Immune Netw. 2022 Feb;22(1):e5
https://doi.org/10.4110/in.2022.22.e5
pISSN 1598-2629·eISSN 2092-6685

IMMUNE NETWORK

Review Article

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Advancements of Common Gamma-Chain Family Cytokines in Cancer Immunotherapy

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OPEN ACCESS

Received: Jan 3, 2022
Revised: Feb 15, 2022
Accepted: Feb 15, 2022
Published online: Feb 22, 2022

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Conflict of Interest
The authors declare no potential conflicts of interest.

ABSTRACT

The approval of immunotherapies such as checkpoint inhibitors (CPIs), adoptive cell therapies and cancer vaccines has revolutionized the way cancer treatment is approached. While immunotherapies have improved clinical outcome in a variety of tumor types, some cancers have proven harder to combat using single agents, underscoring the need for multi-targeted immunotherapy approaches. Efficacy of CPIs and cancer vaccines requires patients to have a competent immune system with adequate cell numbers while the efficacy of adoptive cellular therapy is limited by the expansion and persistence of cells after infusion. A promising strategy to overcome these challenges is combination treatment with common gamma-chain cytokines. Gamma-chain cytokines play a critical role in the survival, proliferation, differentiation and function of multiple immune cell types, including CD8 T-cells and NK cells, which are at the center of the anti-tumor response. While the short half-life of recombinant cytokines initially limited their application in the clinic, advancements in protein engineering have led to the development of several next-generation drug candidates with dramatically increased half-life and bioactivity. When combining these cytokines with other immunotherapies, strong evidence of synergy has been observed in preclinical and clinical cancer settings. This promising data has led to the initiation of 70 ongoing clinical trials including IL-2, IL-7, IL-15 and IL-21. This review summarizes the recent advancements of common gamma-chain cytokines and their potential as a cancer immunotherapy.

Keywords: Cancer; Immunotherapy; Cytokines; IL-2; IL-7

INTRODUCTION

Ipilimumab (anti-CTLA-4) was the first checkpoint inhibitor (CPI) approved for the treatment of melanoma in 2011. In the years since, we have seen an unprecedented shift in how cancer treatment is approached. Checkpoint blockade, adoptive cell therapy (ACT) and cancer vaccines represent some of the major immunotherapy strategies being implemented and investigated for both hematological and solid tumors at various stages of disease.

While these immunotherapies have improved clinical outcomes for some cancer types, they fail in others, highlighting unique challenges for each strategy. CPIs largely rely on

Abbreviations

ACT, adoptive cell therapy; AE, adverse event; aka, also known as; ALC, absolute lymphocyte counts; CPI, checkpoint inhibitor; CR, complete response; CTL, cytotoxic lymphocyte; CTX, cyclophosphamide; DC, dendritic cells; DLT, dose-limiting toxicity; EDB, extra-domain B; FAP, fibroblast activation protein; HD, high-dose; i.v., intravenously; IC, immunocytokine; mM, metastatic melanoma; mRCC, metastatic renal cell carcinoma; MTD, maximum tolerated dose; ORR, overall response rate; OS, overall survival; PEG, polyethylene glycol; PFS, progression-free survival; PK, pharmacokinetics; PR, partial response; Q2W, every 2 weeks; s.c., subcutaneous; SD, stable disease; sip-T, sipuleucel-T; TIL, tumor infiltrating lymphocyte; TME, tumor microenvironment; Tscm, stem-cell memory T-cells; UC, urothelial cancer; VLS, vascular leak syndrome.

Author Contributions

Conceptualization: Wolfarth AA, Dhar S, Ferrando-Martínez S; First Author: Wolfarth AA; Supervision: Lee BH; Validation: Wolfarth AA; Writing - original draft: Wolfarth AA, Dhar S, Goon JB, Ezeanya U, Ferrando-Martínez S; Writing - review & editing: Wolfarth AA, Lee BH.

the presence of CPI-responsive immune cells to be effective, with low clinical efficacy in immunologically 'cold' tumors (1). ACT infuses educated or engineered immune cells into the patient, but the survival and persistence of these adoptively transferred cells is a major limitation (2). Cancer vaccines heavily rely on antigen immunogenicity and memory formation, with low efficacy in immunosuppressive tumor types (3). To overcome these limitations, the combination of these immunotherapies with members of the common gamma-chain family of cytokines represents a promising strategy. Common gamma-chain cytokines, whose biology and immunological effects have been thoroughly reviewed elsewhere (4,5), have strong effects on the survival, proliferation, differentiation and function of lymphocyte subsets capable of mounting an anti-tumor response (6). With current immunotherapies being limited by the number and function of lymphocytes upon treatment, common gamma-chain cytokines can act synergistically to improve clinical efficacies. While their early clinical development was limited due to rapid clearance, many strategies have since been developed to increase their half-life, making their application more practical and reliable. This review will highlight the recent advancements of gamma-chain cytokines being considered as drug candidates and their application as either a monotherapy or in combination with other leading immunotherapies for the treatment of cancer.

IL-2

IL-2 was the first common gamma-chain family cytokine to be discovered as a T-cell growth factor and later understood to function as an inducer of cytotoxic T-cells, NK cells and Treg cells (6). It is mainly produced by activated CD4 T-cells and its receptor is a heterotrimeric complex consisting of IL-2Rα, IL-2Rβ and IL-2Rγ. The IL-2Rβ/γ receptor is expressed on naïve CD8 and memory CD4 and CD8 T-cells. While all 3 subunits of the IL-2 receptor (IL-2Rα/β/γ) are found on activated CD4 and CD8 T-cells (7), high expression of IL-2Rα is a defining characteristic of Tregs (8). The presence of IL-2Rα significantly increases the binding affinity of IL-2 for its receptor. Consequently, low levels of IL-2 are likely to induce an anti-inflammatory response by expanding Tregs. In addition, an immunostimulatory effect requires high-dose (HD) IL-2 and is associated with serious toxicities. Next-generation IL-2 molecules are being developed to harness the immunostimulatory effect of IL-2 while avoiding Treg stimulation and toxicity.

IL-2 as a monotherapy

Proleukin® (Aldesleukin; Chiron, now Clinigen; also known as [aka] HD IL-2) was the first cytokine approved by the Food and Drug Administration (FDA, Silver Spring, MD, USA) in 1992 for metastatic renal cell carcinoma (mRCC) (9) and later (1998) for metastatic melanoma (mM) (10). Longitudinal follow-up studies established that approximately 7% mRCC (17/255) and 6% mM (17/270) patients had complete response (CR) without any further systemic therapy (10,11). However, the required repeated dosing due to short half-life (13–85 min), the black box warning for life-threatening toxicities such as vascular leak syndrome (VLS) and multiple contraindications (12) have spurred the development of next-generation IL-2-based cytokine therapies.

