REVIEW ARTICLE

Cytokines that target immune killer cells against tumors

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T-cell-stimulating cytokines have shown promise as monotherapies or in combination with other therapeutic modalities for immunotherapy of cancer. However, their efficacy is limited due to their short half-life, pleiotropic roles, and induction of severe toxicity even at therapeutic doses. To overcome these major therapeutic barriers, cytokine-based products are being further developed to improve their therapeutic index. These approaches include manipulating their activity to preferentially bind to effector immune cells rather than immune-suppressive cells, prolonging their half-life in vivo and modifying them to target tumors. This review focuses on IL-2, IL-15, and IL-10, which have potent effects on immune cells that mediate effective antitumor responses. We will summarize the recent progress of these cytokines in both preclinical studies and selective clinical applications and will discuss our perspectives on the development of new strategies to potentiate cytokine-based immunotherapy.

Keywords: IL-2; IL-15; IL-10; cytokine therapy; cancer

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INTRODUCTION

Immunotherapy has emerged as a new pillar of cancer treatment. Most studies focus on stimulating T cells by blocking checkpoints or enhancing costimulation signals. Cytokines are a group of major factors that regulate both innate and adaptive immune responses in autocrine and paracrine manners throughout the host immune system. Among the cytokines that have shown natural killer (NK) cell- and $CD8^+$ T-cell-mediated immune responses against tumors, interleukin-2 (IL-2), interleukin-15 (IL-15), and interleukin-10 (IL-10) have recently achieved rapid advances in their development as clinical therapeutics for cancer treatment. The application of these cytokine-based therapeutics for clinical study in cancer treatment has shown encouraging results, such as an increase in NK or/and $CD8^+$ T cells that may play key roles in mediating antitumor effects. On the other hand, after systemic administration of IL-2, IL-15, or IL-10, a short half-life and treatment-related adverse events are major barriers to therapeutic efficacy. In this review, we focus on recent progress in the understanding of therapeutic mechanisms and the development of strategies to overcome these barriers to improve antitumor effects with reduced toxicity.

INTERLEUKIN-2 (IL-2)

IL-2 is a 15.5-kDa glycoprotein and is mainly produced by activated $CD4^+$ T cells.[1–3] Other immune cells, such as activated $CD8^+$ T cells, NK cells, NKT cells, and innate lymphoid cells (ILCs), can also produce IL-2 to a lesser extent.[4,5] The IL-2 receptor is a heterocomplex of three subunits, the IL-2Rα, IL-2Rβ, and IL-2Rγ chains, which are also known as CD25, CD122, and CD132, respectively.[6,7] To activate intracellular signals, IL-2 must bind with the intermediate-affinity (K_d 10^{-9} M) IL-2Rβγ heterodimer or the strong-affinity (K_d 10^{-11} M) IL-2Rαβγ trimer. While low-dose IL-2 primarily stimulates and expands regulatory T cells (Tregs) due to their enriched expression of the high-affinity receptor complex (IL-2Rαβγ), a higher dose of IL-2 allows extra IL-2 to also stimulate $CD8^+$ T cells and NK cells.[8–10] Rosenberg et al. showed that systemic administration of a high dose of IL-2 (HD-IL-2) resulted in an overall objective response rate of 14%, with complete responses seen in 5% of patients with metastatic renal cell carcinoma and malignant melanoma.[11–14] As such, the use of HD-IL-2 has been approved as a first-line immunotherapeutic agent to treat cancer patients. However, systemic HD-IL-2 treatment has been associated with severe host toxicity, including vascular leak syndrome, due to damage to endothelial cells expressing the IL-2 receptor and unwanted inflammatory responses due to elevated proinflammatory cytokines released by peripheral T and NK cells. As such, patients receiving HD-IL-2 treatment need to do so at specialized hospitals.

