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Engineering IL-2 to Give New Life to T Cell Immunotherapy

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Keywords

interleukin-2, IL-2, autoimmunity, cancer, immunotherapy

Abstract

Interleukin-2 (IL-2) is integral to immune system regulation. Its opposing immunostimulatory and immunosuppressive actions make it an attractive therapeutic target for cancer and autoimmune diseases. A challenge in developing IL-2-directed anticancer therapies has been how to stimulate effector T cells (Teffs) without inducing regulatory T cells (Tregs) in the tumor microenvironment; conversely, IL-2 therapy for autoimmune diseases requires Treg induction without further stimulation of Teffs. High-dose IL-2 is approved for melanoma and renal cell carcinoma, but its therapeutic value is limited by a need for frequent dosing at specialist centers, its short half-life, severe toxicity, and a lack of efficacy in most patients. Re-engineered IL-2 therapeutics are designed to have longer in vivo half-lives, target specific IL-2 receptor conformations to stimulate specific T cell subsets, or localize to target tissues to optimize efficacy and reduce toxicity. We discuss recent studies that elucidate the potential of newly engineered IL-2-based therapeutics for cancer and autoimmune diseases.

INTRODUCTION

Interleukin-2 (IL-2) is a cytokine that plays an integral role in the maintenance and homeostasis of the innate and adaptive immune responses. Upon activation by antigen-presenting cells, T effector cells (Teffs) produce IL-2, which, in turn, promotes the survival and/or expansion of multiple lymphocyte populations, including effector and memory T cells, natural killer (NK) cells, and regulatory T cells (Tregs) (1).

IL-2 regulates the balance between immunostimulation and immunosuppression via multiple pathways. IL-2 promotes immune responses by inducing $CD4^+$ T cell proliferation and differentiation into helper T cells, including Th1 and Th2 cells, and increasing the number and activity of $CD8^+$ Teffs and NK cells (1). Yet, IL-2 also simultaneously dampens immune responses by promoting the development and maintenance of immunosuppressive $CD4^+Foxp3^+$ Tregs (1). Specifically, IL-2 induces STAT5 phosphorylation, among other pathways (**Figure 1**), which is essential both for optimal activity of Teffs and for the expression of the Foxp3 transcription factor required for the multiple immunosuppressive functions of Tregs (2).

The pleiotropic effects of IL-2 signaling are mediated through the variable structure of the IL-2 receptor expressed by different immune cells (**Figure 1**) (3). The IL-2 receptor comprises

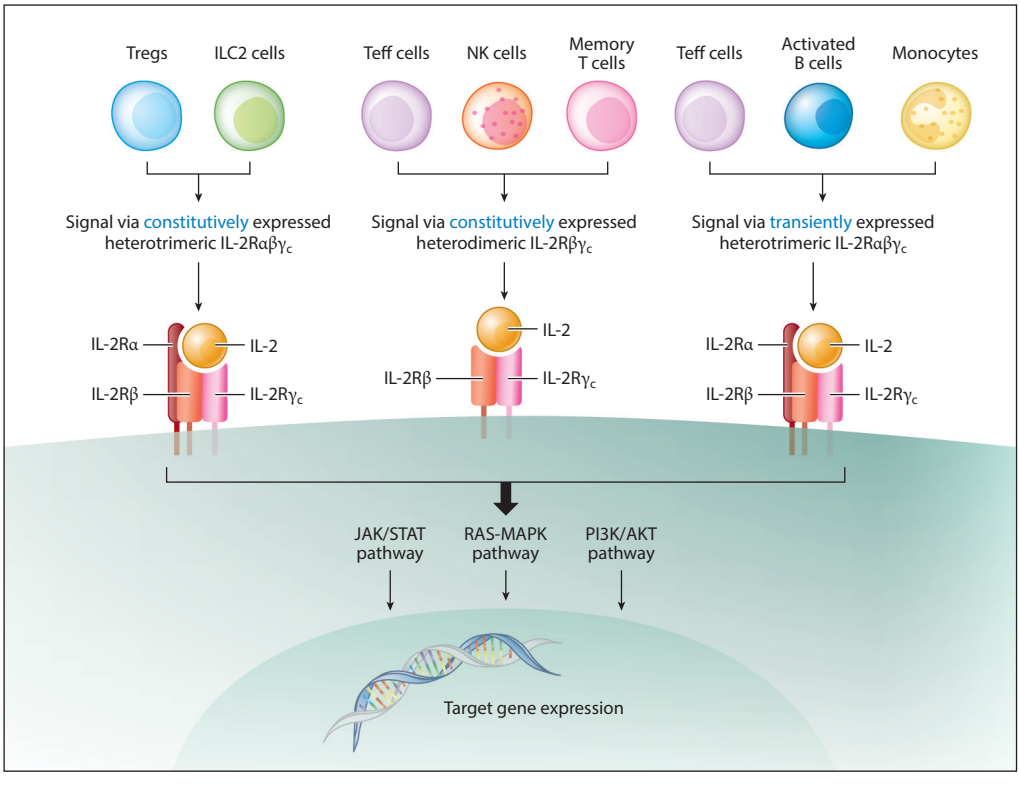

Figure 1

The role of IL-2 in the immune response. IL-2 signaling is mediated by different IL-2R configurations that are expressed by different immune cells. The high-affinity IL-2 receptor, IL-2Rαβγ_c, is constitutively expressed on Tregs and ILC2 cells and transiently expressed on Teffs, activated B cells, and monocytes. The intermediate-affinity IL-2 receptor, IL-2Rβγ_c, is constitutively expressed on Teffs, memory T cells, and NK cells. IL-2 signaling activates the JAK/STAT, RAS-MAPK, and PI3K/AKT pathways, which modulate target gene expression and subsequently differentiation, proliferation, survival, and cellular function. Abbreviations: IL-2, interleukin-2; IL-2R, interleukin-2 receptor; ILC2, type-2 innate lymphoid cells; NK, natural killer; Teffs, effector T cells; Tregs, regulatory T cells.

three subunits: IL-2Rα (CD25), IL-2Rβ (CD122), and the common γ-chain (γ_c; CD132). The intermediate-affinity IL-2 receptor is the heterodimer IL-2R$\beta\gamma_c$, which is expressed by naïve and memory $CD4^+$ and $CD8^+$ T cells, and NK cells; the high-affinity IL-2 receptor is the heterotrimer IL-2R$\alpha\beta\gamma_c$, which is expressed constitutively by Tregs and type-2 innate lymphoid cells, and transiently by Teffs. Stimulation of these two IL-2 receptor conformations leads to different downstream effects, making them promising targets for the development of therapeutics for cancer as well as autoimmune diseases (1).

CONNECTING BIOLOGICAL DIVERGENCE TO THERAPEUTIC RATIONALE

Oncology

The immune system, with its many cell types and functions, can both promote and suppress the initiation and progression of cancer. In general, Teffs and NK cells promote tumor killing, a process inhibited by Tregs (4).

T cells represent up to 10% of cells within the tumor microenvironment (4), with different T cell subsets associated with different outcomes. Typically, $CD8^+$ memory T cells and $CD4^+$ Th1 cells are associated with good cancer prognosis, whereas $Foxp3^+$ Tregs, $CD4^+$ Th2 cells, and Th17 cells are associated with poor cancer prognosis (5). The fact that lymphopenia substantially reduces response to different cancer therapies, including checkpoint inhibitors (CPIs), underlines the central importance of lymphocytes in cancer therapy (6). A key challenge in the therapeutic targeting of IL-2 in oncology is therefore how to direct its activity toward immune supportive Teffs and away from immunosuppressive Tregs.

Autoimmune Diseases

Autoimmune diseases are characterized by the breakdown of immunologic tolerance and exaggerated immune activity, with defects in Tregs contributing to this pathophysiological mechanism. Mice deficient in components of the IL-2/IL-2R pathway develop overactive T cell proliferation and autoimmunity (7), pointing to the nonredundant function of IL-2 in maintaining functional Tregs. This finding generated considerable interest in therapeutically administering IL-2 to control autoimmune disorders.

Foxp3 is highly expressed in Tregs, and mutations in the human *FOXP3* gene are associated with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (IPEX, also known as X-linked autoimmunity-allergic dysregulation syndrome) (8). Foxp3-deficient mice also develop IPEX, and introducing $Foxp3^+$ Tregs from healthy animals resolves the disease, highlighting their central role in preventing autoimmune diseases (9). Importantly, IL-2 directly induces the expression of Foxp3 in Tregs, and administration of IL-2 increases $Foxp3^+$ Treg numbers in mice and humans (10).

