Agonist Antibodies to TNFR Molecules That Costimulate T and NK Cells

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Abstract

Therapy for cancer can be achieved by artificially stimulating antitumor T and natural killer (NK) lymphocytes with agonist monoclonal antibodies (mAb). T and NK cells express several members of the TNF receptor (TNFR) family specialized in delivering a costimulatory signal on their surface. Engagement of these receptors is typically associated with proliferation, elevated effector functions, resistance to apoptosis, and differentiation into memory cells. These receptors lack any intrinsic enzymatic activity and their signal transduction relies on associations with TNFR-associated factor (TRAF) adaptor proteins. Stimulation of CD137 (4-1BB), CD134 (OX40), and glucocorticoid-induced TNFR (GITR; CD357) promotes impressive tumor-rejecting immunity in a variety of murine tumor models. The mechanisms of action depend on a complex interplay of CTL, T-helper cells, regulatory T cells, dendritic cells, and vascular endothelium in tumors. Agonist mAbs specific for CD137 have shown signs of objective clinical activity in patients with metastatic melanoma, whereas anti-OX40 and anti-GITR mAbs have entered clinical trials. Preclinical evidence suggests that engaging TNFR members would be particularly active with conventional cancer therapies and additional immunotherapeutic approaches. Indeed, T-cell responses elicited to tumor antigens by means of immunogenic tumor cell death are amplified by these immunostimulatory agonist mAbs. Furthermore, anti-CD137 mAbs have been shown to enhance NK-mediated cytotoxicity elicited by rituximab and trastuzumab. Combinations with other immunomodulatory mAb that block T-cell checkpoint blockade receptors such as CTLA-4 and PD-1 are also promising. *Clin Cancer Res; 19(5); 1044–53. ©2013 AACR.*

Introduction

TNF receptor family members provide costimulation to T and NK cells

Lymphocyte activation integrates multiple signals carried and delivered across immune synapses. Critical signals for activation are dependent on specific antigens, such as T-cell antigen receptor (TCR) ligation on T cells or on recognition of antibody-coated target cells sensed by FcRγIII (CD16) on natural killer (NK) cells. Costimulatory molecules will subsequently determine the outcome of the primary antigen recognition by providing signals that will amplify, complement, and modulate those elicited from the TCR or CD16. Costimulation (1) is therefore a pathway of intercellular communication that depends on the expression of complementary glycoproteins on the surface of interacting cells.

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Four families of molecules play important roles in immune synapses: the immunoglobulin superfamily, the integrin superfamily, C-type lectins, and the TNF/TNF receptor (TNFR) families. Receptor–ligand interactions in the immune synapse are important for maintaining structure (adhesion), conveying bidirectional biochemical signals for activation or inhibition, reorganizing the cytoskeleton, and reorienting the secretory machinery. The role of the costimulatory members of the TNFR family seems to be related to signaling. However, it should be noted that many molecular players are acting in a structured and concerted fashion at the synapse including receptors, signaling adaptors, cytoskeletal components, and the distribution of lipids in the interacting plasma membranes (2).

T and NK cells express a panoply of cell surface members belonging to the TNFR family (Fig. 1 and Table 1). Some TNFR members such as CD27 are constitutively expressed. However, the expression of other members such as CD137, OX40, and glucocorticoid-induced TNFR (GITR) are expressed at low levels or not at all in the resting state but are upregulated upon activation (color-coded in Fig. 1). The respective ligands for the TNFR molecules are type II transmembrane proteins, primarily expressed in antigen-presenting cells such as macrophages, dendritic cells, and activated B cells (3, 4). Structural studies have shown that TNFR ligands form trimmers and multimerization is essential for cross-linking the receptors (4, 5).

