Protein design and inducible expression allow context-dependent, localized IL-12 activity to enhance solid tumor T-cell therapies

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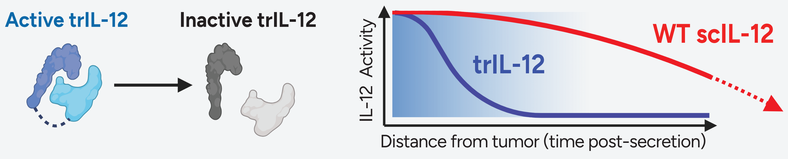
Development of tumor-restricted IL-12 (trIL-12)

- T-cell therapies to treat solid tumors are impaired by insufficient T-cell function, proliferation and survival, in part due to a lack of pro-inflammatory signals in the tumor microenvironment (TME).
- IL-12 is a pleiotropic immune-stimulatory cytokine that can modulate the TME to enhance the cytotoxic activity of T and NK cells; however, systemic exposure of IL-12 causes severe toxicity that has limited its clinical application.
- Leveraging Outpace's OutSmart™ technology, we designed trIL-12 to express from T cells under control of an activation-inducible promoter and auto-inactivate after secretion. Unlike wild-type single-chain IL-12 (WT scIL-12), trIL-12 activity is localized to the region around the producer T cell (**Figure 1**).

Conclusions

- **trIL-12 achieves potent but localized activity *in vitro*, and T cells enhanced with trIL-12 eliminate xenograft tumors while limiting systemic IL-12 exposure *in vivo*.**
- **Collectively, these preclinical data suggest that trIL-12 may enable the development of potent T-cell therapeutics while maintaining a favorable safety profile.**



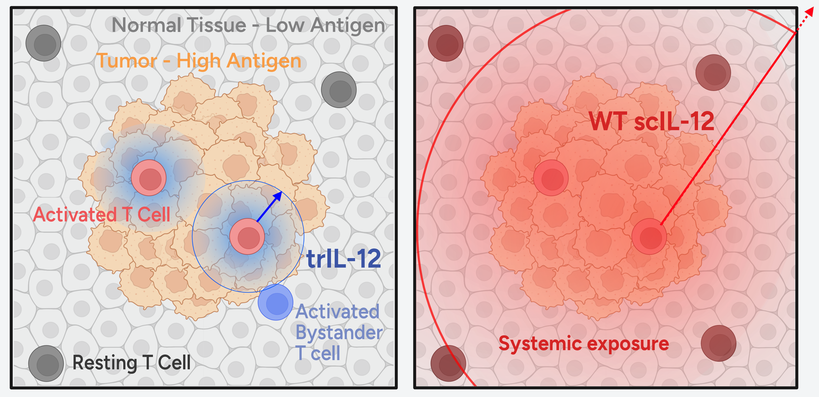


Figure 1. Tumor-restricted IL-12 (trIL-12) concept. trIL-12 is produced after T cells recognize tumor antigen and, unlike WT scIL-12, rapidly dissociates into an inactive form after secretion, restricting IL-12 activity to a tight radius around the producing T cell.

T-cell activation-induced expression of IL-12

Promoter design and IL-12 expression kinetics

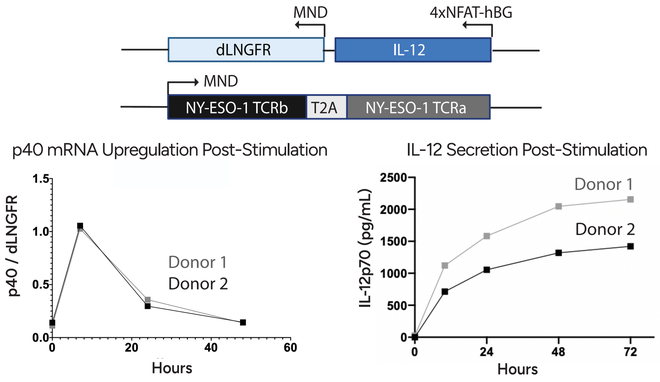
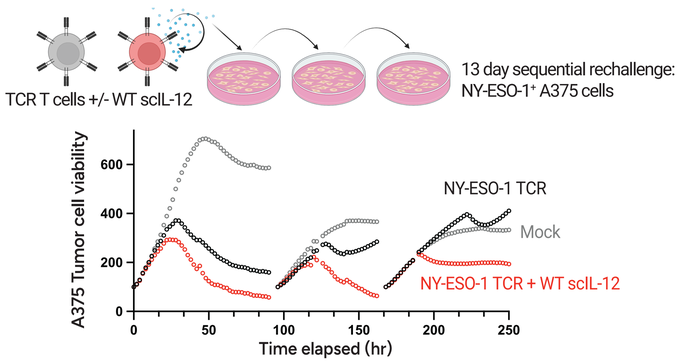


Figure 2. T cells were engineered with activation-induced IL-12 expression under the control of a 4xNFAT-hBG promoter and stimulated with CD3/CD28 TransAct. Kinetics of IL-12 expression was measured by transgene-specific qPCR relative to dLNGFR (left) and MSD (right).

