Tumor Infiltrating Lymphocytes Expressing Membrane-bound IL-2 and IL-12 Exhibit Enhanced Proliferation, Function, and Persistence Without Requiring Exogenous IL-2 Support

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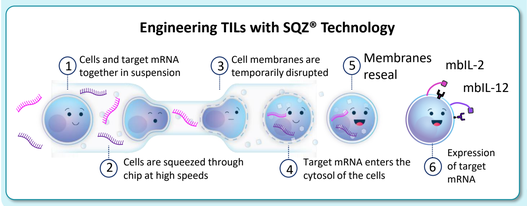
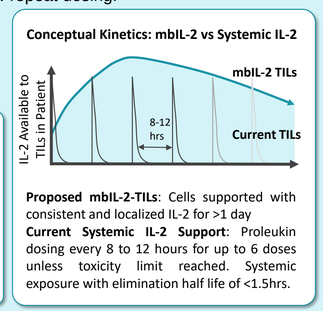
Overview

Background: Tumor Infiltrating Lymphocyte (TIL) therapies have shown significant solid tumor activity in patients, but many current TIL regimens require lymphodepletion and high-dose IL-2 after cell infusion. Removing these requirements that cause systemic toxicity while maintaining TIL product functionality by ***ex vivo* engineering of the TIL product with transient expression of mRNA** could dramatically improve the patient experience, expand the eligible patient population, and allow repeat dosing.

Methods: mRNAs encoding for membrane-bound (mb) IL-2 or IL-12 were delivered directly to the cytosol of TILs expanded from either **melanoma, lung, or ovarian solid tumors** using the microfluidic Cell Squeeze® technology.

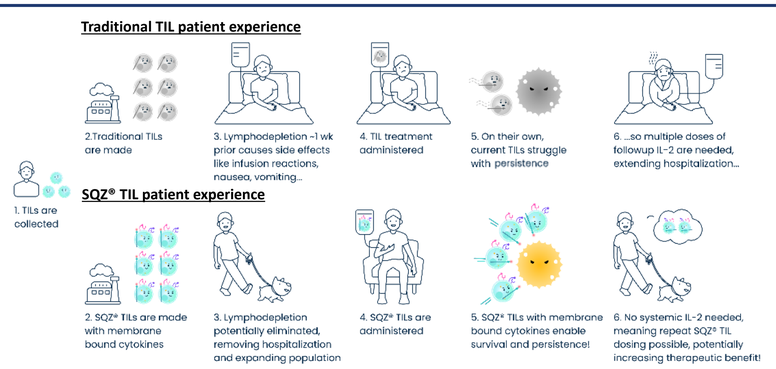




Proposed mbIL-2-TILs: Cells supported with consistent and localized IL-2 for >1 day

Current Systemic IL-2 Support: Proleukin dosing every 8 to 12 hours for up to 6 doses unless toxicity limit reached. Systemic exposure with elimination half life of <1.5hrs.

Vision: SQZ® TILs eliminate need for toxic preconditioning and systemic IL-2 support



Kinetics of mRNA expression, viability, and proliferation in squeezed TILs

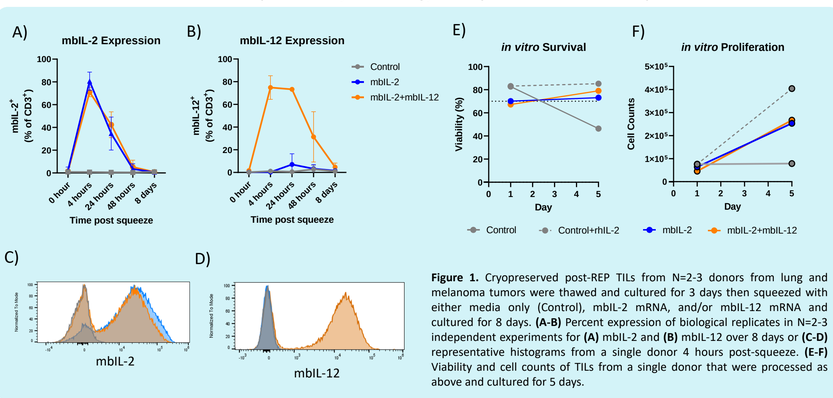


Figure 1. Cryopreserved post-REP TILs from N=2-3 donors from lung and melanoma tumors were thawed and cultured for 3 days then squeezed with either media only (Control), mbIL-2 mRNA, and/or mbIL-12 mRNA and cultured for 8 days. **(A-B)** Percent expression of biological replicates in N=2-3 independent experiments for **(A)** mbIL-2 and **(B)** mbIL-12 over 8 days or **(C-D)** representative histograms from a single donor 4 hours post-squeeze. **(E-F)** Viability and cell counts of TILs from a single donor that were processed as above and cultured for 5 days.

mbIL-2 binds IL-2R and drives STAT5 phosphorylation and proliferation without rhIL-2

