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Review

Rationale for anti-GITR cancer immunotherapy

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Abstract Over the past decade, our understanding of cancer immunotherapy has evolved from assessing peripheral responses in the blood to monitoring changes in the tumour microenvironment. Both preclinical and clinical experience has taught us that modulation of the tumour microenvironment has significant implications to generating robust antitumour immunity. Clinical benefit has been well documented to correlate with a tumour microenvironment that contains a dense infiltration of $CD8^+CD45RO^+$ T effectors and a high ratio of $CD8^+$ T cells to $FoxP3^+$ regulatory T cells (Tregs). In preclinical tumour models, modulation of the Glucocorticoid induced TNF receptor (GITR)/GITR ligand (GITRL) axis suggests this pathway may provide the desired biological outcome of inhibiting Treg function while activating $CD8^+$ T effector cells. This review will focus on the scientific rationale and considerations for the therapeutic targeting of GITR for cancer immunotherapy and will discuss possible combination strategies to enhance clinical benefit.

1. Introduction

The goal of anticancer immunotherapy is to re-establish and enhance the antitumour immune response without causing concomitant autoimmunity that may itself result in significant morbidity. In some cases, it is sufficient to block immunomodulatory molecules responsible for downregulating inflammation, such as programmed cell

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death-1 (PD-1) or programmed cell death-ligand (PD-L1). Other agents may also allow establishment of an immune response in a setting where previously there was little or no evidence of an ongoing antitumour response such as anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4). Despite very promising clinical results, many patients still do not respond to anti-PD-1/PD-L1, anti-CTLA4 blockade or combinations of these agents, substantiating the need for alternative therapeutic strategies to boost antitumour immunity and improve the objective response rate in patients with advanced cancer. One such strategy is targeting GITR, a costimulatory TNF receptor super family member, which affords the potential to expand $CD8^+$ T effector (Teff) memory cell population while promoting the loss or inhibition of Tregs. In this review, we will discuss the scientific and therapeutic rationales for targeting the costimulatory and Treg inhibitory roles of GITR, as a single and combination agent.

2. GITR and GITRL expression

GITR (TNFRSF18/CD357/AITR) is a cell surface receptor constitutively expressed at high levels on Tregs and at low levels on naïve and memory T cells [1–3]. Activation of T cells by a number of different stimuli rapidly increases GITR expression within 24 h, on both Tregs and Teff cells [1,4]. In mature Tregs, FoxP3 promotes high-level GITR expression [5], while in conventional T cells, canonical NFκB signalling induces GITR expression [6], suggesting a cell type-intrinsic regulation of expression. Low to moderate levels of GITR are also detected on innate immune cells following activation [1,2,7]. Within innate cell types, the highest induction is seen on activated natural killer cells with levels comparable to GITR expression on activated Teff cells. Only intermediate levels are seen on activated macrophage and dendritic cells (DCs) [8]. Of all immune subsets studied, activated Tregs exhibit the highest level of GITR, an important distinction that becomes more apparent during the *in vivo* evaluation of GITR modulation.

Its ligand, GITRL (TNFSF18) is also a member of the TNF superfamily and is predominantly expressed by activated antigen presenting cells (APCs), including DCs, macrophage and activated B cells [4,7]. Notably, GITR and GITRL expression are not restricted to haematopoietic cells. For example, GITR has been reported to be expressed at intermediate levels on epidermal keratinocytes and osteoclast precursors, whereas GITRL has been detected at high levels on endothelial cells, particularly following exposure to type I IFN [2]. Given the expression patterns, the GITR/GITRL axis may play a role not only in regulating immune responses but also in mediating leukocyte adhesion and migration.

3. GITR signalling and function

As a member of the TNFR superfamily, GITR represents a class of targets referred to as costimulatory receptors which includes OX40 and 4-1BB (CD137) among others. GITR signalling and function are context and cell type dependent (in depth review by Clouthier and Watts [8]). In the thymus, GITR is expressed during T cell development and plays a crucial role in thymic Treg differentiation and expansion [9]. In the periphery, engagement of GITR on T cells with agonist antibodies, recombinant GITRL or GITRL transfectants, following suboptimal TCR stimulation, enhances T cell activation by upregulating CD25, inducing IL-2 and IFNγ expression, and augmenting proliferation [10–14]. GITR signalling is mediated through the activation of NFκB and members of the MAPK pathway, including ERK, p38 and JNK (reviewed in Snell *et al.* [15]).