Various strategies have been developed to improve IL-2 therapy efficacy and reduce toxicity. For example, one study has demonstrated that injection of a certain monoclonal antibody against IL-2 resulted in the formation of a complex between endogenous IL-2 and this antibody.[15] Furthermore, they demonstrated that this effect not only led to the proliferation of memory $CD8^+$ T cells and NK cells that express the intermediate-affinity receptor IL-2Rβγ but also prevented IL-2 from binding to IL-2Rα on Tregs, a major immunosuppressive cell population within the tumor microenvironment (TME). Moreover, this approach was also tested in a preclinical toxicity study, which showed that the complex of IL-2 and the anti-IL2 antibody prevented vascular leak syndromes such as pulmonary edema by blocking IL-2 from binding to IL-2Rα on lung endothelial cells.[16] Finally, it was reported very recently that the IL-2/anti-IL2 antibody complex can overcome immune checkpoint blockade resistance in murine melanoma models in a $CD8^+$ T-cell-dependent manner when combined with anti-PD-1 antibodies or in both a $CD8^+$ T-cell- and NK cell-dependent manner when combined with anti-CTLA-4

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antibodies.[17] It is likely that anti-CTLA-4 antibodies deplete Tregs, allowing much more IL-2 to stimulate effector cells while mitigating immune suppression by Tregs.

Alternatively, an IL-2 "superkine" was generated based on the IL-2/IL-2R binding structure confirmation. This approach aimed to increase the binding affinity for IL-2Rβ while also eliminating the binding affinity for IL-2Rα and maintaining optimal signal transduction.[18] In line with the IL-2/anti-IL-2 antibody complex, the IL-2 superkine demonstrated improved antitumor activity with an expansion of $CD8^+$ T and NK cells, a decrease in Tregs and reduced toxicity.[18] Later, in an attempt to overcome the short half-life, PEGylated IL-2 with reduced IL-2Rα binding and a prolonged half-life (termed NKTR-214) was generated and tested in both preclinical and clinical studies. In murine melanoma models, NKTR-214 significantly increased the ratio of $CD8^+$ T cells to Tregs and resulted in improved antitumor efficacy compared with recombinant IL-2 (aldesleukin).[19] Notably, in murine breast cancer models, the combination of NKTR-214 and anti-CTLA-4 antibodies generated synergistic and durable antitumor effects.[19] It was reported very recently that NKTR-214 can also synergize with anti-PD-L1 therapy in a variety of murine tumor models.[20] Clinically, preliminary results have reported an increase in NK and $CD8^+$ T cells in the peripheral blood of patients receiving NKTR-214,[21] and another study recently reported a decrease in intratumoral Tregs in a small cohort of cancer patients treated with NKTR-214.[20] In addition, a 23% (6 out of 26 patients) tumor reduction response rate as well as a well-tolerated safety profile was also reported.[22] It is expected that the response rate could increase upon combination with other treatments. However, PEGylated products do not specifically target tumor tissues, and technical improvements must be made to achieve tumor-targeting products with precisely PEGylated sites. Otherwise, variations in clinical outcomes may be obtained due to inconsistently manufactured products. The ongoing clinical trials on the combination therapy of NKTR-214 with immune checkpoints and other therapeutics, as well as its limitations, are reviewed elsewhere.[23]

