

Harnessing the innate immune system to treat cancer: enhancement of antibody-dependent cellular cytotoxicity with anti-CD137 Ab

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Background: Monoclonal antibodies are an invaluable therapeutic class when it comes to cancer therapy. One of the primary mechanisms of anti-tumor activity of monoclonal antibodies is antibody dependent cell-mediated cytotoxicity (ADCC) mediated by natural killer (NK) cells. Innate immune effector cells play important roles in generating and maintaining antitumor immunity to enhance ADCC.

Methods: Efficacy of anti-CD137 mAb was examined by *in vitro* and *in vivo* experiments. Clinical trials of anti-CD137 mAb are also on-going.

Results: CD137 is a costimulatory receptor that belongs to the tumor necrosis factor receptor (TNFR) superfamily. CD137 is expressed on T cell, activated NK cells and other immune cells. We recently showed that an agonistic anti-CD137 mAb activates NK cells, resulting in increased ADCC against cancer cells. Anti-CD137 agonistic mAb therapy may both increase ADCC due to mAb-activated NK cells and promote the proliferation and cytotoxicity of antigen-specific T cells induced by mAb treatment.

Conclusions: We discuss some of the promising strategies that could potentially enhance ADCC with anti-CD137 mAbs.

Keywords: Cancer immunotherapy; CD137; antibody dependent cell-mediated cytotoxicity (ADCC); natural killer cells (NK cells)

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Introduction

Tumor-targeted antibodies are one of the most important developments in the field of cancer therapy in the last 20 years (1). Rituximab was the first monoclonal antibody (mAb) approved in 1997 for the treatment of non-Hodgkin's lymphoma (2,3). Following rituximab, several mAbs have become standard of care for the treatment of both solid tumors and hematological malignancies, including trastuzumab, alemtuzumab, cetuximab, ofatumumab,

pertuzumab (Table 1). Although several effective antibodies have emerged, long-term, durable responses remain elusive and resistance and relapse remain major limitations in cancer therapy (4-6). One of the primary mechanisms of anti-tumor activity of IgG1 monoclonal antibodies is antibody dependent cell-mediated cytotoxicity (ADCC). Many different strategies are under development to stimulate immune effector cells implicated in ADCC in order to enhance the efficacy of tumor-specific mAbs. We

Table 1 Therapeutic antibodies to induce ADCC approved in US

Antibody (company)	Type	Target	Tumor types
Rituximab (Genentech)	Chimeric IgG1	CD20	Non-Hodgkin lymphoma, chronic lymphocytic leukaemia
Trastuzumab (Genentech)	Humanized IgG1	HER2	Breast cancer, gastric cancer
Alemtuzumab (Ilex Pharmaceuticals)	Humanized IgG1	CD52	Chronic lymphocytic leukaemia
Cetuximab (Bristol-Myers Squibb)	Chimeric IgG1	EGFR	Colon cancer, head, and neck cancer
Ofatumumab (Genmab)	Human IgG1	CD20	Chronic lymphocytic leukaemia
Pertuzumab (Genentech)	Humanized IgG1	HER2	Breast cancer
Dinutuximab (United Therapeutics)	Chimeric IgG1	GD2	Neuroblastoma

ADCC, antibody dependent cell-mediated cytotoxicity.

have shown that mAbs directed against CD137 enhance natural killer (NK) cell-mediated ADCC against tumor cells. In this review, we discuss some of the promising novel strategies that could potentially enhance ADCC with anti-CD137 mAbs.

Methods

Efficacy of anti-CD137 mAb was examined by *in vitro* and *in vivo* experiments. Peripheral blood mononuclear cells were cultured with SCC6 cells and cetuximab for 24 hours, and flow cytometry analysis was carried out to detect several markers. Clinical trials of anti-CD137 mAb are also ongoing.