As GITR does not have intrinsic enzymatic activity, the activation of these signalling pathways occurs via recruitment of TRAF family members, most notably TRAF2 and TRAF5 [16,17]. TRAF2/5-dependent NFκB induction following GITR engagement is associated with upregulation of Bcl-x_L expression on activated $CD8^+$ T cells, suggesting a potential role for GITR in enhancing cell survival [16]. Several additional lines of evidence suggest a unique role for GITR on $CD8^+$ T cells. For example, GITR signalling lowers the threshold for CD28 signalling on $CD8^+$ T cells [14], induces expression of 4-1BB in $CD8^+$ memory T cells [18] and promotes survival of bone marrow $CD8^+$ memory T cells [19]. More recently, Kim *et al.* [20] described GITR costimulation that led to TRAF6-dependent NFκB activation and IL-9 production, thereby enhancing the function of DCs and promoting cytotoxic T lymphocyte responses. Together, these data establish the strong costimulatory role for GITR on Teff cells. However, the regulation of differential TRAF recruitment and downstream signalling events is not well understood.

While high GITR expression is clearly a marker for Tregs [21], its function on Tregs is more complex [21,22]. In general, GITR modulation induces Treg expansion [23], inhibits Treg suppressive function [11,13,24] and promotes Teff resistance to Treg suppression [1,12,25–27]. In addition, GITR is frequently found on memory or antigen-experienced Tregs, including $CD25^+$ and $CD25^-$ Tregs. In support of this, Bianchini *et al.* [28] describe a distinct population of human Tregs which are $CD4^+CD25^{low/-}GITR^+$ and also $CD127^{high}CD45RO^+$. The suppressive function of these cells could be inhibited by treatment with a GITR agonist antibody, supporting the translational aspect of GITR modulation from mouse to human while also highlighting the need to look beyond the traditional Treg markers when interrogating the pharmacodynamics of GITR modulation.

One of the complexities in designing therapeutic agents to human GITR is revealed by the structures of the mouse and human ligand. Classical TNFR ligands are trimeric in nature and as such, mediate cross-linking of the receptors which is critical for forward signalling through the receptor [29–31]. Whereas human GITRL shows the predicted trimeric structure [32], crystallisation of murine GITRL led to the identification of a dimeric molecule in two independent studies [33,34]. This raises the question of whether or not a multivalent molecule (trimeric or greater) is needed to achieve effective human GITR oligomerisation and costimulatory function.

4. *In vivo* modulation of GITR in preclinical tumour models

In syngeneic mouse tumour models, GITR modulation shows compelling antitumour activity which is attributed to both its costimulatory role on $CD4^+$ and $CD8^+$ T cells [20,25,35–40] as well as inhibition or depletion of intratumoural Tregs [20,38,39,41,42]. Table 1 summarises the use of different GITR-targeting agents in preclinical tumour models and the intratumoural effects observed that relate to the mechanism of action for the given agent. The most widely used agonist antibody is DTA-1 which is a rat IgG2b generated by the immunisation of rats with a $CD4^+CD25^+$ mouse T cell line [1], while other antibodies to GITR and versions of recombinant GITRL provide additional target validation.

4.1. Costimulatory function of GITR modulation

The costimulatory function of GITR has been elucidated in several studies in which DTA-1 or GITRL-Fc clearly mediates $CD8^+$ T cell expansion and cytokine production *in vivo* [25,37,38,40,42]. DTA-1 further enhances the multifunctionality of $CD8^+$ T cells in an adoptive T cell therapy model [43] and can augment humoural responses in a vaccine model [44]. A recent study also shows a crucial role for IL-9 in DTA-1-mediated rejection of CT26 tumours via the induction of Th9 cells [20]. Furthermore, there is a growing body of data indicating that GITR modulation enhances the Teff to Treg ratio, in part through its costimulatory role of enhancing the $CD8^+$ T cell population, but also through its depletion of Tregs [20,38,41,42].

