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REVIEW ARTICLE

The use of supercytokines, immunocytokines, engager cytokines, and other synthetic cytokines in immunotherapy

Xiaohu Zheng[1,2,3,4]✉, Yaqi Wu[3], Jiacheng Bi[1,2], Yingying Huang[1,2], Ying Cheng[4], Yangyang Li[4], Yuwei Wu[4], Guoshuai Cao[4] and Zhigang Tian[1,2,3,4]✉

Cytokines exert powerful immunomodulatory effects that are critical to physiology and pathology in humans. The application of natural cytokines in clinical studies has not been clearly established, and there are often problems associated with toxicity or lack of efficacy. The key reasons can be attributed to the pleiotropy of cytokine receptors and undesired activation of off-target cells. With a deeper understanding of the structural principles and functional signals of cytokine-receptor interactions, artificial modification of cytokine signaling through protein engineering and synthetic immunology has become an increasingly feasible and powerful approach. Engineered cytokines are designed to selectively target cells. Herein, the theoretical and experimental evidence of cytokine engineering is reviewed, and the "supercytokines" resulting from structural enhancement and the "immunocytokines" generated by antibody fusion are described. Finally, the "engager cytokines" formed by the crosslinking of cytokines and bispecific immune engagers and other synthetic cytokines formed by nonnatural analogs are also discussed.

Keywords: Supercytokines; immunocytokines; engager cytokines; synthetic cytokines; immunotherapy

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INTRODUCTION

Cytokines were the first type of tumor immunotherapy drug to be approved by the United States Food and Drug Administration (FDA). Interferon (IFN)-α was approved in 1986 and interleukin (IL)-2 in 1992, but their development encountered several obstacles. Cytokines are small, soluble signaling proteins with a short half-life. They directly and rapidly initiate the immune response to external stimuli. Cytokines are involved in almost all types of cellular responses, such as the regulation of immune proliferation, differentiation, and effector functions, and are critical to immune cells in the fight against tumor cells and pathogens. Therefore, cytokines regulate a complex network of signals with multipotent, multisource, multiterminal, and multimodal activity. Despite its beneficial effects, cytokine therapy is prone to various side effects and has a narrow therapeutic window, which historically made natural cytokines unsuitable drug candidates, aside from very limited circumstances.

Recent years have marked a broader understanding of cytokine immunobiology and the emergence of new protein engineering and synthetic design technologies. This has led to the removal of adverse biological characteristics induced by certain cytokines through selective and accurate modifications, opening new potential in the field. Most research on cytokine engineering has focused on the receptor interface aiming for enhanced affinity, as attempted for the IL-2 "superkine" [1] and the IL-15 "superagonist" [2]; improving binding selectivity, as implemented for IL-12 partial agonists [3] and the IL-4 superkine [4]; or by interfering with interactions, as implemented for the IL-15 antagonist [4] and IL-13 superkine [4]. Other approaches to improve the efficacy of cytokines include coupling of cytokines to large carrier molecules such as polyethylene glycol (PEG) or albumin or fusion with the fraction crystallized (Fc) portion of immunoglobulins (Igs) [5] (Fig. 1).

In parallel, scientists are also attempting to increase the tolerance of cytokine therapy by protecting normal healthy tissue and preferentially targeting disease sites. Antibodies that target biomarkers specifically expressed at disease sites may be ideal "vectors" for the targeted delivery of cytokines [5]. In many mouse models, antibody–cytokine fusion proteins targeting tumor markers can significantly increase the selective accumulation of corresponding cytokines at the site of tissue remodeling and are under consideration for the treatment of chronic inflammatory diseases, such as tumors [6, 7]. Such antibody–cytokine fusion proteins, known as "immunocytokines", have been recently redeveloped and described as the next generation of cytokine products [8] (Fig. 2).

Bispecific immune engagers with one "arm" that targets tumor cells and another arm that targets immune effector cells have also been generated. The first developed molecules were bispecific T-cell engagers (BiTEs), which are engineered with an arm that targets cluster of differentiation (CD)3 on T cells and another

[1]CAS Key Laboratory of Quantitative Engineering Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China. [2]Shenzhen Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, 518055 Shenzhen, China. [3]Hefei National Laboratory for Physical Sciences at Microscale, CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Basic Medical Sciences, Division of Life Sciences and Medicine, University of Science and Technology of China, 230027 Hefei, China. [4]Hefei TG ImmunoPharma Corporation Limited, Hefei, China. ✉email: ustczxh@ustc.edu.cn; tzg@ustc.edu.cn

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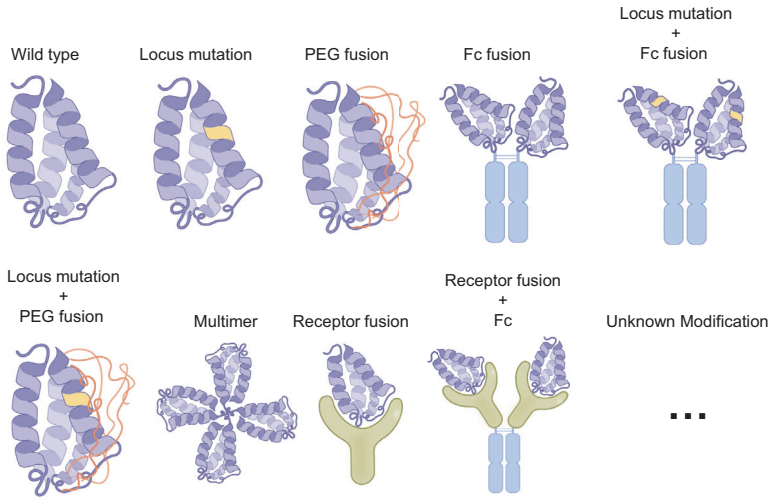


Fig. 1 Supercytokine engineering strategies. Supercytokines can be generated through multiple strategies. The original framework is normally based on a natural cytokine (wild-type), and specific mutations (locus mutations) can be introduced with traditional molecular biology techniques to manipulate intrinsic properties. Alternatively, bioavailability and half-life can be improved with PEGylation (PEG fusion), while targeted delivery is generally achieved by fusion with a target-specific antibody (Fc fusion). Despite traditionally being used as alternatives, all these strategies are now emerging in combination, where a locus mutation version can be fused with an antibody for targeted delivery or PEGylated for extended half-life. Finally, more complex technologies include multimerization (dimer or multimer) to enhance or synergize activity, receptor fusion to modulate activity, and specificity combined with antibody fusion to establish targeted delivery

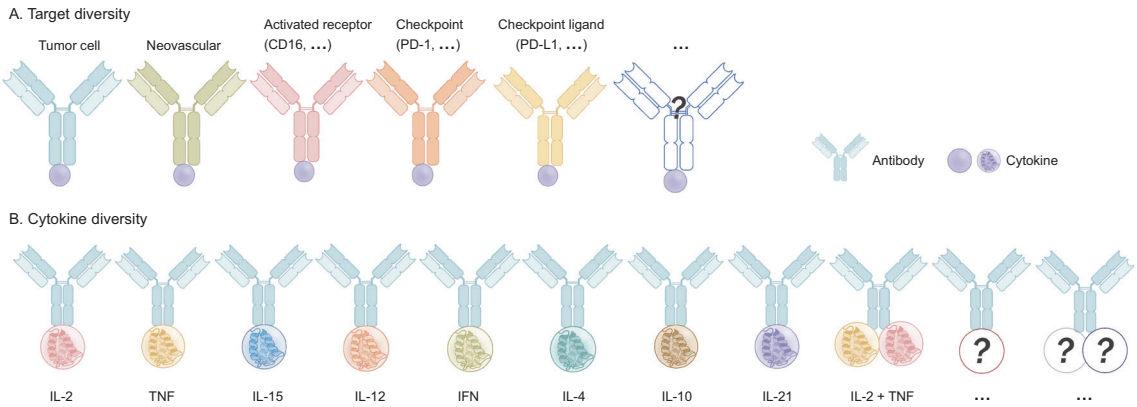


Fig. 2 The breadth of immunocytokine therapies and targets. **A** Immune cytokines are mainly composed of antibody functional regions and cytokine functional regions. The antibody functional region provides specificity with a variety of targets, including tumor cell markers, neovasculature, activated receptors, checkpoints, and checkpoint ligands. **B** Cytokines allow for functional selectivity and diversity. Currently, IL-12, TNF, IL-15, IL-12, IFN, IL-4, IL-10, and IL-21, either alone or in combination, have been tested

variable fragment (Fv) that targets cancer cells. BiTEs such as blinatumomab have exhibited impressive clinical success [9], but the development of BiTEs has been limited by the fact that they cause cytokine release syndrome and diffuse intravascular coagulation and exhibit neurovirulence. Bispecific killer cell engagers (BiKEs) have also been engineered. BiKEs simultaneously target CD16 on natural killer (NK) cells and other targets on cancer cells. They can enhance NK cell activation but cannot support their survival. To eliminate this shortcoming, several research teams have designed agents with novel architectures by fusing BiKEs with cytokines that enhance their performance (e.g., IL-15), which are known as “engager cytokines” (Fig. 3).

Engineered and synthetic cytokines in immunotherapy are expected to continue to improve through advances in molecular, pathophysiological, and synthetic biology approaches. The three major modifications and applications of cytokines were briefly discussed above, but the recent developments of other modifications or applications in the biology of synthetic cytokines will be explored below. Here, we will focus on nonnatural engineered cytokines, fully synthetic analogs, and related functional domains or independent cytokine receptors. Finally, the potential of emerging technologies using immune cells or oncolytic viruses (OVs) that can be combined organically with synthetic cytokines to improve the outcome of immunotherapy is discussed (Fig. 4).

SUPERCYTOKINES

Some intrinsic properties of cytokines greatly hinder their therapeutic use, e.g., short half-life in circulation, off-target effects, and inherent pleiotropic functions. Efforts have been made to enhance the therapeutic potential of cytokines, which mainly include modification of the binding domain, extension of half-life, formation of fusion proteins, and bifunctional cytokines (Fig. 1 and Table 1).

Domain modification

IL-2 superkine. In 1983, the IL-2 gene was cloned, which was considered a landmark achievement in basic research investigating the development of IL-2 drugs [10]. Its role in stimulating the

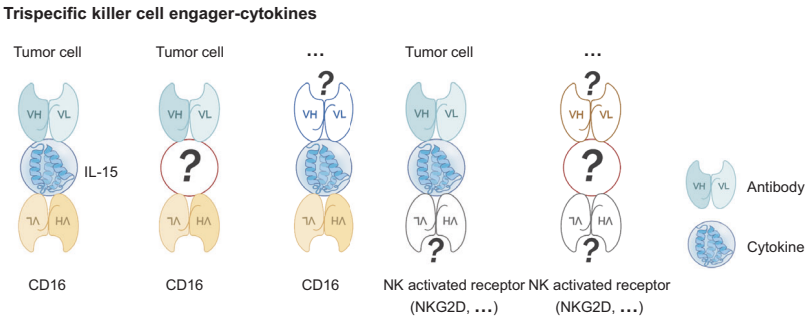


Fig. 3 The architecture of engager cytokines. Trispecific killer cell engager cytokines are mainly composed of a single-chain Fv (scFv) against an immune cell-activating receptor (e.g., CD16), an scFv against a tumor antigen (e.g., CD33), and functional fragments of cytokines (e.g., IL-15) as the linker

proliferation and effector functions of a wide range of leukocytes, including NK cells and T cells, was established rapidly in mice and humans [11]. Rosenberg et al. observed the significant therapeutic effects achieved with recombinant IL-2 in a mouse model of metastatic liver cancer and carried out a clinical study with IL-2 and LAK (also known as NK) cells in 25 patients with metastatic cancer in 1985 [12]. Proleukin® (aldesleukin) is a recombinant IL-2 drug developed by Novartis and Linigen. It was approved by the FDA for the treatment of metastatic kidney cancer and metastatic melanoma. However, due to the possibility of pulmonary edema as a serious side effect, effector-cell-induced cell death, and the accompanying expansion of regulatory T cells (T_{regs}), clinical use of IL-2 drugs remains limited [13, 14].

The IL-2 receptor has three subunits: α (CD25), β (CD122), and γ (CD132). The α subtype has a low affinity with no downstream signal transduction. The β and γ subtypes form a medium-affinity receptor with downstream signal transduction through Janus kinase (JAK), and in the presence of the α subtype, the heterotrimer becomes a high-affinity receptor [15, 16]. Starting in the 1990s, several research institutions and companies began to alter the affinity of IL-2 and different receptor subunits by mutation and molecular modification to increase efficacy and reduce toxicity. In 2000, Shanafelt et al. generated an IL-2 mutant protein (Bayer, BAY50-4798) that selectively binds IL-2Rαβγ to activate T cells with reduced affinity for IL-2Rβγ + and NK cell activation [17, 18]. In 2012, Levin et al. generated an IL-2 superkine with increased binding affinity for IL-2Rβ, which enhanced NK cell and cytotoxic CD8 + T-cell expansion to achieve a potent antitumor response [19]. At the same time, T_{reg} expansion and the incidence of pulmonary edema were reduced. Other companies have engineered various modifications directed at multiple indications based on the broad capabilities and potential of IL-2, e.g., MDNA209, an IL-2 superkine antagonist from Medicenna that has a higher affinity for IL-2Rβ, which suppresses the immune-activating signal and weakens aberrant T-cell function in autoimmune disorders [1].

IL-4 superkine. IL-4 is a critical effective cytokine for the development of immune responses by T helper (Th) 2 cells. Injections of IL-4 direct the differentiation of $CD4^+$ T cells and contribute to antibody formation during vaccination. However, IL-4 has not been used as a therapeutic agent, mainly because of its toxicity [20].

Two types of IL-4 receptors, type-I receptor (γc) and type-II receptor (IL-13Rα1), are expressed as different types of cell-surface receptors and activate different intracellular signaling pathways [21]. Junttila et al. generated two main types of IL-4 superkines: a type-II receptor-selective IL-4 superkine that more effectively activated type-II receptor-positive cells and induced monocytes to differentiate into dendritic cells [22] and a type-I receptor-selective IL-4 superkine, which was conducive to Th2 differentiation and the switching of Ig classes [22]. The type-II receptor is expressed in several types of nonhematopoietic cells, which suggests that the type-I receptor-selective IL-4 superkine has lower toxicity and side effects in clinical studies.

IL-12 partial agonists. Toxicity is the "bottleneck" of the clinical application of IL-12. Garcia and colleagues designed a series of IL-12Rβ1/p40-selective partial agonists that could promote T-cell activation and enhance IFN-γ expression in vitro while reducing the activity of NK cells, which are responsible for inducing the production of toxic cytokines [3].

IL-13 superkines. IL-13 is also critical for the development of Th2 immune responses, but imbalances in its expression have been linked to allergies, fibrosis, and even aggressive types of cancer [23]. It has been recently reported that the tumor microenvironment (TME) distorts the function of Th2 cells to favor their expansion [22, 24].

MDNA132 is a modified IL-13 superkine from Medicenna that specifically targets the "dummy" IL-13 receptor subunit alpha-2 (IL-13Rα2). This receptor shows high expression in various types of solid tumors (breast, colorectal, lung, pancreatic) but not in healthy tissue cells [25]. IL-13Rα2 has been shown to be a viable anticancer drug target [25]. Thus, MDNA132 could serve as a tumor-targeting drug on its own or as a delivery mechanism for other drugs to local tumor tissues. MDNA413 is another IL-13 superkine designed by Medicenna as a type-II receptor (IL-13Rα1)-selective blocker [26]. The type-II receptor is expressed mainly on immunosuppressive cells in the TME and effector cells of Th2 diseases [25, 27]. Thus, MDNA413 may be a new type of immune checkpoint inhibitor.

