

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

Eric Lazear^a, Reza Ghasemi^b, Sarah M. Hein^c, John Westwick^c, Dan Watkins^c, Daved H. Fremont^d, and Alexander Sasha Krupnick^e

^aDepartment of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; ^bDepartment of Medicine, Washington University in St. Louis, St. Louis, MO, USA; ^cCourier Therapeutics, Houston, TX, USA; ^dDepartments of Pathology & Immunology, Biochemistry & Molecular Biophysics, and Molecular Microbiology, Washington University School of Medicine in St. Louis, St. Louis, MO, USA; ^eDepartment of Surgery, University of Virginia, Charlottesville, VA, USA

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.

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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.¹ 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-2R $\beta\gamma$ form of the receptor at baseline but after initial activation display the high-affinity IL-2R $\alpha\beta\gamma$ receptor that is ultimately required for peak effector function. In contrast, vascular endothelium expresses the high-affinity IL-2R $\alpha\beta\gamma$ 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⁺ T_{regs} also express the

high-affinity IL-2R $\alpha\beta\gamma$ receptor at baseline and are preferentially activated by IL-2. T_{regs} 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 T_{reg} activation could greatly enhance therapeutic efficacy.² There are multiple described mutations that decrease IL-2 affinity for IL-2R α , such as the substitution of alanine for arginine (R38A) and lysine for phenylalanine (F42K).³ Alternately, antibody-bound forms of IL-2 have been developed to reduce IL-2R α binding.⁴ Unfortunately, IL-2R α -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-2R β in an effort to remove the preference for IL-2R α .⁵ The IL-2R β -enhanced IL-2 mutants still bind and activate T_{regs} 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

CONTACT Alexander Sasha Krupnick ✉ sasha@couriertherapeutics.com; sashak@virginia.edu Department of Surgery, University of Virginia, PO Box 800679, Charlottesville, VA 22908, USA; Daved H. Fremont ✉ fremont@wustl.edu Department of Pathology & Immunology, Washington University School of Medicine Campus Box 8188, MO 63108, USA.

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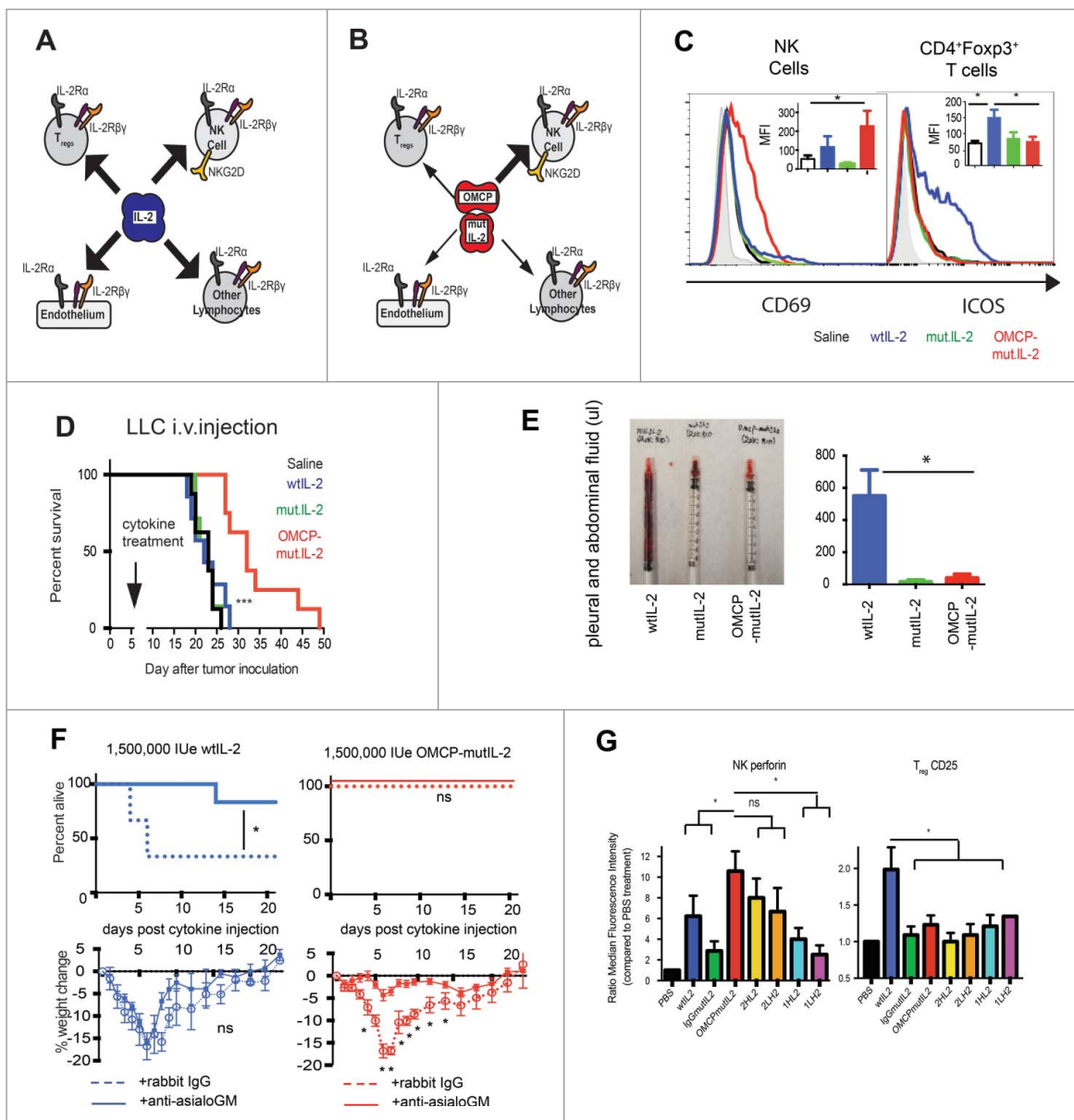