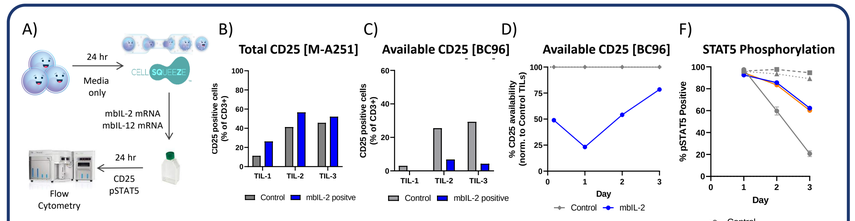


Figure 2. Cryopreserved post-REP TILs from melanoma and ovarian tumors were thawed and cultured for 3 days in rhIL-2 and squeezed with either media only (Control), mbIL-2 mRNA, and/or mbIL-12 mRNA and cultured for 3 days. **(A)** Experimental layout. **(B-C)** Percent expression of N=3 donors for **(B)** Total CD25 [clone M-A251] and **(C)** Available CD25 [clone BC96] at 4 hours post-squeeze and sub-gated on mbIL-2 positive TILs (mbIL-2 positive). **(D)** Available CD25 [clone BC96] in a representative donor over time. **(E)** Phosphorylated STAT5 from an individual donor processed as above and cultured for 3 days.

mbIL-12 TILs upregulate CD62L and CD127, markers of central memory T cells (T_{CM})

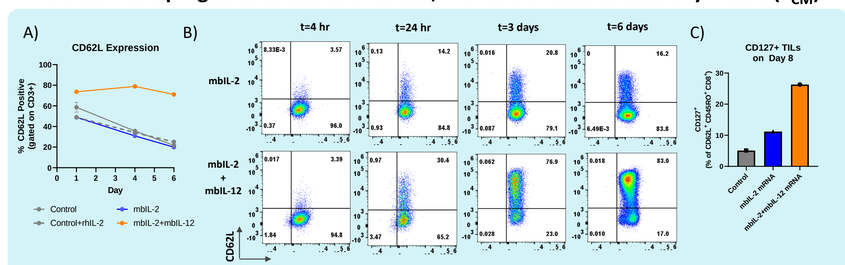


Figure 3. Cryopreserved post-REP TILs from a (A) melanoma tumor or (B) ovarian tumor were thawed and cultured for 3 days in rhIL-2 and squeezed with either media only (Control; solid line) and cultured with or without exogenous cytokine support (Control+rhIL-2; dashed line) or squeezed with mbIL-2 mRNA and/or mbIL-12 mRNA and cultured for 6 days. **(A)** Percent expression of a representative donor from at least N=3 independent experiments for CD62L expression. **(B)** Representative dot plots of a single donor from at least N=3 independent experiments gated on CD8-positive cells and sub-gated on CD45RO (*x-axis*) by CD62L (*y-axis*). **(C)** Fresh post-REP TILs from lung and melanoma tumors were squeeze processed as above and immediately cryopreserved, then thawed and cultured for 8 days. Percent expression of a representative donor for CD127 expression sub-gated on CD8/CD62L/CD45RO triple-positive cells.

mbIL-2/12 TILs release increased IFN-γ during autologous tumor co-culture

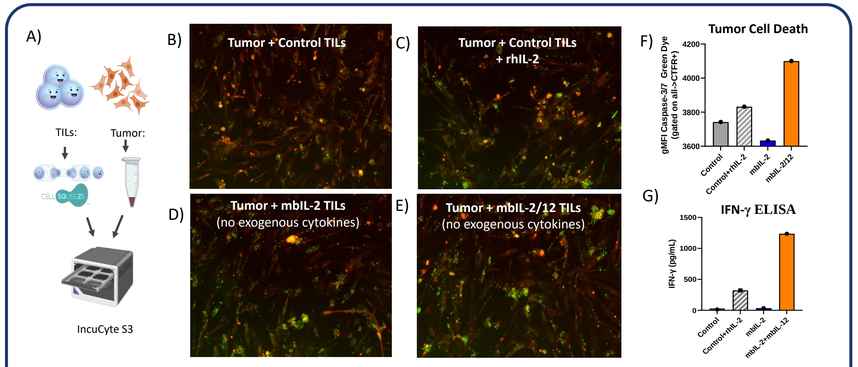


Figure 4. Cryopreserved post-REP TILs from an ovarian tumor were thawed and cultured for 3 days in rhIL-2 and were either not squeeze processed and cultured with (Control+rhIL-2) or without (Control) rhIL-2 support or squeezed with mbIL-2 mRNA (mbIL-2) and/or mbIL-12 mRNA (mbIL-2+mbIL-12) and cultured with donor-matched primary autologous tumor cells. **(A)** Experimental layout, image partially made with BioRender. **(B-E)** Representative images from at least N=3 independent experiments after 24 hours of co-culture between 1E4 tumor cells (red) and 5E4 post-REP TILs (undyed) per well. Images were taken on an IncuCyte S3 fluorescent imager with a Caspase-3/7 dye (green) to mark dying/dead cells. **(F)** Caspase-3/7 Dye brightness when sub-gating for tumor cells (red; CellTrace Far Red; CTFR) via flow cytometry at 24 hr. **(G)** IFN-γ content from the supernatant of wells imaged in (B-E).