IL-12 expression enhances T-cell function *in vitro* and *in vivo*



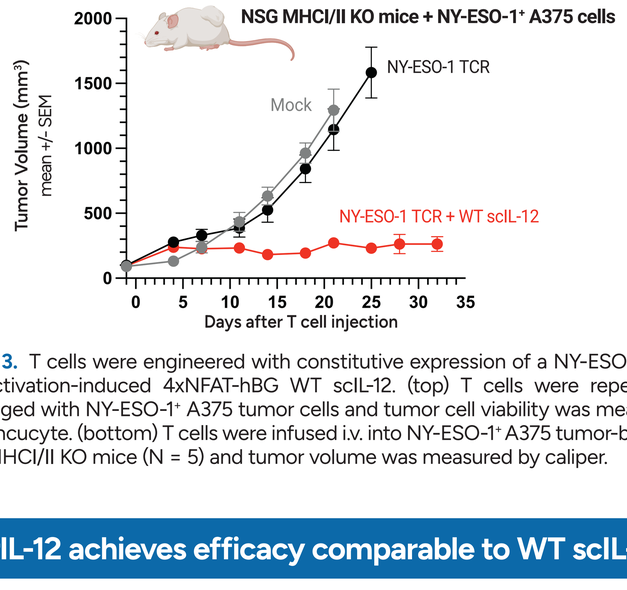


Figure 3. T cells were engineered with constitutive expression of a NY-ESO-1 TCR and activation-induced 4xNFAT-hBG WT scIL-12. (top) T cells were repeatedly challenged with NY-ESO-1+ A375 tumor cells and tumor cell viability was measured using Incucyte. (bottom) T cells were infused i.v. into NY-ESO-1+ A375 tumor-bearing NSG MHCI/II KO mice (N = 5) and tumor volume was measured by caliper.

trIL-12 stability is tuned to rapidly auto-inactivate

Design of mutations and cleavable linker

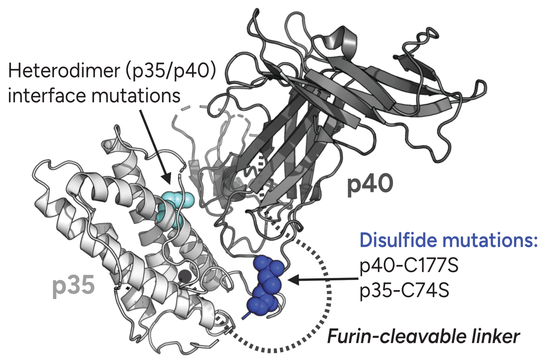
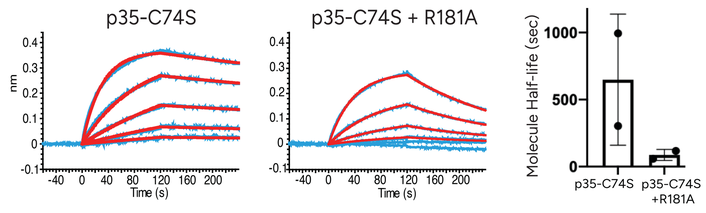


Figure 4. We mutated the residues forming the disulfide bond between the IL-12p40 (C177S) and IL-12p35 (C74S) subunits and added Furin cleavage sites to the linker that connects the subunits in scIL-12. Interface mutations further tune heterodimer stability. Structural model is based on PDB ID 3HMX.

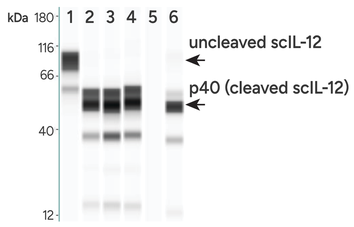
Mutations & cleavable linker create a short molecular half-life



Captured Ligand	KD (nM)	kon (1-M/s)	koff (1/s)	Calculated half-life (s)
p35-C74S	60 ± 40	24000 ± 2400	0.0015 ± 0.001	650 ± 400
p35-C74S + R181A	790 ± 340	11200 ± 560	0.009 ± 0.004	90 ± 40

Figure 5. p40-C177S binding to p35 variants measured by BLI, cleaved p35 captured on Octet tips. Stability of the p35-C74S/p40-C177S heterodimer complex can be tuned by additional mutations (e.g. R181A) that accelerate dissociation and shorten molecular half-life. Without removal of the disulfide bond, the p35-p40 complex is covalently linked and does not dissociate.

