#1165: IMNN-001, IL-12 gene therapy, added to Neo/Adjuvant chemotherapy, safely turns the tumor microenvironment cold-to-hot in newly diagnosed epithelial ovarian cancer (EOC)

Khursheed Anwer[1], Subeena Sood[1], Lauren Musso[2], Danielle Blume[2], Jeff Sparks[1], Ana Limon[2], Stacy R. Lindborg[2], Douglas V. Faller[2]

1. Imunon, Huntsville, AL, USA; 2. Imunon, Lawrenceville, NJ, USA

BACKGROUND

- **Epithelial Ovarian cancer (EOC) remains an unmet medical need,** with 80% of the cases diagnosed in advanced stage (III/IV). >60% of ovarian cancer patients die within 5 years [1].
- **EOC is an immunogenic tumor; however, effective immune therapies** (e.g., Immune checkpoint inhibitors) **remain elusive** due to EOC's marked immunosuppressive tumor microenvironment (TME)[2]. Neo/Adjuvant chemotherapy (N/ACT) and peri-debulking surgery are SoC treatment, followed by maintenance PARPi for patients with homologous recombination deficiency (HRD) who have a complete or partial response to first-line platinum-based chemotherapy[3].
- **IL-12 is a pleiotropic immuno-stimulatory cytokine able to turn "cold" tumor microenvironments to "hot"** by activating both the innate and adaptive immune systems. However, systemic treatment with recombinant hIL-12 is too toxic for use in the clinic.
- **OVATION-2** randomized, controlled Phase I/II (NCT03393884) **study has shown that IMNN-001** (an IL-12 gene therapy)[4] **delivered IP in combination with N/ACT is safe** (with barely detectable systemic exposure) **and improves PFS and OS by 3 and 13 months, respectively**, as compared with the N/ACT alone control[3].
- Herein, we present OVATION-2 translational data on the changes induced by the local administration of IL-12 and its downstream effectors in the TME from paired samples (pre- and post-treatment) from patients treated with IMNN-001 and N/ACT.

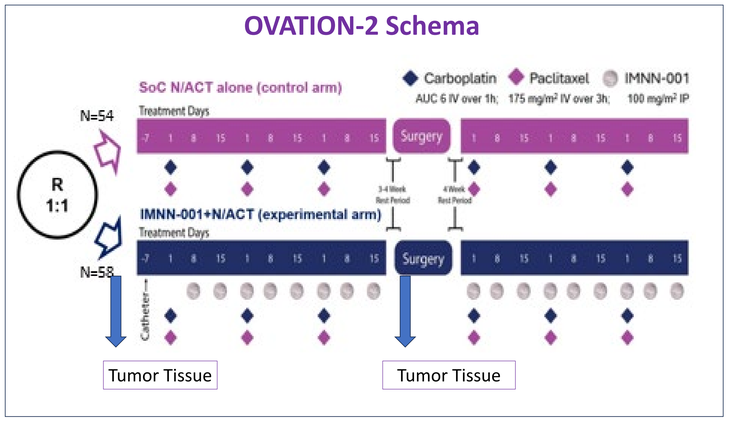


Figure 2. Left: OVATION-2 schema, biological sampling for translational research.

