CD137 Enrichment and Cytokine Programming Improve Ovarian TIL Expansion and Anti-Tumor Function

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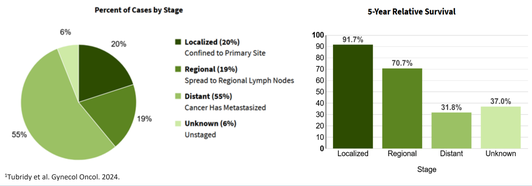
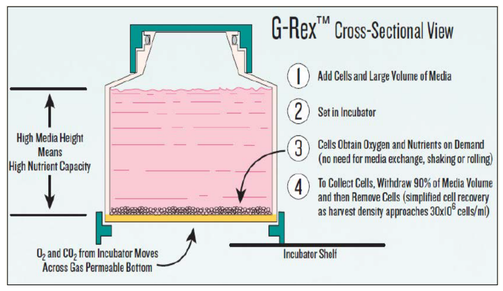
BACKGROUND

- Ovarian cancer remains the deadliest gynecologic malignancy.
- Among immunotherapies, adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TILs) has shown success in melanoma.
- Presence of CD137+ cells have been correlated with improved survival in ovarian cancer patients.[1]
- We developed a new ex vivo expansion platform optimized for ovarian TILs.



[1]Tubridy et al. Gynecol Oncol. 2024.

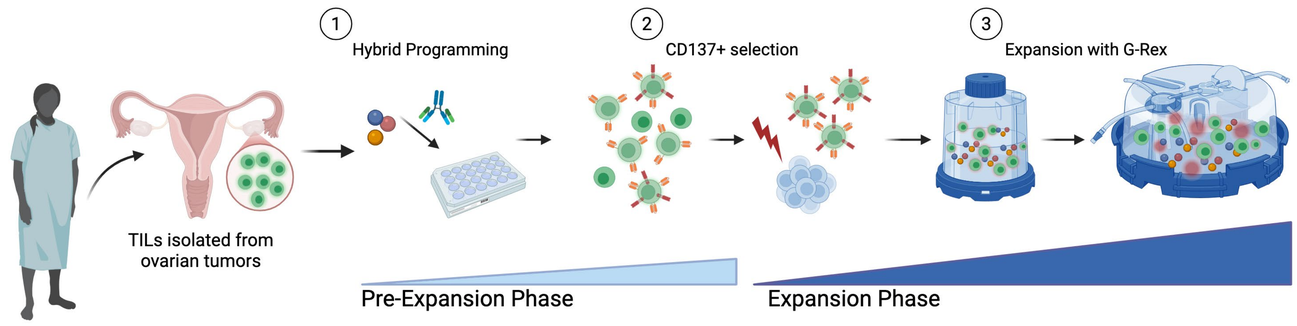


The G-Rex platform enhances T-cell expansion by using a gas-permeable membrane for:

- Increased oxygen delivery,
- Enables large-scale, high-density cell growth
- Maintains high viability and functionality

METHOD

Two-Phase Expansion Protocol (Hybrid programming with CD137 selection)



Method: Ovarian tissue was obtained from eight patients with stage III high-grade epithelial ovarian carcinoma for pathology, next-generation sequencing (NGS), and TIL expansion. The initial priming employed our published hybrid cytokine cocktail, designed to promote effector differentiation while preserving stemness. Following priming, CD137$^+$ (4-1BB$^+$) T cells were isolated based on activation-induced co-stimulatory expression and subjected to rapid expansion with a G-Rex bioreactor system.

