1047 PROTEIN DESIGN AND INDUCIBLE EXPRESSION ALLOW CONTEXT-DEPENDENT, LOCALIZED IL-12 ACTIVITY TO ENHANCE SOLID TUMOR T CELL THERAPIES

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Background IL-12 is a pleiotropic immune-stimulatory cytokine that can modulate the tumor microenvironment to promote innate and adaptive immune responses and support cytotoxic activity of T and NK cells. However, systemic delivery of IL-12 recombinant protein or T cells engineered to secrete IL-12 causes severe toxicity in patients. To overcome these limitations, we leveraged OutSmart™ technology to design a tumor-restricted IL-12 (trIL-12) that rapidly auto-inactivates after secretion from engineered T cells under the control of an inducible promoter. The resulting design achieves safe, local delivery of IL-12 activity from engineered tumor-specific T cells.

Methods trIL-12 was designed via sequence alterations that remove the intermolecular disulfide bond between the p40 (C177S) and p35 (C74S) subunits, and the addition of Furin-cleavable sites on the linker between p40 and p35 in a single-chain (scIL-12) format. The dissociation rate of the cleaved IL-12 heterodimer was measured using bio-layer interferometry. T cells were engineered with lentiviral vectors expressing either wild-type (wt) scIL-12 or trIL-12 under the control of an engineered inducible promoter and a constitutively expressed NY-ESO-1 TCR. IL-12 activity in proximal or distal bystander immune cells was measured by IFN-γ production in T cells co-cultured with engineered T cells producing IL-12, either directly or separated by a transwell membrane. Next, engineered T cells were functionally assessed *in vitro* by repeat challenge with NY-ESO-1^{+} A375 tumor cells. Lastly, T-cell efficacy and systemic IL-12 exposure *in vivo* were evaluated using NSG MHCI/II KO mice engrafted with A375 tumors and treated i.v. with engineered T cells.

Results The inducible promoter enabled minimal basal IL-12 production from T cells and substantially increased IL-12 production after T-cell activation. trIL-12 dissociated into an inactive state post-cleavage with a half-life of ~10 minutes, and it activated proximal but not distal bystander T cells, demonstrating that function is restricted to the site of induced expression. trIL-12 and wt scIL-12 both enhanced T-cell cytotoxicity similarly *in vitro*, and both exhibited potent and comparable anti-tumor efficacy *in vivo*; however, only wt scIL-12, but not trIL-12, was detectable in an active state in peripheral blood, thus demonstrating trIL-12's localized activity that may improve the safety profile.

Conclusions These data demonstrate that trIL-12 can deliver potent IL-12 stimulation at the tumor site while avoiding systemic exposure, potentially improving efficacy for T-cell therapies while maintaining a favorable safety profile that may finally allow effective administration of IL-12.

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