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Stimulating T cells against cancer with agonist immunostimulatory monoclonal antibodies

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Abstract

Elimination of cancer cells through anti-tumor immunity has been a long-sought after goal since Sir F. Macfarlane Burnet postulated the theory of immune surveillance against tumors in the 1950s. Finally, the use of immunotherapeutics against established cancer is becoming a reality in the past 5 years. Most notable are the monoclonal antibodies directed against inhibitory T cell receptors cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1). The next generation of monoclonal antibodies targeting T cells are designed to stimulate co-stimulatory receptors on T cells. Here we review the recent progress on these immunostimulatory agonist antibodies against the costimulatory receptors CD137, GITR, OX40, and CD27.

Keywords

immunooncology; cancer immunotherapy; agonist; immunostimulatory

Introduction

In the past decade, there has been remarkable and stunning progress in our ability to modulate the immune system against human cancers in clinic. This progress is the result of the efforts from the previous few decades focusing on T cell activation and cancer immunoediting (Vesely, Kershaw et al., 2011). We now know there are numerous stimulatory and inhibitory receptors on T cells that help fine-tune the immune response after the T cell receptor (TCR) engages its cognate-major histocompatibility complex (MHC)/peptide ligand (Figure 1). The recent success of cancer immunotherapy is primarily due to monoclonal antibodies (mAbs) directed against inhibitory receptors such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1). These molecules restrain T cell activation and function and are thus, often referred to as immune checkpoints. Therapeutic antibodies targeting these molecules, referred to as immune checkpoint inhibition, are often likened to “releasing the brakes” on the immune system (Alderson, Smith et al., 1994, Sharma and Allison, 2015). Despite recent successes of immune checkpoint inhibition, the majority of patients still fail therapy, necessitating the need for combinatorial therapies. A group of attractive combinatorial targets are T cell activation

receptors, often referred to as costimulatory receptors. Treatment with agonist mAbs against these receptors provide stimulatory signals to T cells to enhance effector function against tumors and can be likened to “pressing on the gas” on the immune system (Figure 2). Here, we review recent progress on targeting T cell costimulatory molecules with agonist antibodies for the treatment of cancer.

CD137

CD137, also known as 4–1BB or tumor necrosis factor receptor 9 (TNFR9), was originally discovered in 1989 as an inducible molecule on the surface of activated CD4⁺ and CD8⁺ T cells (Kwon and Weissman, 1989). It is a member of the TNFR superfamily and is expressed as a homotrimer. Its ligand, CD137L, or called 4–1BBL, is also expressed as a homotrimer on the surface of antigen presenting cells (APCs) (Alderson, Smith et al., 1994). Upon antigen-specific TCR activation, T cells express higher levels of CD137 which when engaged with its ligand CD137L on APCs, augments proliferation, cytokine secretion and survival, thereby enhancing effector functions (Sanmamed, Pastor et al., 2015).

In addition to activated T cells, CD137 is also expressed on regulatory T cells, B cells, myeloid cells and activated natural killer (NK) cells (Melero, Bach et al., 1998, Melero, Johnston et al., 1998, Melero, Murillo et al., 2008, Vinay and Kwon, 2011). Mice-deficient in CD137 were found to have reduced long-lived memory T cells to specific antigens (Willoughby, Kerr et al., 2014). Binding of CD137 to its ligand CD137L results in recruitment of the TNFR-associated factor (TRAF) 1 and TRAF2, resulting in downstream activation of the nuclear factor-kappa-light-chain-enhancer of activated B cells (NF- κ B) and mitogen-activated protein (MAP) kinase signaling pathways (Martinez-Forero, Azpilikueta et al., 2013, Sabbagh, Pulle et al., 2008, Saoulli, Lee et al., 1998). Ultimately, this results in secretion of interleukin-2 (IL-2) and interferon- γ (IFN- γ) as well as upregulation of anti-apoptotic molecules Bcl-xL and Bfl-1 which contribute to T cell expansion, survival and function (So and Croft, 2013).