Interestingly, multivalent entities of anti-GITR (IgM molecule [45]) and GITRL (pentameric GITRL [46]) demonstrate agonistic activity *in vitro*, and while they show *in vivo* antitumour activity, tumour regression is dependent upon *in vivo* depletion of CD4 cells, presumably due to the loss of $CD4^+$ Tregs. In fact, pGITRL only had a transient effect on tumour growth, likely due to the extensive proliferation of Tregs and lack of FcγR engagement which is discussed below. Although little data are available for human cells, Stone *et al.* [47,48] report that a multimeric macaque GITRL (four-trimer) augments anti-CD3 and antigen-induced proliferation of human T cells increases cytokine production and reverses immunosuppression of Tregs *in vitro*.

Another antibody that has been utilised *in vivo* is the rat monoclonal 2F8 which was shown to induce a humoural response in a prime boost model and also to delay the growth of a B16 melanoma tumour [49]. Interestingly, a F $(ab')_2$ version of 2F8 was as effective as the intact antibody in enhancing the humoural response, indicating the Fc is not required [50]. Whether this is a unique feature of 2F8 or GITR biology regulating a humoural response as opposed to antitumour immunity remains to be determined.

4.2. Treg function of GITR modulation

The effect of GITR modulation on Tregs *in vivo* has been challenging to elucidate as effects have largely not been observed in the periphery, requiring deeper characterisation of the tumour microenvironment. Several hypotheses regarding GITR activity on Tregs have been put forward which are not mutually exclusive including FoxP3 destabilisation [42,51], Treg depletion [38,41,42], inhibition of Treg suppression (refs) and resistance of Teff cells to immunosuppression [12,35]. Cohen *et al.* [42] studied FoxP3-GFP mice bearing B16 tumours and demonstrated that DTA-1 treatment resulted in the loss of FoxP3 expression within the intratumoural Treg compartment. Recent data show that *in vitro* stimulation of $CD4^+$ T cells with DTA-1 results in activation of the canonical (p50/RelA) NFkB pathway. Moreover, p50 was shown to mediate site-specific histone de-acetylation and closure of the *FoxP3* locus [39]. These data correlate with *in vivo* observations showing that in a melanoma tumour model treated with DTA-1, there was a reduction in FoxP3 transcripts and $FoxP3^+$ T cells in lung infiltrates [51].

4.3. Significance of Fc effector function

An important consideration when designing agonistic antibodies to TNFRSF proteins is that the Fc of the antibody can interact with both activating and inhibitory Fcγ receptors which may have different outcomes. Engagement of agonistic antibodies targeting CD40, DR4 and DR5 with the inhibitory FcγR, which promotes superclustering of the target, is sufficient to enable *in vivo* efficacy [30,31]. In contrast, antibody binding to the activating FcγR can result in the stimulation of antibody-dependent cell-mediated clearing of target cells. This process can either involve NK cells, which mediate antibody-dependent cellular cytotoxicity (ADCC), or macrophages, which mediate antibody-dependent cellular phagocytosis (ADCP). The use of specific FcγR-deficient mice and chimeric versions of DTA-1, which are not capable of binding to any FcγR, demonstrated that activating FcγRs are required for the ability of DTA-1 to

Table 1
In vivo evaluation of GITR-targeting agents.

