Non-Proliferation Assay

Confirming Feeder Cells for TIL Expansion Are Suitable for Phase II Clinical Trials and Beyond

Chase P. Monckton, Ph.D., Zheng Yang, Ph.D., Trevor Cabreros, Aniha Vijay Kumar, Savannah Ayyoub, Sofia Huertero, and Rob Tressler, Ph.D.

INTRODUCTION

Tumor-infiltrating leukocytes (TILs), which are present in patient tumors, have inherent anti-cancer properties. TIL-based therapies have shown great promise as immunotherapeutic approaches to treat a variety of cancers and the number of TIL-based clinical trials are rapidly increasing. TILs are extracted from a patient's tumor biopsy, expanded ex vivo, and harvested as a patient-specific therapy **(Fig. 1)**. The optimal method for ex vivo expansion requires co-culture with an unrelated secondary cell type (typically non-irradiated peripheral blood mononuclear cells [PBMCs]) as feeder cells. To support the needs of this growing field, we produce cGMP feeder cells for TIL development. However, the presence of feeder cells in the end TIL product could compromise efficacy and safety for the patient due to incompatible immunity, and thus, the non-proliferative capacity of the feeder cells must be assured by testing prior to use for TIL expansion during manufacturing. To facilitate this, we have created a replication competency assay that assesses feeder cell counts in a long-term culture system to confirm non-proliferation of the feeder cells.

METHODS

Feeder cells are thawed and seeded with or without control, non-irradiated PBMC (a proxy for TILs). All conditions are maintained in a standard T cell. Cells are counted and assessed based on the following acceptance criteria:

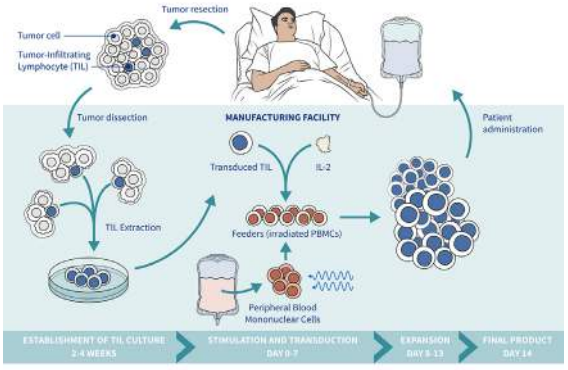
1. A decline of >60% in feeder cell numbers
2. A >1-fold increase in non-irradiated PBMCs

DATA SUMMARY

We can demonstrate that the feeder cells will support TIL expansion, and more importantly, they will die off in the process over a long-term culture period. This is necessary to prevent adverse reactions (i.e., graft vs. host disease) and improve efficacy upon delivery. This assay will yield a set of fold changes for the feeder cell only (for example ~0.1-fold) and the non-irradiated PBMCs in co-culture with the feeder cells (for example >1900-fold); these are calculated by comparing day 14 cell numbers to initial seeding numbers **(Fig. 2)**.

HOW WE CAN SUPPORT YOUR CELL THERAPY NEEDS

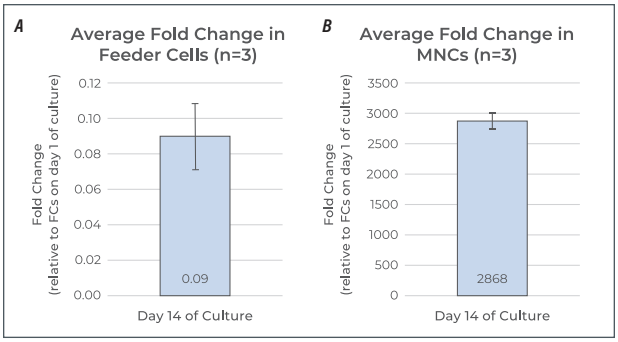
Our feeder cells and complementary non-proliferation assay are powerful tools for enabling TIL-based therapies, which have been shown to increase response rates for cancer patients. This assay was designed and validated in accordance with FDA Q2(R1) *Validation of Analytical Procedures: Text and Methodology Guidance for Industry, November 2005*.



TIL Therapy Manufacturing Process

- Irradiated PBMC and MNCs (proxy for "young TILs") are co-cultured in the presence of anti-CD3 and IL-2.
- The cultures are grown in flasks and incubated at 37° C with 5% CO_2. On day 14, cell concentration is determined.

(Figure 1) *An infographic of the TIL manufacturing method for ACT therapies.*



(Figure 2) Average Fold Change for n=3 Assays.
A) *The FCs has a 0.09-fold change in cell number relative to day 0 cell numbers for the FC-only condition; all pass based on the acceptance criteria.* ***B)*** *Similarly, the MNCs have a 2868-fold change on average relative to day 0 cell numbers of the MNCs only (the FC cell numbers added to the coculture on day 0 were omitted from calculations and considered to be negligible on day 14). Error bars are standard error of the mean.*

1155 Island Avenue, San Diego, CA 92101
info@excellos.com | www.excellos.com