Development of A Feeder-Free Process for IL-2-Independent TIL Expansion

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Abstract AMA362

Background

Tumor-infiltrating lymphocyte (TIL) therapy demonstrates significant promise in treating various solid tumors, recently gaining FDA approval for advanced melanoma. The conventional TIL expansion process relies heavily on high-concentration interleukin-2 (IL-2) and feeder cells (irradiated peripheral blood mononuclear cells from healthy donors), rendering TILs dependent on these factors for survival and function. Consequently, high-dose IL-2 is required post-infusion to maintain TIL potency and persistence. This high-dose IL-2, coupled with the need for high-intensity lymphodepletion to reduce pro-tumor regulatory T cells (Tregs), which are also sensitive to IL-2, is associated with significant toxicity, including high mortality rates and frequent intensive care unit (ICU) admissions. Additionally, the use of feeder cells carries inherent risks such as infection transmission, variability in cultivation and supply chain, and high associated costs.

Methods

- We developed a robust TIL culture platform (DeepTIL®) utilizing a chemically defined medium that eliminates the requirement for high-concentration IL-2 and feeder cells.
- TILs were derived from multiple solid tumors, including melanoma, cervical, ovarian, endometrial, and several other cancers.
- Final harvested TILs were assayed for total viable cells, viability using an automated cell counter.
- Flow cytometry was employed to characterize TILs, including identity, T-cell content, TIL memory subset, and exhaustion status.
- In vitro potency assays were assessed using homogeneous time-resolved fluorescence (HTRF) for interferon-γ (IFN-γ) secretion and a real-time cell analyzer against syngeneic patient-derived organoid (PDO) for cytotoxicity.
- In vivo potency was evaluated using patient-derived xenograft (PDX) models, without IL-2 administration.

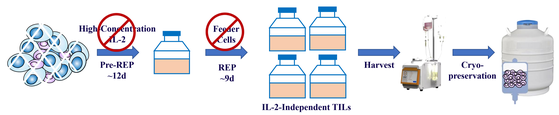


Figure 1. Juncell's DeepTIL® cell expansion platform

Results

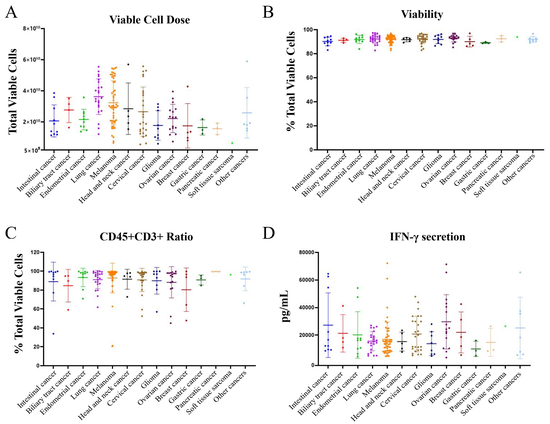


Figure 2. Viable Cell Dose, Viability, Identity, and Potency of TIL Product .

The feeder-free TIL manufacturing process was robust across tumor types, with an average total viable cell number of 28.6 billion (Figure 2A) and viability of 92.02% (Figure 2B). The TIL product was mainly composed of $CD45^+CD3^+$ cells with an average proportion of 91.05% (Figure 2C) and exhibited high IFN-γ secretion levels (Figure 2D).

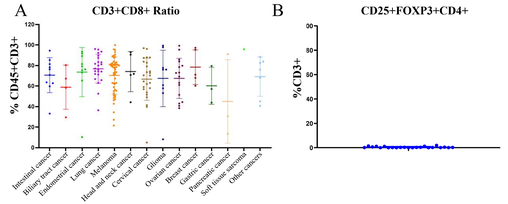


Figure 3. T-Cell Content of TIL Product.

The majority of TILs were $CD8^+$ T cells (Figure 3A). The average proportion of Treg cells was below 0.5% (Figure 3B), since the percentage of CD4+ T cells was generally low, not all samples were tested for Treg cells.

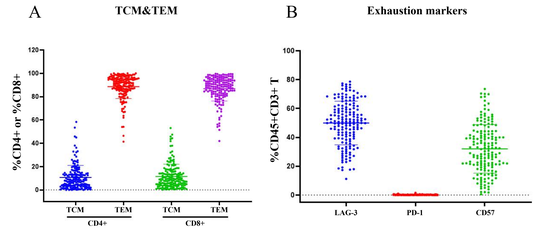
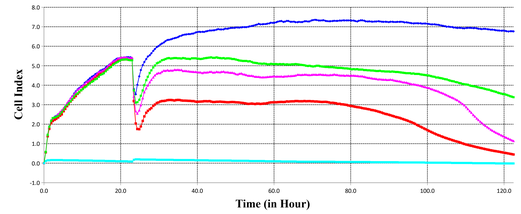


Figure 4. Memory and Exhaustion Phenotypes of $CD3^+$ T Cells.

The $CD3^+$ T cells were predominantly memory T cells, including central memory (TCM, average proportion was 10.11%) and effector memory (TEM, average proportion was 88.06%) T cells (Figure 4A), while the majority displayed a TEM phenotype. The $CD3^+$ T cell expressed moderate levels of LAG-3 and CD57, and a very low level (average proportion was 0.17%) of PD-1 (Figure 4B).



Blue: PDO+TIL (1:0), Green: PDO+TIL(1:2), Pink: PDO+TIL(1:4),
Red: PDO+TIL(1:8), Cyan: PDO+TIL(0:8)

Figure 5. Cytotoxicity of TIL against syngeneic PDO without IL-2 addition.

PDO was derived from the tumor tissue of an endometrial cancer patient. Syngeneic TILs were mixed with PDO at different ratios without any IL-2 addition. Cytotoxicity was measured using a real-time cell analyzer. TILs inhibited PDO growth in a dose-dependent way.

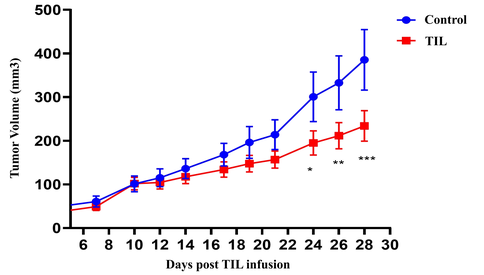


Figure 6. *In Vivo* Anti-tumor Effects of TIL Product Against Syngeneic PDX.

PDX mouse models were established with tumor tissues from a cervical cancer patient using NSG mice (severe combined immunodeficiency and human cytokine-free). Without any IL-2 administration, TIL inhibited tumor growth in PDX models. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

Conclusions

- This feeder-free process enables the expansion of abundant and potent IL-2-independent TILs.
- The TILs exhibit low levels of exhaustion markers like PD-1 and demonstrate robust *in vitro* and *in vivo* functions without the need for IL-2 supplementation.
- This innovative approach has the potential to significantly improve the safety profile and reduce the manufacturing costs of TIL therapy.

Abbreviations:
TIL, Tumor-infiltrating lymphocyte; IL-2, Interleukin-2; Treg, Regulatory T cells; ICU, Intensive Care Unit; HTRF, Homogeneous Time-Resolved Fluorescence; IFN-γ, interferon-γ; PDO, Patient-Derived Organoid; PDX, Patient-Derived Xenograft; TCM, Central Memory T cells; TEM, Effector Memory T cells.

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