

Enhancement of antibody-dependent cell mediated cytotoxicity: a new era in cancer treatment

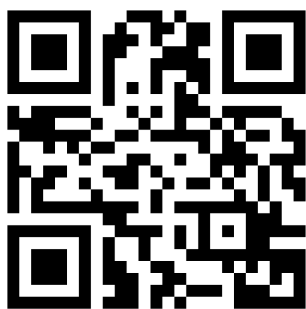
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Abstract: The therapeutic efficacy of some anti-tumor monoclonal antibodies (mAbs) depends on the capacity of the mAb to recognize the tumor-associated antigen and induce cytotoxicity via a network of immune effector cells. This process of antibody-dependent cell-mediated cytotoxicity (ADCC) against tumor cells is triggered by the interaction of the fragment crystallizable (Fc) portion of the mAb with the Fc receptors on effector cells like natural killer cells, macrophages, $\gamma\delta$ T cells, and dendritic cells. By augmenting ADCC, the antitumor activity of mAbs can be significantly increased. Currently, identifying and developing therapeutic agents that enhance ADCC is a growing area of research. Combining existing tumor-targeting mAbs and ADCC-promoting agents that stimulate effector cells will translate to greater clinical responses. In this review, we discuss strategies for enhancing ADCC and emphasize the potential of combination treatments that include US Food and Drug Administration-approved mAbs and immunostimulatory therapeutics.

Keywords: ADCC, NK cell, reovirus, TLR, CD137

Introduction

In the last decade, therapeutic antibodies have become a backbone of routine treatment strategies for a variety of different tumor types (Table 1). Currently, 18 different antibodies have obtained US Food and Drug Administration (FDA) approval for use in oncologic settings.¹ In addition to triggering direct induction of cell death, tumor-targeting antibodies exert antitumor properties through three important innate effector mechanisms: complement-dependent cytotoxicity, antibody-dependent cellular phagocytosis (ADCP), and antibody-dependent cell-mediated cytotoxicity (ADCC). Complement-dependent cytotoxicity is the result of the fragment crystallizable (Fc) region of an antigen-immunoglobulin complex triggering a cascade of more than 30 proteins that culminates in the formation of the membrane-attack complex, an amalgam of subunits that functions to perforate the phospholipid bilayer of the target cell and induce lysis.² ADCP describes target cell elimination by the innate network of phagocytic cells, primarily neutrophils, monocytes, and macrophages. ADCP and ADCC are understood as complementary processes that emphasize the importance of innate immune cells in the therapeutic efficacy of some monoclonal antibodies (mAbs).

Recent work has validated the importance of ADCC in tumor clearance and focused on enhancing ADCC in the cancer setting.³⁻⁶ Originally, ADCC was described as the mechanism by which effector immune cells lyse antibody-coated target cells through the release of cytotoxic molecules like perforin and granzyme.⁷ However, ADCC is now understood as a multitiered process that invokes a network of coordinated immune cells,



Table 1 List of antibodies mentioned in the review

Antibody	Target	Isotype/class	Type	Indication
Rituximab	CD20	IgG1	Chimeric	Non-Hodgkin's lymphoma
Trastuzumab	HER2/neu	IgG1	Humanized	Breast cancer
Alemtuzumab	CD52	IgG1	Humanized	Chronic lymphocytic leukemia
Cetuximab	EGFR	IgG1	Chimeric	Colorectal cancer, head and neck cancer
Panitumumab	EGFR	IgG2	Human	Colorectal cancer
Ofatumumab	CD20	IgG1	Human	Chronic lymphocytic leukemia
Lirilumab	KIR2DL1/2L3	IgG4	Human	Acute myeloid leukemia
Nivolumab	PD-1	IgG4	Human	Renal cell carcinoma, melanoma
Ipilimumab	CTLA4	IgG1	Human	Melanoma
Pembrolizumab	PD-1	IgG4	Humanized	Malignant melanoma
TRX518	GITR	IgG1	Humanized	Melanoma

Abbreviations: CD, cluster of differentiation; CTLA4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; GITR, glucocorticoid-induced tumor necrosis factor receptor; HER2, human epidermal growth factor receptor 2; IgG, immunoglobulin; KIR, killer cell immunoglobulin-like receptor; PD-1, programmed cell-death protein 1.

including monocytes, macrophages, dendritic cells (DCs), and granulocytes. For example, Fc-gamma receptor (Fc γ R) ligation on natural killer (NK) cells can induce the secretion of proinflammatory cytokines like interferon gamma (IFN- γ), which can accelerate DC maturation.^{8,9} Mature DCs enhance antigen presentation and train tumor-specific lymphocytes, producing an immunological memory response.