Next-generation IL-2 candidates have been developed to bypass IL-2Rα binding, a strategy that lowers the risk of toxicity by lowering the therapeutic dose. THOR-707 (SAR444245, Sanofi, Paris, France) is a recombinant IL-2 variant with a polyethylene glycol (PEG) moiety irreversibly bound to a novel amino acid via click chemistry (13), restraining the binding of the α chain while retaining native binding affinity of the βγ receptor (14). Functionally,

THOR-707 elicits NK and CD8 T-cell-mediated anti-tumor activity while limiting proliferation of Tregs and other lymphoid cells that trigger life-threatening complications (15). In an open-label, multicenter phase I/II study (NCT04009681), THOR-707 was administered intravenously (i.v.) as a monotherapy every 2 wk (Q2W). Interim results reported from 28 patients with advanced or metastatic solid tumors showed no dose-limiting toxicity (DLT) or VLS. CD8 T-cells and NK cells increased by a median of 3.1-fold and 7.93-fold after cycle 1, respectively, and were sustained until the next cycle. Despite the modifications, there was a 1.89-fold increase in Tregs. Three patients had confirmed partial response (PR) while 2 had stable disease (SD) and continued for >5 cycles. THOR-707 half-life was about 10 h and there were no reported anti-drug antibodies (against IL-2 or PEG). Overall, these results demonstrate the safety and tolerability of an IL-2 variant that has bias for the IL-2Rβ/γ, leading to successful induction of CD8 T-cells and NK cells while avoiding significant Treg stimulation (16).

NKTR-214 (Bempegaldesleukin, Nektar Therapeutics, San Francisco, CA, USA) is a prodrug consisting of IL-2 conjugated to 6 resealable PEG chains. NKTR-214 retains the amino acid sequence of IL-2 while masking the IL-2Rα chain to mitigate engagement and proliferation of Tregs. In preclinical models, NKTR-214's active conjugated IL-2 was 50-times higher when compared to aldesleukin, without evidence of VLS or pulmonary edema (17). Based on these promising results, a phase I/II trial was designed to study the safety, tolerability, pharmacokinetics (PK) and efficacy in patients with advanced or metastatic solid tumors (NCT02869295). NKTR-214 was administered i.v. at 0.003 to 0.012 mg/kg Q3W or 0.006 mg/kg Q2W until disease progression or unacceptable toxicities occurred (18). NKTR-214-related cytokine (NKTR-214-RC), which consists of various forms of IL-2 conjugated to PEG, remained detectable up to 8–11 days post administration while NKTR-214-active cytokine (NKTR-214-AC) concentrations increased gradually, with Tmax reaching between 24 and 48 h. Neither anti-NKTR-214 nor anti-IL-2 antibodies were detected. Pharmacodynamic analysis showed an increase in CD8 T-cell tumor infiltrating lymphocytes (TILs) within the tumor microenvironment (TME). These encouraging results have given way to combination therapies with CPIs, discussed below (**Table 1**).

ALKS 4230 (Nemvaleukin alfa, Alkermes Inc., Waltham, MA, USA) is a fusion protein of circularly-permuted IL-2 with IL-2Rα, designed to target cells expressing IL-2Rβ/γ and not IL-2Rα. In murine preclinical models the treatment was well tolerated with significantly less proinflammatory plasmatic cytokines compared to rhIL-2. After daily subcutaneous (s.c.) treatment for 5 days, there was a dose-dependent increase of NK and memory CD8 T-cells in the spleen without an increase of Tregs. ALKS 4230 also showed anti-tumor function, with i.v. and s.c. administration in a B16F10 melanoma lung model showing significantly fewer colonies in mice treated with ALKS 4230 compared to the vehicle control (19). In cynomolgus monkeys, 2 s.c. doses led to a 4-fold increase of NK cells, a 6-fold increase of CD8 T-cells and a 2-fold increase in Tregs, peaking between days 6–8 and returning to baseline by day 14 (20). Currently, ALKS 4230 monotherapy is being tested in a phase II trial in melanoma patients (NCT04830124) (**Table 1**).

Another strategy for next-generation IL-2 drug candidates is to target IL-2 to the tumor site. L19-IL2 (Darleukin, Philogen S.p.A, Siena, Italy) is a fully human antibody with the IL-2 moiety fused to the single-chain Fv antibody fragment targeting the alternatively spliced extra-domain B (EDB) of fibronectin. EDB preferentially accumulates around neo-vasculature structures and is usually excluded from healthy human tissues, except female reproductive

Table 1. Summary of ongoing clinical trials involving next-generation gamma-chain cytokines

Drug	Company	Cytokine	Phases	Indications	Immunotherapy combinations	Testing as monotherapy*	Trial identifiers
ALKS 4230	Alkermes, Inc.	IL-2	I, II, III	Advanced solid tumors	Anti-PD-1	Yes	NCT03861793; NCT04592653; NCT02799095; NCT04144517; NCT05092360; NCT04830124
FAP-IL2v	Hoffmann-La Roche	IL-2	I, II	Advanced solid tumors	Anti-PD-1, anti-PD-L1, CXCR4 antagonist, anti-CD40, adenosine receptor antagonist, anti-TIGIT, IL-6R inhibitor	No	NCT03386721; NCT03875079; NCT02627274; NCT03193190
GI-101	GI Innovation, Inc.	IL-2	I, II	Advanced solid tumors	Anti-PD-1	No	NCT04977453
hu14.18-IL2	Apeiron Biologics AG	IL-2	I, II	Melanoma	Anti-PD-1, anti-CTLA-4	No	NCT03958383
L19-IL2	Philogen S.p.A.	IL-2	II, III	Advanced solid tumors	L19-TNFα	Yes	NCT03705403; NCT03567889; NCT04362722
NKTR-214	Nektar Therapeutics	IL-2	I, II, III	Advanced solid tumors	Anti-PD-1, anti-CTLA-4, TLR7/8 agonist, DNA cancer vaccines, Flt3L, poly ICLC	No	NCT03785925; NCT04936841; NCT04730349; NCT03729245; NCT04410445; NCT02983045; NCT03138889; NCT03435640; NCT03548467; NCT03635983; NCT04209114; NCT04540705; NCT04969861; NCT03835533; NCT03282344
RO7284755	Hoffmann-La Roche	IL-2	I	Advanced solid tumors	Anti-PD-L1	No	NCT04303858
THOR-707	Sanofi	IL-2	I, II	Advanced solid tumors	Anti-PD-1	No	NCT05061420; NCT04914897; NCT04913220; NCT04009681; NCT05104567
NT-I7/GX-I7 (efineptakin alfa)	NeoImmuneTech, Inc./Genexine, Inc.	IL-7	I, II	Advanced solid tumors, lymphoma	Anti-PD-1, anti-PD-L1, CD19 CART cells	Yes	NCT04332653; NCT05075603; NCT03687957; NCT03901573; NCT04594811; NCT04984811; NCT04893018; NCT04588038; NCT02659800
N-803	ImmunityBio, Inc.	IL-15	I, II, III	Advanced solid tumors, acute myeloid leukemia	Anti-PD-1, anti-PD-L1, anti-CTLA-4, NK cell adoptive transfers, ab-drug conjugate, anti-PD-L1/anti-TGFβ, DNA vaccines, autologous cancer vaccine, antigen vaccines, anti-VEGF, anti-VEGFR-2, anti-EGFR, anti-CD274	Yes	NCT04290546; NCT03329248; NCT03563157; NCT03136406; NCT03228667; NCT03387111; NCT02138734; NCT03022825; NCT01898793; NCT05096663; NCT02465957; NCT02523469; NCT02890758; NCT03387111; NCT02989844; NCT04847466; NCT04247282; NCT03493945; NCT04927884; NCT04898543; NCT04390399; NCT03520686
NKTR-255	Nektar Therapeutics	IL-15	I, II	Myeloma, lymphoma, HNSCC	Anti-CD38, anti-CD20	No	NCT04136756; NCT04616196