Preclinical studies in mice demonstrated strong anti-tumor response with agonist anti-CD137 mAbs (Melero, Shuford et al., 1997). In fact, stimulation of CD137 is one of the most powerful antibody-based cancer immunotherapeutic strategies in mouse models (Vinay and Kwon, 2012, Wilcox, Flies et al., 2002). In addition, agonist anti-CD137 synergizes with radiotherapy and chemotherapy (Ju, Cheon et al., 2008, Shi and Siemann, 2006). The anti-cancer effect of agonist anti-CD137 is explained not only by enhancing T cell function, but also its effects on NK cells. Stimulation of CD137 on NK cells results in NK cell activation as well as enhanced antibody-directed cellular cytotoxicity (ADCC) in murine models of lymphoma (Muntasell, Ochoa et al., 2017, Ochoa, Minute et al., 2017, Rajasekaran, Chester et al., 2015, Wang, Erbe et al., 2015). Uniquely, there is evidence that CD137 is internalized after ligation with its ligand and continues to signal through endosomal compartments (Martinez-Forero, Azpilikueta et al., 2013). Overall, CD137 is one of the most attractive targets for agonist immunotherapy. Its ability to potentiate T cell responses is underscored by the fact that third-generation chimeric antigen receptor (CAR) T cells use the intracellular CD137 signaling motif to generate the most successful CAR T

cells date and outperforms CAR T cells with CD28 intracellular domains (June, O'Connor et al., 2018).

Two anti-CD137 agonists are furthest along in the treatment of human cancers, urelumab (BMS-663513) and utomilumab (PF-05082566) (Table I). Urelumab is a fully human IgG4 mAb that does not block the interaction between CD137 and its endogenous ligand, CD137L. Initial results in 2008 using urelumab were promising against advanced cancers (Segal, Logan et al., 2017), but a small number of patients experienced liver toxicity at the highest dose of urelumab. Thus, further clinical development was halted until 2012 when the promise of immunotherapy with agents targeting CTLA-4 and PD-1 was finally being realized. In subsequent clinical trials, a lower dose of urelumab was used in combination with rituximab, cetuximab, elotuzumab, and nivolumab that did not show liver toxicity (NCT01471210, NCT01775631, NCT02110082, NCT02252263, and NCT02253992). One particularly exciting preliminary result is the combination of urelumab with rituximab in patients with relapsed or refractory B cell non-Hodgkin lymphoma. These new trials with the lower dose of urelumab that have minimal liver toxicity are promising as we wait for the final results. The bioactivity of urelumab has been examined in many of these trials and found that urelumab in combination with cetuximab (anti-epidermal growth factor receptor) increases dendritic cell (DC) maturation and NK cell cytotoxicity (Kohrt, Colevas et al., 2014, Srivastava, Trivedi et al., 2017).

Utomilumab is a humanized IgG2 mAb that both activates CD137 and blocks engagement with CD137L. Furthermore, it appears that utomilumab has a superior safety profile since there does not appear to be significant liver toxicity. Initial phase I/II clinical trials are encouraging in that there are some durable responses in patients with follicular lymphomas treated with utomilumab (Segal, He et al., 2018, Tolcher, Sznol et al., 2017). With a favorable safety profile and some initial evidence of efficacy, many ongoing trials are using utomilumab in combination with other immunotherapies against a wide array of cancers such as non-small cell lung cancer, renal cell carcinoma, and head and neck cancer (Tolcher, Sznol et al., 2017). Perhaps the most promising malignancy for both urelumab and utomilumab appears to be rituximab-refractory or relapsed B cell lymphomas. We eagerly await the results of these ongoing trials (Table 1).

GITR

Glucocorticoid-induced TNFR-related protein (GITR, TNFSFR18, CD357) is another member of the TNFR superfamily that is induced after TCR engagement in CD4⁺ and CD8⁺ T cells (Gurney, Marsters et al., 1999, Nocentini, Giunchi et al., 1997). GITR was initially discovered in a screen of murine T cell hybridomas treated with dexamethasone (Nocentini, Giunchi et al., 1997), however subsequent studies have found that glucocorticoids have no effect on GITR expression in both mice and humans. In contrast to CD4⁺ and CD8⁺ effector T cells where GITR expression is induced after TCR signaling, regulatory T cells (Tregs) display constitutively high levels of GITR (Shimizu, Yamazaki et al., 2002). Whereas GITR signaling stimulates effector T cell function, it appears to function as an inhibitory receptor on Tregs, thereby limiting the suppressive function of Tregs (McHugh, Whitters et al., 2002). Like the other costimulatory receptors discussed in this review, expression of GITR is

not limited to T cells and can be induced on NK cells, B cells, and myeloid cells (Schaer, Murphy et al., 2012). The binding of GITR with its ligand GITRL results in activation of the NF- κ B and MAPK pathways mediated by TRAF5 and TRAF2 adapter proteins (Snell, McPherson et al., 2010). Ultimately, GITR-induced signaling results in enhanced T cell proliferation and effector function through the production of IL-2 and IFN- γ . In addition, GITR signaling seems to protect against activation-induced cell death (Turk, Guevara-Patino et al., 2004). On CD8⁺ T cells, GITR signaling appears to lower the threshold of CD28 signaling and induces expression of CD137 (4-1BB) on memory CD8⁺ T cells (Lin, Snell et al., 2013, Ronchetti, Nocentini et al., 2007). There also seems to be a unique feature of agonist-GITR mAbs to elicit IL-9 producing CD4⁺ T cells (Th9) which are critical to for CT26 colon carcinoma rejection in mice (Kim, Kim et al., 2015).

