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New emerging targets in cancer immunotherapy: the role of GITR

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ABSTRACT

In the last decade, immunotherapies have revolutionised anticancer treatment. However, there is still a number of patients that do not respond or acquire resistance to these treatments. Despite several efforts to combine immunotherapy with other strategies like chemotherapy, or other immunotherapy, there is an 'urgent' need to better understand the immune landscape of the tumour microenvironment. New promising approaches, in addition to blocking co-inhibitory pathways, such those cytotoxic T-lymphocyte-associated protein 4 and programmed cell death protein 1 mediated, consist of activating co-stimulatory pathways to enhance antitumour immune responses. Among several new targets, glucocorticoid-induced TNFR-related gene (GITR) activation can promote effector T-cell function and inhibit regulatory T-cell (Treg) function. Preclinical data on GITR-agonist monoclonal antibodies (mAbs) demonstrated antitumour activity in vitro and in vivo enhancing $CD8^+$ and $CD4^+$ effector T-cell activity and depleting tumour-infiltrating Tregs. Phase I clinical trials reported a manageable safety profile of GITR mAbs. However, monotherapy seems not to be effective, whereas responses have been reported in combination therapy, in particular adding PD-1 blockade. Several clinical studies are ongoing and results are awaited to further develop GITR-stimulating treatments.

INTRODUCTION

In the last decade, immunotherapies, mainly through antiprogrammed cell death protein 1 (anti-PD-1)/programmed death-ligand 1 and anticytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) monoclonal antibodies (mAbs), have revolutionised anticancer treatment. However, there is still a number of patients that do not respond or acquire resistance to these treatments. According to recent tumour classification by their immune infiltration, some types of cancer potentially respond to immune checkpoint inhibitors (highly immune-infiltrated or 'hot tumour'), while in other tumours available immunotherapies appear not to be effective (non-immune-infiltrated or 'cold tumour'). Despite several efforts to combine immunotherapy with other strategies like chemotherapy, radiotherapy or other immunotherapy aiming to convert 'cold' to 'hot' tumour, there is an 'urgent' need to better understand the immune landscape of the tumour microenvironment and to find alternative approaches to modulate immune function.[1]

New promising approaches, in addition to blocking co-inhibitory pathways, such those CTLA-4 and PD-1 mediated, consist of activating co-stimulatory pathways to enhance antitumour immune responses.[2] One such strategy includes the development of agonist antibodies to target members of the tumour necrosis factor receptor superfamily (TNFRSF) with key role on immune activation and antitumour immune response, like 4-1BB, OX40, CD27 and glucocorticoid-induced TNFR-related gene (GITR).[3] Several data demonstrate that GITR activation can promote effector T-cells function and inhibit regulatory T-cells (Treg) function.[3 4]

In this review, we focus on the GITR/GITR ligand (GITRL) axis.

BIOLOGICAL BACKGROUND

GITR and GITRL expression

GITR (TNFRSF18/CD357/AITR) is a type 1 transmembrane protein belonging to the TNFRSF including OX40, CD27, CD40 and 4-1BB. Human GITR is constitutively expressed at high level on $CD4^+CD25^{+-}$ $FoxP3^+$ Tregs and at low levels on naïve and memory T-cells.[4–7] On activation of $CD8^+$ and $CD4^+$ effector T-cells, GITR expression increases rapidly on both Tregs and effector T-cells, reaching the highest level on activated Tregs.[4 5]

GITR is also expressed on natural killer (NK) cells and at low levels on B cells, macrophages and dendritic cells, and can be upregulated by activation, especially on NK.[8 9]

GITRL is a type 2 transmembrane protein and is also a member of the TNFRSF. It is commonly identified as a trimer, although it can also be present as a monomer or assemble into others multimeric forms.[10]

GITRL is predominantly expressed by activated antigen-presenting cells, including macrophages, B cells, dendritic cells and endothelial cells.[4 8] Notably, GITR and GITRL expression is not restricted to haematopoietic

