TNFR Agonists: A Review of Current Biologics Targeting OX40, 4-1BB, CD27, and GITR

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Abstract

The tumor microenvironment is often immunosuppressive and lacks the appropriate signals necessary for stimulating effective anti-tumor T-cell responses. Therefore, considerable effort in the field of immunotherapy has been dedicated to developing new biologics that target tumor necrosis factor receptors (TNFRs). Signaling through TNFRs elicits a cascade of events critical for overcoming immune suppression, including T-cell activation, differentiation, and the development of long-lived memory cells. In this review, we discuss the biology of TNFRs, the current preclinical studies and clinical trials targeting these receptors, and how they may be combined to synergize with other therapies to improve patient outcomes.

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Background

Immunotherapy is a rapidly evolving field with the goal of using the patients’ immune system to attack cancer. Much attention in the field has been given to inhibitory checkpoints, whereby T cells that have upregulated inhibitory molecules such as CTLA-4, PD-1/PD-L1, TIM-3 (T0cell immunoglobulin mucin-3), and LAG-3 can effectively turn down effector functions such as cytokine release and cytotoxicity—both of which are required to effectively kill tumor cells. This ratcheting down of effector function can be impeded by targeting these inhibitory molecules through the administration of monoclonal antibodies (mAbs) known as checkpoint inhibitors.

However, despite the success of checkpoint blockade with biologics such as anti-CTLA-4 (aCTLA-4) and anti-PD-1 (aPD-1) mAbs, a significant proportion of patients that receive these treatments do not develop therapeutic responses, which highlights the need for more effective therapies.

Optimal T-cell activation requires 2 events: signal 1, which is recognition of the cognate peptide major histocompatibility complex (MHC) by a specific T-cell receptor (TCR), and signal 2, which is ligation of the CD28 costimulatory receptor by its ligands B7.1/B7.2 (CD80/CD86). Together, these are commonly known as T-cell priming. However, in addition to the initial priming event, further signaling is necessary to drive T-cell differentiation and potentiate the development of effector and memory cell subsets; these events enable robust tumor killing activity, in vivo.

The tumor necrosis factor receptors (TNFRs), including glucocorticoid-induced TNFR (GITR; CD357), CD27, OX40 (CD134), and 4-1BB (CD137), are a family of proteins responsible for transducing these additional costimulatory signals. In order to harness this important signaling cascade, agonist mAbs and specific ligand complexes have been developed that can engage with these TNFRs and activate downstream events. In this review, we will discuss the biology of TNFRs, the current preclinical and clinical trials targeting these receptors, and potential synergy with other therapies in order to enhance anti-tumor immunity.

CD27

CD27 costimulation is initiated via its ligand, CD70, a homotrimeric type II membrane protein that is most commonly expressed on antigen presenting cells (APCs) after immune activation.15 Like other TNFR family members, CD27 is expressed on naive CD4+ and CD8+ T cells; however, CD27 differs from other TNFR family members in that it is commonly shed from the cell surface following T-cell activation. Furthermore, this soluble form can be detected and used as a diagnostic marker.7 The cytoplasmic tail of CD27, similar to other costimulatory TNFRs, contains motifs that bind TNFR-associated factors (TRAFs), and the subsequent ubiquitination of TRAFs activates both canonical and noncanonical nuclear factor kappa B (NF-kB) pathways as well as the c-Jun-N-terminal kinase (JNK)-signaling cascade.8,9 Downstream signaling includes expression of the master transcription factor Tbet, which facilitates Th1 differentiation.10-11 These Th1 cells can subsequently promote CD8+ T-cell effector differentiation through promotion of proliferation and survival, however these cells are neither required nor sufficient to support CD8+ T-cell differentiation.12,13 The importance of the CD27 signaling pathway has been demonstrated in vivo utilizing transgenic (Tg) mouse models where either B cells...
TABLE. Immunotherapies Targeting the TNFR Superfamily

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CCR4 indicates CC chemokine receptor 4; GITR indicates glucocorticoid-induced tumor necrosis factor receptor; KIR, killer-cell immunoglobulin-like receptor; TNFR, tumor necrosis factor receptor.

