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REVIEW

Role of IL-2 in cancer immunotherapy

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ABSTRACT

Interleukin-2 (IL-2) is one of the key cytokines with pleiotropic effects on immune system. It has been approved for the treatment of metastatic renal cell carcinoma and metastatic melanoma. Recent progress has been made in our understanding of IL-2 in regulating lymphocytes that has led to exciting new directions for cancer immunotherapy. While improved IL-2 formulations might be used as monotherapies, their combination with other anticancer immunotherapies, such as adoptive cell transfer regimens, antigen-specific vaccination, and blockade of immune checkpoint inhibitory molecules, for example cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) mono-antibodies, would held the promise of treating metastatic cancer. Despite the comprehensive studies of IL-2 on immune system have established the application of IL-2 for cancer immunotherapy, a number of poignant obstacles remain for future research. In the present review, we will focus on the key biological features of IL-2, current applications, limitations, and future directions of IL-2 in cancer immunotherapy.

Abbreviations: ACT, adoptive T cell therapy; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DC, dendritic cells; IL-2, interleukin-2; JAK, Janus family tyrosine kinases; LAK, lymphokine activated killer; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteases; NK, nature killer cell; PD-1, programmed death 1; PD-L1, programmed death ligand 1; PI3K, phosphoinositide 3-kinase; PSA, prostate specific antigen; STAT, signal transducer and activator of transcription; TAAs, tumor-associated antigens; TCR, T cell receptor; Th1, T helper-1; Th2, T helper-2; Th17, T helper-17; TILs, tumor-infiltrating lymphocytes; TNF-α, tumor necrosis factor α; Treg, T regulatory cell

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Introduction

Cancer is one of the most common lethal diseases in the world, with 14 million new cases diagnosed annually and is also the leading cause of deaths worldwide, causing 8.2 million deaths annually as World Health Organization (WHO) reported in the World Cancer Report 2014. Although the identification of a large amount of driver oncogenes and subsequent targeted therapy have resulted in a prolongation of overall survival in these with driver mutations, survival remains dismal as a whole and novel therapeutic approaches are still urgently needed. Recently there has been a breakthrough in harnessing the immune system to treat malignant tumors. Cytokines are small glycoproteins that bind to cell surface receptors and regulate the development, survival, and function of immune cell. Thus, cytokines have been extensively studied as potential therapeutic agents to manipulate the immune response to tumor cells.

IL-2 is one of the key cytokines with pleiotropic effects on the immune system. The discovery of IL-2 as "T-cell growth factor" (TCGF) in 1976 quickly revolutionized the fields of basic immunology research and immunotherapy for human cancers.[1] IL-2 was an early candidate for cancer immunotherapy and was approved for the treatment of metastatic renal cell carcinoma (1992) and later for metastatic melanoma (1998) by FDA. Much progress has been made recently in our understanding of IL-2 in regulating lymphocytes that has led to exciting new directions in cancer immunotherapy (Fig. 1). There are several excellent reviews on IL-2, which examine the molecular biology of its expression, its role in immune cell signaling and immune development, as well as the structural biology of cytokines and their receptors.[2-5] In the present review, we will focus on the key biological features of IL-2, current applications, limitations, and future directions of IL-2 in cancer immunotherapy.

The biology of IL-2 and its receptors

IL-2 is a small 15.5-kDa four α-helical bundle cytokine, which has been one of the most studied cytokines since its discovery about 38 y ago. It is produced predominately by antigen-simulated $CD4^+$ T cells, while it can also be produced by $CD8^+$ cells, natural killer (NK) cells, and activated dendritic cells (DC).[6-8] IL-2 is an important factor for the maintenance of $CD4^+$ regulatory T cells and plays a critical role in the differentiation of $CD4^+$ T cells into a variety of subsets. It can promote $CD8^+$ T-cell and NK cell cytotoxicity activity, and modulate T-cell differentiation programs in response to antigen, promoting naive $CD4^+$ T-cell differentiation into T helper-1 (Th1) and T helper-2 (Th2) cells while inhibiting T helper-17 (Th17) differentiation.[9-11]