IL-15 antagonists. A major focus of IL-15 engineering is the development of antagonists that counteract its immune-activating effects [4]. Pettit and colleagues engineered a point mutant (Q108) of IL-15, which binds weakly to γc and eliminates cytokine-mediated proliferation [28, 29]. Subsequently, Garcia and coworkers designed an IL-15 antagonist (Q101D/Q108D) that could prevent graft rejection and inhibit arthritis progression by inhibiting the proliferation of $CD8^+$ T cells in a mouse model [29–31].

Decoy-resistant IL-18. IL-18 and its receptor (IL-18Rα/Rβ) can activate $CD8^+$ T cells and NK cells to produce IFN-γ. IL-18 has been used in cancer immunotherapy, but a phase II clinical trial failed to demonstrate that IL-18 has any clinical efficacy. Zhou et al. reported an IL-18 that was modified by point mutations into decoy-resistant IL-18 (DR-18), which could not bind to its pseudoreceptor (IL-18BP) but retained signal-transduction functions leading to significant antitumor effects. DR-18 has been

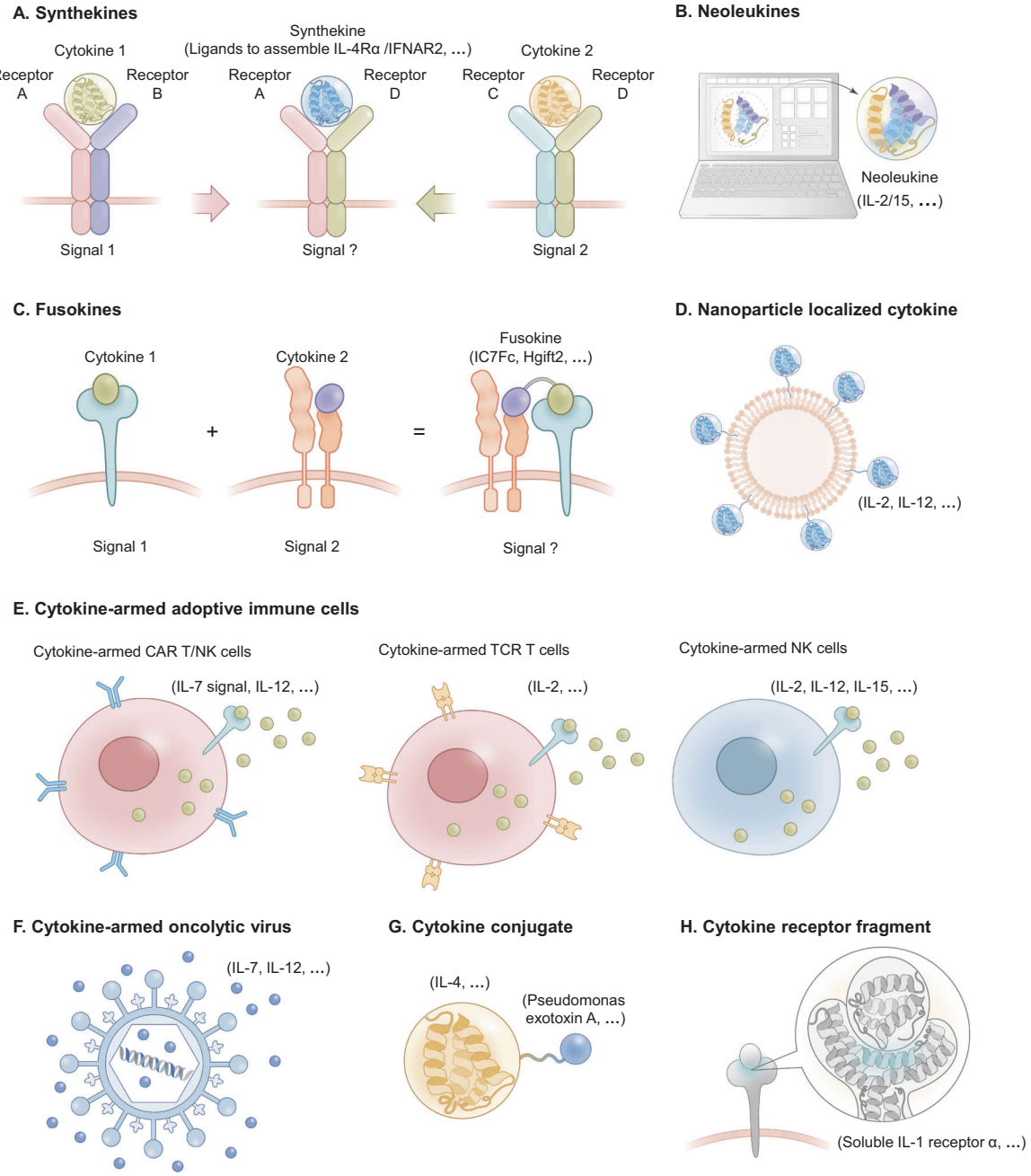


Fig. 4 The architecture of other synthetic cytokines. **A** Synthekines are fused synthetic cytokines that can activate a novel receptor. **B** Neoleukines are produced by a computationally driven design. **C** Fusions of two functional agents are called fusokines. **D** Nanoparticle-localized cytokines are cytokines attached to nanoparticles. **E** Adaptive immune cells with enhanced cytokine signaling are adoptively transferred T/NK cells that are recombinantly or transgenically engineered to ectopically express cytokines. **F** Oncolytic viruses with enhanced cytokine signaling are designed with recombinant or transgenic cytokines. **G** Cytokine conjugates are composed of cytokines and conjugates (e.g., toxin). **H** Cytokine-receptor fragment is composed of the cytokine binding motif of its natural receptor

shown to promote lymphocyte infiltration into tumors (especially that of activated $CD8^+$ T cells) and increase the effector activity of NK cells [32].

"Tunability" of type-I IFNs. Type-I IFNs are a crucial family of antiviral and antiproliferative cytokines currently used in clinical practice. However, side effects and limited efficacy prevent this family from being widely used. These shortcomings can be overcome by structural engineering. Jaitin et al. and collaborators designed YNS (H57Y, E58N, and Q61S)-mutated IFN-α2 with an improved affinity for IFNAR1 and significantly increased antiviral and antiproliferative potency [33]. In addition, an IFN-ω K152R mutant was constructed with a significantly better affinity for IFNAR2 than that of wild-type IFN-α2 [34]. Interestingly, the antiproliferative activity was increased significantly, whereas antiviral activity was increased only slightly upon treatment with the K152R mutant. To decouple the antiviral activity of type-I IFNs from their antiproliferative capacity, two mutants, B9X25 and B9X14, were screened and identified with a 70-fold enhanced antiviral ability [35]. Finally, Levin et al. designed and constructed competitive antagonists of IFN-α2 mutated at the binding site of the IFNAR1 receptor [36].

Table 1. List of supercytokines, immunocytokines, engager cytokines, and other synthetic cytokines currently underevaluation

Types	Classification	Name	Modified type	Disease	Mechanism	Clinical trial	From
Supercytokines	IL-2-based supercytokines	IL-2 mutein, BAY50-4798	Locus mutation	Advanced melanoma and renal cancer	Selectivity for T cells over NK cells	Phase I	Ref. [17], Ref. [18], Bayer
		IL-2 superkine	Locus mutation	Cancer	Induced superior expansion of NK cells and CTL	Preclinical	Ref. [1], Medicenna
		IL-2 superkine antagonist, MDNA209	Locus mutation	Autoimmune diseases	Blocking immune-activating signals	Preclinical	Medicenna
		Long-acting IL-2 superkine, MDNA11	Locus mutation and fusion albumin	Cancer	Preferentially binds the IL-2 beta receptor (IL-2Rβ) and long-acting	Preclinical	Medicenna
		IL-$2^{3\times}$-Fc	Locus mutation and fusion Fc	Solid tumors	Long-acting	Preclinical	Ref. [39]
		PEG-IL-2, NKTR-214	Fusion PEG	Solid tumors	long-acting	Phase I/II	NCT02869295, NCT02983045
		IL-2-mutein-Fc, AMG592	Locus mutation and fusion Fc	Autoimmune diseases	Blocking the immune-activating signal	Phase I	NCT03451422, NCT03410056, NCT03422627
		IL-2 polymer, NKTR-358	Polymer	Autoimmune diseases	Blocking the immune-activating signal	Phase I	NCT03556007
		IgG-(IL-2N88D)2	Locus mutation and fusion Fc	Autoimmune diseases	Expand Treg cells	Preclinical	Roche
		IL-2-mutein-PEG, THOR-707	Locus mutation and fusion PEG	Advanced or metastatic solid tumors	Selective for-CD8 + T cells	Preclinical	Synthorx, NCT04009681
		IL-2-mutein-PEG, SHR-1916	Locus mutation and fusion PEG	Cancer	Promote the proliferation of T and NK cells and antitumor effect	Preclinical	Hengrui
		ALKS 4230	Fusion IL-2Rα domain	Cancer	Selectivity for NK cells	Preclinical	Immuno-Oncology
	IL-4-based supercytokines	IL-4 superkine	Locus mutation		Type-I receptor-selective IL-4 "superkine"	Preclinical	Ref. [22]
		IL-4 superkine	Locus mutation		Type- II receptor-selective IL-4 "superkine"	Preclinical	Ref. [22]
	IL-12-based supercytokines	IL-12 partial agonists	Locus mutation	Cancer	Selectivity for T cells over NK cells	Preclinical	Ref. [3]
	IL-13-based supercytokines	IL-13 superkine, MDNA413	Locus mutation	Cancer	Increased affinity for the IL-13Rα1	Preclinical	Medicenna
		Modified IL-13 Superkine, MDNA132	Locus mutation	Solid tumors	Increased affinity for the IL-13Rα2	Preclinical	Medicenna
	IL-15-based supercytokines	IL-15 antagonist	Locus mutation		Eliminate cytokine-mediated proliferation	Preclinical	Ref. [28], Ref. [29]
		IL-15 antagonist	Locus mutation	Graft rejection, autoimmune diseases	Inhibit CD8 + T -cell proliferation	Preclinical	Ref. [30], Ref. [31]
		Interleukin-15C	Fusion IL-15 receptor a-Fc	Experimental cerebral malaria (ECM)	IL-15C induced NK cells to express IL-10	Preclinical	Ref. [42]
		RLI, IL-15-linker-IL-15Rα sushi domain	Fusion sushi domain	Cancer	Reconstitution of human NK and T cells	Preclinical	Ref. [43], Ref. [44]
		IL-15N72D/IL-15Rα-IgG1-Fc chimera, ALT-803	Locus mutation, IL-15 receptor a-Fc fusion protein	Cancer	Reconstitution and activation of human NK cells	Phase I/II	NCT02191098, NCT01946789, NCT01885897, NCT02099539, NCT02559674, NCT02523469, NCT03022825
		IL-15/IL-15Rα-IgG1-Fc complex	Fusion IL-15 receptor a-IgG1-Fc complex	Cancer	Activated proinflammatory typeII NKT, CD27 + CD11b + NK, and memory T-cell subsets	Preclinical	Ref. [45]
	IL-18-based supercytokines	Decoy-resistant IL-18	Locus mutation	Cancer	Without binding to IL-18BP, activation of CD8 + T and NK cells	Preclinical	Ref. [32]
	Type I interferon-based supercytokines	Mutational IFN-α2	Locus mutation	Antiviral potency, Cancer	Improved affinity for IFNAR1	Preclinical	Ref. [33]

Table 1 continued

Types	Classification	Name	Modified type	Disease	Mechanism	Clinical trial	From
		IFN-ω K152R mutant	Locus mutation	Cancer	Improved affinity for IFNAR2	Preclinical	Ref. [34]
		IFN-α mutant	Locus mutation	Cancer	Improved the antiproliferative activity	Preclinical	Ref. [35]
		IFN-α2 mutant	Locus mutation		Antagonists of IFN-α2	Preclinical	Ref. [36]
	IL-10-based supercytokines	Myeloid-Biased IL-10 variants	Locus mutation	Autoimmune diseases	Inhibiting macrophage activation without stimulating inflammatory CD8 + T-cell activity	Preclinical	Ref. [37]
		Affinity-enhanced IL-10 variant	Locus mutation	Cancer	Improved affinity for IL-10 receptor	Preclinical	Ref. [38]
Types	**Classification**	**Name**	**Cytokine/antigen**	**Disease**	**Treatment method**	**Clinical trial**	**Source**
Immunocytokines	IL-2-based immunocytokines	huKS-IL-2, EpCAM G733-2	IL-2/EpCAM	Solid tumors	Monotherapy, combination with Cyclophosphamide	Phase I	Ref. [54], NCT00132522
		Hu14.18-IL-2	IL-2/GD2	Melanoma, neuroblastoma	Monotherapy, combination with NK cells, combination with GM–CSF and isotretinoin	Phase I/II	NCT00003750, NCT00109863, NCT00082758, NCT00590824, NCT03209869, NCT01334515,
		NHS-IL-2LT	IL-2 mutant (D20T)/ histone complex	Advanced solid tumors	Monotherapy, combination with radiation therapy	Phase I/II	Ref. [57], NCT00879866, NCT01032681,NCT01973608
		CEA-IL2v	IL-2 mutant/CEA	Solid tumors	Combined with PD-L1Ab	Phase I	Roche
		FAP-IL2v	IL-2 mutant/FAP	Solid tumors	Combined with PD-L1Ab or EGFR Ab	Phase Ib	Roche
		DI-Leu16-IL-2	IL-2/CD20	Blood tumors	Monotherapy, following Rituximab blood B-cell depletion	Phase I/II	Provenance Biopharmaceuticals, NCT0215903, NCT1874288, NCT00720135
		CEA-IL2v	IL-2 mutant/CEA	CEA-positive solid tumors	Monotherapy, combined with Atezolizumab	Phase I	NCT02350673, NCT02004106
		L19-IL-2	IL-2/EDB	Advanced solid tumors, diffuse large B-cell lymphoma	Monotherapy, combined with Rituximab	Phase I/II	NCT01198522, NCT01058538, NCT02957019, NCT03705403, NCT02086721, NCT01253096, NCT02076646, NCT01055522,
		F16-IL-2	IL-2/Tenascin C	Advanced solid tumors	Combined with Doxorubicin and prospective therapy, Paclitaxel and prospective therapy, Paclitaxel, Cytarabine, anti-CD33 antibody	Phase I/II	NCT01131364, NCT01134250, NCT02054884, NCT02957032, NCT03207191,
		EGFR Ab-sumIL-2	Supermutant IL-2	EGFR-positive solid tumors	Monotherapy	Pre-clinic	Ref. [66]
		FAP-IL2v	IL-2 mutant/FAP	Solid tumors	Atezolizumab with/without Bevacizumab, Trastuzumab, Cetuximab	Phase I/II	NCT03386721, NCT02627274, NCT03063762
	IL-12-based immunocytokines	NHS-IL-12	IL-12/Histone complex	Solid tumors	Monotherapy, combined with Avelumab	Phase I	NCT01417546
		BC1-IL-2	IL-12/D7	Metastatic RCC, malignant melanoma	Monotherapy	Phase I	NCT00625768
		L19-mIL12	Mouse IL-12/EDB	Glioblastoma	Monotherapy	Pre-clinic	Ref. [72]
	IL-10-based immunocytokines	CmAb-(IL-10)2	IL-10/EGFR	Lung cancer	Monotherapy	Phase I	Ref. [85], Dingfu Target
	IL-21-based immunocytokines	EGFR-IL-21	IL-21/EGFR	Lung cancer	Monotherapy, combined with PD-L1 antibody	Pre-clinic	Ref. [89]
		PD-1-IL-21	IL-21 variants/PD-1	Solid tumors	Monotherapy	Pre-clinic	Ref. [88, 90], Amgen
	IL-15-based immunocytokines	PD-L1-IL-15	IL-15(N72D)/IL-15RαSushi /PD-L1	Lung cancer	Monotherapy	Pre-clinic	Ref. [91], Ref. [92]
		RGD-IL-15	IL-15/IL-15Ra complex/ integrin	Advanced or metastatic solid tumors	Monotherapy	Phase I	Boji Biopharmaceutical Technology Co., Ltd, Ref. [93]

