Targeting of IL-2 to cytotoxic lymphocytes as an improved method of cytokine-driven immunotherapy

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ABSTRACT
The use of high-dose interleukin-2 (IL-2) has fallen out of favor due to severe life-threatening side effects. We have recently described a unique way of directly targeting IL-2 to cytotoxic lymphocytes using a virally encoded immune evasion protein and an IL-2 mutant that avoids off-target side effects such as activation of regulatory T cells and vascular endothelium.

Systemic high-dose IL-2 as a curative form of immunotherapy
High-dose interleukin-2 (IL-2) cytokine therapy was first approved by the FDA for treatment of advanced renal cell carcinoma (RCC) in 1992 and for advanced melanoma in 1998. High-dose IL-2 activates and induces proliferation of natural killer (NK) cells and CD8+ cytotoxic T cells, leading to potent anticancer activity, which results in a durable complete remission in up to 9% of RCC patients with a median durability of 70 mo. Many patients experience remission lasting more than 10 y. This rate is in contrast to the <1% complete remission rate of the anti-PD1 therapy nivolumab, which received Food and Drug Administration (FDA) fast-track approval. Despite highly promising results, high-dose IL-2 therapy is rarely used today because many patients experience severe adverse effects, such as fever, malaise, and systemic capillary leak. As a result, close to 50% of patients discontinue therapy, and a further 2–5% of patients will die as a result of therapy-induced pulmonary edema and cardiovascular instability.

These side effects are the result of off-target signaling via the IL-2 receptor (IL-2R). Cytotoxic lymphocytes (CTLs) express the low-affinity IL-2Rαβγ form of the receptor at baseline but after initial activation display the high-affinity IL-2Rαβγ receptor that is ultimately required for peak effector function. In contrast, vascular endothelium expresses the high-affinity IL-2Rαβγ receptor at baseline and thus high-dose IL-2 results in inflammation, vascular leakage, and the associated adverse events due to direct vascular toxicity. Furthermore, CD4+ Foxp3+ Treg also express the high-affinity IL-2Rαβγ receptor at baseline and are preferentially activated by IL-2. Treg, normally function to downregulate immune responses, and in the context of cancer immunotherapy limit the therapeutic potential of the CTLs (Fig. 1A).

Alternative forms of IL-2 and IL-2 delivery
Previous investigators have hypothesized that a form of IL-2 that prevents Treg activation could greatly enhance therapeutic efficacy. There are multiple described mutations that decrease IL-2 affinity for IL-2Rα, such as the substitution of alanine for arginine (R38A) and lysine for phenylalanine (F42K). Alternately, antibody-bound forms of IL-2 have been developed to reduce IL-2Rα binding. Unfortunately, IL-2Rα-inhibited therapies have reduced NK-cell activation and a correspondingly reduced therapeutic index due to the requirement of the high-affinity receptor for peak cytotoxicity. Another strategy has been to create mutants with increased affinity for IL-2Rβ in an effort to remove the preference for IL-2Rα. The IL-2Rβ-enhanced IL-2 mutants still bind and activate Treg and vascular endothelium and thus still have “off target” side effects. Several groups have attempted to direct cytokine delivery to the tumor bed or vasculature via a targeted antibody–IL-2 conjugate but these therapies may be limited due to the ability of the tumor to escape by shedding, mutating, or downregulating the targeted antigen. Furthermore, the tumor-associated microenvironment actively excludes lymphocytes, thus preventing CTLs from encountering IL-2. Others have extended the half-life of IL-2 by conjugating it with anti-IL-2 antibodies or stabilizing the cytokine in the blood

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stream through the addition of polyethylene glycol.\(^6\) None of these approaches, however, significantly ameliorates \(T_{reg}\) activation with unproven results in human trials.\(^7\)

**Unique method for IL-2 delivery to cytotoxic lymphocytes as a superior form of immunotherapy**

We have recently described a new form of IL-2 immunotherapy through targeted delivery of an IL-2 mutant directly to CTLs. We utilized a high-affinity, virally encoded NKG2D ligand known as orthopoxvirus major histocompatibility complex class I like protein or OMCP\(^8\) (Fig. 1B). Since NKG2D is expressed constitutively on murine NK cells as well as activated murine CD8\(^+\) T cells, the new OMCP-(R38A/F42K)IL-2 protein resulted in preferential binding to and activation of NK cells over Tregs (Fig. 1B and C), improved immunotherapy against both solid and hematopoietic tumors (Fig. 1D), and no systemic capillary leak or animal mortality (Fig. 1E and F).
Limited animal morbidity, manifest as weight loss, was completely dependent on NK activation (Fig. 1F). OMCP offers the highest known NKG2D binding affinity of any known ligand9,10 and has broad applications in both human and veterinary immuno-oncology due to potential reactivity with multiple species. Nevertheless, the use of anti-NKG2D antibodies to target IL-2 to NK cells is also a possibility (Fig. 1G). Taken together our work demonstrates the utility of targeted cytokine delivery for the treatment of cancer immunotherapy and possibly other forms of immune suppression.

Disclosures of potential conflict of interest

E.L. and A.S.K. have filed a patent on data described in this manuscript with technology licensed to Courier Therapeutics (http://www.couriertherapeutics.com). Targeted Cytokine Delivery to Cytotoxic Lymphocytes—Inventors: Alexander S. Krupnick, Daved H. Fremont, Eric Lazear, U.S. Provisional Application number 62/091,898, filed December 15, 2014. EL, ASK, DW, and JW are major shareholders and SH is an employee of Courier Therapeutics.

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