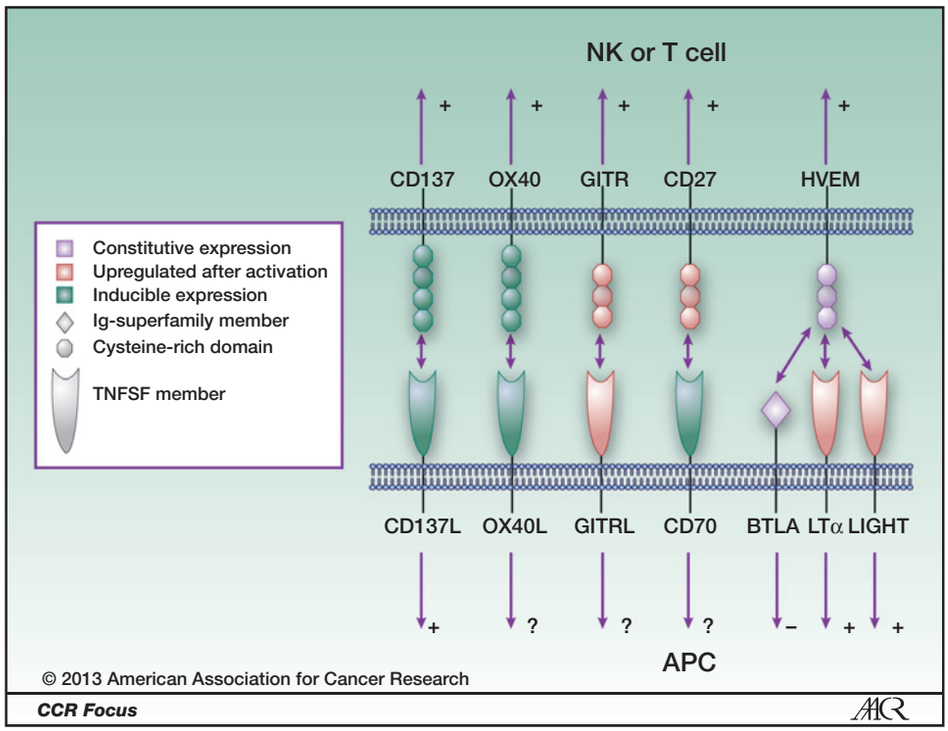

Figure 1. Cell surface–attached costimulatory members of the TNF and TNFR superfamilies. Schematic receptor–ligand pair interactions of the costimulatory members of the TNF and TNFR families at immune synapses. Receptors are color-coded for activation-dependent inducibility and the family of molecules is shape-coded. Plus (+), minus (–), and question mark (?) signs placed at the side of the arrows indicate activatory and inhibitory signals or unknown functional effects. The TNF family with at least 18 described members and TNFR family that encompasses at least 27 members play functions in many other biologic functions beyond costimulation (Table 1) of T and NK responses. It is well known that some of the TNF members act as cell surface–attached molecules and some as soluble cytokines that in some cases can heterotrimerize. Soluble forms of the costimulatory members depicted in the figure have been described but their functional importance remains elusive. We can classify TNFR family members depending on the presence absence of a death domain in the cytoplasmic tail. This death domain recruits apoptosis inducing molecules upon ligation of the receptor and is absent from the costimulatory members whose main function is to convey proinflamatory and activatory signals. The pair CD40/CD40 ligand has not been included, as the main role of CD40 is activating antigen-presenting cells, and has been reviewed in detail in an accompanying review (100).

Knockout mice for TNFR molecules and their ligands show relatively mild phenotypes with partial loss in the ability to fight viral infections controlled by cellular immune response (6). However, cells artificially exposed to a TNFR stimulus via monoclonal antibodies (mAbs) show a highly activated phenotype. Most of the basic knowledge of the TNFR molecules comes from T-cell studies, but additional cell lineages such as NK cells and myeloid cells are known to express TNFR molecules. While the primary function of TNFR family is to provide adequate costimulation, back-signaling by the ligands can convey proinflammatory stimuli (7). Therefore, using artificial ligands such as mAb to engage TNFR molecules forces the receptor system to a point that probably is never reached under physiologic conditions when these molecules are acting confined to immune synapses during transient cell–cell interactions (8).

These families of receptor–ligand pairs are susceptible to multiple layers of regulation because of the following mechanistic facts:

(i) The level of surface expression depends on the activation state of the lymphocyte: for the immunomodulatory mAb to be effective, expression of the target molecule on tumor-infiltrating lymphocytes or other antitumor T cells is critical.
(ii) Differential expression, distribution, and function on naïve versus memory T-cell subsets.
(iii) Differential recruitment to the cytoplasmic tail of members of the TNFR-associated factor (TRAF) family of signaling adaptors whose expression is also regulated upon activation.
(iv) The level of expression of the ligands is controlled by the activation/maturation state of the antigen-presenting cells.
(v) The existence and regulation of negative feedback mechanisms such as deubiquitinases and phosphatases that quench signals from the receptors.

TNFR family member signaling in immune cells

The immunologic outcome of costimulation can be determined by the nature and intensity of reversible biochemical signals. Specifically, integrated signals from multiple accessory receptors dictate, in a coordinated fashion, the intensity, duration, and quality of the immune response (1). Most costimulatory signaling is regulated at the