IL-2 receptor is composed of the three subunits IL-2Rα (CD25), IL-2Rβ (CD122), and IL-2Rγ (CD132) (Fig. 2). The

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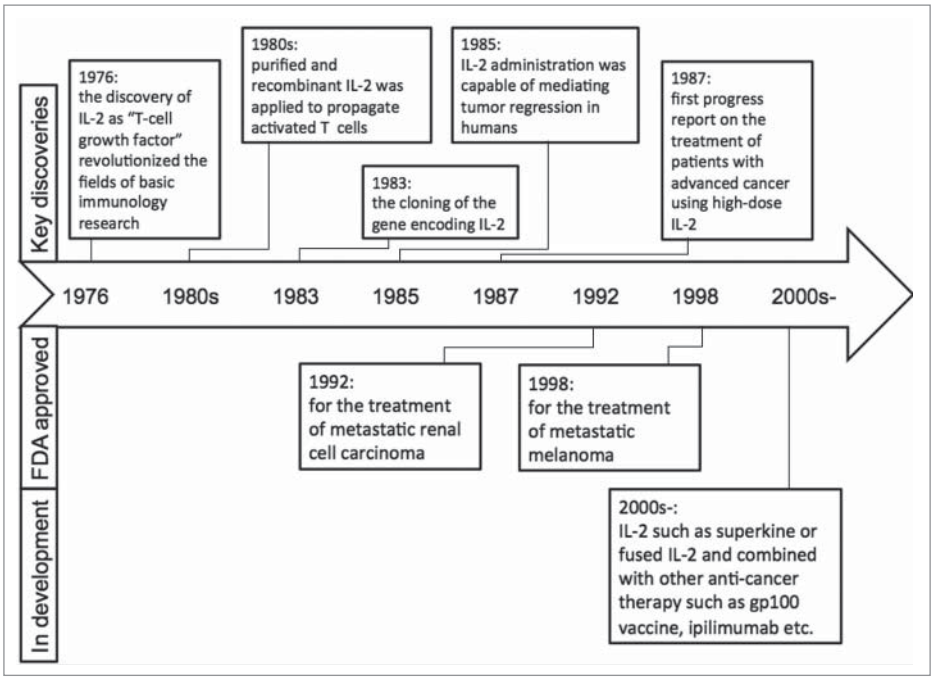

Figure 1. Timeline in understanding the biology and therapeutic application of IL-2.

$\alpha\beta\gamma$ trimeric complex forms the highest affinity receptor. IL-2Rα is unique to IL-2 and is expressed by a number of immune cells including T regulatory cells (Treg), activated $CD4^+$ and $CD8^+$T cells, B cells, mature DCs, endothelial cells, and so on.[12-16] The α chain is overexpressed (8–10-fold) compared with the $\beta\gamma$ chains. It is believed that the α chain functions to bind IL-2 initially, localizing it to the cell surface, effectively increasing its concentration and also inducing a conformational change in IL-2 which then subsequently binds to the $\beta\gamma$ chains on the cell surface.[3,4] The expression of IL-2Rα in naive