mbIL-2/12 TILs exhibit memory-like phenotype change in mouse adoptive transfer

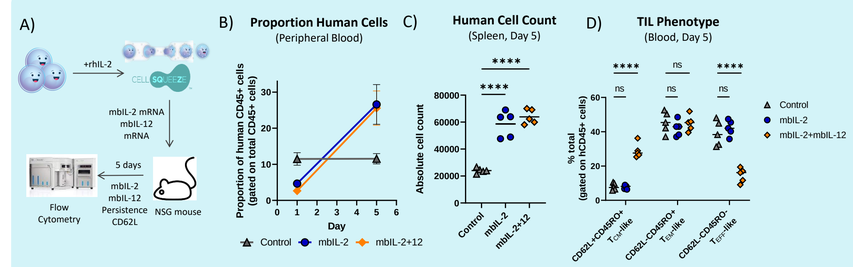


Figure 5. Cryopreserved post-REP TILs from a single ovarian tumor were thawed and cultured for 3 days in rhIL-2 and squeezed with either media only (Control) or mbIL-2 mRNA (mbIL-2) and/or mbIL-12 mRNA (mbIL-2+mbIL-12) and 5M TILs per mouse injected *i.v.* into immunodeficient NOD SCID gamma (NSG) mice. A peripheral bleed was performed at day 1 post-transfer and a terminal bleed and spleen harvest were performed at day 5 post-transfer. Cells were isolated and quantified via flow cytometry. **(A)** Experimental design. **(B)** Percent human CD45-positive cells when sub-gated on total CD45 cells in the blood. **(C)** Absolute cell count of human CD45-positive cells in the spleen on day 5. **(D)** Percent positive for CD62L and/or CD45RO expression when sub-gated on human CD45-positive cells in the blood on day 5. (C-D) Statistical significance was determined via (C) a One-way ANOVA with Dunnett's post-test, or (D) a Two-way ANOVA with Dunnett's post-test. Ns = not significant, **** = p<0.0001. **Note:** This is a representative donor; variability is seen across donors.

Future Targets: Pre-conditioning cytokine IL-7 and pro-survival protein Bcl-2

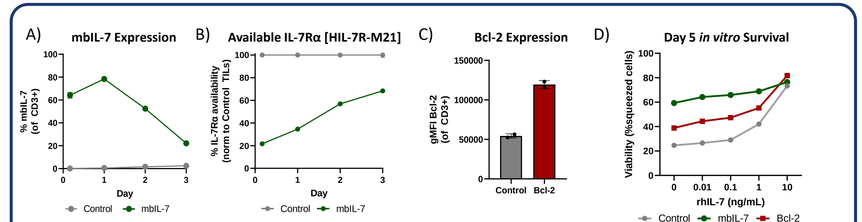
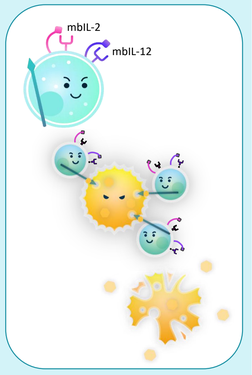


Figure 6. Cryopreserved post-REP TILs from N=1-2 donors from melanoma tumors were thawed and cultured for 3 days in rhIL-2 and squeezed with either media only (Control), mbIL-7 mRNA (mbIL-7), or Bcl-2 mRNA (Bcl-2) and cultured for 1-5 days. **(A)** Percent mbIL-7 expression and **(B)** Available IL-7R-alpha (CD127) in a representative donor over 3 days culture post-squeeze processing. **(C)** Geometric mean fluorescence intensity (gMFI) of Bcl-2 in TILs a single donor measured 24 hours post-squeeze processing. **(D)** Viability of TILs from a single donor measured 5 days post-squeeze processing and cultured with or without rhIL-7.

Engineered mbIL-2/12 Tumor Infiltrating Lymphocytes: Summary

- Through microfluidic **Cell Squeeze®** delivery of mRNAs, we **engineered primary human TILs** with transient expression of membrane-bound cytokines IL-2 and IL-12
- mbIL-2 signals through endogenous IL-2R to phosphorylate STAT5 and drive survival and **proliferation in the absence of exogenous IL-2 support**
- mbIL-12 induces upregulation of CD62L, a marker of central memory T cells which are a T cell subtype known to confer **superior antitumor immunity**
- **mbIL-2/12 TILs upregulate CD62L *in vivo* for at least 5 days** in the blood and spleen of a humanized NSG mouse
- **mbIL-2/12 TILs release more IFN- γ than Control TILs + rhIL-2** (positive control; current clinical standard) when co-cultured with patient-matched tumor cells



Overall, enhanced SQZ® TILs can potentially alleviate current requirements for toxic high-dose IL-2 support and lymphodepleting preconditioning regimens.

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Declaration of Interest: Silver, RJ is an employee and stockholder of SQZ Biotechnologies, Watertown MA, USA