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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 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,

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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