To increase IL-2 delivery to tumor sites, along with the consideration that IL-2-Fc fusion can prolong its half-life,[24] antibody-based tumor-targeted delivery of IL-2 has been attempted by our group and other groups. In mouse studies, it has been shown that when delivered systemically, a tumor-targeting antibody-IL-2 fusion protein can localize to tumor sites, including lung micrometastases.[25] More importantly, the fusion protein alone can induce effective $CD8^+$ T-cell-mediated antitumor immune responses, activate NK cells, and reduce IL-2 retention in peripheral blood, which may lead to lower toxicity.[26] Finally, the fusion protein resulted in significantly improved antitumor effects compared with either antibody or cytokine monotherapy and even better effects than the combination of the antibody and cytokine therapies.[25–28] A tumor-specific antibody fusion with engineered IL-2 has also been developed and demonstrated similar antitumor efficacy to one containing wild-type IL-2 but with reduced toxicity in both immune-competent mice and cynomolgus monkeys.[29] Recently, our group developed a next-generation antibody-IL-2 fusion protein.[30] We engineered IL-2 by introducing several mutations that have been reported to reduce its binding to IL-2Rα (F42A)[31] and increase its binding to IL-2Rβ (super IL-2).[18] The engineered IL-2 was linked to an Fc moiety to generate a super mutant IL2-Fc (termed sumIL-2-Fc). To improve the tumor targeting of sumIL-2-Fc, we generated a heterodimeric sumIL-2 fusion protein that is composed of a sumIL-2 Fc monomer on one arm and an antitumor monoclonal antibody on the other arm, named Ab-sumIL-2. In a mouse study, the prolonged half-life of Ab-sumIL-2 in serum was ~8 h, whereas FDA-approved recombinant IL-2 was cleared from circulation in minutes.[15] Notably, Ab-sumIL-2 not only showed superior antitumor efficacy over fusion proteins containing only F42A or super IL-2 but also achieved improved efficacy when combined with surgery, small molecule targeted therapy, and even immune checkpoint blockade. Cold (fewer TILs) tumors often fail to respond to various immunotherapies, and converting cold tumors to hot ones is challenging. In mice bearing the TUBO mammary tumor model, which is considered a cold tumor due to a lower frequency of $CD8^+$ TILs than murine tumor models such as B16F10 and MC38,[30] antibody-sumIL-2 generated synergistic antitumor effects with an EGFR tyrosine kinase inhibitor (EGFR-TKI), most likely through an increase in TILs. Furthermore, for the treatment of metastatic diseases, we used a 4T1 murine mammary carcinoma model with spontaneous metastases, which allowed us to mimic the clinical setting by surgically removing primary tumors established in the mammary gland. Interestingly, we observed that the treatment regimen of preadministration of Ab-sumIL-2 followed by surgery prolonged mouse survival compared with Ab-sumIL-2 administered as an adjuvant. This antitumor effect was dependent on both $CD4^+$ and $CD8^+$ T cells. In addition, our results showed that Ab-sumIL-2 increased PD-L1 expression on intratumoral DCs and that the combination of Ab-sumIL-2 and anti-PDL-1 therapy could overcome resistance to immune checkpoint blockade. Since IL-2 plays a pivotal role in T-cell survival and proliferation, it is possible that targeting tumors with Ab-sumIL-2 may help expand TILs that are brake-released intratumorally and/or those that are newly recruited into tumors when combined with immune checkpoint blockades.

One of the prospective uses of immune cell growth cytokines is in combination with cell therapy, as a sufficient number of tumor-reactive cells may require exogenous growth factors to sustain their survival and expansion after cell transfer. With respect to this combination strategy, a recent study showed that the combination of an IL-2-Fc fusion protein, antitumor antibody, and adoptive T-cell transfer induced durable tumor control through activation and expansion of tumor-specific $CD8^+$ T cells.[24] Considering that the Ab-sumIL-2 immunocytokine has a longer half-life and less toxicity than unmodified IL-2, tumor-targeted delivery, and preferential binding to $CD8^+$ T cells over Tregs, it is expected that tumor-targeted Ab-sumIL-2 may be further developed as a new agent to improve immunotherapies, including immune checkpoint blockade agents and cell therapies, for cancer patients.