Given this close connection among IL-2, Tregs, and autoimmune disease, there is much interest in using IL-2 to induce Tregs that can suppress autoimmune disease. For example, by boosting Tregs, IL-2 could help to control local inflammation to reduce plaque formation in atherosclerosis, suppress Teff-mediated killing of insulin-producing cells in type 1 diabetes mellitus (T1DM), and control inflammatory joint destruction in rheumatoid arthritis, to name only a few possibilities. Furthermore, IL-2 can also block T follicular helper cells and stimulate T follicular regulatory cell differentiation, which could reduce autoantibody formation and immune complex deposition in systemic lupus erythematosus (SLE) (11). For this approach to be successful, the stimulation of Tregs by IL-2 should ideally not be accompanied by the proliferation and activation of Teffs.

HISTORICAL OVERVIEW OF IL-2 THERAPIES

Oncology

The first proof-of-concept study with recombinant human (rh)IL-2 that demonstrated tumor regression in patients with solid tumors, including melanoma and renal cell carcinoma (RCC), was performed over three decades ago (12).

High-dose (HD)-IL-2 (aldesleukin) was approved by the US Food and Drug Administration (FDA) for the treatment of RCC and melanoma in 1992 and 1998, respectively (13). Its approval was based on objective response rates (ORRs) of 17–20% and complete responses (CRs) lasting up to 91 months (14). However, widespread adoption of HD-IL-2 has been hampered by significant toxicities, including vascular leak syndrome and clinical manifestations of a cytokine storm (11). Because of its short half-life of 13–85 min (15), HD-IL-2 must be administered over 5 consecutive days as an intravenous infusion at a maximum dose of 720,000 international units (IU)/kg every 8 h (15, 16). Consequently, HD-IL-2 causes substantial toxicities, with black box warnings in place for vascular leak syndrome and infection (15). Because of the difficulties with tolerability, patients with a lower performance status are excluded as potential candidates for HD-IL-2 treatment (15). Patients are also required to receive treatment at specialist immunotherapy centers to manage the significant side effects associated with HD-IL-2 treatment (15, 16). Attempts to mitigate toxicity by lowering the dose resulted in a reduction in therapeutic effect (17). Finally, the lack of objective responses to HD-IL-2 in >80% of cancer patients further hinders widespread clinical use. Nevertheless, the pronounced durability of responses observed in patients with RCC and melanoma spurred continued research into IL-2.

IL-2 has been used in combination with adoptive cell therapy (ACT). This involves the systemic infusion of tumor-infiltrating lymphocytes (TILs), isolated from tumor specimens and expanded ex vivo to large numbers, into the cancer-bearing host followed by IL-2 infusions to enhance the antitumor activity of the transferred TILs (18). A series of early clinical studies by Rosenberg et al. more than 20 years ago demonstrated the first objective responses with infused TILs combined with HD-IL-2 in patients with melanoma (19–21), which triggered further research into how ACT could be optimized for cancer treatment. Recent clinical studies have shown promising results in melanoma, cervical cancer, and human papilloma virus (HPV)–associated malignancies and are described later in this review.

Combining rhIL-2 with the anti-disialoganglioside (GD2) monoclonal antibody (mAb) ch14.18 (dinutuximab) was found to augment antibody-dependent cell-mediated cytotoxicity against GD2-expressing neuroblastomas (22). Granulocyte-macrophage colony-stimulating factor (GM-CSF) and isotretinoin were subsequently added to the regimen, leading to significantly improved overall survival versus isotretinoin (23) and a 2015 FDA approval for the maintenance treatment of pediatric patients with high-risk neuroblastoma (24). However, similar to HD-IL-2, the administration schedule is burdensome, involving an intravenous infusion totaling 10–20 h over 4 days. Furthermore, the US label carries black box warnings for life-threatening infusion reactions and neurotoxicity (24). Thus, there is ample room for improvement of IL-2-based therapies.

Autoimmune Diseases

While IL-2 promotes the proliferation and function of Tregs, Teffs, and NK cells, it induces Tregs at much lower concentrations because Tregs constitutively express the high-affinity IL-2R$\alpha\beta\gamma_c$ receptor, whereas Teffs and NK cells mostly express the intermediate-affinity IL-2R$\beta\gamma_c$ receptor (25). Capitalizing on this feature, inducing preferential expansion of Treg populations via

low-dose (LD)-IL-2 is a strategy that has been explored over the last decade for the treatment of autoimmune diseases, with proof-of-concept studies in T1DM, SLE, and graft-versus-host disease (GVHD) demonstrating treatment benefit without the toxicity associated with HD-IL-2 (26–28).

LD-IL-2 complexed with anti-IL-2 mAbs has been shown in preclinical models to preferentially stimulate Tregs without modifying other effector cells (29). The anti-IL-2 mAb JES6 prolonged the half-life of IL-2 (30), while also blocking the interaction of IL-2 with IL-2Rβ, leading to increased Treg proliferation since binding to the high-affinity IL-2R$\alpha\beta\gamma_c$ was preserved (29). However, until now, clinical adoption of antibody/cytokine complexes has been limited by difficulties in manufacturing them and maintaining their stability (31).

Other research included a small study evaluating IL-2 (4.5 million IU three times per week) plus rapamycin in patients with T1DM (32). However, despite an increase in Tregs, treatment showed transient worsening of β cell function in all nine patients. Furthermore, the IL-2Rα-blocking mAb daclizumab was approved for multiple sclerosis in 2016 after efficacy in reducing disease activity was demonstrated in patients with relapsing multiple sclerosis (33). However, toxicities involving the brain, liver, and other organs resulted in its withdrawal in 2018 (34).

ENGINEERED IL-2 THERAPEUTICS IN DEVELOPMENT

There has been a long-felt need in the clinic to provide a therapeutic that isolates varied biologic effects of the IL-2 pathway to address the significant shortcomings of existing IL-2-based therapies. Many strategies are being pursued to optimize the efficacy of drug candidates leveraging the IL-2 pathway, while reducing the associated toxicities. IL-2-based investigational agents in clinical studies in oncology and autoimmune diseases are summarized in **Table 1** and **Figure 2**.

Oncology

PEGylated IL-2 agonists. In general, PEGylation can improve the solubility and pharmacokinetics (PK) of drug molecules through a variety of mechanisms (35). In addition, PEGylation at specific sites of a protein ligand can lead to altered binding of specific molecular domains to specific domains on their cognate receptors. PEGylated IL-2 molecules that preferentially bind to different IL-2R conformations are being explored in oncology to activate the IL-2 system in a controlled way and to tilt the balance in the tumor microenvironment in favor of Teffs.

Bempegaldesleukin. Bempegaldesleukin was designed to improve the half-life, PK, pharmacodynamics, efficacy, and tolerability of IL-2. It is currently the most advanced IL-2 pathway–targeted agent in clinical development for oncology, with multiple active registrational phase III studies. Bempegaldesleukin is an engineered PEGylated IL-2 agonist with an average of six conjugated, releasable polyethylene glycol (PEG) molecules (36). Bempegaldesleukin is an inactive prodrug when administered (i.e., with all six PEG moieties attached), and its bioactivity gradually increases as the PEG molecules are slowly released following administration. Relative to native IL-2, the PEGylated active IL-2 species preferentially bind to IL-2R$\beta\gamma_c$, predominantly expressed on Teffs and NK cells, over IL-2R$\alpha\beta\gamma_c$, expressed predominantly on Tregs (36, 37). The half-life of bempegaldesleukin is 15.5 h in vivo versus aldesleukin's 1.4 h in humans, which leads to the observed controlled IL-2 activity with limited side effects (36, 38). Specifically, the greatly increased duration of drug exposure due to the prodrug design allows for much lower maximum plasma levels, reducing toxicity while increasing efficacy (37).

