Manufacture and Functional Characterization of Tumor-Infiltrating Lymphocyte Product from Endometrial Tumors

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Introduction

- Lifileucel, a tumor-infiltrating lymphocyte (TIL) cell therapy, has been approved for use in patients with advanced melanoma[1] and has shown promise in other solid tumors[2,3]
- Endometrial cancer (EC), one of the most common gynecologic malignancies, has limited options after first-line therapy[4]
- TIL derived from EC tumors contain tumor neoantigen-reactive clones,[5] suggesting that TIL cell therapy may have therapeutic potential in patients with EC
- In this study, we explored the feasibility of manufacturing TIL from EC tumors and characterized the antitumor activity of TIL products

Methods

- Tissue resected from EC tumors was fragmented and placed in medium containing interleukin-2 (IL-2) for 11 days to allow TIL to emigrate from the tumor tissue
- TIL were expanded in the presence of irradiated peripheral blood mononuclear cells, anti-CD3 antibody, and IL-2
- TIL yield, viability, immune phenotype, T-cell receptor clones, and cytotoxic activity were evaluated

Results

- Eleven EC tumor samples were processed (5 deficient mismatch repair [dMMR] and 6 proficient mismatch repair [pMMR]) (**Table 1**). Ten of the 11 EC samples (91%) produced an extrapolated total viable cell (TVC) yield of >1×10^9 with a median yield of 1.07×10^{10} cells and a viability rate of 83% (**Table 2**)
- EC tumor TIL display phenotypes generally comparable with TIL generated from other tumor types (**Figure 1**)
- Most of the putative neoantigen-specific T cells identified by NeoTCR8 score in the tumor digest remained in the final TIL product (**Figure 2**)
- EC TIL product displayed autologous reactivity with upregulated 4-1BB in CD8+ T cells and OX40 in CD4+ T cells and increased production of interferon-γ and tumor necrosis factor-α when stimulated with autologous tumor digests (**Figures 3 and 4**)
- The antitumor activity of TIL was confirmed *in vitro* via a tumor killing assay with autologous tumor endometrial MicroOrganoSpheres™ (MOS) (**Figure 5**)
- Successful full-scale manufacture of endometrial TIL products was achieved with 4 out of 4 EC tumor samples (**Table 3**)

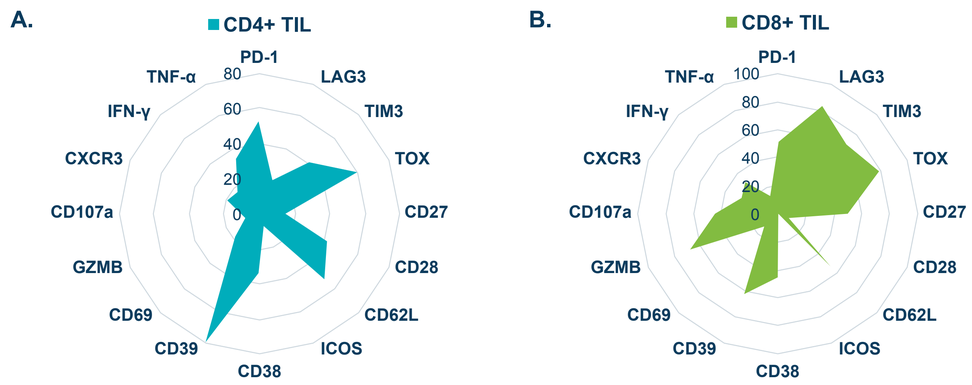
Table 1. Demographic information of patients with EC