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.^{10,11} Expression of Fc γ Rs that recognize the Fc portion of the bound antibodies on the cytotoxic effector cells then initiate ADCC.³ The magnitude of the cytotoxic response is regulated by different classes of activating and inhibiting Fc receptors.⁶ Of the different FcRs: Fc γ R binds to immunoglobulin G (IgG), Fc α R binds to immunoglobulin A, Fc ϵ R binds to immunoglobulin E, and Fc μ / α R binds both immunoglobulin M and immunoglobulin A. Of these, Fc γ Rs are the predominant mediators of ADCC.¹¹ Most hematopoietic cells, except T cells, can express Fc γ Rs.¹² There are three types of Fc γ Rs, and each type of Fc γ R recognizes IgG subclasses with different affinities. Fc γ RI (cluster of differentiation [CD]64), expressed on macrophages and neutrophils, is an activating receptor that binds to human IgG1 and IgG3 with high affinity and mediates phagocytosis of target cells. Signaling from the activating receptors on the phagocytes is attenuated by Fc γ RIIa (CD32A), a low-affinity receptor, and Fc γ RIIb (CD32B), an inhibitory receptor.^{11,13} Fc γ RIIb is a low-affinity activating receptor expressed in neutrophils. Fc γ RIIIa (CD16) is an activating low-affinity receptor expressed on NK cells and macrophages.¹³ However, unlike the other hematopoietic cells, NK cells do not express the inhibitory Fc γ RIIb receptors. Without the influence of Fc-mediated inhibitory signaling, NK cells are free to act as

key mediators of ADCC in the presence of antibody-coated tumor targets.¹¹

Human NK cells comprise around 5% of lymphocytes circulating in the blood and are defined by a CD14⁻CD19⁻CD3⁻CD56⁺ phenotype.^{14,15} They are further subdivided into two subsets defined by their expression of CD16: CD56^{low}CD16⁺ NK cells and CD56^{hi}CD16⁻ NK cells. The CD56^{low}CD16⁺ is the predominant subset in the peripheral blood and displays early cytolytic functions, while the CD56^{hi}CD16⁻ cells are distributed in the tissues and secondary lymphoid organs and display a late response, secreting primarily IFN- γ and tumor necrosis factor (TNF)- α . However, recent data also shows that the dichotomous subset functionality may not be completely polarized: a novel CD56^{low}CD16⁻ subset exhibits cytokine secretory functions very early after activation and thereby aids in cytolytic activity.^{16,17}

NK cell activation is tightly controlled by combinatorial signaling via a network of activating and inhibitory receptors. 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.¹⁸ The interplay of these activating and inhibiting receptors regulates the responses of NK cells when they encounter potential target cells. Efficient cytolytic activity of the NK cells depends on the high-avidity binding of the Fc γ RIIIa to antibodies that are bound to multimeric antigen targets. This binding results in a strong activation signal that overcomes the inhibitory signals, leading to a cytotoxic and cytokine response.

Rituximab was the first mAb approved in 1997 for the treatment of non-Hodgkin's lymphoma. Following rituximab, several mAbs have become standard of care for the treatment of both solid tumors and hematological malignancies,

including trastuzumab, alemtuzumab, cetuximab, panitumumab, and ofatumumab.^{6,19–23} Though clinical trials with mAbs have produced significant clinical benefits, there is a need to increase the potency of these therapies to obtain durable clinical remissions and an increase in survival. In this review, we discuss some of the promising novel strategies that could potentially enhance ADCC of tumor targets.

