Studies of Key Quality Attributes for TIL Product, LN-144

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BACKGROUND

• Adoptive T-cell therapy with autologous tumor infiltrating lymphocytes (TIL) has demonstrated clinical efficacy in patients with metastatic melanoma and other tumors.1,3
• Most reports from clinical studies have included exploratory analyses of the infused TIL products with the intention of identifying quality attributes such as sterility, identity, purity, and potency that could relate to product efficacy and/or safety.4,5
• Here we present the evaluation of three key product parameters from Iovance TIL product LN-144 that may contribute to a future quality control platform for use in the commercial manufacture of TIL.

STUDY OBJECTIVES

• Goal: To fully characterize TIL products for identity, purity, and potency, and thereby
  – Guide the definition of critical quality attributes and
  – Support the establishment of formal release criteria to be implemented in commercial production of TIL products at Iovance.

• Strategy: To develop the following analytical methodologies to support TIL product characterization
  – Phenotypic analysis by flow cytometry for an identity and purity assessment
  – Residual tumor cell detection assay for a measure of purity
  – Interferon-gamma release assay for an assessment of potency

MATERIALS & METHODS

• Identity + Purity Phenotypic characterization: TIL products were stained with anti-CD45, anti-CD2, anti-CD8, anti-CD4, anti-CD45RA, anti-CCR7, anti-CD62L, anti-CD19, anti-CD16, and anti-CD56 antibodies and analyzed by flow cytometry for the quantification of T and non-T cell subsets.

• Purity Residual tumor detection assay: TIL products were stained with anti-MCSP (melanoma-associated chondroitin sulfate proteoglycan) and anti-CD45 antibodies, as well as a Live/Dead fixable Aqua dye, then analyzed by flow cytometry for the detection of melanoma cells. Spiked controls were used to assess accuracy of tumor detection and to establish gating criteria for data analysis.

• Potency IFNγ release assay: TIL products were re-stimulated with anti-CD3/CD28/CD137 coated beads for 18 to 24 hours after which supernatants were harvested for assessment of IFNγ secretion using an ELISA assay.

RESULTS

Identity: The majority (>99%) of melanoma TIL product is composed of CD45+CD3+

Purity: Development of a flow cytometry-based assay for detection of residual tumor cells in TIL products

Potency: IFNγ secretion by TIL (consistently > 1000 pg/ml) demonstrates effector function of TIL product

CONCLUSIONS

• Key product parameters of identity, purity, and potency of TIL products were evaluated.
• TIL products manufactured by Iovance consisted of greater than 99% CD45+CD3+ T cells.
• The majority of CD4+ and CD8+ TIL subsets exhibited an effector-memory phenotype, associated with T-cell cytotoxic function.
• A flow cytometry-based assay to detect contaminant melanoma tumor cells in final TIL product was successfully developed and qualified.
• Applying this assay, contaminant melanoma tumor cells in final TIL product were shown to be below the limits of assay detection.
• IFNγ secretion by final TIL product following anti-CD3/CD28/CD137 re-stimulation may serve as a potency assay for commercially manufactured TIL.
• These data provide the foundation of a quality control platform that will support further development of critical quality attributes for commercial production of TIL products.

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