Journal for ImmunoTherapy of Cancer

Teaching an old dog new tricks: re-engineering IL-2 for immuno-oncology applications

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To cite: Sznol M, Rizvi N. Teaching an old dog new tricks: re-engineering IL-2 for immuno-oncology applications. *Journal for ImmunoTherapy of Cancer* 2023;**11**:e006346. doi:10.1136/jitc-2022-006346

Accepted 05 December 2022

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ABSTRACT

Various approaches are being explored to address the unmet medical need among patients with advanced cancer who do not respond to immune checkpoint inhibitors. Interleukin-2 has become a prominent focus of preclinical and clinical investigation, because of its known clinical activity, the important role of this cytokine in immune biology, and the ability to engineer variant proteins with potentially improved antitumor immunomodulatory activity and reduced toxicity. Bempegaldesleukin, the first of the modified IL-2 agents to reach phase 3 evaluation in combination with an anti-PD-1, did not improve outcome for patients with metastatic melanoma and renal carcinoma. The disappointing data raise important questions about the potential efficacy of other interleukin-2 variants, however, several of the other variants appear to be sufficiently differentiated in anticipated pharmacokinetic properties and immune modulatory effects to warrant continued clinical development.

Interleukin-2 (IL-2) was discovered as a T-cell growth factor in 1976.[1] In addition to interferon-alfa, it was among the first cytokines produced by recombinant technology and tested in clinical trials in the early 1980s. In the 1990s, a high-dose (HD) regimen of human recombinant IL-2, aldesleukin (Proleukin), received regulatory approval for the treatment of metastatic renal cell carcinoma (mRCC) and metastatic melanoma. In melanoma, HD IL-2 produced a 16% objective response rate (ORR) (6% complete response (CR) and 10% partial response (PR)).[2] In mRCC, the ORR was 14% (5% CR and 9% PR).[3] In both studies, patients with CR had durable benefit. However, the HD IL-2 regimen was associated with moderate to severe toxicity including hypotension requiring fluid or pressor support and vascular leak syndrome (VLS), resulting in dose-limiting pulmonary edema, renal failure, transaminitis, diarrhea, and altered mental status. Because of challenges in administration of HD IL-2 and the subsequent development of more effective agents, IL-2 clinical use is limited today.

For more than a decade after the first reports of clinical antitumor activity, many clinical trials of IL-2 were initiated to assess activity and toxicity of different doses and schedules and combinations with other anticancer agents, across a broad range of malignancies. In addition, various agents were combined with IL-2 to mitigate mechanisms of toxicity while attempting to preserve antitumor activity. To our knowledge, none of the many efforts to improve and expand activity or reduce toxicity led to a regulatory approval for standard of care clinical use.

The immunobiology of IL-2 and its receptor is complex and has been covered extensively in multiple prior reviews.[4] From the perspective of clinical development, one of the greatest surprises came from the IL-2 gene knockout mouse, which had the phenotype of bowel autoimmunity and increased activated T-cells and B-cells.[5] It was then recognized that IL-2 was critical for development and maintenance of T-regulatory cells (Treg) and that Treg expressed the high affinity trimeric IL-2 receptor (the alpha chain, CD25, in addition to the beta and gamma chains of the receptor).[6] This new understanding of IL-2 biology led to the hypothesis that IL-2 antitumor effects by activation and expansion of effector T-cells were constrained by concurrent agonist effects on immune inhibitory Treg.