Modifying the antibody: Fc polymorphism and protein engineering

By modulating the strength of the synapse between the antibody Fc and the FcγR, the potency of ADCC can be enhanced. One variant of the FcγRIII, FcγRIIIa, is polymorphic for binding of human IgG. A valine at position 158 on the FcγRIIIa results in a greater affinity for IgG relative to receptors with a phenylalanine in the same position.²⁴ Upon stimulus with IgG, NK cells with a valine-positive FcγRIIIa demonstrate a greater calcium influx and upregulation of surface interleukin (IL)-2 receptor (CD25), a canonical marker of NK cell activation.²⁵ To investigate the effect of these findings clinically, multiple studies stratified patients by FcγRIIIa polymorphism and looked for differing outcomes. As suspected, patients with the high-affinity FcγRIII show increased clinical benefit from mAb therapy.^{26,27} These results suggest that NK cells with the valine-positive FcγRIIIa are advantageous for, and a predictive biomarker of, mAb-mediated cancer immunotherapy.

In contrast to the FcγRIIIa polymorphism, a relatively immutable mediator of ADCC, the glycosylation state of the antibody Fc fragment provides a parameter that can be synthetically optimized to enhance ADCC. Increasingly, posttranslational modifications of protein-based biopharmaceuticals are being utilized to enhance therapeutic activity.²⁸ A common modification, glycosylation, is known to play a critical role in immune cell detection of antibodies. Specifically, glycosylation of the asparagine 297 residue in the C_{H2} domain of the antibody Fc region is accepted as integral to effective FcγRIIIa binding.²⁹ Recent work has investigated the effects of Fc region fucosylation, sialylation, and galactosylation on FcR binding and ADCC within different immune effector subtypes. Generally, low levels of Fc fucosylation enhance mononuclear cell ADCC, while polymorphonuclear cells preferentially kill via highly fucosylated antibodies.³⁰ These insights have led to the generation of glycoengineered antibodies that can elicit up to a tenfold increase in ADCC against cancer cell lines.³¹ Fc-optimization in antibodies against B-cell and ovarian cancer targets

has been shown to improve ADCC in vitro and improve survival and tumor-cell depletion in murine xenograft tumor models.³² Recently, a glycoengineered antibody against CD37, a current antibody target in B cell malignancies, dramatically outperformed an earlier, nonengineered anti-CD37 antibody that is currently in Phase II clinical trials.³³ Obinutuzumab (GA101), a glycoengineered, anti-CD20 mAb, recently outperformed rituximab in a head-to-head comparison in patients with chronic lymphocytic leukemia.³⁴ Obinutuzumab, now approved by the FDA, was designed to have multiple oligosaccharides attached to asparagine 297 in the Fc region.³⁵ The additional glycosylation leads to a greater binding affinity with FcγRIIIa and results in elevated potency and efficacy of ADCC and B-cell depletion.³⁶

Protein engineering focused on optimizing the capacity of the Fc region of the mAb to bind FcγRs is another route to enhancing ADCC effector functions. By generating Fc variants with greater affinity to FcγRs, tumor-targeting antibodies can transmit a more-potent activating signal to NK and phagocytic cells. By utilizing computational designs algorithms and high-throughput screening assays, Fc region mutations have been identified that increase the binding of alemtuzumab, trastuzumab, rituximab, and cetuximab to human FcγRIIIa.³⁷ The designed Fc variants provide substantial enhancement of ADCC relative to nonengineered antibodies and in macaques, a double-mutant S239D/I332E variant of an anti-CD20 mAb proved superior at depleting B cells relative to its nonengineered counterpart. Fc optimization has also been shown to improve survival and enhance tumor-cell depletion in mouse xenograft tumor models for ovarian cancer.³² The Fc-optimized anti-human epidermal growth factor receptor 2 antibody margetuximab has now entered clinical testing in patients with relapsed or refractory advanced breast cancer (NCT01828021).

Effector cell activation: oncolytic viruses

Oncolytic viruses (OVs) selectively kill and replicate within tumor cells but do not harm normal cells. They achieve this by direct oncolytic activity and by inducing an immune response against the infected tumor cells.³⁸ OVs include a diversity of DNA and RNA viruses and fall into two types: 1) viruses that naturally replicate in cancer cells and are nonpathogenic to humans: reovirus, myxomavirus, Newcastle disease virus, and Seneca valley virus; and 2) viruses that are genetically modified to promote tumor selectivity: vesicular stomatitis virus, herpes simplex virus, and vaccine vectors like the vaccinia virus, adenovirus, measles virus, and poliovirus. Because OV-based