In preclinical studies, bempegaldesleukin induced tumor regression by increasing the intratumoral proliferation, activation, and effector function of $CD8^+$ T and NK cells without expanding

Table 1 IL-2 agents in development for cancer and autoimmune diseases

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
CANCER						
PEGylated IL-2 agonists						
Bempegaldesleukin	CD122 preferential PEG IL-2 pathway agonist	Solid tumors (LA/M)	≥2nd line	Phase I/II; NCT02869295 (EXCEL), completed (Oct. 2018)	■ $n = 26$ (39) ■ 54% SD ■ 35% with maximum tumor reductions of 2–30% ■ RCC: PR at first scan after nivolumab follow-on therapy ($n = 3$); 40% tumor reduction at first scan ($n = 1$); SD for 13 months ($n = 1$) ■ Melanoma: SD for 15 months ($n = 1$)	■ $n = 28$ (39) ■ 93% TRAEs; most common were fatigue (71%), flu-like symptoms (68%), pruritus (64%), hypotension (57%), rash (50%), decreased appetite (46%), arthralgia (32%), cough (32%) ■ 21% Grade 3 TRAEs ■ No Grade 4/5 TRAEs ■ RP2D 0.006 mg/kg q3w
Bempegaldesleukin + nivolumab	CD122 preferential PEG IL-2 pathway agonist + anti-PD-1 mAb	Melanoma (U/M)	1st line	Phase III; NCT03635983 (PIVOT IO 001), recruiting (June 2025) NCT04410445 (PIVOT-12), not yet recruiting (July 2027); NCT03729245 (PIVOT-09), recruiting (June 2024); NCT04209114 (PIVOT IO 009), recruiting (Sep. 2024)	N/A	N/A
		Melanoma (adjuvant)	1st line		N/A	N/A
		RCC (M)	1st line		N/A	N/A
		MIBC (LA)	Neoadjuvant/ adjuvant		N/A	N/A
		UC (LA/M)	1st line	Phase II; NCT03785925 (PIVOT-10), active, not recruiting (July 2022);	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
Bempegaldesleukin + nivolumab	CD122 preferential PEG IL-2 pathway agonist + anti-PD-1 mAb + anti-CTLA-4 mAb	Solid tumors (LA/M)	Multiple lines	Phase I/II; NCT02983045 (PIVOT-02), active, not recruiting (Dec. 2021)	Melanoma ($n = 38$): (47) ■ 53% ORR; 34% CR; median TTR 2 mo RCC ($n = 24$): (48) ■ 54% ORR (4/7 PD-L1+; 7/13 PD-L1−); 79% DCR TNBC ($n = 38$): (49) ■ 13% ORR (2/12 PD-L1+; 3/22 PD-L1−); 0% CR; 45% DCR UC ($n = 27$): (50) ■ 48% ORR (6/12 PD-L1+; 5/11 PD-L1−); 19% CR; 70% DCR NSCLC ($n = 6$): (48) ■ 50% ORR (3/5 PD-L1−); 67% DCR	Solid tumors ($n = 162$): (48) ■ Most common TRAEs were flu-like symptoms (63%), fatigue (39%), rash (38%), pruritus (30%); 11% Grade ≥3 TRAEs Melanoma ($n = 41$): (47) ■ 17.1% Grade 3/4 TRAEs; 4.9% Grade≥3 imAEs TNBC ($n = 43$): (49) ■ 26% Grade 3/4 TRAEs; 2.3% Grade 3 imAEs UC ($n = 41$): (50) ■ 15% Grade 3 TRAEs; 0% Grade 4/5 TRAEs
Bempegaldesleukin + nivolumab + ipilimumab					N/A	N/A
Bempegaldesleukin + nivolumab + NKTR-262	CD122 preferential PEG IL-2 pathway agonist + anti-PD-1 mAb + TLR7/8 agonist	Solid tumors (LA/M)	Multiple lines	Phase I/II; NCT03435640 (REVEAL), recruiting (Dec. 2022)	N/A	N/A
Bempegaldesleukin + pembrolizumab	CD122 preferential PEG IL-2 pathway agonist + anti-PD-1 mAb	Solid tumors (LA/M)/ NSCLC (M)	Multiple lines/1st line	Phase I/II; NCT03138889 (PROPEL), recruiting (June 2023)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
Bempegaldesleukin + avelumab	CD122 preferential PEG IL-2 pathway agonist + anti-PD-L1 mAb	HNSCC (LA/M)	1st line	Phase Ib/II; NCT04052204, active, not recruiting (Sep. 2020)	N/A	N/A
Bempegaldesleukin + avelumab + talazoparib	CD122 preferential PEG IL-2 pathway agonist + anti-PD-L1 mAb + PARP inhibitor	CRPC (M)			N/A	N/A
Bempegaldesleukin + avelumab + enzalutamide	CD122 preferential PEG IL-2 pathway agonist + anti-PD-L1 mAb + antiandrogen therapy	CRPC (M)			N/A	N/A
Bempegaldesleukin + VB10.NEO	CD122 preferential PEG IL-2 pathway agonist + neoantigen vaccine	Solid tumors (LA/M)	≥2nd line	Phase I/II; NCT03548467 (DIRECT-01), recruiting (Mar. 2023)	N/A	N/A
THOR-707	PEG-IL-2Rαβ-biased agonist	Solid tumors (A/M)	Multiple lines	Phase I/II; NCT04009681 (HAMMER) (56), recruiting (June 2022)	N/A	N/A
THOR-707 + CPI	PEG-IL-2Rαβ-biased agonist + anti-PD-1/anti-PD-L1				N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
TransCon IL-2 β/γ	PEG-IL-2Rαβ-biased agonist (98)	N/A	N/A	Preclinical	N/A	N/A
Non-PEGylated IL-2 agonists						
MDNA-19	IL-2Rβ-biased agonist (57)	N/A	N/A	Preclinical	N/A	N/A
Neo-2/15	IL-2Rβγ_c-biased agonist (59)	N/A	N/A	Preclinical	N/A	N/A
IL-2 fusion proteins						
ALKS-4230	IL-2v/IL-2Rα fusion protein	Solid tumors (A)	≥2nd line	Phase I/II; NCT02799095 (ARTISTRY-1), recruiting (Sep. 2021)	*n* = 14 (62) ■ 57% SD (1 patient with SD >6 mo)	*n* = 36 (62) ■ Most frequent AEs were pyrexia (75%) and chills (72%); majority Grade 1/2 ■ 31% ≥Grade 3 AEs; mainly transient leukopenia
ALKS-4230 + pembrolizumab	IL-2v/IL-2Rα fusion protein + anti-PD-1 mAb				*n* = 11 (62) ■ 64% SD or better ■ 1 patient with PR (ovarian)	*n* = 20 (62) ■ No new toxicities versus ALKS-4230 monotherapy
ALKS-4230	IL-2v/IL-2Rα fusion protein	Solid tumors (A)	Multiple lines	Phase I/II; NCT03861793 (ARTISTRY-2), recruiting (June 2022)	N/A	N/A
ALKS-4230 + pembrolizumab	IL-2v/IL-2Rα fusion protein + anti-PD-1 mAb					
ALKS-4230 + pembrolizumab	IL-2v/IL-2Rα fusion protein + anti-PD-1 mAb	HNSCC (A/R)	2nd line	Phase II; NCT04144517, recruiting (Sep. 2021)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
IL-2/mAb complexes						
Daromun	Anti-EDB mAb (L19)/IL-2v fused to L19/TNFv	Melanoma (A/M)	Neoadjuvant	Phase III; NCT03567889 (neo-DREAM) (69), recruiting (Dec. 2022)	N/A	N/A
			Multiple lines	Phase II; NCT02076633, completed (May 2015)	$n = 20$ (68) ■ 1 CR; 10 PRs; 5 SD; 4 PD ■ 55% ORR of treated lesions ■ 80% DCR of treated lesions ■ CRs in 32 injected lesions ■ CRs in 7/13 noninjected lesions	$n = 22$ (68) ■ Majority of AEs were Grade 1/2 (two Grade 3 ISR); ISR (73%), fever (59%), headache (50%), edema (36%), erythema (36%) ■ Possible TRAEs: chills (27%), nausea/vomiting (23%), rash (23%), vertigo (18%) ■ No serious AEs
Darleukin	Anti-EDB mAb (L19)/IL-2v	Melanoma (A/M)	≤2nd line	Phase II; NCT01253096, completed (Sep. 2013)	$n = 24$ (99) ■ 25% CRs (≥24 mo responses in 5 patients)[a] ■ 54% ORR in index lesions (44% CRs[a]; 10% PRs[a]; 37% SD; 10% PD)	$n = 25$ (99) ■ Majority of AEs were Grade 1/2 (a few cases of Grade 3 ISR) ■ ISR (76%), fatigue (25%), fever (20%), pruritis (16%), edema (24%), injection pain (~24%) ■ <10% possible TRAEs (chills, headache, pyrosis, dry oral mucosa) ■ No serious AEs
Darleukin + stereotactic ablative body radiotherapy	Anti-EDB mAb (L19)/IL-2v + radiotherapy	NSCLC (M)	Multiple lines	Phase II; NCT03705403 (IMMUNOSABR) (100), recruiting (Dec. 2023)	N/A	N/A
Darleukin + rituximab	Anti-EDB mAb (L19)/IL-2v + anti-CD20 mAb	DLBCL (R/R)	2nd line	Phase I/II; NCT02957019, recruiting (Dec. 2019)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
APN-301	Anti-GD2 mAb/IL-2v	Neuro-blastoma (R/R)	Multiple lines	Phase II; NCT00082758, completed (May 2012)	$n = 13$ (measurable disease by standard radiographic criteria) (72) ■ No responses (23% SD; 77% PD) $n = 23$ (measurable disease by ^{123}I-MIBG) (72) ■ 22% CR (17% SD; 61% PD)	$n = 38$ (72) ■ Grade 3/4 nonhematologic AEs were elevated transaminases (45%), pain (42%), acute vascular leak syndrome (32%), hyperbilirubinemia (21%), allergic reaction (11%), rash (5%), hypoxia (3%)
		Melanoma (A/M)	1st line	Phase II; NCT00590824, completed (Sep. 2018)	$n = 18$ (74) ■ Median RFS 6 mo (95% CI: 2–NR); 24-mo RFS 39% (95% CI: 18–60%) ■ Median OS 62 mo (95% CI: 14–NR); 24-mo OS 65% (95% CI: 40–82%)	$n = 18$ (74) ■ TRAEs that required treatment modification: hypotension ($n = 3$), syncopal episode ($n = 1$), elevated AST ($n = 2$), elevated bilirubin ($n = 1$), elevated creatinine with decreased urine output ($n = 1$), pain ($n = 1$)
APN-301 + nivolumab + ipilimumab + radiation	Anti-GD2 mAb/ IL-2v + anti-PD-1 mAb + anti-CTLA-4 mAb	Melanoma (A/M)	Multiple lines	Phase I/II; NCT03958383, suspended due to COVID-19 (Aug. 2025)	N/A	N/A
APN-301 + sargramostim + isotretinoin	Anti-GD2 mAb/ IL-2v + GM-CSFv + stereoiso-mer of retinoic acid	Neuro-blastoma (R/R)	Multiple lines	Phase II; NCT01334515, completed (Sep. 2019)	$n = 14$ (measurable disease by standard radiographic criteria) (73) ■ No responses $n = 31$ (measurable disease by ^{123}I-MIBG) ■ 16% ORR (10% CR; 6% PR)	$n = 51$ (73) ■ 86% Grade ≥3 TRAEs; non-hematologic AEs in >10% of patients: elevated transaminases (41%), pain (33%), elevated bilirubin (27%), fever (25%), capillary leak syndrome (16%), hypokalemia (16%), hypotension (12%), hypoxia (12%)

