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

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Methods

Manufacturing

Tumors were harvested from patients with metastatic melanoma treated at Melanoma Institute Australia (MIA). Tumor samples were shipped fresh (2°C–8°C) (“Fresh”) or cryopreserved prior to shipment (“Frozen”). Fresh tumor fragments were processed using the Rapid Expansion Protocol (REP) and the 20-day REP expansion process, including an 11-day pre-REP Rapid Expansion Protocol (pre-REP) and 11-day REP duration. Frozen tumor processing was executed with a pre-REP Auration of 7 days and REP duration of 14 days.

Dose (Total Viable Cells) and Purity

Fresh REP and pre-REP samples were analyzed for total nucleated cells, total viable cells, and purity (% viability) determined by sytox orange dye exclusion 2 (viable) vs 3 (nonviable) cell population (CD34+) counterstain using the NucleoCounter® NC-301™ (Chemesense, Lachat, Denmark) automated cell counter.

Identity

Fresh pre-REP and REP samples were analyzed for identity by immunofluorescence staining. Percentage of cells was determined as the CD4+/CD3+ (double-positive) population of viable cells.

Purity

The ability of the harvested tumor product to secrete interferon-γ (IFN-γ) and Granzyme B upon stimulation was measured following co-culture with antibody-coated beads (IFN-γ: anti-CD3, anti-CD28, and anti-CD137; Granzyme B: anti-CD3, anti-CD28, and anti-CD137 (Chemesense, Lachat, Denmark)) automated cell counter.

Phenotype

The recruited harvested TIL product pre-REP was analyzed for expression of surface markers using 2-color flow cytometry panels.

Statistical Analysis

Differences in phenotype, late-stage apoptosis, CD107a granulation, and Granzyme B release were analyzed by the unpaired Student t-test, and P < 0.05 was considered statistically significant.