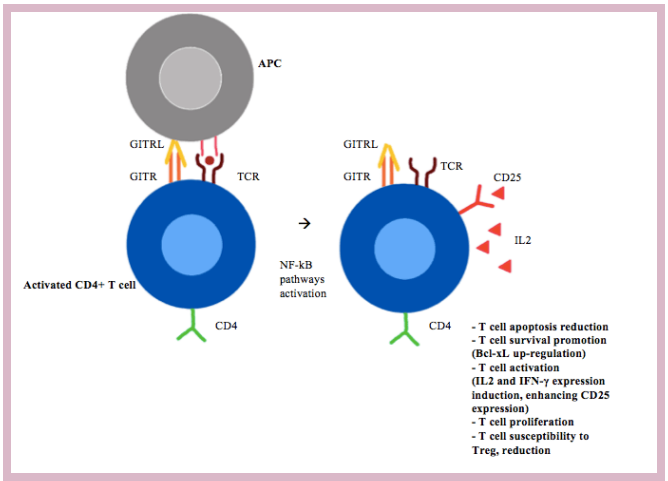

Figure 1 CD4$^+$ T-cell GITR/GITRL activation. APCs, antigen-presenting cells; GITR, glucocorticoid-induced TNFR-related gene; GITRL, GITR ligand; IFN, interferon; IL, interleukin; NF-κB, nuclear factor-κB; TCR, T-cell receptor; Treg, regulatory T-cell.

cells. GITR expression has been described on epidermal keratinocytes and osteoclast precursors and GITRL expression on endothelial cells, especially after type I interferon (IFN) exposure.[6]

Recently, another GITR endogenous ligand has been described: SECTM1A, which is expressed both as a transmembrane protein and as a secreted protein. In mouse, SECTM1A is able to activate both GITR and CD7, but its role is not yet defined.[11]

GITR signalling and function

GITR, as other molecules of the TNFRSF, can act as a co-stimulatory receptor, thus representing a potential

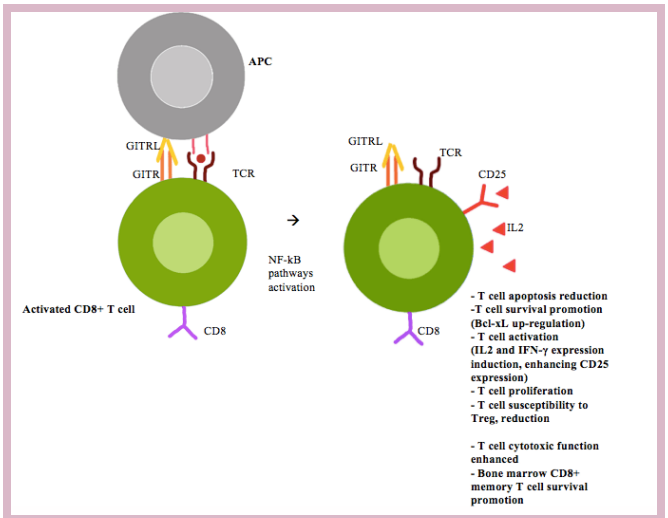

Figure 2 CD8$^+$ T cell GITR/GITRL activation. APCs, antigen-presenting cells; GITR, glucocorticoid-induced TNFR-related gene; GITRL, GITR ligand; IFN, interferon; IL, interleukin; NF-κB, nuclear factor-κB; TCR, T-cell receptor; Treg, regulatory T-cell.

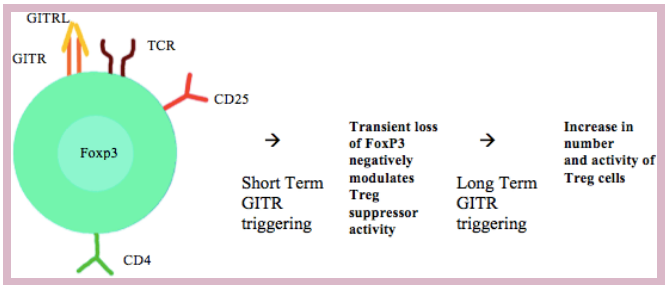

Figure 3 Treg GITR/GITRL activation. GITR, glucocorticoid-induced TNFR-related gene; GITRL, GITR ligand; TCR, T-cell receptor; Treg, regulatory T-cell.

target to enhance immunotherapy, in particular immune checkpoint inhibitors.