In the clinic, the antitumor effects of IL-2 were surpassed by the far more effective immune checkpoint inhibitors (ICI), particularly antagonists of the programmed death receptor 1 and its ligands (PD1-PD-L1 pathway). Two independent paths of investigation led to a resurgence of interest in clinical development of IL-2. The first was a demonstration that wild type IL-2 could combine with anti-PD-L1 to clear infection in a difficult mouse model of lymphocytic choriomeningitis virus (LCMV).[7] In this model, IL-2 was shown to expand a subset of exhausted

virus-specific CD8 T-cells and improve their responsiveness to antigen despite a concurrent increase in blood and tissue Treg. The second was promising antitumor activity of a pegylated IL-2, NKTR-214 (bempegaldesleukin) in preclinical mouse models.[8]

NKTR-214 is an engineered aldesleukin prodrug with six pegylated surface lysines that extend its half-life and reduce binding to the alpha chain of the IL-2 receptor.[8 9] After administration, serial shedding of PEG chains increases binding to the IL-2 receptor. Because of its limited activation of the high affinity trimeric IL-2 receptor (IL-2Rαβγ) expressed on Tregs, NKTR-214 was predicted to reduce IL-2's preferential expansion of Treg compared with the expansion and activation of T cells and NK cells, which only express the intermediate affinity dimeric IL-2 receptor lacking the α subunit (IL-2Rβγ). In mouse models, NKTR-214 increased Treg in peripheral tissues but depleted Treg in the tumor microenvironment through a mechanism that involved CD8 T cell production of interferon-gamma and tumor necrosis factor-α.[10] In addition, direct binding of IL-2 to the trimeric IL-2 receptor on endothelial cells had been proposed as a potential mechanism of IL-2 induced VLS, and also supported a non-α receptor binding IL-2 design to reduce toxicity.[11] Preliminary clinical data from NKTR-214 in combination with anti-PD-1 appeared promising in patients with immunotherapy naïve melanoma. Subsequently, the immense creativity of a modern generation of protein engineers and capital investment were unleashed to create many second-generation IL-2 molecules which are either in the clinic or soon-to-enter clinical development.

At the 2022 European Society for Medical Oncology (ESMO) Congress, the first phase 3 trials of NKTR-214 in combination with nivolumab were reported in melanoma and renal cell carcinoma.[12 13] Both trials were negative and no improvement over standard of care was observed leading to discontinuation of NKTR-214 development. In patients with metastatic melanoma, the ORR, median PFS, and median overall survival for NKTR-214+Nivo vs Nivo alone were 27.7 vs 36% (p=0.03), 4.17 vs 4.99 months (HR=1.09, p=0.398), and 29.67 vs 28.88 months (HR=0.94, p=0.636).

The results for NKTR-214 have understandably called into question the potential viability of the many other engineered IL-2 molecules in clinical development. Was this a failure for IL-2 or a failure specific to NKTR-214 engineering? In a report based on a standard of care registry database, administration of HD aldesleukin to patients with disease progression after treatment with immune checkpoint inhibitors continued to show a comparable level of durable response as in the 1980s, with the caveat that most patients in this series had only received single agent anti-PD-1.[14] In contrast NKTR-214 demonstrated no monotherapy objective responses in ICI-resistant/refractory patients enrolled in phase 1 studies, and in phase 3 studies, there was no numeric increase in response rate with NKTR-214+nivolumab compared with nivolumab alone.[15] Additionally, NKTR-214 cytokine mediated toxicities were similar to aldesleukin, which precluded dose escalation. Although the dose taken forward for phase 2 and 3 trials was calibrated to allow safe outpatient administration, strict management guidelines to reduce agent-induced hypotension were required including intravenous fluid administration before each dose of NKTR-214.[16] Additionally, in the phase 3, melanoma data presented at the 2022 European Society for Medical Oncology (ESMO) Congress, grade 3–4 treatment emergent adverse events were observed in 21.7% with NKTR-214+nivolumab vs 11.5% with nivolumab alone.

