Combination intraperitoneal chemoimmunotherapy triggers a T-cell chemotactic locoregional response in patients with recurrent platinum-sensitive ovarian cancer

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Background

Most epithelial ovarian tumors show some level of infiltration by T lymphocytes and increased prevalence of CD8+ tumor-infiltrating lymphocytes (TIL) in the tumor microenvironment (TME) correlates with improved outcomes in patients with epithelial ovarian cancer (EOC).

The field of cancer immunotherapy has rapidly evolved and made a significant impact on many tumor types, including gynecologic tumors. However, single agent immune therapies have not yet demonstrated significant clinical success in epithelial ovarian cancer. Most of the limitations for previously tested immune interventions are largely due to the complex locoregional environment that is dominated by a number of immune suppressive mechanisms.

We hypothesize that a combination of intraperitoneal (IP) chemotherapy (via immunogenic cell death-inducing cisplatin) with dual agent immunotherapy using IV pembrolizumab (anti-PD1) and IP rintatolimod (dsDNA and TLR-3 agonist) promotes increased T cell chemotaxis and cytolytic function for improved clinical outcomes.

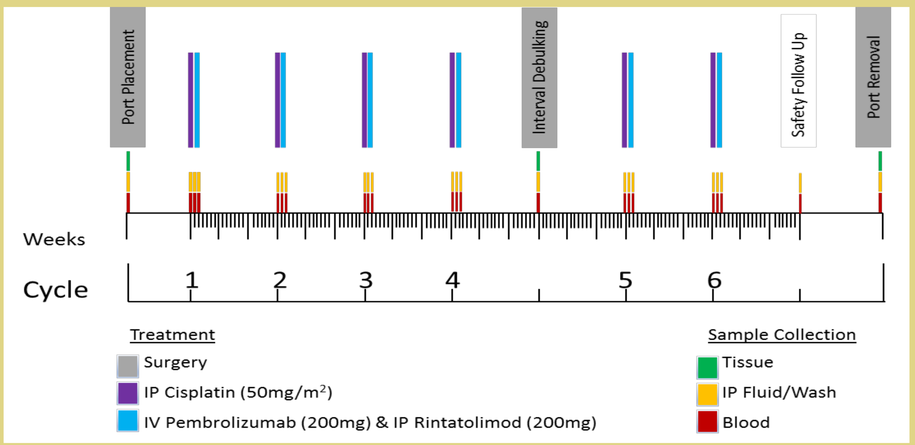
Methods

To test our hypothesis, we have performed translational studies focused on the immune TME, using a longitudinal collection of biospecimens, including plasma, PBMC, IP washes and tumor tissue collected from patients treated in an investigator initiated, phase II, efficacy/safety trial (NCT03734692) combining intraperitoneal (IP) chemotherapy (cisplatin) with dual agent immunotherapy using IV pembrolizumab (anti-PD1, Keytruda®, provided by Merck) and IP rintatolimod (Ampligen®, a dsRNA acting as toll-like receptor 3 -TLR-3- agonist, provided by AIM ImmunoTech).

Each treatment cycle includes: day one IP cisplatin 50mg/m^2, day two IV pembrolizumab 200mg followed by IP rintatolimod 200mg. Tumor tissue is collected at IP port placement and at time of debulking surgery. IP washes are collected before cisplatin (Day 1), before pembrolizumab, after pembrolizumab but before rintatolimod and after rintatolimod (Day 2), and on Day 3. Blood samples are collected on each day of treatment.

RNA sequencing of IP wash cells was performed using the Novogene platform. The Mesoscale Delivery (MSD) platform was used to profile different biomarkers in the peritoneal samples throughout treatment.