Table 1. Members of TNFR superfamily

Without death-domain (costimulatory and proinflamatory)	OX40 (CD34; TNFRSF4)	Immunostimulatory mAbs under clinical development
	CD40 (TNFRSF5)	
	CD27 (TNFRSF7)	
	CD30 (TNFRSF8)	
	CD137 (4-1BB; TNFRSF9)	
	HVEM (CD270) TNFRSF14)	
	GITR (CD357; TNFRSF18)	
	TNFR1B (CD120b; TNFRSF1B)	
	Lymphotoxin-β receptor (CD18; TNFRSF3)	
	DCR3 (TNFRSF6B)	
	DCR1 or TRAILR3 (CD263; TNFRSF10C)	
	RANK (CD265; TNFRSF11A)	
	Fn14 or TWEAKR (CD266; TNFRSF12A)	
	TACI (CD267; TNFRSF13B)	
	BAFFR (CD268; TNFRSF13C)	
	BCM or BCMA (CD269; TNFRSF17)	
	TRADE (TNFRSF19)	
	EDA2R (TNFRSF27)	
Death-domain (apoptosis inducing)	TNFR1A (CD120a) TNFRSF1A)	
	FAS or APO-1 (CD95; TNFRSF6)	
	DR4 or APO-2 or TRAILR (CD261; TNFRSF10A)	
	DR5 or KILLER (CD 262; TNFRSF10B)	
	DCR2 or TRAILR4 (CD264; TNFRSF10D)	
	Osteoprotegerin (TNFRSF11B)	
	NGFR (CD271; TNFRSF16)	
	DR6 (CD358; TNFRSF21)	
	APO-3 or DR3 (TNFRSF25)	

transcriptional level; however, additional mechanisms such as chromatin remodeling, stability of mRNAs, and miRNAs are very likely to play a role.

The TNFR family is a large group of over 27 members that share sequence homology with the TNF and lymphotoxin receptors (Table 1). Some of the members of the TNFR family were originally discovered in T cells (Fig. 1). Biochemically, TNFR family member signaling begins by multimerization of the receptors that eventually lead to the formation of multiprotein complexes important for conveying downstream signaling (9, 10). The cytoplasmic tail of these molecules contains TRAF-binding domains that recruit TRAFs upon receptor–ligand binding (Fig. 2). TRAF2, TRAF1, and TRAF5 are the primary TRAF adaptors reported to interact with the intracellular tails of the costimulatory receptors of the TNFR family (CD137, CD134, and GITR; ref. 11). In addition, TRAF3 and TRAF6 may play a role for some of these receptors (12). TRAF molecules form heterodimers that associate with the receptors and signaling from homo and heterotrimers reportedly has different quantitative and qualitative outcomes (11). For instance, TRAF1 is upregulated upon T-cell activation and would displace homotrimers of TRAF2 generating TRAF2:TRAF1 heterotrimers with functional consequences.

TRAF2 has been reported to exert E3 ubiquitin ligase activity through its RING domain (refs. 13, 14; Fig. 2). TRAF2 is constitutively associated with cIAP1 and 2, which are endowed with E3 ubiquitin ligase activities (15). Upon ligation of the TNFR molecules, TRAF2-associated E3 activity forms polyubiquitin chains linked via their lysine 63 residue (16). These polyubiquitins become attached to TRAF2 and additional protein substrates and may act as second messengers. K-63-polyubiquitins act as docking sites for downstream signaling molecules through recruitment of the TAB1/2-TAK1 complexes that ultimately activate the mitogen-activated protein kinase (MAPK) pathway to form Fos/Jun AP1 transcription factors. In addition, polyubiquitination promotes NEMO- IKK-β complexes to unleash the canonical NF-κB pathway transcription factors (Fig. 2). K63 ubiquitin chains are kept at bay by specific deubiquitinases such as CYLD and A20 whose functional control is not well understood (ref. 17; Fig. 2). It is clear, however, that the deficiency of these enzymes in mice causes autoimmunity and hyperinflammation. TRAF5 also contains a RING ubiquitin ligase catalytic domain and presumably operates in a similar manner. TRAF1 is induced upon T-cell activation and complexes with the receptor. Even though the biochemical functions of TRAF1 are not well understood (18), this adaptor is known to be critical for optimal T-cell memory (19).