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
APN-301 + NK cells	Anti-GD2 mAb/ IL-2v + GM-CSFv + haploidentical NK cells	Neuroblastoma (R/R)	Multiple lines	Phase I; NCT03209869, suspended due to COVID-19 (Sep. 2021)	N/A	N/A
RG-7461	Anti-FAP mAb/IL-2v	Solid tumors (A/M)	1st line	Phase I; NCT02627274, recruiting (Dec. 2021)	$n = 35$ (75) ■ 3 patients with objective response >6 mo	$n = 35$ (75) ■ Most frequent AEs (>30%; mostly Grade 1/2) were: pyrexia, IRR, fatigue/asthenia, nausea, diarrhea, decreased appetite, elevated transaminases
RG-7461 + trastuzumab	Anti-FAP mAb/IL-2v + anti-HER2 mAb	HER2$^+$ breast cancer (LA/M/R)	≥3rd line		N/A	N/A
RG-7461 + cetuximab	Anti-FAP mAb/IL-2v + anti-EGFR mAb	HNSCC (R/M)	≥2nd line		N/A	N/A
RG-7461 + atezolizumab	Anti-FAP mAb/ IL-2v + anti-PD-L1 mAb	HNSCC, esophageal cancer, cervical cancer (A/M)	≥2nd line	Phase II; NCT03386721, recruiting (Jan. 2022)	N/A	N/A
RG-7461 + pembrolizumab	Anti-FAP mAb/ IL-2v + anti-PD-1 mAb	Melanoma (A/M)	1st line or 2nd line	Phase I; NCT03875079, recruiting (Apr. 2022)	N/A	N/A
RG-7461 + atezolizumab ± bevacizumab	Anti-FAP mAb/ IL-2v + anti-PD-L1 mAb ± anti-VEGF mAb	RCC (A/M)	1st line	Phase I; NCT03063762, active, not recruiting (Mar. 2021)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
Cergutuzumab amunaleukin	Anti-CEA mAb/IL-2v	Solid tumors (A/M)	2nd line	Phase I; NCT02004106, completed (Aug. 2016)	$n = 21$ (PET substudy) (77) ■ 14% SD, 86% PD	$n = 22$ (PET substudy) (77) ■ Most frequent TRAEs were IRR (63%), pyrexia (54%), fatigue (46%), nausea (46%) ■ 70% (14/20) with antidrug antibodies
Cergutuzumab amunaleukin + atezolizumab	Anti-CEA mAb/ IL-2v + anti-PD-L1 mAb	Solid tumors (LA/M)	2nd line	Phase I; NCT02350673, completed (Dec. 2019)	N/A	N/A
PD1-IL2v	Anti-PD-1 mAb/IL-2v (101)	N/A	N/A	Preclinical	N/A	N/A
Other						
Oncoquest-L vaccine	Vaccine of patient-derived tumor cells + HD-IL-2	Follicular lymphoma (A/M)	1st line	Phase II; NCT02194751, not yet recruiting (Jan. 2023)	N/A	N/A
		CLL	1st line	Phase I; NCT01976520, active, not recruiting (Jan. 2022)	N/A	N/A
Lifileucel	Adoptive cell therapy + IL-2 infusion	Melanoma	≥2nd line	Phase II; NCT02360579, active, not recruiting (Dec. 2024)	$n = 66$ (79) ■ 24% ORR (3% CRs; 33% PRs; 44% SD; 14% PD) ■ 80% DCR	$n = 66$ (79) ■ 97% Grade 3/4 AEs [thrombocytopenia (82%), anemia (56%), febrile neutropenia (55%)] ■ 2 deaths
		Cervical cancer	≥2nd line up to 4th line	Phase II; NCT03108495, recruiting (Dec. 2026)	$n = 27$ (80) ■ 44% ORR (11% CRs; 33% PRs; 41% SD; 15% PD)	$n = 27$ (80) ■ 96% Grade 3/4 AEs [anemia (56%), thrombocytopenia (44%), febrile neutropenia (30%); neutropenia (30%)] ■ No deaths

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
		HNSCC	≥2nd line up to 4th line	Phase II; NCT03083873, recruiting (June 2022)	N/A	N/A
		TNBC	≥2nd line up to 4th line	Phase II; NCT04111510, recruiting (Jan. 2022)	N/A	N/A
		Ovarian cancer, osteosarcoma, other bone/soft tissue sarcomas	≥2nd line	Phase II; NCT03449108, recruiting (Dec. 2021)	N/A	N/A
Lifileucel + pembrolizumab	Adoptive cell therapy + IL-2 infusion + anti-PD-1 mAb	Solid tumors	≥1st line up to 4th line, CPI-naïve	Phase II; NCT03645928, recruiting (Dec. 2024)	N/A	N/A
Orthogonal IL-2/IL-2Rβ mutant pairs	Orthogonal IL-2v/IL-2Rβ mutant pairs (77)	N/A	N/A	Preclinical	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
Camidanlumab tesirine	Anti-IL-2Rα mAb/PBD conjugate	HL (R/R)	≥3rd line	Phase II; NCT04052997, recruiting (May 2024)	N/A	N/A
		NHL or HL (R/R)	≥2nd line	Phase I; NCT02432235, completed (Oct. 2019)	Classical HL ($n = 75$) (102) ■ 71% ORR (40% CR; 31% PR; 11% SD; 15% PD) NHL ($n = 41$) (103) ■ 32% ORR at ≥60 μg/kg ■ No responses at ≤60 μg/kg T cell lymphoma ($n = 19$) (103) ■ Overall ($n = 19$): 42% ORR (5% CR; 37% PR; 5% SD; 47% PD) ■ 60 and 80 μg/kg groups ($n = 13$): 54% ORR (8% CR; 46% PR)	Classical HL ($n = 77$) (102) ■ Most common all-grade AEs (≥20%) were fatigue, maculopapular rash, pyrexia, increased GGT, nausea, elevated transaminases, cough ■ 66% Grade ≥3 AEs ■ Potential TRAEs were edema or effusion (25%), skin-related toxicity (65%), abnormal LFT (39%) ■ 1 death (possibly treatment-related) NHL ($n = 44$) (103) ■ Most common AEs (≥20%) were diarrhea (25%), fatigue (25%), pyrexia (25%), decreased platelet count (21%) ■ 77% Grade ≥3 AEs ■ 16% imAEs
		AML or ALL (R/R)	≥2nd line	Phase I; NCT02588092, terminated due to slow enrollment (Aug. 2018)	$n = 16$ (104) ■ 2 CRs with incomplete hematologic recovery	$n = 35$ (AML, $n = 34$; ALL, $n = 1$) (104) ■ 57% possible TRAEs [maculopapular rash (17%), increased GGT (17%), decreased appetite (14%), diarrhea (11%), fatigue (11%), rash (11%), increased AST (11%)] ■ 86% Grade ≥3 AEs [febrile neutropenia (26%), lymphopenia (14%), neutropenia (14%), thrombocytopenia (14%), fatigue (14%), pneumonia (11%), increased GGT (11%), hypophosphatemia (11%)]