therapy targets multiple oncolytic pathways simultaneously, it has a lower probability of inducing resistance compared to therapeutics that individually target pathways (eg, small-molecule inhibitors). Preclinical studies in murine models have demonstrated that OV_s act as powerful inducers of antitumor immunity by triggering strong inflammatory responses and a CD8⁺ T cell-mediated adaptive immune response.^{39–43} In clinical trials, OV_s demonstrated limited toxicity and immune responses to the tumor, but overall antitumor efficacy has been limited.^{44,45} A major challenge in OV therapy is that the immune response elicited by OV_s may also become detrimental to the therapeutic outcome. Viral neutralization by natural antibodies after systemic administration of the virus may lead to rapid clearance of the virus, thus impairing therapy.⁴⁶ However, recent studies demonstrated that immune cells like NK cells can themselves be carriers of reovirus, thereby playing a vital role in enhancing tumor immunity. Here we discuss the possibility of combining the immunotherapy approaches with reovirus therapy to enhance the combined therapeutic potential of both the agents.

Reovirus

Reovirus is a naturally occurring double-stranded oncolytic virus. Reovirus commonly infects the respiratory and gastrointestinal tracts of humans but is not associated with any known human disease.⁴⁷ Reovirus targets and kills tumor cells through selective replication in cells with an activated Ras pathway.⁴⁸ Though Ras mutations occur in only 30% of human tumors, aberrant signaling in the Ras pathway is due to mutations occurring downstream of Ras, making non-Ras mutants suitable targets for reovirus therapy.^{49–53} The mechanism of antitumor activity of reovirus is due to its direct oncolytic activity on the tumor through induction of apoptotic pathways, as well as by activation of antitumor immunity.^{43,54–57} Recent studies have shown that reovirus administration causes secretion of proinflammatory cytokines and chemokines.^{57,58}

Preclinical studies with reovirus as monotherapy have demonstrated the therapeutic benefits of reovirus in treating solid tumors like melanoma, glioma, and ovarian, breast, and colon cancers, as well as in a range of hematological malignancies like myelomas and lymphomas.^{48,58–61} Further, combination therapy of reovirus and vascular endothelial growth factor inhibitors in a mouse model of melanoma demonstrated that NK cells, CD4⁺ T cells, and CD8⁺ T cells were required for tumor regression. In another study, it was demonstrated that tumor infection by reovirus activates DCs that in turn induce NK cell recruitment, activation, and increased

NK cell-mediated cytotoxicity.^{62,63} Recently, Hamano et al⁶⁴ have demonstrated in a preclinical model that reovirus can augment trastuzumab-induced cytotoxicity in gastric cancer cells. Further, Adair et al⁶⁵ interestingly show that reoviruses are protected from neutralizing mAbs by binding to NK cells, and these cells can also carry the reovirus to the targets facilitating direct cytotoxic killing of tumor cells. Further, NK cells, upon binding to reovirus, exhibit an upregulation of activation markers CD69 and CCR7, and killed tumor targets in a Type 1 interferon-dependent manner.⁶⁶ Thus, systemic administration of reovirus by immune-cell carriage in combination with mAb therapies like rituximab or cetuximab can facilitate the delivery of reovirus directly to tumor target and result in increased NK-mediated ADCC.

Currently, reovirus is being used in clinical trials as monotherapy or in combination with other anticancer agents. Phase I trials performed using intratumoral administrations have proved that reovirus is well tolerated as monotherapy.^{67,68} Other Phase I/II trials tested the systemic administration of reovirus as monotherapy in patients with advanced malignancies and found that intravenous injections were safe and well tolerated and encouragingly showed tumor reduction.^{69–72} The majority of ongoing clinical trials with reovirus involve combinatorial treatment strategies. Reovirus is currently being tested in combination with radiotherapy or multiple-chemotherapy agents like docetaxel, apaclitaxel, and carboplatin.^{73–75} The latter studies demonstrated that reovirus administration does not increase toxicity associated with radiation and chemotherapies. These recent clinical data support the prospect of using reovirus in combination with mAbs for antitumor therapies. A summary of active clinical trials testing reovirus is presented in Table 2.