Results

Antibody dependent cellular cytotoxicity (ADCC)

ADCC plays an important role in the efficacy of IgG1 Abs in cancer therapy (7,8). The specificity of ADCC is conferred by the binding of the antibody through its fragment antigen-binding portion to the tumor-associated antigen on the target cell. Following binding of the mAb to the tumor antigen, the Fc portion of the mAb interacts with the Fc receptor (FcR) on the surface of effector cells (i.e., NK cells and macrophages), and then initiates ADCC. The magnitude of the cytotoxic response is regulated by different classes of activating and inhibiting FcRs. Most hematopoietic cells, except T cells, can express FcγRs (9). FcγRIIIa (CD16) is an activating low-affinity receptor expressed on NK cells and macrophages (10). On the other hand, NK cells do not express the inhibitory FcγRIIb receptors. Without the influence of Fc-mediated inhibitory signalling, NK cells are free to act as key mediators of

ADCC in the presence of antibody-coated tumor targets.

Human NK cells comprise around 5% of lymphocytes circulating in the blood and are defined by a CD3–CD56+ phenotype. They are further subdivided into two subsets defined by their expression of CD16: CD56lowCD16+ NK cells and CD56hiCD16– NK cells (11). The CD56lowCD16+ is the predominant subset in the peripheral blood and displays early cytolytic functions, while the CD56hiCD16– cells are distributed in the tissues and secondary lymphoid organs and display a late response, secreting primarily IFN-γ (11).

CD137

CD137 (4-1BB) is a costimulatory receptor that belongs to the TNF receptor superfamily (8,12–14). The cDNA of CD137 was cloned in 1989 as an inducible gene from stimulated T cells (15). Follow-up studies showed that CD137 is also detectable on Tregs, DCs and NK cells. The functional role of CD137 in enhancing cytotoxic T cell responses was established in 1997 (16). Melero *et al.* (17) first reported that the administration of anti-CD137 mAbs could eradicate established tumors in mice. The immune response induced by anti-CD137 mAbs is mediated by CD8+ cells and accompanied by a marked augmentation of tumor-selective cytolytic T-cell activity. Interestingly, the efficacy of anti-CD137 mAbs was long lasting and generated memory responses as mice survived rechallenge with the same tumor. The role of CD137 in anti-tumor responses was also demonstrated in CD137^{−/−} mice (18). The knockout mice displayed increased metastasis in the lungs and shorter survival time compared with wild type mice. CD137 is a promising therapeutic target for cancer immunotherapy.