All TNFR are characterised by their ability to bind TNF ligand and activate the transcription nuclear factor-κB (NF-κB) pathways via TNF receptor-associated factors (TRAFs), a family of six proteins that are recruited to further transduce signals within the cell. In particular, the activation of GITR signalling pathways, mediated by TRAF2/5-NF-κB, results in reduced T-cell apoptosis and promotes T-cell survival, at least in part by upregulating the expression of the Bcl-xL prosurvival molecule.[12]

In the periphery, after T-cell receptor (TCR) stimulation, the GITRL or agonist antibodies on conventional T-cells increases T-cell activation by inducing interleukin (IL)-2 and IFN-γ expression, enhancing CD25 expression and stimulating cell proliferation (figure 1).[12–14] Furthermore, GITR co-stimulation enhances CD8$^+$ T-cell cytotoxic function, and promotes survival of bone marrow CD8$^+$ memory T-cell (figure 2).[15]

Although GITR is highly expressed in (CD4$^+$CD25$^+$ FoxP3$^+$) Treg cells, its function on these cells is more complex (figure 3).[3]

In vitro and in vivo, GITR signalling, especially mediated by agonist mAb, can inhibit Treg ability to suppress effector T-cells, either by rendering effector T-cells less susceptible to Treg immunosuppressive activities or by directly inhibiting Tregs.[16 17] This last mechanism could be due to the transient loss of FoxP3 on Tregs, although it has been observed only in Tregs from tumour-bearing mice and not in Tregs from naïve mice.[18]

Interestingly, the GITR/GITRL axis effect on Treg seems to be inhibitory in the short-term, while the long-term over stimulation in vivo favours the expansion and the activity of Treg in mice.[16]

In addition, GITR co-triggering of conventional T-cells stimulates IL-10 production, favouring differentiation of conventional CD4$^+$ T-cells into T-helper 2 and Treg cells, these findings sustain the role of GITR in the balancing between T-helper and Treg cells.[19]

Differently, the role of GITR in NK remains to be determined because of contradictory data as to whether GITR engagement increases[8] or decreases NK cell activity.[20]

In summary, while commonly Treg cells antagonise effector T-cells, thereby limiting antitumour activity, GITR

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Table 1 Main characteristics of the agonist GITR mAb

Compound	Phase	Treatment arm (no. of pts)	DLT, n	TRAEs any grade, n (%)	TRAEs, any grade, in ≥5% pts	TRAEs G3-4, n (%)	Serious TRAEs, n (%)	Confirmed ORR, n (%)	Confirmed DCR, n (%)
MEDI-1873[44]	I	Monotherapy (40)	3	82.5%*	Headache, IRR†	G3: 22.5%* No G4-5	Not reported	0	42.5%*
AMG-228[45]	I	Monotherapy (30)	0	18 (60%)	Fatigue (13%), IRR (7%), fever (7%), decreased appetite (7%), hypophosphataemia (7%)	G3-4: 0 G5: 1	2 (7%)	0	7 (23%)
BMS-986156[46]	I–IIa	Monotherapy (34)	0	20 (59%)	Fever (18%), nausea (15%), fatigue (12%), chills (9%), lipase increased (6%), arthralgia (6%), vomiting (6%), malaise (6%), IRR (6%), diarrhoea (6%)	0	0	0	11 (32%)
		Combination therapy: BMS-986156+nivolumab (258)	1‡	170 (66%)	Fatigue (15%), fever (11%), IRR (10%), nausea (8%), chills (8%), diarrhoea (6%), asthenia (5%), arthralgia (5%)	24 (9.3%)	7 (2.7%)	21 (8%)	105 (42%)
TRX-518[47]	I	Monotherapy (43)	0	16 (37%)	Fatigue (11.6%)†	Not reported	0	0	4 (9%)
MK-4166[48]	I	Monotherapy (48)	1	Not reported	Fatigue, IRR, nausea, abdominal pain, pruritus†	6 (5%)	Not reported	4 (9%)	Not reported
		Combination therapy: MK-4166+pembrolizumab (65)§	0					4/45 (9%)¶ 9/13 (69%)**	Not reported