Effector cell activation: toll-like receptor agonists

Toll-like receptors (TLRs) are expressed by both effector cells of the immune system and cancer cells.⁷⁶ TLR family of receptors (TLR1–TLR10) recognize highly conserved structural motifs known as pathogen-associated molecular patterns, which are exclusively expressed by microbial pathogens, or damage-associated molecular patterns, which are endogenous molecules released from necrotic or dying cells. On binding, they initiate signaling cascades that result in a variety of cellular responses, including proinflammatory cytokine expression.⁷⁷ TLR agonists have immune stimulatory effects through the induction of costimulatory molecules like CD80 and CD86 on DCs and the production of inflammatory cytokines like TNF- α and IL-2. But they

Table 2 Ongoing active clinical trials with reovirus

Trial number	Phase	Title
NCT01274624	Phase I	Study of REOLYSIN® in combination with FOLFIRI and bevacizumab in FOLFIRI naïve patients with KRAS mutant metastatic colorectal cancer
NCT02101944	Phase I	Viral protein production after dexamethasone, wild-type reovirus, and carfilzomib in treating patients with multiple myeloma
NCT01533194	Phase I	Reo viral therapy in treating patients with relapsed or refractory multiple myeloma
NCT01280058	Phase II	Carboplatin and paclitaxel with or without reo viral therapy in treating patients with recurrent or metastatic pancreatic cancer
NCT00861627	Phase II	REOLYSIN® in combination with paclitaxel and carboplatin for non-small cell lung cancer with KRAS or EGFR activation
NCT00998192	Phase II	A study of REOLYSIN® in combination with paclitaxel and carboplatin in patients with squamous cell carcinoma of the lung

Abbreviations: EGFR, epidermal growth factor receptor; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog; FOLFIRI, Folinic acid, Fluorouracil, Irinotecan.

may also induce several immune suppressive factors like IL-10 and programmed cell-death protein-1 ligand (PD-L1) that makes them poor candidates as standalone tumor therapeutics.^{78,79}

Only a few TLR agonists are currently licensed by the FDA for use in cancer patients: *Bacillus Calmette–Guérin* (TLR2/TLR4), monophosphoryl lipid A (TLR4), and imiquimod (TLR7).^{80,81} However, recent in vitro studies have demonstrated that TLR8 stimulation through its agonist VTX-2337 enhanced the activation and function of NK cells in the presence of cetuximab-coated head and neck cancer cells.⁸² Similarly the TLR3 ligand polyinosinic:polycytidylic acid (polyI:C) increased the cetuximab-dependent ADCC by NK cells against head and neck cancer cell lines. During cetuximab-induced ADCC, the percentage of activated NK cells (CD107a⁺granzyme B⁺) increased significantly in presence of both the agonist and cetuximab, compared to either of them alone.⁸³ Thus these TLR agonists in combination with cetuximab can enhance cetuximab induced ADCC against head and neck cancer.

In another study involving TLR9, it has been demonstrated that CpG-containing oligodeoxynucleotides (CpG ODN), the TLR9 agonist, can directly promote the secretion of cytokines by NK cells exposed to antibody-coated tumor cells by activating TLR9.⁸⁴ Further, Sommariva et al⁸⁵ have demonstrated in an in vivo advanced ovarian xenograft model that mice treated with a combination of CpG ODN and cetuximab had a significantly increased median survival rate relative to

monotherapy with either agent. CpG ODNs can also activate NK cells through indirect activation of plasmacytoid DCs that stimulate IFN- γ production by T cells.⁸⁶ CpG ODNs can also induce CD20 expression on malignant B cells.⁸⁷ Thus the activating effect of CpG ODN on the effector cells as well as on the tumor cells can have a synergistic effect when used in combination with mAbs. It has been shown in preclinical studies that CpG ODNs enhance antitumor activity of rituximab in treating lymphomas^{88,89} and trastuzumab in treating breast cancer.^{87,90}

Effector cell activation: agonistic and antagonistic mAbs

The importance of utilizing mAb therapy to elicit ADCC-mediated tumor clearance was initially established by studies exploring the mechanism of action of rituximab. One of the primary mechanisms by which rituximab exerts its antitumor effects is by making the CD20-expressing tumor a more attractive target for NK cell lysis. In the decades following the introduction of rituximab, subsequent mAbs have been developed that augment ADCC. A particularly promising strategy for enhancing ADCC via mAb therapy is targeting the costimulatory pathways that activate NK cell cytotoxicity. One molecule that has demonstrated strong preclinical success in this approach is CD137.