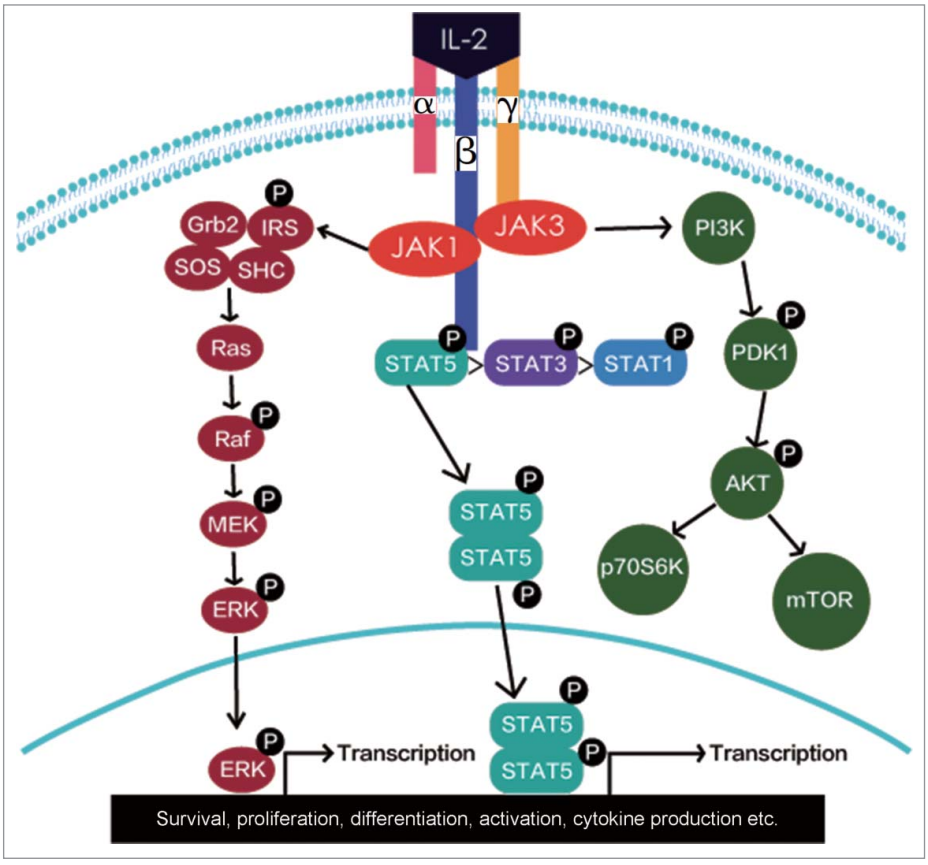

Figure 2. Signaling pathways of IL-2.

T cells can be triggered rapidly by T cell receptor (TCR) and co-stimulatory signals followed by a positive IL-2/IL-2Rα feedback loop.[2] Unlike naive T cells, NK cells, and memory phenotype $CD8^+$ cells express high levels of βγ and some NK cells can also express α chain after the stimulation by IL-2. Of note, Tregs, which act to dampen the immune response, constitutively express high levels of α chain.[17] This enables them to consume IL-2 more efficiently than effector $CD4^+$, $CD8^+$, and NK cells, even at a low level.[18]

The β chain is shared with the IL-15 receptor and the γ chain can be partnered with several other cytokines (e.g., IL-15 and IL-21) receptor chains and is critically involved in signaling. Both of them belong to hematopoietin receptor family.[19] IL-2Rβ is mainly expressed by multiple lymphoid populations such as Treg, memory $CD8^+$ T cells, NK cells, and NKT cells. Similar to the IL-2Rβ subunit, the IL-2Rγ subunit is expressed mostly by haematopoietic cells.[20,21] Interestingly, the IL-2Rγ subunit is stored intracellularly and its expression by $CD4^+$ is triggered only upon activation.[22]

The signaling pathways of IL-2

IL-2 binds to its receptors at different affinities.[23] The isolated IL-2Rα binds IL-2 with low-affinity (K_d-$10^{-}8$ M) without transducing a signal and the heterodimeric IL-2Rβγ binds IL-2 with intermediate-affinity (K_d-$10^{-}9$ M) and transduces intracellular signals. When all three IL-2 receptor subunits form an IL-2Rαβγ trimeric complex, its binding affinity to IL-2 is greater than binding to either a single IL-2 receptor subunit or the IL-2Rβγ heterodimer.[24-26] Binding of IL-2 to the IL-2Rβγ or IL-2Rαβγ complex leads to the activation of multiple signaling pathways with initial signal transduction involving the recruitment of Janus family tyrosine kinases (JAK1 and JAK3) to the cytoplasmic domains of IL-2Rβγ or IL-2Rαβγ. The activation of JAK kinases results in the recruitment and phosphorylation of signal transducer and activator of transcription 1 (STAT1), STAT3, STAT5A, and STAT5B. Then, three major downstream signaling pathways including the STAT signaling pathway, the phosphoinositide 3-kinase (PI3K-AKT) signaling pathway, and the mitogen-activated protein kinase (MAPK) signaling pathway are activated (Fig. 2).[2] These pathways have mediated the survival, proliferation, differentiation, activation, cytokine production etc. in different types of immune cells.[2,18]

