in mice[57]. Patterns of CD70 expression are similar with CD70 absent on peripheral human DCs but upregulated following exposure to TNF-α, irradiation or TLR agonists[55,66-68]. Like OX40L, other cell types can express CD70 including B and NK cells, myocytes, and CD4+ and CD8+ T cells[55,66,69,70]. In mice, an antigen-presenting cell population of non-hematopoietic origin that constitutively expresses CD70 persists in the lamina propria which likely supports local T-cell effector responses[71]; it is not known whether this same cell type exists in humans. Some of the interactions that may occur in vivo between CD27/CD70 and OX40/OX40L-expressing cells in humans are indicated in Figure 2.

Signaling by OX40 and CD27

OX40

For both OX40 and CD27, and in common with other members of the TNFRSF, transmembrane signaling is largely mediated through members of the TNF receptor associated factor (TRAF) family. TRAFs are trimeric proteins that interact with short motifs found in the cytoplasmic tails of ligand-bound trimeric TNFRSF receptors[72-74]. For OX40, initial interactions between the receptor and ligand stimulate movement of OX40 and TRAF2 into lipid rafts, an essential step for subsequent activation of NF-kB[75]. OX40 also binds to TRAFs 1, 3 and 5 with all four TRAF-binding sites largely overlapping at sequence GGSFRTPIQEE in the human protein[76-78]; it is not known whether TRAFs other than TRAF2 also co-localize with OX40 in rafts, nor what governs the likelihood of any given TRAF interacting with OX40. For CD40 a hierarchy of binding affinity exists in which TRAF2 binds more strongly than TRAFs 1, 3 or 6, and a similar hierarchy likely dictates patterns of TRAF binding to other members of the TNFRSF[72]. While both TRAF2 and TRAF5 can activate NF-kB signals downstream of OX40[76], knock-down of TRAF2 ablates canonical NF-kB activation and PI3K and Akt recruitment in vitro[75,79], suggesting that TRAF2 engagement is a non-redundant event for some aspects of OX40-induced signaling. Interestingly, others have reported that OX40L-driven activation of the alternative NF-kB pathway at late time points is dependent on TRAF6[78], and it may be that different TRAF proteins confer distinct signaling events. Further downstream, the group of M. Croft have characterized an OX40-activated signalosome complex which includes TRAF2, IKKα, β and γ, PKCθ, RIP1, CARMA1, BCL1, MALT1, the p85 subunit of PI3K and also Akt. Interestingly this complex forms and drives NF-kB activation independent of antigen although OX40-driven Akt phosphorylation is antigen dependent[79].

CD27

CD27 similarly binds to TRAFs 2, 3 and 5 and binding of all three TRAFs is through the REEEGSTIPIQEDYR region of CD27[80,81]. NF-kB activation by CD27 is also driven by TRAF2, and to a lesser extent TRAF5, and these same TRAFs are responsible for triggering JNK activation following CD27 engagement[81]. Like OX40, CD27 triggers both the alternative and

canonical NF-kB pathways, and activation of the alternative pathway by both OX40 and CD27, is inhibited by TRAF3[82].

While the canonical and alternative NF-kB signalling pathways are usually considered distinct[83], downstream of CD27 these pathways are tightly linked. Simplistically canonical NF-kB activation relies on activation of an IKKβ-containing complex leading to release of an active p65/relA transcriptional unit, whereas the alternative pathway is dependent on NIK stabilization, downstream IKKα activation and release of a RelB/p52 dimer[83]. Downstream of CD27 however NIK is recruited to the receptor within minutes, and is required for activation of both NF-kB pathways[84]. For OX40, signaling is more conventional with alternative, but not canonical, NF-kB activation being dependent on NIK[85].

Receptor engagement can also effect reverse signaling through CD70 and OX40L. For instance, OX40L signals induce *c-jun* and *c-fos* transcription in human endothelial cells, promote immunoglobulin secretion from human B cells and augment human DC activation in vitro[60,86], although it should be pointed out that absence of OX40/OX40L signaling does not detectably hinder humoral responses in humans or mice[34,87]. Similarly, reverse signaling by CD70 enhances aspects of human B cell activation and NK cell survival and function in an allogeneic tumor setting[88,89]. These data support the concept that reverse signaling via CD70 might be a potential target for cancer therapy although this assertion is tempered with some caution given that CD70 is also aberrantly expressed by malignant B-cell tumors, in which it confers a pro-proliferative signal to tumor cells[90]. Another point to consider is that agents designed to target OX40 or CD27 may conceivably exert additional biological effects through reducing the availability of OX40 and CD27, and thereby preventing reverse signaling through OX40L or CD70.

Functions of OX40 and CD27 – insights from the clinic

OX40

In vitro studies have shown that stimulation with OX40L enhances proliferation and expression of effector molecules and cytokines by human T cells, although the effects on CD8+ T cells are largely indirect, being due to enhanced T-cell help provided by the CD4+ population[91,92]. Further insights into the role of OX40 in human cells have been provided by the recent characterization of a 19-year old patient with an R65C mutation in OX40 CRD1 that precludes OX40-OX40L signaling[34]. This patient presented with Kaposi's sarcoma (KS), a rare tumor of endothelial cell origin and which arises secondary to chronic human herpes virus 8 (HHV8) infection. KS is often associated with underlying HIV infection and endothelial cells taken from KS sites in HIV patients express abundant OX40L[34], suggesting that enhanced susceptibility to KS as a result of OX40 deficiency may result from locally defective endothelial-driven co-stimulation. This patient also presented with a history of susceptibility to the macrophage-infecting parasite *Leishmania,* potentially indicating a further requirement for OX40 in T cell-mediated activation of macrophages[34].

While overall immune cell frequencies and antibody responses were normal in this patient, a skewed lymphocyte repertoire was apparent with increased frequencies of naïve B, CD4+ and CD8+ T cells and a corresponding decrease in memory B and T cells notably in the T_{EM} compartment. Further analysis showed Treg frequencies (defined as CD4+CD25^{hi}CD127^{lo}) to be substantially diminished while follicular T helper cell numbers (CD4+CD45RA-CXCR5+; important for supporting B-cell function) were unaltered. While CD4+ T-cell recall responses to a number of different antigens were significantly decreased, EBV-reactive CD8+ T cells were detected at normal frequencies confirming that OX40 is redundant for at least some CD8+ T-cell responses and perhaps providing some explanation for the lack of overt susceptibility to standard childhood infections in this patient[34].