Preclinical studies using agonist anti-GITR mAbs have shown antitumor effect against of number of murine cancers. The most commonly used mAb, clone DTA-1 has been used to successfully treat mice against B16 melanoma, CT26 colon carcinoma and sarcomas (Cohen, Diab et al., 2006, Ko, Yamazaki et al., 2005, Zhou, L'Italien et al., 2007). Synergistic effects beyond monotherapy were also achieved with CTLA-4, Toll-like-receptor (TLR) ligands and vaccines. This antitumor effect of agonist anti-GITR mAbs is thought to be due to enhancing effector T cell function as well as inhibiting Tregs. The anti-GITR mAb DTA-1 has been reported to directly deplete Tregs through an Fc γ R-dependent mechanism (Coe, Begom et al., 2010). With this compelling, preclinical data, humanized agonist anti-GITR antibodies were developed for clinical use.

Currently there are several agents targeting GITR in ongoing clinical trials (Knee, Hewes et al., 2016) (Table 1). The first agonist anti-GITR mAb, (TRX518) against malignant melanoma showed little toxicity, but also little efficacy. Repeat phase I trial with TRX518 against multiple advanced cancers started in 2015 with no results reported yet. Subsequently, a number of agonist anti-GITR mAbs have entered phase I/II clinical trials including INCAGN01876 (Incyte), MK-4166 (Merck), AMG 228 (Amgen) and BMS-986156 (BMS). Furthermore, Medimmune with Astra-Zeneca has developed a hexamerix GITRL protein (MEDI1873) currently in phase I clinical trials that was designed to maximize stimulating effector T cells while inhibiting or depleting regulatory T cells. Results from these trials are still pending.

OX40

OX40, also known as CD134 or TNFRSF4, is a type I transmembrane protein and a member of TNFR superfamily. It was initially discovered using a panel of monoclonal antibodies generated against rat CD4⁺ T cell blasts. One antibody in the panel, MRC-OX40, increased CD4⁺ T-cell proliferation under either concanavalin A or alloantigen stimulation *in vitro* and the target was subsequently named OX40 (Mallett, Fossum et al., 1990, Paterson, Jefferies et al., 1987). In addition to CD4⁺ T cells, OX40 is also expressed on CD8⁺ T cells following activation (Mallett, Fossum et al., 1990). In mice, Tregs constitutively express OX40, while in humans, OX40 expression is upregulated on Tregs upon activation (Valzasina, Guiducci et al., 2005). NK, NKT cells and neutrophils also express OX40 (Baumann, Yousefi et al., 2004).

OX40L, the ligand of OX40, also known as TNFSF4 and CD252, was first identified as a new glycoprotein on T-cell leukemia virus type-I transformed lymphocytes (Tanaka, Inoi et al., 1985) and later found to bind OX40 (Baum, Gayle et al., 1994, Godfrey, Fagnoni et al., 1994). OX40L is not constitutively expressed but, rather is induced on activated APCs including DCs (Ohshima, Tanaka et al., 1997), B cells (Stuber, Neurath et al., 1995) and macrophages (Weinberg, Wegmann et al., 1999). The expression of OX40L on APCs is in line with its function in controlling the extent of T cell priming following recognition of antigen (Gramaglia, Jember et al., 2000, Gramaglia, Weinberg et al., 1998). OX40 ligation with OX40L recruits TRAF2 and TRAF3 to the intracellular domain of OX40, leading to activation of both the canonical and non-canonical NF- κ B pathways (Kawamata, Hori et al., 1998). Downstream signaling ultimately leads to the expression of pro-survival molecules including Bcl-xL and Bcl-2, increased cytokine production associated with enhanced T-cell expansion, differentiation, and the generation of long-lived memory cells (Rogers, Song et al., 2001, Song, So et al., 2005).