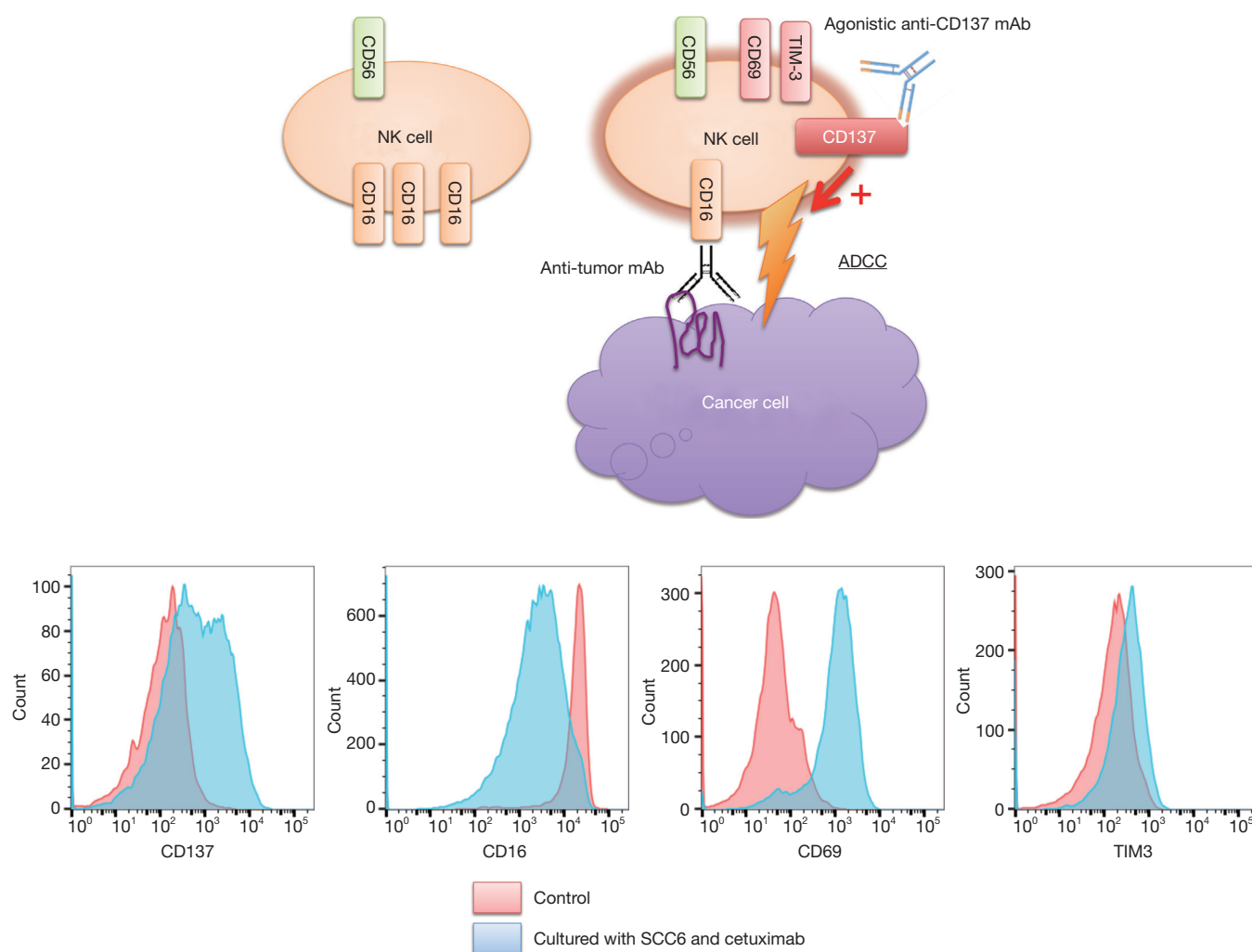


Figure 1 Enhancement of antibody-dependent cellular cytotoxicity with anti-CD137 Ab. NK cells express CD56 and CD16. When NK cells meet cancer cells coated with antibody, NK cells are activated and induce ADCC. In addition, CD137 expression as well as several activation markers (CD69, Tim3 and so on) are increased. Agonistic CD137 Ab enhances ADCC and can lead to a high efficacy in cancer patients. NK, natural killer; ADCC, antibody dependent cell-mediated cytotoxicity.

Enhancement of ADCC by anti-CD137 Ab

NK cells initiate innate immune responses toward tumor (11,19). Expression of CD137 with several other activation markers (CD69, Tim3) on NK cells increases significantly when NK cells are cultured with mAbs bound to tumor cells (Figure 1) (20-22). We found NK cells in circulation and in the tumors that have upregulated CD137. It was observed that there is a variation between patients in the level of CD137 expression on NK cells after exposure to rituximab in B-cell lymphoma patients (20). Differences in histology and percentage of circulating tumor cells may partially

account for this variance, given the observed correlation between circulating tumor cells and CD137 expression levels. In head and neck cancer patients, we also observed a significant increase in NK cell CD137 expression following cetuximab treatment, normally occurring within 24 hours and in some patients, CD137 was elevated as long as 1 week following therapy (22). In addition, patients with either V/V or V/F FcR mutations demonstrated greater CD137 upregulation compared with patients with two low-affinity alleles. We expect anti-CD137 mAb to preferentially target activated NK cells, including NK cells implicated in tumor-directed ADCC, while sparing inactive or resting

NK cells, thus limiting the potential off-target toxicity of anti-CD137 mAb.