CD27

Similarly CD27 agonists (either CD70 or agonist anti-CD27 mAb) costimulate human αβ as well as γδ T cells to undergo proliferation and cytokine production in vitro[44,67,93], although CD27-driven CD8+ T-cell activation can occur directly without a requirement for CD4+ T cells[67]. The capacity of CD27 to directly stimulate CD8+ T cells, coupled with its constitutive

expression on naïve T cells, may make it essential for the clearance of EBV infection since patients which lack functional CD27 or CD70 typically present with EBV infection in childhood and associated Hodgkin's lymphoma[35,94-96], whereas OX40 deficiencies had no apparent effect on the EBV-reactive CD8+ T cell population (see above). Furthermore, patients with defects in CD27 (see also Figure 1) or CD70 report a history of persistent infections throughout childhood[95,96] suggesting a more severe phenotype compared with OX40 deficiency. Investigation of the lymphocyte compartment in CD27/CD70-deficient patients revealed a paucity of memory B, CD4+ and CD8+ T cells to varying extents and hypogammaglobulinemia (consistent with CD27 signals supporting IgG production in vitro[51]), although the frequency of Tregs was unchanged[95,96]. Interestingly, and despite the clear lack of EBV immunity in these patients, T-cell proliferative responses to other mitogens were largely normal[95,96] suggesting that CD70, which is upregulated on B cells in response to EBV infection[95], is important for T cell-mediated immunosurveillance of B cells when a robust immune response is required to counter massive B-cell proliferation such is the case during EBV infection. In contrast, the ability of these patients to mount successful T-cell responses to other common pathogens and childhood vaccines suggests that in situations where antigen is presented by DCs, the absence of CD27-CD70 may not be limiting[95].

Manipulating OX40 and CD27 signals in cancer

OX40

A number of pre-clinical studies support that mAbs targeting OX40 and CD27 can promote T-cell activation and anti-tumor immunity (reviewed in[97] and[43]). For agonistic anti-mouse OX40 mAb (clone OX86; rat IgG1) anti-tumor activity was shown to be due firstly, to restored local DC function as a consequence of Treg inhibition and, secondly, to direct agonist activity towards the effector population[98,99]. A study in 2014 further identified that OX86 depletes tumor-resident Tregs but not CD8+ T cells, likely due to the preferential expression of OX40 on Tregs compared with effector T cells in the tumor[100]; a version of this antibody optimized to bind activatory Fc receptors (FcRs), which have been associated with mAb-mediated cell depletion in other settings, similarly caused tumor rejection and a higher local CD8+/Treg ratio largely as a result of Treg loss[100]. Given the higher expression of OX40

on Tregs compared with effector T cells in human tumors (see above), anti-OX40 mAbs with depleting activity may be similarly effective in the clinic. However, purely agonistic mAbs, with no depleting activity may also be of benefit. In the clinic anti-OX40 clone 9B12 has been shown to induce expression of the proliferation marker Ki-67 on effector CD4+ and CD8+ T cells yet does not do so on Tregs, and Treg depletion was not noted[101]. Nonetheless, augmented tumor-specific immune responses have been observed with this antibody in two melanoma patients[101].

The effects of OX40 agonists on human Tregs remain unclear. OX40 is required for local Treg maintenance in a murine model of colitis and enforced OX40 stimulation has been reported to enhance human Treg (and effector T cell) proliferation[102, 103]. However, others have shown that brief exposure of Tregs to agonist anti-OX40 mAbs directly inhibits their suppressive activity, and that Tregs are more sensitive than other T-cell subsets to apoptosis induced by prolonged exposure to high dose anti-OX40 mAbs[104]. It is therefore possible that agonistic anti-OX40 mAbs may promote expansion of Tregs (although for 9B12 this appears not to be the case[101]), suppress their function or induce their apoptosis.

CD27

Enforced CD27 signals similarly prevent tumor growth. For instance, CD70 over-expressing mice, and mice constitutively expressing CD70 on CD11c-positive cells, mount greater tumor-specific CD8+ T-cell responses and reject tumor cells more effectively than their wild-type counterparts[105, 106]. Furthermore, around a third of patients with germline deficiencies in CD27 or CD70 present with Hodgkin's lymphoma or diffuse large B cell lymphoma[35, 95, 96], and somatic mutations or deletions in CD70 are frequently observed in diffuse large B cell and Burkitt lymphomas[107, 108], confirming that CD27/CD70 signals also contribute to anti-tumor immunity in humans. We and others have shown that agonist mAb targeting CD27 bypass the requirement for CD40 signaling and CD4+ T-cell help to promote CD8+ T-cell dependent tumor rejection in mice[109-111], consistent with the dependency of CD40-mediated DC licencing and subsequent CD8+ T-cell priming on CD70[112-115]. Within the tumor environment an agonist anti-mouse CD27 mAb promotes the local accumulation of tumor-

specific and IFN-γ producing CD8+ T cells[111], and varlilumab, an anti-human CD27 mAb, similarly causes CD8+ T-cell dependent tumor rejection in human-CD27 transgenic mice and enhances the expansion of human T cells in vitro[93,116]. As is becoming increasingly apparent for mAb therapies, the efficacy of varlilumab is dependent on its interaction with FcRs in the host[116].

With regards the effects of CD27 stimulation on Tregs, varlilumab only moderately increased the proliferation of human Tregs although this was likely secondary to increased availability of IL-2 in this in vitro mixed culture assay[93]. In vivo, enforced stimulation with agonist anti-CD27 mAbs reduces Treg frequency in a murine tumor[111] and varlilumab similarly reduces the frequency of Tregs in the peripheral blood of cancer patients[117]. Of note, the most suppressive human Tregs express the highest levels of CD27[118,119] and it seems likely that these would therefore be preferentially depleted by appropriate anti-CD27 mAb although this remains conjecture.

Intriguingly, varlilumab acts as a direct T-cell agonist and also depletes human lymphoma cells in immune-deficient mice[93,120]; whether the mechanism of action is linked to the relative expression levels of CD27 on the target cell population, and/or the local FcR milieu remains to be clarified. Clinically, early indications are that varlilumab is well tolerated and exhibits biological activity consistent with activation of the CD27 pathway, including chemokine induction, particularly IP-10 (CXCL10), and expansion of activated and effector T cells[117]. Importantly this first-in-human trial shows that varlilumab is clinically active, achieving responses similar to those observed with anti-OX40[101].

CD70

Also of relevance here, CD70 is aberrantly expressed by some tumor types including renal-cell carcinoma, in which CD70 drives terminal differentiation of responding T cells[121] and in CLL in which CD70 confers proliferative signals to the tumor[90]. In addition, human CML stem cells receive proliferative signals via the Wnt pathway following CD27 engagement[122]

and AML stem cells were recently shown to express both CD27 and CD70, an interaction which promotes their survival[123]. Encouragingly, a blocking anti-CD70 mAb prevented the growth of AML blast cells in a xenotransplantation model[123]. These studies suggest that mAbs that either block CD70 signals or deplete CD70+ tumor cells would be an appropriate therapeutic strategy for some tumors. To date, an anti-CD70 mAb (MDX-1203; Table 1) is well tolerated in renal carcinoma and NHL patients with some evidence for improved stabilization of disease at higher doses[124].

