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Abstract # e15052

Characterization of Two Potential Immunotherapeutics in Tumor Infiltrating Lymphocytes (TILs) Using Flow Cytometry and Meso Scale Discovery

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Abstract

Background: 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 after 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-related 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, IFNg 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, IFNg, TNFa, IL-4, IL-10 and IL-13 production was observed. Compound B did not show immunomodulatory properties.

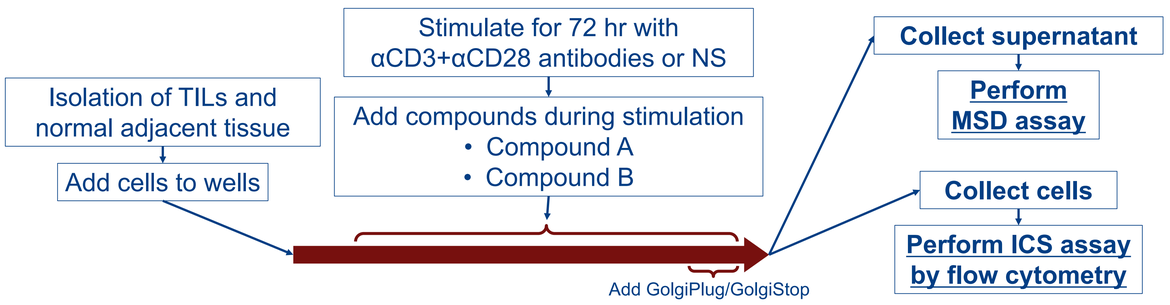
Conclusions: This work provides an example of the utility of flow cytometry in compound evaluation. In this case, preliminary data suggests that Compound A, but not Compound B, was able to modulate the response in TIL samples *in vitro*, and supports the continued investigation of Compound A as an immunotherapeutic. 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 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) were tested *in vitro* using TILs from subjects with NSCLC
  - By flow cytometry, the expression of functional markers CD25, IL-2, IFNg and Ki67 in NK, NKT, CD4 T and CD8 T cells was measured
  - By MSD assay, the concentration of various cytokines were measured in cell culture supernatant after treatment with the compounds

Methods

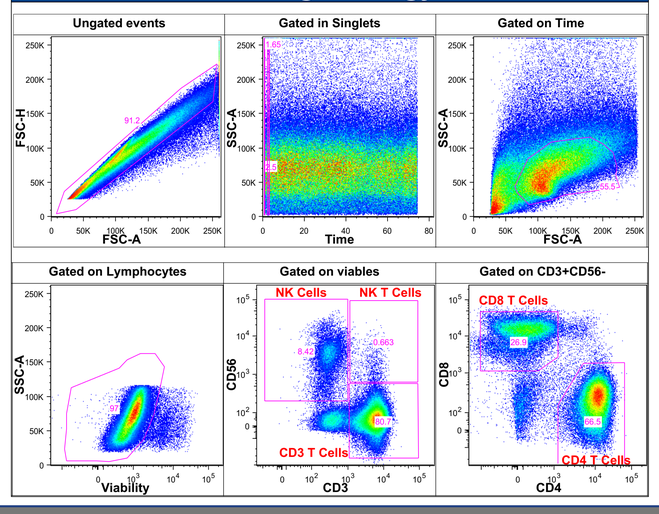
Experimental summary



Intracellular cytokine staining (ICS) assay

Panel
Live/Dead
CD3
CD4
CD8
CD25
CD56
CD57
IFN-g
IL-2
Ki67

Gating strategy



Reportable Results	
Cell subsets	NK cells
	NKT cells
	Total T cells
	CD4 T cells
	CD8 T cells
Functional markers measured in all cell subsets	CD25
	IL-2
	IFNɣ
	Ki67

Dot plots derived from PBMC samples.

Meso Scale Discovery (MSD) assay

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

Results

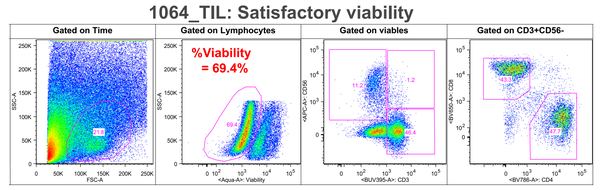
Tumor samples

- TIL and normal adjacent tissue samples were obtained from two NSCLC donors (Subject ID 1064 and 1071)
- Cells isolated by mechanical disaggregation
- Variable number of TILs at different viability were obtained with the two samples

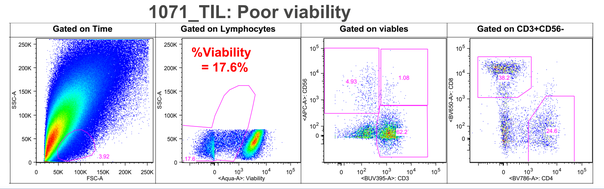
Sample ID	SAMPLE	Sample mass	# cells (x10e6)	Viability (%)
1064_TIL	TUMOR	0.6962	5.5	20
1064_ADJACENT_TISSUE	ADJACENT TISSUE	2.3831	11.6	41
1071_TIL	TUMOR	1.5951	16.4	17
1071_ADJACENT_TISSUE	ADJACENT TISSUE	1.3561	3.7	23

Dot plots derived from TIL samples.