trIL-12 is cleaved efficiently after production by T cells



1. p40-G6S-p35 (WT scIL-12)
2. p40-furin-p35 (WT scIL-12-furin)
3. p40-C177S-furin-p35-C74S (trIL-12)
4. p40-C177S-furin-p35-C74S + R181A
5. Mock
6. 500 ng recombinant WT IL-12 (p70)

Figure 6. T cells were engineered with activation-inducible WT scIL-12 or trIL-12 variants and stimulated with CD3/CD28 TransAct. Secreted cleaved or uncleaved scIL-12 in T-cell supernatant was immunoprecipitated using a p40-specific antibody, separated under reducing conditions by SDS-PAGE, and detected by Western blot (rabbit anti-p40 primary, anti-rabbit HRP secondary).

trIL-12 activity is limited to the site of production

trIL-12 enhances T-cell function *in vitro*

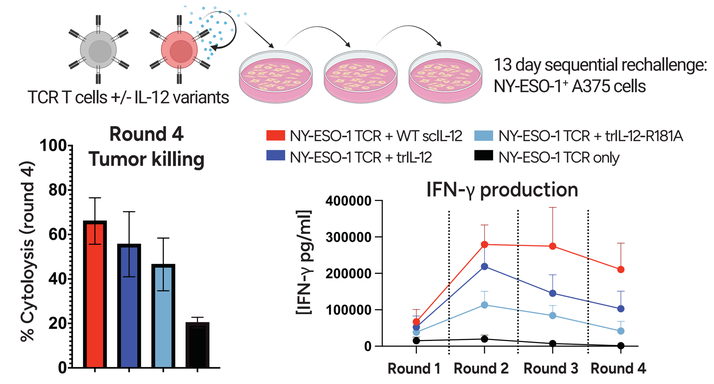


Figure 7. T cells were engineered with constitutive expression of a NY-ESO-1 TCR and activation-induced trIL-12 expression. T cells were challenged with A375 tumor cells every 3 or 4 days for a total of 4 rounds. Target cell viability was measured using Incucyte, and cytokine production was measured using MSD. N=3 donors.

trIL-12 activates proximal, but not distal, bystander T cells

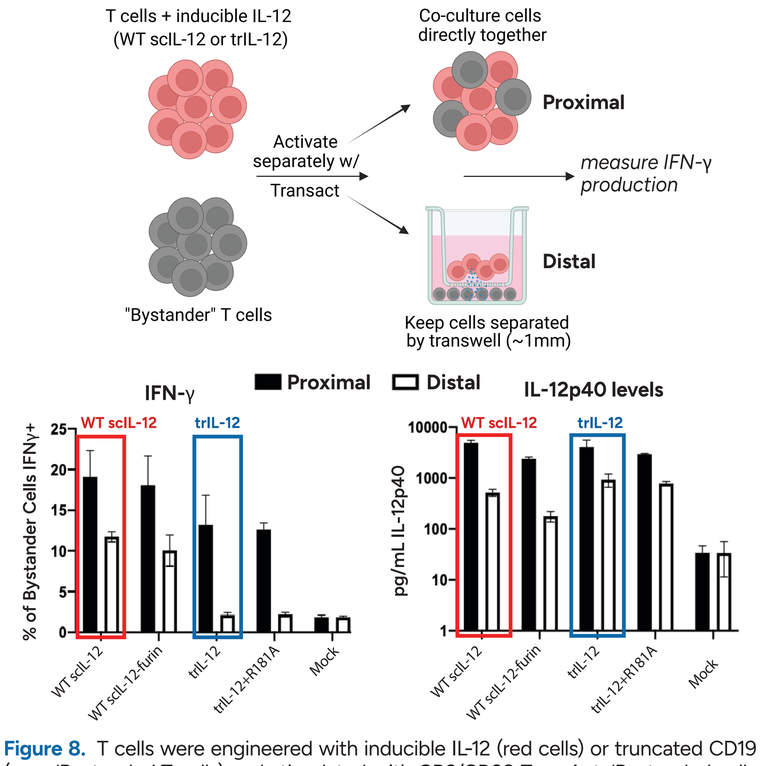
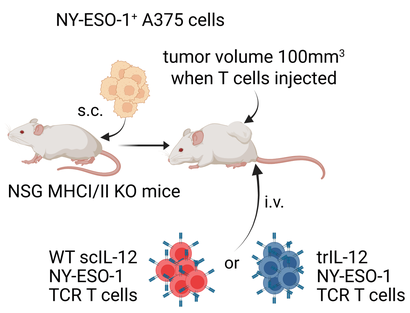