*a*The letter “a” preceding a molecule indicates an antibody to that molecule.
T cells express both activating and inhibitory receptors. These receptors can be targeted in order to increase T-cell activation, thus allowing for downstream effector functions, including proliferation and tumor killing. Inhibitory receptors can be targeted using blocking antibodies; this prevents negative signals from being transduced, thereby reversing the suppression of T-cell activation. Also referred to as checkpoint inhibitors, several of these blocking antibodies are already approved for use (red) and others are currently in development (black). In addition, activating receptors can be targeted via agonist antibodies that transduce positive signals, thus providing T-cell costimulation. Currently under investigation are many agonist antibodies that target activating receptors within the tumor necrosis factor receptor family.

Current Clinical and Preclinical Biologics
Varilumab is a first-in-class fully human agonist IgG1 mAb that is directed against CD27 (aCD27), which has been shown to induce T-cell proliferation and production of Th1 effector cytokines such as interferon-gamma (IFN-gamma) in vitro and in vivo in preclinical work. Varilumab has been tested in a phase I clinical trial as a monotherapy (NCT01460134) (Table) in patients with advanced B-cell lymphomas or solid tumors. In patients with B-cell lymphomas, no dose-limiting toxicities (DLTs) were reported, and furthermore, the 1 patient that experienced a complete response (CR) was subsequently found to have the highest CD27 expression level of all patients included, as determined by immunohistochemistry (IHC). In patients with solid tumors, 1 DLT and 1 partial response (PR) in a patient with metastatic renal cell cancer were reported. Of note, evidence of CD27 stimulation as detected by chemokine induction and T-cell stimulation was found at all dosing levels.

In murine models, treatment with aPD-1 combined with anti-CD27 (aCD27) resulted in tumor eradication in 100% of adjuvant treatment-induced tolerance. This treatment induced tolerance. Also referred to as checkpoint inhibitors, several of these blocking antibodies are already approved for use (red) and others are currently in development (black). In addition, activating receptors can be targeted via agonist antibodies that transduce positive signals, thus providing T-cell costimulation. Currently under investigation are many agonist antibodies that target activating receptors within the tumor necrosis factor receptor family.
of mice due to the ability of aCD27 to stimulate CTLs in lieu of CD4+ T cell help. This response was far superior to treatment with aPD-1/aCTLA-4 dual therapy. Interestingly, varilumab is being tested in combination with aPD-1 mAb (nivolumab) for safety, tolerability, and efficacy in a dose escalation and expansion phase I/II trial for patients with advanced refractory solid tumors (NCT02335918). Preliminary results from the 36 patients enrolled indicate that the combination is well-tolerated and show clinical activity in a subset of patients, including 3 patients that had an objective PR by Response Evaluation Criteria In Solid Tumors (RECIST) criteria. One of these patients experienced a 94% decrease in target lesion diameter and a progression-free survival (PFS) of 19+ months. Interestingly, compared to their baseline biopsy, posttreatment these patients had an increase in PD-L1 expressing tumors that correlated with increased CD8+ T-cell infiltration and decreased circulating regulatory T cells (Tregs). Another clinical trial testing the varilumab and nivolumab combination is slated to begin enrolling patients with aggressive B-cell lymphomas December 2017 (NCT03038672). In addition to response rates and survival times, this study will measure CD27 expression and investigate changes in peripheral and intratumoral immune cells by mass cytometry, which has the capability of evaluating over 40 markers on an individual cell, and by IHC. This additional monitoring may shed light on how T-cell costimulation synergizes with checkpoint blockade (NCT03038672).