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
		Solid tumors (LA/M)	≥2nd line	Phase I; NCT03621982, recruiting (July 2021)	N/A	N/A
AUTOIMMUNE DISEASES						
Low-dose IL-2	rhIL-2	Autoimmune and inflammatory diseases (stable/moderately active disease)	2nd line	Phase II; NCT01988506 (TRANSREG), recruiting (Feb. 2022)	$n = 46$ (85) ■ Significant improvement in CGI score at mo 3 and 6 ($p < 0.001$) and CGI severity at mo 6 ($p < 0.01$) versus baseline ■ Significant decrease in patients with fatigue ($p = 0.002$) or arthralgia ($p = 0.00015$) at mo 3 versus baseline	$n = 46$ (85) ■ Most common TRAEs were ISR (87%), fatigue (39%), headache (26%), influenza-like syndrome (24%), nausea (20%) ■ No antidrug antibodies ■ 13% serious AEs (none treatment-related)
		Type 1 diabetes (newly diagnosed)	2nd line	Phase II; NCT02411253 (DIABIL-2), recruiting (July 2022)	N/A	N/A
		SLE (moderate to severe)	2nd line	Phase II; NCT02955615 (LUPIL-2), completed (Feb. 2019)	N/A	N/A
IL-2 conjugates and fusion proteins						
NKTR-358	PEG-IL-2Rα-biased agonist	Healthy volunteers	N/A	Phase II; NCT04133116, completed (Mar. 2020)	N/A	$n = 100$ (89) ■ Majority of AEs were Grade 1/2 ISRs ■ No antidrug antibodies
		SLE (minimal to moderate)	2nd line	Phase I; NCT03556007, completed (Aug. 2019)	N/A	$n = 48$ (90) ■ Majority of TRAEs were Grade 1 ISRs
		Psoriasis (active)	1st line	Phase I; NCT04119557, suspended to accrual due to COVID-19 (Apr. 2021)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
		Atopic dermatitis (active)	1st line	Phase I; NCT04081350, suspended to accrual due to COVID-19 (Apr. 2021)	N/A	N/A
		SLE	N/A	Phase II; NCT04433585 (ISLAND-SLE), not yet recruiting (Apr. 2023)	N/A	N/A
THOR-809	PEG-IL-2Rα-biased agonist (91)	N/A	N/A	Preclinical	N/A	N/A
Efavaleukin alfa (AMG592)	IL-2v/human Fc fusion protein	Healthy volunteers	N/A	Phase I	N/A	$n = 64$ (105) ■ Most common AE was Grade 1 erythema ■ No serious AEs
		SLE (active)	≥2nd line	Phase Ib; NCT03451422, recruiting (May 2021)	N/A	N/A
		RA (active)	2nd line	Phase I/II; NCT03410056, terminated (May 2020)	N/A	N/A
		GVHD (chronic)	2nd line	Phase I/II; NCT03422627, recruiting (June 2023)	N/A	N/A
RG-7835 (RO7049665)	IL-2Rα-biased (N88D)/IgG1 fusion protein	Ulcerative colitis (active)	2nd line	Phase I; NCT03943550, recruiting (Apr. 2021)	N/A	N/A
		Healthy volunteers	N/A	Phase I; NCT03221179, completed (July 2019)	N/A	N/A
CC-92252	IL-2 mutein/Fc fusion protein	Psoriasis	N/A	Phase I; NCT03971825, recruiting (Jan. 2021)	N/A	N/A

(*Continued*)

Table 1 (*Continued*)

Agent	Mechanism of action	Indication	Line of treatment	Phase; ClinicalTrials.gov identifier (trial name), trial status (actual or estimated completion date)	Efficacy data	Safety data
IL-2/mAb complexes						
F5111.2	Anti-IL-2 mAb/IL-2v (97)	N/A	N/A	Preclinical	N/A	N/A
Other						
NNC0361–0041	Recombinant plasmid encoding IL-2, PPI, TGF-β1, and IL-10	Type 1 diabetes (newly diagnosed)	2nd line	Phase I; NCT04279613, not yet recruiting (Dec. 2022)	N/A	N/A
MDNA-209	IL-2Rβ antagonist (106)	N/A	N/A	Preclinical	N/A	N/A

[a]Assessed using immune-related response criteria.

Abbreviations: A, advanced; AEs, adverse events; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AST, aspartate aminotransferase; CEA, carcinoembryonic antigen; CGI, clinical global impression; CI, confidence interval; CLL, chronic lymphocytic leukemia; CPI, checkpoint inhibitor; CTLA-4, cytotoxic T lymphocyte antigen-4; CR, complete response; CRPC, castration-resistant prostate cancer; DCR, disease control rate; DLBCL, diffuse large B cell lymphoma; EDB, extra domain B; EGFR, epidermal growth factor receptor; FAP, fibroblast activation protein; GGT, gamma-glutamyltransferase; GM-CSFv, granulocyte-macrophage colony-stimulating factor variant; GVHD, graft-versus-host disease; HD-IL-2, high-dose interleukin-2; HL, Hodgkin's lymphoma; HNSCC, head and neck squamous cell carcinoma; IL-2R, interleukin-2 receptor; IL-2v, interleukin-2 variant; IL-10, interleukin-10; imAEs, immune-mediated adverse events; IRR, infusion-related reaction; ISR, injection site reaction; LA, locally advanced; LFT, liver function test; M, metastatic; mAb, monoclonal antibody; MIBC, muscle-invasive bladder cancer; MIBG, metaiodobenzylguanidine; mo, months; N/A, not applicable; NHL, non-Hodgkin's lymphoma; NK, natural killer; NR, not reached; NSCLC, non–small cell lung cancer; ORR, objective response rate; OS, overall survival; PARP, poly ADP ribose polymerase; PBD, pyrrolobenzodiazepine; PD, progressive disease; PD-1, programmed cell death protein-1; PD-L1, programmed death ligand-1; PEG, pegylated; PPI, preproinsulin; PR, partial response; q3w, every 3 weeks; R, recurrent; RA, rheumatoid arthritis; RCC, renal cell carcinoma; RFS, recurrence-free survival; rhIL-2, recombinant human IL-2; RP2D, recommended phase II dose; R/R, relapsed/refractory; SD, stable disease; SLE, systemic lupus erythematosus; TGF-β1, transforming growth factor-β1; TLR, toll-like receptor; TNBC, triple-negative breast cancer; TNFv, tumor necrosis factor variant; TRAEs, treatment-related adverse events; TTR, time to response; U, unresectable; UC, urothelial carcinoma; VEGF, vascular endothelial growth factor.

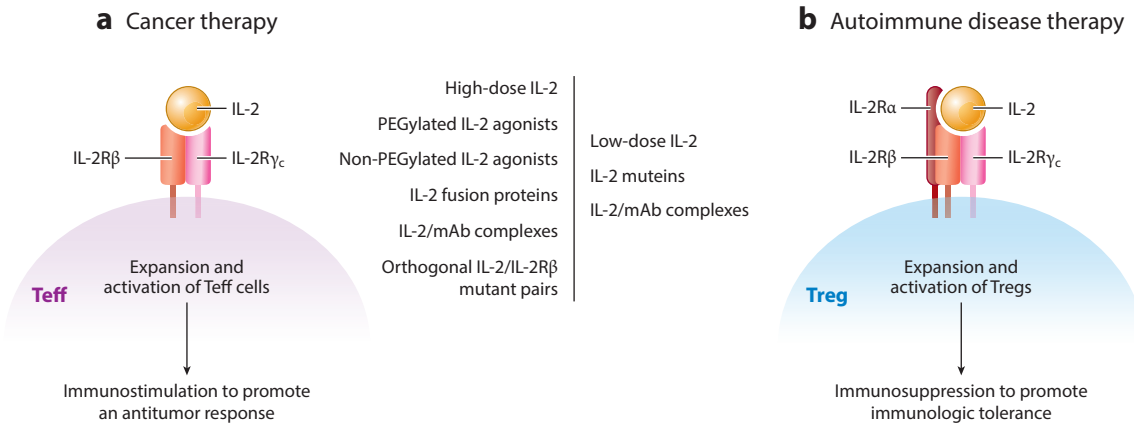

Figure 2

Mechanism of action of IL-2 agents for the treatment of cancer and autoimmune diseases. (*a*) Cancer can be treated using agents that target the intermediate-affinity IL-2Rβγ_c receptor that is constitutively expressed on Teffs, memory T cells, and NK cells, while (*b*) autoimmune diseases can be treated using agents that target the high-affinity IL-2Rαβγ_c receptor that is constitutively expressed on Tregs. Receptor stimulation by IL-2 triggers the differentiation, proliferation, and function of each immune cell population, leading to immunostimulation by Teffs, memory T cells, and NK cells for the treatment of cancer and immunosuppression by Tregs for the treatment of autoimmune diseases. Abbreviations: IL-2, interleukin-2; IL-2R, interleukin-2 receptor; mAb, monoclonal antibody; NK, natural killer; Teffs, effector T cells; Tregs, regulatory T cells.

