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Blood 142 (2023) 3432–3433

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Outsmart™ IL-2/15, a Novel Cytokine Designed to Enhance ROR1-Specific CAR T Anti-Tumor Activity While Minimizing Treg Activation and Systemic Cytokine Exposure

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Introduction: Effective treatment with chimeric antigen receptor T (CAR-T) cells against solid and some liquid tumors will require the safe production of immune-modulating cytokines to promote CAR-T cell expansion and to stimulate endogenous anti-tumor immune responses within the tumor microenvironment. OutSmart™ IL-2/15 is a computationally designed, CD8α-targeted, IL-2-based cytokine produced by engineered T cells via a T cell activation-dependent promoter. Here, we demonstrate that inducible production of CD8-targeted IL-2/15 drives proliferation and anti-tumor efficacy of ROR1-specific CAR-T cells, stimulates bystander CD8 $^+$ T and NK cells, and minimizes activation of immune suppressive T regulatory cells (Tregs).

Methods and Results: Wild-type (wt) IL-2 was modified using Rosetta protein design software package to eliminate IL-2Rα binding while retaining native IL-2R$\beta\gamma$ binding interfaces. Out of 10,355 designs, 38 were prioritized based on *in silico* analysis and ML-guided structure prediction, and evaluated for IL-2R binding as recombinant proteins. Consistent with the design criteria, none of the designs bound IL-2Rα, and 33 (87%) retained IL-2R$\beta\gamma$ binding and signaling activity. pSTAT5 analysis showed wt IL-2 highly activated CD4 $^+$CD25 $^+$FoxP3 $^+$ Treg cells, whereas the novel IL-2 designs showed a >3-log reduction Treg stimulation. These designs were iteratively optimized to generate a version exhibiting improved thermostability and IL-2R$\beta\gamma$ binding affinity equivalent to wt IL-2. To increase the potency against immune effector cells, but not Tregs, an anti-CD8α VHH was tethered to the modified IL-2 molecules (CD8-IL-2/15) to mimic high-affinity IL-2Rα binding and selectively activate CD8 $^+$ T and NK cells. This prosthetic binding interaction dramatically increased CD8 $^+$ T cell stimulation and CD8α $^+$ NK cell activation (>2-logs and 1-log by pSTAT5, respectively), while retaining its reduced capacity to stimulate Tregs. CD8-IL-2/15 gene expression was subsequently placed under the control of a T cell activation-dependent promoter and integrated into a lentiviral construct with a constitutively expressed ROR1-specific CAR. T cells transduced with a lentivirus expressing CD8-IL-2/15 and a ROR1-targeting CAR showed inducible cytokine production following exposure to ROR1 $^+$ tumor cells (H1975) leading to enhanced CAR-T cell proliferation and anti-tumor killing during repeat tumor challenge assays *in vitro*. Using immune-deficient mice (NSG) engrafted with H1975 tumors, inducible CD8-IL-2/15 enhanced ROR1-specific CAR-T cells showed durable tumor control at low doses (1e6 - 4e6 CAR T cells/animal), which was comparable to wt IL-2, whereas mock T cells and ROR1 CAR only T cells failed to control tumor growth (**Figure 1**). In addition, CAR-T expansion with CD8-IL-2/15 increased >4-fold over wt IL-2 ($p<0.0001$), measured by cell counts in peripheral blood.

Conclusions: In summary, OutSmart™ IL-2/15 is a genetic module that produces CD8-IL-2/15 in response to T cell activation, promoting robust CAR-T expansion and enhanced anti-tumor efficacy. Furthermore, local production of a CD8-IL-2/15 enhances the effector function of bystander CD8 $^+$ T cells and NK cells, but only minimally activates Treg cells due to removing the IL-2Rα binding interface. Inducible production of CD8-IL-2/15 dramatically improves the potency of a ROR1 CAR for treating ROR1 $^+$ solid and liquid cancers. More generally, OutSmart™ protein design methods and control technologies can be applied to create other designed cytokines for oncology and beyond.

