Tumor-infiltrating lymphocytes (TIL) with inducible and membrane-bound IL-12 exhibit superior anti-tumor activity *in vitro*

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Background

- Tumor-infiltrating lymphocyte (TIL) cell therapy has shown clinical benefit for patients with solid tumors[1,2]
  - However, an immunosuppressive tumor microenvironment (TME) may abrogate the full potential of TIL cell therapy[3]
- The proinflammatory cytokine IL-12, known to increase IFN-γ production and promote type 1 immune responses, reshapes the TME and has the potential to augment anti-tumor activity[4]
- Engineered TIL secreting IL-12 showed clinical benefit, although circulating IL-12–related AEs limited its further development[5]
  - Therefore, enabling IL-12 expression on the surface of therapeutic T cells has the potential to attenuate IL-12 toxicity[6]
- Here, we report on the development, characterization, and cytotoxic activity of genetically engineered TIL with inducible and membrane-bound IL-12, incorporated into a 22-day TIL manufacturing process

Methods

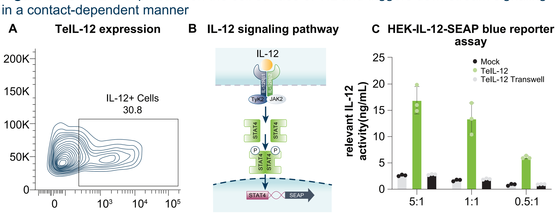
- Tissues from several solid tumor histologies including non-small cell lung cancer, breast cancer, head & neck cancer, and ovarian cancer were fragmented and cultured for 11 days (pre-rapid expansion protocol [pre-REP])
- The tumor tissue cultures were then transduced with a lentivirus containing a gene encoding membrane-bound IL-12 via an EF-1α promoter (TeIL-12; constitutively expressing TeIL-12 TIL) or an NFAT-promoter (NFAT-TeIL-12; inducible TeIL-12 TIL), and underwent a subsequent 11-day expansion period via a rapid expansion protocol (REP) (**Figure 1**).
  - Mock (without transduction) and pLenti-control (pLV ctrl) virally transduced TIL were simultaneously evaluated as controls

Figure 1. Generation of TeIL-12 genetically engineered TIL

- The expression, biological function, and shedding of IL-12 molecules were assessed *in vitro*
- TeIL-12 genetically engineered TILs were characterized by flow cytometry
- *In vitro* cytotoxic activity of TeIL-12 genetically engineered TIL was evaluated using the KILR®-THP-1 (Eurofins DiscoverX, Fremont, CA, USA) and xCELLigence® (Agilent Technologies, Santa Clara, CA) assays

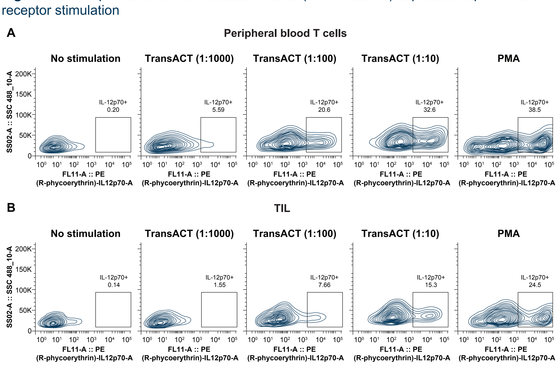
Results

Figure 2. TeIL-12 is expressed on the cell surface of TIL and triggers downstream signaling in a contact-dependent manner



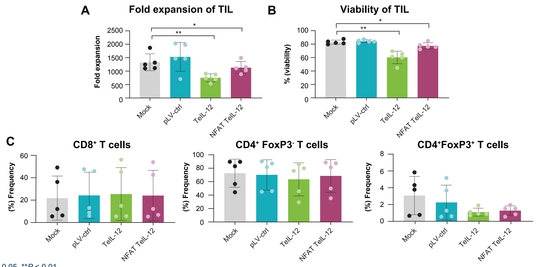
A. Surface expression of TeIL-12 on REP-TIL was examined by flow cytometry. **B.** TeIL-12–expressing TIL can activate downstream signaling through IL-12 receptor on **C.** HEK-IL-12-SEAP blue reporter cells, but activation was disrupted when cell-cell interaction was blocked with a transwell, indicating a lack of IL-12 shedding