intratumoral Tregs, with a marked elevation of the CD8:Treg ratio in the tumor by day 7 of treatment in a melanoma mouse model (37). Mirroring these results, a first-in-human study showed that bempegaldesleukin increased peripheral and intratumoral $CD8^+$ T cell proliferation, number, and effector function without increasing intratumoral Tregs, and without causing serious toxicity, in previously treated patients with advanced solid tumors. Bempegaldesleukin transiently increased Tregs in peripheral blood but not in the tumor (39), which may reduce autoimmune phenomena in patients receiving concomitant immunostimulatory therapies.

Preclinical studies have demonstrated activity of bempegaldesleukin in settings of anti-PD-1 and anti-CTLA-4 CPI therapy, vaccination, and ACT (40, 41). All studies found a consistent increase in the number and function of Teffs and superior antitumor activity, without increases in intratumoral Tregs and without significant toxicities. Furthermore, three in vivo studies have shown enhanced antitumor activity of bempegaldesleukin in combination with radiotherapy, radiotherapy plus anti-CTLA-4 therapy, and radionuclide therapy in mouse models of brain melanoma metastases, non–small cell lung cancer (NSCLC), and head and neck squamous cell carcinoma (HNSCC), respectively (42–44). Finally, bempegaldesleukin recently showed activity in a preclinical model of cancer that was resistant to anti-PD-1 therapy due to a lack of β-2-microglobulin. This defect is a known mechanism of anti-PD-1 resistance in human cancer and leads to a loss of expression of major histocompatibility complex (MHC) class I on the cell surface, which in turn leads to a loss of recognition by Teffs. Bempegaldesleukin was revealed to induce $CD4^+$ T cells and NK cells that could recognize and destroy the tumor cells in an MHC class I–independent manner (45). Taken together, these results demonstrate the synergy of bempegaldesleukin with other immunomodulating approaches, including CPI therapy, to increase tumor control.

To this end, the PIVOT-02 trial of bempegaldesleukin and nivolumab in patients with locally advanced/metastatic solid tumors established the recommended phase II dose as 0.006 mg/kg

bempegaldesleukin every 3 weeks plus nivolumab 360 mg every 3 weeks (46). Data have also been reported for patients with melanoma (47), RCC (48), triple-negative breast cancer (49), urothelial cancer (UC) (50), and NSCLC (48). The phase II dose was well tolerated and elicited deep and durable responses in the first-line melanoma setting, with an ORR of 53% ($n = 20/38$) and a CR rate of 34% ($n = 13/38$) observed after 18.6 months of follow-up (47). Responses in melanoma, UC, and other tumor types occurred regardless of baseline PD-L1 expression (47, 49, 50). In contrast to HD-IL-2, multiple cycles of bempegaldesleukin can be easily administered in the outpatient setting and, with the renewed generation of antigen-specific lymphocytes with each subsequent cycle, greater tumor shrinkage in a larger proportion of patients is achieved in combination with PD-1 checkpoint blockade compared with reported results for PD-1 checkpoint blockade alone. Furthermore, bempegaldesleukin plus nivolumab elicited the conversion of PD-L1-negative tumors to PD-L1-positive tumors in 7 of 10 patients (50), which is important because increased tumor PD-L1 expression is associated with improved responses to CPIs (51). This unique feature of bempegaldesleukin is particularly promising for the treatment of tumors such as advanced cisplatin-ineligible UC, where there is a high unmet need for novel treatments for patients with low-PD-L1-expressing tumors (52).

THOR-707. THOR-707 is a PEGylated IL-2 variant that lacks binding affinity for IL-2Rα, achieved through the attachment of one PEG molecule at an unnatural amino acid introduced in the IL-2 molecule (53, 54). THOR-707 demonstrated an extended half-life and high AUC in mice and nonhuman primates versus HD-IL-2, with repeated dosing at 0.1 mg/kg showing similar PK profiles when dosed every 2, 3, or 4 weeks in nonhuman primates (55). Activation and proliferation of $CD8^+$ effector and memory cells and NK cells were also observed, with minimal Treg expansion (53, 55). In CT-26 syngeneic mouse tumors, THOR-707 elicited the infiltration of $CD8^+$ T cells, increasing the proportion and repertoire diversity of TILs (54, 55). Furthermore, vascular leak syndrome was not observed at any dose level (53, 54). Antitumor activity was observed in combination with anti-PD-1 therapy (54, 55). The phase I/II HAMMER study of THOR-707 as a single agent or in combination with pembrolizumab began in 2019 in patients with advanced/metastatic solid tumors (56).

Non-PEGylated IL-2 agonists. Non-PEGylated rhIL-2 agonists in the early stages of development include MDNA-19, which is targeted to IL-2Rβ, and Neo-2/15, which binds to IL-2R$\beta\gamma_c$. A nonhuman primate study demonstrated expansion of $CD4^+$ T cells, $CD8^+$ T cells, and NK cells after treatment with MDNA-19, with limited effects on eosinophils and Tregs and an extended half-life versus rhIL-2 (57). MDNA-19 is expected to enter clinical trials in 2021 (58). For Neo-2/15, results so far have demonstrated a higher $CD8^+$ T cell:Treg ratio compared with wild-type mouse IL-2 and little or no immunogenicity in mice. Dose-dependent delays in tumor growth were observed in melanoma and colon cancer mouse models with single-agent Neo-2/15, with superior therapeutic activity and reduced toxicity shown following combination treatment with anti-TRP1 mAb TA99 in the melanoma model compared with wild-type mouse IL-2 (59). Most recently, Neo-2/15 targeted to engineered chimeric antigen receptor T cells (CAR-T cells) increased CAR-T cell expansion and prolonged survival in a B cell tumor xenograft model compared to CAR-T cells and nontargeted Neo-2/15 (60).

IL-2 fusion proteins. ALKS-4230 is an engineered protein of circularly permuted IL-2 fused to the extracellular domain of IL-2Rα, which inhibits interaction with IL-2Rα and preferentially binds to IL-2R$\beta\gamma_c$ (61). In mice, ALKS-4230 stimulated greater expansion of NK cells and $CD8^+$ memory T cells compared with rhIL-2 at doses that did not expand or activate Tregs, and it

demonstrated superior antitumor efficacy in a B16F10 lung tumor model. The half-life of ALKS-4230 was four- to fivefold longer than that of rhIL-2, and absorption was prolonged. Recent data from ARTISTRY-1, a phase I/II study investigating ALKS-4230 as monotherapy and in combination with pembrolizumab in patients with advanced solid tumors, have shown preliminary clinical benefit and acceptable tolerability profiles (62). ALKS-4230 monotherapy at 6 μg/kg elicited a partial response in one patient with metastatic urethral melanoma who had previously relapsed on adjuvant nivolumab and demonstrated durable responses in heavily pretreated patients with ovarian cancer combined with pembrolizumab (63). Other ongoing combination trials include the phase II ARTISTRY-2 study in patients with advanced/metastatic solid tumors and a phase II trial in patients with HNSCC.

IL-2/mAb complexes. Complexing IL-2 with a mAb can prevent the resulting complex from interacting with IL-2Rα to preferentially stimulate intermediate-affinity IL-2R$\beta\gamma_c$-expressing cells, as demonstrated in preclinical experiments with recombinant IL-2 complexed with the anti-IL-2Rα mAb S4B6. IL-2/S4B6 complexes expanded memory $CD8^+$ T cells and NK cells by 20–40-fold and Tregs by only 2–5-fold, while causing less endothelial cell damage and vascular leak syndrome in the lungs and liver of animals than HD-IL-2 (64). However, clinical evaluation of this approach has not yet been undertaken.