Kohrt *et al.* (20-22) demonstrated that an anti-CD137 agonistic mAb enhances ADCC and thus the therapeutic activity of anti-tumor mAbs including rituximab, trastuzumab and cetuximab (*Figure 1*). Human NK cells up-regulate CD137 after encountering mAbs and tumor cells *in vitro* and in the patients, and subsequent stimulation of these NK cells with anti-CD137 mAb enhances mAb-dependent cytotoxicity against tumor cells (22). Therefore, sequential administration of therapeutic antibodies followed by anti-CD137 mAb with a 24-hour time delay would be better than concurrent administration. Despite the predominant role of NK cells and the innate immune response underlying the mechanism of synergy, we observed a role for CD8⁺ T cells in the antitumor response. In the combination of anti-CD20 mAb followed by anti-CD137 mAb, increased late relapses were noted when CD8⁺ T cells were depleted. *In vivo*, the combination of anti-CD137 mAb and cetuximab increases recruitment of NK cells and dendritic cells to EGFR-expressing tumors, augmenting the innate immune response locally and secondarily enhancing DC function and adaptive immunity with increased tumor infiltration by CD8⁺ T cells. The systemic nature of the T cell response observed in the mouse model is consistent with findings in the peripheral blood of patients with head and neck carcinoma. Our results further support the tight interplay between the innate and adaptive immune response following mAb therapy. We hypothesize that anti-CD137 agonistic mAb therapy may both stimulate ADCC due to mAb-activated NK cells and promote the proliferation and cytotoxicity of antigen-specific T cells induced by mAb treatment.

Clinical application of anti-CD137 Abs

Two anti-CD137 mAbs have entered clinical testing. Urelumab (BMS-663513) is a fully human IgG4 mAb developed by Bristol-Myers Squibb, and PF-05082566 is a fully human IgG2 mAb developed by Pfizer. They are agonistic mAbs, which bind to the extracellular domain of human CD137.

NCT00309023 study was a first-in-human open-label, ascending, multidose phase I-II trial of urelumab conducted in patients with locally advanced or metastatic solid tumors (23). This study suggested that urelumab was tolerable. Based on the phase I study, a randomized, multi-dose, open-label, phase II study of urelumab as a second-

line monotherapy was designed in patients with metastatic melanoma. However, the study was terminated in May 2009 due to fatal hepatotoxicity. Following the first clinical trial, several combination therapies with chemotherapy (NCT00351325), chemoradiation (NCT00461110), ipilimumab (NCT00803374), rituximab (NCT01775631), cetuximab (NCT02110082), and elotuzumab (NCT02252263) have been launched as phase I or I/II studies with a lower dose of urelumab.

Clinical trials of PF-05082566 are also on-going. NCT01307267 is an open-label, dose escalation study that was conducted in patients with advanced malignancies. This study suggested that PF-05082566 was well tolerated, with evidence of disease stabilization in multiple patients.

Other candidates in combination therapy to enhance ADCC

NK cell activation is tightly controlled by combinatorial signalling via a network of activating and inhibitory receptors (7,24). The NKp receptors and leukocyte immunoglobulin-like receptors are solely activating receptors, while the killer cell immunoglobulin-like receptors (KIRs) and CD94-NKG2A receptor family contain both inhibitory and activating receptors (25). The interplay of these activating and inhibiting receptors regulates the responses of NK cells when they encounter potential target cells.

KIR signalling

The KIR family constitutes one of the key mediators of NK cell activation. Inhibitory KIR molecules bind to the self-major histocompatibility complex (MHC) class I ligands (HLA-A, HLA-B, HLA-C) and upon binding transduce inhibitory signals that abrogate the effects of activating receptors (26,27). Because MHC class I is expressed on virtually all healthy cells, KIR molecules are considered to be one of the primary mechanisms responsible for NK cell tolerance to self. Reducing KIR-mediated inhibitory signalling in NK cells via antibody blockade has been shown to increase NK cell cytotoxicity and survival of leukemia-bearing mice. In addition, we reported that blockade of the interface of inhibitory KIRs with MHC class I antigens on lymphoma cells by anti-KIR antibodies prevents a tolerogenic interaction and augments NK-cell spontaneous cytotoxicity (28). Lirilumab, a KIR-blocking mAb (IPH2102/BMS-986015) is currently being tested in clinical trials. Early-phase clinical trials of lirilumab in patients with

multiple myeloma demonstrated increased patient-derived NK cell cytotoxicity *ex vivo* but failed to produce any objective responses (29,30). A trial of lirilumab in patients with acute myeloid leukemia (AML) in first complete remission further validated anti-KIR therapy as safe and tolerable, but only produced transient NK activation (31).