Like wild-type IL-2, NKTR-214 exposure in humans appears to be much lower than what was achieved in mouse tumor models. NKTR-214 activates and expands NK cells, and although a clear role for NK cells in the antitumor effects of IL-2 has not been established, increasing data support a major role for NK cells in mediating IL-2 toxicities.[17–19] Therefore, NKTR-214's failure in the phase 3 trial could be explained by inadequate exposure particularly within the tumor microenvironment, or inadequate expansion of tumor-specific T cells, or inadequate selectivity for tumor-specific CD8 T-cells over Treg. Another consideration is that CD25 binding may be important for efficacy. A recent paper showed that IL-2 binding to CD25 was required for its synergistic effect with PD-1 blockade in the difficult to-treat LCMV mouse model.[20] Since October of 2022, two other non-alpha IL-2 variants, NL-201 and Thor-707 (SAR444245) were discontinued or deprioritized from clinical development.

Various protein engineering approaches have been used to improve the properties of IL-2 and could perhaps overcome the limitations presented by NKTR-214. In addition to development of other non-alpha IL-2 variants, prodrug molecules have been created which are activated specifically within the tumor microenvironment, and IL-2 variants have been attached to tumor targeting molecules such as fibroblast activation protein α. Non-alpha IL-2s have also been directed to specific T cell subsets with 'bispecific' antibodies, referred to as cis-targeting, for example, to CD8 T-cells or T cells expressing PD-1.[21 22] A recent publication indicates that cis-targeting of non-alpha IL-2 to T-cells may bypass the requirement for CD25 binding.[22] Finally, a novel IL-2 molecule with reduced binding to the gamma IL-2 receptor chain (and therefore preferential binding to cells expressing the alpha and beta chains) recently entered clinical trials.[23] These approaches are expected to increase IL-2 effect within the tumor microenvironment and/or bias IL-2 away from interaction with NK cells±Tregs.

Perhaps the biggest hurdle for clinical development of all the novel IL-2 molecules is the lack of predictive biomarkers to identify patients who can respond to IL-2 alone or who require an IL-2 effect to enable response to immune checkpoint inhibitors. The actual clinical significance of IL-2-mediated effects on Treg remain undefined, and perhaps diminishes in importance if the specific IL-2 variant sufficiently expands tumor-specific CD8 T cells

and/or restores the functionality of the tumor-specific exhausted CD8 T cell pool, particularly within the tumor microenvironment. In addition to biological design, optimizing the dose and duration of exposure for a particular IL-2 variant may be necessary for clinical success.

Despite the concerns, many of the IL-2 variant molecules are significantly differentiated from NKTR-214 to justify continued clinical development. Moreover, based on prior preclinical data, there are many opportunities to explore in the clinic that go beyond just combination of the novel IL-2 molecules with immune checkpoint inhibitors, for example, combinations with other cytokines, cell therapies, and bispecific CD3 (T cell) or NK cell engagers. Clinical data and correlative studies from ongoing and pending trials, which address drug exposure and confirm IL-2 immune modulatory effects in the tumor, are necessary to determine if we can indeed teach this old dog new tricks.

Contributors Both authors contributed equally to the conception of the manuscript, to the analysis of external data and writing of this manuscript, and both authors approved the final version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests MS consults for and owns options in companies developing Il-2 products. NR is employed by a company developing an Il-2 variant.

Patient consent for publication Not applicable.

Provenance and peer review Commissioned; externally peer reviewed.