GITR agonist	Description	Models	Intratumoural changes	Reference
DTA-1	Rat IgG2b	MethA	• Eradication of established tumours • Increase infiltration of $CD4^+$ and $CD8^+$ T cells • Response was not seen in IFNγ deficient mice	Ko et al. 2005, J Exp Med
DTA-1	Rat IgG2b	MB49	• Eradication of established tumours • Increase infiltration of $CD4^+$ and $CD8^+$ T cells • Decrease in $CD4^+FoxP3^+$ Tregs	Coe et al. 2010, Canc Imm Immunother
DTA-1	Rat IgG2b	B16	• Delay in tumour cell growth • Rejection requires $CD4^+$, $CD8^+$, and $NK1.1^+$ cells • Rejection is dependent on IFNγ and FasL and independent of perforin	Ramirez-Montagut et al. 2006, J Imm
DTA-1	Rat IgG2b	B16	• Inhibition of tumour growth • Decrease of $FoxP3^+$ Tregs • Increase in $CD8^+CD44^{hi}CD62L^{lo}$ cells • Increase in $CD8^+$ $IFN\gamma^+$ cells and $CD8^+$ proliferating cells	Cohen et al. 2010, PLoS One
DTA-1	Rat IgG2b	B16	• Inhibition of tumour growth and induction of concomitant immunity • Enhancement of tumour-specific $CD8^+$ CTL • Depletion of $CD8^+$ T cells impairs secondary challenge with same tumour	Cote et al. 2011, J Imm
DTA-1	Rat IgG2b	B16	• Conversion of Treg to Teff cell phenotype (loss of FoxP3, Helios and IL-10 expression but increase in IFNγ and Eomes in DTA-1 treated Tregs)	Schaer et al. 2013, Canc Imm Res
DTA-1	Rat IgG2b	B16-OVA	• Reduction in number of tumour foci in the lung • Decrease in $FoxP3^+$ Tregs • Increase in $CD4^+$ $IL9^+$ (Th9) cells	Xiao et al. 2015, Nat Comm
DTA-1	Rat IgG2b	B16F10-OVA	• Decrease in $FoxP3^+$ Tregs • Increase in $CD8^+$/Treg ratio	Kim IK et al. 2015, Nat Med
DTA-1	Rat IgG2b	CT26	• Tumour regression is dependent upon GITR-induced Th9 cells • Increase in tumour-specific $CD8^+$ CTLs via IL9 activation of tumour-infiltrating DCs	Kim IK et al. 2015, Nat Med
DTA-1	Rat IgG2b	CT26	• Tumour rejection is dependent on $CD4^+$ effector T cells • $CD4^+$ T cells play a central role in orchestrating multiple effector cells of anti-GITR mAb-induced tumour rejection, including $CD8^+$ T cells and NK cells	Zhou et al. 2007, J Imm
DTA-1	Mouse IgG2a	Colon26	• Eradication of established tumours • Depletion of $CD4^+FoxP3^+$ Tregs, dependent on activating FcγR • Increase in $CD8^+$/Treg ratio	Bulliard et al. 2013, J Exp Med
DTA-1 N297A	Mouse IgG2a-N297A	Colon26	• No regression of tumours • No depletion of Tregs	Bulliard et al. 2013, J Exp Med
G3c	Rat IgM	Colon26	• No effect on tumour growth • Increased number of Tregs	Nishioka et al. 2008, Imm Letters

Table 1 (*continued*)

GITR agonist	Description	Models	Intratumoural changes	Reference
mGITRL Fc	Dimeric mouse GITRL (Fc isotype unknown)	Colon26 RENCA	• Tumour regression in both models • Increased TILs and Granzyme B with Fc-mGITRL and DTA-1 • Increase in $CD11b^+$ macrophage with Fc-mGITRL • Depletion of $CD8^+$ T cells significantly reduced the effect of Fc-mGITRL	Hu et al. 2008, Clin Canc Res
pGITRL	Pentameric mouse GITRL (no Fc)	MC38	• Delayed tumour growth • Depletion of $CD4^+$ T cells led to complete regression with pGITRL • Combination of CD4 depletion and pGITRL led to loss of Tregs and increased $CD8^+$ T cells	Kim YH et al. 2015, J Imm
mGITRL	Plasmids encoding mouse GITRL	CMS5	• Delay in tumour growth • Depletion of $CD8^+$ T cells abolished effect of GITRL	Nishikawa et al. 2008, Cancer Res

NK, natural killer; TIL, tumour infiltrating lymphocyte.

regress tumours [41,52]. Detailed immunophenotyping of the tumour infiltrating lymphocytes (TILs) showed that Tregs were rapidly depleted in the tumour microenvironment but not in the periphery. The level of antigen expression contributes to the efficiency of ADCC [53], and indeed, intratumoural Tregs have the highest level of GITR expression compared to both peripheral Tregs and activated Teff cells [38,41,42]. Antibody/FcγR-mediated loss of Tregs within the tumour microenvironment is observed for several other targets expressed at high level on intratumoural Tregs including CTLA4 [41,54,55] and OX40 [56].