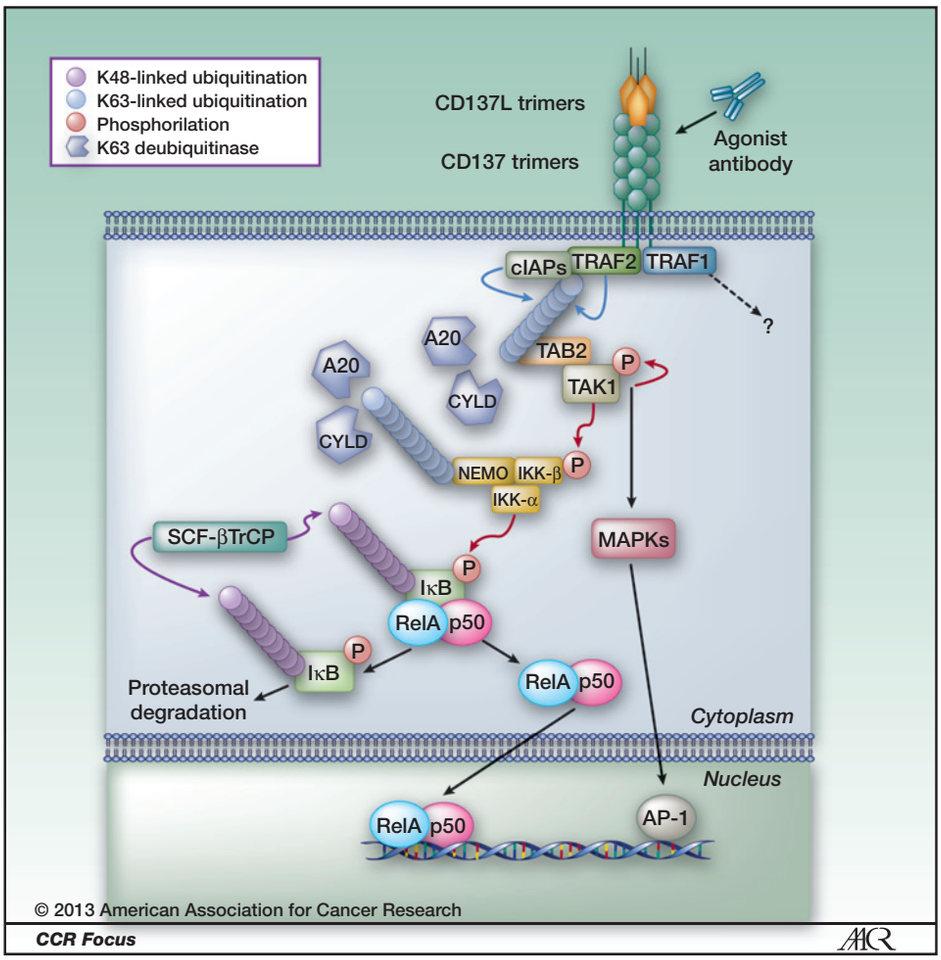

Figure 2. Early signal transduction events from CD137. Schematic representation of TRAF2 and TRAF1 recruitment by CD137 surface molecules perturbed by the natural ligand or agonist mAb. TRAF2 has associated ubiquitin ligase activity (E3) that dictates self-ubiquitination and presumably ubiquitination of other protein targets. These events lead to recruitment of TAB1/2-TAK1 complexes that downstream activate NF-κB and MAPK. Signals controlled or modulated by TRAF1 are less well understood. K63 polyubiquitin chains are removed by deubiquitinases (i.e., CYLD and A20), which keep the pathway under control, and therefore offer potential therapeutic targets.

CD137-based cancer immunotherapy

CD137 (4-1BB, TNFRSF9) is a surface protein originally discovered on activated, but not resting, T cells by Kwon and Weissman (20, 21). CD137 has only one confirmed ligand (CD137-Ligand, TNFSF9) expressed primarily on macrophages, activated B cells, and dendritic cells. In the mouse, NK cells express CD137 when activated by cytokines in contrast to human cells in which surface expression requires ligation of CD16 (22). Expression of CD137 is also found on activated B cells, dendritic cells, myeloid precursors, mast cells, and endothelial cells in tumors or inflamed tissues (8). CD137 and CD137-Ligand deficiency do not cause an overt immune deficiency but only mild alterations in T-cell activation and memory. Mice deficient in CD137 signaling weakly control virulent viral infections (23, 24).

CD137 agonists such as mAb and soluble forms of the ligand have been shown to enhance cytokine production, proliferation, and cytolytic effector functions, and protect lymphocytes from programmed cell death by upregulating BCL-xL and downregulating B-cell lymphoma 2 interacting mediator of cell death (25, 26). CD137 is also expressed by activated regulatory T cells (Treg) and ligation of CD137 on Tregs limits the suppressive function by a mechanism yet to be elucidated (27). Paradoxically, CD137 ligation on Tregs can cause promitogenic effects. Importantly, ligation of CD137 on NK cells enhances cytokine release (including IFN-γ; ref. 28) and potentiates antibody-dependent cellular cytotoxicity (ADCC; refs. 29, 30).

CD137 ligation was first used to treat mouse tumors by means of agonist antibodies (31). As a monotherapy, CD137 mAbs are effective in controlling tumor growth or promoting complete rejection in a variety of transplantable rodent tumors including sarcomas, matocytomas, colon carcinomas, and lymphomas. The mechanism clearly involves enhancement of CTL function (31, 32) and cross-priming of tumor antigens by dendritic cells (ref. 33; Fig. 3). Interestingly, CD4+ T cells seem to be first stimulated and then eliminated by activation-induced cell death (AICD; ref. 34). This is one of the explanations for the paradox that the very same mAbs, which successfully treat tumors, can ameliorate autoimmune diseases by removing autoreactive CD4+ T lymphocytes (35).

