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Abstract #440

Utilizing Stim-R™ Technology to Reduce Irradiated Feeder Cell Use in the Tumor Infiltrating Lymphocyte Culture Process

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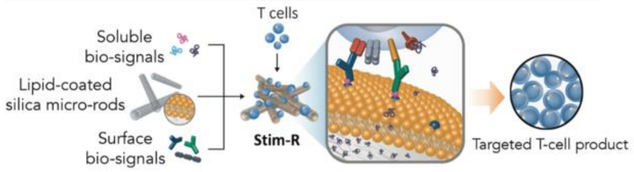
Background

- Tumor-infiltrating lymphocyte (TIL) therapy can mediate effective responses in solid tumors and is currently FDA approved for metastatic melanoma in patients previously treated with other therapies.
- Conventional TIL manufacturing processes require the use of "feeder cells", irradiated allogeneic peripheral blood mononuclear cells (PBMCs).
  - Sourcing feeder cells from qualified donors may incur logistical and financial obstacles[1].
  - Feeder cells introduce donor-to-donor variability[1].
- Stim-R™ is a customizable and degradable biomimetic emulating physiologic, cell-like presentation of signal molecules (**Fig. 1**) and may be used as a replacement for feeder cells during TIL manufacturing.

Methods

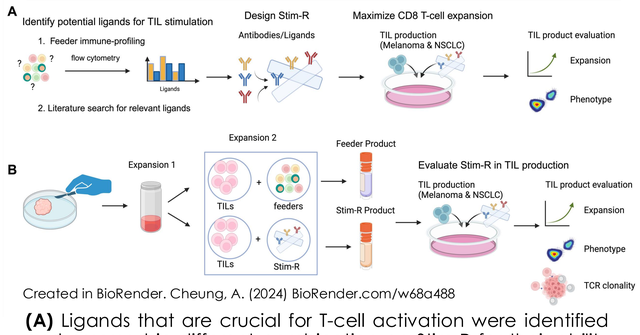
Figure 1: The Stim-R technology is a programmable cell-signaling platform for feeder cell replacement



The Stim-R technology comprises biodegradable lipid-coated silica micro-rods that can present multiple signals in precise densities and stoichiometries[2] in a way that mimics physiologic presentation.

- Feeder cells were analyzed using flow cytometry to assess the expression of potential T-cell stimulating ligands[3,4,5]. Based on these data, Stim-R formulations were designed to present these ligands in various combinations and stoichiometries (**Fig. 2A**).
- Control TILs were produced using Lyell's Epi-R™ P2[6] protocol at research scale. Stim-R was utilized as a replacement for feeder cells in "expansion 2" (**Fig. 2B**). A matrix of assays was used to compare the performance of Stim-R to feeder cells:
  - TIL expansion and phenotype measured by automated cell counter & spectral cytometer.
  - T-Cell Receptor (TCR) sequencing to assess polyclonality and retention of putative tumor-reactive cells (PTRCs) in products. (PTRCs are cells that have TCRs identified from high-frequency T-cell clones in the starting tumor material)[7].

Figure 2: Stim-R for TIL formulation design and screening workflow to assess product attributes[8,9]



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(A) Ligands that are crucial for T-cell activation were identified and assessed in different combination on Stim-R for their ability to expand CD8⁺ T cells in "expansion 2". Ref. (**Fig. 3 & 4**)

(B) An optimized formulation of Stim-R was further benchmarked against the standard Epi-R P2 protocol in terms of CD8 T-cell stemness, polyclonality, and the retention of PTRCs. Ref. (**Fig. 5, 6 & 7**)

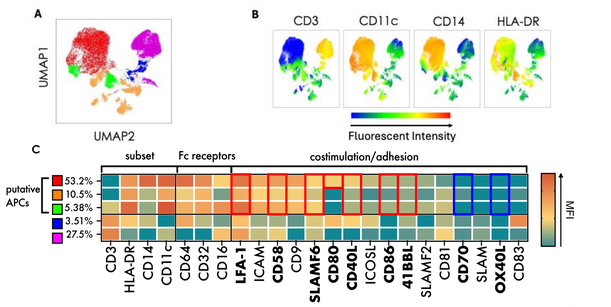
Results

Key Findings:

Stim-R (EXT221) is able to replace feeder cells in "expansion 2" of Epi-R P2 and produce TIL products with:

- Comparable expansion of CD8⁺ T cells.
- Similar stemlike characteristics measured by the percentage of $CD62L^+CD27^+$ and $CD39^-CD69^-$ cells in the CD8⁺ T-cell population.
- Comparable polyclonality, measured by the Simpson Clonality Index.
- Proportional putative tumor reactive cells (PTRCs).