INTERLEUKIN-15 (IL-15)

IL-15 has a heterotrimeric receptor including IL-15Rα and IL-2Rβγ, which is also used by IL-2. In contrast to IL-2, IL-15 is mainly produced by monocytes, macrophages, and dendritic cells,[32] with a pivotal role in NK cell development and in the homeostasis of memory $CD8^+$ T cells. Its effects on other types of immune cells, such as ILCs, were also identified in recent studies.[33] Importantly, IL-15 does not stimulate Tregs because it does not bind to the IL-2Rα chain, which is required for the formation of the high-affinity receptor complex (IL-2Rαβγ) on Tregs to stimulate immunosuppressive signals. Of note, IL-15 associated with IL-15α on the cell surface of monocytes or DCs, rather than the IL-15 monomer, can provide strong signals in *trans* to NK and $CD8^+$ memory T cells, leading to enhanced antitumor effects.[34–36] As such, several versions of the IL-15/IL-15Ra complex have been developed for clinical applications. For example, an IL-15 superagonist, termed ALT-803, consisting of human IL-15 covalently linked to the sushi domain of human IL-15Rα, has been used in combination with anti-PD-1 antibodies in a phase Ib trial for patients with metastatic non-small-cell lung cancer. The preliminary results showed promising antitumor activity in 6 out of 21 patients with a tolerable safety profile.[37] In addition, based on the observations of effective antitumor effects in preclinical studies,[38–42] clinical trials have been initiated for combinations of recombinant IL-15 (rhIL-15) with both anti-CTLA-4 and anti-PD-1 therapy, with a CD40 agonist and with monoclonal antibodies including anti-CD52 and anti-CD20 antibodies.[33] As a whole, collective clinical studies have

suggested that the combination of IL-15 with other immune therapeutics to increase NK and $CD8^+$ T-cell-mediated antitumor immunity may improve efficacy, but the risk of IL-15 therapy-induced adverse events should be kept in mind since IL-15 can activate NK and T cells in peripheral blood (reviewed elsewhere).[33]

To improve IL-15 efficacy with reduced toxicity, several novel strategies have been very recently developed and tested in preclinical studies. For example, since IL-2 and IL-15 share the same receptor, IL-2Rβγ, computational approaches to design proteins mimics of IL-2 and IL-15 that bind human and mouse IL-2Rβγ chains but do not bind IL-2Rα or IL-15Rα were utilized, and the resulting protein was termed Neo-2/15.[43] The resulting product can recapitulate the natural signaling function of IL-2 and IL-15 but does not carry the adverse effects associated with IL-2Rα or IL-15Rα binding. This product has shown superior therapeutic activity with reduced toxicity in the treatment of murine tumors, suggesting the possibility of creating superior therapeutic candidates in the future.[43] Another study examined the tumor-targeted delivery of an IL-15 superagonist by using CAR-T cells, which were manipulated by protein nanogels to selectively release IL-15 into the TME.[44] As such, this approach would not only increase the therapeutic window for cytokine-based therapy but also achieve the combined therapeutic efficacy of both cytokine and T-cell therapy. This strategy may be feasible to utilize in the next generation of cytokines by engineering the cytokine itself, creating a "pro-cytokine" that is only activated in the TME rather than systemically. This would subsequently potentiate intratumoral effects while mitigating the induction of host toxicity.

INTERLEUKIN-10 (IL-10)

In contrast to IL-2 and IL-15, IL-10 was initially identified as an inhibitory cytokine that is produced by Th2 cells and can inhibit Th1 cell cytokine production.[45,46] Later, it was found that IL-10 can indeed be expressed not only by immune-suppressive Tregs (both $Foxp3^+$ and $Foxp3^-$)[47,48] but also by other immune cells.[49–52] In addition, the production of IL-10 by normal human epithelial cells and human melanoma cells has also been reported.[53,54] Much of the known roles of IL-10 are related to its immune regulatory function. In line with this, it has been demonstrated that the genetic ablation of *Il10* or deficiency of the IL-10 receptor (IL-10R) is associated with inflammatory pathology and autoimmune diseases, including inflammatory bowel disease, rheumatoid arthritis, and psoriasis. The IL-10R is a heterodimer of two subunits termed IL-10Rα and IL-10Rβ, also known as IL-10R1 and IL-10R2, respectively. IL-10 initially binds IL-10R1, and the subsequent conformational change mediates IL-10 binding to IL-10R2.[55] The phosphorylation and activation of intracellular STAT3 are major signaling events leading to IL-10-mediated anti-inflammatory responses,[56,57] although the activation of STAT1 and STAT5 pathways has also been studied.[58–60] The deletion of STAT3 in myeloid cells can cause enterocolitis and aberrant inflammation in mice.[61,62] Mechanistically, the anti-inflammatory roles of IL-10 include inhibition of proinflammatory cytokine production, limitation of antigen presentation through downregulation of MHCII and costimulatory molecules, and maintenance of Foxp3 expression and the immune-suppressive function of Tregs, among others.[56,63–65] As with other IL-10 family cytokines, IL-10-mediated anti-inflammatory mechanisms are still mainly understood in the context of inflammatory disease models,[66,67] and the application of IL-10-based treatment has also been mainly used for inflammatory and autoimmune diseases.[68]