Disclosures Moffett: *Outpace Bio:* Current Employment. **Weitzner:** *Outpace Bio:* Current Employment. **Davenport:** *Outpace Bio:* Current Employment. **Tait:** *Outpace Bio:* Current Employment. **Tan:** *Outpace Bio:* Current Employment. **Baker:** *Outpace Bio:* Current Employment. **Crowl:** *Outpace Bio:* Current Employment. **Obenza:** *Outpace Bio:* Current Employment. **Hammerson:** *Outpace Bio:* Current Employment. **Kirkpatrick:** *Outpace Bio:* Current Employment. **Sample:** *Outpace Bio:* Current Employment. **Jones:** *Outpace Bio:* Current Employment. **Hermans:** *Outpace Bio:* Current Employment. **Langan:**

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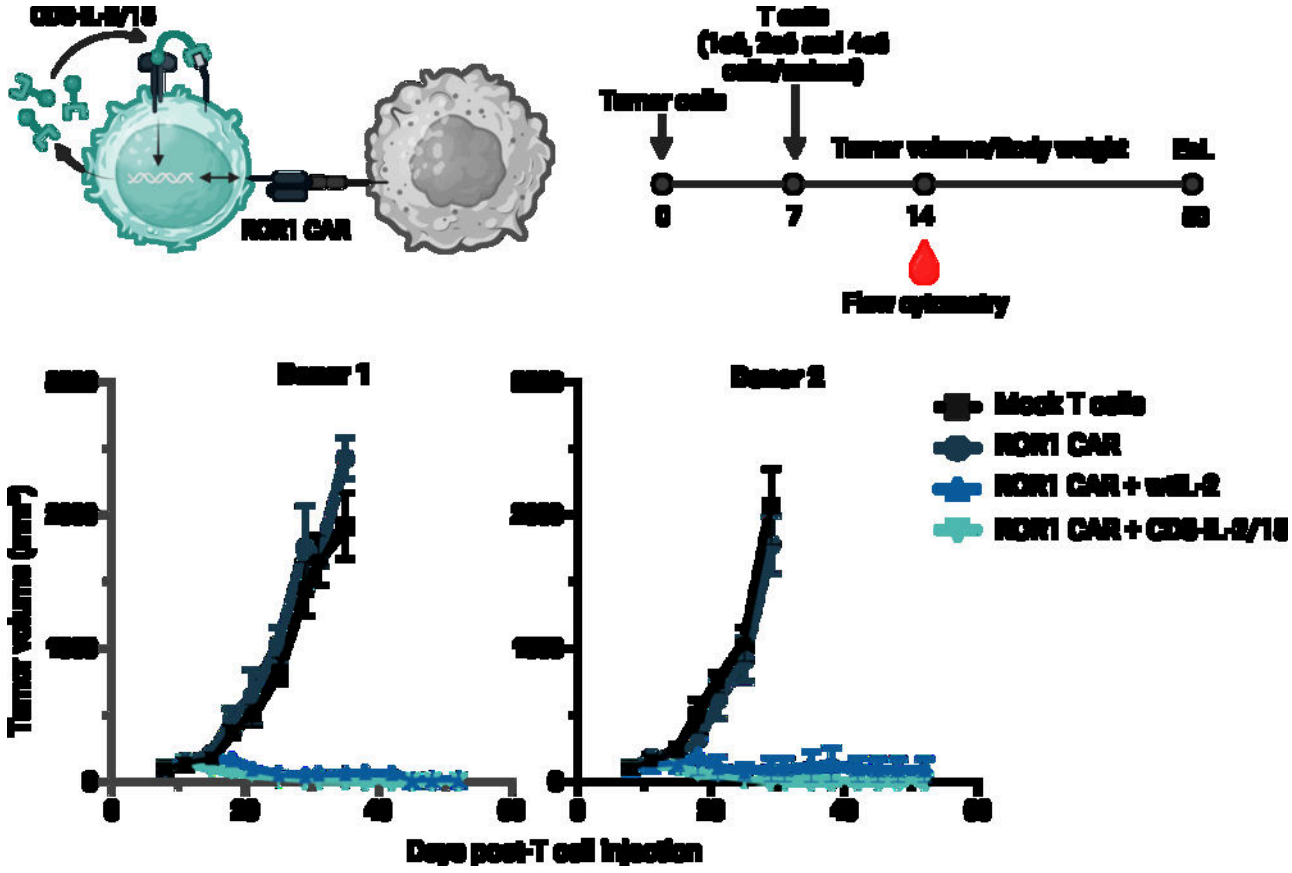

Figure 1

https://doi.org/10.1182/blood-2023-182417