GITR

Glucocorticoid-induced tumor necrosis factor receptor (TNFR) family related gene (GITR) is another member of the TNFR superfamily (TNFRSF18) that is upregulated upon cellular activation (32). GITR ligand (GITRL) is frequently expressed on leukemia cells in AML and chronic lymphocytic leukemia, and impairs the reactivity of NK cells that express GITR (33). GITRL also inhibits the rituximab-induced ADCC of NK cells (34). The anti-GITR mAb TRX518 blocks the interaction of GITR, expressed on NK cells, and its ligand GITRL, thereby increasing the cytotoxicity of NK cells. TRX518 is a promising candidate for combination with other mAbs where it can augment NK cell-mediated ADCC. A phase I study with TRX518 (NCT01239134) is being conducted in melanoma patients.

CD27

CD27, or TNFRSF7, is a costimulatory receptor that is expressed on the surface of T cells, B cells, and NKs, providing a target for enhancing antitumor immunity (35). CD27 signaling is mediated by its cognate ligand, CD70, and CD27-CD70 interactions have been shown to accelerate NK-mediated tumor clearance while generating an adaptive immune response (36). Anti-CD27 therapy dramatically increased ADCC; suggesting innate effectors are integral to the mechanism of 1F5 activity. A phase I trial in patients with solid tumors and hematologic cancers (NCT02270372) is currently ongoing with the anti-CD27 varilumab from Celldex.

BTK inhibitor

Ibrutinib is an irreversible inhibitor of Bruton's tyrosine kinase (BTK) with promising activity in CD20+ B-cell malignancies and recent US Food and Drug Administration (FDA) approval in mantle cell lymphoma (37). We found that FcR-stimulated NK cells that have been exposed to rituximab-coated lymphoma cells express moderate levels of BTK (38). Ibrutinib inhibited both rituximab- and trastuzumab-induced NK cell cytokine secretion and cytotoxicity. We demonstrate that the abrogation of both rituximab's and trastuzumab's antitumor efficacy is a

result of ibrutinib's inhibition of FcR-stimulated NK cell function, specifically ADCC. Selective BTK inhibitors or alternative ibrutinib dosing schedules (e.g., sequential versus concurrent), may preserve the anti-lymphoma efficacy of both agents.

Conclusions

Immunomodulatory antibodies have revolutionized cancer immunotherapy. Most cancer immunotherapy strategies stimulate the patient's immune system to overcome immunosuppression induced by tumor cells and generate an anti-tumor immune response. The clinical data and recent FDA approvals of ipilimumab, pembrolizumab, nivolumab and blinatumomab validate mAb-mediated cancer immunotherapy as a valuable therapeutic strategy. Agents that augment the antitumor functions of innate immune cells represent a promising new class of immunotherapeutics. Therapies that enhance the antitumor properties of innate effector cells can be combined with antitumor mAbs. One of the most promising findings is the anticancer efficacy of agonistic anti-CD137 mAb. The strong preclinical successes underscore the importance of CD137 in cancer therapy, especially in combination therapeutic strategies. The clinical trials which validate the synergy between established mAbs and co-stimulatory or disinhibitory therapies must emphasize the collection of high-quality biomarkers and immunologic data to better understand this relationship. We believe anti-CD137 mAbs hold great clinical promise. Their clinical potential should be tested in conjunction with other FDA-approved immunomodulators and antibody therapeutics. It is anticipated that "Combination Cancer Immunotherapy" with CD137 will make significant contributions to the field of cancer immunotherapy.

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None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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