*The number of pts is not reported.
†No other data available.
‡DLT occurred at the combination dose of BMS-986156 800 mg+nivolumab 240 mg.
§Of whom, 45 pts were in the dose escalation cohort and 20 pts were in an expansion cohort (treatment-naïve and pretreated melanoma).
¶ORR in the dose escalation cohort.
**ORR in the immune-checkpoint inhibitor-naïve pts with melanoma (13 pts).
DCR, disease control rate; DLT, dose-limiting toxicity; GITR, glucocorticoid-induced TNFR-related gene; IRR, infusion-related reaction; mAb, monoclonal antibody; ORR, overall response rate; pts, patients; TRAEs, treatment-related adverse events.

Table 2 Ongoing clinical trials testing GITR-stimulating treatments

ClinicalTrial.gov identifier	Tumour type	Setting (early or advanced disease, first, second or more lines if metastatic)	Phase	Treatment arms	Target accrual	Status (at submission date)
NCT02437916: AMG228	Melanoma non-small cell Lung cancer squamous cell Carcinoma of the head and neck transitional cell Carinoma of bladder Colorectal cancer	Advanced tumour	I	AMG228 Part 1 and part 2 of the study will both be with single agent AMG228 in different selected tumour types	30	Terminated (business decision)
NCT04225039: Anti-GITR Agonist INCAGN1876	Glioblastoma	Second line	II	A: INCAGN01876+INCMGA00012+rt stereotactic radiosurgery, not surgery B: INCAGN01876+INCMGA00012+rt stereotactic radiosurgery, followed by surgery	32	Not yet recruiting
NCT03707457: Anti-GITR Monoclonal Antibody MK-4166	Glioblastoma	Second line	I	A: Nivolumab+anti-GITR monoclonal antibody MK-4166 B: Nivolumab+IDO1 inhibitor C: Nivolumab+ipilimumab	30	Recruiting
NCT02132754: Anti-GITR Monoclonal Antibody MK-4166	Advanced malignancies	Second or more lines	I	▶ Experimental: MK-4166 ▶ Experimental: MK-4166+pembrolizumab	113	Completed
NCT04021043: Anti-GITR Agonistic Monoclonal Antibody BMS-986156	Advanced or metastatic Lung/ chest or liver cancers	Advanced disease	I/II	I: Ipilimumab+BMS-986156+nivolumab II: Ipilimumab+BMS-986156+nivolumab+SBRT III: BMS-986156+nivolumab+SBRT	60	Recruiting
NCT02598960: Anti-GITR Agonistic Monoclonal Antibody BMS-986156	Advanced solid tumours	Second or more lines	I/II	▶ Experimental: BMS-986156: dose escalation followed by dose expansion ▶ Experimental: BMS-986156+nivolumab (nivo): dose escalation followed by dose expansion ▶ Experimental: BMS986156+Nivo: cohort expansion	331	Active not recruiting
NCT01239134: Anti-GITR mAb TRX518	Stage iii or Iv Malignant melanoma or other solid tumours	Second or more lines	I	Part A: a single ascending dose study of TRX518 Part B: a dose-escalation study of multidose TRX518 monotherapy Part C: an expansion cohort of multidose TRX518 monotherapy at the maximum tolerated dose	10	Completed
NCT02628574: Anti-GITR mAb TRX518	Advanced solid tumours	Advanced disease	I	▶ A /B TRX518 monotherapy ▶ C TRX518 with gemcitabine ▶ D TRX518 with pembrolizumab ▶ E TRX518 with nivolumab	146	Active, not recruiting
NCT03861403: Anti-GITR mAb TRX518	Advanced solid tumours	Second or more lines	Ib/IIa	▶ TRX518+cyclophosphamide ▶ TRX518+cyclophosphamide+avelumab	125	Active, not recruiting