4-1BB
4-1BB (CD137) has broad expression, which includes a vast array of cell types within the hematopoietic system, those of neuronal origin, as well as subtypes of lymphomas and leukemias. In particular, both CD4+ and CD8+ T cells transiently upregulate 4-1BB following activation. When 4-1BB ligand (4-1BL; CD137L) engages 4-1BB, the receptor trimerizes and initiates a signaling cascade that activates downstream pathways including JNK and NF-kB. In a way similar to the action of CD27, the activation of these pathways induces increased proliferation, survival, and execution of effector functions such as cytokine release. In addition, costimulatory signals transduced through 4-1BB aide DC maturation. Melero et al first demonstrated that treatment with 4-1BB agonists could eradicate tumors in vivo, an effect mediated primarily by CTLs, which marked 4-1BB as an attractive immunotherapy candidate.

Current Clinical and Preclinical Biologics
Urelumab, an engineered IgG4 mAb, and utomilumab, an IgG2 isotype mAb, are both fully human agonist anti-4-1BB mAbs (a4-1BB) currently in clinical trials. Urelumab’s initial translation to the clinic was stalled due to adverse events that included grade 4 liver toxicities, which resulted in termination of the trials (NCT00309023, NCT00612664). Further investigation identified interleukin (IL)-27 expression by myeloid cells in the liver as the process responsible for recruitment and activation of T cells, which was mediating liver damage. Subsequently, in a phase I safety trial including patients with either advanced solid tumors or relapsed/refractory B cell non-Hodgkin lymphoma (NCT01471210), urelumab was found to have a maximum tolerated dose of 0.1 mg/kg given every 3 weeks. Now, it is once again being investigated, this time in concert with other immunotherapies. For instance, urelumab is being evaluated for safety when given in combination with checkpoint inhibition (aPD-1/nivolumab; NCT02253992, NCT02845323, NCT02658981), cell-specific depleting antibodies (Abs) (aCD20/rituximab; NCT01775631), blocking Abs (aEGFR/cetuximab; NCT02110082), adoptive cell therapy (NCT02652455), or mAbs that modulate natural killer (NK) cell responses (aKIR/lirilumab, anti-SLAMF7/elotuzumab; NCT02252263).

Utomilumab was first evaluated in a phase I trial in combination with rituximab. No severe adverse events were reported and 2 patients achieved PRs and 2 patients achieved CRs that were durable for >2 years (NCT01307267). Utomilumab is now under investigation in combination with other mAbs, including costimulatory molecules such as an OX40 agonist mAb (aOX40; NCT02315066) and checkpoint inhibitors such as aPD-1 mAb (NCT02179918); and, in a triple combination, with avelumab (aPD-L1) plus aOX40 mAb (NCT02554812).

Additionally, new technology is being developed whereby checkpoint mAbs and agonist mAbs are bound to nanoparticles; this is termed an “immunoswitch.” In one trial, the immunoswitch bound with a4-1BB and aPD-L1 was superior in terms of reducing tumor burden and increasing survival compared to the control treatment, which was soluble a4-1BB and aPD-L1 given concurrently. Also in development is PRS-343, a 4-1BB agonist mAb fused to a variant of trastuzumab, which is a mAb targeting HER2, in an effort to focus immune responses toward tumor cells and away from healthy cells. PRS-343 was shown to reduce tumor growth and to elicit increased tumor infiltrating lymphocytes in a humanized mouse model. It will be interesting to see what other novel agents are capable of harnessing T-cell costimulation while also preventing the inhibitory effects of immune checkpoints.

