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

1. Results

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

- The Epi-R P2 protocol shortened TIL manufacturing time by less than 25 days.
- Epi-R P2 showed improved expansion kinetics since 2 weeks and selected significantly more TIL compared with the Epi-R P1 initial expansion phase (approximately 2 weeks) in CRB melanoma, metastatic melanoma, CRC, and NSCLC (Figure A), as well as in CRP-reactive melanoma (Figure B).
- In the selected tumor types (n = 21), the Epi-R P2 initial expansion phase resulted in a median fold of approximately 4-10 x 10^7 cells.
- After a second expansion step, the Epi-R P2 protocol produced an average of 4-10 x 10^7 cells in different tumor types including CRP-reactive melanoma, metastatic melanoma, CRC, and NSCLC (Figure C).

Figure 1: Comparison of TIL manufacturing protocols

Standard

Epi-R P1

Epi-R P2

Time

+++***

+++***

Media

Abbrevial/Optimizer

Epi-R™

Epi-R™

ATCC

Epi-R™

Epi-R™

Genotypic

Polyclonality

Epi-R™

Epi-R™

Table 1: Comparison of TIL manufacturing protocols

Table 2: Comparison of TIL manufacturing protocols

Results

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Figure 2: Epi-R P2 produced more TIL in a shorter time compared with Epi-R P1

Figure 3: Epi-R P2 derived TIL demonstrated comparable product profiles with Epi-R P1-derived TIL

- Epi-R P1 and Epi-R P2 products showed a similar proportion of CD62L+CD27+CD8+ T cells in TIL products derived from CRP-reactive melanoma, NSCLC, and CRC (data not shown).
- Epi-R P2 TIL cells had retained or improved expression of immunoregulatory markers CD20 (Figure A) and reduced activation markers (data not shown) across all tumor types.
- Epi-R P2 TIL cells retained expression of stem-like markers including CD133 and CD105 (Figure B).

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

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

- The putative tumor-reactive clones preserved using the Epi-R P2 protocol exhibited strong cytotoxicity of autologous NSCLC tumor cells.
- MHC-I blockade prevented TIL reactivity and effector functions.

Figure 6: MHC-I blockade prevented TIL reactivity and effector functions

- Figure 7A shows representative flow cytometry of Epi-R P2TIL incubated with or without MHC-I-block and autologous NSCLC tumor cells.
- CD39+ cells from four tumor samples (three NSCLC and one metastatic breast cancer) demonstrated a preserved CD8+ T cell population across a range of products involved in T-cell dysfunction, inflammation, activation, and exhaustion.

Figure 8: 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 polyvalency (Figure A) and the top 50 clone frequencies in TIL products (Figure B).

Figure 9: Epi-R P2 protocol preserved polyvalency

Figure 10: Top 50 clone frequencies in TIL products

<table>
<thead>
<tr>
<th>Clone Size (log10)</th>
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<th>Epi-R P2</th>
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Conclusions

- Epi-R P2 demonstrated successful expansion from both immunologically hot (melanocytic melanomas and NSCLC) and cold (CRC) tumors and comparable product profiles to Epi-R P1 using a shortened process.
- After a second expansion step, the Epi-R P2 protocol produced an average of 4-10 x 10^7 cells in different tumor types including CRP-reactive melanoma, metastatic melanoma, CRC, and NSCLC.
- Epi-R P2 showed improved expansion kinetics since 2 weeks and selected significantly more TIL compared with the Epi-R P1 initial expansion phase (approximately 2 weeks) in CRB melanoma, metastatic melanoma, CRC, and NSCLC (Figure A), as well as in CRP-reactive melanoma (Figure B).
- In the selected tumor types (n = 21), the Epi-R P2 initial expansion phase resulted in a median fold of approximately 4-10 x 10^7 cells.

Acknowledgments

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