A different approach to cancer therapy is the intralesional administration of targeted agents, with the goal of killing tumor cells directly as well as stimulating a local and systemic antitumor immune response (65). Daromun is an engineered immunocytokine combining the anti–extra domain B (EDB) mAb L19 and the IL-2 agent L19-IL-2 (darleukin) with L19-TNF-α (fibromun), an immunocytokine in which L19 is fused to human tumor necrosis factor-α. The L19 antibody recognizes EDB on the tumor angiogenesis marker fibronectin, which is present in the newly formed vasculature of most tumors but absent in almost all healthy tissues (66). In syngeneic immunocompetent mouse models of cancer, complete remissions were observed following daromun administration, whereas the two components alone did not lead to cures (67). These results led to a phase II trial of intralesional daromun in patients with unresectable metastatic melanoma, in which 32 lesions in 20 patients exhibited CRs. CRs were also observed in 54% of noninjected lesions, demonstrating the ability of daromun to trigger systemic immune responses (68). The phase III neo-DREAM trial is now under way in patients with resectable stage IIIB/C melanoma (69). Darleukin is also being clinically investigated separately in NSCLC and lymphoma.

APN-301 is an anti-GD2 mAb covalently linked to IL-2, which has demonstrated potential anticancer activity in melanoma and neuroblastoma mouse models (70, 71). Phase II clinical studies of APN-301 are now complete. APN-301 as a single agent demonstrated a 22% ($n = 5/23$) CR rate in patients with recurrent/refractory neuroblastoma, with an observed duration of response of up to 35 months (72). In the same population, APN-301 in combination with GM-CSF and isotretinoin elicited a 16% ORR (73). In patients with resectable recurrent stage III/IV melanoma, 33% ($n = 6/18$) of those receiving APN-301 remained recurrence free, with 24-month recurrence-free and overall survival rates of 39% and 65%, respectively (74). Both regimens exhibited acceptable tolerability profiles across indications.

RG-7461 (RO6874281) is a recombinant fusion protein comprising a mAb directed against the tumor-associated fibroblast activation protein linked to an rhIL-2 variant that does not bind to IL-2Rα (75). The antibody portion mediates the retention and accumulation of the molecule in malignant tissue given the strong expression of fibroblast activation protein on tumor-associated fibroblasts; administration is accompanied by the activation and intratumoral accumulation of $CD8^+$ T and NK cells but reduced activity of Tregs (75). In a phase I trial, single-agent RG-7461

demonstrated an acceptable safety profile; rapidly expanded Teffs and NK cells, but not Tregs, in peripheral blood and tumors; and elicited long-lasting (>6 months) objective responses in three patients with metastatic solid tumors (75). Several phase I/II trials of RG-7461 in combination with other targeted therapies are currently recruiting.

Cergutuzumab amunaleukin (CA) is an anticarcinoembryonic antigen (CEA) mAb linked to rhIL-2 with three mutations that prevent binding to IL-2Rα. CEA is a glycoprotein that is highly expressed in certain solid tumor types, including colorectal cancer (95%), pancreatic cancer (90%), gastric cancer (80%), NSCLC (60%), and breast cancer (40%), compared with low levels in normal tissue (76). As a fusion protein, CA is designed for tumor targeting and an extended half-life compared with IL-2. Indeed, in human CEA-transgenic C57BL/6 mice, CA demonstrated superior PK and tumor targeting compared with a wild-type IL-2-based CEA immunocytokine, strongly expanded NK and $CD8^+$ T cells in the blood and tumor tissue, and demonstrated increased survival in syngeneic MC38-CEA and PancO2-CEA models, without preferentially activating Tregs (76). Selective and targeted tumor accumulation was confirmed in a recent positron emission tomography imaging study (77). Preliminary phase I data in patients with CEA-positive advanced/metastatic solid tumors confirmed the expansion of $CD8^+$ T cells and NK cells and the intratumoral accumulation of CA (78). Final results from this study, as well as results from a combination study with atezolizumab, have not yet been published.

Adoptive cell therapy. Lifileucel is a cryopreserved autologous TIL therapy that comprises a TIL infusion followed by up to six doses of IL-2. Lifileucel has recently demonstrated encouraging efficacy in phase II trials in patients with previously treated metastatic melanoma and recurrent, metastatic, or persistent cervical cancer. In melanoma, an ORR of 36% was observed (median duration of response: not reached at 18.7 months of median study follow-up), and in cervical cancer a 44% ORR (11% CRs) was reported (median duration of response: not reached) (79, 80). Other phase II trials of lifileucel are recruiting in different malignancies, including HNSCC and triple-negative breast cancer.

Recent proof-of-concept data have been published by the National Cancer Institute for an ACT for the treatment of HPV-associated epithelial cancers. This approach mediated the regression of HPV-associated cervical cancer, oropharyngeal cancer, and anal cancer in a phase II trial, with an overall ORR of 24% ($n = 7/29$) and CRs ongoing 67 and 53 months after treatment in two patients with cervical cancer (81).

Engineered IL-2 represents a potential advancement over the use of native IL-2 (aldesleukin) for ACT. Bempegaldesleukin showed superior activity over aldesleukin in a preclinical model of ACT (41). In an alternative approach, modified IL-2 receptors can be directly engineered into T cells and complemented with administration of a matched, engineered IL-2 protein variant, which is discussed in the next section. Together, the advancement of industrial-scale TIL manufacturing, expansion into cancers beyond melanoma, and engineered IL-2 may help T cell–based therapy gain a firmer foothold in solid tumor therapeutics.

Orthogonal IL-2/IL-2Rβ mutant pairs. A new approach to recombinant IL-2 therapy has been to develop orthogonal IL-2/IL-2Rβ mutant pairs where a mutant IL-2 molecule selectively binds to a mutant IL-2Rβ to transmit native IL-2 signals, but neither mutant binds to its wild-type counterpart. This approach is thought to alleviate toxicity and activation of Tregs induced when using native rhIL-2, by instead administering mutant IL-2 to patients receiving adoptive transfer of T cells engineered to express a matching, mutant IL-2Rβ. Sockolosky et al. (82) have recently developed orthogonal murine IL-2/IL-2Rβ mutant pairs which expanded T cells and promoted

antitumor responses in an in vivo melanoma model. Additionally, the transfer of orthoIL-2Rβ into T cells demonstrated selective cellular targeting of orthoIL-2 to engineered $CD4^+$ or $CD8^+$ T cells, as well as $CD4^+$ Tregs, but not to endogenous T cells expressing native IL-2Rs (82). This approach, which has yet to be validated in the clinic, is aimed at specifically enriching orthoIL-2Rβ-engineered T cells by administration of orthoIL-2.

Autoimmune Diseases

Unlike in oncology, the overall goal of targeting IL-2 is to dampen the immune response, with a focus on selectively enhancing immunosuppressive Tregs via the high-affinity IL-2R$\alpha\beta\gamma_c$ while limiting Teff cell proliferation driven by engagement of the intermediate-affinity receptor IL-2R$\beta\gamma_c$.

Low-dose IL-2. Following a short-term study of LD-IL-2 in patients with SLE that showed increased Tregs and decreased disease activity (83), a recent phase II pilot study reported significant response rates with LD-IL-2 versus placebo (66% versus 37%; $p = 0.027$) as well as complete remission in 54% of patients with lupus nephritis (84). There were no serious infections reported, in contrast to the increased infection risk associated with standard SLE therapies. The recent TRANSREG study of LD-IL-2 aimed to establish which autoimmune diseases could be selected for further clinical development. LD-IL-2 was administered to 46 patients with one of 11 autoimmune diseases, including rheumatoid arthritis, ankylosing spondylitis, SLE, psoriasis, and Crohn's disease. LD-IL-2 led to Treg expansion and activation in all patients without impacting Teffs, and there were no serious drug-related adverse events. Clinical Global Impression and disease-specific scores also improved over the course of treatment, indicating potential clinical benefit (85). These results warrant further clinical investigation of LD-IL-2 versus standard treatments in various autoimmune conditions. The phase II DIABIL-2 trial is recruiting patients to assess LD-IL-2 in T1DM, and the LUPIL-2 phase II study in SLE was completed in 2019 with results eagerly awaited. However, determining the most appropriate dose of IL-2 to optimize clinical efficacy in autoimmune conditions remains a challenge, as its short half-life means that an inconvenient dosing schedule is required (1 million IU for 5 days, then every week or every 2 weeks for up to 12 months). Since native IL-2 also stimulates Teffs in a dose-dependent manner, IL-2 conjugates and fusion proteins and IL-2/antibody complexes are being explored to more selectively expand Tregs with minimal impact on Teffs (86).