4.4. Preclinical combination of anti-GITR with other treatment modalities

While GITR modulation demonstrates promising anti-tumour activity in a variety of preclinical models, the most robust activity is seen with smaller tumours which are more sensitive to immunotherapy in general and may be less likely to predict results in the clinical setting. For larger established tumours, combination strategies using mechanistically different therapies are being investigated. Blocking the PD-1/PD-L1 axis to overcome T cell exhaustion provides an opportunity to enhance anti-GITR therapy. Indeed, anti-PD-1 plus anti-GITR therapy in an ovarian model (1D8) led to increased overall survival with 20% of mice tumour free 90 days following treatment [57]. TILs from the responding mice demonstrated a dramatic increase in $CD4^+$ and $CD8^+$ effector memory ($CD44^+CD26L^-$) T cells with a concomitant decrease in $CD4^+FoxP3^+$ Tregs and myeloid-derived suppressor cells. Triple combination of cisplatin or paclitaxel with anti-PD-1 and anti-GITR further improved response to >80% of mice that were tumour free at day 90, in both the ovarian and a breast cancer model. The dramatic improvement in response with the triple combination highlights the potential benefit for more complex combination strategies in the clinic that target different mechanisms.

Another strategy is to promote T cell priming and infiltration through the combination of anti-GITR with anti-CTLA4 treatment. Using MethA and CT26 tumour models under conditions where monotherapy was ineffective, anti-GITR and anti-CTLA4 combination demonstrated robust, synergistic antitumour activity [25,27]. Mechanistic evaluation indicated that anti-CTLA4 treatment enhanced the proliferation and infiltration of $CD8^+$ Teff cells into the tumour, while anti-GITR treatment reduced the accumulation of intratumoural Tregs [26,27]. The combination thus led to a higher frequency of proliferating $CD8^+$ Teff cells producing greater quantities of IFNγ. Translating these findings to the human setting, Pedroza-Gonzalez *et al.* [58] reported that intratumoural Tregs from hepatic cellular carcinoma or liver metastases from colorectal cancer patient samples express high levels of both GITR and CTLA-4. *Ex vivo* stimulation of the TILs with a combination of low-dose soluble GITRL and anti-CTLA-4 restored T cell function by a largely additive effect on proliferation and TNFα production compared to either monotherapy.

Tumours exhibiting low antigen-burden may require T cell priming prior to or in conjunction with anti-GITR therapy. Using intratumoural delivery of adenovirus expressing interferon alpha (IFNα), coadministration of anti-GITR intraperitoneally enhanced antitumour activity and led to an abscopal effect [59]. The combination strategy synergistically increased the number of Teff cells in the tumour while reducing the number of Tregs, in part due to a potentially novel mechanism of CCR5 downregulation. In a unique cellular therapy, DCs were engineered to secrete either GITRL-Fc or GITR Ab and

coadministered with antigen-loaded DCs. Coinjection of the two DC populations into the same site led to dramatic antitumour activity and prolonged survival [60]. A follow-on study in which human DCs coexpressed human GITRL-Fc (hIgG1) and anti-human CTLA-4 heavy and light chains demonstrated enhanced induction of tumour antigen-specific CTLs [61] which led to the initiation of a phase I vaccine study in metastatic melanoma. As another approach to cell therapy combinations with GITR agents, adoptive T cell therapy models demonstrate that DTA-1 enhances the multifunctionality of $CD8^+$ T cells (cytokine production and proliferation) while also reducing the number of $FoxP3^+$ Tregs, leading to improved overall survival [43,62].

