Successful Manufacturing of Tumor-Infiltrating Lymphocyte (TIL) Cell Therapy from Cryopreserved Melanoma Tumors Shipped from Australia

Background: Lifileucel TIL cell therapy has demonstrated safety and activity in treating patients with advanced melanoma (Sarnaik AA et al. J Clin Oncol 2021) using fresh (non-cryopreserved) tumors shipped to a central GMP manufacturing facility, primarily in the United States. Australia has the highest rate of melanoma in the world. However, shipping duration from Australia to the US can be extended and may impact the ability to manufacture TIL from a fresh tumor sample. The current study was designed to determine the feasibility of receiving fresh tumors from Australia versus freezing tumors at the clinical site prior to shipment, as well as determining whether TIL can be manufactured from tumors in each of these conditions.

Methods: Tumors were resected from 4 patients with melanoma at the Melanoma Institute Australia. For each tumor, samples were shipped fresh overnight (2–8° C) and also cryopreserved (frozen) prior to shipping. Upon receipt, fresh tumor samples were processed using a Gen 2 (22-day) manufacturing process. Frozen tumor process was executed with the pre-Rapid Expansion Protocol (pre-REP) duration of 7 days (vs 11 days for “fresh” control) and REP duration of 14 days (vs 11 days for “fresh” control). Final harvested TIL products were characterized for the following quality attributes: total viable cells (TVC), purity (% viability), identity (% $CD45^+CD3^+$), and activity ($IFN\gamma$ release). Extended phenotypic characterization and clonotype distribution by T-cell repertoires of the final products were analyzed.

Results: Shipping duration of fresh tumor samples from Australia to the US was between ~72–120 hours. Logistical challenges included a limited window for scheduling resections to ensure receipt of fresh tumors in the US. TIL manufacturing success rate was 75% (3/4) for fresh tumors (67% [2/3] for those with transport time ≥96 hours) and 100% (4/4) for frozen tumors (Table 1). Frozen tumors produced a lower pre-REP TVC than fresh tumors, but this was offset by higher REP yield than fresh tumors. $CD4^+/CD8^+$ ratio was generally lower in frozen than fresh conditions. Phenotypic characterization and clonotyping are ongoing.

Conclusions: Time to ship fresh tumors from Australia (≥3 days) was substantially longer than our experience with tumors shipped within the US (<1–2 days, data on file), and logistical challenges may limit the utility of this treatment using fresh tumors. Tumors that were frozen locally in Australia prior to shipment consistently produced sufficient dose for TIL treatment (4/4 samples); TIL manufacturing using fresh tumors was less reliable (3/4 samples). Additional tumor samples will be analyzed to continue optimizing the TIL manufacturing process using samples from Australia.

Table 1. Comparison of Fresh and Frozen Tumor TIL Product Attributes

Tumor ID	Number of Fragments		Pre-LOVO TVC, × 10^9 (Number of Doublings)		Purity (% Viability)		Identity (% $CD45^+CD3^+$)		Activity (IFN-γ release, pg/mL)		$CD4^+/CD8^+$	
	Fresh (D0)	Frozen (D0)	Fresh (D22)	Frozen (D21)	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
M1211	40	84	0.2 (-)	4 (9.9)	-*	85.1	-*	82.3	-*	3855	-*	59/35
M1213	40	38	56 (5.4)	16 (9.2)	81.2	80.8	95.2	95.8	5358	5078	74/18	43/54
M1214	19	36	64 (5.6)	33 (11.6)	81.6	85.0	98.1	97.9	3106	2900	6/93	3/97
M1219	40	77	12 (10.2)	27 (10.8)	87.2	85.1	99.0	98.3	6355	8949	74/22	72/26

*Not tested.

IFN, interferon; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocytes; TVC, total viable cells