IL-2 conjugates and fusion proteins. An alternative approach to stimulating IL-2-mediated effects has been the generation of IL-2 variants in which the protein is altered by changing one or more key amino acids or by conjugation or fusion to proteins or other macromolecules to create a molecule with novel properties. Early work focused on IL-2 muteins with attenuated binding to IL-2Rβ to mitigate IL-2-induced toxicity. Although these agents did not demonstrate decreased toxicity in clinical trials, they showed significant selectivity for high-affinity IL-2Rs and Tregs (86, 87), which prompted further refinement of this approach.

NKTR-358 is a PEGylated IL-2 molecule that selectively induces the proliferation and activation of Tregs. PEGylation attenuates the affinity of NKTR-358 for IL-2Rβ relative to recombinant IL-2, rendering Tregs with high-affinity receptors more sensitive to NKTR-358 than Teffs (88). In a BALB/c mouse model, a single injection of NKTR-358 stimulated a greater magnitude and duration of Treg mobilization than five daily administrations of recombinant IL-2 and suppressed antigen-driven inflammation. Efficacy was also observed in an SLE mouse model (88). Initial results from a first-in-human study showed that NKTR-358 is safe and well tolerated and

that it elicits substantial dose-dependent expansion of Tregs with no measurable changes in $CD4^+$ and $CD8^+$ T cells (89). These results have been extended in a recent phase I study in patients with SLE, in which NKTR-358 increased the mean peak Treg:CD8 ratio 12-fold in the 24 μg/kg group (90). A phase II study of NKTR-358 in SLE has recently been initiated, with other phase I studies in psoriasis and atopic dermatitis ongoing.

THOR-809 is a covalently bound mono-PEGylated IL-2 variant with a PEG molecule attached to an unnatural amino acid, introduced at a site on IL-2 where affinity at the βγ chain of the IL-2 receptor is reduced, and is designed to increase half-life and enhance selectivity for IL-2Rα$\beta\gamma_c$ (91). In C57BL/6 mice and cynomolgus monkeys, a single subcutaneous dose of THOR-809 conferred the expansion and activation of Tregs, but not Teffs, and increased markers of Treg differentiation and function (91). Further studies are awaited.

Efavaleukin alfa (AMG592) is another IL-2 fusion protein being pursued in the clinic. This is a variant form of IL-2 fused to a human Fc molecule, which increases its stability and half-life (92). In a first-in-human trial, dose-dependent expansion of Tregs (a four- to fivefold increase) peaked at day 8 and was elevated above baseline until day 29. The phenotype of expanded Tregs included elevated IL-2Rα and Foxp3, as well as higher proportions of PD-1 (93). Phase I/II trials are ongoing in SLE and GVHD.

RG-7835 (RO7049665) is a conjugate of the IL-2 mutein N88D, which has reduced binding to IL-2Rβγ, fused with human IgG1. In cynomolgus monkeys, RG-7835 elicited a 10–14-fold increase in $CD4^+$ and $CD8^+$ $Foxp3^+$ Tregs with no effect on $CD4^+$ or $CD8^+$ memory T cells (94). A phase I trial has been completed in healthy subjects, and a phase Ib trial in ulcerative colitis is under way.

CC-92252 (DEL-106) is an IL-2 mutein Fc fusion protein. A phase I randomized study of CC-92252 in healthy adult subjects and adults with psoriasis is currently recruiting.

IL-2/mAb complexes. The IL-2/JES6–1 complex is an anti–mouse IL-2 mAb which has provided preclinical proof-of-concept evidence that certain IL-2/IL-2R mAb complexes increase the number of IL-R2α-expressing $Foxp3^+$ Tregs in mice without significantly affecting $CD8^+$ T cells (29). Several preclinical studies have shown promising results for IL-2/JES6–1 in the treatment of autoimmune diseases, including T1DM and GVHD (95, 96).

The anti-rhIL-2 mAb F5111.2 complexed with IL-2 has recently demonstrated similar findings in animal models of T1DM, autoimmune encephalomyelitis, and GVHD. A substantial increase of Foxp3, CD25, and p-STAT5 signals occurred without an effect on Teffs (97). Further studies are warranted to translate the application of IL-2/F5111.2 for the treatment of autoimmune diseases in the clinic.

FUTURE DIRECTIONS

After many years of limited use of IL-2 for the treatment of metastatic melanoma and RCC, multiple re-engineered IL-2 molecules are currently in clinical development, and many more are moving through preclinical studies toward clinical testing. In oncology, the focus is on improved PK, reduced toxicity and frequency of administration, reduced induction of Tregs, and increased activation of Teffs and NK cells for strong therapeutic efficacy, especially in combination with CPIs. Future work will focus on these important questions: What is the impact of partial versus complete loss of IL-2Rα binding on antitumor efficacy versus the risk of increased autoimmunity? Does tumor targeting of IL-2 increase its tolerability while maintaining its efficacy? What are the impacts of IL-2 modifications on the induction of antidrug antibodies, and do those impair therapeutic efficacy? Can engineered IL-2-based regimens induce meaningful clinical activity in

cancers beyond those that are responsive to current forms of immunotherapy, such as melanoma, RCC, and UC? Is engineered IL-2 critical for the success of ACT in solid tumors? What are the best combinations of engineered IL-2 with other immunologic or conventional modalities? And what pretreatment biomarkers can predict outcome in patients receiving these regimens?

In autoimmune disease, the use of low-dose native IL-2 to induce Tregs is continuing while new, engineered IL-2 molecules are emerging. Development of the new molecules seeks to improve ease of administration, tolerability, and the differential induction of Tregs without activation of autoreactive Teffs. Future work will focus on convenient dosing and scheduling, preventing induction of antidrug antibodies in settings of chronic administration, the theoretical long-term risk of infection or malignancy, combinations with other immunosuppressive agents, and expansion into other autoimmune and inflammatory conditions.

CONCLUSION

IL-2-targeted therapy has been rejuvenated by the development of re-engineered agents that promote or block the actions of IL-2 with improved efficacy and tolerability profiles versus historical use of the unmodified cytokine. Achieving the optimal balance between activating Teffs and activating Tregs has been an ongoing challenge for IL-2-targeted therapies in cancer and autoimmune diseases, but new strategies directed toward different IL-2R conformations are showing promising results in the clinic. With results from ongoing trials anticipated to establish the role of newly engineered agents in the treatment of cancer and autoimmune diseases, as well as their potential in other indications where the modulation of lymphocytes could have clinical benefit, an exciting future lies ahead for IL-2 therapeutics.

SUMMARY POINTS

1. IL-2 is a cytokine that regulates the balance between immunostimulation and immunosuppression to preserve immune homeostasis.
2. IL-2 stimulates immunity by inducing the proliferation and activity of effector and memory $CD4^+$ and $CD8^+$ T cells as well as NK cells, but it also suppresses immunity by promoting the proliferation and activity of $CD4^+Foxp3^+$ Tregs.
3. The downstream effects of IL-2 are mediated by the different conformations of the IL-2 receptor. These include the intermediate-affinity heterodimer IL-2Rβγc, mostly expressed by $CD4^+$ and $CD8^+$ T cells and by NK cells, and the high-affinity heterotrimer IL-2Rαβγc, mostly expressed by Tregs and type-2 innate lymphoid cells and transiently expressed by activated T cells.
4. The variable structures of the IL-2 receptor make them attractive targets for the treatment of cancer via the stimulation of Teffs and the treatment of autoimmune diseases via the suppressive action of Tregs.
5. Historical approaches have centered on rhIL-2, which has demonstrated activity in cancer and autoimmune disease, but were limited by either toxicity or inconvenient dosing regimens.
6. Re-engineering IL-2 to preferentially bind to different IL-2 receptor conformations has generated novel IL-2 formulations, including PEGylated IL-2 agonists, IL-2/mAb

complexes, and IL-2 fusion proteins, that selectively enhance different immune cell populations and have demonstrated promising activity in preclinical and clinical studies.

7. Ongoing studies aim to establish the role of engineered IL-2 therapeutics in the treatment of cancer and autoimmune diseases, as well as other diseases where the modulation of lymphocyte populations could have clinical benefit.

DISCLOSURE STATEMENT

The authors are employees of Nektar Therapeutics and receive salary- and equity-based remuneration. Nektar Therapeutics is developing bempegaldesleukin (NKTR-214), NKTR-358, and NKTR-262, either alone or in collaboration with third parties.

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