A: IMNN-001 Formulation and Delivery

B: IL-12 MoA

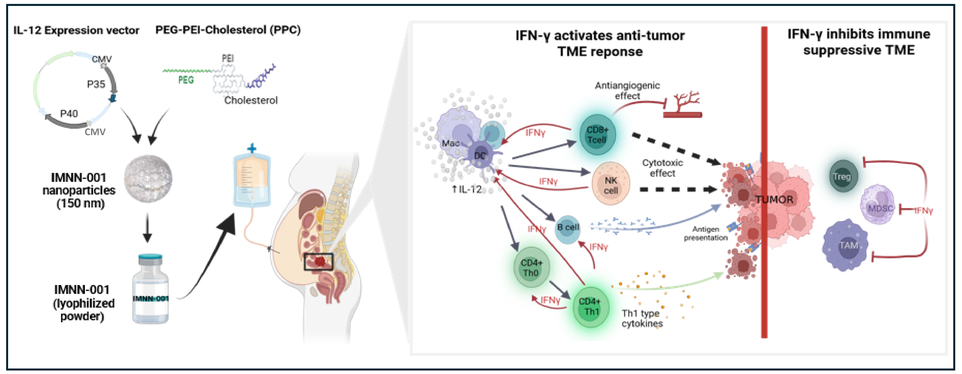


Figure 1 A: IMNN-001, comprised of a plasmid expressing the p35 and p45 subunits of hIL-12 encased in a synthetic lipopolymer delivery system composed of a polyethylenimine (PE) backbone covalently linked to polyethylene glycol (PEG) and cholesterol, is delivered intraperitoneally in the clinic. **B:** The expression of hIL-12 by cells in the tumor microenvironment (TME), through its downstream mediators (IFN-γ and other cytokines), activates the innate and adaptive immune systems and inhibits immune suppressive cells, turning the TME from cold to hot and providing anti-tumor activity.