In the context of tumor models, IL-10 has shown a paradox in terms of immunological response, likely resulting from its pleiotropic action on a variety of immune cells, particularly within the complicated TME. Indeed, in most studies of antitumor immunity, endogenous IL-10 is considered a main factor contributing to the immune-suppressive TME.[47,69–71] In patients, it has been reported that tumor-derived IL-10 can inhibit T-cell proliferation, likely by impairing the functions of antigen-presenting cells, including DCs and monocytes.[72,73] Interestingly, it has been reported that melanoma patient-derived serum amyloid A-1 (SSA-1) protein can induce IL-10 production by immune-suppressive neutrophils, but SSA-1 can also promote the interaction between these neutrophils and invariant NKT cells, resulting in greatly decreased IL-10 secretion and reduced suppressive function of the neutrophils.[74] This study suggested that IL-10 may be a key factor in modulating the plasticity of certain immune cell subsets, such as neutrophils, in tumor immunity. Moreover, blockade of IL-10 signaling has been shown to enhance antitumor immunity. For example, in mouse studies, blockade of the IL-10R significantly improved chemotherapeutic efficacy in a $CD8^+$ T-cell-dependent manner.[69] In addition, blockade of IL-10 can increase the effects of anti-PD-1 therapy on melanoma patient-derived tumor-antigen-specific $CD8^+$ T-cell expansion and function.[70] Finally, it was reported that there is a positive correlation between serum levels of IL-10 and tumor progression.[75,76]

However, paradoxically, increasing evidence demonstrates that IL-10 can induce antitumor effects in an immune-dependent manner.[77–83] Particularly, recent preclinical studies report that systemic delivery of a pegylated form of IL-10 (PEG-IL10), initially designed to prolong the half-life of IL-10, can inhibit tumor growth by enhancing intratumoral $CD8^+$ T-cell proliferation and function.[81,82] A phase I clinical trial with PEG-IL10 has also shown encouraging antitumor activity in the treatment of patients with advanced solid tumors.[84] Furthermore, the combination of PEG-IL10 treatment and anti-PD-1 therapy was tested as a treatment for patients with solid tumors. Recent preliminary results reported that the combination of PEG-IL10 and anti-PD-1 therapy had a 42% overall response rate among 19 patients. In a subsequent phase Ib trial, the results showed that the objective response rates were 43% among 28 patients with non-small-cell lung cancer and 40% among 35 patients with renal cell carcinoma.[85] However, PEG-IL10-induced toxicity has been observed in both preclinical and clinical studies. Systemic administration of PEG-IL10 to treat murine tumors showed an increase in immune cell infiltration and pathological immune responses in several normal organs, possibly due to off-tumor delivery of PEG-IL10.[81] Moreover, patients receiving the highest doses of PEG-IL10 showed treatment-related adverse events,[84] in line with other clinical studies that showed that systemic administration of rIL-10 promotes proinflammatory cytokine production, potentially limiting therapeutic effects.[86,87]