CD137

CD137 is a member of TNF receptor superfamily and is upregulated on NK cells after Fc γ RIIIa (CD16) ligation.⁹¹ Administration of agonistic anti-CD137 mAbs has been shown to amplify antitumor immune responses in a variety of different murine cancer models.⁹² On NK cells, activation of CD137 increases proliferation, degranulation, and IFN- γ secretion, leading to enhanced ADCC.⁹³ The ability of anti-CD137 mAbs to enhance ADCC makes them ideal candidates for combination therapeutic strategies. We have previously demonstrated that targeting CD137 concomitantly with rituximab or trastuzumab administration accelerates tumor clearance in murine xenograft models of lymphoma and breast cancer.^{94,95} Recently, we combined cetuximab and anti-CD137 antibody therapy to obtain complete tumor resolution and prolonged survival in xenograft models of epidermal growth factor receptor-expressing cancer cells, head and neck cancer cells, and wild-type Kirsten rat sarcoma 2 viral oncogene homolog (KRAS-WT) and KRAS-mutant colorectal cancer.⁹⁶ An anti-CD137 antibody, urelumab, is currently in clinical trials with rituximab for patients with non-Hodgkin's lymphoma (NCT01775631) and with

cetuximab in patients with colorectal cancer or head and neck cancer (NCT02110082).

KIR signaling

The killer cell immunoglobulin-like receptor (KIR) family constitutes one of the key mediators of NK cell activation. Inhibitory KIR molecules bind to the self-major histocompatibility complex class I ligands (HLA-A, HLA-B, HLA-C) and upon binding transduce inhibitory signals that abrogate the effects of activating receptors.⁹⁷ Because major histocompatibility complex 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 signaling in NK cells via antibody blockade has been shown to increase NK cell cytotoxicity and survival of leukemia-bearing mice.⁹⁸ A fully human mAb that binds KIR2DL1, KIR2DL2, and KIR2DL3 receptors enhanced NK cell-mediated lysis of tumor cells, including autologous acute myeloid leukemia

(AML) blasts, but did not induce killing of normal peripheral blood mononuclear cells.⁹⁹

Based on these results, a KIR-blocking mAb, lirilumab (IPH2102/BMS-986015), was developed and 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.¹⁰⁰ A trial of lirilumab in patients with AML in first complete remission further validated anti-KIR therapy as safe and tolerable, but only produced transient NK activation.¹⁰¹

There is also interest in using anti-KIR antibodies in combinatorial checkpoint blockade strategies. Immune checkpoints are inhibitory pathways that downregulate activated immune cells. During tumor genesis, cancer cells express proteins that activate immune checkpoint pathways and induce immune suppression, thereby evading targeting and removal by the immune system. Checkpoint blockade is a therapeutic strategy by which these inhibitory signals are

Table 3 A list of therapeutic reagents mentioned in the article in clinical trials

Agent	Target	Cancer	Status	Phase	Notes	Reference
Obinutuzumab	CD20	Chronic lymphocytic leukemia	Recruiting	Phase III	A safety study of obinutuzumab alone or in combination with chemotherapy in patients with chronic lymphocytic leukemia	NCT01905943
Obinutuzumab	CD20	Chronic lymphocytic leukemia	Active	Phase I	A study comparing RO5072759 (GA101) 1,000 mg versus 2,000 mg in patients with previously untreated chronic lymphocytic leukemia (GAGE trial)	NCT01414205
Margetuximab	HER2	Breast cancer	Recruiting	Phase II	Phase II study of the monoclonal antibody MGAH22 (margetuximab) in patients with relapsed or refractory advanced breast cancer	NCT01828021
Hiltonol (poly-ICLC)	TLR3	Melanoma, head and neck cancer, breast cancer	Recruiting	Phase II	Treatment of solid tumors with intratumoral Hiltonol® (poly-ICLC)	NCT01984892
Urelumab	CD137	B-cell non-Hodgkin's lymphoma	Recruiting	Phase I	Combination study of urelumab and rituximab in patients with B-cell non-Hodgkin's lymphoma	NCT01775631
Urelumab	CD137	Colorectal cancer/ head and neck cancer	Recruiting	Phase I	Combination study of urelumab and cetuximab in patients with advanced/metastatic colorectal cancer or advanced/metastatic head and neck cancer	NCT02110082
Lirilumab	KIR	Multiple myeloma	Recruiting	Phase I	A Phase I open label study of the safety and tolerability of elotuzumab (BMS-901608) administered in combination with either lirilumab (BMS-986015) or urelumab (BMS-663513) in subjects with multiple myeloma	NCT02252263
Nivolumab	KIR	Renal cell carcinoma, melanoma	Recruiting	Phase I	A Phase I study of an anti-KIR antibody in combination with an anti-PD-1 antibody in patients with advanced solid tumors	NCT01714739
Ipilimumab	CTLA4	Melanoma	Recruiting	Phase I	Safety study of BMS-986015 (anti-KIR) in combination with Ipilimumab in subjects with selected advanced tumors	NCT01750580
TRX518	GITR	Malignant melanoma	Recruiting	Phase I	Trial of TRX518 (anti-GITR mAb) in stage 3 or 4 malignant melanoma	NCT01239134