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REFERENCES

1 Morgan DA, Ruscetti FW, Gallo R. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science* 1976;193:1007–8.
2 Atkins MB, Lotze MT, Dutcher JP, *et al*. High-Dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol* 1999;17:2105–16.
3 Fyfe G, Fisher RI, Rosenberg SA, *et al*. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. *J Clin Oncol* 1995;13:688–96.
4 Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nat Rev Immunol* 2018;18:648–59.
5 Sadlack B, Merz H, Schorle H, *et al*. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 1993;75:253–61.
6 Malek TR. The main function of IL-2 is to promote the development of T regulatory cells. *J Leukoc Biol* 2003;74:961–5.
7 West EE, Jin H-T, Rasheed A-U, *et al*. Pd-L1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells. *J Clin Invest* 2013;123:2604–15.
8 Charych DH, Hoch U, Langowski JL, *et al*. NKTR-214, an engineered cytokine with biased IL2 receptor binding, increased tumor exposure, and marked efficacy in mouse tumor models. *Clin Cancer Res* 2016;22:680–90.
9 Charych D, Khalili S, Dixit V, *et al*. Modeling the receptor pharmacology, pharmacokinetics, and pharmacodynamics of NKTR-214, a kinetically-controlled interleukin-2 (IL2) receptor agonist for cancer immunotherapy. *PLoS One* 2017;12:e0179431.
10 Sharma M, Khong H, Fa'ak F, *et al*. Bempegaldesleukin selectively depletes intratumoral Tregs and potentiates T cell-mediated cancer therapy. *Nat Commun* 2020;11:661.
11 Krieg C, Létourneau S, Pantaleo G, *et al*. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. *Proc Natl Acad Sci U S A* 2010;107:11906–11.
12 Diab A, Gogas HJ, Sandhu SK, *et al*. 785O pivot io 001: first disclosure of efficacy and safety of bempegaldesleukin (BEMPEG) plus nivolumab (NIVO) vs NIVO monotherapy in advanced melanoma (MEL). *Annals of Oncology* 2022;33:S901–S409.
13 Tannir N, Formiga MN, Agarwal N, *et al*. LBA68 Bempegaldesleukin (BEMPEG) plus nivolumab (NIVO) compared to the investigator's choice of sunitinib or cabozantinib in previously untreated advanced renal cell carcinoma (RCC): Results from a phase III randomized study (PIVOT-09). *Annals of Oncology* 2022;33:S1433–69.
14 Buchbinder EI, Dutcher JP, Daniels GA, *et al*. Therapy with high-dose interleukin-2 (HD IL-2) in metastatic melanoma and renal cell carcinoma following PD1 or PDL1 inhibition. *J Immunother Cancer* 2019;7:49.
15 Bentebibel S-E, Hurwitz ME, Bernatchez C, *et al*. A first-in-human study and biomarker analysis of NKTR-214, a novel IL2Rβγ-Biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov* 2019;9:711–21.
16 Diab A, Tannir NM, Bentebibel S-E, *et al*. Bempegaldesleukin (NKTR-214) plus nivolumab in patients with advanced solid tumors: phase I dose-escalation study of safety, efficacy, and immune activation (PIVOT-02). *Cancer Discov* 2020;10:1158–73.
17 Assier E, Jullien V, Lefort J, *et al*. Nk cells and polymorphonuclear neutrophils are both critical for IL-2-induced pulmonary vascular leak syndrome. *J Immunol* 2004;172:7661–8.
18 Carson WE, Yu H, Dierksheide J, *et al*. A fatal cytokine-induced systemic inflammatory response reveals a critical role for NK cells. *J Immunol* 1999;162:4943–51.
19 Peace DJ, Cheever MA. Toxicity and therapeutic efficacy of high-dose interleukin 2. in vivo infusion of antibody to NK-1.1 attenuates toxicity without compromising efficacy against murine leukemia. *J Exp Med* 1989;169:161–73.
20 Hashimoto M, Araki K, Cardenas MA, *et al*. PD-1 combination therapy with IL-2 modifies $CD8^+$ T cell exhaustion program. *Nature* 2022;610:173–81.
21 Moynihan KD, Pappas D, Park T, *et al*. Abstract 3518: AB248 is a CD8+ T cell selective IL-2 designed for superior safety and anti-tumor efficacy. *Cancer Res* 2022;82:3518–18.
22 Codarri Deak L, Nicolini V, Hashimoto M, *et al*. PD-1-cis IL-2R agonism yields better effectors from stem-like $CD8^+$ T cells. *Nature* 2022;610:161–72.
23 Emmerich J, Bauer M, Semana M, *et al*. Abstract 1744: STK-012, an alpha/beta selective IL-2 mutein for the activation of the antigen-activated T cells in solid tumor. *Cancer Res* 2021;81:1744–44.