Agonist anti-OX40 mAbs have been reported to reverse CD4⁺ T-cell tolerance by overturning the anergic state induced by antigenic peptides under non-inflammatory conditions (Bansal-Pakala, Jember et al., 2001). Engagement of OX40 increases tumor immunity against multiple transplantable syngeneic tumors including sarcomas, melanoma, colon carcinoma, and glioma in experiments using *in vivo* gene transfer of OX40 ligand to tumor cells or administration of OX40L-Fc or OX40 agonist mAbs (Andarini, Kikuchi et al., 2004, Kjaergaard, Tanaka et al., 2000, Weinberg, Rivera et al., 2000). However, anti-OX40 administration shows very limited impact on the growth of poorly immunogenic tumors (Kjaergaard, Tanaka et al., 2000). In this context, combinational strategies could be important to increase OX40 agonist antitumor efficacy. For example, in preclinical studies, OX40 stimulation has been demonstrated to enhance antitumor effects when combined with multiple therapeutic strategies including cytokines (Redmond, Triplett et al., 2012, Ruby, Montler et al., 2008), adjuvants (Gough, Crittenden et al., 2010, Houot and Levy, 2009, Voo, Foglietta et al., 2014), vaccinations (Murata, Ladle et al., 2006), chemotherapy (Hirschhorn-Cymerman, Rizzuto et al., 2009), or radiotherapy (Young, Baird et al., 2016). In addition, anti-OX40 antibodies have been combined with immunomodulatory antibodies against other costimulatory receptors (Lee, Myers et al., 2004, Morales-Kastresana, Sanmamed et al., 2013, Pan, Zang et al., 2002), or blocking coinhibitory pathways (Linch, Kasiewicz et al., 2016, Messenheimer, Jensen et al., 2017, Redmond, Linch et al., 2014) to treat lymphomas, sarcomas, colon metastases, and spontaneous hepatocellular carcinoma.

One of the main advantages of targeting OX40 is that OX40 signaling can prevent Treg-mediated suppression of antitumor immune responses. Three potential mechanisms have been described. First, OX40 signaling reduces the induction of adaptive Tregs. Mice-deficient in OX40 had normal development of naturally arising CD4⁺Foxp3⁺ Tregs *in vivo*, but OX40 costimulation prevents the induction of inducible Foxp3⁺ Tregs from either naïve or effector T cells *in vitro* (So and Croft, 2007, Vu, Xiao et al., 2007). Second, OX40 signaling reduces Treg suppressive activity. Triggering OX40 signaling on Tregs using either agonist antibody or OX40L overexpressed on APCs inhibits Treg capacity to suppress, allowing for greater effector T-cell proliferation and production of IL-2 and other cytokines (Valzasina, Guiducci et al., 2005, Vu, Xiao et al., 2007). For example, in mice bearing CT26

transplanted tumors, intratumoral injection of agonist anti-OX40 mAb resulted in reduced Treg function, more infiltrating DCs and an influx of tumor-specific cytotoxic T lymphocytes (Piconese, Valzasina et al., 2008). Thirdly, anti-OX40 mAbs can directly deplete Tregs. In a recent report, agonist anti-OX40 OX86 administration resulted in the depletion of intratumoral Tregs in an Fc γ R-dependent manner, which correlated with tumor regression (Bulliard, Jolicoeur et al., 2014).

The murine anti-human OX40 mAb (clone 9B12) was the first OX40 agonistic reagent tested in a clinical trial of 30 patients with advanced solid tumors. In this phase I study, although none of the patients showed an objective response by RECIST criteria, some immune responses like Ki67-staining by antigen-experienced CD4⁺ and CD8⁺ T cells in blood was increased, suggesting enhanced activation of T cells. In addition, upregulation of OX40 by tumor-infiltrating Tregs was detected. Overall, agonist anti-OX40 mAb 9B12 was well tolerated with mild to moderate side effects (Curti, Kovacsovic-Bankowski et al., 2013). To increase the anti-tumor response, combinations of 9B12 with chemotherapy and/or radiotherapy in clinical trials are currently ongoing in different solid tumors. However, elevated levels of neutralizing human anti-mouse immunoglobulin antibodies were detected in treated patients and is considered one of the most important limitations in this clinical trial. Thus, the development of fully human OX40 agonists may overcome this limitation. Currently, agonistic OX40 monoclonal antibodies (e.g. MOXR0916, PF-04518600, MEDI0562, MEDI6469, INCAGN01949, and GSK3174998) are being evaluated in several Phase I/II clinical trials either as monotherapy or in combination with other immunomodulating agents. In addition, OX40L-Fc has demonstrated promising results in preclinical model and MEDI-6383, a human OX40L-Fc is also being evaluated in clinical trials currently (Table 1).