Tumor ID	Tumor site	Age	Race/ Ethnicity	Pathology	FIGO grade	TNM classification	MMR	MLH1 promoter methylation
END22061	Uterus	61	White	Endometrioid adenocarcinoma	FIGO grade II	pT3a, pN0	pMMR	
END22062	Uterus	68	White	Endometrioid adenocarcinoma	FIGO grade II	pT1b, pN0	dMMR	81%
END22063	Uterus	67	White	Endometrioid adenocarcinoma	FIGO grade II	pT1b, pN0	dMMR	97%
END22091	Uterus	83	White	Endometrioid carcinoma	FIGO grade II	pT1a, pN0	pMMR	
END22093	Uterus	63	White	Endometrioid carcinoma	FIGO grade II	pT1b, pN0	pMMR	
END22094	Uterus	66	White	Endometrioid adenocarcinoma	FIGO grade I	pT1b, pN0	pMMR	
END22095	Uterus	81	White	Serous carcinoma	High grade (grade III)	pT3a, pN0	pMMR	
END22096	Uterus	45	White	Endometrioid carcinoma	FIGO grade II	pT3b, pNx	dMMR	Positive
END22098	Uterus	67	Hispanic	Endometrioid carcinoma	FIGO grade II	pT1a, pN0	dMMR	Positive
END22100	Uterus	69	White	Endometrioid carcinoma	FIGO grade II	pT1a, pN0	dMMR	N/A (MLH1 intact)
END22102	Uterus	84	White	Endometrioid carcinoma	FIGO grade III	pT1a, pNx	pMMR	

Table 2. Summary of product attributes

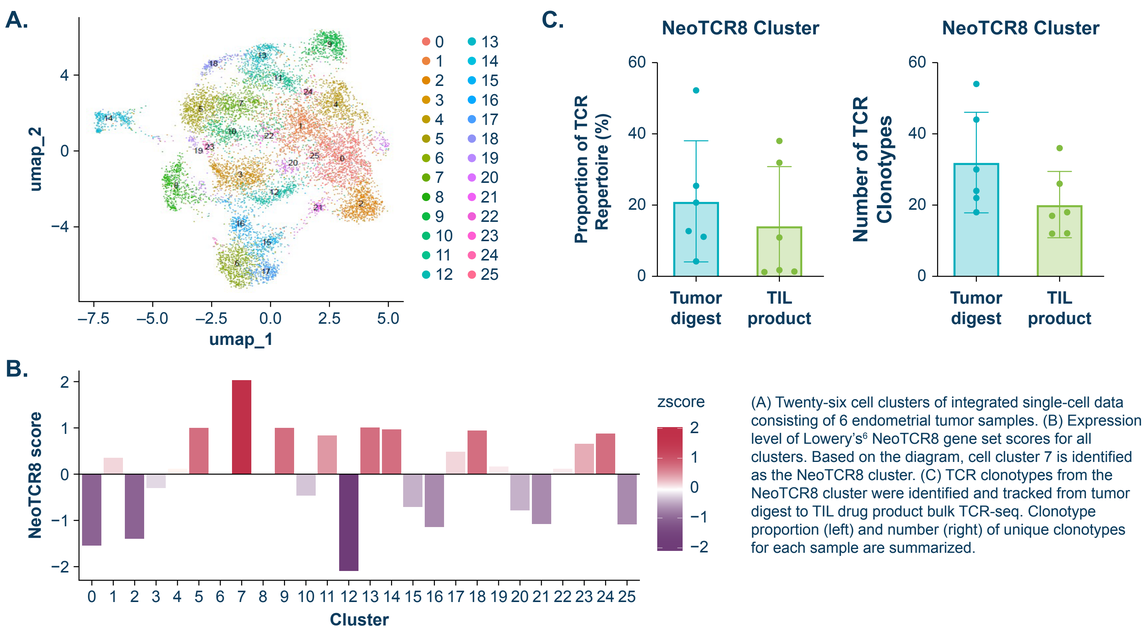
Tumor ID	Tumor size (mg)	Total # of fragments	TVC (extrapolation)	Viability (NC200)	CD45+CD3+%	CD4+CD3+%	CD8+CD3+%
END22061	1083	63	2.71E+10	90.1	96.6	75.0	23.0
END22062	1100	48	1.24E+10	82.7	98.1	86.2	12.9
END22063	1032	52	N/A	N/A	N/A	N/A	N/A
END22091	1013	50	6.64E+09	72.4	98.3	76.2	22.9
END22093	1010	30	8.60E+09	77.1	97.3	91.7	6.4
END22094	1050	53	1.94E+10	87.9	90.2	67.0	31.7
END22095	1131	48	8.10E+09	82.8	97.8	81.6	17.1
END22096	1400	59	9.77E+09	84.9	93.0	78.8	19.9
END22098	1400	80	3.53E+10	86.6	97.3	34.4	63.1
END22100	1327	50	3.80E+09	69.3	95.6	49.4	48.5
END22102	1150	29	1.16E+10	72.3	94.6	51.3	46.5
			1.07E+10	82.7	97.0	75.6	23.0