Figure 8. T cells were engineered with inducible IL-12 (red cells) or truncated CD19 (gray 'Bystander' T cells) and stimulated with CD3/CD28 TransAct. 'Bystander' cells and IL-12-producing T cells were co-cultured either directly together (proximal) or separately in transwells (distal). Intracellular IFN-γ production in response to IL-12 signaling was measured in the 'Bystander' population by flow cytometry, and secreted IL-12p40 was measured by MSD. N=2 donors.

trIL-12 achieves efficacy comparable to WT scIL-12 while limiting systemic exposure in a xenograft tumor model



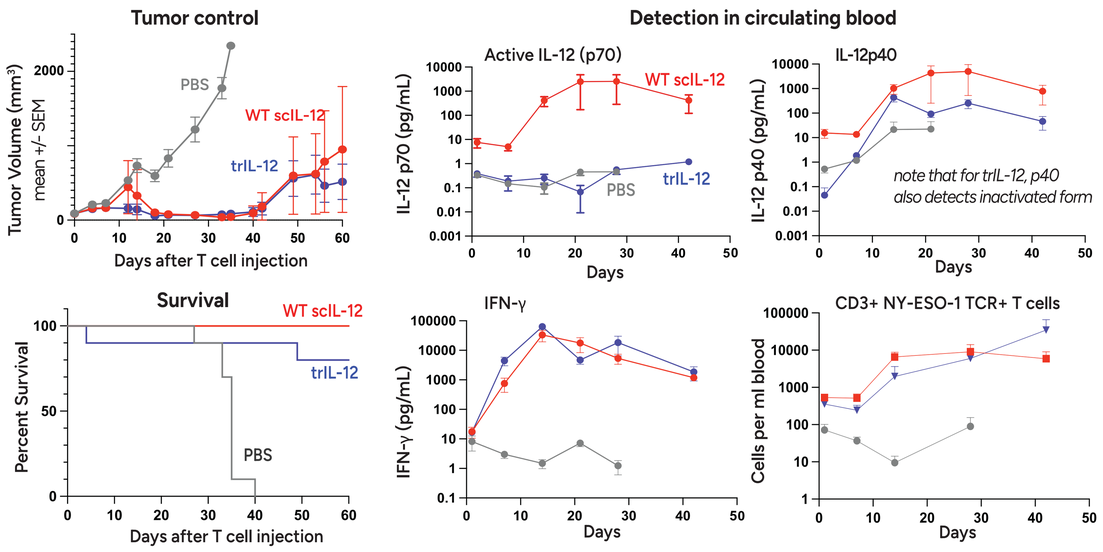


Figure 9. NY-ESO-1 TCR T cells with activation-induced WT scIL-12 (red) or trIL-12 (blue) were infused i.v. into A375 tumor-bearing NSG MHCI/II KO mice (N=10). Tumor volume was measured by caliper (twice-weekly); peripheral T-cell counts were measured by flow cytometry (weekly) and serum cytokine concentrations were measured by MSD.

Anti-tumor efficacy in a B16 syngeneic tumor model

trIL-12 maintains efficacy and reduces systemic IL-12 and IFN-γ exposure

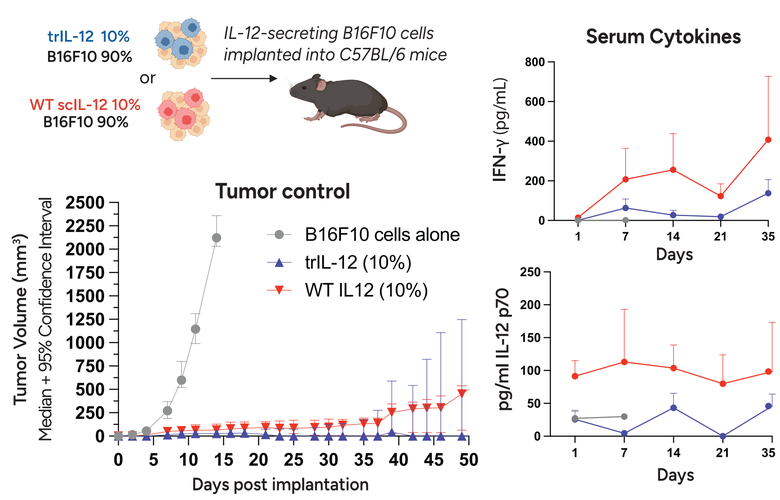


Figure 10. B16F10 tumor cells were engineered to express mouse surrogates of either WT scIL-12 or trIL-12. C57/BL6 mice (N=10) were implanted with a mixture of 90% parental B16F10 and 10% cytokine-expressing B16F10 cells. Tumor growth was measured using calipers and serum cytokines were measured using MSD.

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