The application of IL-2 in cancer immunotherapy

IL-2 as monotherapy

In 1985, 25 previously treated patients with metastatic cancer were treated with increasing high dose (HD) IL-2 at an escalated dose of 60,000–600,000 IU/kg until intolerable toxicity. In this first series of 25 patients, 4 of 7 patients with metastatic melanoma and 3 of 3 patients with metastatic renal cancer showed regression of metastatic tumor.[27] The study first demonstrated that IL-2 was capable of mediating tumor regression in humans, and thus it was further evaluated in the subsequent studies in these two kinds of cancer types. In a phase II trial, multiple cycles of HD IL-2 at a dose of 600,000–720,000 IU/kg with up to 15 bolus infusions were administered every 8 h or as many as the patient could tolerate in 255 patients with metastatic renal cell carcinoma, which showed a complete response of 7% and an overall response rate of 15%.[28] Hence, IL-2 was approved for metastatic renal cell carcinoma in 1992 and later it was approved for metastatic melanoma in 1998 by FDA. Although IL-2 has been demonstrated capable of mediating tumor regression, it is insufficient to improve patients' survival due to its dual functional properties on T cells and severe adverse effect in high dose. Nowadays, IL-2 monotherapy is not the optimal and standard treatment in both metastatic renal cell carcinoma and metastatic melanoma. Efforts to further improve the efficacy of IL-2 therapy are focused on its combination with other anticancer immunotherapies.

IL-2 combined with other cytokines

Though HD IL-2 monotherapy showed promising results in metastatic renal cell carcinoma and melanoma, the toxicity and cost limited its application in a large population. Thus, some investigators evaluated the efficacy of regimens containing low-dose IL-2 combined with other cytokines, such as interferon α (IFN-α). Several phase II trials evaluated HD bolus IL-2 alone, intravenous (IV) IL-2 and IFN, and subcutaneous IL-2 and IFN in patients with metastatic renal cell carcinoma and showed a similar response rates and median overall survivals.[29-31] The addition of IV IFN to HD IL-2 did not seem to improve efficacy with an increasing toxicity. Furthermore, in a randomized phase III trial, patients with advanced renal cancer were assigned to receive either low-dose IL-2 and IFN every 6 weeks or HD IL-2 every 12 weeks. The results showed that HD IL-2 produced a statistically significant improvement in response rate (23.2% vs. 9.9% $p = 0.018$) and response duration (median 24 vs. 15 mo) compared with low-dose IL-2 and IFN-α.[32] Other two randomized studies also demonstrated that there were no significant differences in overall survival between HD IL-2 and IL-2 combined with IFN.[33,34] Taken together, these results indicated that HD IL-2 is superior to both lower doses of IL-2 or IL-2 and IFN in terms of response rates and duration of response.

IL-2 combined with other cell-based immunotherapy

As mentioned above, IL-2 can promote the activation and cell growth of T and NK cells. Thus, early combination strategies were initiated to investigate IL-2 incorporating immune cells such as lymphokine activated killer (LAK) cells and T cells. Compared with HD IL-2 monotherapy, co-administration of LAK cells with IL-2 yielded a clinical response rate of 20–35%, however, mostly with a transient response in solid tumors.[35-37] Another study focused on utilizing an adoptive T cell therapy (ACT) that combines the infusion of *ex vivo* expanded tumor-infiltrating T cells (TILs) with HD IL-2 regimen in patients with metastatic melanoma.[38] In this approach, HD IL-2 is used to expand TILs from tumor fragments to large numbers for a period of 5–6 weeks. Then, these TILs undergo further rapid expansion in the presence of HD IL-2, feeder cells, and anti-CD3 for an additional 2 weeks to reach billions of cells for later infusion.[39] The promising results were reported in numerous phase II clinical trials, with an approximately 50% clinical

response rate and 13% of durable complete regression in patients with metastatic melanoma.[40,41] Although IL-2-based TIL therapy is very promising, TILs expanded in the presence of IL-2 exhibit a more differentiated phenotype that can shorten their long-term persistence and survival *in vivo*. These drawbacks may compromise clinical benefits of this treatment. To improve the quality of TILs, some researchers attempted to use other cytokines such as IL-7, IL-15, and IL-21 to grow TILs. These studies have shown that these cytokines could maintain the expression of CD28 by $CD8^+$ TILs more efficiently than IL-2 during the rapid expansion of TILs.[42]

