105P Immune biomarker analysis of the OVATION-2 trial, a randomized Phase I/II study of IL-12 gene therapy IMNN-001 in combination with Neo/Adjuvant Chemotherapy (N/ACT) in newly-diagnosed advanced Epithelial Ovarian Cancer (EOC)

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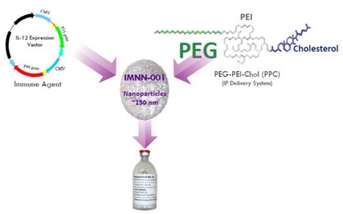
BACKGROUND

- Ovarian cancer remains one of the leading cause of death among gynecologic cancers with approximately 20,000 new cases each year in the US, 80% of which are diagnosed in advanced stage (III/IV), and >60% die within 5 years of diagnosis[1]
- Neo/Adjuvant (N/ACT) peri-debulking surgery is SoC treatment for newly-diagnosed advanced Epithelial Ovarian Cancer (EOC)
- Immunotherapy is an attractive approach for the treatment of EOC due to its multifaceted, highly immunosuppressive ("cold") tumor environment[2]. However, the addition of immune checkpoint inhibitors (ICIs) to SoC Chemotherapy has only modestly improved ORR[3-7]
- IL-12 is a pleiotropic immuno-stimulatory cytokine with activity on both the innate and adaptive immune systems and is able to turn "cold" tumor microenvironments to "hot". However systemic treatment with IL-12 is too toxic for use in the clinic.
- The randomized, controlled Phase I/II OVATION-2 study has shown that IL-12 gene-therapy with IMNN-001 delivered IP in combination with N/ACT is safe and improves PFS and OS by 3 and 13 months respectively, as compared with the chemo-alone control[8].
- Herein, we present translational studies data on the localized vs systemic levels of IL-12 and its effects on immune downstream effectors.

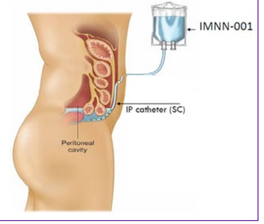
What is IMNN-001?

IMNN-001 is an IL-12 DNA-based plasmid encased in a lipopolymer nanoparticle delivery system enabling efficient cell transfection and durable, local secretion of the IL-12 protein at the tumor site[9].

IL-12 coding plasmid in lipid nanoparticle



Intraperitoneal delivery



OVATION-2 trial (NCT03393884) n=112 (ITT)

SAFETY

- Most common TEAE related to IMNN-001 were abdominal pain and pyrexia
- NO CRS or higher incidence of immune events in IMNN-001 arm vs control

EFFICACY (all favor IMNN-001 arm)

METHODS

- **Human IL-12, IFN-γ, TNF-α and VEGF analysis:** Blood and peritoneal fluid (lavage or ascites) samples were collected before and after IMNN-001 dosing and analyzed by Luminex assay using Millipore kits for IL-12 and TNF-α, IFN-γ and VEGF. Concentrations were expressed over 100 mg total protein (quantified with a Pierce BCA protein assay kit (Rockford, IL)). Patients with baseline and at least one post-treatment sample and samples with total protein >1 mg/ml were included.
- **Immune markers expression in tumor:** pre & post-treatment tissue samples were analyzed by cyclic immunofluorescence analysis (Phenocycler-fusion) for the expression of CD8, CD11c, CD44, CD4, HLA-DR, CD45, CD45RO, Ki67, CD14, CD3e, CD20, CD56, HLA-A, CD68, CD163, CD11b, CD16, Pan CK, FOXP3, PD-L1, PD-1, IDO-1

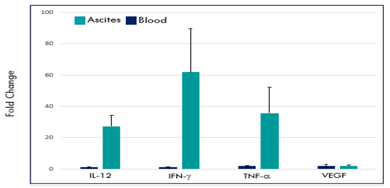
CONCLUSIONS

- IMNN-001 generates IL-12, IFN-γ and TNF-α locally in the tumor microenvironment, while limiting cytokine expression systemically, likely contributing to the efficacy, and safety seen in the clinic.
- IMNN-001 creates a "hot" anti-tumor microenvironment by recruiting CD8+ T cells, macrophages and dendritic cells in the tumor microenvironment and decreasing Treg suppressor cells.
- This biomarker research confirms IMNN-001's MoA and selective local immune activation at the tumor site. Together with the excellent safety and activity observed in the clinic, these results warrant further investigation. A Phase III trial (OVATION-3, NCT06915025) is currently enrolling.