HNSCC, head and neck squamous cell carcinoma.
*Whether or not the drug is being tested as the only immunotherapy in the trial.

tissues (21,22). In a first-in-human phase I/II clinical trial in advanced solid tumors, the maximum tolerated dose (MTD) was found to be 22.5 Mio IU and the half-life was 2–3 h, with manageable and reversible toxicities (23). Pharmacodynamic analysis showed an increase in CD8 T-cells and NK cells, while Tregs were markedly diminished. At the time of publication, 2 patients who had received 4 out of 6 total cycles of L19-IL2 remained progression-free for, approximately, 30 months. L19-IL2 is also currently being tested in phase II trials in combination with Rituximab (NCT02957019), radiation therapy (NCT02086721) and dacarbazine (NCT01055522), and in a phase III trial in combination with targeted TNF (NCT02938299). A recently developed novel format of L19-IL2, the L19L19-IL2 (antibody in a single-chain diabody), showed superior tumor-specific biodistribution, but it is still in preclinical development (24).

https://doi.org/10.4110/in.2022.22.e5

Hu14.18-IL2 (APN301, Apeiron Biologics AG, Wien, Austria) is a humanized immunocytokine (IC) comprised of human IL-2 linked to hu14.18 mAb, which recognizes the GD2 disialoganglioside expressed on melanoma, neuroblastoma, and certain sarcomas. The half-life of hu14.18-IL2 was reported to be 3.1 h (25) with daily dosing needed to maintain *in vivo* activity. Phase II trials with hu14.18-IL2 i.v. resulted in 1 PR in a melanoma patient (NCT00109863) (26) and 5 CRs in a pediatric oncology study of neuroblastoma (NCT00082758) (27). Most common DLTs included fever, chills, nausea, anaphylactoid reactions and neuropathic pain. Based on a superior clinical response in patients with low tumor burden (minimal residual disease) in the neuroblastoma cohort, a pilot phase II trial was conducted in 23 patients with completely resectable recurrent stage III or stage IV melanoma (NCT00590824). Out of 18 patients, 6 remained recurrence-free for a median of 5.6 months with a 24-month overall survival (OS) rate of 65% (28).

While initial studies testing aldesleukin revealed life-threatening toxicities, recent studies prove that next-generation IL-2 drug candidates have improved efficacy and are better tolerated. Accordingly, several trials are underway to test the safety and tolerability of these IL-2 drug candidates in combination with other immunotherapies.

IL-2 in combination with CPIs

Several trials investigating the combination of next-generation IL-2 drug candidates with CPIs are ongoing (**Table 1**). The PIVOT-10 study published preliminary results testing NKTR-214, the pro-IL-2 molecule that targets IL-2Rβ, in combination with nivolumab in patients with locally advanced or metastatic solid tumors. Results from this study showed that the combination of NKTR-214 and nivolumab was well tolerated and had encouraging response rates. Nivolumab monotherapy is approved for patients with urothelial cancer (UC) that have progressed following platinum-containing chemotherapy, after an overall response rate (ORR) of 19.6% observed in the CHECKMATE-275 trial (29). Of the 27 evaluable patients with UC, the combination treatment showed an investigator-assessed ORR of 48%, including 5 CR (30), regardless of baseline PD-L1 expression. These results highlight the potential of NKTR-214 to improve the ORR historically seen with nivolumab monotherapy and could represent a promising treatment option for cisplatin-ineligible UC patients.

A second next-generation IL-2 drug candidate tested in combination with a CPI is fibroblast activation protein (FAP)-IL2v (simlukafusp alfa, Hoffmann-La Roche, Basel, Swiss). This IL-2 variant has increased affinity for IL-2Rβγ and is conjugated to an antibody targeting FAPα. When combined with anti-muPD-L1 in a murine Panc02 pancreatic model, median survival was significantly longer when compared to either single agent therapy (31). Results from a phase II study combining FAP-IL2v with atezolizumab showed an ORR of 27% in patients with recurrent or metastatic cervical squamous cell carcinoma, with an acceptable safety profile (32).

RO72284755 (Hoffmann-La Roche) is a recombinant fusion protein with an antibody directed against PD-1 linked to IL-2v. This drug candidate is currently being tested in the clinic as a monotherapy or in combination with atezolizumab in patients with advanced and/or metastatic solid tumors (NCT04303858). GI-101 (GI Innovation, Inc., Seoul, Korea) is a bispecific CD80-IgG4-IL2 variant that targets CTLA-4-expressing cells with preferential binding for IL-2Rβ. Preclinical models showed significant proliferation of NK cells and CD8 T-cells and improved tumor control as a monotherapy and in combination with pembrolizumab (33). In a CT26 tumor model, the TME showed increased M1 macrophages and CD8 central memory cells, with no Treg cell proliferation (34). This data led to the

KEYNOTE-B59 trial involving patients with advanced solid tumors to determine the safety, tolerability and the recommended phase 2 dose (NCT04977453) (35).

IL-2 in combination with ACT

While infusion of aldesleukin is common practice after ACT to improve T-cell activity *in vivo*, DLTs associated with HD IL-2 limit its use (36). Most recent clinical trials combining ACT with IL-2 have involved patients with advanced melanoma and have sought to improve toxicities by lowering the dose, administering subcutaneously or by using a modified IL-2 protein. Several trials have reported a favorable toxicity for aldesleukin given after TIL infusion as s.c. low-dose, (37,38), s.c. regular dose (39) or i.v. low-dose (39), with pharmacodynamic analysis showing some indirect evidence of TIL engraftment (38). However, to this date, these trials have failed to prove that combination treatment provides a clinical improvement over TIL infusion alone.

Regarding next-generation IL-2 drug candidates, while preclinical data from NKTR-214 suggests potential efficacy (40), the development has not reached clinical stages.