IL-2 combined with chemotherapeutic agents

IL-2 combined with chemotherapeutic agents (so-called biochemotherapy [BCT]) including cisplatin and dacarbazine has been extensively investigated in patients with metastatic melanoma over the past two decades.[43] Composite results from a variety of inpatient regimens show a response rate about 50%, with 10% to 20% complete responses and a median survival of 11 to 12 mo. Despite promising antitumor activity reported in initial studies, BCT regimens have consistently failed to produce statistically significant benefits in overall survival in randomized phase III trials. Of seven previously reported phase III trials involving a spectrum of BCT combinations,[44-50] only a single-institution trial comparing sequential administration of cisplatin, vinblastine, and dacarbazine (CVD) followed by IL-2 and IFN with CVD reported an increase in overall survival with a statistically marginal significance. Moreover, two meta-analyses of the literature encompassing 18 trials and more than 2,600 patients in which BCT (including IFN, IL-2, or IL-2 plus IFN regimens) was compared with chemotherapy alone showed higher response rates, but no survival advantage, for the BCT regimens.[51,52] Although BCT produced slightly higher response rates and longer median progression-free survival than CVD alone, this was not associated with either improved overall survival or durable responses. Considering the extra toxicity and complexity, this concurrent BCT regimen cannot be recommended for patients with metastatic melanoma. New combinations of IL-2 with other chemotherapeutic agents should be investigated.

IL-2 combined with targeted therapy

Targeted therapy has deeply revolutionized the current strategy for cancer treatments, especially after the discovery of BCR-ABL in leukemia and EGFR mutation in non-small-cell lung cancer (NSCLC). Unfortunately, not all of patients would benefit from targeted therapy and nearly all patients who initially respond to targeted inhibitors inevitably develop acquired resistance to the treatment.[53-55] In advanced NSCLC, the imbalance of the IL-2/IL-2R system, with the decline in IL-2 levels and the significantly high-soluble IL-2 receptor (sIL-2R) concentrations, has been observed and associated with poor prognosis.[56] On the other hand, the role of IL-2 activation in the restoration of the immunocompetence of lymphocytes against lung cancer has been demonstrated.[57] Other authors found that EGFR-TKI affects the cancer-related networks of pro-inflammatory cytokines and activates the lymphocytic responses, which suggested a possible synergism between the EGFR molecular pathway inhibition and immune system modulation in tumor shrinkage.[58,59] In a phase II study,[60] 70 consecutive patients with advanced NSCLC were divided into gefitinib (G) and gefitinib + IL-2 (GIL-2) group. The author observed a significant higher overall response rate (16.1% vs. 5.1%, $p < 0.001$) and a similar disease control rate (41.9% vs. 41%, $p > 0.05$). The median time to progression was similar (3.5 vs. 4.1 mo, $p > 0.05$) while the median OS was significantly prolonged in the GIL-2 group (20.1 vs. 6.9 mo, $p = 0.002$), which showed that IL-2 might improve the outcome of EGFR-TKI. A recent retrospective analysis examined the safety and efficacy of HD-IL2 following TKI therapy in patients with metastatic renal cell carcinoma,[61] which showed that prior TKI did not affect the effect of subsequent HD IL-2 therapy. These results suggested the combination of IL-2 could increase the efficacy of targeted inhibitors. However, there is still lack the randomized compared study in patients with driver mutations. Thus, whether other targeted inhibitors combined IL-2 have this effect remains unknown and requires further investigation.

