Epi-R™ P2 Protocol Produces a Scalable Polyclonal TIL Product With a Greater Expansion Success Rate Across Hot and Cold Tumors in Shorter Culture Time

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Abstract 379

Objectives

TIL therapy

- Tumor-infiltrating lymphocyte (TIL) therapy has shown promising efficacy in select solid tumor types, including in patients treated with checkpoint inhibitors (CPI)[1–3]
- TIL products with higher proportions of stem-like T cells, tumor antigen recognition, and clonal diversity may result in improved clinical outcomes[4,5]
- Conventional TIL production protocols include long culture times (4–6 weeks) that may reduce TIL stemness and TCR diversity[6,7]
- Shorter culture duration may be correlated with longer telomere length, increased stemness, improved persistence, and positive clinical outcomes in patients with metastatic melanoma[6,7]
- Epi-R manufacturing protocols control T-cell activation and differentiation by optimizing cell culture media and other manufacturing steps, resulting in preservation of stem-like qualities (**Table 1**)[8,9]

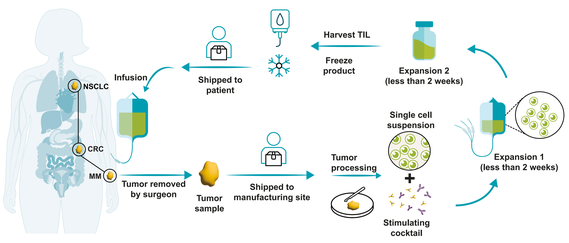
Table 1: Comparison of TIL manufacturing protocols

	Standard	Epi-R P1	Epi-R P2
Time	++++	+++	++
Media	AIM-V™/Optimizer	Epi-R™	Epi-R™
Allogeneic feeder	++++	++++	+
CD8/CD4	++	++++	++++
Stemness	+	++++	++++
Polyclonality	++	++	+++

Methods

- Tissue from 21 donors across tumor types, including CPI-refractory metastatic melanoma, NSCLC, and CRC
  - TIL were produced from these tumors using either the Epi-R P1 or Epi-R P2 protocols (**Figure 1**)
- Characteristics of the resulting products (Epi-R P1 and Epi-R P2) were compared using a matrix of assays including:
  - Flow cytometry to measure markers of stemness
  - Co-culturing with an autologous tumor cell line/tumor digest
  - TCRβ sequencing to assess polyclonality

Figure 1: Planned manufacturing schematic for Lyell's Epi-R P2 protocol



Results

Epi-R P2 protocol reduced the duration of culture time without compromising the favorable phenotype of the product

- The Epi-R P2 protocol shortened TIL manufacturing time to less than 25 days
- Epi-R P2 initial expansion phase lasted less than 2 weeks and yielded significantly more TIL compared with the Epi-R P1 initial expansion phase (approximately 2 weeks) in CPI-naïve metastatic melanoma, CRLM, NSCLC (**Figure 2A**), and in CPI-refractory metastatic melanoma (**Figure 2B**)
  - In the selected tumor types (n = 21), the Epi-R P2 initial expansion phase resulted in a median fold expansion of 37.44, with a median yield of 1.4 x 10^9 T cells
- After a second expansion step, the Epi-R P2 protocol produced an average of 6.0 x 10^{10} T cells in different tumor types including CPI-refractory metastatic melanoma, metastatic CRC, and NSCLC (**Figure 3**)

Figure 2: Epi-R P2 produced more TIL in a shorter amount of time

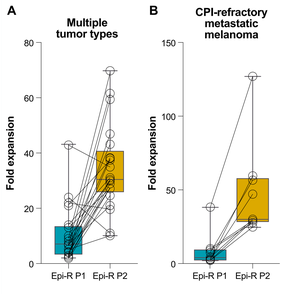
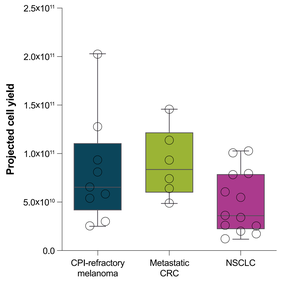


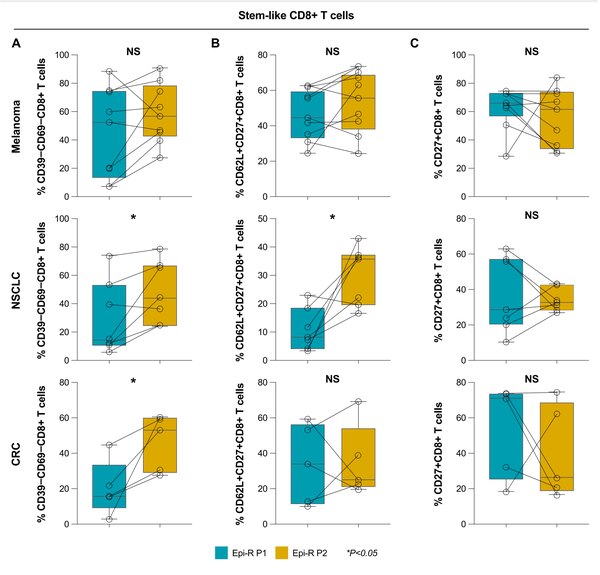
Figure 3: Epi-R P2 produced more TIL across multiple tumor types