Figure 1: Trial schema depicting treatment and biospecimen collection timeline



Results

- To date 24 patients have been enrolled in the trial with 17 evaluable for response.
- Sequential sampling of the IP cavity showed an increase in cellularity immediately after treatment consistent with an "acute" pro-inflammatory reaction
- MSD measurements in IP washes revealed an acute increase in granzyme B, perforin, TNF alpha, CXCL9, CXCL10, and CXCL11 after treatment showing in Figure 2 ($p<0.05$). Longitudinal data revealed a progressive increase in some biomarkers ($p<0.05$).
- RNA sequencing data showed a significant upregulation acutely in STAT1 and downstream targets, CXCL9, 10, 11 and TH1 type response genes ($p<0.05$)
- As would be expected in response to TH1 signaling activation, CXCL12, a protumor chemokine, showed intra-cycle increase post-treatment

Results

Longitudinal profiling of IP washes shows a gradual, durable response over time in T lymphotactic CXCR3 ligands and markers of type 1 immunity

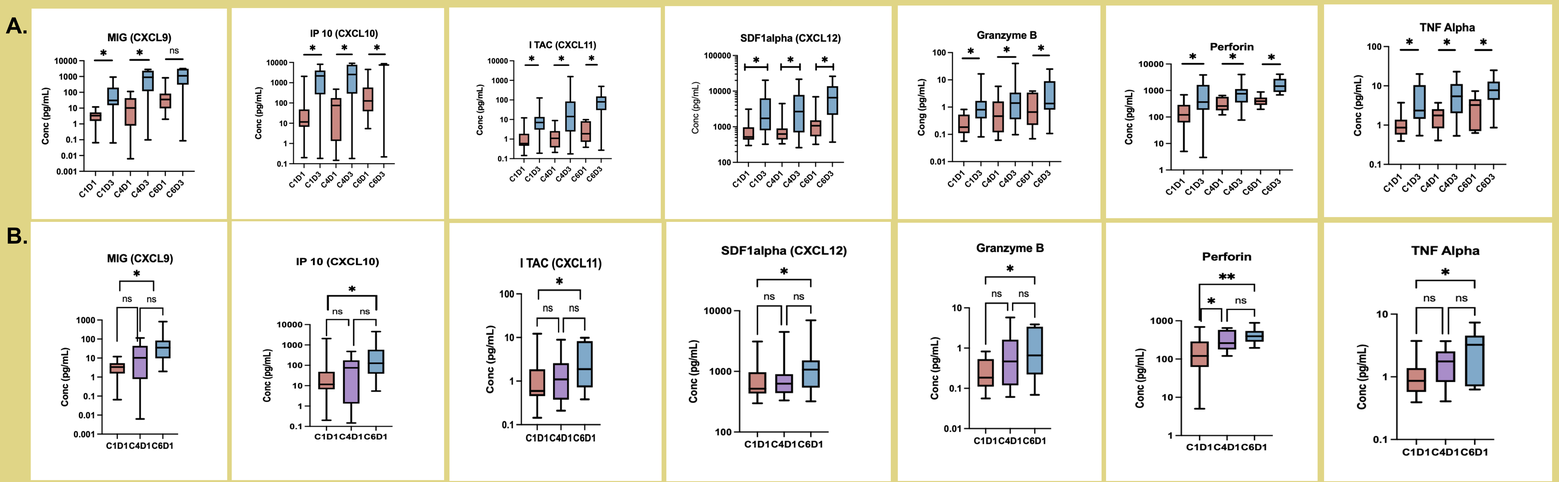
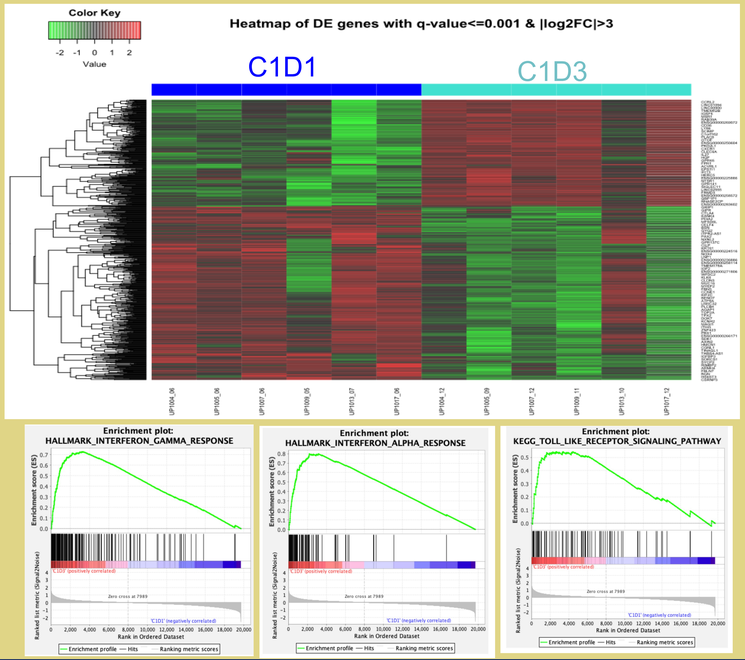