IL-2 combined with peptide vaccines

Theoretically, IL-2 has a synergistic effect with cancer vaccines in the treatment of human malignancies.[62] When IL-2 is administered in conjunction with cancer vaccines such as recombinant viruses, naked DNA, or peptide antigens, it can dramatically enhance antitumor effects. A previous phase II study demonstrated that patients with metastatic melanoma receiving HD IL-2 plus the gp100 peptide vaccine had a higher response rate than expected among patients who are treated with IL-2 alone.63 A recent phase III trial further confirmed this result.[64] In this trial, patients with advanced melanoma were randomly assigned to receive HD IL-2 alone or gp100 plus incomplete Freund's adjuvant (Montanide ISA-51) once per cycle, followed by IL-2. The vaccine plus IL-2 group had a significant improvement in centrally verified overall clinical response (16% vs. 6%), longer progression-free survival (median 2.2 vs. 1.6 mo; $p = 0.008$) and overall survival (median 17.8 vs. 11.1 mo; $p = 0.06$) compared with the IL-2 group. These studies illustrated that the addition of cytokines could enhance the effect of vaccine therapy in patients with melanoma and highlighted the potential of using rational combinations of immune agents in treating patients with metastatic cancer.

IL-2 combined with immune checkpoint inhibitors

Tumor cells can escape from the immune system via several mechanisms. One important way is by adapting immune inhibitory pathways called immune checkpoints. Some checkpoints are co-stimulatory, which are required for T-cell activation such as CD28 and its ligands B7.1 (CD80) and B7.2 (CD86). Other checkpoints inhibit T-cell activation such as CTLA-4 and PD-1 immune checkpoints.[65-67] CTLA-4 is capable of suppressing effector immune responses on T cells and multiple animal models have suggested enhanced antitumor immunity with CTLA-4 blockade.[68-70] IL-2 administration may also mediate antitumor

effects. In addition, IL-2 also stimulates T-regulatory cells that constitutively express CTLA-4 and can suppress immune reactions. Hence, IL-2 might enhance antitumor reactivity in the presence of CTLA-4 blockade. In fact, a phase I/II study had assessed the antitumor activity and autoimmune toxicity of CTLA-4 blockade in combination with IL-2.[71] Disappointingly, the objective response rate is not superior to single administration and there is no evidence to support a synergistic effect of CTLA-4 blockade plus IL-2 although durable cancer regressions were seen in patients treated with this combination. Interestingly, a recent study also suggested that the efficacy of CTLA-4 blockade was significantly improved by recombinant IL-2 in mouse and elevated serum IL-2Rα predicted resistance to CTLA-4 blockade in patients with advanced melanoma.[72] Hence, we suppose that only patients presenting a high baseline sIL-2Ra concentration might benefit from CTLA-4 blockade in combination with IL-2. To date, the combination of IL-2 with CTLA-4 inhibitors seems have no extra benefit for cancer immunotherapy. However, whether IL-2 has a synergistic antitumor effect with other immune checkpoint inhibitors (such as PD-1/PD-L1 antibody, nivolumab, or pembrolizumab) need more basic and clinic research.

The limitations of IL-2 immunotherapy against cancer

Undoubtedly, IL-2 showed great potential in treating metastatic cancers. However, its application in the clinic remains relatively restricted due to several shortcomings. First, IL-2 has the dual functional properties that it can act on both Tregs as well as effector T cells.[5] As a result, some studies have used IL-2 to enhance antitumor immune responses and other studies have used IL-2 to dampen autoimmune responses. Furthermore, both HD and low-dose IL-2 therapy preferentially induce the expansion of $CD4^+CD25^+Foxp3^+$ Treg and the Treg level remains elevated after each cycle of HD IL-2 therapy.[73-75] A study by Sim et al. has shown that HD IL-2 induces a large expansion of a specific $CD4^+CD25^+Foxp3^+$ Treg subset that expresses ICOS. These $ICOS^+$ Tregs had a higher proliferative capacity in response to IL-2 and displayed a more immunosuppressive phenotype. Patients who showed no response to HD IL-2 had significantly greater expansion of $ICOS^+$ Treg after the first cycle of therapy compared with these who responded,[75] which suggested an inhibitory role of these cells that could be a crucial limiting factor in preventing antitumor lymphocyte activity and tumor eradication during HD IL-2 therapy. Tregs would undergo a rapid reconstitution during HD IL-2 and TIL therapy, which was found to be associated with poor clinical response. Meanwhile, the reconstitution of endogenous Tregs was correlated with the dose of IL-2 doses during TIL therapy.[76]