Abbreviations: CD, cluster of differentiation; CTLA4, cytotoxic T-lymphocyte-associated protein 4; GITR, glucocorticoid-induced tumor necrosis factor receptor; HER2, human epidermal growth factor receptor 2; KIR, killer cell immunoglobulin-like receptor; mAb, monoclonal antibody; PD-1, programmed cell-death protein 1; TLR, toll-like receptor.

blocked by antibodies. With the immune suppression in the tumor microenvironment removed, the tumor is targeted for clearance. A trial testing lirilumab and nivolumab, a fully human IgG4 antibody that inhibits the checkpoint marker programmed cell-death protein 1 (PD-1), is currently recruiting patients with advanced solid tumors (NCT01714739). Lirilumab is also being tested in combination with the anti-CTLA4 antibody, ipilimumab in patients with advanced melanoma, non-small cell lung cancer, and castrate-resistant prostate cancer (NCT01750580).

PD-1/PD-L1 axis

PD-1 and its ligands (PD-L1 and PD-L2) constitute another important signaling pathway in regulating NK cell activation and downstream ADCC. PD-1 and PD-L1 blockade is currently thought to primarily influence T-cell subsets, but following activation, PD-1 is expressed on NK cells and ligation of PD-1 transmits a negative regulatory signal, limiting NK cell cytotoxicity.¹⁰² In histologically diverse tumors, PD-L1 can be upregulated, providing a potent mechanism of immunosuppression in the tumor microenvironment.¹⁰³ Tumors can also induce PD-L1 expression on immune subsets. In murine cancer models, tumor-derived interleukin IL-18 was shown to induce PD-L1 expression on a subset of immature NK cells, which then trafficked to lymph nodes and suppressed the DC/NK cell crosstalk necessary for the development of mature, cytotoxic NK cells.¹⁰⁴ Preclinically, antibodies that block PD-1 signaling have been shown to enhance NK cell cytotoxicity against autologous, primary multiple myeloma tumors.¹⁰⁵ In late 2014, the FDA approved the first anti-PD-1 mAb (pembrolizumab) based on a study that reported an overall response rate of 26% in ipilimumab-refractory advanced melanoma patients.¹⁰⁶ Currently, clinical trials are being considered that combine PD-1 blockade and therapeutic, tumor-targeting antibodies.¹⁰⁴

GITR/GITRL

Glucocorticoid-induced TNF receptor (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 and upregulate its expression following activation.^{107,108} GITRL also inhibits the rituximab-induced ADCC of NK cells.¹⁰⁹ 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. Thus, 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 patients with stage 2 or stage 4 melanoma.

Conclusion

The combination of tumor-targeting mAbs and ADCC-enhancing immunomodulators is a promising treatment strategy for oncology patients. As more tumor-associated antigens are identified and immune effectors' activating pathways are better understood, the applicability of this approach will only increase. Though preliminary data from work with oncolytic viruses, TLR agonists, engineered mAbs, and immunostimulatory mAbs is encouraging, substantial clinical evidence is needed to validate these therapies. The results of ongoing clinical trials are eagerly awaited (Table 3). Hopefully, clinical studies validate this novel therapeutic strategy and lead to increased patient survival.

Disclosure

The authors report no conflicts of interest in this work.

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