CD27

CD27, also known as TNFRSF7, is a type I membrane protein and a member of TNFR family. CD27 was first cloned and characterized in 1987 as a novel T cell differentiation antigen by using an antibody directed against human T cells (van Lier, Borst et al., 1987). Murine and human CD27 are expressed on T cells, B cells, and NK cells (Gravestein, Nieland et al., 1995, Klein, Rajewsky et al., 1998, Sugita, Robertson et al., 1992, van Lier, Borst et al., 1987). A unique feature of CD27 among TNFR family members is its constitutive expression on the majority of T cells, which suggests that CD27 may play a role during T cell priming. Naive T cells constitutively expresses CD27, and TCR signaling further upregulates the CD27 expression (de Jong, Loenen et al., 1991). Interestingly, besides $\alpha\beta$ T cells, CD27 is also highly expressed on a subset of $\gamma\delta$ T cells, V γ 9V δ 2 T cells, that is thought to have antitumor activity (DeBarros, Chaves-Ferreira et al., 2011). CD27 is also expressed on subsets of NK cells. In mice, CD27 expression differentiates the mature CD11b^{high} NK cell pool into two functionally distinct subsets. The CD27^{high} NK cell subset has a greater effector function compared to CD27^{low} NK cells (Hayakawa and Smyth, 2006). While in humans, the loss of CD27 on NK cells is associated with enhanced cytotoxicity (Vossen, Matmati et al., 2008). Importantly, CD27 is expressed by both effector and regulatory T-cell populations at sites of inflammation and cancer, indicating that both T cell populations are potential targets for CD27 immunomodulation.

CD70 is the only known ligand of CD27. The interaction between CD27 and its ligand CD70 provides a costimulatory signal for T cell expansion. Under physiologic conditions, the tight regulation of CD70 expression ensures the transient availability of this costimulatory signal on activated APCs, T cells, and NK cells. On human peripheral DCs, CD70 is absent at steady-state, but is upregulated by TNF- α , TLR agonists or irradiation (Huang, Wang et al., 2011, Krause, Bruckner et al., 2009, Oosterhoff, Heusinkveld et al., 2013).

On T cells, the simultaneous engagement of the TCR by MHC/peptide and CD27 by CD70 provides an important signal for T cell expansion. Mice constitutively expressing CD70 on B cells or DCs had higher number of peripheral T cells were a greater percentage were activated effector T cells (Arens, Tesselaar et al., 2001, Keller, Schildknecht et al., 2008). CD70-CD27 interaction leads to recruitment of TNFR-associated factor (TRAF) proteins TRAF2 and TRAF5 to the intracellular domain of CD27 (Akiba, Nakano et al., 1998, Gravestein, Amsen et al., 1998). Subsequent activation of NF- κ B and c-Jun pathways promotes CD8⁺ T cell priming, proliferation, survival, and cytotoxicity (Carr, Carrasco et al., 2006, Ramakrishnan, Wang et al., 2004, Rowley and Al-Shamkhani, 2004, Taraban, Rowley et al., 2006). In addition to the function on T cells, CD70/CD27 also promotes germinal center formation of B cells at the centroblast stage (Xiao, Hendriks et al., 2004). Additionally, a subset of NK cells express CD27, and engagement of CD27 results in NK cell proliferation and IFN- γ production (Takeda, Oshima et al., 2000).