A summary of current clinical trials targeting OX40 and CD27/CD70 can be found in Table 1.

Combining OX40 and CD27 mAbs with other agents

While agonist mAbs targeting OX40 and CD27 can impart tumor protection in mice these have limited effects in poorly immunogenic settings[110,125]. There is therefore increasing interest in combining these mAbs with additional strategies to e.g. enhance antigen availability, augment inflammation, or inhibit immunosuppressive signals. Thus, co-targeting toll-like receptors, or the addition of IFN-α, profoundly enhances the effects of agonist CD27 mAb for expansion of antigen-specific CD8+ T cells in mice dependent on the direct effects of type I IFN on CD8+ T cells[126]. In addition, agonist anti-OX40 and anti-CD27 mAbs synergize with PD-L1 blockade to enhance the proliferation and function of exhausted CD8+ T cells[127]. Finally, given the preferential activation of human CD4+ T cells by OX40 yet the ability of CD27 agonists to directly promote CD8+ T-cell responses[67,92], it may be that coincident OX40 and CD27 activation would also synergize to promote stronger T-cell immunity compared with activation achieved through either of the individual receptors. In these exciting times for cancer immunotherapy, combining mAbs targeting CD27, OX40 and/or checkpoint-blocking mAbs thus represents a particularly promising approach to achieve even greater therapeutic success in a variety of cancers.

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Figure 1. Structures of human OX40 and CD27 and locations of clinically-described mutations. Schematic of full-length human OX40 and CD27 (including the signal peptides), showing CRDs 1, 2, 3 and in the case of OX40 also CRD 4 in progressively lighter shades of blue with positions of the CRDs shown as defined from crystallographic studies. Residues within the extracellular region of the receptor but which do not fall within a CRD are indicated by a vertical line, the transmembrane regions are shown in grey and cytoplasmic residues are represented in pink. Amino acid numbers are indicated and positions of cysteine residues within the CRDs are shown by a horizontal line. Positions of mutations described in patients, and their impact on receptor expression where known are also shown. Why a heterozygous C10X mutation should result in absent CD27 expression is unclear; the C10X heterozygous mutation in the patient's father resulted in only partial loss of CD27. It is likely that the patient's mother, who also exhibited defects in CD27 expression, carries an unidentified mutation elsewhere in the genome (no mutations could be identified in the CD27 locus) which causes sub-optimal CD27 expression. Thus, in reality the patient is likely to be a compound heterozygote having inherited the C10X mutation from her father and an unknown mutation from her mother (see reference 35).

Figure 2. Diagrammatic representation of potential interactions between OX40/OX40L and CD27/CD70-expressing human cells in an inflamed environment. For simplicity tumor cells are not shown here although some tumor types may additionally express CD27 and CD70 (see the accompanying text for details).

References

1. Lafferty KJ, Prowse SJ, Simeonovic CJ, Warren HS. Immunobiology of tissue transplantation: a return to the passenger leukocyte concept. *Annu Rev Immunol*. 1983;1:143-173.
2. Mueller DL, Jenkins MK, Schwartz RH. Clonal expansion versus functional clonal inactivation: a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu Rev Immunol*. 1989;7:445-480.
3. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA. CD28 Costimulation: From Mechanism to Therapy. *Immunity*. 2016;44(5):973-988.
4. Camerini D, Walz G, Loenen WA, Borst J, Seed B. The T cell activation antigen CD27 is a member of the nerve growth factor/tumor necrosis factor receptor gene family. *J Immunol*. 1991;147(9):3165-3169.
5. Mallett S, Fossum S, Barclay AN. Characterization of the MRC OX40 antigen of activated CD4 positive T lymphocytes--a molecule related to nerve growth factor receptor. *EMBO J*. 1990;9(4):1063-1068.
6. Dougall WC, Kurtulus S, Smyth MJ, Anderson AC. TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy. *Immunol Rev*. 2017;276(1):112-120.
7. Wikenheiser DJ, Stumhofer JS. ICOS Co-Stimulation: Friend or Foe? *Front Immunol*. 2016;7:304.
8. Ward-Kavanagh LK, Lin WW, Sedy JR, Ware CF. The TNF Receptor Superfamily in Co-stimulating and Co-inhibitory Responses. *Immunity*. 2016;44(5):1005-1019.
9. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*. 1995;182(2):459-465.
10. Kamphorst AO, Wieland A, Nasti T, et al. Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. *Science*. 2017;355(6332):1423-1427.
11. Hui E, Cheung J, Zhu J, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science*. 2017;355(6332):1428-1433.
12. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008;322(5899):271-275.
13. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. *Immunol Rev*. 2008;224:166-182.
14. Lo B, Zhang K, Lu W, et al. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science*. 2015;349(6246):436-440.
15. Lo B, Fritz JM, Su HC, Uzel G, Jordan MB, Lenardo MJ. CHAI and LATAIE: new genetic diseases of CTLA-4 checkpoint insufficiency. *Blood*. 2016;128(8):1037-1042.
16. Kitchens WH, Dong Y, Mathews DV, et al. Interruption of OX40L signaling prevents costimulation blockade-resistant allograft rejection. *JCI Insight*. 2017;2(5):e90317.
17. Bodmer JL, Schneider P, Tschopp J. The molecular architecture of the TNF superfamily. *Trends Biochem Sci*. 2002;27(1):19-26.
18. van Lier RA, Borst J, Vroom TM, et al. Tissue distribution and biochemical and functional properties of Tp55 (CD27), a novel T cell differentiation antigen. *J Immunol*. 1987;139(5):1589-1596.
19. Borst J, Sluyser C, De Vries E, Klein H, Melief CJ, Van Lier RA. Alternative molecular form of human T cell-specific antigen CD27 expressed upon T cell activation. *Eur J Immunol*. 1989;19(2):357-364.
20. Compaan DM, Hymowitz SG. The crystal structure of the costimulatory OX40-OX40L complex. *Structure*. 2006;14(8):1321-1330.
21. Obmolova G, Teplyakov A, Malia TJ, et al. Epitope-dependent mechanisms of CD27 neutralization revealed by X-ray crystallography. *Mol Immunol*. 2017;83:92-99.