Continued

Table 2 Continued

ClinicalTrial.gov identifier	Tumour type	Setting (early or advanced disease, first, second or more lines if metastatic)	Phase	Treatment arms	Target accrual	Status (at submission date)
NCT02740270: GWN323 (Anti-GITR)	Advanced cancer or lymphomas	Advanced disease	I/Ib	A : Drug: GWN323 B: Drug: GWN323 Drug: PDR001	92	Active, not recruiting
NCT03295942: OMP-336B11	Locally advanced or metastatic solid tumours	Second or more lines	Ia	OMP-336B11	24	Terminated (sponsor decision)
NCT02583165: MEDI1873	Advanced solid tumours	Advanced disease	I	MEDI1873	40	Completed
NCT03799003: ASP1951 GITR Agonistic Antibody	Advanced solid tumours	Second or more lines	Ib	▶ ASP1951 monotherapy escalation ▶ ASP1951 monotherapy expansion ▶ ASP1951 optional monotherapy retreatment period ▶ ASP1951+pembrolizumab combination escalation ▶ ASP1+pembrolizumab combination expansion ▶ ASP1951+pembrolizumab optional retreatment period	435	Recruiting
NCT02553499: MK-1248	Advanced solid tumour	Second or more lines	I	▶ Experimental: MK-1248 ▶ Experimental: MK-1248+pembrolizumab	37	Terminated (enrolment prematurely discontinued due to programme prioritisation, not due to any safety concerns)
NCT02697591: INCAGN01876	Advanced or metastatic solid tumours	Second or more lines	I/II	Initial cohort dose of INCAGN01876 monotherapy at the protocol-defined starting dose, with subsequent cohort escalations based on protocol-specific criteria	100	Active not recruiting
NCT03277352: INCAGN01876	Advanced or metastatic malignancies	Second or more lines	I/II	INCAGN01876+pembrolizumab+epacadostat	10	Acrive not recruiting
NCT03126110: INCAGN01876	Advanced or metastatic malignancies	Second or more lines	I/II	▶ Experimental: INCAGN01876+nivolumab ▶ Experimental: INCAGN01876+ipilimumab ▶ Experimental: INCAGN01876+nivolumab+ipilimumab	285	Recruiting

GITR, glucocorticoid-induced TNFR-related gene.

activation on effector T-cells increase effector function by limiting the sensitivity of these cells to Treg suppression.

MODULATION OF GITR IN PRECLINICAL TUMOUR MODELS

Antitumour activity of GITR mAb

In recent years, GITR has been largely studied as a pharmacological target.

Co-activation of GITR by agonist mAbs can increase immune response, inflammation and thereby antitumour response.[9] Differently, GITR inhibition, through antagonist mAbs could inhibit T-cell activation and immune response.[6] Consequently, GITR agonist mAbs has been further developed as antitumour agents.

In tumour models, the antitumour activity of GITR mAbs is mainly based on the ability to enhance $CD8^+$ and $CD4^+$ effector T-cell activity and on the inhibition/depletion of tumour-infiltrating Tregs.[21–24]

Importantly, GITR is not expressed on the tumour itself, but it is expressed on tumour-infiltrating lymphocytes (TILs) of several human cancer types including lung cancers, renal cell carcinoma, head and neck carcinoma and melanoma.[25]

The most widely used molecules to trigger GITR are agonist antibodies like DTA-1 (a rat IgG2b)[5] or recombinant version of GITRL, like GITR-Fc.