Recent reports have shown that CD137 is present on the surface of capillaries in the tumor bed but not in healthy vasculature. One of the reasons hypothesized for the ectopic CD137 expression on vascular cells is hypoxia (36). CD137,

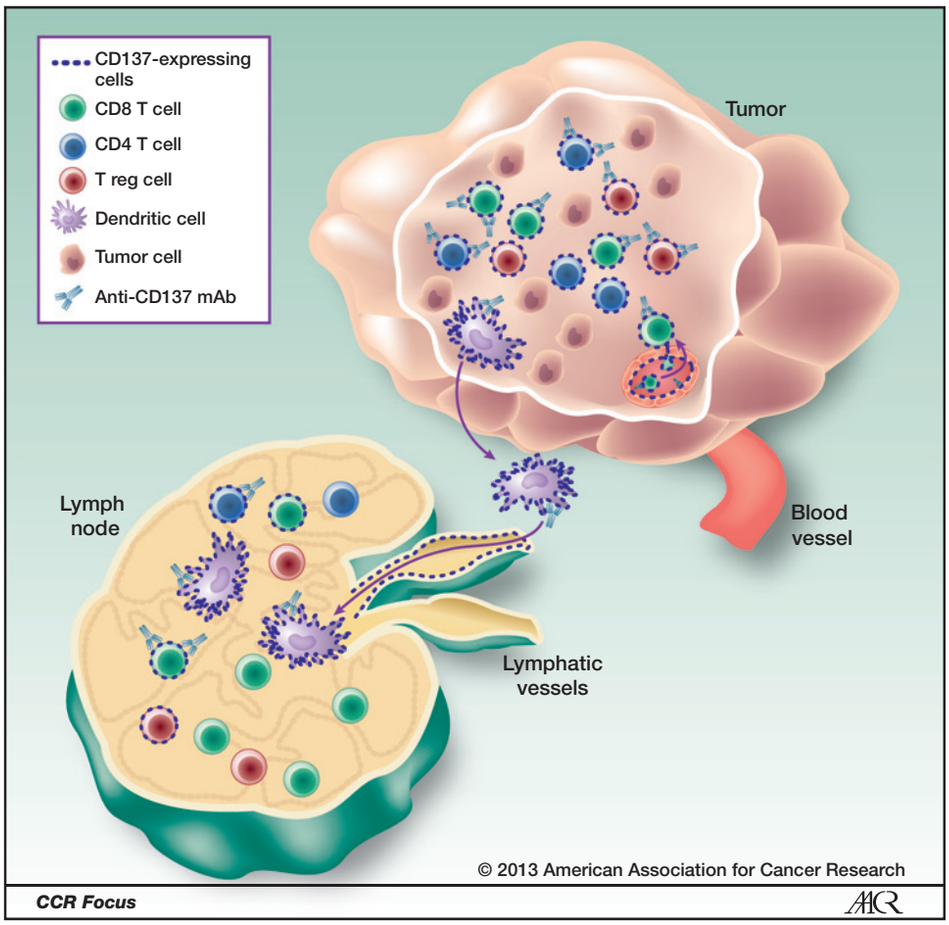

Figure 3. Schematic representation of the sites and cells on which agonist anti-CD137 mAbs act as example of TNFR mAb mechanism. The main mechanism of action is costimulation of CD8+ CTLs cross-primed by dendritic cell against tumor antigens. CD137 expression on dendritic cells, activated CD4 T cells (including Tregs), and on tumor blood and lymphatic vessels could offer additional sites and mechanisms of action. Similar mode of action settings can be envisioned for other TNFR members suitable for targeted cancer immunotherapy.

on endothelial cells, promotes leukocyte infiltration by upregulating the expression of adhesion molecules. Interestingly, CD137 expression by effector and Treg in the tumor microenvironment is dependent on the hypoxia-inducible factor-1α (HIF-1α) pathway, which senses hypoxia. Local costimulation may be effective in treating tumors because of the selective CD137 expression at this hypoxic peritumoral location. This point opens up the possibility for local or targeted delivery of CD137 agonists (37).

Apart from immunostimulatory mAbs, CD137-based immunotherapy has been achieved by transfecting tumor cells to express CD137 ligand (38) or membrane-bound single chain antibodies (39). Multimerizing RNA aptamers (but not monomers), binding selectively to CD137, have also shown antitumor efficacy (40), particularly when targeted to surface tumor antigens (41).

A potentially promising aspect of anti-CD137 mAb immunotherapy is combination with other treatments (both conventional and immunotherapeutic). Combination with chemotherapy (42, 43) and radiotherapy (44) is clearly synergistic in preclinical models and likely dependent on eliciting immunogenic cell death with subsequent cross-priming of tumor antigens. Synergistic combinations with vaccines (45) and virotherapy (46, 47) also rely on the principle that CD137 costimulation must act on ongoing tumor-specific immune responses encompassing CD137+ activated lymphocytes. Recently, some intriguing preclinical studies have shown that anti-CD137 mAb therapy synergizes with NK-mediated ADCC elicited by antibodies targeting the surface antigens CD20 or HER-2 (30, 48).