IL-2 in combination with cancer vaccines

IL-2 in combination with various cancer vaccines has shown little promise in completed clinical trials. Multiple trials have seen the expansion of Tregs, even with low-dose IL-2. For melanoma patients receiving a vaccine of HLA-class I peptides and low-dose cyclophosphamide (CTX), the depletion of Tregs was transient after CTX treatment and returned to baseline levels by 8 wk after vaccination. At 13 and 15 wk, the patients received low-dose IL-2 with the goal of boosting vaccine-induced immune responses. Pharmacodynamic analysis 1 wk after IL-2 treatment showed a significant increase of Tregs without impairing the expansion and functionality of peptide-specific CD8 T-cells (41). However, this CD8 T-cell increase was ultimately not associated with an improvement of disease-free or OS compared to the observation group (42). In a different approach involving patients with solid tumors, IL-2 was administered with GM-CSF and a vaccine containing mutant ras peptides. Patients receiving IL-2 alone or in combination with GM-CSF had lower vaccine-induced immune responses compared to patients that received GM-CSF alone, with no difference in progression-free survival (PFS) or OS observed (43). Low-dose IL-2 has also been tested in combination with a therapeutic lysate-loaded dendritic cells (DC) vaccine in a small cohort of 10 patients with ovarian cancer. After initial surgery and chemotherapy, patients received the DC vaccine followed by 2 wk of daily IL-2 injections. Pharmacodynamics analysis 8 wk post-vaccination showed that 5/10 patients had significantly increased NK cell activity and 3/10 patients had increased IFN-γ-secreting T-cells. While combining IL-2 with DC vaccination was safe, the increase of specific immunity observed in this small cohort was not associated with clinical outcomes (44). More recent studies combining IL-2 with cancer vaccines have uncovered new combinations with encouraging results. Combining low-dose IL-2 with a whole-tumor lysate-pulsed DC vaccine, CTX, bevacizumab and acetylsalicylic acid showed encouraging responses in a small cohort of ovarian cancer patients, with a significant increase in IFN-γ-secreting T-cells and prolonged survival (45).

IL-2 concluding remarks

In summary, considerable efforts are underway to develop next-generation IL-2 drug candidates. These drug candidates have a longer half-life, are well tolerated and have the ability to increase cytotoxic T-cells without stimulating Tregs. Several clinical trials are underway to test these candidates as monotherapies or in combination with other immunotherapies for the treatment of cancer (**Table 1** and **Fig. 1**).

https://doi.org/10.4110/in.2022.22.e5

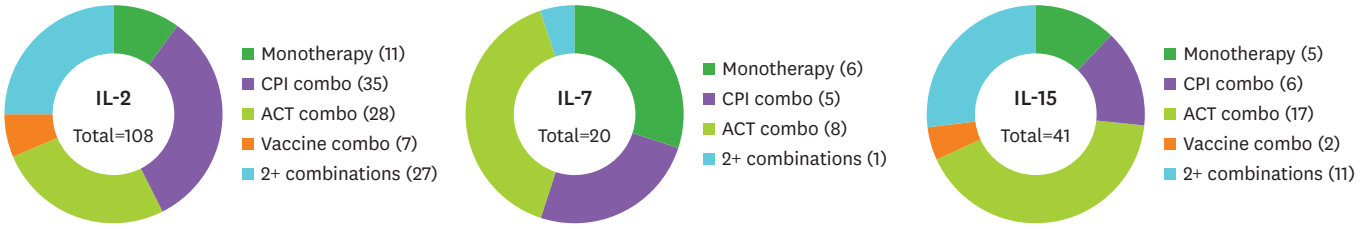

Figure 1. Combination strategies for gamma-chain cytokines in ongoing clinical trials. Trials involving IL-2, IL-7, IL-15 and IL-21 were identified on www.clinicaltrials.gov and organized by combination strategy. Only ongoing trials involving cancer patients were included. The number of trials for each strategy are indicated as (number). The 2+ combinations represents trials combining a gamma-chain cytokine with 2 or more immunotherapy strategies. Only one active trial was found for IL-21, which involves CART cells co-expressing IL-15 and IL-21 (NCT04715191).

IL-7

IL-7 is unique among the common gamma-chain family of cytokines because it is not produced by T-cells and, instead, is produced constitutively by non-immune stromal cells found in primary and secondary lymphoid structures. IL-7 is a non-redundant cytokine critical for the survival and differentiation of thymocytes and for the survival and proliferation of peripheral T-cells (46,47). IL-7 also has an important role in the formation of secondary and tertiary lymphoid structures (48). IL-7 has a heterodimeric receptor consisting of the IL-7Rα and the common gamma-chain (IL-2Rγ). In humans, IL-7R is expressed on all CD4 and CD8 T-cell subsets, with naïve and memory subsets having the highest expression (49). Importantly, Tregs have very low IL-7R expression, making IL-7 a straightforward candidate for cancer immunotherapy. IL-7 can boost naïve T-cells and support T-cell memory subsets important for the formation of durable anti-tumor immunity. In addition, the strong synergistic potential of IL-7 combined with current immunotherapies is being investigated in multiple ongoing clinical trials.

IL-7 as a monotherapy

There are currently 2 IL-7 drug candidates in the clinical stage: CYT107 (Cytheris, now RevImmune) and NT-I7 (efineptakin alfa, NeoImmuneTech, Inc., Rockville, MD, USA; aka GX-I7, Genexine, Inc., Seoul, Korea). In the first-in-human trial with CYT99 007 (unglycosylated version of CYT107), patients with refractory cancers were treated with doses ranging from 3–60 μg/kg Q1D for 14 days (NCT00062049). Frequent dosing was required because this unglycosylated form of IL-7 had a short half-life of 6-10 h (50). Pharmacodynamic data showed significant age-independent increases in circulating CD4 and CD8 T-cells, increased T-cell receptor diversity, decreased Treg frequency, and the dose was well tolerated. Following this, a randomized placebo-controlled phase IIa trial (ELYPSE, NCT01368107) was designed to study the effect of CYT107, a glycosylated rhIL-7 with a half-life of 8.7–34.6 h, in restoring CD4 lymphopenia in metastatic breast cancer patients before or during chemotherapy (51). Patients received 10 μg/kg Q1W of CYT107 or placebo for 3 wk. While there was no difference in PFS or OS between the placebo and the drug treatment cohorts (52), the treatment was safe and well tolerated and biomarker analysis revealed a significant increase of naive and memory CD4 and CD8 T-cell subsets compared to placebo, with a 2-fold increase of CD8 T-cells. Although there was a transient increase of Tregs at day 21, this coincided with a significant increase in all CD4 T-cells. Importantly, total CD4 T-cells remained higher through day 57, while Treg levels did not.