The advent of immunotherapy has not abrogated the need for traditional cancer therapies. Indeed, administration of radiation therapy (RT) and chemotherapeutic drugs remains the current standard of care for numerous cancer types. Preclinical models are helping to investigate whether adding a4-1BB to either RT or chemotherapy boosts treatment efficacy. For instance, highly radiosen-
sitive gliomas were treated in murine models with RT in combination with systemic a4-1BB, which lead to increased T-cell infiltrate, reduced tumor burden, and increased survival.48 These outcomes were also observed in a murine glioma model following treatment of focal RT and CTLA-4 blockade—an effect that was enhanced by the addition of a4-1BB. This illustrated the importance of providing costimulation to bring about optimal therapeutic outcomes.49 In addition, multiple murine tumor models have demonstrated tumor regression due to treatments that combine a4-1BB with such chemotherapies as 5-fluorouracil,50 cisplatin,51 and cyclophosphamide.52

Other combination immunotherapy strategies include the use of therapeutic cancer vaccines or oncolytic viruses that either express T-cell–modulating agents themselves or are administered in conjunction with immunotherapies. An adenovirus expressing CD40L and 4-1BBL is currently being developed, and early results show its ability to induce T-cell expansion and tumor regression.53 Another strategy is DC-based vaccination, whereby DCs are pulsed with tumor antigens in order to prime T cells; which induces anti-tumor effects, including tumor regression and Th1 cytokine production (IL-2 and IFN-gamma). When DC-based vaccination was combined with a4-1BB in multiple murine models, greater anti-tumor responses were observed compared to DC-based vaccination alone.54-56 Bartkowski et al57 reported that the addition of a4-1BB to DC-based vaccination was more efficacious in promoting tumor regression when given with an HPV+ peptide-based cancer vaccine than the addition of aCTLA-4. A melanoma-specific tyrosinase related protein-2 (Trp2) peptide vaccine, when combined with TLR9 stimulation and a4-1BB, promoted increased T-cell infiltration and tumor eradication.58 However, in this climate of investigating new combinatorial strategies, more is not always better. McKee et al59 recently found that while the addition of a4-1BB to an alpha-galactosylceramide–loaded irradiated tumor-cell vaccine led to increased protection against tumor rechallenge, the effects were diminished upon co-administration of aPD-1, as evidenced by a reduction in effector CD8+ T cells. Although the authors do not provide a mechanism for this seeming anomaly, one could postulate that repeated T-cell activation could lead to activation-induced cell death.

In addition to the development of agonist a4-1BB mAbs, research has been focused toward developing both soluble and tumor-expressing 4-1BBL agonists. Multiple preclinical studies have shown that tumor cells engineered to express 4-1BBL elicited anti-tumor immune responses.60 Tetramers composed of 4-1BBLs bound to streptavidin (SA-4-1BBL) were tested in a murine model. SA-4-1BBL not only induced anti-tumor responses, but also induced levels of T-cell proliferation comparable to those produced with agonist a4-1BB mAb treatment.60 The authors also showed that the effect was not Fc-receptor or complement dependent; however, they did find a dramatic increase in non-specific T-cell activation.60 Furthermore, these experiments also revealed that SA-4-1BBL treatment created less toxicity than a4-1BB, highlighting one prominent advantage of this strategy. Moreover, the addition of 4-1BBL to therapeutic cancer vaccines lead to increased efficacy and tumor eradication.61,62 Lastly, the Gilboa laboratory has engineered a novel method to trigger 4-1BB through the use of aptamers, which are single-stranded oligonucleotides designed to bind target proteins.63 These aptamers can be enhanced by conjugation to a wide range of molecules; these could include small interfering ribonucleic acids (siRNAs) or even additional aptamers that are targeting motifs associated with tumor tissue.64-66