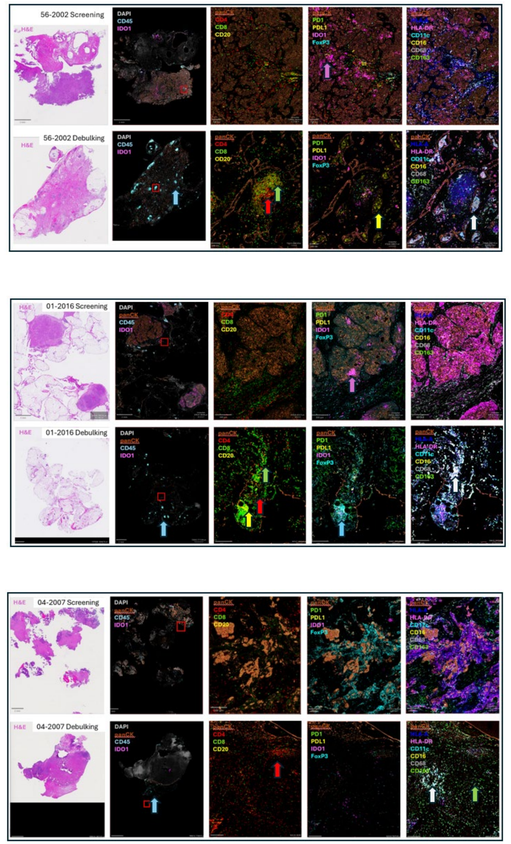
RESULTS

IMNN-001 treatment selectively induces IL-12, IFN-γ and TNF-α at local level vs systemic exposure

Fold changes in cytokine/VEGF levels in ascites/washes and blood samples before and 24 hours after IP administration of IMNN-001 at 100 mg/m² in OVATION-2. The data are Mean ± SE of ascites/washes collected at different time points from 8-10 patients and blood samples from 15 patients.



Treatment-induced changes in the immune microenvironment



Images of FFPE tissue at screening (upper rows in each panel) and at IDS (lower rows) from 3 patients treated with IMNN-001 and N/ACT in the study. H&E staining is shown in the first column of each panel. The 2nd column shows multiplex-Phenocycler color assay for markers indicated in the legend to analyze changes on hematopoietic infiltration (CD45+ cells). Selected area (red square) from the tissue section at 20× magnification is shown in the last 3 columns with stains indicating lymphocyte infiltration (CD4+, CD8+, CD20+, 3rd column), expression of immunoregulatory cell markers (FoxP3+, IDO1+, PD-L1+, 4th column) and macrophages, monocytes and dendritic cells markers (CD68+, CD163+, CD16, CD11c, last column), all with pan-cytokeratin staining (maroon) to identify tumor cells.

Overall, these representative images show that after chemotherapy and IL-12 treatment, decreased tumor cells, increased lymphocyte infiltration (CD4+, CD8+, and CD20+ cells), as well as immunostimulatory macrophages (CD68+, PD-L1+ and dendritic cells (CD163+), are observed. Additionally, examples of the presence of immunosuppressive IDO1+ T-cells is appreciated at screening as well as the presence of FoxP3+ exhausted T-cells at debulking in some cases.

This cellular composition and distribution at the time of IDS indicate immune engagement with the tumor, evidenced by tumor-infiltrating lymphocytes (TILs), immunostimulatory PD-L1+ macrophages, dendritic cells and para-tumoral regulator T-cells. The lack of cytokeratin-stained tumor cells in the IDS specimens, together with the dense infiltration of foamy macrophages, indicate profound tumor destruction.

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P.H.T receives consulting fees and owns stock from Imunon.