Figure 1. Phenotype of endometrial TIL



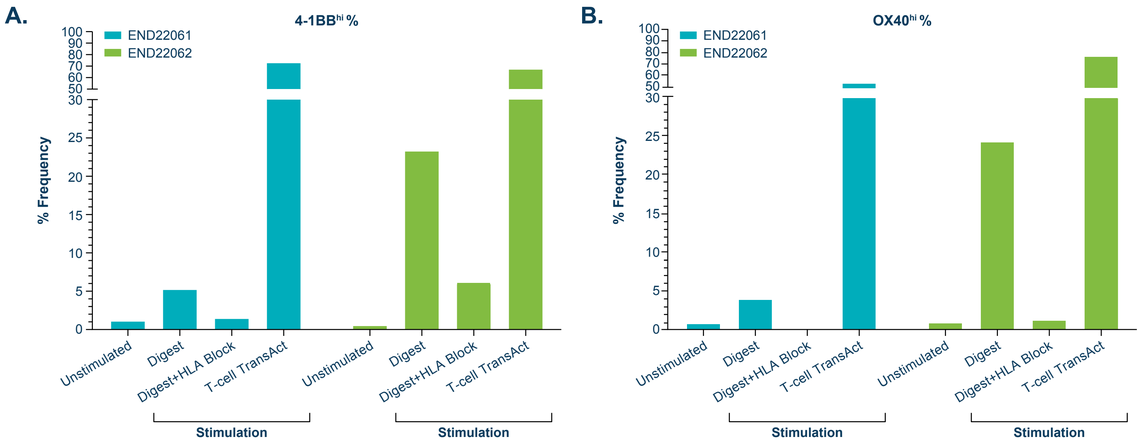
Cellular phenotypic analysis of marker expression related to exhaustion, activation, memory, and function in both (A) CD4+ T and (B) CD8+ T subsets. Data were generated from 5 EC TIL samples.

Figure 2. NeoTCR8 analysis of endometrial TIL



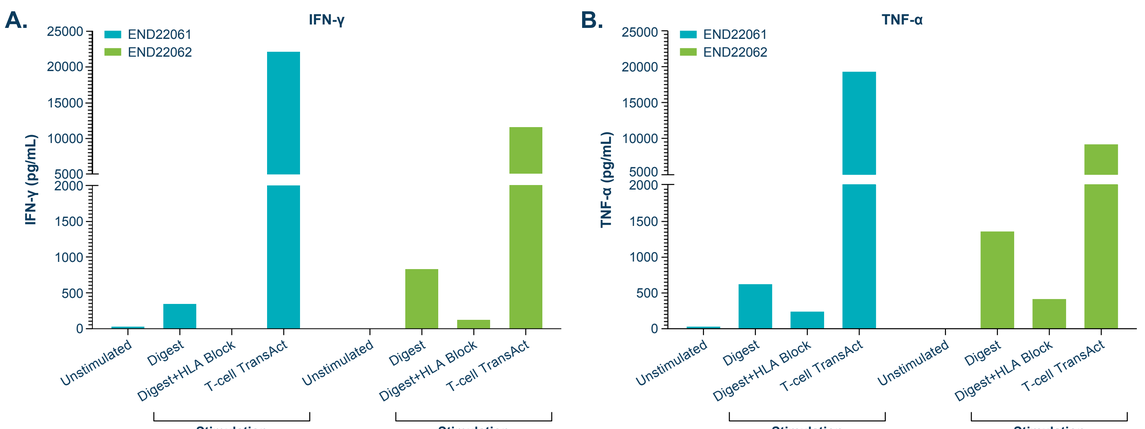
(A) Twenty-six cell clusters of integrated single-cell data consisting of 6 endometrial tumor samples. (B) Expression level of Lowery's[6] NeoTCR8 gene set scores for all clusters. Based on the diagram, cell cluster 7 is identified as the NeoTCR8 cluster. (C) TCR clonotypes from the NeoTCR8 cluster were identified and tracked from tumor digest to TIL drug product bulk TCR-seq. Clonotype proportion (left) and number (right) of unique clonotypes for each sample are summarized.