OX40

OX40 (CD134) is up-regulated on CD4+ and CD8+ T cells 12 to 24 hours following TCR ligation; it is downregulated 48 to 96 hours later.66-71 Similarly, OX40 ligand (4X40L) is transiently upregulated on activated APCs. Although OX40 expression is transient on both CD4+ and CD8+ T cells, CD4+ T cells maintain this expression for a longer duration than CD8+ T cells.72 Notably, TCR ligation alone is insufficient to induce optimal upregulation of OX40. Instead, IL-2/IL-2R signaling augments OX40 expression through JAK3-mediated activation of STAT3 and STAT5.73,74 Some subsets of CD4+ T cells, like follicular helper T cells, constitutively express OX40, and their fate is initially determined by the interaction between primed CD4+ T cells and DCs, whereby the OX40:OX40L interaction leads to the upregulation of CXCR5.75 Murine Tregs constitutively express OX40; however, on human Tregs, OX40 is only induced after activation.76 Importantly, administration of OX40 agonists enhances CD4+ and CD8+ T cell expansion—presumably due to increased T cell survival as opposed to increased proliferation.77,78 OX40 signaling also promotes T-cell differentiation; for example, OX40 ligation on primed CD8+ T cells leads to increased granzyme B expression and cytolytic activity, thus skewing toward an effector phenotype.79,80 Soluble OX40L agonists are also being investigated as biologics. Several preclinical models have highlighted an Fc-OX40L fusion protein’s anti-tumor effects, including proliferation of tumor-infiltrating T cells, tumor regression, and increased survival.81-83

Additionally, tumor-specific T cells accumulate at the tumor site in vivo.84 The formation of antigen-specific memory T cells is dependent upon TRAF2 binding the cytoplasmic tail of OX40, which signals downstream through NF-kB.85 The NF-kB inducing kinase was revealed to be necessary for OX40 costimulation via the noncanonical NF-kB pathway.86 Anergy induction can occur due to continuous strong TCR stimulation by tumor-expressed self-antigens.

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in the absence of costimulation. However, OX40 ligation reversed CD8+ T-cell anergy of tumor-reactive cells, which lead to their increased survival and tumor regression in murine models.87 Furthermore, OX40 engagement on intratumoral FoxP3+ Tregs decreases their immunosuppressive effects within the tumor microenvironment.20,88,91 This reduction in Treg effectiveness may be due to prevention of naïve CD4+ T-cell to Treg conversion,92 reduction in FoxP3 expression,93 and/or FC receptor (FcR)-mediated depletion of intratumoral Tregs.93, 94 However, there are reports that the cytokine milieu of the microenvironment can drive Treg expansion.93, 95 Despite these observations of varying effects on Tregs by antibody OX40 (mOX40) mAb, which are likely due to differences in tumor models and the inflammatory milieu present in these different systems, administration of agonist aOX40 mAb is extremely promising due to its ability to elicit anti-tumor responses, thus warranting further investigations in clinical trials.

Current Clinical and Preclinical Biologics

The first-in-human phase I clinical trial with an agonist aOX40 mAb was tested as a monotherapy in patients with advanced cancer (NCT01644968). Since this agent was originally developed as a mouse anti-human aOX40 mAb, patients received only 3 doses because they generated human anti-mouse antibodies against the murine IgG1 domain. However, after only one treatment cycle, an impressive 12 of 30 patients experienced regression of at least one metastatic lesion.96, 97 In addition, the treatment was generally well tolerated, and the only grade 3/4 event reported was transient lymphopenia.97 Recapitulating preclinical results, patients treated with aOX40 mAb also had a significant increase in proliferation among effector CD4+ and CD8+ T cells, but not CD4+FoxP3+ Tregs.97 Preliminary phase I results testing the humanized IgG1 agonist aOX40 mAb, MEDI0562, in patients with advanced solid tumors revealed no DLTs and of 32 patients evaluable for response, an objective response (irRECIST and RECIST 1.1) was observed in one patient with a response duration of 16 weeks (NCT02705482).98