22. Teplyakov A, Obmolova G, Malia TJ, Gilliland GL. Crystal structure of CD27 in complex with a neutralizing noncompeting antibody. *Acta Crystallogr F Struct Biol Commun*. 2017;73(Pt 5):294-299.
23. Han BK, Olsen NJ, Bottaro A. The CD27-CD70 pathway and pathogenesis of autoimmune disease. *Semin Arthritis Rheum*. 2016;45(4):496-501.
24. Loenen WA, De Vries E, Gravestein LA, Hintzen RQ, Van Lier RA, Borst J. The CD27 membrane receptor, a lymphocyte-specific member of the nerve growth factor receptor family, gives rise to a soluble form by protein processing that does not involve receptor endocytosis. *Eur J Immunol*. 1992;22(2):447-455.
25. Kato K, Chu P, Takahashi S, Hamada H, Kipps TJ. Metalloprotease inhibitors block release of soluble CD27 and enhance the immune stimulatory activity of chronic lymphocytic leukemia cells. *Exp Hematol*. 2007;35(3):434-442.
26. Burchill MA, Tamburini BA, Kedl RM. T cells compete by cleaving cell surface CD27 and blocking access to CD70-bearing APCs. *Eur J Immunol*. 2015;45(11):3140-3149.
27. Hintzen RQ, de Jong R, Hack CE, et al. A soluble form of the human T cell differentiation antigen CD27 is released after triggering of the TCR/CD3 complex. *J Immunol*. 1991;147(1):29-35.
28. Laustsen JK, Rasmussen TK, Stengaard-Pedersen K, et al. Soluble OX40L is associated with presence of autoantibodies in early rheumatoid arthritis. *Arthritis Res Ther*. 2014;16(5):474.
29. Ruf M, Mittmann C, Nowicka AM, et al. pVHL/HIF-regulated CD70 expression is associated with infiltration of CD27+ lymphocytes and increased serum levels of soluble CD27 in clear cell renal cell carcinoma. *Clin Cancer Res*. 2015;21(4):889-898.
30. Huang J, Jochems C, Anderson AM, et al. Soluble CD27-pool in humans may contribute to T cell activation and tumor immunity. *J Immunol*. 2013;190(12):6250-6258.
31. Banner DW, D'Arcy A, Janes W, et al. Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell*. 1993;73(3):431-445.
32. Muller N, Wyzgol A, Munkel S, Pfizenmaier K, Wajant H. Activity of soluble OX40 ligand is enhanced by oligomerization and cell surface immobilization. *FEBS J*. 2008;275(9):2296-2304.
33. Wyzgol A, Muller N, Fick A, et al. Trimer stabilization, oligomerization, and antibody-mediated cell surface immobilization improve the activity of soluble trimers of CD27L, CD40L, 41BBL, and glucocorticoid-induced TNF receptor ligand. *J Immunol*. 2009;183(3):1851-1861.
34. Byun M, Ma CS, Akcay A, et al. Inherited human OX40 deficiency underlying classic Kaposi sarcoma of childhood. *J Exp Med*. 2013;210(9):1743-1759.
35. Alkhairy OK, Perez-Becker R, Driessen GJ, et al. Novel mutations in TNFRSF7/CD27: Clinical, immunologic, and genetic characterization of human CD27 deficiency. *J Allergy Clin Immunol*. 2015;136(3):703-712 e710.
36. Fujita T, Ukyo N, Hori T, Uchiyama T. Functional characterization of OX40 expressed on human CD8+ T cells. *Immunol Lett*. 2006;106(1):27-33.
37. Xie F, Wang Q, Chen Y, et al. Characterization and application of two novel monoclonal antibodies against human OX40: costimulation of T cells and expression on tumor as well as normal gland tissues. *Tissue Antigens*. 2006;67(4):307-317.
38. Montler R, Bell RB, Thalhofer C, et al. OX40, PD-1 and CTLA-4 are selectively expressed on tumor-infiltrating T cells in head and neck cancer. *Clin Transl Immunology*. 2016;5(4):e70.
39. Lai C, August S, Albibas A, et al. OX40+ Regulatory T Cells in Cutaneous Squamous Cell Carcinoma Suppress Effector T-Cell Responses and Associate with Metastatic Potential. *Clin Cancer Res*. 2016;22(16):4236-4248.
40. Timperi E, Pacella I, Schinzari V, et al. Regulatory T cells with multiple suppressive and potentially pro-tumor activities accumulate in human colorectal cancer. *Oncoimmunology*. 2016;5(7):e1175800.
41. Piconese S, Timperi E, Pacella I, et al. Human OX40 tunes the function of regulatory T cells in tumor and nontumor areas of hepatitis C virus-infected liver tissue. *Hepatology*. 2014;60(5):1494-1507.

42. Baumann R, Yousefi S, Simon D, Russmann S, Mueller C, Simon HU. Functional expression of CD134 by neutrophils. *Eur J Immunol*. 2004;34(8):2268-2275.
43. Croft M. The role of TNF superfamily members in T-cell function and diseases. *Nat Rev Immunol*. 2009;9(4):271-285.
44. DeBarros A, Chaves-Ferreira M, d'Orey F, Ribot JC, Silva-Santos B. CD70-CD27 interactions provide survival and proliferative signals that regulate T cell receptor-driven activation of human gammadelta peripheral blood lymphocytes. *Eur J Immunol*. 2011;41(1):195-201.
45. Mahnke YD, Brodie TM, Sallusto F, Roederer M, Lugli E. The who's who of T-cell differentiation: human memory T-cell subsets. *Eur J Immunol*. 2013;43(11):2797-2809.
46. Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun Ageing*. 2008;5:6.
47. Romero P, Zippelius A, Kurth I, et al. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. *J Immunol*. 2007;178(7):4112-4119.
48. Vossen MT, Matmati M, Hertoghs KM, et al. CD27 defines phenotypically and functionally different human NK cell subsets. *J Immunol*. 2008;180(6):3739-3745.
49. Jung J, Choe J, Li L, Choi YS. Regulation of CD27 expression in the course of germinal center B cell differentiation: the pivotal role of IL-10. *Eur J Immunol*. 2000;30(8):2437-2443.
50. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. *Blood*. 2005;105(11):4390-4398.
51. Morimoto S, Kanno Y, Tanaka Y, et al. CD134L engagement enhances human B cell Ig production: CD154/CD40, CD70/CD27, and CD134/CD134L interactions coordinately regulate T cell-dependent B cell responses. *J Immunol*. 2000;164(8):4097-4104.
52. Ruprecht CR, Gattorno M, Ferlito F, et al. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. *J Exp Med*. 2005;201(11):1793-1803.
53. Gros A, Robbins PF, Yao X, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. *J Clin Invest*. 2014;124(5):2246-2259.
54. Ito T, Wang YH, Duramad O, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med*. 2005;202(9):1213-1223.
55. Krause P, Bruckner M, Uermosi C, Singer E, Groettrup M, Legler DF. Prostaglandin E(2) enhances T-cell proliferation by inducing the costimulatory molecules OX40L, CD70, and 4-1BBL on dendritic cells. *Blood*. 2009;113(11):2451-2460.
56. Ohshima Y, Tanaka Y, Tozawa H, Takahashi Y, Maliszewski C, Delespesse G. Expression and function of OX40 ligand on human dendritic cells. *J Immunol*. 1997;159(8):3838-3848.
57. Karulf M, Kelly A, Weinberg AD, Gold JA. OX40 ligand regulates inflammation and mortality in the innate immune response to sepsis. *J Immunol*. 2010;185(8):4856-4862.
58. Krimmer DI, Loseli M, Hughes JM, et al. CD40 and OX40 ligand are differentially regulated on asthmatic airway smooth muscle. *Allergy*. 2009;64(7):1074-1082.
59. Kim S, Han S, Withers DR, et al. CD117(+) CD3(-) CD56(-) OX40Lhigh cells express IL-22 and display an LTi phenotype in human secondary lymphoid tissues. *Eur J Immunol*. 2011;41(6):1563-1572.
60. Wang Q, Chen Y, Ge Y, et al. Characterization and functional study of five novel monoclonal antibodies against human OX40L highlight reverse signalling: enhancement of IgG production of B cells and promotion of maturation of DCs. *Tissue Antigens*. 2004;64(5):566-574.
61. Fujita T, Kambe N, Uchiyama T, Hori T. Type I interferons attenuate T cell activating functions of human mast cells by decreasing TNF-alpha production and OX40 ligand expression while increasing IL-10 production. *J Clin Immunol*. 2006;26(6):512-518.
62. Kashiwakura J, Yokoi H, Saito H, Okayama Y. T cell proliferation by direct cross-talk between OX40 ligand on human mast cells and OX40 on human T cells: comparison of gene expression profiles between human tonsillar and lung-cultured mast cells. *J Immunol*. 2004;173(8):5247-5257.
63. Kondo K, Okuma K, Tanaka R, et al. Requirements for the functional expression of OX40 ligand on human activated CD4+ and CD8+ T cells. *Hum Immunol*. 2007;68(7):563-571.