Preclinical toxicity of CD137 ligation, mainly mediated by polyclonal T-cell infiltrates in the liver (dominated by CD8 T cells), results in mild and reversible transaminase elevations (49). In addition, TNF-α–mediated myelosuppression has been reported (49, 50). Fully human and chimeric mAbs against CD137 have been produced (BMS-663513, PF-05082566, GTC biotherapeutics). These reagents effectively upregulate cellular immune functions and show tolerable toxicity levels in non-human primates (51).

Clinical trials have only been carried out to completion with BMS-663513 (Table 2). This drug has been used in phase I and in multiple-dose phase II clinical trials. Indications of objective clinical activity in melanoma were reported (50). About 10% of the patients developed liver inflammation limiting treatment and 2 fatalities were reported because of liver toxicity at doses greater 1 mg/kg. This speaks to the need to reconsider dose ranges for agonist antibodies, which likely require lower doses for efficacy compared with antagonist antibodies. Both BMS and

Table 2. Clinical trials with antiCD137, antiOX40, antiCD27, and antiGITR mAb

	Study agent (ClinicalTrial.Gov identifier)	Indication disease	Status (Oct 2012)	Doses	Phase
Anti-CD137	BMS-663513 (NCT00309023)	Metastatic solid tumors	Terminated	BMS-663513 (0.3, 1, 3, 6, 10, 15 mg/kg)	I/II
	BMS-663513 (NCT00351325)	Metastatic solid tumors	Terminated	BMS-663513 (0.3, 1, 3, 10 mg/kg) every 3 or 6 wk	I
	BMS-663513 (NCT00461110)	Metastatic Non–small cell lung cancer	Terminated	BMS-663513 (0.3, 1, 3, 6, 10 mg/kg)	I
	BMS-663513 (NCT00612664)	Unresectable stage III or IV Malignant Melanoma	Completed	BMS-663513 (0.1,1,5 mg/kg)	II
	BMS-663513 plus Ipilimumab (NCT00803374)	Unresectable stage III or IV Malignant Melanoma	Withdrawn	BMS-663513 (0.1, 0.3, 1, 3, 10 mg/kg) Ipilimumab (10 mg/kg)	I
	PFZ-05082566 plus rituximab (NCT01307267)	Series A. Solid tumor Series B. Non-Hodgkin's lymphoma	Recruiting	A. PFZ-05082566 Dose escalation B. Rituximab 375 mg/m^2 PFZ-05082566 Dose escalation	I
	BMS-663513 (NCT01471210)	Metastatic solid tumors	Recruiting	BMS-663513 (0.03,0.1,0.3 mg/kg)	I
Anti-OX40	Anti-OX40 plus cyclophosphamide (NCT01303705)	Metastatic prostate Cancer	Recruiting	Anti-OX40 (0.4 mg/kg) Cyclophosphamide (300, 600, and 900 mg/m^2)	I/II
	Anti-OX40 plus radiation (NCT01642290)	Metastatic breast Cancer	Recruiting	Anti-OX40 (0.4 mg/kg)	I
	Anti-OX40 plus KLH and Tetanus toxoid vaccine (NCT01644968)	Metastatic solid tumors	Active, not recruiting	Anti-OX40 (0.1, 0.4, 2 mg/kg d1, 3, 5)	I
Anti-CD27	CDX-1127 (NCT01460134)	Hematologic malignancies metastatic solid tumors	Recruiting	CDX-1127 (0.1–10 mg/kg)	I
Anti-GITR	TRX518 (NCT01239134)	Unresectable stage III or IV Malignant Melanoma	Recruiting	Dose escalation	I

Pfizer have resumed clinical trials, implementing dose-escalation studies that focus on safety (NCT01471210 and NCT01307267).

In the trial with PF-05082566 sponsored by Pfizer (NCT01307267), a combination with rituximab is formally planned as an extension to exploit the ADCC-potentiating effect. If active and safe doses are clinically defined, this will open opportunities for local delivery and combinatorial approaches.

OX40-based cancer immunotherapy

OX40 (CD134 or TNFRSF4) is a costimulatory molecule discovered on the surface of activated CD4+ T cells in rats (52). Expression of OX40 was later found to be restricted to activated CD4+ T cells 24 to 72 hours after TCR engagement. Subsequent studies revealed that OX40 is also found on CD8+ T lymphocytes and other cells such as NK, NKT, and neutrophils (53). CD4+ Foxp3+ Tregs constitutively express OX40 in mice, but human cells upregulate its expression.