The anti-tumor effect of CD27 pathway has been demonstrated in multiple mouse tumor models. In a mouse B cell lymphoma model, blocking CD27 costimulation by using a blocking mAb against CD70 completely abolished the anti-CD40 mAb therapeutic effect due to severely impairing CD8 T-cell expansion. Furthermore, it was shown in two different lymphoma models that agonistic anti-CD27 mAb given as a single reagent could completely protect tumor-bearing mice (French, Taraban et al., 2007). In an established mouse melanoma model, agonistic anti-CD27 antibody as monotherapy led a substantial reduction in the outgrowth of both subcutaneous tumors and experimental lung metastases. Some potential mechanisms include survival of tumor-specific CD8⁺ T cells, reducing the frequency of regulatory T cells within tumors, and enhancing the function of NK cells and CD8⁺ CTLs (Roberts, Franklin et al., 2010). In a recent study, the potential of anti-CD27 monotherapy for cancer immunotherapy was demonstrated in murine models of lymphoma (Turaj, Hussain et al., 2017). In this study, an extended panel of immunomodulatory mAbs, either as a single agent or combined with the direct tumor-targeting mAb anti-CD20, was used to treat murine lymphoma. Among all the immunomodulatory mAbs tested, including mAbs against costimulatory receptors OX40, 4-1BB, GITR, CD27 or checkpoint blockers TIGIT, PD-L1, PD-1, or CTLA-4, only anti-4-1BB and anti-CD27 showed efficacy as single reagent. Combination of anti-4-1BB/CD20 didn't show any synergistic effect and combination of anti-CD20 with anti-OX40, GITR, TIGIT, PD-L1, PD-1, or CTLA-4 only showed a moderate benefit compared with anti-CD20 therapy alone. Remarkably, combination of anti-CD20/CD27 showed a complete cure. This effect was apparent in multiple lymphoma models, as well as in a human CD27 transgenic mice using the anti-human CD27 mAb, varlilumab. Single-cell RNA sequencing demonstrated that anti-CD27 stimulated CD8⁺ T and natural killer cells to release myeloid chemo-attractants and IFN- γ ,

to elicit myeloid infiltration and macrophage activation. This study demonstrates the therapeutic advantage of using an immunomodulatory mAb to regulate lymphoid cells, which then recruit and activate myeloid cells for enhanced killing of mAb-opsonized tumors.

Varlilumab (CDX-1127) is a fully human mAb that targets and stimulates CD27. In both *in vitro* assays and *in vivo* preclinical models, variliumab showed direct antitumor activity against a CD27-expressing lymphoma or leukemia by stimulating effector T cells and depleting T regulatory cells (Ramakrishna, Sundarapandiyam et al., 2015, Vitale, He et al., 2012). In addition, varlilumab showed synergistic effects when combined with either anti-CD20 or PD-1/PD-L1 blockade in pre-clinical lymphoma and melanoma tumor models. A Phase I study of varlilumab in patients with both solid cancers and hematologic malignancies demonstrated that varlilumab is well tolerated and has anti-tumor efficacy (Burris, Infante et al., 2017). Currently, there are more clinical trials of varlilumab as a cancer therapeutic that are ongoing (Table 1).

Emerging agonists: ICOS and CD40

In addition to CD137, GITR, OX40 and CD27, there are additional costimulatory receptors being targeted for cancer immunotherapy (Cabo, Offringa et al., 2017, Marin-Acevedo, Dholaria et al., 2018). One such receptor is inducible costimulator (ICOS or CD278) which is expressed on activated CD4⁺ T cells and member of the B7-CD28 family of proteins (Hutloff, Dittrich et al., 1999). Engagement of ICOS with its ligand ICOSL results in greater T cell proliferation and cytokine production (Simpson, Quezada et al., 2010). Of note, its levels are increased in response to anti-CTLA-4 treatment and thus current efforts are underway to use anti-CTLA-4 in combination with agonist anti-ICOS.

Agonist mAbs directed against TNFR family member CD40 have also emerged as a potentially potent anti-tumor therapy, especially in combination with either CTLA-4 or PD-1 blockade (Vonderheide and Glennie, 2013). CD40 is expressed on APCs including B cells and its ligand CD40L (CD152) is expressed on activated T cells. Initial safety concerns including the association of thromboembolic events and cytokine storm release have limited clinical development of agonist anti-CD40 (Dempke, Fenchel et al., 2017). Nevertheless, a series of agonist anti-CD40 reagents are currently in clinical trials and more are in clinical development.

CD28 superagonist as a cautionary tale

The excitement of cancer immunotherapy has been a boom for both academic and biotechnology firms in the development of novel targets for immunomodulation. Importantly, this has provided many patients with advanced or metastatic cancer a chance for survival when standard therapies fail. But it is important to remember that agonists stimulating the immune system can have dangerous side effects. The most infamous of these cases is the development of superagonist anti-CD28 TGN1412 (Hunig, 2012, Hunig, 2016). In March 2006, during a phase I clinical trial, six healthy volunteers who were given TGN1412 developed profound cytokine storm and nearly died. This occurred at a time before any successes with immunotherapy and sent shockwaves throughout hospitals,

doctor's offices, board rooms, and of course, patient's homes. The lesson was clear: stimulate the immune response at your own risk and with great caution.