Table 1 continued

Types	Classification	Name	Modified type	Disease	Mechanism	Clinical trial	From
	TNF-based immunocytokines	L19-TNF	TNF/edb	Advanced solid tumors	Monotherapy, combined with melphalan, Doxorubicin	Phase I/II, III	EudraCR N.O.2016-003239-38, NCT03779230
	Two cytokine payloads	IL-2-F8-TNFmut	IL-2, TNF mutant	Solid tumors	Monotherapy	Pre-clinic	Ref. [94]
Engager cytokines	TriKE	CD16-IL-15-CD33	IL-15/CD16, CD33	Blood tumors	Monotherapy	Phase I/II	Ref. [108, 109, 111, 1107]; GT Biopharma, Inc.; NCT03214666
		CD16-IL-15-CD19	IL-15/CD16, CD19	Blood tumors	Monotherapy	Pre-clinic	Ref. [110]
Types	**Classification**	**Mechanism**	**Structure**	**Disease**	**Treatment method**	**Clinical Trial**	**Source**
Other synthetic cytokines	Synthekines	Fusion of two unrelated dominant-negative cytokine subunit	Ligands to assemble IL-4Rα /IFNAR2 or IL-2Rβ/IL-4Rα	Tumors	Monotherapy, combined therapy	Pre-clinic	Ref. [112]
	Neoleukines	De novo design of potent mimics of cytokine A and cytokine B	Selective mimics of IL-2 and IL-15: neoleukin 2/15	Tumors	Monotherapy, combined therapy	Pre-clinic	Ref. [114]
	Fusokines	IL-6 and CNTF-based synthekines	IC7Fc: IL-6, domain of CNTF, Fc	Type-2 diabetes	Monotherapy	Pre-clinic	Ref. [115]
		A specific immunosuppressant of CCR2	GM–CSF(N-terminal)-CCL2 (GMME1) Fusokine	Autoimmune diseases	Monotherapy	Pre-clinic	Ref. [116]
		Fusions of two functional agents of GM–CSF and IL-2	hGIFT2 (GM–CSF-IL-2 fusokine)	Tumors	Monotherapy	Pre-clinic	Ref. [119]
		Fusions of two functional agents of GM–CSF and IL-4	GIFT4 (GM–CSF-IL-4 Fusokine)	Tumors	Monotherapy	Pre-clinic	Ref. [120]
	Nanoparticle-localized cytokines	Anchoring IL-2 and anti-4-1BB on the surface of liposomes	IL-2- and 4-1BB-coated nanoparticles	Tumors	Monotherapy	Pre-clinic	Ref. [121]
		TRAIL-presenting nanocages	TTPNs (Trimeric TRAIL-presenting nanocages)	Tumors	Monotherapy	Pre-clinic	Ref. [123]
		IL-12-mediated nanoparticle	DMP-pIL12 complex	Tumors	Monotherapy	Pre-clinic	Ref. [128]
	Cytokine-armed adaptive immune cells	IL-7 signaling armed CAR-T cells	Continuous activated IL-7R	Tumors	Monotherapy	Pre-clinic	Ref. [129]
		IL-12 armed CAR-T cells	Secreting IL-12	Tumors	Monotherapy	Pre-clinic	Ref. [131]
		IL-2 signaling armed engineered T cells	Secreting IL-2	Tumors	Monotherapy	Pre-clinic	Ref. [11, 136]
		Cytokines armed NK cells	Secreting IL-2, IL-12, IL-15, and stem cell factor (SCF)	Tumors	Monotherapy	Pre-clinic	Ref. [139–141]
	Cytokine-armed oncolytic virus	IL-7 and/or IL-12 armed oncolytic virus	The expression of IL-7 and IL-12	Tumors	Monotherapy	Pre-clinic	Ref. [145]
		Superagoinst IL-15 armed oncolytic virus	The expression of Superagoinst IL-15	Tumors	Monotherapy	Pre-clinic	Ref. [146]
	Cytokine conjugate	IL-4 trojan horse	IL-4/pseudomonas exotoxin A	Recurrent glioblastoma multiforme (rGBM)	Monotherapy	Pre-clinic	Ref. [147, 148]
	Cytokine-receptor fragment	IL-1 receptor antagonist	Soluble IL-1 receptor α	Rheumatoid arthritis (RA)	Monotherapy	Approved by the FDA	Ref. [149]
		PD-L1AbxTGFβR	PD-L1 antibody and TGF-β-neutralizing trap	Tumors	Monotherapy	Phase I/II, III	Merck, Hengrui

IL-10. IL-10 has immunostimulatory and anti-inflammatory properties, and its expression is often abnormally regulated in diseases. Garcia and colleagues demonstrated IL-10 pleiotropy by determining the structure of the IL-10 receptor (IL-10R) complex. The hexamer structure shows that IL-10 and IL-10Rα form a composite surface that binds to a shared signaling receptor, IL-10Rβ [37]. The data showing this structural feature made it possible to design selective agonists. Multiple IL-10 variants with different affinities for IL-10β binding were engineered and revealed significant differences in response thresholds between populations of immune cells. Myeloid-biased IL-10 variants work by inhibiting macrophage activation without stimulating the inflammatory activity of $CD8^+$ T cells [37]. Moraga and coworkers used yeast display technology to generate an IL-10 mutant with enhanced affinity for its IL-10 receptor. Compared with wild-type cytokines, the affinity-enhanced IL-10 variant had a more potent immune-activating effect and activated signal transducer and activator of transcription (STAT)1 and STAT3 in human monocytes and $CD8^+$ T cells at a higher level [38]. In addition, compared with wild-type IL-10, the engineered IL-10 variant stimulated greater amplification ability and cytolytic activity in chimeric antigen receptor (CAR) T cells [38]. These studies offer new opportunities to reevaluate IL-10 therapies and provide a blueprint for modulating the pleiotropic effects of IL-10.

Modification of long half-life

Long-lived IL-2. The half-life of IL-2 in human serum is only 5–7 min [1]. Increasing the half-life and biological activity are the other important directions of IL-2 modification. PEGylated human recombinant IL-2 (PEG-IL-2) and Fc-IL-2 [39] show prolonged plasma clearance and improved antitumor activity at low doses. NKTR-214 (Nektar Therapeutics) is PEG-IL-2 with a favorable binding affinity to IL-2β. NKTR-214 has been used in combination with the anti-programmed cell death protein (PD)-1 antibody nivolumab in clinical trials (NCT0363598, see www.clinicaltrials.gov/) [40]. Amgen designed AMG592, an IL-2 mutein-Fc fusion protein that exhibits selective activity on T_{regs} via decreased CD122 affinity and increased CD25 dependence. These molecules showed a longer half-life than wild-type IL-2 for the treatment of chronic graft-versus-host disease (NCT03422627) and active systemic lupus erythematosus (NCT03451422) and are currently in phase-I/II clinical trials [1]. Nektar Therapeutics engineered an IL-2 polymer (NKTR-358), which has a high affinity for IL-2αβγ and a lower affinity for IL-2βγ. This new protein could induce the selective expansion of T_{regs} and prolong their half-life if given subcutaneously once or twice a month, thus providing the *rationale* for a phase-I clinical trial (NCR03556007) [1]. MDNA11 (Medicenna) is a long-acting IL-2 superkine that has been designed to preferentially bind to IL-2Rβ and fused with human recombinant albumin. The terminal half-life of MDNA11 was 24-fold longer than that of wild-type IL-2 in mice. MDNA11 triggered an effective antitumor response and tumor growth control in syngeneic mouse tumor models [41].

Complex formulations

IL-15 superagonist. IL-15 is essential for the development and function of NK cells and memory $CD8^+$ T cells. It is being studied as an immunotherapeutic agent for cancer and microbial infections [42]. Based on the natural transpresentation of IL-15, this cytokine and its soluble high-affinity receptor (IL-15Rα) bind to form an IL-15 superagonist.

Currently, three structural configurations of the IL-15 superagonist are under investigation. In 2006, RLI (IL-15-linker-IL-15Rα sushi domain) was engineered by a French research team that linked human IL-15 with the high-affinity IL-15 binding ("sushi") domain of human IL-15Rα [43, 44]. RLI transmits its activation signal via IL-15Rβ/γc, which enhances the reconstitution of $CD8^+$ T cells and NK cells in humanized mice [43]. Soon after the development of RDL1, Rubinstein et al. generated the IL-15/IL-15Rα-Fc complex by noncovalent coupling of the soluble IL-15 and IL-15Rα-IgG1-Fc to form a chimeric protein [45]. Specifically, this IL-15 superagonist preferentially activates the proinflammatory subsets of type II NK, CD27 + CD11b + NK, and memory T cells.

Activated NK cells can significantly increase the expression of activating receptors (NKp46, NKG2D) and effector functional molecules (IFN-γ, perforin, granzyme) [45–47]. In addition, ALT-803 was engineered by coupling the human IL-15 mutant (IL-15N72D) and IL-15Rα sushi domain linked with the Fc domain of IgG1 [2, 48]. ALT-803 has shown significant antitumor effects by activating NK cells and memory T cells in mouse models and has entered phase-I/II clinical studies [49]. In addition, combined therapy with ALT-803 and immune checkpoint inhibitors, such as anti-PD-1, presented enhanced efficacy in refractory solid tumors [50, 51]. For instance, ALT-803 in combination with nivolumab was found to be safe for clinical use in a phase Ib trial for the treatment of non-small-cell lung cancer (NSCLC). Finally, ALT-803 has been reported to reactivate antitumor activity in patients with PD-1 monoclonal antibody-relapsed disease and refractory disease [52].

IMMUNOCYTOKINES

Antibodies can be "armed" with cytokines to form immunocytokines. The choice of cytokine is dependent upon the nature of the disease. Recently, the selection of cytokines has expanded from tumor necrosis factor (TNF), IL-2, and IL-12 to include IL-21 and IL-10. In addition, the antigens targeted by immunocytokines have expanded from targeting tumor cells and angiogenesis to targeting immune checkpoints in tumor-infiltrating immune cells. In addition, the architecture of immunocytokines is developing (mainly as the number of functional units has increased), with the emergence of two antigen-specific antibodies and one cytokine fusion protein as well as one antibody and two different cytokine-like fusion proteins (Fig. 2 and Table 1).

Antibody x cytokine

IL-2-based immunocytokines. Systemic injection of IL-2 is an untargeted immunotherapy approach leading to severe side effects, including hypotension, capillary leak syndrome, and severe influenza-like symptoms, which severely limit its clinical implementation [8, 53]. Therefore, the fusion of IL-2 with an antibody for targeted localization at the lesion site is a natural choice to reduce the side effects.

The first IL-2-based immunocytokines were developed around the turn of the century. Significant efficacy was achieved using antibodies against a specific epithelial cell adhesion protein (EpCAM G733-2) and ganglioside GD2 in mouse models, such as colon cancer [54, 55]. Gillies et al. designed a cH14.18-IL-2 fusion protein that targets ganglioside GD2 IgG with IL-2 linked to the C-terminus of the Ig heavy chain [56]. This immunocytokine has since demonstrated strong antitumor activity in several tumor models in mice. Hu14.18-IL-2 is a humanized product that has entered phase II clinical trials for the treatment of melanoma and neuroblastoma. Subsequently, the authors continued to improve its structure, mainly by constructing a low-toxicity IL-2 mutant (D20T, IL-2LT), upon which NHS-IL-2LT (Selectikine) was designed. NHS-IL-2LT has shown significant efficacy in the Lewis lung carcinoma (LLC) model in mice [54]. Currently, NHS-IL-2LT (targeting necrotic DNA) is being used as a single agent in patients with advanced solid tumors in a phase-I/II clinical study [57].

Recently, Roche developed immunocytokines targeting FAP and CEA that contain an IL-2 variant that does not bind to IL-2Rα, reducing undesired T_{reg} activation. Phase I clinical trials of CEA-IL2v showed local accumulation in tumors, and it has been combined with atezolizumab (anti-PD-L1) in a phase Ib clinical study [58, 59]. FAP-IL2v is also in clinical trials in combination with cetuximab

(anti-epidermal growth factor receptor (EGFR)) and atezolizumab [60]. For blood malignancies, the anti-CD20 immunocytokine DI-Leu16-IL-2 (Provenance Biopharmaceuticals) is currently in a phase-I/II clinical trial in patients with B-cell lymphoma [7, 61].

Neri and colleagues together with Philogen have focused on immunocytokines as promising preclinical and clinical agents. They conjugated IL-2 to antibodies targeting components of the extracellular matrix (e.g., F8, L19, F16) to form a noncovalent homodimer [8, 62]. In preclinical studies, compared with untargeted IL-2 treatment, treatment with L19-IL-2 significantly promoted the infiltration of mouse immune cells (e.g., NK cells, T cells) into tumor tissues and showed significantly stronger tumor growth inhibition [63, 64]. In phase-I clinical study, L19-IL-2 showed encouraging antitumor activity as a single or combined treatment for metastatic solid tumors. In addition, several phase II and III clinical trials evaluating the IL-2 fusion protein alone or in combination with another drug (e.g., L19-TNF, anti-CD20 antibody) are underway [65]. Finally, Sun et al. designed an Ab-sumIL-2 fusion molecule consisting of a supermutant IL-2 (sumIL-2) conjugated to an EGFR antibody to promote antitumor activity by binding specifically to tumor-infiltrating cytotoxic T lymphocytes (CTLs), which has been defined as part of the next generation of IL-2 agents [66].

TNF-based immunocytokines. TNF (or more specifically TNF-α) is a homotrimeric proinflammatory cytokine that promotes vascular permeability and blood clotting and attracts immune cells to prevent microbial infection against tumors. TNF stimulation also directly induces the apoptosis of tumor cells [67]. Recombinant TNF (Beromun™) has been approved for preoperative tumor reduction or local treatment in patients with unresectable soft-tissue sarcoma [68]. Systemic administration of TNF causes serious systemic adverse reactions, such as shock and organ failure, so its clinical application is greatly limited [69].

Scientists from Philogen have constructed L19-targeted immunocytokines presenting an scFv-TNF homotrimeric structure. Compared with untargeted TNF therapy, L19-TNF treatment showed stronger tumor potential for growth inhibition in mouse models of fibrosarcoma and colorectal cancer. Moreover, L19-TNF was localized preferentially in tumor tissue [70, 71]. Interestingly, in a glioblastoma mouse model, intravenous administration of L19-mIL12 or L19-mTNF achieved a cure in a certain percentage of tumor-bearing mice, whereas L19-IL-2 did not. The therapeutic activity of the former was dependent on $CD4^+$ and $CD8^+$ T cells. Mechanistically, these two immunocytokines promoted the accumulation of localized lymphocytes and effector cytokines in the tumor, and L19-mTNF also strongly induced tumor necrosis [72].