Figure 3: Putative Antigen Presenting Cells (APCs) in bulk feeder cell population express various costimulatory and adhesion molecules potentially important for T-cell activation

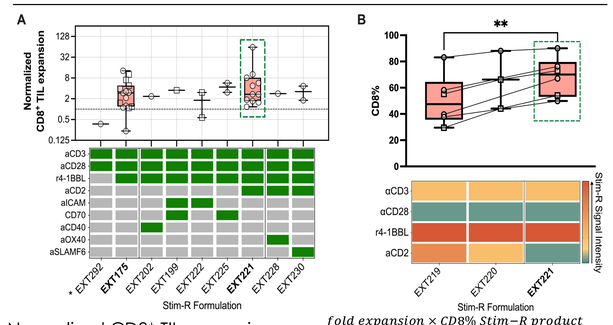


Feeder cells pooled from three healthy donors were profiled using flow cytometry.

(A) & **(B)** Putative APCs, the hypothetical stimulatory population in the feeder cells, were identified based on high HLA-DR and low CD3 expression.

(C) Heat map of the expression of various differentiation, Fc receptors, and costimulatory/adhesion molecules on the surface of the APC population.

Figure 4: Screening based on CD8 T-cell expansion identified an optimal combination of ligands

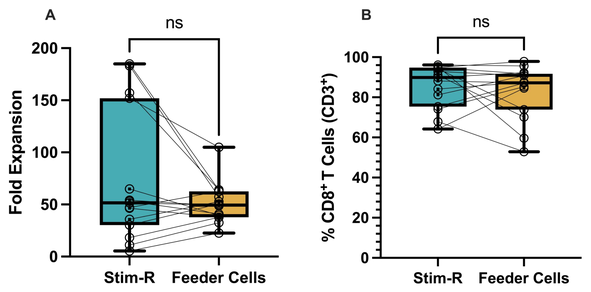


$$\text{Normalized CD8}^+\text{ TIL expansion} = \frac{fold\ expansion \times CD8\%\ Stim\text{-}R\ product}{fold\ expansion \times CD8\%\ Epi\text{-}R\ P2\ feeder\ product}$$

(A) Preliminary experiments identified r4-1BBL signaling as critical to TIL expansion (EXT175). Subsequently, the addition of aCD2 signaling allowed for consistent normalized CD8⁺ TIL expansion over a diverse set of tumors (EXT221). The * designates that EXT292 was tried in n=3 additional tumors but expansion was not high enough to obtain a CD8⁺ percentage from the TIL product.

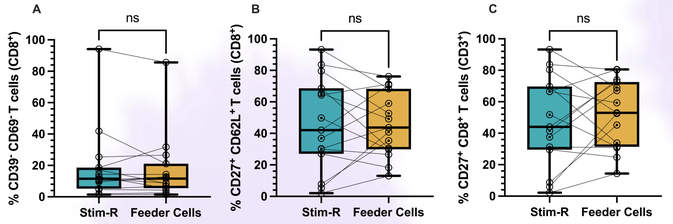
(B) Titration of aCD2 on Stim-R was performed to optimize aCD2 intensity. EXT221 was identified as the optimal formulation for feeder replacement based on higher CD8 T cell percentage in the expanded product.

Figure 5: Utilizing Stim-R in "expansion 2" of Epi-R P2 maintains equivalent CD8⁺ T-cell expansion



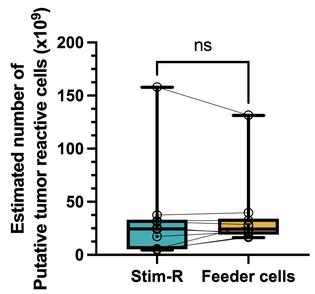
(A) & **(B)** Comparison of fold expansion and CD8⁺ percentage of TIL products stimulated with either Stim-R or Feeder cells in "expansion 2" of TIL production. Statistical significance was determined via paired t-test, n=14.

Figure 6: Utilizing Stim-R in "expansion 2" of Epi-R P2 maintains comparable stemness of expanded product



(A), **(B)** & **(C)** Comparison of stemness phenotype of TIL products stimulated with either Stim-R or Feeder cells in "expansion 2" of TIL production. Statistical significance was determined via a paired t-test, n=14.

Figure 7: Using Stim-R in place of feeder cells in the "expansion 2" step of Epi-R P2 results in a polyclonal product that retains a similar number of estimated putative tumor reactive cells



Range of Simpson Clonality

Stim-R products	Feeder cell products
0.02 - 0.138	0.04 – 0.236

Estimated number of PTRCs in TIL products that were activated with either Stim-R or feeder cells in "expansion 2." A paired t-test was run; no statistical difference was observed (n=10). Estimated number of PTRCs was calculated by extrapolating the number of PTRCs detected to a clinical scale production. Both methods of stimulation resulted in a polyclonal product; the range of Simpson Clonality across all samples in both groups is shown.

Conclusions

- A Stim-R formulation engaging CD2, CD3, CD28 and 4-1BB, designated "EXT221", was identified based on its ability to mediate robust CD8⁺ TIL expansion in "expansion 2" of the Epi-R P2 protocol.
- Stemlike qualities, polyclonality, and number of PTRCs were similar in the TIL products expanded with Stim-R compared to control TILs.
- Our results suggest that Stim-R is a viable replacement for feeder cells in "expansion 2" of the Epi-R P2 manufacturing protocol, and has the potential to overcome limitations that may arise in feeder cell-mediated TIL expansion.

Acknowledgments

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