NT-I7 is the only next-generation long-acting IL-7 drug candidate in the clinical stage. NT-I7 is a recombinant human IL-7-hyFc fusion protein consisting of the homodimeric human IL-7

built on the 'hyFc' (hybrid Fc) platform (Genexine, Inc.), where the Fc fusion fragment is composed of a hybridizing IgD/IgG4 immunoglobulin domain. This structural modification significantly increases stability and potency, generating a long-acting molecule that overcomes the major limitations encountered with early forms of unmodified recombinant gamma-chain cytokines. In a trial of 30 healthy individuals (NCT02860715), GX-I7 (aka NT-I7 in the Americas and Europe) was administered intramuscularly in doses up to 60 μg/kg. GX-I7 was well tolerated and showed an impressive half-life of 63.26 h. GX-I7 treatment significantly increased absolute lymphocyte counts (ALC) that peaked by day 21 (53). The administration of GX-I7 led to a dose-dependent increase in NK cells and all analyzed CD4 and CD8 T-cell subsets, but there was no dose-dependent increase in B-cells. Based on preclinical studies that demonstrated the ability of NT-I7 to significantly reduce tumor growth and increase CD8 TILs in murine solid tumor models (54), a clinical trial testing GX-I7 in patients with solid tumors was established to determine safety and tolerability (NCT03478995). GX-I7 was safely administered at dose levels up to 1,200 μg/kg with a notable half-life up to 147 h (55). The most common treatment-related adverse events (AEs) were injection site reactions, which were mild to moderate and resolved without intervention. Interim data showed an increase of ALC in both lymphopenic and non-lymphopenic patients, with a 3–4-fold increase of CD4 and CD8 T-cells.

NT-I7 preclinical data also showed the potential of combining NT-I7 with standard of care for high-grade gliomas (56). Based on these encouraging findings, NT-I7 is being tested as a monotherapy in patients with high-grade gliomas after concurrent radiation and temozolomide (NCT03687957; NCT02659800). Interim data has proven that NT-I7 is able to significantly boost ALC even in the presence of adjuvant chemotherapy. Interestingly, biomarker analysis showed that NT-I7 leads to the preferential increase of CD8 stem-cell memory T-cells (Tscm), a self-renewing subset with better anti-tumor activity compared to other memory CD8 T-cell subsets (57). In addition to testing NT-I7 monotherapy with standard of care in high-grade glioma patients, NT-I7 monotherapy is being tested in patients with locally recurrent squamous cell carcinoma of head and neck undergoing salvage surgery (NCT04588038) and Kaposi's sarcoma with or without HIV (NCT04893018).

IL-7 in combination with CPIs

IL-7 reliably boosts T-cell numbers and therefore has great potential to synergize with CPIs to improve efficacy. Two combination trials involving CYT107 are in early stages and results are not available yet, but this synergy has already been highlighted by interim results from 2 ongoing clinical trials combining NT-I7 with pembrolizumab in patients with advanced solid tumors. In a trial testing GX-I7 in combination with pembrolizumab (NCT03752723) in patients with metastatic triple negative breast cancer who failed standard chemotherapy, GX-I7 was given Q12W and pembrolizumab Q3W. The treatment was well tolerated, with up to a 7-fold increase in peripheral ALC at all dose levels tested, regardless of concurrent CTX. Clinical outcome data is encouraging, with 4 out of 6 patients achieving SD at the 1,200 μg/kg dose level (58). A second trial testing NT-I7 1,200 μg/kg Q6W in combination with pembrolizumab in patients with advanced solid tumors (NCT04332653) has also published interim results for 2 arms of the study involving advanced recurrent/refractory pancreatic cancer and MSS-colorectal cancer. Preliminary data shows that NT-I7 significantly boosts peripheral T-cell counts, with a significant increase in the CD8:Treg ratio. Among the CD8 T-cell subsets, the most substantial fold-change increase occurred in the self-renewing Tscm subset, which increased 50-fold in peripheral blood compared to baseline after a single NT-I7 dose. Preliminary clinical outcome data from the ongoing trial is encouraging, with 4 PR

(out of 34 evaluable patients) achieved at the time of analysis in a difficult to treat patient population (59,60). Following this encouraging data, NeoImmuneTech, Inc. has initiated clinical trials to test NT-I7 in combination with nivolumab (NCT04594811) and atezolizumab (NCT03901573 and NCT04984811). It is worth noting that, due to the significant increase in the next-generation cytokine half-life, all trials involving NT-I7 (and GX-I7) have a dosing schedule between Q3W and Q12W while increasing clinical efficacy.

IL-7 in combination with ACT

While combination of IL-7 with ACT is usually in the context of *in vitro* stimulation of CART cells prior to infusion (61), there has been increasing interest in adding IL-7 expression to the CART design. This strategy has rendered encouraging results in both preclinical (62-65) and clinical settings (NCT03198546). In this clinical study tumors were determined to express either GPC3 or MSLN and subsequently treated with anti-GPC3 or anti-MSLN-7×19-CART cells. No grade 2–4 AEs occurred, and clinical outcomes included 1 CR, 1 PR and 2 SD out of 6 patients. Clinical trials testing the coexpression of IL-7 and CCL19 in CART cells are also underway (NCT03929107, NCT04381741).

Preclinical studies combining ACT with IL-7 administration have also produced encouraging data. In mice inoculated with a B-cell lymphoma cell line, rhIL-7 significantly increased the expansion and persistence of antigen-specific CD4 T-cells and significantly prolonged mouse survival, preventing the relapse typically seen with ACT alone in this model (66). In addition, preclinical data using next-generation long-acting IL-7 has shown that NT-I7 enhances CART expansion, persistence and anti-tumor activity *in vivo* (67). After these encouraging results, NeoImmuneTech, Inc. has started a Phase 1b trial (NCT05075603) to evaluate the safety, tolerability and efficacy of NT-I7 post-Kymriah® in subjects with relapsed/refractory large B-cell lymphoma (**Table 1**).