In phase-I/II clinical studies, L19-TNF did not show any efficacy as a monotherapy [73]. In contrast, combination therapy comprising L19-TNF and melphalan induced an objective response in 89% of advanced melanomas. In patients with inoperable metastatic melanoma, the potent anticancer activity of L19-TNF was also observed when combined with L19-IL-2 in the lesion [74]. Interestingly, tumor regression was also observed in noninjected lesions, which suggests that L19-TNF activates systemic antitumor immunity [65]. The combination is currently in a phase III clinical trial (NCT02938299).

IL-12-based immunocytokines. IL-12 is characterized by a heterodimer structure. IL-12 can activate T cells and NK cells and promote a Th1-mediated adaptive immune response. Preclinically, recombinant IL-12 has been shown to exert a significant antitumor effect on various types of malignancies and has been associated with a strong antigen-specific immune response and memory generation. However, its strong toxicity severely limits its clinical use [8].

Halin et al. constructed an IL-12-L9 immunocytokine by linking the p40 domain and p35 domain of IL-12 and fusing them to the scFv of the L19 antibody. Compared with untargeted recombinant IL-12, IL-12-L19 showed stronger inhibition of tumor progression and increased leukocyte infiltration into tumor tissues [75]. IL-12-L19 combined with L19-TNF further improved these therapeutic effects [76]. Recently, Neri and colleagues achieved a cure in a proportion of mice with glioblastoma following intravenous administration of L19-mIL12 by promoting immune cell infiltration and cytokine secretion in tumor tissues [72]. Some scientists are also exploring the construction of F8-IL-12 and CD30-IL-12 immunocytokines, which have been shown to activate antitumor effector functions in T cells in vivo and in vitro [77, 78].

NHS-IL-12 is a DNA-histone H1 complex-targeting immunocytokine developed by Merck. Currently, it is being evaluated in phase-I trials as a monotherapy or in combination with a PD-L1 antibody (avelumab). BC1-IL-12 can recognize the fibronectin 7 domain and is composed of the IL-12 heterodimer and complete IgG1. In a phase-I clinical study, six of 11 patients with malignant melanoma achieved disease stabilization for ≥4 months. All patients showed good tolerance and increased serum levels of IFN-γ [79].

IL-10-based immunocytokines. IL-10 is a classic immunosuppressive cytokine, and IL-10 deficiency is associated with various autoimmune diseases [80]. Interestingly, IL-10 also exerts antitumor effects through immunomodulation. Studies have shown that PEGylated IL-10 exerts antitumor effects by enhancing the proliferation and function of tumor-infiltrating $CD8^+$ T cells [81, 82]. However, untargeted IL-10 treatment carries great risks of toxicity via induced $CD8^+$ T-cell infiltration in healthy organs, as shown in mouse models [83]. In addition, high doses of PEGylated IL-10 have been associated with significant treatment-related side effects in clinical studies [84]. Fu et al. constructed a cetuximab (anti-EGFR)-based IL-10 fusion protein CmAb-(IL-10)2 to extend the half-life of IL-10 and to target the TME [85]. CmAb-(IL-10)2 has a bispecific antibody-like structure whereby Fc fuses to the fragment antigen-binding (FAB) portion of cetuximab and the IL-10 dimer, respectively. The fusion protein was found to have better activity and antitumor effects than those of nontargeted IL-10. Researchers have revealed that its mechanism of action involves the prevention of dendritic cell-mediated apoptosis of tumor-infiltrating $CD8^+$ T cells by regulating IFN-γ production [85]. In 2020, the National Medical Products Administration of China approved the clinical application of the EGFR monoclonal antibody/IL-10 fusion protein produced by Suzhou Dingfu Target Biotechnology.

IL-21-based immunocytokines. IL-21 cooperates with IL-7 or IL-15 to promote the proliferation and survival of memory and naive $CD8^+$ T cells [86]. More importantly, tumor-responsive T cells activated IL-21 have shown better antitumor efficacy in vivo than those activated by other γc cytokines [87]. IL-21, like most cytokines, acts on a wide range of cell types despite its short half-life and toxicity profile [88]. Designing fusion proteins that extend the half-life of IL-21 and achieve the targeted delivery of IL-21 to tumors is extremely important.

Deng et al. developed a novel tumor-targeting fusion protein (EGFR-IL-21) that could extend the half-life of IL-21 and improve its antitumor efficacy [89]. Compared with the IL-2 fusion protein cetuximab (EGFR-IL-2), EGFR-IL-21 showed lower toxicity in vivo. Mechanistically, EGFR-IL-21 can selectively amplify functional CTLs in the TME, coordinate the treatment of programmed death-ligand 1 (PD-L1) antibodies, and overcome the tolerance of immune checkpoint blockade in advanced tumor-bearing mice [89].

Recently, scientists have investigated molecules that link IL-21 with immune checkpoint antibodies (e.g., PD-1) in preclinical studies. In cancer patients, tumor-infiltrating $CD8^+$ T cells express high levels of PD-1; thus, a fusion protein should selectively target the tumor-responsive population of $CD8^+$ T lymphocytes. In addition, such a fusion protein can increase the proportion of

individuals who respond to PD-1 antibody therapy. Using structural guidance engineering, scientists from Amgen designed multifusion proteins with different IL-21 variants that target PD-1 [90]. The bifunctional fusion proteins blocked the interaction between PD-1 and PD-L1 while delivering IL-21 cytokines to PD-1-expressing T cells.

Targeted delivery of IL-21 improves T-cell function and is superior to anti-PD-1 monotherapy. One of the highly attenuated IL-21 mutants (R9E:R76A) was fused with PD-1 antibody and showed protective effects in a humanized mouse model of cancer that was resistant to anti-PD-1 monotherapy [90]. Wang et al. constructed a fusion protein connecting two IL-21 molecules with an anti-PD-1 scFv. In established tumor-bearing mice, PD-1AB21 treatment showed a powerful antitumor effect and was superior to the combination of PD-1 inhibition and IL-21 infusion [88]. Interestingly, PD-1AB21 promoted the generation of T memory stem cells and the expansion of tumor-specific CD8 + T cells with a memory phenotype [88].

IL-15-based immunocytokines. IL-15 superagonists (e.g., ALT-803) have achieved remarkable clinical efficacy [52]. Recently, scientists have developed customized IL-15 tumor-targeting therapeutic agents. ImmunityBio constructed mouse N-809, which consists of an IL-15(N72D)/IL-15Rα sushi superagonist complex and intact anti-PD-L1 ScFv domains [91]. Knudson et al. revealed that N-809 could block PD-1/PD-L1 interactions and induce IL-15-dependent immune effects. Compared with N-803 and αPD-L1, N-809 was well tolerated and reduced the lung metastasis of 4T1-derived tumors. Mechanistically, compared with N-803+αPD-L1, N-809 enhanced the infiltration of $CD8^+$ T cells in the TME and NK and $CD8^+$ T-cell activation in the draining lymph nodes and TME [91, 92]. In addition, N-809 reduced the number of T_{regs}, M2-like macrophages, and mononuclear myeloproliferative suppressor cells in the TME [91].

Chen et al. constructed an integrin-targeting fusion protein, PFC-1, which comprised an IL-15/IL-15Ra complex, an Fc domain, and an RGD polypeptide. PFC-1 showed strong antitumor effects and lymphocyte infiltration in the tumor [93]. BJ-001, a PFC-1-based drug from Boji Biopharmaceutical Technology, has entered phase-I clinical trials for the treatment of locally advanced or metastatic solid tumors.

Dual cytokine fusion molecules: cytokine1–antibody–cytokine2

IL-2- and TNF-based immunocytokines. Studies by Neri and colleagues have involved the simultaneous delivery of two cytokine "payloads" to the TME to elicit synergistic anticancer effects. They designed IL-2- and TNF-based immunocytokines and found that in combination, these could eradicate tumors in mice and achieve complete remission in patients with stage IIIB/C melanoma [65, 94, 95]. Based on these preliminary observations, the authors constructed a fusion protein comprising fibronectin-targeting IL-2-F8-TNF^{mut}, also termed a "potency-matched dual cytokine fusion". From a pharmaceutical perspective, combining IL-2 and TNF components into a single agent was attractive because only one product had to be developed to deliver two synergistic payloads. This dual cytokine fusion protein exhibited in vivo antitumor activity in immunocompetent mouse cancer models of WEHI-164, CT26, LLC, and F9 teratocarcinoma, either as a monotherapy or in combination with mouse PD-L1-specific monoclonal antibodies. The authors found that PD-L1 antibodies could aggregate in the TME and induce many NK cells and T cells to infiltrate tumor tissue [94].

ENGAGER CYTOKINES

Trispecific killer cell engagers

Bispecific immune engagers are designed such that one arm targets tumor antigens and the other arm targets T cells or NK cells. Specifically, BiTEs are engineered molecules in which one arm targets CD3 on T cells and an Fv on the other arm targets cancer cells. Combining these two specificities results in an agent that can induce targeted T-cell-mediated killing of identified tumor cells. Binding to CD3 and tumor antigens also leads to the formation of lytic "immune synapses" between T cells and tumor cells. BiTEs are completely free of the constant region of parental antibodies, and their small size (~55 kDa) and high flexibility enable close interaction between immune effector cells and cancer cells, thereby promoting simultaneous binding of target antigens to each cell [96]. BiTEs are considered novel bispecific antibodies with excellent efficacy [96]. In preclinical models, BiTEs exhibited strong antitumor activity superior to that of conventional monoclonal antibodies and other forms of bispecific antibodies. In vitro and in vivo studies have indicated that BiTEs can induce T-cell-mediated tumor cell killing at very low concentrations (10–100 pg/mL) and at a very low ratio of effector:target cells (<1:90) without the addition of agents that can elicit T-cell-activated costimulatory signaling. The most well-characterized BiTE is blinatumomab [97, 98]. In patients with non-Hodgkin's lymphoma, a very low dose of blinatumomab (which targets CD19 and CD3) can kill tumor cells in the blood, and patients exhibit a partial or complete response [99]. In patients with relapsed or refractory precursor B-cell lymphoblastic leukemia, intravenous infusion of blinatumomab has reportedly resulted in complete remission in 43% of patients [100, 101]. Blinatumomab has been approved for the treatment of r/r acute lymphocytic leukemia [9, 96]. Notably, it induces the activation and proliferation of T cells, which can cause cytokine release syndrome, diffuse intravascular coagulation, and nervous system events, including epilepsy and encephalopathy [102].

BiKEs are another type of engineered molecule in which one arm targets CD16 on NK cells and an Fv on the other arm targets cancer cells. BiKEs target CD16 and activate NK cells without the need for additional stimulatory agents. In this manner, NK cells and tumor cells are clustered together and form a lytic immune synapse, which, in turn, leads to continued activation of NK cells and further tumor cell lysis [103, 104]. CD16 BiKEs have been used to target $CD19^+$, $CD33^+$, $CD20^+$, $EpCAM^+$, and $CD133^+$ tumor cells. Interestingly, the CD16-CD33 BiKE has been shown to effectively activate NK cells in patients with myelodysplastic syndrome and to target $CD33^+$ myelodysplastic syndrome cells and immunosuppressive $CD33^+$ cells [104, 105]. In those and other studies, BiKEs could induce autologous NK cells to attack tumor cells, overcoming the immunosuppression that is common in these situations. The CD16-CD33 BiKE also enhances the production of TNF-α and IFN-γ and the degranulation of NK cells [106]. BiKEs can enhance the activation and function of NK cells on tumor cells, but its progress is limited by its inability to support the long-term survival of NK cells.

In recent years, several research teams have investigated further molecular optimization of BiKEs to promote survival signals in NK cells, which has led to the development of trispecific killer cell engagers (TriKEs). One type is BiKEs associated with a cytokine (e.g., IL-15) [107], which are called engager cytokines. Vallera et al. [107] first synthesized a TriKE consisting of a single-chain Fv (scFv) against CD16, an scFv against CD33, and IL-15 as the linker (i.e., 161533 TriKE). The advantage of this design is that one arm targets tumor antigens, one arm binds to NK cell receptors (CD16), which bring NK cells and tumor cells together to form an immune synapse, and the modified IL-15 crosslinker can stimulate NK cell survival and proliferation, further promoting NK cell activity and tumor cell death. The 161533 TriKE has exhibited significant activity against myeloid malignancies in in vitro and in vivo models by inducing greater NK cell expansion, cytotoxicity, and IFN-γ production [108]. Vallera et al. [107] also designed a TriKE molecule (TriKETM) containing an scFv against CLEC12A (an IL-15 receptor that sustains NK cell survival) and a heavy-chain camelid

single-domain antibody (sdAb) against CD16 that activates NK cells. The CLEC12A TriKE induces NK cell proliferation in vitro and greatly exhibits cytotoxic activity against AML cell lines and patient-derived AML cells. In vivo, the CLEC12A TriKE significantly reduced the tumor burden in preclinical mouse models. These results suggest the clinical potential of CLEC12A TriKE for the treatment of AML. In addition, 161533 TriKE can restore tumor-induced NK cell dysfunction [109]. Both our research team [110, 111] and Felices et al. [110, 111] developed the CD19-targeting 161519 TriKE. Compared with 1619 BiKE, 161519 TriKE significantly inhibited tumor growth and prolonged the overall survival of a B-cell lymphoma mouse model [110] (Fig. 3 and Table 1).

OTHER SYNTHETIC CYTOKINES (FIG. 4 AND TABLE 1)

Synthekines

The fusion of two dominant-negative cytokine variants, each of which binds only one receptor subunit, generates synthekines [112]. The latter recruits two receptor subunits. Synthekines drive the formation of cytokine-receptor dimers, which are not formed by endogenous cytokines and are not found in nature, to activate different signaling cascades [113].

Ligands that assemble IL-4Rα/IFNAR2 or IL-2Rβ/IL-4Rα heterodimers. Garcia and colleagues demonstrated that various unnatural heterodimers of cytokine receptors can cause differential signaling outputs. They designed synthekine ligands to assemble IL-4Rα/IFNAR2 or IL-2Rβ/IL-4Rα receptor heterodimers that do not occur naturally and trigger signaling and functional responses different from those activated by the endogenous cytokines IL-2, IL-4, and IFN [112] in vitro. Synthekine ligands that enable the dimerization of JAK/STAT cytokine receptors to receptor tyrosine kinases also induce signaling reactions [112]. Synthekines represent a new family of synthetic ligands with predefined receptors that allow a "freer" combination of the dimer signaling receptors encoded in the human genome.

Neoleukines

In a recent breakthrough, a computationally driven design produced neoleukin 2/15, which selectively has the function of natural cytokines IL-2 and IL-15 but has a completely unrelated amino acid sequence and topological structure [114]. Neoleukin 2/15 selectively binds the IL-2 receptor βγc heterodimer (IL-2Rβγc) but not IL-2Rα or IL-15Rα. The design is stable and can bind human and mouse IL-2Rβγc. It has a higher affinity for these receptors than do the natural cytokines and induces downstream cell signaling independent of IL-2Rα and IL-15Rα. Neoleukin 2/15 shows superior therapeutic activity to IL-2 in mouse models of melanoma and colon cancer, with lower toxicity and immunogenicity [114].

Fusokines

Fusions of two functional agents are called fusokines. Engineered chimeric cytokines can drive gain-of-function activity for immune cells.