5. Clinical testing of anti-GITR biologics

5.1. Considerations for indication selection

A growing number of clinical studies document the favourable prognostic importance of a $CD8^+CD45RO^+$ memory cell infiltrate in a variety of cancer types [63,64]. Based on preclinical data, the emerging hypothesis is that a GITR agonist antibody with the ability to bind activating FcγR should shift the balance in the $CD8^+$ Teff/Treg ratio to impart robust antitumour immunity. The clinical utility of anti-GITR antibodies as single agents may vary depending upon the pretreatment balance of Tregs to Teffs, the presence of NK cells and macrophages capable of ADCC or ADCP, respectively, as well as the subsets of Tregs present in the tumour.

Selection of tumour types most likely to benefit from treatment with agents targeting Tregs, including anti-GITR antibodies, may be guided by data suggesting that functional Tregs are a component of the TIL population [21]. In an early study by Curiel *et al.* [65], Tregs derived from ascites of ovarian patients were shown to be $CD4^+CD25^+GITR^+CTLA4^+FOXP3^{hi}$, functionally suppressive and associated with a high risk of mortality. Since that publication, numerous reports have described the association of tumour infiltrating Tregs with poor prognosis in a variety of indications including hepatocellular (HCC), lung, renal cell carcinoma, cervical, and melanoma [63,66]. Thymus-derived Tregs ($CD4^+FoxP3^+Helios^+$) which are most prominent in glioblastoma are $GITR^+$ [67]. Furthermore, Tregs found within HCC tumours and in metastatic colorectal cancer sites have a higher surface expression of GITR than circulating Tregs, suggesting that strategies directed at elimination of Tregs may have a preferential effect within the tumour microenvironment [68], provided innate immune cells expressing activating FcγR are also present.

However, data from colorectal, breast, head and neck, lymphoma and even ovarian have reported both positive and negative prognostic outcomes associated with intratumoural Tregs [63,66]. In depth evaluation of these reports indicates several factors may contribute to these seemingly disparate findings including methodologies for identifying Tregs and the site of the tumour [69]. Of 58 studies evaluated, 50 used FoxP3 as a sole identifier for Tregs. The challenge with this is approach is that human Teff cells can transiently express FoxP3 upon activation [70,71]. The inclusion of other markers such CD4, CD8, CD25 and CCR4 alongside FoxP3 has provided better prognostic associations. Utilizing advanced technologies that incorporate evaluation of GITR expression to assess Treg and Teff composition pre- and post-treatment might provide better mechanistic insight into GITR modulation and potential patient stratification.

The tumour type and role of disease-causing inflammation may also be relevant to the prognostic importance of Tregs. In colorectal cancer, Tregs are thought to control a Th17-mediated pro-inflammatory response that supports tumour growth suggesting a favourable role in this setting [72]. Similarly, high Treg content in stromal TILs associated with gastric cancer of the cardia appears to result in prolonged disease-free survival [73]. Based on these findings, careful consideration should be given to the indication before employing the use of Treg-targeting agents.

5.2. Potential for toxicity

The immune-related adverse events (IRAEs) observed with checkpoint inhibitors (anti-PD-1, PD-L1 and CTLA-4) are similar, but combination treatment may increase both the frequency and severity of adverse events (AEs). The overall incidence of treatment-related grade 3/4 AEs with CTLA-4 and PD-1 blockade monotherapy is 27% and 16%, respectively. In combination, ipilimumab and pembrolizumab enhanced toxicity causing a 55% rate of high-grade AEs [74]. Similarly, due to the elimination of Tregs, agents targeting GITR may increase the frequency of IRAEs. Preclinical differences in CTLA-4- and GITR-mediated toxicity support the possibility that strategies targeting GITR will be better tolerated than CTLA-4 blockade. CTLA-4 knockout mice have a fatal, early-onset defect in T cell tolerance impacting both conventional T cells as well as Tregs [75]. In contrast, GITR-null mice develop normal lymphoid organs and do not develop overt autoimmunity [1,76]. While DTA-1 can exacerbate pre-existing inflammation and autoimmune disease [1,2], the use of DTA-1 in tumour models has not led to autoimmunity across a number of models and mouse strains [25,27,60] except for very mild hypopigmentation during melanoma therapy [36,42].

