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Abstract #4067

Development of Tumor-restricted IL-12 With Antigen-dependent Expression and Localized IL-12 Activity

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Introduction

IL-12 is an immune-stimulatory cytokine that can modulate the tumor microenvironment (TME) to enhance the cytotoxic activity of T and NK cells;[1] however, the potent activity of the cytokine needs to be localized to the TME to avoid systemic toxicity.[2] Tumor-specific T cells could be ideal vehicles for IL-12 delivery; but expression of wild-type IL-12 by T cells caused severe toxicity in a previous clinical trial.[3] Using Outpace's OUTSMART™ technology, we designed a tumor-restricted IL-12 (trIL-12) that is under control of an T-cell activation-dependent promoter and auto-inactivates within minutes after secretion.

Methods

T cells were engineered via lentiviral vectors (LVV) to express wild-type single-chain IL-12 (WT scIL-12) or trIL-12 under the control of an activation-inducible promoter; a second LVV introduces an NY-ESO-1 TCR. Kinetics of IL-12 expression was measured by qPCR and MSD technology; kinetics of IL-12p70 heterodimer dissociation was measured using Octet bio-layer interferometry. T-cell cytotoxicity and cytokine production was evaluated in vitro after repeated stimulation with NY-ESO-1–expressing A375 cells using Incucyte and MSD. IL-12 activity on bystander cells was measured after co-culture with IL-12–expressing T cells by detection of IFN-γ using flow cytometry. In vivo T-cell function of trIL-12–engineered NY-ESO-1 TCR T cells was measured in NSG MHCI/II KO mice bearing A375 xenografts. trIL-12 activity in a fully immune-competent mouse model was measured in B6 mice implanted with B16F10 tumor cells engineered to express murine surrogates of trIL-12. All graphs show mean value +/- standard error of the mean.

References

1. Tait Wojno, E.D., C.A. Hunter, and J.S. Stumhofer, *Immunity*, 2019. 50(4): p. 851-870.
2. Nguyen, K.G., et al., *Front Immunol*, 2020. 11: p. 575597.
3. Zhang, L., et al., *Clin Cancer Res*, 2015. 21(10): p. 2278-88.

Results

Expression of WT scIL-12 under the control of an activation-inducible promoter peaks within 6 hours after activation and produces sufficient IL-12 to improve T-cell function in vitro and in vivo. trIL-12 was created by inclusion of a cleavable linker and elimination of the covalent disulfide bond between the p35 and p40 subunits of IL-12p70, resulting in dissociation of the functional cytokine within 10 minutes post-cleavage. Additional mutations further reduce the IL-12p70 half-life. trIL-12 activates the IL-12–producing T cells, as well as proximal bystander T-cells in direct co-culture, but it does not activate distal bystander T-cells separated by Transwell membranes. In xenograft mouse models, trIL-12–expressing T cells display potent anti-tumor activity and cytokine production without showing systemic IL-12 accumulation. Furthermore, in fully immune competent mice, expression of a murine trIL-12 surrogate by tumor cells led to generation of potent anti-tumor responses without systemic trIL-12 accumulation and reduced systemic IFN-γ.