IC7Fc (IL-6 and ciliary neurotrophic factor (CNTF)). Scientists have been searching for new drugs that effectively restore the control of blood sugar in patients with type-2 diabetes mellitus. IL-6 and CNTF have been reported to control body weight and enhance insulin sensitivity. Unfortunately, IL-6 is a proinflammatory cytokine, whereas CNTF induces the production of antibodies that cause side effects; these issues limit the translational potential of these agents. Febbraio et al. constructed a novel protein that performs both functions while eliminating their negative effects. The domain of CNTF-bound LIFR was replaced with IL-6 to form a new synthetic protein: IC7. Then, the Fc domain of IgG was fused to IC7 to obtain a cytokine similar to CNTF but dependent on IL-6R-IC7Fc for activity. Intraperitoneal injection of IC7FC significantly reduced the total body weight and fat weight in a mouse model [115]. In addition, IC7FC could reduce fasting blood glucose levels and enhance glucose tolerance. These results suggested that IC7FC could improve physiological indices. Febbraio et al. are actively promoting a phase-I clinical trial of IC7FC, which they present as a very promising therapy for type-2 diabetes mellitus in the future [115].

Granulocyte–macrophage colony-stimulating factor–chemokine ligand 2 (GM–CSF–CCL2) fusokine. C–C motif chemokine receptor 2 (CCR2) is a chemokine receptor that is widely expressed in lymphocytes and involved in autoimmune diseases. Therefore, CCR2 is a promising biological target for immunosuppression due to its direct role in autoimmune diseases (e.g., rheumatoid arthritis) [116]. Stagg et al. synthesized a novel fusion protein comprising GM–CSF and CCl2 N-terminal truncation protein (GMME1) and investigated its use as a specific immunosuppressant of CCR2. GM–CSF has a fairly long plasma half-life (reportedly 6 h), which can significantly extend the bioavailability of the C-terminal CCl2 fragment in vivo [117, 118]. The mechanism of action of GMME1 is dependent upon binding to CCR2, which subsequently leads to abnormal activation of mitogen-activated protein kinase- and caspase-3-induced cell death. This action depletes the CCR2-positive lymphocytes and monocytes involved in the initiation and progression of autoimmune diseases. When GMME1 was administered to mice with the symptoms of inflammatory arthritis, the clinical symptoms were significantly improved, foot thickness was reduced to normal levels, titers of anti-collagen antibody and rheumatoid factor were reduced, and levels of intraarticular and systemic proinflammatory cytokines were reduced [116].

hGIFT2 (GM–CSF-IL-2 fusokine). Penafuerte et al. designed a GM–CSF/IL-2 fusion protein exhibiting novel antitumor properties in vivo compared to that of the two individual cytokines combined [119]. This human GM–CSF/IL-2 fusion protein (known as "hGIFT2") induced NK cell activation in vitro and significantly increased the secretion of RANTES and IFNγ compared with that in response to IL-2 and GM–CSF monotherapy or combination therapy. In addition, hGIFT2-treated NK cells expressed higher levels of the active receptors NKP44, NKP46, and CD226, as well as functionally related molecular receptors such as CD69, CD107A, and IL-2Rβ [119]. Interestingly, hGIFT2 can promote NK cell maturation by downregulating CD117 expression and upregulating CD11b expression. This altered phenotype is conducive to greater cytotoxicity of tumor cells. At the molecular level, hGIFT2 leads to effective activation of the IL-2 and GM–CSF receptors downstream of JAK1/2, resulting in the phosphorylation of STAT1, STAT3, and STAT5 [119]. In conclusion, the fusokine hGIFT2 has unique biochemical characteristics different from those of IL-2 and GM–CSF and is an effective tool for NK cell activation and maturation, which may be used for tumor immunotherapy.

GIFT4 (GM–CSF-IL-4 fusokine). Deng et al. reported a new fusion cytokine with strong antitumor activity developed by the N-terminal coupling of GM–CSF with IL-4, called GIFT4 [120]. B cells expressing GM–CSF and IL-4 receptors and treated with GIFT4 show hyperphosphorylation of STAT, which is associated with the acquisition of a unique phenotype and function. In wild-type C57BL/6J mice, administration of GIFT4 promoted the proliferation of numerous B cells in vivo and inhibited the growth of B16F0 melanoma cells. In addition, B16F0 melanoma cells embedded with GIFT4 expression were designed to be directly rejected by the immune system [120]. When B16F0 cells expressing GIFT4 were implanted into B-cell-deficient mice, this effect was eliminated, thereby demonstrating a B-cell-dependent antitumor effect. hGIFT4-pretreated B cells can also assist in the

activation of CTLs and promote their specific killing of melanoma cells in vivo and in vitro. In conclusion, GIFT4 can mediate the expansion of B cells and has a strong antigen-specific effect [120]. GIFT4 may become an efficacious immunotherapy tool and define the previously unrecognized potential of B cells in melanoma immunotherapy.

Nanoparticle-localized cytokines

For specific targeted applications, cytokines can be coated on nanoparticles, which can provide effective cytokine delivery to tumors via passive delivery of cells owing to enhanced permeability and retention.

IL-2- and anti-CD137 (4-1BB)-coated nanoparticles. Immune-stimulating drugs such as the agonist 4-1BB and IL-2 can produce efficacious antitumor immunity, but they also elicit serious systemic toxicity, which limits their clinical application [121]. To overcome this issue, Zhang et al. anchored IL-2 and anti-CD137 on the surface of PEGylated liposomes, leading to rapid local accumulation of these immune agonists at the tumor site while reducing systemic exposure. In multiple tumor models, the immunoliposomes exerted antitumor activity equivalent to that achieved with free IL-2/anti-CD137 but without any obvious systemic toxicity. The immunoliposomes promoted cytotoxic lymphocyte infiltration in the tumor tissue and produced proinflammatory cytokines and granzymes, which completely changed the tumor immune microenvironment [121, 122]. Thus, liposomal-anchored delivery can provide a broad approach for the development of immune agonists that still exhibit strong activity on target cells but have reduced systemic toxicity.

Trimeric tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-presenting nanocages (TTPNs). Based on a rational design, high-resolution structural analysis, and activity analysis, Kih et al. designed, optimized, and tested the presentation strategy of trimer ligands on nanocages. Nanocages comprise 24 subunits that form by self-assembly of ferritin heavy chains, with a spherical cage-like structure. Based on this technology, they designed nanocages to deliver a TNF superfamily member, TRAIL, in its natural trimer-like structure [123]. The nanocages containing the trimer TRAIL were obtained by inserting sufficient spacing to mimic the natural structure of the TRAIL complex to provide optimal access to the target receptor. Preclinical studies demonstrated the efficacy of TTPNs as antitumor agents, showing a 330-fold increase in affinity, a 62.5-fold increase in apoptotic activity, and a significant improvement in their pharmacokinetic properties and stability compared with those of monomeric TRAIL. TTPNs also show >90% stability for ≤1 month, while ~50% of the monomeric form of TRAIL aggregates show >90% stability for only 2 days. The binding affinity of TTPNs with their receptors is consistent with that of natural TRAIL, and TTPNs can induce tumor cell apoptosis in vivo, thereby effectively inhibiting tumor growth [123]. Although TRAIL is used here as a proof of concept, all members of the TNF superfamily share the homologous TNF domain. Hence, other TNF superfamily ligands can be delivered on this "bionic" platform.

IL-12-mediated nanoparticles. Clinical trials of the recombinant IL-12 protein have been halted due to serious systemic toxicity and lower-than-expected efficacy [124, 125]. Instead, the delivery of IL-12 via gene therapy is an ideal way to maintain low expression levels that eventually drop to baseline [126]. To optimize *IL-12* therapy, more efficient and less toxic gene vectors are urgently needed. Although replication-deficient adenoviruses have some important advantages as vectors for gene therapy, their clinical applicability is limited by rapid inactivation, transduction efficiency, and adverse systemic side effects. Their use also leads to the serious concern of endogenous virus recombination, carcinogenic effects, and immune responses during treatment [127]. Liu et al. developed a nonviral gene-delivery system that uses nanoparticles to achieve targeted delivery of therapeutic genes in vivo while overcoming physical and biological barriers [128]. They established a novel gene-delivery system by self-assembly of methoxy (poly)ethylene glycol-poly(L-lactide) and 1,2-diolyl-3-trimethylammonium propane. The DMP-pIL12 complex significantly inhibited tumor growth in subcutaneous and peritoneal mouse models by promoting apoptosis, inhibiting angiogenesis, and reducing proliferation. When delivered in this way, IL-12 continues to activate the immune system with significantly lower toxicity [128]. Thus, the IL-12 plasmid and DMP complex may be used as a new drug for the clinical treatment of colorectal cancer.

Cytokine-loaded adaptive immune cells

Successful adoptive T-cell/NK cell immunotherapy for solid tumors requires improved expansion and activity of tumor-targeted or nonengineered T cells/NK cells. The availability of recombinant or transgenic cytokines may improve their potency. Cytokine (e.g., IL-7, IL-12, IL-2) signaling has been introduced into these T cells/NK cells [129].

CAR-T cells with enhanced IL-7 signaling. Adoptive immunotherapy using chimeric antigen receptor-modified T cells has achieved remarkable clinical results in the treatment of refractory leukemias and lymphomas, but challenges remain for successfully transforming these into solid tumors [129]. Shum et al. constructed an artificial cytokine receptor C7R with persistent signaling that can effectively trigger the IL-7 signaling axis but is insensitive to extracellular cytokines [129]. This synthetic IL-7R was developed from a variant in patients with acute lymphoblastic leukemia (ALL) that forms a homodimer due to the insertion of proline and/or cysteine residues across the membrane region and propagates a signal that does not require IL-7 stimulation [130]. This strategy tightly enhances the function of GD2-CAR-T cells but avoids the stimulation of "bystander" lymphocytes. Coexpression of C7R and GD2 CAR can increase the proliferation, survival, and antitumor activity of adoptive T cells with repeated exposure to the TME without changing the status of autologous T cells. In addition, GD2-CAR-T cells coexpressing C7R are active against xenograft mouse models of in situ glioblastoma and metastatic neuroblastoma [129]. Therefore, the developed C7R system can deliver powerful IL-7 stimulation to CAR-T cells to support their clinical development and may enhance antigen-specific T cells in the treatment of cancer.

CAR-T cells with enhanced IL-12 signaling. A recent approach to improve the efficacy of CAR-T cells is the ectopic expression of the stimulatory cytokine IL-12 [131]. Preclinical studies by Pegram et al. showed that CD19-specific CAR-modified T cells secreting IL-12 could safely eradicate disease without whole-body irradiation, chemotherapy, and/or additional cytokine support [132]. In a mimetic tumor model, they demonstrated that tumor elimination requires $CD4^+$ and $CD8^+$ T-cell subsets, autocrine IL-12, and subsequent secretion of IFNγ by CAR-T cells. Interestingly, the CAR-T cells that secreted IL-12 gained the ability to resist Treg suppression [132]. Therefore, based on these preclinical data, adoptive transfer therapy via IL-12 secretion from CAR-T cells would reduce or eliminate the need for potentially dangerous pretreatment regimens (e.g., chemotherapy) to achieve an optimal antitumor response in cancer patients [131]. This might lead to a promising adoptive cell therapy approach for cases of regulatory T-cell-mediated inhibition.

Engineered T cells with enhanced IL-2 signaling. Adoptive transfer of tumor-reactive T cells has been developed as a clinically useful therapy [133]. However, the widespread use of adoptive T-cell

transfer (ACT) in the treatment of cancer has several limitations, including the insufficient invasion of transferred cells into the lesion and the inability of transferred T cells to persist and maintain functionality in the body [11, 134, 135]. In clinical practice, the concurrent administration of IL-2 improves the survival, function, and antitumor activity of transplanted T cells. However, its pleiotropy (which simultaneously stimulates and suppresses immune responses and systemic toxicity) severely limits its clinical use [11]. Sockolosky et al. designed an orthogonal (ortho) IL-2 cytokine-receptor complex that transmits natural IL-2 signals but does not interact with their natural cytokines and receptors. Specifically, introducing *ortho*IL-2Rβ into T cells allowed *ortho*IL-2 to selectively target engineered $CD4^+$ cells and CD8 + T cells in vivo and in vitro with limited off-target effects and negligible toxicity. The *ortho*IL-2 signal was effective in a preclinical mouse cancer model treated with adoptive cells and may, therefore, represent a synthetic approach to achieve selective enhancement of engineered cells [136].

NK cells with enhanced cytokine signaling. Cytokine transgenes are effective approaches for the genetic manipulation, engineering, and enhancement of NK cells against tumors [137, 138]. Nonengineered allogeneic and autologous NK cells have been widely used in various clinical trials to treat malignancies [139]. The use of allogeneic NK cells has shown efficacy preliminarily in eliminating cancers, including metastatic melanoma, renal cell carcinoma, and AML. In preclinical studies, our research team and others have demonstrated that transfection or introduction of NK cells with cytokines such as IL-2, IL-12, IL-15, and stem cell factors can enhance their proliferation, survival, or targeting. This strategy has been shown to restore cytotoxicity and antitumor activity in vitro and in vivo [140, 141], but whether these methods will be successful in clinical trials requires further exploration.

Cytokine-armed OVs

Oncolytic viruses (OVs) are naturally occurring or genetically modified viruses that have been developed as an immunotherapy approach. OVs can selectively replicate in the TME, destroy cancer cells and expose tumor antigens to antigen-presenting cells [142]. In addition, the release of damage-associated molecular patterns can enhance antitumor immunity. OV-mediated cancer cell death is considered a type of immunogenic cell death, and tumor-selective OVs can be used as vectors to deliver immunomodulators to further modify the TME. To improve the potential efficacy of oncolytic therapy, multiple strategies have been devised to enhance antitumor immunity, such as OVs carrying genes encoding proinflammatory cytokines [143, 144].

IL-7 and/or IL-12 OVs. Systemic IL-12 treatment is highly toxic to the body. Several IL-12-expressing OVs (OV-IL12s) have been genetically engineered for local production of IL-12 and are in preclinical testing stages in various cancer models. Nakamura and colleagues established an oncolytic vaccinia virus (VV) platform that coexpresses IL-7 and IL-12 to alter the immune state within tumors as a monotherapy approach and a promising cotreatment with immune checkpoint blockade [145]. They found that the VVs encoding IL-7 and IL-12 completely changed the tumor immune microenvironment by improving the inflammatory immune state, eliciting a stronger systemic antitumor effect, and significantly increasing the sensitivity of solid tumors to systemic antitumor PD-1 anti-CTLA4 [145]. These findings provide strategies for overcoming immunotherapy-resistant tumors and provide a theoretical basis for further evaluation in humans.

Superagonist IL-15 OVs. The Bartlett research team constructed a novel oncolytic VV that expresses the superagonist IL-15 of the IL-15 and IL-15Rα fusion protein [146]. The virus was named vvDD-IL-15-Rα. It has a replication efficiency similar to that of the parent virus vvDD and can prolong the survival of mice suffering from MC38 cell-derived colon cancer or ID8 cell-derived ovarian cancer. This antitumor activity is highly dependent on $CD8^+$ T cells and, to a lesser extent, on $CD4^+$ T cells and NK cells [146]. A combination of oncolytic cell immunotherapy and anti-PD-1 antibodies was also significantly better than either monotherapy.