Signaling through OX40 increases T-cell survival, promotes clonal expansion, and augments proinflammatory cytokine production (54). Ligation of OX40 is known to recruit TRAF2 and TRAF3, leading to activation of the canonical and noncanonical NF-κB pathways (55, 56). OX40-mediated NF-κB activation subsequently leads to enhanced expression of antiapoptotic molecules such as Bcl-2, Bcl-xl, and survivin, which provide the basis for clonal expansion and expanded memory pool of activated T cells (57).

Given that OX40 engagement can expand T-cell populations, promote cytokine secretion, and support T-cell memory, agonists including mAbs and soluble forms of OX40L have been used successfully in a variety of preclinical tumor models. The first studies, in which anti-OX40 antibodies showed antitumor activity, were pioneered by Weinberg and colleagues. Mirroring early studies where mAbs recognizing inhibitory and costimulatory molecules induce tumor immunity in rodents [anti-CTLA4 and 4-1BB (31, 58)], an anti-OX40 antibody was shown to be effective in a number of tumor models including MC303 sarcomas, CT26 colon carcinomas, SM1 breast cancer, and small B16 melanoma (59). Subsequent studies confirmed these observations in additional preclinical models (60–62).

As a monotherapy, OX40 engagement has been effective in eradicating primarily immunogenic tumors, while failing to provide adequate antitumor immunity in established and more clinically relevant poorly immunogenic tumors. Therefore, a variety of combinatorial strategies to increase anti-OX40 antibody therapy have been explored. Given that OX40 ligation upregulates cytokine receptors on T cells,

OX40 antibodies synergize with cytokines such as interleukin (IL)-2 or IL-12 alone or with vaccination (63, 64). Combining OX40 agonists with granulocyte macrophage colony-stimulating factor (GM-CSF)–secreting syngeneic irradiated tumor cells or DNA vaccination promotes the expansion of tumor-specific T cells, leading to protection or eradication of established cancers [Murata and colleagues (65) and unpublished data]. Furthermore, anti-OX40 antibodies have been combined with other clinically relevant mAbs against inhibitory and costimulatory molecules to treat lymphomas and sarcomas (66, 67).

Modalities with direct cytolytic capability, such as chemotherapy or radiation, have proven particularly effective in treating established tumors when used concomitantly with OX40 agonists (68, 69). In combination with cyclophosphamide, engagement of OX40 not only expands antitumor T-effector cells but also reduces Foxp3+ Tregs by promoting activation-induced cell death. Of interest, elimination and deactivation of Tregs by anti-OX40 antibodies has been important in the antitumor response in some preclinical models (62, 68, 70, 71). Furthermore, given that OX40 ligation can potently stimulate CD4+ T cells, adoptive transfer of antimelanoma CD4+ T cells can eliminate very advanced melanomas when combined with an anti-OX40 antibody and cyclophosphamide. The potency of the therapy is in part attributed to the newly described ability of OX40 engagement to trigger a cytolytic program in CD4+ T cells (72, 73).

Given the substantial evidence from mouse models showing that OX40 agonists can potentiate an antitumor immune response in multiple settings, a clinical grade reagent is now being developed and tested. A mouse anti-human OX40 mAb has shown activity in non-human primates with induction of enlarged lymph nodes and spleens and increased T-cell responses (74). This antibody was further tested in phase I clinical trials in 30 patients where the mouse anti-human OX40 antibody was given on days 1, 3, and 5 at 0.1, 0.4, and 2.0 mg/kg (Table 2). The antibody was well tolerated with minimal toxicity and observation of some tumor size reduction, although none of the patients showed an objective response by Response Evaluation Criteria in Solid Tumors (RECIST) criteria. However, specific proliferation and activation of T cells against keyhole limpet hemocyanin (KLH) or tetanus toxin were observed when these model antigens were coinjected the anti-OX40 antibodies (75). Given that patients showed elevated levels of neutralizing human anti-mouse antibodies, the clinical effectiveness of this antibody is significantly limited. For that reason, humanized anti-OX40 antibodies are being prepared for future clinical trials.

GITR-based cancer immunotherapy

The GITR (TNFRSF18) was originally discovered in T-cell hybridomas that were treated with dexamethasone (76). Glucocorticoid treatment, however, was later shown to have no effect on GITR expression in human T cells and was not necessary in mice (77, 78). GITR is upregulated in T cells 24 to 72 hours after activation, although basal expression of GITR is found both in human and mouse T cells (79). GITR expression has also been found in NK cells, eosinophils, basophils, macrophages, and B cells, particularly upon activation (80).

Similarly to OX40 and CD137, GITR modulates T-cell activation by providing a costimulatory signal. Unique for a TNFR family member, GITR signals through a complex of a single TRAF5 and TRAF2 molecules, suggesting a nonredundant role for this molecule (11). GITR, as a costimulatory molecule, increases proliferation, activation, and cytokine production of CD4+ and CD8+ T cells after TCR engagement. Furthermore, it seems that GITR engagement supports a T-helper 1 cell (T_H1) response in CD4+ T cells in a variety of disease models (80).