Epi-R P2-derived TIL demonstrated comparable product profiles compared with Epi-R P1-derived TIL

- Epi-R P1 and Epi-R P2 protocols showed a similar proportion of CD8+ T cells in TIL products derived from CPI-refractory melanoma, NSCLC, and CRC (data not shown)
- Epi-R P2 T cells had retained or improved expression of stemness markers (CD39–CD69–; **Figure 4A**) and reduced differentiation (data not shown) across all tumor types
- Epi-R P2 T cells retained expression of stem-like markers including CD62L and CD27 (**Figure 4B–C**)

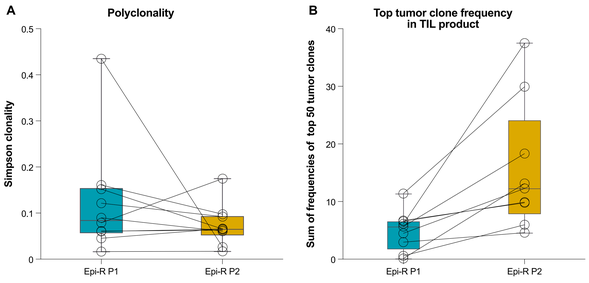
Figure 4: CD8+ product profiles of Epi-R P1 and Epi-R P2 TIL were comparable



Epi-R P2 reduced terminally differentiated cells and preserved the number of tumor-reactive clones present in the product

- The Epi-R P2 protocol preserved polyclonality (**Figure 5A**) and the top 50 clone frequencies in TIL products (**Figure 5B**)

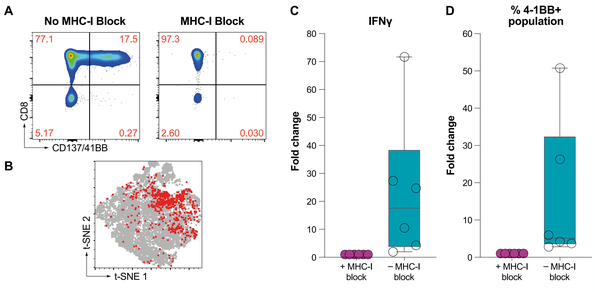
Figure 5: Tumor-reactive clones were preserved with the Epi-R P2 protocol



MHC-I blockade prevented TIL reactivity and effector functions

- **Figure 6A** shows representative flow cytometry of Epi-R P2 TIL incubated with or without MHC-I block and autologous NSCLC tumor cells
- CD3+ cells from four tumor samples (three NSCLC and one metastatic breast cancer) demonstrated clustering based on the expression of markers involved in T-cell differentiation, stemness, activation, and exhaustion (**Figure 6B**)
- CD8+ T cells demonstrated a greater fold change in IFNγ secretion and co-stimulatory molecules (**Figure 6C–D**)
- Specificity of the TIL reactivity activation and effector functions were completely blockable by anti–MHC-I antibody (**Figure 6C–D**).

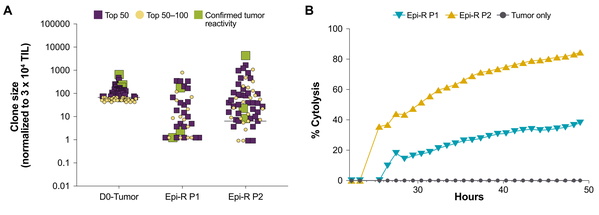
Figure 6: MHC-I blockade prevented TIL activation and cytokine secretion



Clones retained during the Epi-R P2 protocol demonstrated cytotoxicity against autologous tumor cell lines

- The putative tumor-reactive clones preserved using the Epi-R P2 protocol exhibited strong cytotoxicity of an autologous tumor cell line (**Figure 7**)

Figure 7: Epi-R P2 TIL preserved tumor-reactive clones and demonstrated cytotoxicity when cultured with autologous tumor cells



Conclusions

- Epi-R P2 demonstrated successful TIL expansion from both immunologically hot (metastatic melanoma and NSCLC) and cold (CRC) tumors and comparable product profiles to Epi-R P1 using a shortened process
- Epi-R P2 retained all the improved product characteristics associated with Epi-R P1 across several tumor types, including a greater proportion of stem-like T cells and an increase in the magnitude of the most frequent clones that were present in the baseline tumor
- The Epi-R P2 protocol is a promising method for manufacturing TIL products with retained antitumor activity in both immunologically hot and cold tumors

Abbreviations

4-1BB, tumor necrosis factor ligand superfamily member 9; CD, cluster of differentiation; CRLM, colorectal cancer liver metastases; CPI, checkpoint inhibitor; CRC, colorectal cancer; IFNγ, interferon gamma; MHC, major histocompatibility complex; MM, metastatic melanoma; NS, not significant; NSCLC, non-small cell lung cancer; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte; t-SNE, t-distributed stochastic neighbor embedding.

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