Cytokine conjugates

IL-4-toxins. Overexpression of IL-4 receptors is a key feature of a wide variety of tumors and has been found to be commonly associated with suppression of the immune response and poor survival outcomes [27, 147]. MDNA55 is a genetic fusion developed by Medicenna that contains two molecules: a highly specific circular IL-4 superkine and the catalytic domain of the *Pseudomonas* exotoxin A. The IL-4 superkine acts as a carrier to deliver potent bacterial toxins to the TME [148]. MDNA55 is in phase II clinical trials (www.ClinicalTrials.gov/, identifier: NCT02858895) for the treatment of recurrent glioblastoma multiforme (rGBM). The curative effects of MDNA55 in four clinical trials involving 118 patients with rGBM were positive; thus, MDNA55 has been granted the Fast Track Designation (FDA) and Orphan Drug Status (FDA and the European Medicines Agency [EMA]) for the treatment of rGBM (from the website of Medicenna Inc) (Table 1).

Cytokine-receptor fragments

IL-1 receptor antagonists. Anakinra is a modified soluble IL-1 receptor α, which blocks IL-1 receptors. Anakinra blocks IL-1α and IL-1β binding to the endogenous IL-1 receptor [149]. The safety and benefits of anakinra in the inflammatory process have been demonstrated in severe rheumatoid arthritis in adults and global juvenile idiopathic arthritis [150]. Anakinra has also been reported to improve systemic lupus erythematosus. Anakinra has been approved by the FDA for the treatment of rheumatoid arthritis [113].

Preclinical studies have shown that anakinra reduces adverse remodeling in mouse models of ischemic cardiomyopathy and is beneficial for the remission of heart failure [151]. In clinical studies, antagonists of IL-1 receptors are being studied as novel therapies for acute myocardial infarction and chronic heart failure in adults. Two small clinical trials showed that anakinra improved exercise performance in patients with chronic heart failure [152]. Further studies will be needed to determine whether blockade of IL-1 receptors is a viable therapeutic target for heart failure in adults.

PD-L1Ab×TGFβR. M7824 (Merck) is an innovative bifunctional fusion protein that can elicit synergistic antitumor activity by targeting the PD-L1 and TGF-β pathways. It consists of two parts: one is the IgG1 mAb against human PD-L1; the other is a TGF-β-neutralizing trap consisting of the extracellular domain of human TGF-βRII. In a preclinical tumor model in mice, the response rate of M7824 was an astonishing 100%. In syngeneic mouse models, M7824 was more effective in inhibiting tumor growth and metastasis than either anti-PD-L1 antibodies or TGF-β traps alone. In a mouse model of breast cancer, none of the mice treated with M7824 died for 218 days compared with the median survival of 21 days in the control group [153]. The clinical efficacy of M7824 as a second-line treatment for advanced NSCLC has been investigated (NCT02517398). In the total population, the objective response rate was 21.3%, the disease control rate was 40%, and the median duration of response was 14.1 months. At a dose of 1200 mg, the objective response rate of patients with PD-L1 positivity reached 37.0%, whereas the objective response rate of patients with high PD-L1 expression was ≤85.7% [154]. However, Merck (Germany) recently announced the termination of a phase III study of M7824 in lung cancer. After analyzing the interim data, the researchers concluded that M7824 could not outperform Keytruda and decided to terminate the trial early.

CONCLUDING REMARKS

Herein, we have reviewed recent advances in cytokine-based immunodrugs. The first type involves modification of cytokines to form supercytokines; the second type focuses on the fusion of modified cytokines and therapeutic antibodies to form immunocytokines; the third type centers on the fusion of modified cytokines and bispecific immune engagers to form engager cytokines; and finally, the other types of synthetic cytokines are still in the exploratory stage. Each of these entities has its own unique characteristics (Fig. 5).

Currently, most research on supercytokines is at the preclinical stage. Immunocytokine studies started later but have been progressing rapidly. In recent years, several immunocytokine agents have entered clinical trials in China and the United States (Table 1). The synthesis of novel immunocytokines is an important developmental direction of cytokine-based immunotherapy. Engager cytokines can also be understood as having a specific immunocytokine structure. Currently, there are few studies on engaging cytokines, but this topic is worthy of further study. We may also need to reestablish cytokines as "superassistants" to facilitate new immunotherapies. One of the biggest obstacles to the use of cytokines as immunotherapy agents is their toxicity, which is determined by their individual properties. Several researchers have modified cytokines to improve their targeting, efficacy, and long-term efficacy (Fig. 1). Reducing the biological activity or function of cytokines may be a promising way to reduce their toxicity and facilitate a supporting role in immunotherapy. To date, the modifications made to cytokines have been simple molecular biology changes or protein fusion. In the future, engineering and synthetic immunology technologies should be investigated in cytokine modification studies, such as those described next.

OUTLOOK

Supercytokines allow the transformation of a "cold tumor" to a "hot tumor"

Only 9% of patients with nonsquamous NSCLC with PD-L1-negative (<1%) tumors have been reported to achieve a clinical response to anti-PD-1 (nivolumab) treatment alone. However, the combination of ALT-803 (IL-15 superagonist) and nivolumab achieved a 30% clinical response rate in PD-L1-negative patients. These data suggest that the use of IL-2Rβγ agonists may help overcome resistance to immune checkpoint therapies such as anti-PD-1 monoclonal antibodies.

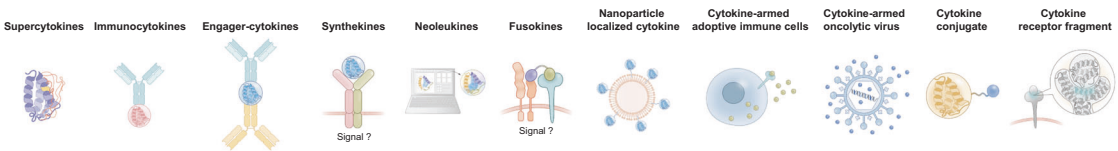
There are several possible mechanisms for increasing response rates. The systemically upregulated expression of proinflammatory cytokines such as IFN-γ is conducive to mobilizing the systemic effector immune response, which promotes the transformation of cold tumors to hot tumors. Another possibility is that ALT-803 drives the survival and expansion of neoantigen-specific T cells, resulting in specific killing. ALT-803 also drives substantial migration and expansion of NK cells. This is noteworthy, as major histocompatibility complex class I antigens are not expressed in a large proportion of NSCLC tumors, making this tumor type sensitive to NK cell killing. Broadly speaking, supercytokines such as ALT-803 can broaden the range of benefits of immune checkpoint therapy.

Supercytokines may act as useful assistants in ACT therapy

IL-2 has been widely used for in vitro amplification and in vivo persistence of adoptive transfer of CAR-T cells or tumor-infiltrating lymphocytes. IL-15 may be superior to IL-2 due to its unique role in activating NK cells and $CD8^+$ T cells without inducing T_{regs}. A combination of cytokines and tumor-infiltrating lymphocytes is being evaluated in multiple cancer types in multiple clinical trials, including different doses of IL-2 (NCT02414945, NCT04052334). IL-15 and IL-2 have been used as adjuvants for NK cell infusion (NCT03669172, NCT03213964). Clinical data have suggested that the adoptive transfer of NK cells causes minor "cytokine storms" and other side effects compared to those induced by T-cell-based immunotherapy. However, the weak viability of NK cells is a disadvantage. Administration of IL-2 or IL-15 may allow the adoptive transfer of NK cells to overcome these difficulties and prolong their presence in the body to exert their biological function. Therefore, supercytokines could act as superassistants in ACT therapy in the future.

The fusion of immune checkpoints and cytokines is a powerful immunocytokine (Fig. 2)

Previously, the concept of immunocytokines was attributed mainly to the formation of fusion proteins by combining cytokines with antibodies against tumors or lesions, which was conducive to enhancing the local effect of cytokines. Recently, scientists have evaluated the fusion of cytokines with antibodies against immune checkpoints in preclinical and clinical studies. Antibody therapy against immune checkpoints has shown great clinical success. Immunotherapies such as PD-1 blockade can significantly enhance endogenous antitumor immunity and improve the survival of cancer patients; however, only a small proportion of



Element	Cytokine	Cytokine Antibody[1]	Cytokine Antibody[1] Antibody[2](Anti-CD16)	Cytokine	Cytokine	Cytokine	Cytokine Nanoparticle	Cytokine Cytokine receptor T/NK cell	Cytokine Oncolytic virus	Cytokine Conjugate	Receptor fragment
Long half-life	++	++	++	+	+	+	++	++	++	+	-
off-target effect	++	+	+	++	++	++	+	+	++	+++	+
Regional delivery	-	++	++	-	-	-	++	++	++	+	-
Targeting-immune cell effect	-	+	++	-	-	-	-	++	-	-	-
Polyfunctionality	+	++	+++	+	++	+++	+	++	++	++	+
Technological difficulty factor	+	+	++	++	++	++	+	++	+	+	++
Mono-therapy	+	++	++	+	+	+	+	+	+	+	+
Combined therapy	++	-	-	+	+	+	-	-	-	-	-
Clinic trial	+	++	+	-	-	-	-	-	-	-	-

"-", negative, "+", weakly positive, "++", intermediately positive, "+++", strongly positive

Fig. 5 Specific characteristics of supercytokines, immunocytokines, engager cytokines, and other synthetic cytokines. Detailed comparison of the framework, element, in vivo half-life, off-target effects, local distribution in vivo, targeting immune cell effect, polyfunctionality, technical difficulties of drug development, treatment method, and clinical study phase of all chemokine-based therapies to show their respective characteristics. "-", negative, "+", weakly positive, "++", intermediately positive, "+++", strongly positive

patients respond to such therapies. The efficacy of immune checkpoint inhibitors may be extended by cytokines. Mechanistic studies have shown that immune checkpoint proteins are highly expressed in local immune cells of the TME. Thus, the fusion proteins described above can also act as targeted cytokines facilitating aggregation to the lesion. These bifunctional fusion proteins block immune checkpoints and simultaneously deliver cytokines to T cells with high levels of immune checkpoint expression, enabling them to survive or function efficiently. Such fusion proteins can also promote the expansion of tumor neoantigen-specific T cells. More interestingly, an IL-21 fusion protein promoted the memory phenotype of T cells and the proliferation of a population of T memory stem cells. These events have not been observed during anti-immune checkpoint therapy using a single agent. Thus, this engineering strategy has great potential to improve the therapeutic efficacy of immune checkpoint blockade.

Cytokine selection is a checkpoint in the success of immunocytokine treatment

Recently, Neri and colleagues found that intravenous administration of L19-mIL12 or L19-mTNF cured a certain percentage of tumor-bearing mice in a model of glioblastoma, whereas L19-IL-2 did not achieve similar outcomes. Upon analyzing the underlying mechanism, they found that the number of proinflammatory cytokines and tumor-infiltrating lymphocytes increased following treatment with L19-TNF or L19-IL-12. Glioblastoma presents with a strong immunosuppressive microenvironment and very little infiltration by immune effector cells. Therefore, L19-IL-2 is not suitable for the treatment of glioblastoma because the main function of IL-2 is to promote the survival and proliferation of lymphocytes. In conclusion, the selection of cytokines for immunocytokine therapy needs to be based on tumor type and the characteristics of the immune microenvironment.

Expanding the cytokine spectrum aids the development of immunocytokines as therapeutic agents (Fig. 2)

Supercytokines and immunocytokines have been in development for a long time, but they have not revolutionized the treatment of cancer or other diseases. Many studies have been based on IL-2- and IL-15-engineered supercytokines and immunocytokines. Thus, the potential of the chosen cytokine may determine the efficacy of the fusion protein. Expanding the cytokine spectrum is also needed. Recently, IL-21 has garnered significant attention. Fu et al. developed an EGFR-IL-21 fusion protein and found that EGFR-IL-21 showed lower toxicity in vivo than the IL-2 fusion protein containing cetuximab (EGFR-IL-2). IL-21 promotes the memory formation of T cells, which is very important for chronic diseases. Thus, broadening the cytokine spectrum may lead to unexpected advances in the development and use of supercytokines and immunocytokines.

Expanding the targeting of engager cytokines (Fig. 3)

Currently, the main engager cytokines are TriKEs, which activate NK cells by targeting CD16. CD16 is an important active receptor on cytotoxic NK cells, and our research team proposes that there are several critical activation receptors on NK cells, including NKG2D, NKp30, and NKp46. There is a major group of $CD16^{-}CD56^{bright}$ NK cells in local organ tissues that have a strong capacity to secrete effector cytokines. The development of engager cytokines that target the aforementioned broad-spectrum activation of receptors on NK cells may yield unexpected positive results in vivo.

REFERENCES

1. Mizui M. Natural and modified IL-2 for the treatment of cancer and autoimmune diseases. Clin Immunol. 2019;206:63–70.
2. Han KP, Zhu X, Liu B, Jeng E, Kong L, Yovandich JL, et al. IL-15:IL-15 receptor alpha superagonist complex: high-level co-expression in recombinant mammalian cells, purification and characterization. Cytokine. 2011;56:804–10.
3. Glassman CR, Mathiharan YK, Jude KM, Su L, Panova O, Lupardus PJ, et al. Structural basis for IL-12 and IL-23 receptor sharing reveals a gateway for shaping actions on T versus NK cells. Cell. 2021;184:983–99.e924.
4. Spangler JB, Moraga I, Mendoza JL, Garcia KC. Insights into cytokine-receptor interactions from cytokine engineering. Annu Rev Immunol. 2015;33:139–67.
5. Abbas AK, Trotta E, R Simeonov D, Marson A, Bluestone JA. Revisiting IL-2: biology and therapeutic prospects. Sci Immunol. 2018;3:eaat1482.
6. Carnemolla B, Borsi L, Balza E, Castellani P, Meazza R, Berndt A, et al. Enhancement of the antitumor properties of interleukin-2 by its targeted delivery to the tumor blood vessel extracellular matrix. Blood. 2002;99:1659–65.
7. Gillies SD, Lan Y, Williams S, Carr F, Forman S, Raubitschek A, et al. An anti-CD20-IL-2 immunocytokine is highly efficacious in a SCID mouse model of established human B lymphoma. Blood. 2005;105:3972–8.
8. Murer P, Neri D. Antibody-cytokine fusion proteins: a novel class of biopharmaceuticals for the therapy of cancer and of chronic inflammation. N. Biotechnol. 2019;52:42–53.
9. May MB, Glode A. Blinatumomab: a novel, bispecific, T-cell engaging antibody. Am J Health-Syst Pharm: AJHP. 2016;73:e6–13.
10. Taniguchi T, Matsui H, Fujita T, Takaoka C, Kashima N, Yoshimoto R, et al. Structure and expression of a cloned Cdna for human interleukin-2. Nature. 1983;302:305–10.
11. Rosenberg SA. IL-2: the first effective immunotherapy for human cancer. J Immunol. 2014;192:5451–8.
12. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N. Engl J Med. 1985;313:1485–92.
13. Kammula US, White DE, Rosenberg SA. Trends in the safety of high dose bolus interleukin-2 administration in patients with metastatic cancer. Cancer. 1998;83:797–805.
14. Sim GC, Radvanyi L. The IL-2 cytokine family in cancer immunotherapy. Cytokine growth factor Rev. 2014;25:377–90.
15. Wang X, Rickert M, Garcia KC. Structure of the quaternary complex of interleukin-2 with its alpha, beta, and gamma c receptors. Science. 2005;310:1159–63.
16. Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat Rev Immunol. 2018;18:648–59.
17. Shanafelt AB, Lin Y, Shanafelt MC, Forte CP, Dubois-Stringfellow N, Carter C, et al. A T-cell-selective interleukin 2 mutein exhibits potent antitumor activity and is well tolerated in vivo. Nat Biotechnol. 2000;18:1197–202.
18. Margolin K, Atkins MB, Dutcher JP, Ernstoff MS, Smith JW, Clark JI, et al. Phase I trial of BAY 50-4798, an interleukin-2-specific agonist in advanced melanoma and renal cancer. Clin Cancer Res. 2007;13:3312–9.
19. Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature. 2012;484:529–33.
20. O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science. 2010;327:1098–102.
21. Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD. Interleukin-4 and interleukin-13 signaling connections maps. Science. 2003;300:1527–8.
22. Junttila IS, Creusot RJ, Moraga I, Bates DL, Wong MT, Alonso MN, et al. Redirecting cell-type specific cytokine responses with engineered interleukin-4 superkines. Nat Chem Biol. 2012;8:990–8.
23. Zhang L, Ying Y, Chen S, Arnold PR, Tian F, Minze LJ, et al. The transcription factor RelB restrains group 2 innate lymphoid cells and type 2 immune pathology in vivo. Cell Mol Immunol. 2021;18:230–42.
24. Gurram RK, Zhu J. Orchestration between ILC2s and Th2 cells in shaping type 2 immune responses. Cell Mol Immunol. 2019;16:225–35.
25. Yin Y, Boesteanu AC, Binder ZA, Xu C, Reid RA, Rodriguez JL, et al. Checkpoint blockade reverses anergy in IL-13Rα2 humanized scFv-based CAR T cells to treat murine and canine gliomas. Mol Ther oncolytics. 2018;11:20–38.
26. Junttila IS. Tuning the cytokine responses: an update on interleukin (IL)-4 and IL-13 receptor complexes. Front Immunol. 2018;9:888.
27. Wang L, Liu Z, Wang L, Wu Q, Li X, Xie D, et al. Spatial distribution of IL4 controls iNKT cell-DC crosstalk in tumors. Cell Mol Immunol. 2020;17:496–506.
28. Ring AM, Lin JX, Feng D, Mitra S, Rickert M, Bowman GR, et al. Mechanistic and structural insight into the functional dichotomy between IL-2 and IL-15. Nat Immunol. 2012;13:1187–95.
29. Pettit DK, Bonnert TP, Eisenman J, Srinivasan S, Paxton R, Beers C, et al. Structure-function studies of interleukin 15 using site-specific mutagenesis, polyethylene glycol conjugation, and homology modeling. J Biol Chem. 1997;272:2312–8.