IL-7 in combination with cancer vaccines

While preclinical data supports the combination of IL-7 with cancer vaccines (68,69), clinical data is still scarce. CYT107 has been combined with a tumor lysate-pulsed DC vaccine in pediatric patients with metastatic and recurrent sarcoma. Following standard therapy, patients received the DC vaccination and an autologous lymphocyte infusion, with or without administration of CYT107 Q2W. While standard of care includes alkylators that induce prolonged lymphopenia, patients receiving CYT107 had significantly increased lymphocyte recovery with increased CD4 and CD8 T-cells at 6-wk post treatment. Notably, CYT107 led to decreased Treg frequencies compared to baseline. While CYT107 increased the number of circulating lymphocytes, patients treated with CYT107 did not have a significantly different OS compared to patients in the control arm (70). Additionally, CYT107 has been tested in combination with sipuleucel-T (sip-T), an autologous cellular vaccine that is the only FDA-approved immunotherapy for castration-resistant metastatic prostate cancer. After administration of sip-T, patients either received CYT107 weekly for a total of 4 doses or were assigned to an observation group. The treatment was well tolerated and led to a significant increase of peripheral T-cells and NK cells, with increased intracellular pro-inflammatory cytokines. Encouraging data showed that CYT107 treatment increased the PSA doubling time compared to the observation group. Due to production issues of CYT107, patient enrollment was ended prematurely, and the small sample size did not allow for proper assessment of the clinical efficacy of sip-T in combination with IL-7 (71).

https://doi.org/10.4110/in.2022.22.e5

IL-7 concluding remarks

In summary, preclinical studies testing IL-7 as a monotherapy or in combination with CPIs, ACT and cancer vaccines have yielded consistently positive results. The ability to increase T-cell numbers safely and significantly in the blood, lymphoid tissues and tumor, together with a safe and well tolerated profile, makes IL-7 a particularly attractive cytokine for cancer immunotherapy. When unmodified, the clinical efficacy of IL-7 remained uncertain, likely due to the short half-life. By significantly increasing the half-life of IL-7, NT-I7 (aka GX-I7) has allowed for more infrequent dosing (up to 12 wk) with consistent bioactivity, generating encouraging preliminary clinical efficacy in a range of tumor types and in combination with a CPI (**Table 1** and **Fig. 1**).

IL-15

IL-15 is important for NK, NKT and CD8 memory T-cell function and homeostasis (72). It is expressed as a heterodimer with IL-15Rα, mainly by monocytes, macrophages and DCs which then present it to neighboring cells that express IL-15Rβ/γ (73). While usually presented in trans, the IL-15Rα can also be cleaved to form a soluble heterodimer superagonist (hetIL-15) with increased stability and bioactivity compared to the IL-15 monomer (74). The IL-15Rβ/γ is expressed on NK cells and CD8 T-cells and receptor binding with IL-15/IL-15Rα has been shown to increase the proliferation and cytotoxic function of these cells (72,73). IL-15 is often compared to IL-2 as they share the same IL-2/15Rβ and gamma-chain receptor complex and both expand activated T-cells and NK cells. But unlike IL-2, IL-15 is dispensable for Treg generation and is more important for the maintenance of CD8 memory T-cells (73). However, it should be noted that trans-presentation of the IL-15/IL-15Rα complex, but not IL-15 alone, can influence CD4 T-cell function and differentiation (75). Given its ability to expand and enhance the function of effector lymphocytes important for the anti-tumor response, IL-15 is actively being investigated for cancer immunotherapy, with 2 next-generation IL-15 candidates being tested in the clinic (**Table 1**).

IL-15 as a monotherapy

A first-in-human phase I study was designed to investigate the safety and tolerability of recombinant human IL-15 (rhIL-15, National Cancer Institute, Bethesda, MD, USA) in patients with malignant melanoma or metastatic renal cell cancer (NCT01021059). rhIL-15 was administered i.v. at 0.3–3 μg/kg per day for 12 consecutive days. Correlative analysis showed a substantial increase in NK and γδ T-cells, followed by CD8 memory T-cells, with limited expansion of Tregs (76). The average half-life across the doses was approximately 2.5 h with a non-linear Cmax suggesting clearance by the high-affinity IL-15 receptor and, possibly, IL-15R-independent mechanisms, and an MTD of 0.3 μg/kg per day. The most common AEs were fevers, rigors, and hypotension related to the marked elevation in IFNα, IL-6, IL-8, and IL-2Rα associated with i.v. administration, and clinically, there were no responses. To circumvent these limitations, daily s.c. rhIL-15 treatment was investigated in patients with solid tumors (NCT01727076). The regimen was well tolerated and achieved robust NK cell expansion while there was less effect on CD8 T-cells (77). Out of 18 evaluable patients, 7 patients had SD while 11 patients stopped treatment because of disease progression. Although this route of administration was better tolerated, PK analysis showed that the plasmatic concentration of rhIL-15 significantly decreased after 24 h with daily injections required.

https://doi.org/10.4110/in.2022.22.e5

In addition to a short half-life, native rhIL-15 has limited immunomodulatory effects because it is usually trans-presented to CD8 T- and NK cells in a complex with the IL-15Rα. N-803 (ALT-803, ImmunityBio, Inc., Culver City, CA, USA) contains 2 molecules of an optimized amino acid–substituted (N72D) IL-15 "superagonist" and 2 molecules of the IL-15α receptor "sushi" domain fused to a dimeric human IgG1 Fc (IL-15N72D:IL-15RαSu/IgG1 Fc complex), with a half-life of 29.3 h (78,79). Following pre-clinical studies using N-803 in murine models of solid tumors (80) that showed immunomodulatory and anti-tumor activity, a first-in-human trial was initiated to test the safety, tolerability, PK and immunologic effects in a dose escalating schedule via i.v. and s.c. administration (NCT01946789). Subcutaneous weekly administration of N-803 at 20 μg/kg showed low Cmax values between 8–24 h, robust NK but moderate CD8 T-cell expansion, no indication of neutralizing antibodies and was well tolerated in patients (81). These encouraging findings have bolstered some of the current trials of N-803 in combination with CPIs, adoptive NK and T-cell therapies, and antitumor antibodies among others (**Table 1**). Nevertheless, recent findings suggest that engagement of the IL-2Rβ expressing lymphocyte subsets by the precomplexed rhIL-15/IL-15Rα molecules may lead to diminished biological responses after repeated dosing (82).

NKTR-255 (Nektar Therapeutics) is a PEG-conjugated rhIL-15 that retains the same receptor binding affinity of the native IL-15 molecule. Preclinical studies in cynomolgus monkeys testing a single dose of NKTR-255 at 0.1 mg/kg showed a half-life of 30.5 h, with significant expansion of both NK and effector CD8 T-cells with equal efficacy. Interestingly, no expansion of CD4 T- or B-cells was observed (83). NKTR-255 is currently being investigated in a phase I open-label study for patients with relapsed or refractory multiple myeloma and non-Hodgkin's lymphoma, either alone or in combination with Rituximab or Daratumumab (NCT04136756).

IL-15 in combination with CPIs

A clinical trial is currently underway to test the combination of nivolumab with N-803 for the treatment of non-small cell lung cancer. Data from the phase 1b trial demonstrated the combination is safe and well tolerated, with no reported DLTs. Two out of 21 participants experienced grade 3 lymphocytopenia that spontaneously resolved. Pharmacodynamic analysis showed a 7-fold increase of NK cells 7 days after starting treatment. Post-hoc analysis of clinical responses showed 29% (6/21) of patients had an objective response. Notably, 10/11 patients that progressed after previous anti-PD-1 therapy achieved disease control (84). Results from the phase Ib trial demonstrate combination of N-803 with nivolumab is safe and tolerable, with the phase II study currently underway (NCT02523469).