Conclusions

We are now in the next phase of immune-oncology, where T cells can be modulated by both blocking antibodies against inhibitory receptors and stimulating antibodies against activating receptors. The number of agonists stimulating T cells is growing and exciting early clinical trial data shows great promise. However, most of these agents are in phase I trials and there is no significant efficacy data from phase II or phase III clinical trials yet. Identifying the most appropriate cancer population subsets and what combinatorial therapy is most effective with the least tolerable toxicity remains an ongoing challenge. But what is certain is that costimulatory agonists such as those targeting CD137, GITR, OX40, and CD27 will be among our new weapons against cancer.

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Figure 1:
Regulation of T cells by modulating TCR signals through co-stimulatory and co-inhibitory ligands and receptors.

Figure 2:

An immune synapse between effector T cells and an antigen-presenting cell (APC). Co-stimulatory ligands and receptors belonging to B7/CD28, and TNF/TNFR families are expressed on antigen presenting cells and T cells. The agonist monoclonal antibodies under clinical development can mimic the ligand to engage the co-stimulatory receptors.

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Table 1:

Ongoing clinical trials with costimulatory agonist monoclonal antibodies for cancer immunotherapy.

Reagent	Company	Diseases	Phase	Status	Treatment	# ClinicalTrials.gov	First posted
<i>OX40 agonists</i>							
9B12	AgonOx/MedImmune	Solid tumors	I	Completed	As a single agent	NCT01644968	2012
GSK3174998	GlaxoSmithKline	Solid tumors	I	Recruiting	As a single agent or combined with pembrolizumab (a-PD-1)	NCT02528357	2015
		Solid tumors	I	Recruiting	Combined with GSK1795091 (TLR4 agonist)	NCT03447314	2018
INCAGN01949	Incyte Agenus	Solid tumors	I/II	Recruiting	As a single agent	NCT02923349	2016
		Solid tumors	I/II	Recruiting	Combined with nivolumab(a-PD-1) +/-ipilimumab (a-CTLA4)	NCT03241173	2017
MEDI-0562	MedImmune	Solid tumors	I	Completed	As a single agent	NCT02318394	2014
		Solid tumors	I	Recruiting	Combined with tremelimumab (a-CTLA4) or durvalumab (a-PD-L1)	NCT02705482	2016
MEDI-6469	MedImmune	Solid tumors/B cell lymphoma	I/II	Terminated	As a single agent or combined with tremelimumab (a-CTLA4), durvalumab (a-PD-L1), or rituximab (a-CD20)	NCT02205333	2014
		Head and neck	I	Active, not recruiting	Given prior to surgery	NCT02274155	2014
		Colorectal cancer	I	Active, not recruiting	As a single agent	NCT02559024	2015
MEDI-6383 (OX40L-Fc)	MedImmune	Solid tumors	I	Active, not recruiting	As a single agent or combined with MEDI-4736 (a-PD-L1)	NCT02221960	2014
MOXR0916	Genentech	Solid tumors	I	Active, not recruiting	As a single agent	NCT02219724	2014
		Solid tumors	I	Active, not recruiting	Combined with atezolizumab(a-PD-L1)	NCT02410512	2015
		Urothelial carcinoma	II	Active, not recruiting	Combined with atezolizumab (a-PD-L1)	NCT03029832	2017
PF-04518600	Pfizer	Solid tumors	I	Recruiting	As single agent or combined with PF-05082566 (a-4-1BB)	NCT02315066	2014
		Renal cell carcinoma	II	Recruiting	Combined with axitinib (tyrosine kinase inhibitor)	NCT03092856	2017
		Solid tumors	I/II	Recruiting	Combined with avelumab (a-PD-L1)	NCT03217747	2017
		Leukemia	I/II	Recruiting	As single agent or combined with avelumab (a-PD-L1 mAb) +/- azacitidine (analog of cytidine), or with utomlumab (a-41BB mAb)	NCT03390296	2018
BMS-986178	Bristol-Myers Squibb	Solid tumors	I/II	Recruiting	As a single agent or combined with nivolumab (a-PD-1) +/- ipilimumab (a-CTLA4)	NCT02737475	2016
		B-Cell Non-Hodgkin Lymphomas	I	Not yet recruiting	Combined with radiation therapy and TLR9 agonist	NCT03410901	2018
<i>CD27 agonists</i>							
Varilumab	Celldex Therapeutics	Hematologic cancers and solid tumors	I	Completed	As single agent	NCT01460134	2011
		Ovarian or Breast Cancer	I	Completed	Combined with ONT-10 (MUC1-based cancer vaccine)	NCT02270372	2014
		Melanoma	II	Recruiting	Combined with Glembaumumab Vedotin (cancer cell targeted toxic drug)	NCT02302339	2014