5.3. Therapeutic agents in development and ongoing clinical trials

A number of agents have entered the clinic or will do so in the near future (Table 2). These include traditional bivalent antibodies and multivalent GITR ligand fusion

Table 2
Clinical trials with GITR-targeting agents.

Drug	Indication	Phase (start date)	NCT number
TRX518 (GITR, Inc.)	Melanoma	Phase I	NCT01239134
Aglycosyl human IgG1	(lead)	(10/2010)	NCT02628574
	Solid tumours	Phase I (12/2015)	
MK-4166 (Merck)	Solid	Phase I	NCT02132754
(±pembrolizumab)	tumours	(06/2014)	
MK-1248 (Merck)	Solid	Phase I	NCT02553499
(±pembrolizumab)	tumours	(11/2015)	
AMG 228 (Amgen)	Solid tumours	Phase I (04/2015)	NCT02437916
BMS-986156 (BMS)	Solid	Phase I	NCT02598960
(±nivolumab)	tumours	(10/2015)	
INCAGN01876	Solid	Phase I/II	NCT02697591
(Incyte/Agenus)	tumours	(06/2016)	
Human IgG1			
MEDI1873 (AZ)	Solid	Phase I	NCT02583165
Hexameric GITRL protein	tumours	(11/2015)	
Human IgG1			
GWN323 (NVS)	Solid	Phase I/Ib	NCT02740270
(±PDR001)	tumours	(07/2016)	
Human IgG1			

proteins. The antibody isotype, ADCC capability and valence are likely to impact the balance of Treg depletion and Teff stimulation. TRX518, a humanised non-depleting antibody, was the first antibody to enter the clinic in 2010 for malignant melanoma. The first study was a single-ascending dose escalation showing little toxicity or efficacy. A new dose escalation study with repeat-dosing opened in December 2015. In contrast, INCAGN01876 and GWN323, both human IgG1, are capable of engaging Fcγ receptors and have demonstrated ADCC competency *in vitro* ([77]; unpublished data). At least four other anti-GITR antibodies are in development although limited information is available with respect to isotype and effector function. A novel agent, MEDI1873, is a hexameric GITRL molecule with a human IgG1 Fc domain [78]. This design may provide an opportunity to maximise multimerisation of GITR and its costimulatory function on Teff cells while depleting or inhibiting Tregs. A phase I trial in select advanced solid tumours is currently open.

Agonists against other TNFR family members have faced some hurdles in the clinic with respect to toxicity (4-1BB) or minimal early efficacy (OX40). However, increased understanding of target biology in preclinical models and