Figure 3. NFAT promoter drives inducible TeIL-12 (NFAT-TeIL-12) expression upon T-cell receptor stimulation



Inducible surface expression of TeIL-12 on transduced **A.** peripheral blood T cells and **B.** TIL was examined by flow cytometry after stimulation with increasing titers of TransACT and the T-cell activator PMA

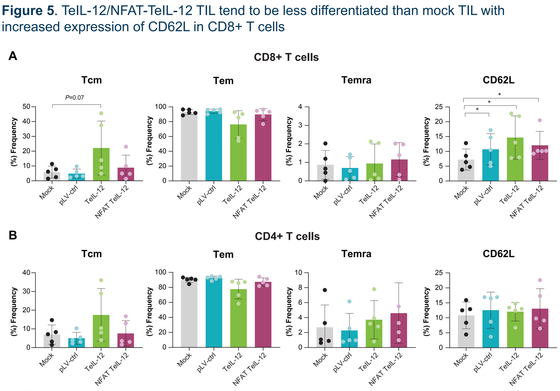
Figure 4. Expansion, viability, and T-cell subsets in TIL product



*P < 0.05, **P < 0.01.

A. Fold expansion, **B.** viability, and **C.** distribution of T-cell subsets ($CD8^+$ T cells, $CD4^+FoxP3^-$ T cells, and $CD4^+FoxP3^+$ T cells) in mock, pLenti-control, TeIL-12 transduced REP-TIL, and NFAT-TeIL-12 transduced REP-TIL

Figure 5. TeIL-12/NFAT-TeIL-12 TIL tend to be less differentiated than mock TIL with increased expression of CD62L in CD8+ T cells

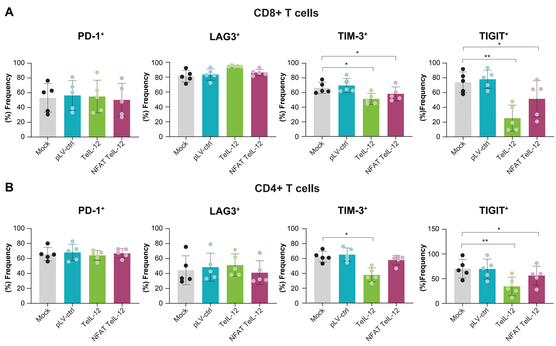


*P < 0.05.

T-cell differentiation status was examined in mock, pLenti-control, TeIL-12, and NFAT-TeIL-12 transduced TIL. Frequencies of Tcm ($CD45RA^-CCR7^+$), Tem ($CD45RA^-CCR7^-$), Temra ($CD45^+CCR7^-$), and $CD62L^+$ cells in **A.** $CD8^+$ and **B.** $CD4^+$ TIL are shown

Results

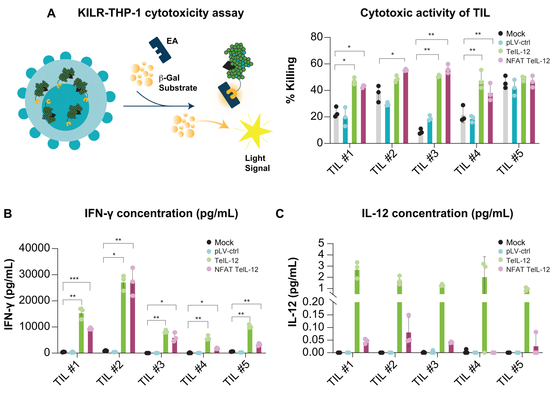
Figure 6. TeIL-12/NFAT-TeIL-12 TIL show reduced TIM-3 and TIGIT expression compared with mock TIL



*P < 0.05, **P < 0.01.