To overcome its short half-life and allow tumor-targeted delivery of IL-10, we recently generated a bispecific fusion protein targeting both an oncogenic receptor and IL-10R.[88] For proof-of concept studies, we chose the FDA-approved anti-epidermal growth factor receptor (anti-EGFR) antibody cetuximab (Erbitux) for targeted delivery of IL-10 to $EGFR^+$ tumors. We generated an antioncogenic receptor antibody-based fusion protein, termed CmAb-$(IL10)_2$, and tested whether it could overcome the short half-life of rIL-10 while minimizing off-tumor toxicity, evaluated its antitumor effects and elucidated the mechanisms by which CmAb-$(IL10)_2$ improves $CD8^+$ T-cell-mediated antitumor responses. Our results showed that the half-life of CmAb-$(IL10)_2$ is ~40 h. Notably, at least 10% of the initial dose of CmAb-$(IL10)_2$ was detected and retained in serum up to 4 days after administration. By comparison, in mice, PEG-IL10, which was specially designed to prolong the circulation time of IL-10, was detected at less than 10% of the initial dose in serum 24 h after i.v. adminstration.[89] Importantly, our results in mouse tumor models suggest that systemic delivery of CmAb-$(IL10)_2$ can target IL-10 to and retain it within tumor tissue, leading to superior antitumor effects over nontargeted IL-10 fusion proteins.[88]

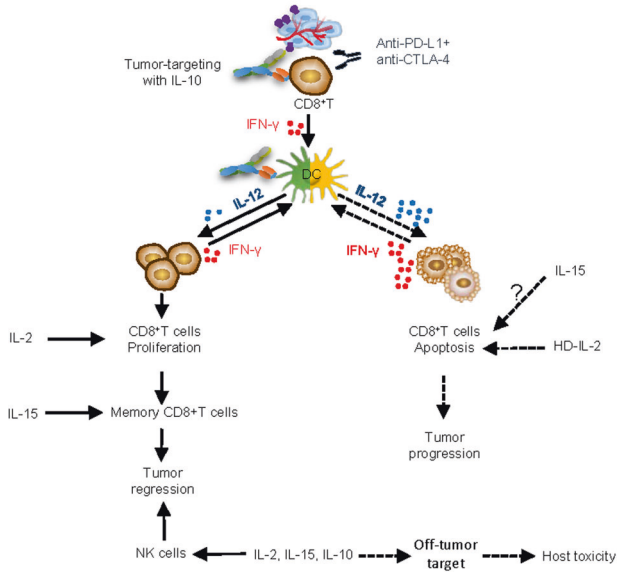

Fig. 1 Distinct and cooperative roles of IL-10, IL-2, and IL-15 in antitumor immunity. Tumor-targeted delivery of IL-10 inhibits intratumoral $CD8^+$ T-cell apoptosis, which may offer a strong rationale for combining IL-10-based strategies with immunotherapies that can potently boost T-cell proliferation and function, such as IL-2 and immune checkpoint blockade therapy, and the addition of IL-15 may further boost NK and memory $CD8^+$ T-cell immunity to achieve synergistic antitumor effects. Nontargeted delivery of cytokines induces host toxicity, limiting the therapeutic index

