Background & Objectives

- Inter-K Peptide Therapeutics has developed two synthetic compounds that modulate kinase activity. To investigate the potential of these compounds as cancer immunotherapeutics, a flow cytometric intracellular cytokine staining (ICS) assay was developed to investigate the effect of the compounds on immune cells isolated from tumors from subjects with non-small cell lung cancer (NSCLC).
- In addition, the concentration of various cytokines was measured in cell culture supernatant following treatment with the compounds.

Methods

- After optimizing the assay in PBMC from healthy donors, clinical PBMC specimens were assayed. Tumor-infiltrating lymphocytes (TILs) were isolated from two resected tumors by mechanical disaggregation. TILs were stimulated with or without anti-CD3/CD28 antibodies with and without Inter-K Compound A or Compound B for 72 hours. Following stimulation, cell culture supernatants were analyzed for 10 proinflammatory cytokines using the Meso Scale Discovery (MSD) assay.
- Following stimulation, TILs were analyzed by flow cytometry with a 10-color immunophenotyping panel to measure the expression of functional markers CD25, IL-2, IFNγ and Ki67 in NK, NKT, CD4 T and CD8 T cells.

Results

- Immunomodulation induced by Compound A treatment included an over two-fold increase in CD25 expression in CD4 T cells, statistically significant increases in IL-6 and IL-8 production while a decrease in Ki67 expression in CD8 T cells and a reduction in IL-2, IFNγ, TNFa, IL-4, IL-10 and IL-13 production was observed. Compound B did not show immunomodulatory properties.

Conclusions

- This work provides an example of the utility of flow cytometry in compound evaluation. In this case, preliminary data suggests that Compound A, but not Compound B, was able to modulate the response in TIL samples in vitro, and supports the continued investigation of Compound A as an immunotherapeutic. Although TIL specimens are challenging to work with due to tumor availability and low cellular recovery, it is important that compound characterization be conducted in TIL samples as they are the target of the candidate immunotherapy.

Background & Objectives

- Inter-K Peptide Therapeutics has developed synthetic peptide-based immune-therapeutic candidates to treat a range of diseases.
- The immunotherapeutic potential of two compounds (Compound A and Compound B) was tested in vitro using TILs from subjects with NSCLC.
- By flow cytometry, the expression of functional markers CD25, IL-2, IFNγ and Ki67 in NK, NKT, CD4 T and CD8 T cells was measured.
- By MSD assay, the concentration of various cytokines was measured in cell culture supernatant following treatment with the compounds.

Methods

- Isolation of TILs and normal adjacent tissue
- Add cells to wells
- Stimulate for 72 hr with anti-CD3+anti-CD28 antibodies or NS
- Add compounds during stimulation
  - Compound A
  - Compound B
- Perform MSD assay
- Collect supernatant
- Collect cells
- Perform ICS assay by flow cytometry
- Add GolgPlug/GolgStop

Intracellular cytokine staining (ICS) assay

- NK cells
- NKT cells
- Total T cells
- CD4 T cells
- CD8 T cells
- Functional markers measured in all cell subsets
- CD25
- IL-2
- IFNγ
- Ki67

Meso Scale Discovery (MSD) assay

- V-PLEX Proinflammatory Panel 1 Human Kit was used to quantify cytokines:
  - IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNFα

Results

Tumor samples

- With subject 1071, treatment with Compound A resulted in decreased expression of CD25 in total T cells and CD8 T cells, and an increased expression of CD25 in CD4 T cells.
- With subject 1064, fewer tests were performed due to insufficient cells, no major trends were observed (data not shown).

Flow cytometry (ICS assay) data

- With subject 1071, treatment with Compound A resulted in a decreased expression of Ki67 in total T cells and CD8 T cells.
- No statistically significant differences were identified in this graph.

Conclusions

- Compound A (but not Compound B) was able to modulate the response in TIL samples in vitro, and this work supports the continued investigation of Compound A as an immunotherapeutic.
- Variable viability and difference responses were observed with the two TIL samples tested.
- Although tumor availability and low cellular recovery represents a challenge for compound characterization, it is important that analysis be conducted in TIL in addition to healthy donor PBMC and cell lines.