Figure 3. TIL cell activation after co-culture with autologous tumor digest



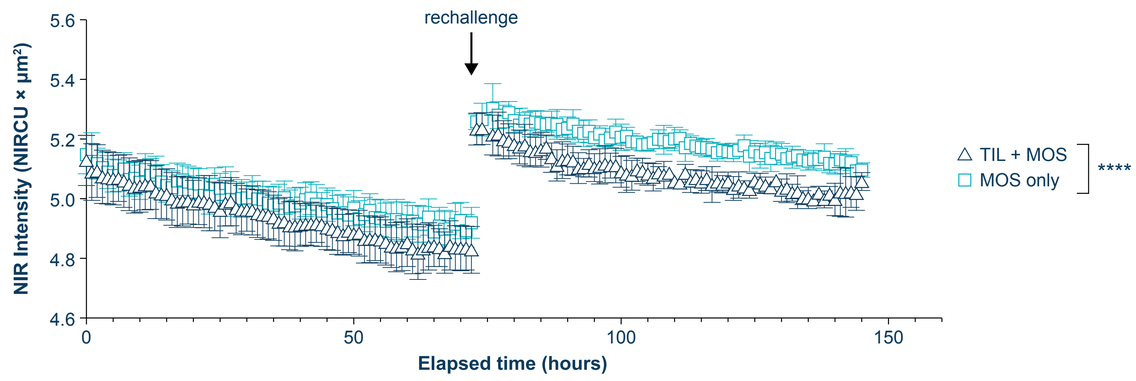
Endometrial TIL (derived from two patients) were co-cultured with a matched tumor digest. Twenty-four hours after stimulation, (A) 4-1BB activation in CD8 T cells and (B) OX40 expression in CD4 T cells were assayed by flow. HLA-I/II antibodies were used as a T-cell activation block. TransACT was used as a positive T-cell activation control.

Figure 4. Cytokine production after co-culture with autologous tumor digest



Cell supernatant from co-culture assay was harvested. (A) IFN-γ and (B) TNF-α expressions were quantified by the BioPlex assay.

Figure 5. TIL-mediated autologous antitumor killing using MicroOrganoSpheres™ (MOS)



TIL and MOS were co-cultured at a ratio of 5:1 and evaluated on the Incucyte® S3 Live-Cell Imager. MOS were labeled with BioTracker NIR680 before co-culture. MOS killing was assessed for 144 hours in a time series and quantified using the average (mean ± SEM) integrated NIR intensity (NIRCU x µm²). MOS killing was assessed for 72 hours and rechallenged with MOS and evaluated for another 72 hours. Data were log transformed and evaluated using a two-way ANOVA using repeated measures. ****p<0.0001.

Table 3. Successful full-scale manufacture of endometrial TIL product

Final product attributes	Run 1	Run 2	Run 3	Run 4	Expected results
	END22111	END22133	END22134	END22135	
Purity (cell viability, %)	88.5	95.5	89.1	89.5	≥70%
Dose (TVC)	5.9 x 10^9	77.1 x 10^9	2.7 x 10^9	68.3 x 10^9	1 x 10^9 – 150 x 10^9 cells
Identity (% CD45+/CD3+)	96.2	99.4	96.1	99.2	≥90%
Cellular impurity (% TROP2+ or EPCAM+ tumor cells)	0.06	0.04	0.06	0.08	N/A
Potency (Dynabead-based IFN-γ release)	9410	3940	12,620	6475	N/A

Conclusions

- **Successful *ex vivo* expansion of TIL from dMMR and pMMR EC tumors was accomplished**
- **EC TIL products demonstrated antitumor activity**
- **These data support clinical investigation of TIL cell therapy in patients with EC**

References

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Conflicts of Interest

- All Authors – Employment: Iovance Biotherapeutics. Stock or Stock Options: Iovance Biotherapeutics. Travel, Accommodations, Expenses: Iovance Biotherapeutics.

Abbreviations

ANOVA, analysis of variance; EC, endometrial cancer; FIGO, International Federation of Gynecology and Obstetrics; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; LAG3, lymphocyte activation gene-3; MMR, mismatch repair; MOS, MicroOrganoSpheres™; N/A, not applicable; NIR, near-infrared spectroscopy; PD-1, programmed cell death protein-1; pNx, no lymph node was collected; SEM, standard error of the mean; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocytes; TNF-α, tumor necrosis factor-alpha; TVC, total viable cells.