RESULTS

Table 1. Patient Clinical Characteristics

	Histology	Stage at Diagnosis	Initial TIL Sample	Matched TIL Sample
OVA 1	high grade serous carcinoma	3b	Pre-chemo	Post-adjuvant 4C carbo/taxol
OVA 2	high grade serous carcinoma*	3C	Pre-chemo	Post Adjuvant 3C carbo/taxol + Recurrance 4C Mirv/Bev
OVA 3	high grade serous carcinoma	3C	Primary Post 3C carbo/taxol	
OVA 4	high grade serous carcinoma	3C	Primary Post 3C carbo/taxol	
OVA 5	endometrioid adenocarcinoma	3C	Pre-chemo	Post-adjuvant 3C carbo/taxol
OVA 6	high grade serous carcinoma	4b	Pre-chemo	Post-adjuvant 4C carbo/taxol
OVA 7	high grade carcinoma*	3C	Pre-chemo	Post-adjuvant 4C carbo/taxol
OVA 8	endometrioid adenocarcinoma	3A	Pre-chemo	
OVA 10	high grade serous carcinoma	3C	Pre-chemo	
OVA 11	high grade serous carcinoma	3C	Pre-chemo	

*platinum refractory

Table 1. Clinical characteristics from ovarian patients used to develop TILs including if TILs expanded after therapy and what therapy was received.

Table 2. Optimization of GREX Expansion Phase

Tumor	Programming Condition	Number of Cells Activated (x10^6)	Total Days of Procedure	Number of Cells After Expansion (x10^9)
Ovarian-5 Post	Conventional	N/A	25	2.5
	Hybrid CD137 Sorted	5.0	33	16.1
Ovarian-6	IL2 Low	2.2	34	18.5
	Hybrid	2.2	34	16.8
Ovarian-6 Post	NCI IL2^{hi}	N/A	24	2.1
	Hybrid CD137 Sorted	8.0	31	15.4
Ovarian-7	NCI IL2^{hi}	N/A	24	2.1
	Hybrid CD137 Sorted	4.0	22	2.4
Ovarian-7 Post	NCI IL2^{hi}	N/A	24	2.1
	Hybrid CD137 Sorted	6.0	22	3.2

Table 2. TILs were expanded using the method above which consistently produced 15-20 billions of viable TILs with >90% viability and approximately 2500-fold expansion.

Figure 1: Two-phase expansion enriches memory-like, less exhausted CD8$^+$ TILs.

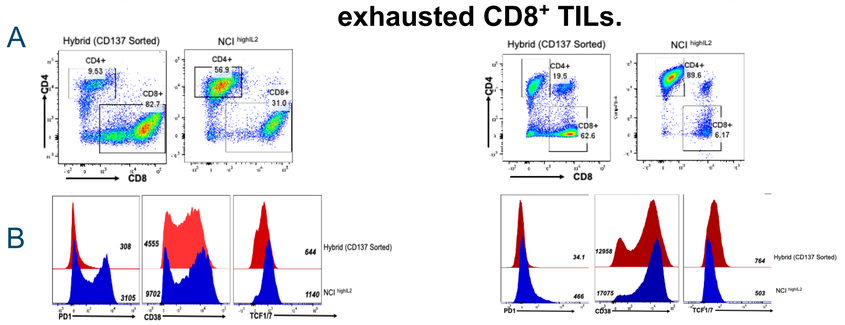


Figure 1: Flow cytometry was performed on matched samples from ovarian tumor tissue, peripheral blood, and two ex vivo expanded TIL populations derived from the same patient (D0 tumor TILs). Expanded TIL groups included: (1) NCI IL2 ^{high} TILs, generated using the traditional NCI high-dose IL-2 protocol (6000 U/ml), and (2) Hybrid (CD137-sorted) TILs, enriched for CD137$^+$ tumor-reactive cells and subsequently expanded in GREX flasks. (A) Representative plots show the distribution of CD4$^+$ and CD8$^+$ T cells following Hybrid versus NCI expansion for two patients. Hybrid expansion yielded a higher proportion of CD8$^+$ T cells (62.6%) compared to Conventional NCI IL-2 expansion (6.2%), which was predominantly CD4$^+$. (B) Histograms of PD-1, CD38, and TCF1/7 demonstrate that Hybrid-expanded TILs exhibited reduced PD-1 expression but increased CD38 and TCF1/7 compared to Conventional NCI IL2 ^{high} TILs suggestive of preserved stem-like and memory potential.