The agonist aOX40 mAb MEDI0562 is also being tested in combination with either aPD-L1 mAb (durvalumab) or aCTLA-4 mAb (tremelimunab) (NCT02705482). The combination of aOX40 and aCTLA-4 mAbs is akin to “stepping on the gas” with costimulatory OX40 agonists while simultaneously “removing the brakes” with checkpoint blockade. This rationale has been tested in preclinical models including a poorly immunogenic prostate cancer model and a sarcoma model. Findings from both models demonstrate that combined aOX40/ aCTLA-4 mAb therapy improved tumor regression and survival.99 Interestingly, the addition of IL-4 blockade to this combination therapy further enhanced the anti-tumor response, likely due to suppression of an aberrant Th2 CD4 T cell response induced following aOX40/aCTLA-4 therapy,99 Th1 polarization could also be restored when aOX40/aCTLA-4 therapy was combined with tumor antigen-specific vaccinations, which lead to enhanced IFN-gamma production and tumor regression in a mammary carcinoma model.100

Other clinical trials combining agonist aOX40 mAbs with checkpoint blockade include the investigation of MOXRO0916, currently being tested in phase I/II clinical trials in combination with aPD-L1 mAb atezolizumab (NCT02410512, NCT03029832). No DLTs have been reported (NCT02410512), and the dose-expansion phase has been set for MOXRO0916 300 mg + atezolizumab 1200 mg every 3 weeks.101 This study also includes triple dose escalation + expansion arms investigating the agonist/ checkpoint blockade in combination with bevacizumab, a recombinant humanized anti-VEGF monoclonal antibody (NCT02410512). GSK3174998 is a humanized IgG1 agonist aOX40 mAb being evaluated as a monotherapy with aPD-1 pembrolizumab (NCT02528357); initial monotherapy cohorts were completed without dose limiting toxicities.102

The fourth OX40 agonist currently being developed is PF-04518600 (NCT02315066, NCT02554812, NCT03092856). In monotherapy cohorts, similar to other OX40 agonists currently in clinical trials, PF-04518600 was also well tolerated, and 27 of 48 patients achieved either PR (2 patients) or SD (25 patients).102 PF-04518600 is being tested in combination with a4-1BB agonists (NCT02315066) and as well as a phase 1b/II study of various combinations that all include aPD-L1 mAb (avelumab); combination A (avelumab and 4-1BB agonist, PF-05082566); combination B (avelumab and OX40 agonist, PF-04518600); combination C (avelumab and anti-cytokine colony stimulating factor 1 mAb, PD 0360324); and combination D (avelumab and 4-1BB and OX40 agonists, NCT02554812). In addition to combinations with additional immunotherapies, PF-04518600 will be tested for efficacy when combined with the tyrosine kinase inhibitor axitinib in patients with kidney cancer (NCT03092856).

Another interesting combination involves GR-MD-02, a drug that specifically inhibits galectin-3. Galectin-3 is upregulated in numerous cancers and its expression correlates with increased metastatic potential and poor patient outcomes. Additionally, galectin-3 can play a role in immune suppression by interfering with TCR accessibility and recruitment of myeloid derived suppressor cells.104 Preclinical testing revealed that the addition of GR-MD-02 to an agonist OX40 mAb or to checkpoint inhibitors including aCTLA-4 or aPD-1 mAbs led to increased survival and tumor regression compared to immunotherapy alone.104 These data provided the rationale for further testing of GR-MD-02 plus checkpoint blockade (ipilimumab or pembrolizumab)
in phase I clinical trials for patients with advanced cancer (NCT02117362, NCT02575404).

**GITR**

GITR (CD357) is constitutively expressed at high levels on Tregs and minimally expressed on naive and memory T cells. However, similar to OX40, GITR is upregulated on effector T cells 24 hours post activation due to signaling cascades downstream of NF-kB. Additionally, there is an increase of FoxP3-mediated GITR expression on Tregs upon activation. Interestingly, activated Tregs are the immune subset with the highest GITR expression, and thus are crucial to study, to further understand the potential modes of action for GITR biology currently under development. GITR is expressed on NK cells and moderately expressed on activated macrophages and DCs. The ligand for GITR, GITRL, is expressed on activated APCs and on endothelial cells, which can express high levels of GITRL in the presence of type I interferon. GITR engagement by GITRL enhances T cell proliferation and effector function by upregulating CD25 and inducing cytokine (IL-2/IFN-gamma) expression.