64. Jacquemin C, Schmitt N, Contin-Bordes C, et al. OX40 Ligand Contributes to Human Lupus Pathogenesis by Promoting T Follicular Helper Response. *Immunity*. 2015;42(6):1159-1170.
65. Deng Y, Tsao BP. Genetic susceptibility to systemic lupus erythematosus in the genomic era. *Nat Rev Rheumatol*. 2010;6(12):683-692.
66. Huang J, Wang QJ, Yang S, et al. Irradiation enhances human T-cell function by upregulating CD70 expression on antigen-presenting cells in vitro. *J Immunother*. 2011;34(4):327-335.
67. Polak ME, Newell L, Taraban VY, et al. CD70-CD27 interaction augments CD8+ T-cell activation by human epidermal Langerhans cells. *J Invest Dermatol*. 2012;132(6):1636-1644.
68. Oosterhoff D, Heusinkveld M, Lougheed SM, et al. Intradermal delivery of TLR agonists in a human explant skin model: preferential activation of migratory dendritic cells by polyribosinic-polyribocytidylic acid and peptidoglycans. *J Immunol*. 2013;190(7):3338-3345.
69. Seko Y, Ishiyama S, Nishikawa T, et al. Expression of tumor necrosis factor ligand superfamily costimulatory molecules CD27L, CD30L, OX40L and 4-1BBL in the heart of patients with acute myocarditis and dilated cardiomyopathy. *Cardiovasc Pathol*. 2002;11(3):166-170.
70. Kashii Y, Giorda R, Herberman RB, Whiteside TL, Vujanovic NL. Constitutive expression and role of the TNF family ligands in apoptotic killing of tumor cells by human NK cells. *J Immunol*. 1999;163(10):5358-5366.
71. Laouar A, Haridas V, Vargas D, et al. CD70+ antigen-presenting cells control the proliferation and differentiation of T cells in the intestinal mucosa. *Nat Immunol*. 2005;6(7):698-706.
72. Pullen SS, Labadia ME, Ingraham RH, et al. High-affinity interactions of tumor necrosis factor receptor-associated factors (TRAFs) and CD40 require TRAF trimerization and CD40 multimerization. *Biochemistry*. 1999;38(31):10168-10177.
73. McWhirter SM, Pullen SS, Holton JM, Crute JJ, Kehry MR, Alber T. Crystallographic analysis of CD40 recognition and signaling by human TRAF2. *Proc Natl Acad Sci U S A*. 1999;96(15):8408-8413.
74. Park YC, Burkitt V, Villa AR, Tong L, Wu H. Structural basis for self-association and receptor recognition of human TRAF2. *Nature*. 1999;398(6727):533-538.
75. So T, Soroosh P, Eun SY, Altman A, Croft M. Antigen-independent signalosome of CARMA1, PKCtheta, and TNF receptor-associated factor 2 (TRAF2) determines NF-kappaB signaling in T cells. *Proc Natl Acad Sci U S A*. 2011;108(7):2903-2908.
76. Kawamata S, Hori T, Imura A, Takaori-Kondo A, Uchiyama T. Activation of OX40 signal transduction pathways leads to tumor necrosis factor receptor-associated factor (TRAF) 2- and TRAF5-mediated NF-kappaB activation. *J Biol Chem*. 1998;273(10):5808-5814.
77. Arch RH, Thompson CB. 4-1BB and Ox40 are members of a tumor necrosis factor (TNF)-nerve growth factor receptor subfamily that bind TNF receptor-associated factors and activate nuclear factor kappaB. *Mol Cell Biol*. 1998;18(1):558-565.
78. Xiao X, Balasubramanian S, Liu W, et al. OX40 signaling favors the induction of T(H)9 cells and airway inflammation. *Nat Immunol*. 2012;13(10):981-990.
79. So T, Choi H, Croft M. OX40 complexes with phosphoinositide 3-kinase and protein kinase B (PKB) to augment TCR-dependent PKB signaling. *J Immunol*. 2011;186(6):3547-3555.
80. Yamamoto H, Kishimoto T, Minamoto S. NF-kappaB activation in CD27 signaling: involvement of TNF receptor-associated factors in its signaling and identification of functional region of CD27. *J Immunol*. 1998;161(9):4753-4759.
81. Akiba H, Nakano H, Nishinaka S, et al. CD27, a member of the tumor necrosis factor receptor superfamily, activates NF-kappaB and stress-activated protein kinase/c-Jun N-terminal kinase via TRAF2, TRAF5, and NF-kappaB-inducing kinase. *J Biol Chem*. 1998;273(21):13353-13358.
82. Hauer J, Puschner S, Ramakrishnan P, et al. TNF receptor (TNFR)-associated factor (TRAF) 3 serves as an inhibitor of TRAF2/5-mediated activation of the noncanonical NF-kappaB pathway by TRAF-binding TNFRs. *Proc Natl Acad Sci U S A*. 2005;102(8):2874-2879.
83. Hoffmann A, Baltimore D. Circuitry of nuclear factor kappaB signaling. *Immunol Rev*. 2006;210:171-186.