T-cell exhaustion status was examined in mock, pLenti-control, TeIL-12, and NFAT-TeIL-12 transduced TIL Frequencies of PD-1$^+$, LAG3$^+$, TIM-3$^+$, and TIGIT$^+$ cells in **A.** $CD8^+$ and **B.** $CD4^+$ TIL are shown

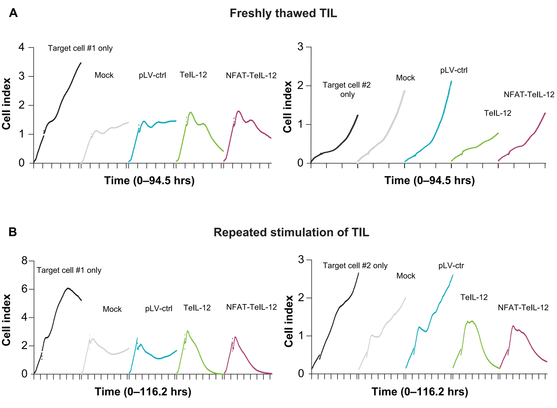
Figure 7. KILR-THP-1 assay shows enhanced cytotoxic activity of TeIL-12/NFAT-TeIL-12 TIL



*P < 0.05, **P < 0.01, ***P < 0.001

A. Cytotoxicity evaluation by the KILR-THP-1 assay. Cryopreserved control and genetically engineered TIL were thawed and cocultured for 24 hours with KILR-THP-1 cells (Eurofins DiscoverX, Fremont, CA, USA) at a 10:1 E:T cell ratio to measure cytotoxicity in an allogeneic setting. Concentrations of **B.** IFN-γ and **C.** soluble IL-12 in the coculture supernatant were examined by ELISA

Figure 8. xCELLigence assay shows enhanced cytotoxic activity of TeIL-12/NFAT-TeIL-12 TIL



A. Cytotoxicity assessment by the xCELLigence assay. Cryopreserved control and genetically engineered TIL were thawed and added into E-plates, pre-seeded with cells from two different tumor cell lines: Target cells #1 (left) and Target cells #2 (right), at a 3:1 E:T cell ratio to measure cytotoxicity in an allogeneic setting. **B.** To evaluate serial cytotoxic activity, TIL were stimulated every 3 days with TransACT (1:100); 1 day after repeated stimulation (× 3), TIL were washed and assessed for cytotoxic activity

Conclusions

- Both TeIL-12 and NFAT-TeIL-12 show superior *in vitro* cytotoxic activity with pronounced IFN-γ production; superior anti-tumor cytotoxicity was maintained after repeated TransACT stimulation
- Both TeIL-12 and NFAT-TeIL-12 show a favorable T cell phenotype with increased expression of CD62L and decreased expression of TIM-3 and TIGIT
- TeIL-12 shows a good safety profile with minimal shedding of IL-12; however, NFAT-TeIL-12 demonstrates the potential for a better safety profile due to less shedding of IL-12 relative to TeIL-12 in co-culture systems
- Considering the systemic toxicities observed due to secreted IL-12 in a prior NFAT-IL-12 TIL trial with a TIL product manufactured using a different process[5], a tethered IL-12 combined with a NFAT-inducible construct offers a double safety TIL design feature to ensure NFAT-TeIL-12 TIL are safe in future first-in-human clinical trials
- IL-12 expression systems were successfully incorporated into the 22-day TIL manufacturing process
- Overall, the efficacy and safety data support further clinical development of NFAT-TeIL-12 alone or in combination with other approaches to enhance TIL potency

References

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Abbreviations

CCR7, chemokine (C-C motif) receptor 7; ELISA, enzyme-linked immunosorbent assay; E:T, effector to target ratio; IFN-γ, interferon gamma; IL-12, interleukin 12; LAG3, lymphocyte activation gene 3; NFAT, nuclear factor of activated T cells; PMA, phorbol 12-myristate 13-acetate; PD-1, programmed cell death protein-1; REP, rapid expansion protocol; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; Tcm, central memory T cells; Tem, effector memory T cells; Temra, effector memory RA T cells; TIL, tumor-infiltrating lymphocytes; TME, tumor microenvironment.

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Disclosures

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