Figure 2: CD137$^+$-selected TILs exhibit enhanced tumor control in the platinum-resistant PE-04 ovarian cancer cell line.

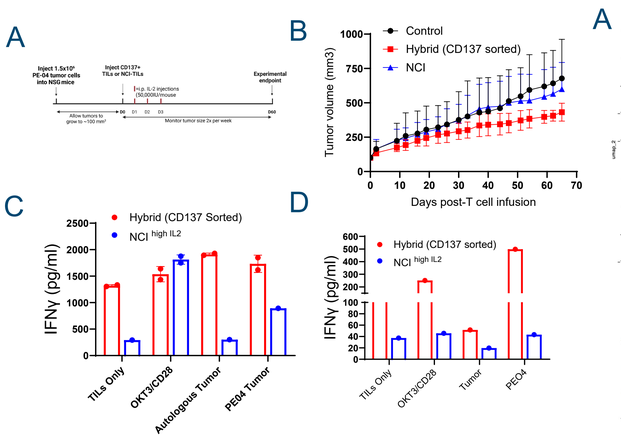


Figure 2: (A) NSG mice were injected with PE-04 tumor cells and allowed to grow until tumors reached a volume of ~100 mm³. Mice were then received either CD137-selected TILs or conventional NCI-expanded TILs, along with IL-2 (50,000 U/mouse for three consecutive days). Tumor growth was monitored following infusion, and cumulative tumor growth curves are shown. (C, D) IFN-γ release was measured in two independent patient samples following co-culture of expanded TILs with autologous tumor targets and ovarian tumor cell lines. Hybrid CD137-sorted TILs produced stronger IFN-γ responses than conventional NCI IL-2–expanded TILs, indicating a higher frequency of tumor-reactive clonotypes within the Hybrid population.

Figure 3: Metabolic programming and CD137$^+$ selection preferentially expand CD8$^+$ TILs and hyper-expand defined TCR clonotypes.