84. Ramakrishnan P, Wang W, Wallach D. Receptor-specific signaling for both the alternative and the canonical NF-kappaB activation pathways by NF-kappaB-inducing kinase. *Immunity*. 2004;21(4):477-489.
85. Murray SE, Polesso F, Rowe AM, et al. NF-kappaB-inducing kinase plays an essential T cell-intrinsic role in graft-versus-host disease and lethal autoimmunity in mice. *J Clin Invest*. 2011;121(12):4775-4786.
86. Matsumura Y, Hori T, Kawamata S, Imura A, Uchiyama T. Intracellular signaling of gp34, the OX40 ligand: induction of c-jun and c-fos mRNA expression through gp34 upon binding of its receptor, OX40. *J Immunol*. 1999;163(6):3007-3011.
87. 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(6):699-708.
88. Al Sayed MF, Ruckstuhl CA, Hilmenyuk T, et al. CD70 reverse signaling enhances NK cell function and immunosurveillance in CD27-expressing B-cell malignancies. *Blood*. 2017.
89. Arens R, Nolte MA, Tesselaar K, et al. Signaling through CD70 regulates B cell activation and IgG production. *J Immunol*. 2004;173(6):3901-3908.
90. Lens SM, Drillenburg P, den Drijver BF, et al. Aberrant expression and reverse signalling of CD70 on malignant B cells. *Br J Haematol*. 1999;106(2):491-503.
91. Baum PR, Gayle RB, 3rd, Ramsdell F, et al. Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34. *EMBO J*. 1994;13(17):3992-4001.
92. Serghides L, Bukczynski J, Wen T, et al. Evaluation of OX40 ligand as a costimulator of human antiviral memory CD8 T cell responses: comparison with B7.1 and 4-1BBL. *J Immunol*. 2005;175(10):6368-6377.
93. Ramakrishna V, Sundarapandiyan K, Zhao B, Bylesjo M, Marsh HC, Keler T. Characterization of the human T cell response to in vitro CD27 costimulation with varlilumab. *J Immunother Cancer*. 2015;3:37.
94. van Montfrans JM, Hoepelman AI, Otto S, et al. CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia. *J Allergy Clin Immunol*. 2012;129(3):787-793 e786.
95. Izawa K, Martin E, Soudais C, et al. Inherited CD70 deficiency in humans reveals a critical role for the CD70-CD27 pathway in immunity to Epstein-Barr virus infection. *J Exp Med*. 2017;214(1):73-89.
96. Abolhassani H, Edwards ES, Ikinciogullari A, et al. Combined immunodeficiency and Epstein-Barr virus-induced B cell malignancy in humans with inherited CD70 deficiency. *J Exp Med*. 2017;214(1):91-106.
97. Aspeslagh S, Postel-Vinay S, Rusakiewicz S, Soria JC, Zitvogel L, Marabelle A. Rationale for anti-OX40 cancer immunotherapy. *Eur J Cancer*. 2016;52:50-66.
98. Weinberg AD, Rivera MM, Prell R, et al. Engagement of the OX-40 receptor in vivo enhances antitumor immunity. *J Immunol*. 2000;164(4):2160-2169.
99. Piconese S, Valzasina B, Colombo MP. OX40 triggering blocks suppression by regulatory T cells and facilitates tumor rejection. *J Exp Med*. 2008;205(4):825-839.
100. Bulliard Y, Jolicoeur R, Zhang J, Dranoff G, Wilson NS, Brogdon JL. OX40 engagement depletes intratumoral Tregs via activating FcgammaRs, leading to antitumor efficacy. *Immunol Cell Biol*. 2014;92(6):475-480.
101. Curti BD, Kovacsovics-Bankowski M, Morris N, et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res*. 2013;73(24):7189-7198.
102. Griseri T, Asquith M, Thompson C, Powrie F. OX40 is required for regulatory T cell-mediated control of colitis. *J Exp Med*. 2010;207(4):699-709.

103. Hippen KL, Harker-Murray P, Porter SB, et al. Umbilical cord blood regulatory T-cell expansion and functional effects of tumor necrosis factor receptor family members OX40 and 4-1BB expressed on artificial antigen-presenting cells. *Blood*. 2008;112(7):2847-2857.
104. Voo KS, Bover L, Harline ML, et al. Antibodies targeting human OX40 expand effector T cells and block inducible and natural regulatory T cell function. *J Immunol*. 2013;191(7):3641-3650.
105. Arens R, Schepers K, Nolte MA, et al. Tumor rejection induced by CD70-mediated quantitative and qualitative effects on effector CD8+ T cell formation. *J Exp Med*. 2004;199(11):1595-1605.
106. Keller AM, Schildknecht A, Xiao Y, van den Broek M, Borst J. Expression of costimulatory ligand CD70 on steady-state dendritic cells breaks CD8+ T cell tolerance and permits effective immunity. *Immunity*. 2008;29(6):934-946.
107. de Miranda NF, Georgiou K, Chen L, et al. Exome sequencing reveals novel mutation targets in diffuse large B-cell lymphomas derived from Chinese patients. *Blood*. 2014;124(16):2544-2553.
108. Scholtysik R, Nagel I, Kreuz M, et al. Recurrent deletions of the TNFSF7 and TNFSF9 genes in 19p13.3 in diffuse large B-cell and Burkitt lymphomas. *Int J Cancer*. 2012;131(5):E830-835.
109. French RR, Taraban VY, Crowther GR, et al. Eradication of lymphoma by CD8 T cells following anti-CD40 monoclonal antibody therapy is critically dependent on CD27 costimulation. *Blood*. 2007;109(11):4810-4815.
110. Ahrends T, Babala N, Xiao Y, Yagita H, van Eenennaam H, Borst J. CD27 Agonism Plus PD-1 Blockade Recapitulates CD4+ T-cell Help in Therapeutic Anticancer Vaccination. *Cancer Res*. 2016;76(10):2921-2931.
111. Roberts DJ, Franklin NA, Kingeter LM, et al. Control of established melanoma by CD27 stimulation is associated with enhanced effector function and persistence, and reduced PD-1 expression of tumor infiltrating CD8(+) T cells. *J Immunother*. 2010;33(8):769-779.
112. Taraban VY, Rowley TF, Al-Shamkhani A. Cutting edge: a critical role for CD70 in CD8 T cell priming by CD40-licensed APCs. *J Immunol*. 2004;173(11):6542-6546.
113. Feau S, Garcia Z, Arens R, Yagita H, Borst J, Schoenberger SP. The CD4(+) T-cell help signal is transmitted from APC to CD8(+) T-cells via CD27-CD70 interactions. *Nat Commun*. 2012;3:948.
114. Taraban VY, Rowley TF, Tough DF, Al-Shamkhani A. Requirement for CD70 in CD4+ Th cell-dependent and innate receptor-mediated CD8+ T cell priming. *J Immunol*. 2006;177(5):2969-2975.
115. Taraban VY, Martin S, Attfield KE, et al. Invariant NKT cells promote CD8+ cytotoxic T cell responses by inducing CD70 expression on dendritic cells. *J Immunol*. 2008;180(7):4615-4620.
116. He LZ, Prostak N, Thomas LJ, et al. Agonist anti-human CD27 monoclonal antibody induces T cell activation and tumor immunity in human CD27-transgenic mice. *J Immunol*. 2013;191(8):4174-4183.
117. Burris HA, Infante JR, Ansell SM, et al. Safety and Activity of Varlilumab, a Novel and First-in-Class Agonist Anti-CD27 Antibody, in Patients With Advanced Solid Tumors. *J Clin Oncol*. 2017:JCO2016701508.
118. Koenen HJ, Fasse E, Joosten I. CD27/CFSE-based ex vivo selection of highly suppressive alloantigen-specific human regulatory T cells. *J Immunol*. 2005;174(12):7573-7583.
119. Coenen JJ, Koenen HJ, van Rijssen E, Hilbrands LB, Joosten I. Rapamycin, and not cyclosporin A, preserves the highly suppressive CD27+ subset of human CD4+CD25+ regulatory T cells. *Blood*. 2006;107(3):1018-1023.
120. Vitale LA, He LZ, Thomas LJ, et al. Development of a human monoclonal antibody for potential therapy of CD27-expressing lymphoma and leukemia. *Clin Cancer Res*. 2012;18(14):3812-3821.
121. Wang QJ, Hanada K, Robbins PF, Li YF, Yang JC. Distinctive features of the differentiated phenotype and infiltration of tumor-reactive lymphocytes in clear cell renal cell carcinoma. *Cancer Res*. 2012;72(23):6119-6129.

