Tumor Infiltrating Lymphocytes Show *in vitro* Cytotoxic Activity Against Tumor Cells in Multiple Cancers

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Conflict of Interest:
Datar Cancer Genetics Limited offers commercial services in the domain of oncology.

BACKGROUND

- Considerable interest in recruiting immune cells of cancer patients to detect and destroy malignant cells.
- Approaches include activation of dendritic cells, chimeric antigen receptor T-cells, check point inhibitors and Tumor Infiltration Lymphocytes (TILs).
- TILs have been successfully evaluated in few cancers e.g., Cervix and Melanoma.

RATIONALE

- To explore the feasibility of using TILs for treatment of various solid organ cancers we hypothesized that their *in vitro* evaluation would establish preliminary viability of the approach.

APPROACH

- We isolated TILs from 9 patients with various solid organ cancers,
- Explant cultures generated from 3 mm^3 sections of tumor tissue,
- Growth medium for TIL propagation contained TCGF, IL2,
- Sufficient TILs expanded in 3-4 weeks of culture,
- *In vitro* antitumor activity of TILs assayed by live cell imaging and Interferon Gamma Release (IGR) test.

DEMOGRAPHICS

Table 1. Cancer Types

Cancer Type	Number
Breast	4
Head and Neck	3
Cervix	2

Table 2. Gender.

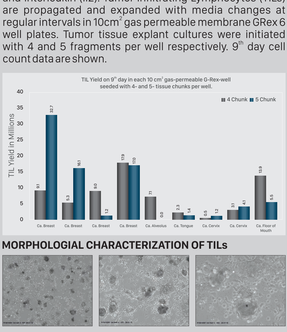
Gender	Number
Male	2
Female	7

Table 3. Age

Age	Number
Minimum	41
Maximum	62
Median	51

EXPLANT CULTURE AND YIELD OF TILs

Figure 1. TIL yield from tumor explant culturing.
Surgically resected human tumor tissue samples were are aseptically transferred to research laboratory and processed aseptically. Explant tissues fragments were transferred to growth media containing T cell growth factor and interleukin (Il2). Tumor Infiltrating Lymphocytes (TILs) are propagated and expanded with media changes at regular intervals in $10cm^2$ gas permeable membrane GRex 6 well plates. Tumor tissue explant cultures were initiated with 4 and 5 fragments per well respectively. 9^{th} day cell count data are shown.



MORPHOLOGIAL CHARACTERIZATION OF TILs

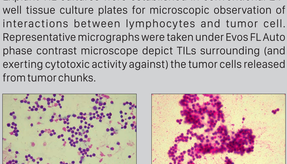


Figure 2. Microscopy of TIL cultures.

Explant TIL cultures were established in conventional 24-well tissue culture plates for microscopic observation of interactions between lymphocytes and tumor cell. Representative micrographs were taken under Evos FL Auto phase contrast microscope depict TILs surrounding (and exerting cytotoxic activity against) the tumor cells released from tumor chunks.

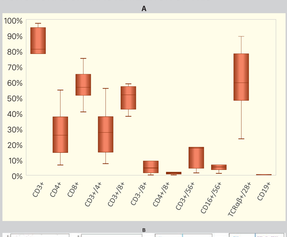


Figure 3. Verification of Purity of TILs by Cytopathology.

Established human bulk TILs are cultured for ≥2 weeks. Cytocentrifuged smears were prepared from TILs and stained with Hematoxylin and Eosin. Morphology of TILs were examined by microscopy for presence of residual tumor cells. We were unable to detect the presence of residual viable tumor cells in any of the established bulk TIL cultures.

FUNCTIONAL CHARACTERIZATION OF TILs

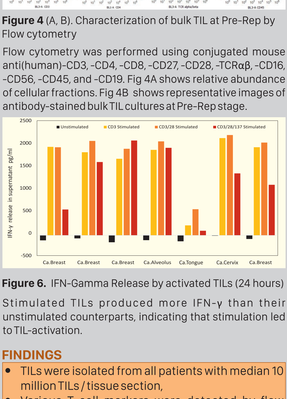


Figure 4 (A, B). Characterization of bulk TIL at Pre-Rep by Flow cytometry

Flow cytometry was performed using conjugated mouse anti(human)-CD3, -CD4, -CD8, -CD27, -CD28, -TCRαβ, -CD16, -CD56, -CD45, and -CD19. Fig 4A shows relative abundance of cellular fractions. Fig 4B shows representative images of antibody-stained bulk TIL cultures at Pre-Rep stage.

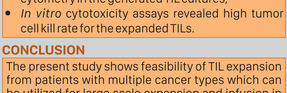


Figure 6. IFN-Gamma Release by activated TILs (24 hours)

Stimulated TILs produced more IFN-γ than their unstimulated counterparts, indicating that stimulation led to TIL-activation.

FINDINGS

- TILs were isolated from all patients with median 10 million TILs / tissue section,
- Various T cell markers were detected by flow cytometry in the generated TIL cultures,
- *In vitro* cytotoxicity assays revealed high tumor cell kill rate for the expanded TILs.

CONCLUSION

The present study shows feasibility of TIL expansion from patients with multiple cancer types which can be utilized for large scale expansion and infusion in future clinical trials.