The DTA-1 mAb has demonstrated in vivo antitumour activity in multiple syngeneic mouse tumour models (eg, melanoma,[24] cervical[26]) enhancing $CD8^+$ and $CD4^+$ T-cell proliferation and cytokine induction. A recent study reported that GITR agonists can also increase cellular metabolism to support $CD8^+$ T-cell effector function and proliferation.[27]

The intermediate role of $CD8^+$ and $CD4^+$ T-cells in tumour rejections seems to be crucial.

Regressing tumour-bearing mice, treated with DTA-1, were found infiltrated by a large number of $CD4^+$ and $CD8^+$ T-cells, including those secreting IFN-γ. However, the treatment resulted in tumour regression only in IFN-γ-intact mice but not IFN-γ-deficient mice.[28 29] The effect of DTA-1 was lost/decreased in the absence of $CD8^+$ T and NK cells.[4]

Moreover, GITR engagement by DTA-1 promoted the differentiation of IL-9-producing $CD4^+$ T-helper cells, thus enhancing immune-mediated tumour response.[30]

The additional crucial concomitant mechanism to inhibit tumour growth, following DTA-1—GITR triggering *is the reduction of Treg activity and number*. Such a reduction can occur via Treg-specific and tumour-specific antibody-dependent cell cytotoxicity (ADCC): $GITR^+$ Tregs specific for tumour antigens, through the Fc domain of anti-GITR mAbs, are recognised and killed by myeloid and NK cells present in the tumour.[22 23]

GITR has a higher expression in tumour infiltrating Treg compared with peritumoral region in several tumour like renal, colorectal and hepatocarcinoma.[31–33]

$FoxP3^+$ Treg reduced accumulation in tumours has been also hypothesised as a result of reduced trafficking or loss of FoxP3 expression in intratumour Treg and their 'conversion' into activated T-cells.[24]

However, Mahne *et al* reported that mDTA-1 depletes rather than converts intratumour Tregs. In tumour-bearing mice, Treg depletion together with GITR triggering were necessary to revert intratumour $CD8^+$ T-cell exhaustion, thus improving antitumour efficacy.[34]

Vence *et al* confirmed that tumours with high expression of $CD8^+$ and $CD4^+$, after GITR mAb treatment, have the better response, mainly lung cancer, renal cancer and melanoma.[25]

Moreover, preliminary results showed a better suppression of tumour growth with intratumour compared with intravenous injection. In fact, the intratumour injection was able to induce a systemic antitumour immune reaction, exerting its effect on injected and on un-injected tumours.[35]

Combination of GITR mAb with immune-modulating therapies

GITR, like other co-stimulating molecules, has a key role on T-cell activation and its activity can potentiate, in a synergic effect, other anticancer therapies.

Combined treatment with anti PD-1 and GITR-agonist mAbs was able to achieve long-term survival in mouse model of ovarian and breast cancer, stimulating IFN-γ producing conventional T-cells and inhibiting immunosuppressive Tregs and myeloid-derived suppressor cells.[4 36] The treatment combination manages to rescue $CD8^+$ T-cell dysfunction and to induce proliferation of precursor effector memory T-cell phenotype in a CD226-dependent manner.[37] Durable responses were also reported adding cytotoxic chemotherapy or radiotherapy to anti-PD-1/GITR mAbs.[36 38 39]

Co-administration of GITR mAbs and anti-CTLA-4 resulted in an 80% tumour-response in CT26 (colon carcinoma) and CMS5a (fibrosarcoma) mice tumour models reducing intratumour Treg (via GITR) and stimulating $CD8^+$ T-cells (via CTLA-4).[37]

Targeting GITR together with an OX40 agonist (OX40 ligand fusion protein), showed unexpectedly a synergistic antitumour effect on CT26 tumour-bearing mice, although the toxic profile of the combination could represent a limit to clinical development.[40]

The synergistic and complimentary antitumour effect obtained combining GITR mAbs and vaccines was reported[13] in cervical cancer[41] and in melanoma.[42] Moreover, adding chemotherapy (gemcitabine) to the combination of vaccine and GITR mAb was able to decrease tumour-suppressive environment and to induce a long-lasting memory immune response.[43]

In conclusion, in preclinical tumour models co-activating GITR through agonist mAb was able to induce antitumour responses. In particular DTA-1 mAb demonstrated in vivo antitumour activity in multiple mouse tumour models, enhancing $CD8^+$ and $CD4^+$ T-cell proliferation/cytokine induction, and *reducing Treg activity and number,* especially via ADCC. Moreover, GITR agonist

mAbs best antitumour responses were achieved in combination with other immune-modulating therapies.