Initial studies with an agonist monoclonal rat anti-mouse GITR antibody (DTA-1) show that it can potently stimulate effector T cells, while decreasing the suppressive function of Treg leading to autoimmunity (81–83). Subsequently, it was shown that DTA-1 overrides the suppressive effects of Tregs on T-effector cells (84). Thus, anti-GITR can potentially overcome tolerance to self- and tumor-antigens, making it an attractive target for development as a cancer immunotherapy. Indeed, DTA-1 has been shown to be effective in treating small-established B16 tumors (85, 86) and 8-day established Meth-A sarcomas (87), CT26 (88), and A20 lymphoma (unpublished data). An interesting antitumor property of DTA-1 is its capacity to promote concomitant immunity (89), suggesting the potential for GITR-induced tumor immunity in treating metastatic disease.

DTA-1 has also been successful as an immunologic adjuvant in variety of combinatorial settings. Notably, DTA-1 has shown to substantially enhance the potency of xenogenic DNA vaccines in a melanoma model in which protection is marginal (90). Similarly, dendritic cells engineered to express a melanoma antigen showed higher therapeutic potency when coadministered with DTA-1 or when dendritic cells are engineered to secrete DTA-1 or soluble GITRL (91). Moreover, an adenovirus-based vaccine against human papillomavirus failed to provide complete protection unless it was combined with GITR engagement (92).

A humanized agonist anti-human GITR mAb (TRX518) has been developed by Tolerex Inc. (now GITR Inc), and similarly to DTA-1, provides potent costimulation to human lymphocytes *in vitro*. A dose-escalation phase I clinical trial has been initiated at Memorial Sloan-Kettering Cancer Center (New York, NY) using TRX518 (Table 2).

Future Perspective

While it is clear that agonist antibodies against members of TNFR family can significantly increase antitumor immune responses based on preclinical data, these agents are not realistically expected to induce complete regressions in patients with cancer as monotherapies. Therefore, combinatorial modalities should be explored in future clinical trials. One attractive strategy is to combine cytolytic chemotherapeutic agents with TNFR agonists. In addition to

directly killing tumor cells, these agents can lead to release of self-antigens and TLR agonists that can expand antitumor T cells. In one study, stereotactic radiation is being combined with anti-OX40 in patients with metastatic breast cancer (NCT01642290). Furthermore, anti-OX40 is being combined with cyclophosphamide and radiation in patients with metastatic prostate cancer (NCT01303705).

Another interesting approach is the combination of agonist anti-TNFR antibodies in combination with checkpoint-blockading antibodies, such as anti-CTLA-4 (ipilimumab) or anti-PD-1 (93). Anti-CTLA-4 and anti-PD-1 have shown antitumor activity in about 20% to 30% of patients tested (94–98). Given the nonredundant signaling of the TNFR and checkpoint blockade pathways, it is conceivable that combinations of agonist and antagonist antibodies against these pathways can synergize to yield higher response rates.

Safety is a concern when considering agonist immunomodulatory antibody therapy. While the phase I anti-human OX40 antibody was well tolerated with low toxicity, trials of anti-CD137 mAb were temporarily suspended after fatal hepatic events were observed. Such studies have now been successfully reopened using lower doses of agonist antibody therapy. Conversely, in some models, the use of TNFR antibodies can cause hyperactivation and death of antigen-specific effector T cells (99, 90) with the potential of hampering antitumor immunity. Therefore, careful design of future clinical trials, identification of biomarkers, and lessons from preclinical studies will be necessary to guide future therapies in our quest to develop potent and well-tolerated treatments.

Disclosure of Potential Conflicts of interest

I. Melero has a commercial research grant, honoraria from speakers bureau, and is a consultant/advisory board member of Bristol Myers Squibb. J.D. Wolchok has a commercial research grant from Novartis and Bristol-Myers Squibb and is a consultant/advisory board member of Bristol-Myers Squibb and Merck. No potential conflicts of interest were disclosed by the other authors.

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.F. Sanmamed
Study supervision: J.D. Wolchok

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Clinical Cancer Research

Correction: Agonist Antibodies to TNFR Molecules That Costimulate T and NK Cells

In the print version of this article (Clin Cancer Res 2013;19:1044–53), which was published in the March 1, 2013, issue of *Clinical Cancer Research* (1), a National Clinical Trial identification number and the associated drug, cyclophosphamide, were listed incorrectly.

The original text from page 1051 reads as follows:

Furthermore, anti-OX40 is being combined with cyclophosmamide and radiation in patients with metastatic prostate cancer (NCT01301705).

The correct information is:

Furthermore, anti-OX40 is being combined with cyclophosphamide and radiation in patients with metastatic prostate cancer (NCT01303705).

The online version of this article reflects the correct number and drug name.

Reference

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