30. Ferrari-Lacraz S, Zheng XX, Kim YS, Li Y, Maslinski W, Li XC, et al. An antagonist IL-15/Fc protein prevents costimulation blockade-resistant rejection. J Immunol. 2001;167:3478–85.
31. Ferrari-Lacraz S, Zanelli E, Neuberg M, Donskoy E, Kim YS, Zheng XX, et al. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. J Immunol. 2004;173:5818–26.
32. Zhou T, Damsky W, Weizman OE, McGeary MK, Hartmann KP, Rosen CE, et al. IL-18BP is a secreted immune checkpoint and barrier to IL-18 immunotherapy. Nature. 2020;583:609–14.
33. Jaitin DA, Roisman LC, Jaks E, Gavutis M, Piehler J, Van der Heyden J, et al. Inquiring into the differential action of interferons (IFNs): an IFN-alpha2 mutant with enhanced affinity to IFNAR1 is functionally similar to IFN-beta. Mol Cell Biol. 2006;26:1888–97.
34. Thomas C, Moraga I, Levin D, Krutzik PO, Podoplelova Y, Trejo A, et al. Structural linkage between ligand discrimination and receptor activation by type I interferons. Cell. 2011;146:621–32.
35. Brideau-Andersen AD, Huang X, Sun SC, Chen TT, Stark D, Sas IJ, et al. Directed evolution of gene-shuffled IFN-alpha molecules with activity profiles tailored for treatment of chronic viral diseases. Proc Natl Acad Sci USA. 2007;104:8269–74.
36. Levin D, Schneider WM, Hoffmann HH, Yarden G, Busetto AG, Manor O, et al. Multifaceted activities of type I interferon are revealed by a receptor antagonist. Sci Signal. 2014;7:ra50.
37. Saxton RA, Tsutsumi N, Su LL, Abhiraman GC, Mohan K, Henneberg LT, et al. Structure-based decoupling of the pro- and anti-inflammatory functions of interleukin-10. Science. 2021;371:eabc8433.
38. Gorby C, Sotolongo Bellón J, Wilmes S, Warda W, Pohler E, Fyfe PK, et al. Engineered IL-10 variants elicit potent immunomodulatory effects at low ligand doses. Sci Signal. 2020;13:eabc0653.
39. Vazquez-Lombardi R, Loetsch C, Zinkl D, Jackson J, Schofield P, Deenick EK, et al. Potent antitumour activity of interleukin-2-Fc fusion proteins requires Fc-mediated depletion of regulatory T-cells. Nat Commun. 2017;8:15373.
40. Charych DH, Hoch U, Langowski JL, Lee SR, Addepalli MK, Kirk PB, et al. NKTR-214, an engineered cytokine with biased IL2 receptor binding, increased tumor exposure, and marked efficacy in mouse tumor models. Clin Cancer Res. 2016;22:680–90.
41. To MD, Merchant F, Merchant N. MDNA11 is a long-acting IL-2 superkine that potentiates durable immune response in mouse tumor models and non-human primates. J Clin Oncol. 2021;39:e14546.
42. Burrack KS, Huggins MA, Taras E, Dougherty P, Henzler CM, Yang R, et al. Interleukin-15 complex treatment protects mice from cerebral malaria by inducing interleukin-10-producing natural killer cells. Immunity. 2018;48:760–72. e764.
43. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A, et al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med. 2009;206:25–34.
44. Mortier E, Quéméner A, Vusio P, Lorenzen I, Boublik Y, Grötzinger J, et al. Soluble interleukin-15 receptor alpha (IL-15R alpha)-sushi as a selective and potent agonist of IL-15 action through IL-15R beta/gamma. Hyperagonist IL-15 x IL-15R alpha fusion proteins. J Biol Chem. 2006;281:1612–9.
45. Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD, et al. Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}. Proc Natl Acad Sci USA. 2006;103:9166–71.
46. Guo Y, Luan L, Rabacal W, Bohannon JK, Fensterheim BA, Hernandez A, et al. IL-15 superagonist-mediated immunotoxicity: role of NK cells and IFN-γ. J Immunol. 2015;195:2353–64.
47. Dubois S, Patel HJ, Zhang M, Waldmann TA, Müller JR. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8 +/CD44high T cells and its antitumor action. J Immunol. 2008;180:2099–106.
48. Xu W, Jones M, Liu B, Zhu X, Johnson CB, Edwards AC, et al. Efficacy and mechanism-of-action of a novel superagonist interleukin-15: interleukin-15 receptor αSu/Fc fusion complex in syngeneic murine models of multiple myeloma. Cancer Res. 2013;73:3075–86.
49. Wong HC, Jeng EK, Rhode PR. The IL-15-based superagonist ALT-803 promotes the antigen-independent conversion of memory CD8(+) T cells into innate-like effector cells with antitumor activity. Oncoimmunology. 2013;2:e26442.
50. Guo Y, Luan L, Patil NK, Sherwood ER. Immunobiology of the IL-15/IL-15Rα complex as an antitumor and antiviral agent. Cytokine Growth Factor Rev. 2017;38:10–21.
51. Redman JM, Steinberg SM, Gulley JL. Quick efficacy seeking trial (QuEST1): a novel combination immunotherapy study designed for rapid clinical signal assessment metastatic castration-resistant prostate cancer. J Immunother Cancer. 2018;6:91.
52. Wrangle JM, Velcheti V, Patel MR, Garrett-Mayer E, Hill EG, Ravenel JG, et al. ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial. Lancet Oncol. 2018;19:694–704.
53. Baldo BA. Side effects of cytokines approved for therapy. Drug Saf. 2014;37:921–43.
54. Dolman CS, Mueller BM, Lode HN, Xiang R, Gillies SD, Reisfeld RA. Suppression of human prostate carcinoma metastases in severe combined immunodeficient mice by interleukin 2 immunocytokine therapy. Clin Cancer Res. 1998;4:2551–7.
55. Sabzevari H, Gillies SD, Mueller BM, Pancook JD, Reisfeld RA. A recombinant antibody-interleukin 2 fusion protein suppresses growth of hepatic human neuroblastoma metastases in severe combined immunodeficiency mice. Proc Natl Acad Sci USA. 1994;91:9626–30.
56. Gillies SD, Reilly EB, Lo KM, Reisfeld RA. Antibody-targeted interleukin 2 stimulates T-cell killing of autologous tumor cells. Proc Natl Acad Sci USA. 1992;89:1428–32.
57. Albertini MR, Yang RK, Ranheim EA, Hank JA, Zuleger CL, Weber S, et al. Pilot trial of the hu14.18-IL2 immunocytokine in patients with completely resectable recurrent stage III or stage IV melanoma. Cancer Immunol, immunotherapy: CII. 2018;67:1647–58.
58. Klein C, Waldhauer I, Nicolini VG, Freimoser-Grundschober A, Nayak T, Vugts DJ, et al. Cergutuzumab amunaleukin (CEA-IL2v), a CEA-targeted IL-2 variant-based immunocytokine for combination cancer immunotherapy: overcoming limitations of aldesleukin and conventional IL-2-based immunocytokines. Oncoimmunology. 2017;6:e1277306.
59. Ribba B, Boetsch C, Nayak T, Grimm HP, Charo J, Evers S, et al. Prediction of the optimal dosing regimen using a mathematical model of tumor uptake for immunocytokine-based cancer immunotherapy. Clin Cancer Res. 2018;24:3325–33.
60. Herter S, Morra L, Schlenker R, Sulcova J, Fahrni L, Waldhauer I, et al. A novel three-dimensional heterotypic spheroid model for the assessment of the activity of cancer immunotherapy agents. Cancer Immunol, Immunotherapy: CII. 2017;66:129–40.
61. Bachanova V, Lansigan F, Quick DP, Vlock D, Gillies S, Nakamura R. Remission induction in a phase I/II study of an anti-CD20-interleukin-2 immunocytokine DI-Leu16-IL2 in patients with relapsed B-cell lymphoma. Blood. 2015;126:1533.
62. Bootz F, Neri D. Immunocytokines: a novel class of products for the treatment of chronic inflammation and autoimmune conditions. Drug Discov Today. 2016;21:180–9.
63. Schliemann C, Palumbo A, Zuberbühler K, Villa A, Kaspar M, Trachsel E, et al. Complete eradication of human B-cell lymphoma xenografts using rituximab in combination with the immunocytokine L19-IL2. Blood. 2009;113:2275–83.
64. Wagner K, Schulz P, Scholz A, Wiedenmann B, Menrad A. The targeted immunocytokine L19-IL2 efficiently inhibits the growth of orthotopic pancreatic cancer. Clin Cancer Res. 2008;14:4951–60.
65. Danielli R, Patuzzo R, Di Giacomo AM, Gallino G, Maurichi A, Di Florio A, et al. Intralesional administration of L19-IL2/L19-TNF in stage III or stage IVM1a melanoma patients: results of a phase II study. Cancer Immunol, Immunotherapy: CII. 2015;64:999–1009.
66. Sun Z, Ren Z, Yang K, Liu Z, Cao S, Deng S, et al. A next-generation tumor-targeting IL-2 preferentially promotes tumor-infiltrating CD8(+) T-cell response and effective tumor control. Nat Commun. 2019;10:3874.
67. Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer. 2009;9:361–71.
68. Gamm H, Lindemann A, Mertelsmann R, Herrmann F. Phase I trial of recombinant human tumour necrosis factor alpha in patients with advanced malignancy. Eur J Cancer. 1991;27:856–63.
69. van Horssen R, Ten Hagen TL, Eggermont AM. TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist. 2006;11:397–408.
70. Balza E, Mortara L, Sassi F, Monteghirfo S, Carnemolla B, Castellani P, et al. Targeted delivery of tumor necrosis factor-alpha to tumor vessels induces a therapeutic T cell-mediated immune response that protects the host against syngeneic tumors of different histologic origin. Clin Cancer Res. 2006;12:2575–82.
71. Borsi L, Balza E, Carnemolla B, Sassi F, Castellani P, Berndt A, et al. Selective targeted delivery of TNFalpha to tumor blood vessels. Blood. 2003;102:4384–92.
72. Weiss T, Puca E, Silginer M, Hemmerle T, Pazahr S, Bink A, et al. Immunocytokines are a promising immunotherapeutic approach against glioblastoma. Sci Transl Med. 2020;12:eabb2311.
73. Spitaleri G, Berardi R, Pierantoni C, De Pas T, Noberasco C, Libbra C, et al. Phase I/II study of the tumour-targeting human monoclonal antibody-cytokine fusion protein L19-TNF in patients with advanced solid tumours. J Cancer Res Clin Oncol. 2013;139:447–55.
74. Papadia F, Basso V, Patuzzo R, Maurichi A, Di Florio A, Zardi L, et al. Isolated limb perfusion with the tumor-targeting human monoclonal antibody-cytokine fusion protein L19-TNF plus melphalan and mild hyperthermia in patients with locally advanced extremity melanoma. J Surg Oncol. 2013;107:173–9.