**Current Clinical and Preclinical Biologies**

Initial preclinical studies were conducted using an agonist rat anti-mouse IgG2a aGITR mAb, (clone DTA-1). Agonist DTA-1 administration expands the number of effector CD8+ T cells with increased cytokine production; and at the same time, it abates Treg-mediated immune suppression. Initial hypotheses regarding this mechanism include reduced FoxP3 expression and Treg depletion due to FcγR activity. Recently, new evidence by Mahne et al shed light on this outstanding question using a Treg fate-mapping approach, wherein they found that Tregs were indeed depleted due to DTA-1 treatments. Furthermore, highly activated Tregs were preferentially targeted. A 5-part dose-escalation study is currently recruiting patients with advanced solid tumors to determine the maximum tolerated dose (MTD) of MK-4166, which binds an epitope closely approximating the epitope bound by DTA-1. One cohort of the study will determine the MTD for MK-4166 in combination with pembrolizumab (NCT02132754). An additional GITR agonist mAb, MK-1248, is also being evaluated alone or in combination with pembrolizumab in a phase I trial (NCT02553499). The first GITR agonist (TRX518) to be evaluated in humans is a humanized aglycosyl IgG1 nondepleting mAb; it was evaluated in a phase I study in stage III/IV melanoma patients. While the dose escalation study found little toxicity (up to 8 mg/kg), the study also showed little efficacy with only 4 patients achieving the best clinical response of stable disease (n=28) (NCT01239134). A subsequent 2-part phase I open-label trial of TRX518 is being conducted in patients with advanced solid tumors (NCT02628574). Moreover, the role of antibody-dependent cell-mediated cytotoxicity in depleting Tregs in addition to GITR agonism will be interrogated by 2 humanized IgG1 aGITR mAbs both of which engage Fc receptors and went into phase I trials in 2016 (INCAGN01876, NCT02697591 and MEDI1873, NCT02583165). A phase I/II trial combining INCAGN01876 with nivolumab, ipilimumab, or triple combination is currently recruiting patients (NCT03126110). Another GITR agonist, BMS-986156, is being tested in a phase I trial in combination with nivolumab (NCT02598960). In addition to agonist mAbs, the use of GITRL is also being explored as a therapeutic to trigger GITR mediated costimulation. For example, MEDI1873 is a hexameric GITRL molecule that was designed to contain an IgG1 Fc domain in an effort to deplete Tregs. It is currently being tested in a phase I trial (NCT02583165).

**Conclusions**

The use of checkpoint blockade has been extensively researched in multiple cancer types and has achieved impressive results. Importantly, this reinvigoration of T cells assumes the presence of tumor-specific neoantigens capable of eliciting priming of tumor-specific T cells. Indeed, the FDA recently approved the use of pembrolizumab as a second-line treatment for all metastatic solid tumor types classified as high microsatellite instability or deficient DNA mismatch repair. Tumors with these types of mutation are associated with an increased level of neoantigens that may be recognized by tumor-reactive T cells. However, these treatments have not been successful for all patients, which highlights the critical need for additional therapeutic options. In addition to “removing the brakes” on T cells with checkpoint blockade, numerous studies are actively testing whether “stepping on the gas” by the addition of TNFR costimulatory agonists will increase tumor-specific T cell proliferation and cytokytic function, thus leading to tumor eradication (Figure). Many of the TNFR agonist mAbs/ligands currently under investigation have shown potential to stimulate T cells; however, some efficacy may be attributed to the depletion of Tregs. In addition, the sheer number of biologics coming down the pipeline warrants the rational design of clinical trials based upon the biology of the respective TNFR family members, to select optimal combinations of these new immunotherapies to bring about the best possible patient outcomes.

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