Another major drawback is the severe toxicities of HD IL-2 therapy. Due to rapid elimination and metabolism via the kidney, IL-2 has a short serum half-life of several minutes. Thus, to achieve an optimal immune-modulatory effect, IL-2 should be given in a high dose, which will inevitably result in severe toxicities. HD IL-2-induced severe toxicities including vascular leak syndrome (VLS), pulmonary edema, hypotension, and heart toxicities.[77-79] Several mechanisms have been proposed but still not clearly clarified. It is believed that the induction of pro-inflammatory cytokines such as IL-1, IL-6, tumor necrosis factor α (TNF-α), and IFNγ were potential contributors to IL-2-induced VLS. In addition, Krieg et al. reported that binding of IL-2 to the high-affinity IL-2Rα-expressing endothelial cells induced an acute vasodilation effect and VLS.[16] Other studies have also suggested that elevated levels of eNOS, angiopoietin 2, or a protein fragment of the IL-2 molecule designated as permeability-enhancing peptide may lead to VLS.[80-82]

Strategies to improve efficacy of IL-2 immunotherapy

IL-2 mutants

Ideally, we hope the IL-2 could efficiently activate NK cells and T effector cells without Treg expansion. To achieve this goal, IL-2 mutants were created, which had different binding properties for the IL-2 receptor components. Initial mutational approaches to improve IL-2 efficacy for effector T cells focused on enhancing binding to the α chain of the IL-2 receptor.[83-86] Disappointingly, this was not as successful as envisioned because these mutants might actually downregulate immune responses *in vivo* when delivered systemically due to the global stimulation of Tregs that express the α chain component of the high affinity IL-2 receptor. Recently, two novel IL-2 mutants, namely F42K and R38A, have been described and characterized.[87,88] These IL-2 mutants have changed IL-2Rα binding domains that greatly decrease their binding affinity to IL-2Rα while having an affinity similar to that of native IL-2 to the IL-2Rβγ complex. Moreover, these mutants can activate LAK cells without the production of high levels of pro-inflammatory cytokine (IFNγ, IL-1β, TNF-α) and prevent VLS.[88] Furthermore, some other studies have suggested that IL-2 mutants have less effect in stimulating a large expansion of Treg when compared with native IL-2. These findings are crucial and encouraging since expansion of Treg by wild-type IL-2 is another main limitation of HD IL-2 therapy. A more recent paper identified IL-2 mutants using yeast displaying a higher affinity to the β chain.[89] One of these mutants, termed as "superkine" or "super-2" reflecting its enhanced agonist properties, also showed improved antitumor activity and exhibited less VLS compared to native IL-2. The higher affinity of the mutated IL-2 for the β chain of the IL-2 receptor may be important to explain the reason that modified IL-2 might function *in vivo*. Others have explored an alternative strategy in which IL-2 mutants were engineered to carry four point mutations that limit their interaction with IL-2Rα to avoid the expansion of Tregs. Taken together, these studies clearly showed that it was feasible to structurally alter IL-2 to accentuate or reduce particular biologic properties to modify the function of IL-2, which might be an important breakthrough in the use of IL-2 for cancer immunotherapy.

Antibody–cytokine fusions or immunocytokines

Another novel approach attempts to deliver IL-2 to tumor sites by genetically fusing cytokines with antibodies (also called immunocytokine), or antibody components such as a single-chain variable fragment (scFv), which could bind tumor-