Figure 1: Kinetics of activation-induced IL-12 expression

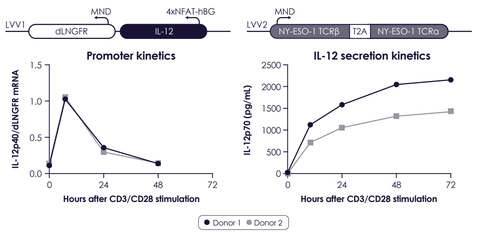


Figure 2: Inducible IL-12 expression enhances T-cell function

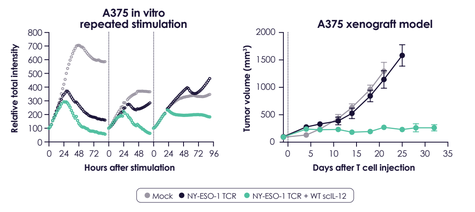


Figure 3: Inhibition of heterodimerization reduces IL-12 stability

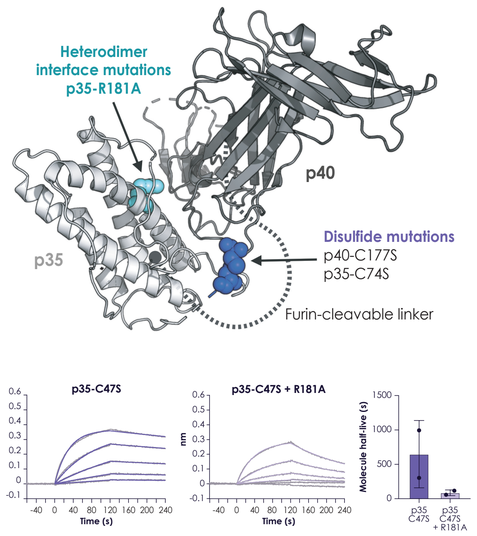


Figure 4: Destabilized IL-12 enhances T-cell function

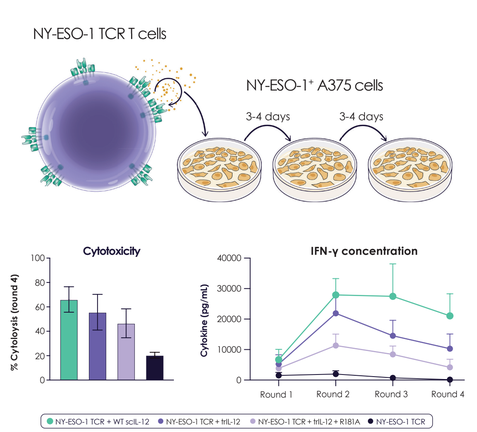


Figure 5: trIL-12 activates proximal but not distal bystanders

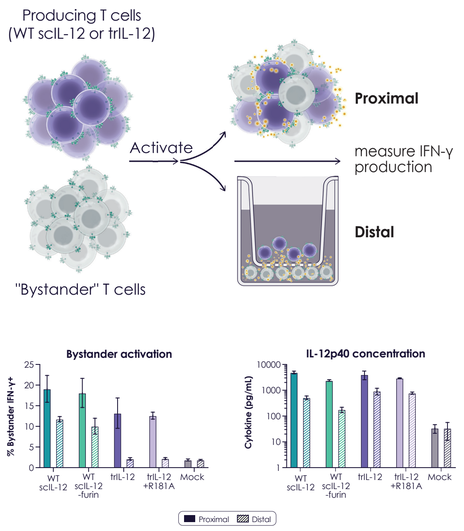


Figure 6: trIL-12 enhances T-cell function without systemic accumulation

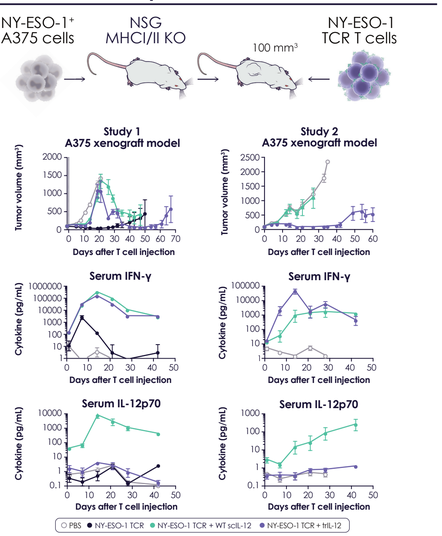
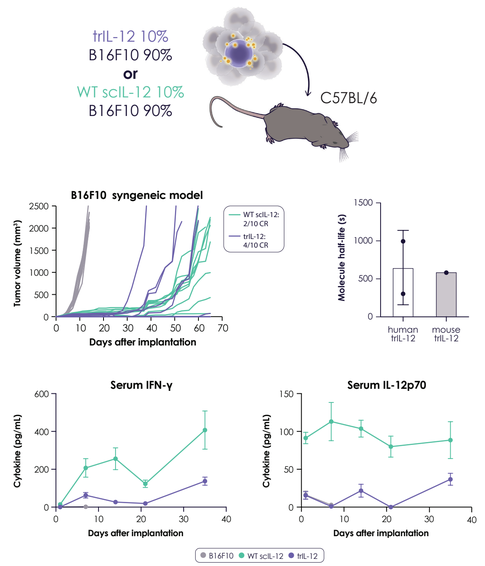


Figure 7: trIL-12 maintains anti-tumor efficacy while reducing systemic cytokine levels in a syngeneic mouse model



Conclusions

trIL-12–engineered T cells generate potent anti-tumor activity in vitro and in vivo. Unlike WT scIL-12, trIL-12 activity is localized to the region around the producing T cell and systemic IL-12 exposure is not observed in vivo. Collectively, these preclinical data suggest that trIL-12 may enable the development of potent T-cell therapeutics while maintaining an acceptable safety profile.

Abbreviations:
CR: complete response; IL-12: interleukin-12; IFN-γ: interferon gamma; KO: gene knockout; LVV: lentiviral vector; MHC: major histocompatibility complex; MSD: Mesoscale Discovery; NY-ESO-1: New York esophageal squamous cell carcinoma 1; scIL-12: single-chain IL-12; TCR: T-cell receptor; TME: tumor microenvironment; trIL-12: tumor-restricted IL-12; qPCR: quantitative polymerase-chain reaction; WT: wild-type.

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