Ultimately, although IL-10 is generally known as an immunosuppressive cytokine, it has also been shown to promote $CD8^+$ T-cell responses in cancer treatment.[68,80–82] We speculate that the dose and administration schedule of exogenous IL-10 and the abundance, activation status, and expression of the IL-10R on $CD8^+$ TILs may contribute to IL-10-mediated antitumor effects. However, it remains elusive how the immune-suppressive effects of IL-10 can contribute to $CD8^+$ T-cell-mediated immune responses against tumors. As such, we further investigated the therapeutic mechanisms of CmAb-$(IL10)_2$ in cancer treatment. We revealed a new mechanism by which CmAb-$(IL10)_2$ suppresses DC-mediated antigen-specific $CD8^+$ T-cell apoptosis, leading to higher levels of intratumoral, tumor-specific $CD8^+$ T cells in a DC-IL10R signal-dependent manner.[88] The molecular mechanism linking this role of DCs to T-cell enhancement is that CmAb-$(IL10)_2$ regulates the IL-12/IFN-γ production axis to suppress IFN-γ-mediated apoptosis of antigen-specific $CD8^+$ T cells (as denoted in Fig. 1). Our finding is in support of recent studies showing that apoptosis of $CD8^+$ TILs may be a critical barrier to effective immunotherapy[90] and further suggests that IL-10-based treatment may provide a novel strategy to improve immunotherapy for cancer. In support of our findings, we observed that the combination of CmAb-$(IL10)_2$ with anti-CTLA-4 and anti-PD-L1 blockade therapy in the treatment of advanced murine tumors had greatly improved therapeutic efficacy compared with either IL-10 or immune checkpoint blockade therapy alone. Moreover, this improved efficacy was associated with a decrease in tumor-specific $CD8^+$ TIL apoptosis.[88] Taken together, these results suggest that immunotherapies aimed at stimulating effector cells to produce high levels of IFN-γ, such as checkpoint blockade therapies that can significantly increase the expression of IFN-γ by TILs,[91,92] would be ideal candidates for rational combination with IL-10-based therapies to prevent T-cell apoptosis. In future studies, since anti-CTLA-4 and anti-PD-1 therapies induce different antitumor effects through specific mechanisms,[93] it will be necessary to dissect the role of IL-10 in combination with either anti-CTLA-4 or anti-PD-1 antibodies to better design an optimal treatment regimen. A major issue with immune checkpoint blockade therapy, particularly when combining anti-CTLA-4 and anti-PD-L1 strategies together, is the frequency of immune-related adverse events.[94,95] Although we did not observe toxicity in mice treated with the combination of CmAb-$(IL10)_2$ and anti-CTLA-4 and anti-PD-L1 antibodies, whether it would have limited toxicity in humans remains to be determined.

CONCLUSIONS AND PERSPECTIVES

Checkpoint blockade and adoptive T-cell transfer immunotherapies for cancer treatment have shown success in the clinic. Strategies aimed at improving cytokine-based therapy along with reducing toxicity are rapidly being developed, mainly through engineering better cytokines and optimizing combination therapy regimens. Clinically, NKTR-214, an engineered IL-2 molecule, has shown evidence of antitumor activity and tolerability in a first-in-human phase I study as a monotherapy in patients with advanced solid tumors.[96] This led to the further combination of NKTR-214 with immune checkpoint blockade therapy, which is already under ongoing clinical trials.

Furthermore, our strategy of tumor-targeted delivery of IL-10 potentiates intratumoral $CD8^+$ T-cell-mediated antitumor immunity without inducing host toxicity in mice. We thus expect CmAb-$(IL10)_2$ to be a good candidate for further development, by which it can ultimately be tested in clinical studies as monotherapy or in combination immunotherapy strategies for cancer. The feasibility of generating new fusion proteins by integrating tumor-targeting molecules other than anti-EGFR antibodies or by integrating cytokines other than IL-10 would allow a broad application of this approach for the treatment of different types of tumors. Moreover, our discovery that IL-10 inhibits T-cell apoptosis offers a strong rationale for combining IL-10-based strategies with immunotherapies that can potently boost T-cell proliferation and function, such as IL-2 and immune checkpoint blockade therapy, and the addition of IL-15 may further boost NK and memory $CD8^+$ T-cell immunity to achieve synergistic antitumor effects (as denoted in Fig. 1). Given that the goal of enhancing immunotherapy to increase efficacy while minimizing therapy-induced adverse effects is important, the key next step for further development of cytokine therapy is to fully understand the mechanisms of each factor-induced response. Based on the knowledge obtained, a potential next generation of cytokine-based agents and a strategy for rational combination therapy could be generated and will ultimately provide therapeutic benefits for cancer patients.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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