associated antigens (TAAs).[90,91] The major advantage of this approach is that it could improve the half-life of cytokine and enhance the immune-modulatory effect of cytokines with less toxicity. For example, IL-2 was conjugated to an antibody reactive with ganglioside 2 (GD2), which would eventually accumulate at the tumor site due to the binding of the antibody to the GD2 antigen on the tumor. As a consequence, the local concentration of IL-2 is increased at the tumor site. Currently, two immunocytokines, Hu14.18-IL2 and L19-IL2 (Darleukin), are in phase II clinical studies. Hu14.18-IL2 consists of an IgG antibody that recognizes GD2, and L19-IL2 is a diabody with two human IL-2 molecules that are genetically fused to the C-terminus of each scFv domain. Pre-clinical studies have demonstrated encouraging therapeutic outcome *in vivo*.[92-95] In the phase I clinical trials, Hu14.18-IL2 and L19-IL2 have shown a mild and reversible toxicity profiles.[96,97] In addition Hu14.18-IL12 showed that 58% of melanoma patients achieved stable disease after the first cycle of treatment in the phase I clinical study. Currently, L19-IL2 is in phase II trials to validate its efficacy in patients with metastatic melanoma in combination with dacarbazine (NCT02076646 and NCT01253096).

Protease activated cytokines

Severe toxicities limit the wide application of HD IL-2 in clinical practice. Recently, a new strategy was developed to reduce its adverse effects. This strategy employs a fusion protein (FP) in which IL-2 is joined covalently to a specific inhibitory binding component separated by a protease cleavage site. The local concentration of the specific binding inhibitor is extraordinarily high and specially binds to IL-2. However, after cleavage by proteases that are over-expressed locally at the tumor site [such as matrix metalloproteases (MMPs) or prostate-specific antigen (PSA)], IL-2 will be available to interact with high-affinity receptors on immune cells. These receptors are 10- to 1,000-fold more avid than the specific binding of the isolated α chain. Moreover, immune cells can produce additional cytokines after stimulation. This approach successfully demonstrated that the FP could reduce tumor growth in a mouse colon cancer model *in vivo*.[98] In this study, antibody/IL-2 complexes might be able to bind preferentially to cells that express particular combinations of receptors, which is meaningful since it might preferentially stimulate effector cells such as $CD8^+$ cells and NK cells via eliminating inhibitory components. As a result, specificity will increase, which might further increase its efficacy. This method could not only increase the efficacy of immunotherapy by preferentially altering the tumor microenvironment and enhancing particular subsets of immune cells but also reduce toxicity since it could specially identify tumor cells.

Future directions of IL-2-based immunotherapy

Despite the comprehensive studies of IL-2 on immune system have established the application of IL-2 for tumor immunotherapy, a number of poignant obstacles remain for future research. First, the dose and timing of these new IL-2-based reagents, the immunogenicity of the novel molecules, and their effective combination remain unclear. Secondly, although a lot of studies have focused on the role of IL-2 on T-cells and NK cells, the IL-2R can be expressed by other haematopoietic cells, in particular B cells that can express IL-2Rα along with the β and γ subunits. The role of intermediate and high-affinity IL-2R signaling in B cells and different B cell subsets needs to be clarified. Thirdly, the role of IL-2 in regulating $CD4^+$ T cell lineage commitment into different effector types, especially the switch between Treg and Th17 differentiation still remain controversial.[99] Lastly, successful immunotherapies might be a combination, which includes not only one of effective cytokines, but also other immunologic approaches. In the combination, which one could bring survival benefit and will be transformed into clinical application need further research in the future.

Conclusions

IL-2 plays a critical role in the activation of immune system that could be a useful way to eradicate cancer. As monotherapy, IL-2 has been demonstrated capable of mediating tumor regression and was approved for metastatic renal cell carcinoma and metastatic melanoma by FDA. Nevertheless, IL-2 as monotherapy is insufficient to improve patients' survival due to its dual functional properties on T cells and severe adverse effect in high dose. The complexity of IL-2 or IL-2 mutants with one or more of these other common γ chain cytokine family members, named as "superkines" may stimulate unique and more potent signaling effects on lymphocytes through the simultaneously triggering of multiple signaling complexes. Their alone or combinations with other anticancer immunotherapies, such as adoptive cell transfer regimens, antigen-specific vaccination, and blockade of immune checkpoint inhibitory molecules (e.g., CTLA-4 and PD-1/PD-L1 antibodies), have shown to overcome these drawbacks and bring some survival benefit in patients with advanced cancer. These strategies might hold the promise of treating metastatic cancer in the future.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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