75. Morillon YM 2nd, Su Z, Schlom J, Greiner JW. Temporal changes within the (bladder) tumor microenvironment that accompany the therapeutic effects of the immunocytokine NHS-IL12. J Immunother Cancer. 2019;7:150.
76. Halin C, Gafner V, Villani ME, Borsi L, Berndt A, Kosmehl H, et al. Synergistic therapeutic effects of a tumor targeting antibody fragment, fused to interleukin 12 and to tumor necrosis factor alpha. Cancer Res. 2003;63:3202–10.
77. Pasche N, Wulhfard S, Pretto F, Carugati E, Neri D. The antibody-based delivery of interleukin-12 to the tumor neovasculature eradicates murine models of cancer in combination with paclitaxel. Clin Cancer Res. 2012;18:4092–103.
78. Heuser C, Diehl V, Abken H, Hombach A. Anti-CD30-IL-12 antibody-cytokine fusion protein that induces IFN-gamma secretion of T cells and NK cell-mediated lysis of Hodgkin's lymphoma-derived tumor cells. Int J Cancer. 2003;106:545–52.
79. Xu C, Zhang Y, Rolfe PA, Hernández VM, Guzman W, Kradjian G, et al. Combination therapy with NHS-muIL12 and avelumab (anti-PD-L1) enhances antitumor efficacy in preclinical cancer models. Clin Cancer Res. 2017;23:5869–80.
80. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy-review of a new approach. Pharmacol Rev. 2003;55:241–69.
81. Berman RM, Suzuki T, Tahara H, Robbins PD, Narula SK, Lotze MT. Systemic administration of cellular IL-10 induces an effective, specific, and long-lived immune response against established tumors in mice. J Immunol. 1996;157:231–8.
82. Emmerich J, Mumm JB, Chan IH, LaFace D, Truong H, McClanahan T, et al. IL-10 directly activates and expands tumor-resident CD8(+) T cells without de novo infiltration from secondary lymphoid organs. Cancer Res. 2012;72:3570–81.
83. Mumm JB, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, et al. IL-10 elicits IFNγ-dependent tumor immune surveillance. Cancer Cell. 2011;20:781–96.
84. Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJ, van der Poll T. Proinflammatory effects of IL-10 during human endotoxemia. J Immunol. 2000;165:2783–9.
85. Qiao J, Liu Z, Dong C, Luan Y, Zhang A, Moore C, et al. Targeting tumors with IL-10 prevents dendritic cell-mediated CD8(+) T cell apoptosis. Cancer Cell. 2019;35:901–15.e904.
86. Zeng R, Spolski R, Finkelstein SE, Oh S, Kovanen PE, Hinrichs CS, et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J Exp Med. 2005;201:139–48.
87. Moroz A, Eppolito C, Li Q, Tao J, Clegg CH, Shrikant PA. IL-21 enhances and sustains CD8+ T cell responses to achieve durable tumor immunity: comparative evaluation of IL-2, IL-15, and IL-21. J Immunol. 2004;173:900–9.
88. Li Y, Cong Y, Jia M, He Q, Zhong H, Zhao Y, et al. Targeting IL-21 to tumor-reactive T cells enhances memory T cell responses and anti-PD-1 antibody therapy. Nat Commun. 2021;12:951.
89. Deng S, Sun Z, Qiao J, Liang Y, Liu L, Dong C, et al. Targeting tumors with IL-21 reshapes the tumor microenvironment by proliferating PD-1intTim-3-CD8+ T cells. JCI Insight. 2020;5:e132000.
90. Shen S, Sckisel G, Sahoo A, Lalani A, Otter DD, Pearson J, et al. Engineered IL-21 cytokine muteins fused to anti-PD-1 antibodies can improve CD8+ T cell function and anti-tumor immunity. Front Immunol. 2020;11:832.
91. Knudson KM, Hicks KC, Ozawa Y, Schlom J, Gameiro SR. Functional and mechanistic advantage of the use of a bifunctional anti-PD-L1/IL-15 superagonist. J Immunother Cancer. 2020;8:e000493.
92. Spitzer MH, Carmi Y, Reticker-Flynn NE, Kwek SS, Madhireddy D, Martins MM, et al. Systemic immunity is required for effective cancer immunotherapy. Cell. 2017;168:487–502.e415.
93. Chen S, Huang Q, Liu J, Xing J, Zhang N, Liu Y, et al. A targeted IL-15 fusion protein with potent anti-tumor activity. Cancer Biol Ther. 2015;16:1415–21.
94. De Luca R, Neri D. Potentiation of PD-L1 blockade with a potency-matched dual cytokine-antibody fusion protein leads to cancer eradication in BALB/c-derived tumors but not in other mouse strains. Cancer Immunol, Immunotherapy: CII. 2018;67:1381–91.
95. Schwager K, Hemmerle T, Aebischer D, Neri D. The immunocytokine L19-IL2 eradicates cancer when used in combination with CTLA-4 blockade or with L19-TNF. J investig Dermatol. 2013;133:751–8.
96. Goebeler ME, Bargou RC. T cell-engaging therapies - BiTEs and beyond. Nat Rev Clin Oncol. 2020;17:418–34.
97. Dreier T, Baeuerle PA, Fichtner I, Grün M, Schlereth B, Lorenczewski G, et al. T cell costimulus-independent and very efficacious inhibition of tumor growth in mice bearing subcutaneous or leukemic human B cell lymphoma xenografts by a CD19-/CD3- bispecific single-chain antibody construct. J Immunol. 2003;170:4397–402.
98. Löffler A, Gruen M, Wuchter C, Schriever F, Kufer P, Dreier T, et al. Efficient elimination of chronic lymphocytic leukaemia B cells by autologous T cells with a bispecific anti-CD19/anti-CD3 single-chain antibody construct. Leukemia. 2003;17:900–9.
99. Bargou R, Leo E, Zugmaier G, Klinger M, Goebeler M, Knop S, et al. Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. Science. 2008;321:974–7.
100. Labrijn AF, Janmaat ML, Reichert JM, Parren P. Bispecific antibodies: a mechanistic review of the pipeline. Nat Rev Drug Discov. 2019;18:585–608.
101. Topp MS, Gökbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, et al. Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16:57–66.
102. Barrett DM, Teachey DT, Grupp SA. Toxicity management for patients receiving novel T-cell engaging therapies. Curr Opin pediatrics. 2014;26:43–9.
103. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer. 2016;16:7–19.
104. Hodgins JJ, Khan ST, Park MM, Auer RC, Ardolino M. Killers 2.0: NK cell therapies at the forefront of cancer control. J Clin Investig. 2019;129:3499–510.
105. Wiernik A, Foley B, Zhang B, Verneris MR, Warlick E, Gleason MK, et al. Targeting natural killer cells to acute myeloid leukemia in vitro with a CD16 x 33 bispecific killer cell engager and ADAM17 inhibition. Clin Cancer Res. 2013;19:3844–55.
106. Gleason MK, Ross JA, Warlick ED, Lund TC, Verneris MR, Wiernik A, et al. CD16xCD33 bispecific killer cell engager (BiKE) activates NK cells against primary MDS and MDSC CD33+ targets. Blood. 2014;123:3016–26.
107. Vallera DA, Felices M, McElmurry R, McCullar V, Zhou X, Schmohl JU, et al. IL15 trispecific killer engagers (TriKE) make natural killer cells specific to CD33+ targets while also inducing persistence, in vivo expansion, and enhanced function. Clin Cancer Res. 2016;22:3440–50.
108. Sarhan D, Brandt L, Felices M, Guldevall K, Lenvik T, Hinderlie P, et al. 161533 TriKE stimulates NK-cell function to overcome myeloid-derived suppressor cells in MDS. Blood Adv. 2018;2:1459–69.
109. Schmohl JU, Felices M, Oh F, Lenvik AJ, Lebeau AM, Panyam J, et al. Engineering of anti-CD133 trispecific molecule capable of inducing NK expansion and driving antibody-dependent cell-mediated cytotoxicity. Cancer Res Treat. 2017;49:1140–52.
110. Cheng Y, Zheng X, Wang X, Chen Y, Wei H, Sun R, et al. Trispecific killer engager 161519 enhances natural killer cell function and provides anti-tumor activity against CD19-positive cancers. Cancer Biol Med. 2020;17:1026–38.
111. Felices M, Kodal B, Hinderlie P, Kaminski MF, Cooley S, Weisdorf DJ, et al. Novel CD19-targeted TriKE restores NK cell function and proliferative capacity in CLL. Blood Adv. 2019;3:897–907.
112. Moraga I, Spangler JB, Mendoza JL, Gakovic M, Wehrman TS, Krutzik P, et al. Synthekines are surrogate cytokine and growth factor agonists that compel signaling through non-natural receptor dimers. eLife. 2017;6:e22882.
113. Scheller J, Engelowski E, Moll JM, Floss DM. Immunoreceptor engineering and synthetic cytokine signaling for therapeutics. Trends Immunol. 2019;40:258–72.
114. Silva DA, Yu S, Ulge UY, Spangler JB, Jude KM, Labão-Almeida C, et al. De novo design of potent and selective mimics of IL-2 and IL-15. Nature. 2019;565:186–91.
115. Findeisen M, Allen TL, Henstridge DC, Kammoun H, Brandon AE, Baggio LL, et al. Treatment of type 2 diabetes with the designer cytokine IC7Fc. Nature. 2019;574:63–8.
116. Rafei M, Berchiche YA, Birman E, Boivin MN, Young YK, Wu JH, et al. An engineered GM-CSF-CCL2 fusokine is a potent inhibitor of CCR2-driven inflammation as demonstrated in a murine model of inflammatory arthritis. J Immunol. 2009;183:1759–66.
117. Stagg J, Wu JH, Bouganim N, Galipeau J. Granulocyte-macrophage colony-stimulating factor and interleukin-2 fusion cDNA for cancer gene immunotherapy. Cancer Res. 2004;64:8795–9.
118. Burgess AW, Metcalf D. Serum half-life and organ distribution of radiolabeled colony stimulating factor in mice. Exp Hematol. 1977;5:456–64.
119. Penafuerte C, Bautista-Lopez N, Boulassel MR, Routy JP, Galipeau J. The human ortholog of granulocyte macrophage colony-stimulating factor and interleukin-2 fusion protein induces potent ex vivo natural killer cell activation and maturation. Cancer Res. 2009;69:9020–8.
120. Deng J, Yuan S, Pennati A, Murphy J, Wu JH, Lawson D, et al. Engineered fusokine GIFT4 licenses the ability of B cells to trigger a tumoricidal T-cell response. Cancer Res. 2014;74:4133–44.
121. Kwong B, Gai SA, Elkhader J, Wittrup KD, Irvine DJ. Localized immunotherapy via liposome-anchored Anti-CD137 + IL-2 prevents lethal toxicity and elicits local and systemic antitumor immunity. Cancer Res. 2013;73:1547–58.
122. Zhang Y, Li N, Suh H, Irvine DJ. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. Nat Commun. 2018;9:6.
123. Kih M, Lee EJ, Lee NK, Kim YK, Lee KE, Jeong C, et al. Designed trimer-mimetic TNF superfamily ligands on self-assembling nanocages. Biomaterials. 2018;180:67–77.
124. Robert M, Miossec P. Reactivation of latent tuberculosis with TNF inhibitors: critical role of the beta 2 chain of the IL-12 receptor. Cell Mol Immunol. 2021;18:1644–51.
125. Zhang X, Smith SM, Wang X, Zhao B, Wu L, Hu X. Three paralogous clusters of the miR-17~92 family of microRNAs restrain IL-12-mediated immune defense. Cell Mol Immunol. 2021;18:1751–60.
126. Melero I, Mazzolini G, Narvaiza I, Qian C, Chen L, Prieto J. IL-12 gene therapy for cancer: in synergy with other immunotherapies. Trends Immunol. 2001;22:113–5.

127. Duffy MR, Parker AL, Bradshaw AC, Baker AH. Manipulation of adenovirus interactions with host factors for gene therapy applications. Nanomed. 2012;7:271–88.
128. Liu X, Gao X, Zheng S, Wang B, Li Y, Zhao C, et al. Modified nanoparticle mediated IL-12 immunogene therapy for colon cancer. Nanomed: Nanotechnol, Biol, Med. 2017;13:1993–2004.
129. Shum T, Omer B, Tashiro H, Kruse RL, Wagner DL, Parikh K, et al. Constitutive signaling from an engineered IL7 receptor promotes durable tumor elimination by tumor-redirected T cells. Cancer Discov. 2017;7:1238–47.
130. Shochat C, Tal N, Bandapalli OR, Palmi C, Ganmore I, te Kronnie G, et al. Gain-of-function mutations in interleukin-7 receptor-α (IL7R) in childhood acute lymphoblastic leukemias. J Exp Med. 2011;208:901–8.
131. Geering B, Fussenegger M. Synthetic immunology: modulating the human immune system. Trends Biotechnol. 2015;33:65–79.
132. Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood. 2012;119:4133–41.
133. Kalos M, June CH. Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity. 2013;39:49–60.
134. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348:62–8.
135. Yee C, Thompson JA, Byrd D, Riddell SR, Roche P, Celis E, et al. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. Proc Natl Acad Sci USA. 2002;99:16168–73.
136. Sockolosky JT, Trotta E, Parisi G, Picton L, Su LL, Le AC, et al. Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. Science. 2018;359:1037–42.
137. Sivori S, Vacca P, Del Zotto G, Munari E, Mingari MC, Moretta L. Human NK cells: surface receptors, inhibitory checkpoints, and translational applications. Cell Mol Immunol. 2019;16:430–41.
138. Li H, Er Saw P, Song E. Challenges and strategies for next-generation bispecific antibody-based antitumor therapeutics. Cell Mol Immunol. 2020;17:451–61.
139. Sun H, Sun C, Xiao W, Sun R. Tissue-resident lymphocytes: from adaptive to innate immunity. Cell Mol Immunol. 2019;16:205–15.
140. Cheng M, Chen Y, Xiao W, Sun R, Tian Z. NK cell-based immunotherapy for malignant diseases. Cell Mol Immunol. 2013;10:230–52.
141. Habif G, Crinier A, André P, Vivier E, Narni-Mancinelli E. Targeting natural killer cells in solid tumors. Cell Mol Immunol. 2019;16:415–22.
142. Wu C, Wu M, Liang M, Xiong S, Dong C. A novel oncolytic virus engineered with PD-L1 scFv effectively inhibits tumor growth in a mouse model. Cell Mol Immunol. 2019;16:780–2.
143. Varadé J, Magadán S, González-Fernández Á. Human immunology and immunotherapy: main achievements and challenges. Cell Mol Immunol. 2021;18:805–28.
144. He Y, Hwang S, Ahmed YA, Feng D, Li N, Ribeiro M, et al. Immunopathobiology and therapeutic targets related to cytokines in liver diseases. Cell Mol Immunol. 2021;18:18–37.
145. Nakao S, Arai Y, Tasaki M, Yamashita M, Murakami R, Kawase T, et al. Intratumoral expression of IL-7 and IL-12 using an oncolytic virus increases systemic sensitivity to immune checkpoint blockade. Sci Transl Med. 2020;12:eaax7992.
146. Kowalsky SJ, Liu Z, Feist M, Berkey SE, Ma C, Ravindranathan R, et al. Superagonist IL-15-armed oncolytic virus elicits potent antitumor immunity and therapy that are enhanced with PD-1 blockade. Mol Ther: J Am Soc Gene Ther. 2018;26:2476–86.
147. Joshi BH, Leland P, Asher A, Prayson RA, Varricchio F, Puri RK. In situ expression of interleukin-4 (IL-4) receptors in human brain tumors and cytotoxicity of a recombinant IL-4 cytotoxin in primary glioblastoma cell cultures. Cancer Res. 2001;61:8058–61.
148. Achrol AA, Manish; Bankiewicz, Krystof; Bexon, Martin; Bisdas, Sotirios; Brem, Steven; Brenner, et al. Intratumoral delivery of MDNA55, an interleukin-4 receptor targeted immunotherapy, by MRI-guided convective delivery for the treatment of recurrent glioblastoma: 2018; https://digitalcommons.psjhealth.org/other_pubs/47.
149. Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. Nature. 1990;343:341–6.
150. Eloseily EM, Weiser P, Crayne CB, Haines H, Mannion ML, Stoll ML, et al. Benefit of anakinra in treating pediatric secondary hemophagocytic lymphohistiocytosis. Arthritis Rheumatol. 2020;72:326–34.
151. Mezzaroma E, Toldo S, Farkas D, Seropian IM, Van Tassell BW, Salloum FN, et al. The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. Proc Natl Acad Sci USA. 2011;108:19725–30.
152. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. Circulation Res. 2002;91:988–98.
153. Lan Y, Zhang D, Xu C, Hance KW, Marelli B, Qi J, et al. Enhanced preclinical antitumor activity of M7824, a bifunctional fusion protein simultaneously targeting PD-L1 and TGF-β. Sci Transl Med. 2018;10:eaan5488.
154. Paz-Ares L, Kim TM, Vicente D, Felip E, Lee DH, Lee KH, et al. Bintrafusp Alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in second-line treatment of patients with NSCLC: results from an expansion cohort of a phase 1 trial. J Thorac Oncol. 2020;15:1210–22.

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AUTHOR CONTRIBUTIONS

Z.T. and X.Z. conceived and conducted the project. Z.T. and X.Z. supervised the project. Yaqi. W., Z.T., J.B., Y.H, Y.C., Y.L., Yuwei. W., G.C., and X.Z. wrote the paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Xiaohu Zheng or Zhigang Tian.