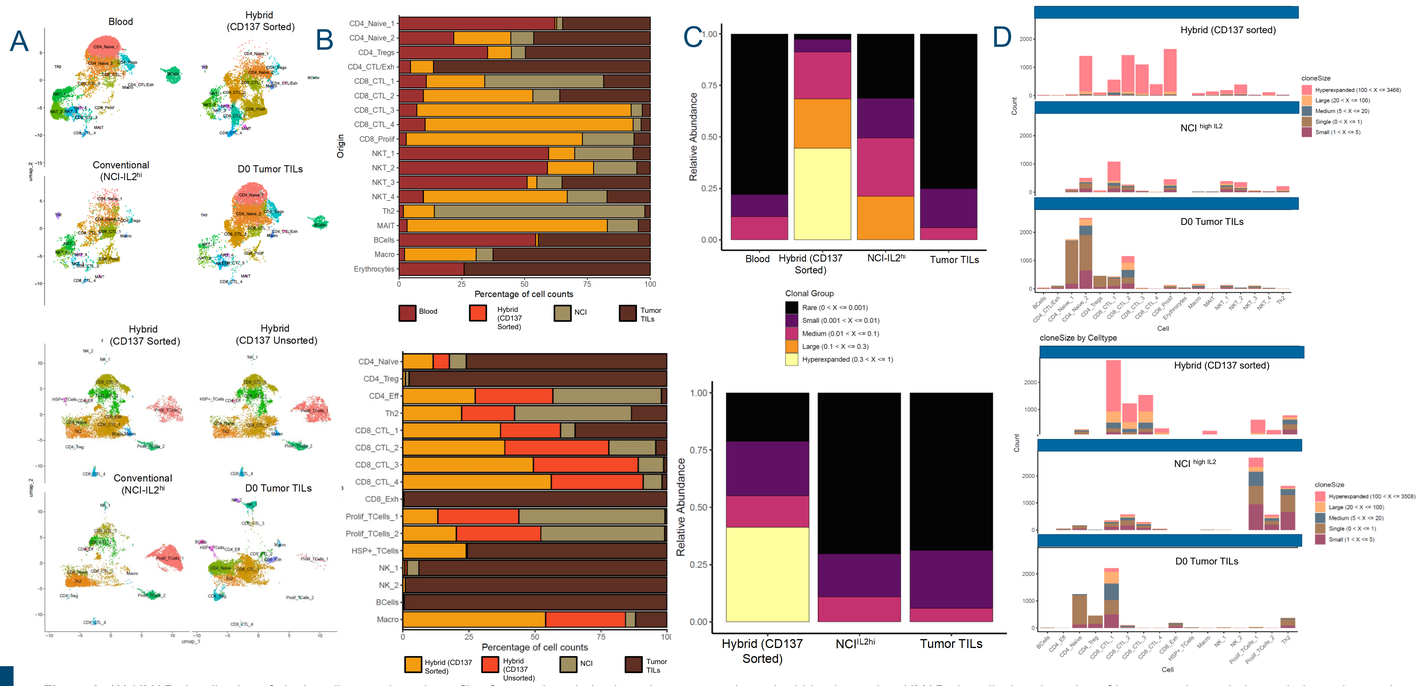


Figure 3: (A) UMAP visualization of single-cell transcriptomic profiles from patient-derived ovarian tumor and matched blood samples. UMAP plots display clustering of immune subsets, in in two independent patient samples. (B) The accompanying bar plot quantifies cell-type composition, showing Hybrid TILs enriched for CD8 CTLs and proliferative subsets, while Conventional NCI-IL2^{hi} expansion favored CD4 naïve and Treg populations. (C) Clonal homeostasis analysis showing the relative abundance of TCR clonotypes stratified into rare ($0 < X \leq 1 \times 10^{-4}$), small ($1 \times 10^{-4} < X \leq 1 \times 10^{-3}$), medium ($1 \times 10^{-3} < X \leq 1 \times 10^{-2}$), large ($1 \times 10^{-2} < X \leq 0.1$), and hyperexpanded ($0.1 < X \leq 1$) groups. Conventional NCI-expanded TILs retained a substantial pool of rare and small clones, while Hybrid-expanded TILs were relatively enriched in large and hyperexpanded clonotypes. (D) Bar plots show TCR clone size distribution stratified by cell type for CD137-sorted hybrid expansion, high IL-2 NCI protocol expansion, and baseline tumor-derived T cells. Clone sizes were defined as: hyperexpanded ($100 < X \leq 3468$), large ($20 < X \leq 100$), medium ($5 < X \leq 20$), small ($1 < X \leq 5$), and singletons ($0 < X \leq 1$). Tumor samples contained predominantly small and singleton clones across multiple subsets, particularly CD4$^+$ T cells and Tregs. CD137-sorted expansion led to marked enrichment of hyperexpanded clonotypes within CD8$^+$ effector subsets, highlighting the selective outgrowth of dominant clones. NCI high IL-2 expansion yielded a broader distribution with small-to-medium clonotypes alongside expanded effector CD8$^+$ T cells.

CONCLUSIONS

- TIL Expansion Protocol allows for over 2500-fold, rapid expansion of TILs from ovarian patient tumor samples, before and after exposure to chemotherapy.
- Hybrid cytokine programming combined with CD137+ sorting improves CD8+ T cell expansion and reduces exhaustion markers CD38, PD-1.
- CD137+ sorting allows for hyper-expansion of select clones, which improves tumor control.
- Expanded TILs are over 95% viable and metabolically active upon re-stimulation.
- This approach holds significant promise for enhancing the efficacy of TIL-based ACT for ovarian cancer.

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