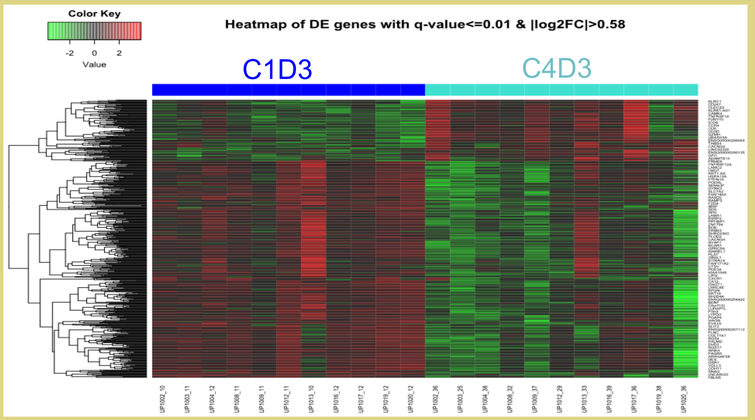
Figure 2: A. Intra-cycle increase from day 1 to day 3 of cycles 1, 4 and 6 ($p<0.05$, Student t test). B. Change in baseline (ie Day 1) of cycles 1 (red), 4 (purple) and 6 (blue) $p<0.05$ (ANOVA).

RNA Sequencing data showed an upregulation acutely in genes associated with anti-tumor immunity, T lymphotactic chemokines and TH1 type response

767 DE genes before and after chemoimmunotherapy – GSEA analysis shows enrichment in IFN α and IFN γ response



1434 DE genes show "chronic" response during treatment



Acute (log2fold)		Chronic (log2fold)	
CXCL10	4.677805	CXCL9	1.747529
CXCL11	4.609135	CCL5	2.882486
STAT1	2.27351	IFNG	2.94892
RIGI	3.323878	Tbet	1.912535
IL27	3.828912	CD8a	2.008109

Conclusions

- We are conducting a Phase II clinical trial that tests a novel, triple drug combination for recurrent, platinum sensitive epithelial ovarian cancer.
- Intensive sampling of the peritoneal cavity through IP washes provides unique opportunities for phenotypic analyses of cells and secreted factors found in the tumor microenvironment.
- MSD profiling of IP washes shows an acute locoregional response with an increase in biomarkers associated with T cell chemotaxis and cytolytic function.
- Longitudinal comparison of these biomarkers showed a gradual, durable response over time in T lymphotactic CXCR3 ligands and cytolytic factors.
- RNA sequencing data shows upregulation of genes important for T lymphotaxis and function via TCR engagement with cognate tumor antigens
- GSEA demonstrates an acute enrichment interferon IFN α and IFN γ response
- Studies using multiplex tumor tissue profiling and RNAseq of tissue are ongoing

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