122. Schurch C, Riether C, Matter MS, Tzankov A, Ochsenbein AF. CD27 signaling on chronic myelogenous leukemia stem cells activates Wnt target genes and promotes disease progression. *J Clin Invest*. 2012;122(2):624-638.
123. Riether C, Schurch CM, Buhrer ED, et al. CD70/CD27 signaling promotes blast stemness and is a viable therapeutic target in acute myeloid leukemia. *J Exp Med*. 2017;214(2):359-380.
124. Owonikoko TK, Hussain A, Stadler WM, et al. First-in-human multicenter phase I study of BMS-936561 (MDX-1203), an antibody-drug conjugate targeting CD70. *Cancer Chemother Pharmacol*. 2016;77(1):155-162.
125. Kjaergaard J, Tanaka J, Kim JA, Rothchild K, Weinberg A, Shu S. Therapeutic efficacy of OX-40 receptor antibody depends on tumor immunogenicity and anatomic site of tumor growth. *Cancer Res*. 2000;60(19):5514-5521.
126. Dong H, Franklin NA, Ritchea SB, Yagita H, Glennie MJ, Bullock TN. CD70 and IFN-1 selectively induce eomesodermin or T-bet and synergize to promote CD8+ T-cell responses. *Eur J Immunol*. 2015;45(12):3289-3301.
127. Buchan SL, Manzo T, Flutter B, et al. OX40- and CD27-mediated costimulation synergizes with anti-PD-L1 blockade by forcing exhausted CD8+ T cells to exit quiescence. *J Immunol*. 2015;194(1):125-133.

Table 1. Ongoing clinical trials targeting OX40, CD27 and CD70 in cancer.

Target	Agent	Delivered with	Trial number	Tumor type
OX40	MEDI6469 (9B12)	Vaccine or adjuvant	NCT01644968	Various advanced
		Surgical resection	NCT02274155	Head and neck
		Surgical resection	NCT02559024	Colorectal
		Radiation	NCT01862900	Breast
		Radiotherapy and cyclophosphamide	NCT01303705	Prostate
	MEDI0562	Monotherapy	NCT02318394	Solid tumors
		Anti-CTLA-4 or anti-PD-L1	NCT02705482	Solid tumors
	PF-04518600	Alone or with anti-4-1BB	NCT02315066	Solid tumors
		Anti-PD-L1 +/- anti-4-1BB	NCT02554812	Solid tumors
		Tyrosine-kinase inhibitor	NCT03092856	Renal
	INCAGN0194	Alone	NCT02923349	Solid tumors
	BMS-986178	Alone or with anti-PD-1/anti-CTLA-4	NCT02737475	Solid tumors
	MOXR0916 (RG7888)	Alone	NCT02219724	Solid tumors
		Anti-PD-L1	NCT03029832	Urothelial
		Anti-PD-L1 +/- anti-VEGF	NCT02410512	Solid tumors
	GSK3174998	Alone or with anti-PD-1	NCT02528357	Solid tumors
	MEDI6383	Alone or with anti-PD-L1	NCT02221960	Solid tumors
CD27	Varlilumab (CDX-1127)	Alone	NCT01460134	Hematologic or solid
		Peptide vaccine and adjuvant	NCT02924038	Glioma
		Peptide vaccine	NCT02270372	Ovarian and breast
		Anti-PD-1	NCT02335918	Refractory
		Anti-PD-1	NCT03038672	B-cell lymphoma
		Anti-tumor mAb-drug conjugate	NCT02302339	Melanoma
CD70	SGN-CD70A	Alone	NCT02216890	CD70+ renal or lymphoma
	SGN-75	Alone	NCT01015911	CD70+ renal or NHL
	ARGX-110	Alone	NCT01813539	CD70+ advanced tumors
		Alone or with chemotherapy	NCT02759250	Nasopharyngeal
	MDX-1203	Alone	NCT00944905	CD70+ renal or NHL