Figure 1. Targeted delivery of IL-2. (A) Proposed model of competition between NK cells and stromal cells for IL-2. (B) NKG2D targeted OMCP-mutIL-2 preferentially delivers cytokine to NK cells. Width of arrow indicates proposed strength of IL-2 signaling. (C) *In vitro* flow cytometrically evaluated activation of A/J spleen-derived lymphocyte subsets after 36 h of culture in 100 IUe/mL of cytokines or OMCP-mutIL-2 construct. Graph demonstrating one representative histogram plot and median fluorescent intensity (MFI) \pm S.E.M. across 5–7 separate experiments. (D) Survival of C57BL/6 mice treated with 750,000 IUe of cytokine 5 d after injection of 1×10^5 LLC intravenously (E) ascites (representative syringe—left; average from all mice in the group—right) in A/J mice treated with 200,000 IUe of either wild-type IL-2 (blue) or OMCP-mutIL-2 (red). (F) Animal mortality and morbidity, as assessed by survival (top) and weight loss (bottom) of A/J mice treated with supratherapeutic doses of wild-type IL-2 or OMCP-mutIL-2. NK cells were either depleted with anti-asialo GM (solid line) or not (dashed line). (G) In order to compare antibody-mediated delivery of mutIL-2 we engineered four anti-human NKG2D single chain variable fragment domains based on the described sequence of the KYK1 and KYK2 antibodies.¹¹ 1HL2 and 1LH2 are derived from the KYK1 antibody and 2HL2 and 2LH2 from the KYK2 antibody. The binding coefficients of OMCP, KYK1, and KYK2 are 0.1 nM, 27 nM, and 6 nM, respectively. Similar to what is described for *in vitro* human studies NK activation was measured by expression of intracellular perforin, whereas T_{reg} activation by surface CD25 levels, both expressed as relative ratio to saline control. After 48 h in 100 U/mL NK cells treated with 2HL2 and 2LH2 antibodies synthesized as much perforin as OMCP-mutIL-2 treated cells but lower levels of perforin were evident in 1HL2 and 1LH2 treated NK cells. Higher levels of CD25 were evident in wild-type IL-2 treated cultures over all constructs, * $p < 0.05$; *** $p < 0.001$; ns = $p > 0.05$. Data from Figure A-F modified from Ghasemi et. al., Nature Communications 2016; 7:12878; <http://dx.doi.org/10.1038/ncomms12878>.

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 OMCP⁸ (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 T_{regs} (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 ligand^{9,10} 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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ORCID

Eric Lazear  <http://orcid.org/0000-0001-6173-8904>

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