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Reagent	Company	Diseases	Phase	Status	Treatment	# ClinicalTrials.gov	First posted
<i>CD137 agonists</i>		Melanoma	I/II	Terminated (Feasibility concerns)	Combined with ipilimumab (a-CTLA4) <i>c/</i> CDX-1401 (vaccine) and poly-ICLC)	NCT02413827	2015
		Renal cell carcinoma	I/II	Terminated (Portfolio re-prioritization)	As a single agent or combined with sunitinib (receptor tyrosine kinase inhibitor)	NCT02386111	2015
		Solid tumors	I/II	Terminated (Portfolio re-prioritization)	Combined with atezolizumab (a-PD-L1)	NCT02543645	2015
		Solid tumors	I/II	Active, not recruiting	Combined with nivolumab (a-PD-1)	NCT02335918	2015
		Glioma	I	Recruiting	Combined with IMA950 peptides and poly-ICLC	NCT02924038	2016
		B-cell lymphoma	II	Recruiting	Combined with nivolumab (a-PD-1)	NCT03038672	2017
		B-cell lymphoma	II	Recruiting	Combined with rituximab (a-CD20)	NCT03307746	2017
Urelumab	Pfizer	Diffuse large B-cell lymphoma	I	Recruiting	Combined with avelumab (a-PD-L1), and rituximab (a-CD20) or azacitidine	NCT02951156	2016
		Advanced cancers	I	Active, not recruiting	As a single agent and in combination with rituximab (a-CD20)	NCT01307267	2011
		Solid tumors	I	Recruiting	Combined with mogamulizab (a-CCR4)	NCT02444793	2015
		Solid tumors	I/II	Recruiting	Combined with avelumab (a-PD-L1) <i>C/</i> PF-04518600 (a-OX40)	NCT02554812	2015
		Breast Cancer	I	Recruiting	Combined with trastuzumab emtansine(a-HER2/neu + tubulin inhibitor) or trastuzumab (a-HER2/neu)	NCT03364348	2017
		Colorectal Cancer	II	Recruiting	Combined with cetuximab (a-EGFR) and irinotecan (topoisomerase inhibitor)		
	Bristol-Myers Squibb	Glioblastoma	I	Recruiting	As a single agent or combined with nivolumab (a-PD-1)	NCT02658981	2016
		Leukemia	II	Withdrawn	Combined with rituximab (a-CD20)	NCT02420938	2015
		Solid tumors	II	Recruiting	As a single agent or combined with nivolumab (a-PD-1)	NCT02534506	2015
<i>GITR agonist</i>		Urothelial carcinoma	II	Not yet recruiting	Combined with nivolumab (a-PD-1)	NCT02845323	2016
		Melanoma	I	Recruiting	Combined with nivolumab (a-PD-1)	NCT02652455	2017
	Amgen	Solid tumors	I	Terminated	As a single agent	NCT02437916	2015
	Bristol-Myers Squibb	Solid tumors	I/II	Active, not recruiting	As a single agent or combined with nivolumab (a-PD-1)	NCT02598960	2015
	Novartis	Lymphomas	I	Recruiting	As a single agent or combined with PDR001 (a-PD-1)	NCT02740270	2016

Reagent	Company	Diseases	Phase	Status	Treatment	# ClinicalTrials.gov	First posted
INCAGN01876	Incyte	Solid tumors	I/II	Recruiting	As a single agent	NCT02697591	2016
		Solid tumors	I/II	Recruiting	Combined with nivolumab (a-PD-1) and/or ipilimumab (a-CTLA4)	NCT03126110	2017
		Solid tumors	I/II	Active, not recruiting	Combined with pembrolizumab (a-PD-1) and/or epacadostat (IDO inhibitor)	NCT03277352	2017
MEDI-1873	MedImmune	Solid tumors	I	Active, not recruiting	As a single agent	NCT02583165	2015
MK-1248	Merck	Solid tumors	I	Active, not recruiting	As a single agent or combined with pembrolizumab (a-PD-1)	NCT02553499	2015
MK-4166	Merck	Solid tumors	I	Recruiting	As a single agent or combined with pembrolizumab (a-PD-1)	NCT02132754	2014
TRX518	Leap Therapeutics	Solid tumors	I	Recruiting	As a single agent	NCT02628574	2015
OMP-336B11	OncoMed	Solid tumors	I	Recruiting	As a single agent	NCT03295942	2017