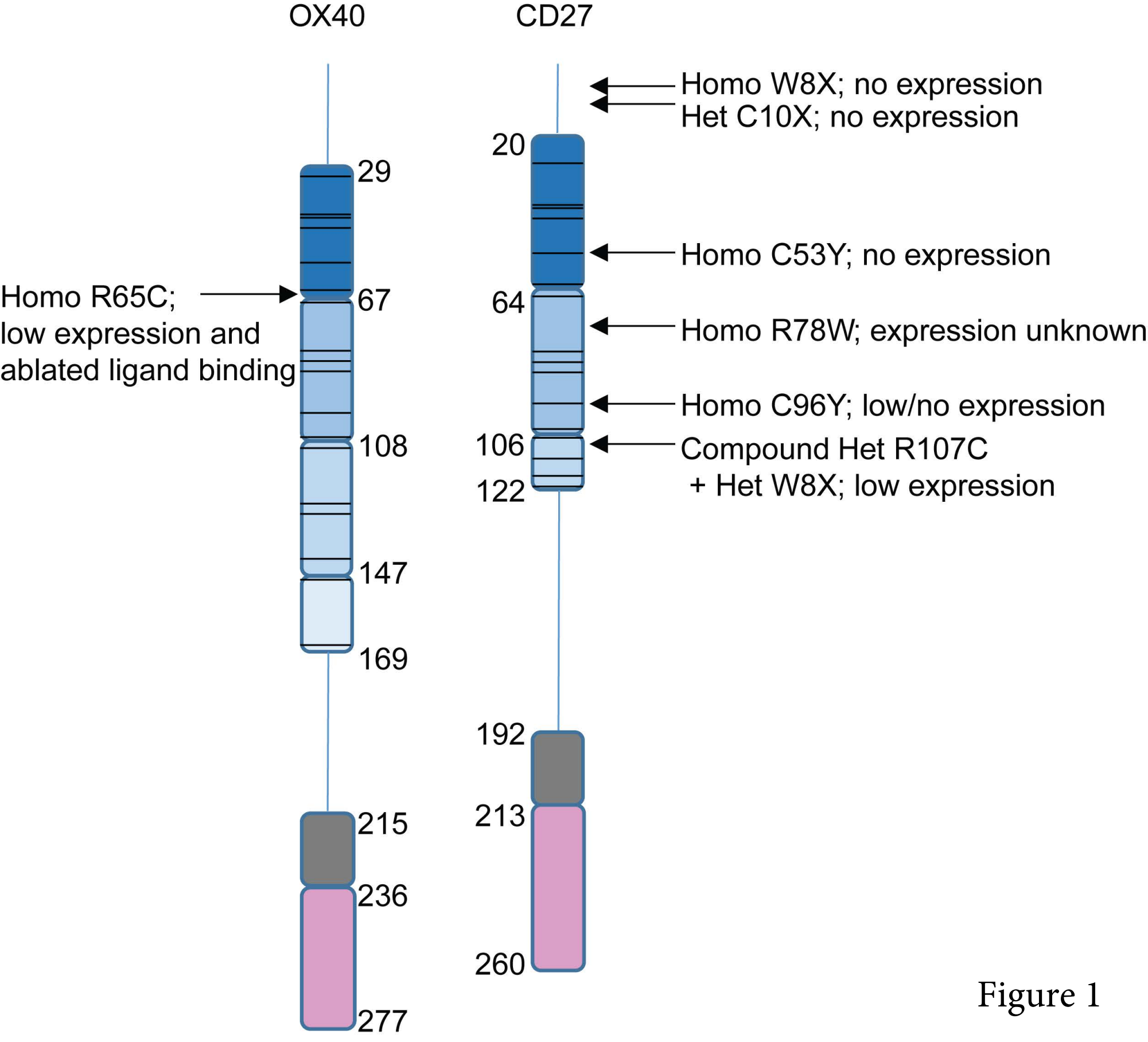
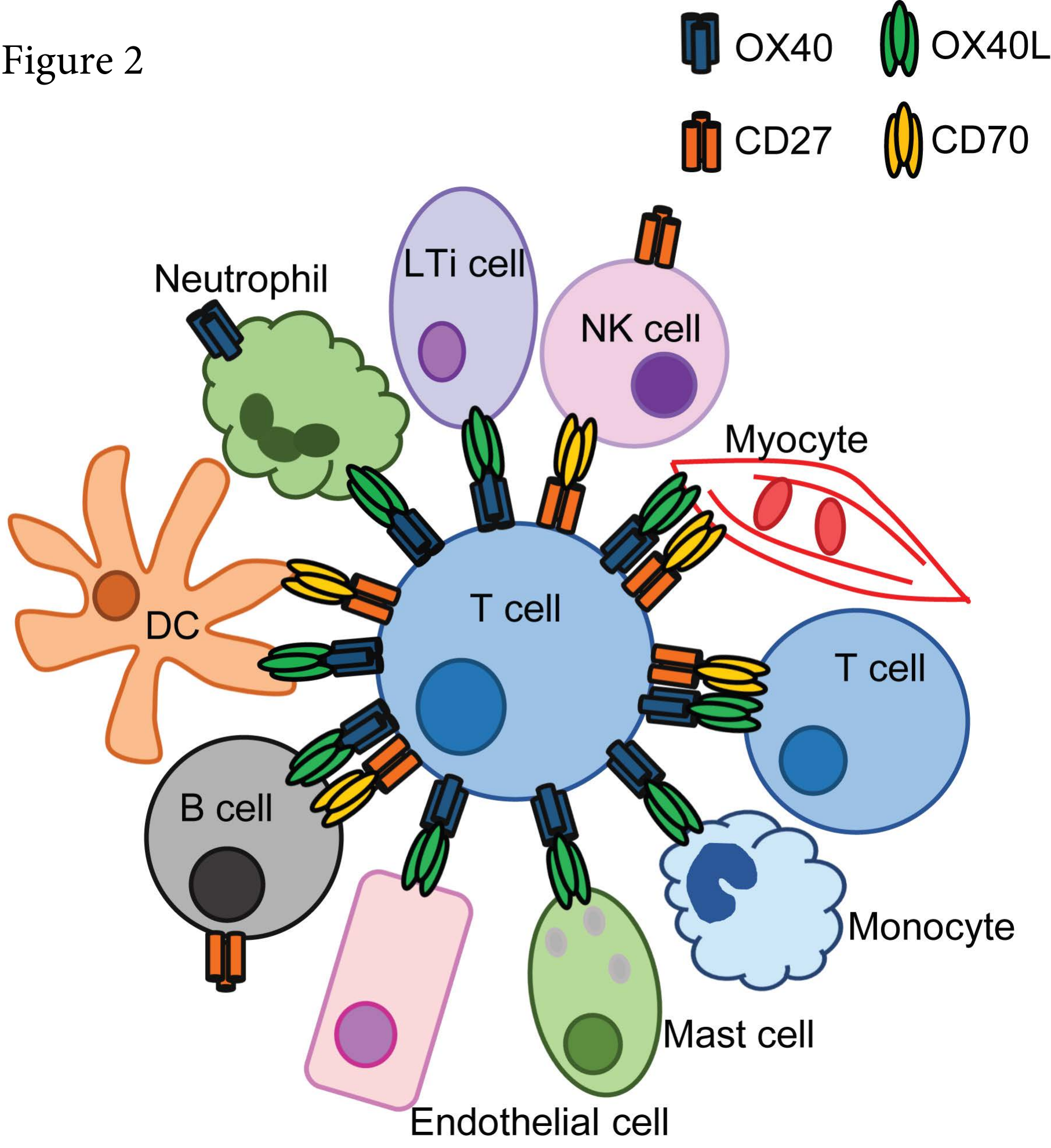

OX40
CD27
Homo W8X; no expression
Het C10X; no expression
20
29
Homo C53Y; no expression
Homo R65C;
low expression and
ablated ligand binding
64
67
Homo R78W; expression unknown
Homo C96Y; low/no expression
106
108
Compound Het R107C
+ Het W8X; low expression
122
147
169
192
213
215
236
260
277

Figure 1

Figure 2


OX40
OX40L
CD27
CD70
Neutrophil
LTi cell
NK cell
Myocyte
DC
T cell
T cell
B cell
Monocyte
Mast cell
Endothelial cell