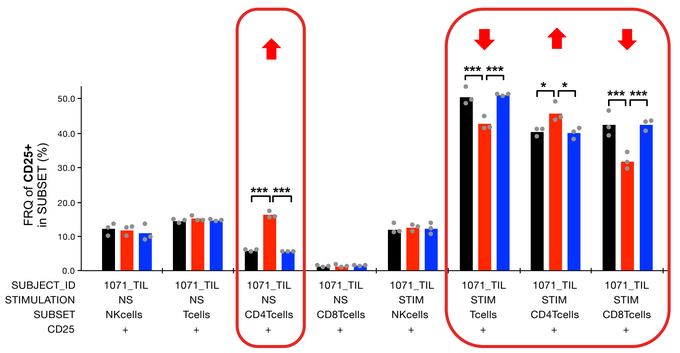
1064_TIL: Satisfactory viability



1071_TIL: Poor viability



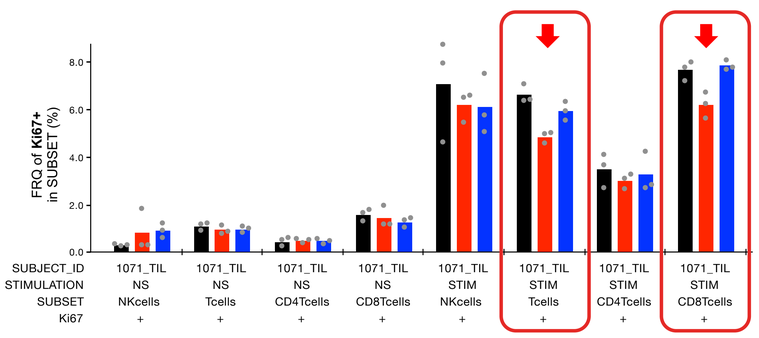
Flow cytometry (ICS assay) data



- 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

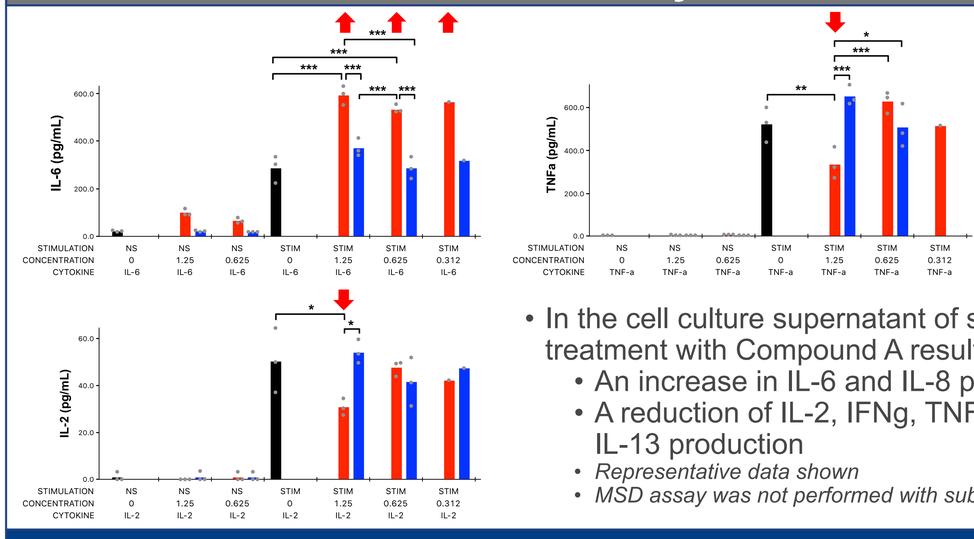
Bar Chart Legend
- COMPOUND: Vehicle CONCENTRATION: 0
- COMPOUND: Compound A CONCENTRATION: 1.25
- COMPOUND: Compound B CONCENTRATION: 1.25

Statistical significance was calculated using Ordinary One-way ANOVA with Tukey's multiple comparisons test to compare all possible pairs of groups.



- 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*
- With subject 1064, fewer tests were performed due to insufficient cells, no major trends were observed (data not shown)

MSD assay data



Bar Chart Legend
- COMPOUND: Vehicle
- COMPOUND: Compound A
- COMPOUND: Compound B

Statistical significance was calculated using Ordinary One-way ANOVA with Tukey's multiple comparisons test to compare all possible pairs of groups.

- In the cell culture supernatant of subject 1071, treatment with Compound A resulted in
  - An increase in IL-6 and IL-8 production
  - A reduction of IL-2, IFNg, TNFa, IL-4, IL-10 and IL-13 production
  - *Representative data shown*
  - *MSD assay was not performed with subject 1064*

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