Based on this success, ImmunityBio, Inc. is developing N-809, a first-in-class molecule where N-803 is fused to 2 anti-PD-L1 domains. N-809 showed superior tumor control in murine models and surviving mice were protected from rechallenge (85). Other promising next-generation molecules, like the IL-15 superagonist receptor linker IL-15 (RLI), also showed increased survival and tumor control in murine models of cancer (86). However, while preclinical studies continue to highlight the potential of this combination, these molecules have not yet reached the clinical stage.

IL-15 in combination with ACT

IL-15, like IL-7, has been mostly tested for the preconditioning of cells before adoptive transfer. While results have not been very promising for NK cell therapy (87,88), preclinical data for the preconditioning of CART cells with IL-15 has been more positive. In a murine cancer model it was shown that IL-7/IL-15-generated CART cells showed increased tumor

control and significantly prolonged survival when combined with anti-PD-1 therapy compared to IL-2/IL-15-generated CART cells (89). Similar to IL-7 studies, engineering cells to express IL-15 *in vivo* is also being tested. In a study involving patients with CD19-positive cancers, NK cells engineered to co-express CD19-targeting CAR and IL-15 were infused after lymphodepleting chemotherapy. The therapy was well tolerated with no cytokine release syndrome, neurotoxicity or GvHD developed after infusion of the CAR-NK cells. Clinical efficacy data from the 11 patients treated was encouraging, with 7 patients in complete remission and responses seen within 30 days of treatment (90). IL-15 expression has also been added to NKT cells expressing a GD2-targeting CAR. Preliminary data from a study involving pediatric patients with relapsed or resistant neuroblastoma showed infusion of the autologous NKT cells was well tolerated with no DLTs. Although the reported data was only for 3 patients, pharmacodynamic analysis showed elevated NKT cells compared to baseline and one patient experienced an objective response with lesion regression (91). Although study sizes are small, this clinical data supports further investigation of IL-15 expressing adoptively transferred cells for cancer immunotherapy.

Multiple preclinical studies testing the combination of IL-15 with ACT have been conducted by ImmunityBio, Inc. They have demonstrated the ability of N-803 to enhance the anti-tumor function of PD-L1 t-haNK cells, a novel NK cell line engineered to express CD16 and a PD-L1-targeting CAR (92). N-820, a fusion protein of N-803 and 4 single-chains of rituximab, when combined with ex vivo expanded NK cells significantly increased the anti-tumor response against CD20+ Burkitt lymphoma (93). Multiple clinical trials testing N-803 in combination with several immunotherapies, including t-haNK cell infusion, are ongoing (**Table 1**).

IL-15 in combination with cancer vaccines

Results from a phase Ib trial combining N-803 with BCG vaccination in bladder cancer were recently published (94). The combination was well tolerated for all doses tested, with MTD not reached and no treatment-related AEs at or exceeding grade 3. Although this single-arm study was small, with the enrollment of only 9 patients, no patients experienced disease progression 6 years after treatment. Pharmacodynamic analysis showed elevated IL-6 in urine and serum after the combination therapy, but this did not correlate with dose levels of N-803. Two trials combining N-803 with BCG vaccination are ongoing, testing in both BCG-naïve and BCG-unresponsive bladder cancer patients (NCT02138734; NCT03022825). Additional strategies, still in preclinical development, include an autologous cell-based cancer vaccine secreting IL-15 in complex with IL-15Rα. Mice receiving inactivated cancer cells secreting IL-15:IL-15Rα had significant tumor control in both prophylactic and therapeutic cancer models (95), highlighting the promising potential of IL-15 to improve the anti-tumor response elicited by cancer vaccines.

IL-15 concluding remarks

Next-generation IL-15 drug candidates have produced promising results in both clinical and preclinical settings. Complexing IL-15 with the IL-15Rα significantly extends the half-life and promotes NK cell expansion and function. Combining N-803 with nivolumab achieved clinical responses in patients that had previously progressed with anti-PD-1 therapy. In addition, the ability of IL-15 to expand NK cells has led to its application in the setting of NK cell adoptive transfers, with IL-15 expressing NK cells increasing in number after infusion. Boosting CD8 memory T-cells has also prompted the combination of IL-15 with cancer vaccines, with preclinical studies showing increased antigen-specific CD8 T-cells. These data have prompted intense investigation of these combinations in the clinic (**Table 1** and **Fig. 1**).

IL-21

IL-21 is produced mainly by CD4 T-cells but is also secreted by NKT cells and CD8 T-cells. IL-21 can affect the homeostasis and function of several immune cells, including B-cells, T-cells, NK cells, macrophages, DCs and mast cells, all of which express the heterodimeric receptor composed of IL-21Rα and the common gamma-chain. IL-21 is well known for enhancing the survival, proliferation and differentiation of B-cells, germinal center functions and plasma cell differentiation. Interestingly, IL-21 can have a pro-apoptotic effect on B-cells in the absence of co-stimulation, making IL-21 important for the prevention of autoreactive antibody production. IL-21 can further promote the expansion of CD8 memory T-cells in the presence of IL-7 or IL-15, improves NK cell viability and function, memory T-cell persistence and cytotoxic CD8 T-cell function (6,96). Notably, IL-21 does not promote Treg differentiation, although the presence of Tregs can mitigate the IL-21-induced expansion of CD8 T-cells (97). With the ability to improve the function of multiple immune cells important for the anti-tumor response, IL-21 is a relevant cytokine to be considered for cancer immunotherapy.

IL-21 as a monotherapy

Phase I and phase IIa clinical trials were initiated to study the safety and efficacy of rIL-21 (98-100). A dosing regimen of rIL-21 at 30 μg/kg/dose (i.v. bolus injection in 8-wk cycles) was established as the recommended dose. DLTs included hyponatremia, hypophosphatemia and increased liver transaminases, although there was no evidence of VLS (NCT00095108). The half-life of rIL-21 was 3.09 h and biomarker analysis showed significant increases in CD8 T-cells and soluble CD25 with a concomitant increase in IFN-γ, perforin, and granzyme B mRNA expression. About 10% of patients developed anti-drug antibodies, although these were not causatively associated with any AEs. Anti-tumor activity was observed in the mM cohort of 48 patients as 1 CR, 1 PR and 20 SD, while in the renal cancer cohort, out of 19 patients 4 PR and 13 SD were recorded. Despite these promising Phase I/II results, a recent report identified comparable efficacy between rIL-21 and dacarbazine in refractory or mM patients (99).

Other clinical strategies include IL-21 in combination with sorafenib in mRCC (NCT00389285) and with anti-CD20 mAb rituximab for non-Hodgkin's lymphoma (NCT00347971). Preclinical studies have attempted to address the short half-life of IL-21 using fusion cytokines in the form of IL-21-αHSA, αCD20-IL-21 and GIFT-21 (101-104). Also in preclinical development is an Erbitux-based IL-21 fusion protein (Erb-IL21, IL-21 fused to the Fc portion of IgG1) that specifically targets murine tumors expressing chimeric EGFR (105). However, none of these next-generation IL-21 drug candidates are in clinical stages yet.