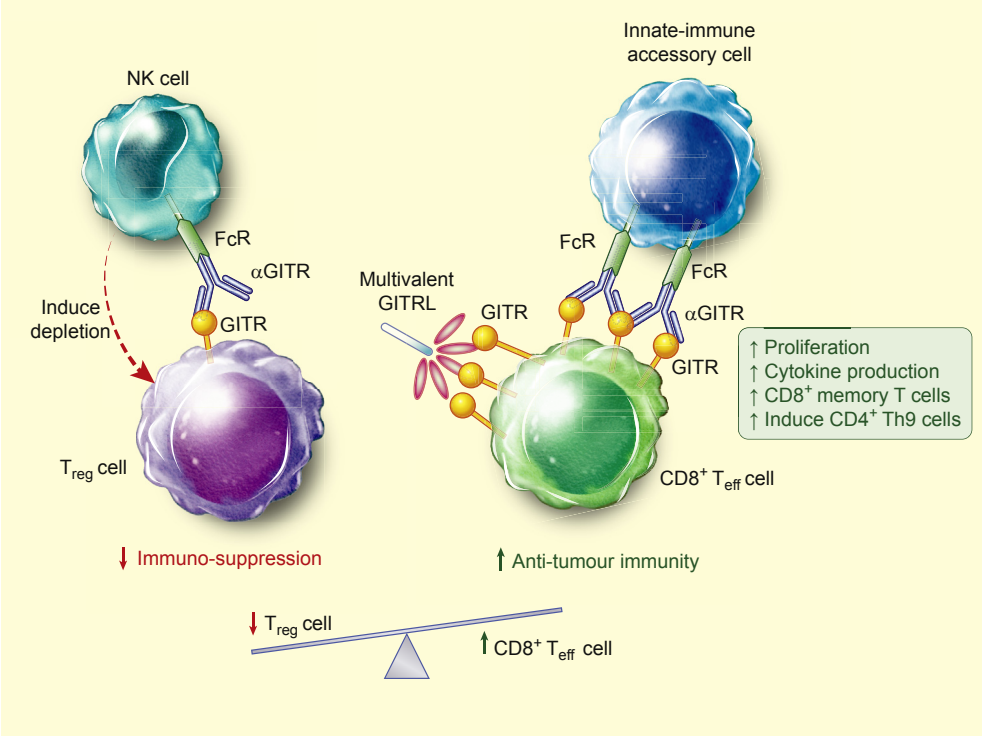

Fig. 1. Model for GITR modulation of antitumour immunity. In the tumour microenvironment, engagement of GITR on Tregs by antibodies that can also bind to activating FcγR on NK cells (or macrophage) leads to Treg depletion. The biased depletion of Tregs is due, in large part, to the fact that activated Tregs express the highest level of GITR. The costimulatory function of GITR modulation, shown in the right portion of the figure, is mediated by oligomerisation of GITR on Teff cells. Clustering of GITR can be induced either by treatment with soluble, multivalent GITRL or with agonist antibody/FcR mediated cross-linking via coengagement of inhibitory or activating $Fc\gamma R^{+}$ immune cells. The downstream signalling following GITR multimerisation can lead to a variety of costimulatory functions, depending on the targeted cell type. The combination of reduced immunosuppression via Treg depletion and enhanced costimulatory function of $CD8^{+}$ Teff cells leads to improved antitumour immunity. NK, natural killer.

biophysical characteristics of the therapeutic agent have reinvigorated efforts for both OX40 [79] and 4-1BB [80]. Interestingly, while GITR, OX40 and 4-1BB share similarities in their expression patterns and downstream signalling pathways, they also exhibit unique characteristics, suggesting they are not redundant molecules.

Based on the clinical experience with antibodies targeting CTLA-4 and the PD-1/PD-L1 axis along with the preclinical data for GITR, the expectation is that GITR-targeting therapies will require combination strategies with other immunomodulatory agents to overcome the immunosuppressive tumour microenvironment of solid tumours. As is shown in Table 2, several companies are already designing combination strategies of anti-GITR with anti-PD-1 agents.

6. Conclusions and future perspectives

Deeper mechanistic understanding of GITR-targeting agents in preclinical models reveals a previously underappreciated role of the Fc effector function to promote Treg depletion while further highlighting the importance of GITR oligomerisation to achieve downstream costimulatory signals (Fig. 1). With currently eight different molecules registered in ClinicalTrials.gov and employing different modalities, the translational relevance of targeting GITR in human tumours can now be evaluated.

Furthermore, the ability of GITR modulation to enhance the CD8/Treg ratio via two seemingly disparate mechanisms (Treg depletion and costimulation of Teff cells) provides a novel combination partner with checkpoint inhibitors to increase the response rate and duration of response. Recognizing that the immune system is dynamic, the success of any of these agents, especially as combination partners, will depend heavily on the interrogation of the tumour microenvironment and pharmacodynamics of these agents. Long-term depletion of Tregs or sustained blockade of checkpoint inhibitors may be counterproductive to achieving the right balance of immune stimulation versus immune suppression. Nonetheless, this is an exciting time for the field. With the explosion of new immunomodulatory agents in the clinic and sophisticated technologies to enable better immune monitoring within the tumour, we have the opportunity to significantly advance the regulation of tumour immunity and hopefully, transform the lives of cancer patients.

Conflict of interest statement

All authors are employed by and shareholders of Novartis. Novartis AG holds patent applications related to aspects of the discussed subject matter.

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