CLINICAL TRIALS WITH GITR MONOCLONAL ANTIBODIES

MEDI1873, a GITR-ligand/IgG1 agonist fusion protein, was tested in a phase I trial reporting G3 treatment-related adverse events (TRAEs) in the 22.5% of patients and no G4-5 TRAEs (table 1). Pharmacodynamics analysis confirmed that MEDI1873 increased $CD4^+Ki67^+$ T-cells and induced a >25% decrease in $GITR^+/FoxP3^+$ T-cells in the evaluable patients. Stable disease (42.5%), durable in the 17.5% of patients, was the best response in this heavily pretreated population, supporting further clinical trials.[44]

The phase I trial with *AMG 228*, an agonistic human IgG1 GITR-mAb, reported a favourable safety profile, but no evidence of T-cell activation or antitumour activity, at least as monotherapy.[45]

BMS-986156, a fully human IgG GITR-mAb, has been tested as monotherapy and in combination therapy with nivolumab in a phase I/IIa trial. None of the 34 patients in the monotherapy arm experienced a dose-limiting toxicity (DLT) or grade G3-5 TRAEs, a patient out of 258 had a DLT in combination with nivolumab 240 mg. No responses were seen with monotherapy, although an objective response rate (ORR) of 9% (18 out of 200 evaluable patients) across all tumour types was achieved in the combination arm.[46]

No responses were reported in the phase I trial with *TRX518*, a fully humanised Fc-dysfunctional aglycosylated IgG1κ GITR-mAb, in monotherapy. Pharmacodynamics data and subsequent in vitro and in vivo investigation highlighted the possible *mechanisms of tumour resistance to anti-GITR monotherapy* and its possible overcome combining anti PD-1/PD-L1 therapy. In a murine model, DTA-1 early treatment delayed tumour growth, preventing intratumour Treg accumulation and $CD8^+$-not exhausted T-cell upregulation. Differently, in advanced tumours microenvironment, high Treg expression increases dysfunctional $CD8^+$ T-cells that shows an exhausted profile and fail to upregulate markers of activation and cytotoxicity. Thus, adding PD-1 blockade was able to counteract $CD8^+$ T-cells exhaustion, resulting in better tumour control.[47] Preliminary evaluations of tumour response among the first patients enrolled in the phase I combinational trial were encouraging (NCT02628574).

MK-4166, a humanised IgG1 agonist GITR mAb, in combination with pembrolizumab, an anti PD-1 mAb, demonstrated a good safety profile and potential activity, in particular among patients with melanoma naïve to treatments.[48]

Others compounds under investigation (table 2) are ASP1951 (PTZ-522),[49] a tetravalent monospecific (TM) anti-GITR agonist antibody (NCT03799003); INCAGN01876, a humanised IgG1 mAb (NCT03126110) and GWN323 (NCT02740270).

CONCLUSIONS AND FUTURE PERSPECTIVES

GITR can act as a co-stimulatory receptor, representing a potential target to enhance immunotherapy efficacy. Preclinical data confirmed GITR triggering could increase $CD8^+$ and $CD4^+$ effector T-cell activity and reduce tumour-infiltrating Tregs. GITR mAbs have a manageable safety profile. However, they seem not to be effective as monotherapy, whether responses have been reported in phase I/II trials combination therapy with immune checkpoint inhibitors. In particular, adding PD-1 blockade may have a synergistic and complimentary antitumour effect, by converting $CD8^+$ T-cells exhaustion.

Several clinical studies are ongoing, especially in combination with other treatments and results are awaited to further develop GITR-stimulating treatment.

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