IL-21 in combination with CPIs

While there are currently no clinical trials testing IL-21 in combination with CPIs, there are multiple preclinical studies supporting its combination. Erb-IL21 combined with anti-CTLA-4 or anti-PD-L1 improved tumor control and mouse survival in CPI-resistant tumors (105). Murine IL-21 administered in combination with anti-CTLA-4 or anti-PD-1 in multiple cancer models, has rendered mixed results (106). Additional preclinical studies have investigated the effect of IL-21 on NK cells and its ability to enhance CPI therapy through an NK-cell dependent mechanism. By treating mice bearing MHCI-deficient MC38 cells with CD8-depleting antibodies and a combination of intratumoral anti-PD-1, anti-Tim-3 and rIL-21, the triple combination significantly improved tumor control and survival (107). Lastly, PD-1Ab21 is an IC that combines IL-21 with a single-chain anti-PD-1 antibody that directs IL-21 to PD-1+ T-cells. PD-1Ab21 elicited significant tumor control and increased expansion of

https://doi.org/10.4110/in.2022.22.e5

tumor-specific memory T-cells (108). The existing preclinical work demonstrates the ability of IL-21 to synergize with CPIs to increase the number and function of NK and CD8 T-cells in the tumor, supporting further investigation of this combination.

IL-21 in combination with ACT

While there have not been many attempts to combine IL-21 with ACT, there is evidence to support the culturing of cytotoxic lymphocytes (CTLs) with IL-21 prior to infusion. Chapuis et al. (109) demonstrated that culturing donor-derived WTI-specific CTLs with IL-21 led to increased persistence and CTLs cultured with IL-21 had a less differentiated phenotype and enhanced survival *in vivo*. Although all 3 treated patients had a high risk of relapse, all continued to be in complete remission when the study was published. A similar strategy was then applied to a patient with mM that was refractory to monoclonal CTL and anti-CTLA-4 applied as monotherapies. Infusion of donor-derived IL-21-primed MART1-specific T-cells and administration of anti-CTLA-4 led to a CR and the patient remained disease free for at least 5 years (110).

IL-21 in combination with cancer vaccines

While there is a strong rationale to combine IL-21 with cancer vaccines, evidence to support its combination remains limited to preclinical studies. Recently, a preclinical study involving a therapeutic bladder cancer vaccine revealed that mice treated with irradiated MB49 bladder cancer cells expressing IL-21 had significantly decreased tumor growth and significantly more CD4 and CD8 T-cells in the spleen and tumor. Interestingly, they found that if the cancer vaccine expressed both IL-21 and GM-CSF, the anti-tumor response was even greater (111). In addition, incorporating IL-21 expression into a B16F10-ESTAT-6-gpi tumor vaccine has shown decreased tumor growth in a murine melanoma model (112). There is also evidence that the co-expression of IL-21 with IL-7 in a whole cell cancer vaccine has synergistic effects in a melanoma model (69).

IL-21 concluding remarks

As IL-21 was the most recently discovered common gamma-chain family cytokine, there is less clinical development. Like other unmodified recombinant cytokines, IL-21 half-life is short. However, several preclinical studies have demonstrated IL-21 can increase the function of NK and CD8 T-cells in the tumor and often works in synergy with other immunotherapies. While there are currently no next-generation IL-21 drug candidates in clinical trials, the development of candidates with increased half-life continues with promising results.

CONCLUSION

When considering the broad importance of gamma-chain cytokines on immune cell development, survival and function, administering these cytokines in clinical settings where enhanced anti-tumor immune responses is desired is a reasonable and promising approach. Early application of these cytokines in the clinic revealed rapid clearance *in vivo*, requiring frequent injections and with some showing toxic side effects at HDs. Fortunately, several next-generation drug candidates have been developed that are safer, have an increased half-life or are able to target the cytokine to specific cells (**Table 2**). In addition, the advancement of engineering cells to express various gamma-chain cytokines has proven promising for ACT and cancer vaccine settings. An aspect of common gamma-chain cytokine biology that is outside the scope of this review, but could have important implications

Table 2. Comparison of unaltered and next-generation gamma-chain cytokines

Variables	Half-life	Dosing	Safety	CD8 T-cell/NK cell increase
IL-2				
Unaltered (Aldesleukin)	13–85 min	Daily	Low-dose expands Tregs HD has life-threatening toxicities	N/A
Next-Gen (ALKS 4230; FAP-IL-2v; GI-101; hu14.18-IL2; L19-IL2; NKTR-214; RO72284755; THOR-707)	Up to 10–17 h	Q2W–Q3W	Decreased Treg engagement and increased potency allow for lower doses and a better safety profile	Up to 3X CD8 T-cells Up to 8X NK cells
IL-7				
Unaltered (CYT-107)	6–10 h (unglycosylated) 9–35 h (glycosylated)	Q1W–Q2W	Good safety profile (no MTD reached)	Up to 2X CD8 T-cells
Next-Gen (NT-I7/GX-I7 (efineptakin alfa))	33–147 h	Q3W–Q12W	Good safety profile (no MTD reached)	Up to 4X CD8 T-cells (preferential TSCM expansion)
IL-15				
Unaltered (rhIL-15)	2.5 h	Daily	Marked elevation of inflammatory cytokines with i.v. injection, better safety profile when administered s.c.	Up to 1.5–2X CD8 T-cells Up to 2–3X NK cells
Next-Gen (N-803; NKTR-255)	Up to 30 h	Q1W–Q4W	Well tolerated	Up to 2.5X NK cells
IL-21				
Unaltered (rIL-21)	3 h	Daily	Serious dose-limiting toxicities	Up to 2.5X NK cells
Next-Gen	N/A	N/A	N/A	N/A

N/A, not applicable.

in cancer immunotherapy, is the soluble form of each cytokine receptor. The generation of soluble cytokine receptors and their ability to regulate the immune response has been comprehensively reviewed elsewhere (113). It is worth noting that while all cytokines reviewed in this manuscript are currently being tested in ongoing clinical trials (**Table 1**), IL-4 and IL-9 also utilize the gamma-chain receptor subunit. However, their role in the anti-tumor response is less understood and concerns about potential pro-tumorigenic effects have hindered their clinical development (114-116).

As various cancer immunotherapies continue to be tested in the clinic, disappointing clinical efficacy following monotherapy approaches has revealed that many tumor types will require a combination of strategies to completely eliminate the tumor. With the backing of strong preclinical and clinical data, next generation gamma-chain cytokines have earned the distinction of being a critical component in future combination immunotherapy strategies.

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