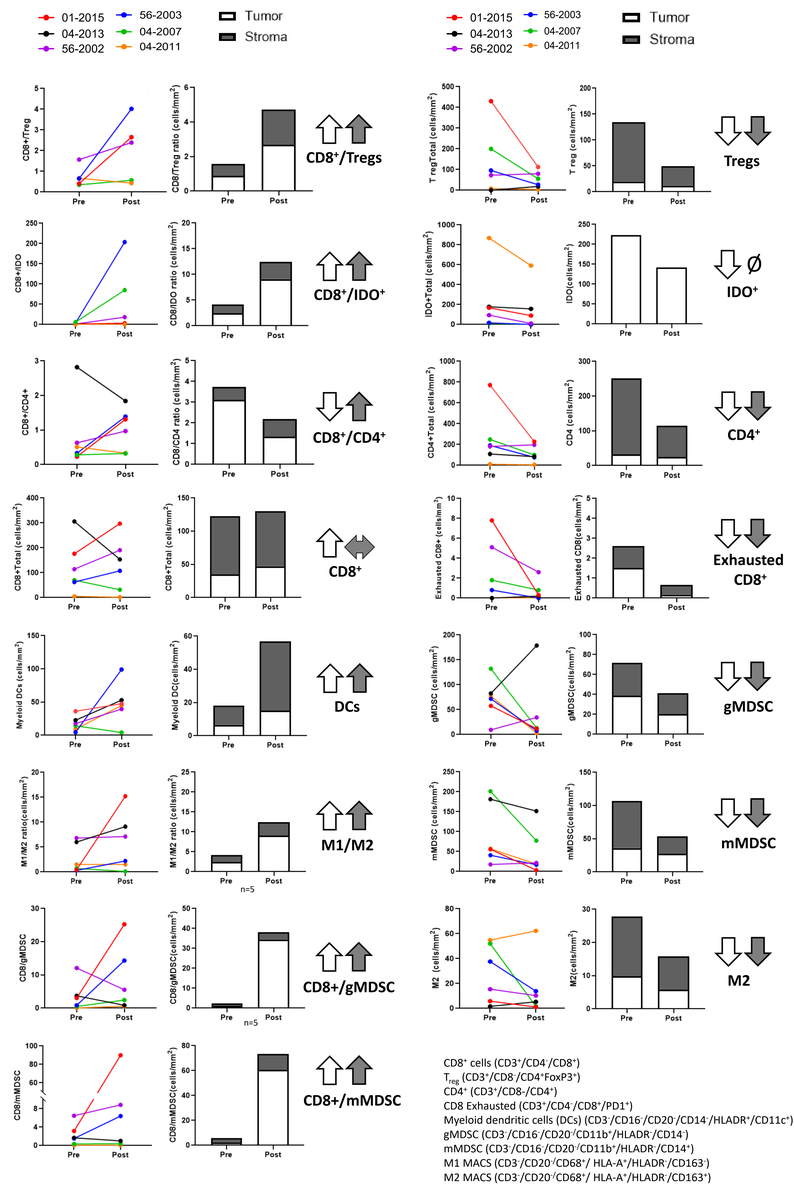
CONCLUSIONS

- IMNN-001 creates a "hot" anti-tumor microenvironment in EOC by: i) increasing the recruitment of CD8+, myeloid dendritic cells and M1 macrophages in 50-80% of the paired samples in tumor and stroma; and ii) decreasing immunosuppressive markers (IDO, Treg, exhausted CD8, M2 macrophages) in 65-80% of the tumor and stroma samples.
- These results, including induction of favorable ratios of CD8+/Tregs and CD8+/CD4+ cells, - both associated with improved patient outcomes[5-6]- as well as CD8+/IDO+ cells, are consistent with the translational results of the previous Phase 1 OVATION-1 study[9] and with the efficacy seen in the clinic in the OVATION-2 study[3].
- This biomarker research confirms local immune activation at the tumor site by IMNN-001. 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

Biomarkers of immune stimulation

Biomarkers of immune suppression



CD8⁺ cells (CD3⁺/CD4⁻/CD8⁺)
T_{reg} (CD3⁺/CD8⁻/CD4⁺FoxP3⁺)
CD4⁺ (CD3⁺/CD8⁻/CD4⁺)
CD8 Exhausted (CD3⁺/CD4⁻/CD8⁺/PD1⁺)
Myeloid dendritic cells (DCs) (CD3⁻/CD16⁻/CD20⁻/CD14⁻/HLADR⁺/CD11c⁺)
gMDSC (CD3⁻/CD16⁻/CD20⁻/CD11b⁺/HLADR⁻/CD14⁺)
mMDSC (CD3⁻/CD16⁻/CD20⁻/CD11b⁺/HLADR⁻/CD14⁺)
M1 MACS (CD3⁻/CD20⁻/CD68⁺/ HLA-A⁺/HLADR⁻/CD163⁺)
M2 MACS (CD3⁻/CD20⁻/CD68⁺/ HLA-A⁺/HLADR⁻/CD163⁺)

Figure 3. Line graphs: Quantitative changes of the density of immune cells in 6 paired patient samples obtained from IP tumor/tissue blocks pre- and post-treatment with IMNN-001+N/ACT (at interval debulking surgery) from the OVATION-2 study. Staggered columns represent the density of immune cells found in pathologist-annotated tumor and stroma tissue on each sample (n=5-6). Numbers are normalized according to the total number of cells and area analyzed.

METHODS

Immune marker expression: pre- & post-treatment tissue samples were analyzed by cyclic immunofluorescence analysis (Phenocycler-fusion) for the expression of CD8, CD11c, CD44, CD4, HLA-DR, CD14, CD3, CD20, CD45, HLA-A, CD68, CD163, CD11b, CD16, Pan CK, FOXP3, PD-1 and IDO-1. The whole slide phenocycler-fusion images were analyzed using Qupath Version 5.1. Cell segmentation was first processed with the StarDist package using the DAPI channel to obtain an initial segmentation of the nuclei followed by an expansion of these regions to define the cell bounds. Training was then performed on positive and negative cell distributions to generate marker positivity classifiers for each channel. These classifiers were then combined into composite classifiers to determine the full phenotypic profiles of each cell. Then pixel classifiers were generated using the PAN-CK channel in order to define the tumor and stroma regions of each slide. A set of cell populations defined on a phenotypic map of co-expressions was then quantified in total, tumor, and stroma regions as well as normalized by tissue area to total cells/mm² [7,8].

REFERENCES

(1) Siegel et al., *Cancer Statistics*, 2023; (2) Blanc-Durand, *Front Immunol*, 2023; (3) Thaker et al.,*Gynecol Oncol*, 2025; (4) Anwer et al., *Gene Ther*, 2009; (5) Preston et al., PLoS One 2013; (6) Sato et al., PNAS 2005; (7) Bankhead, P. et al. Sci. Rep. 7, 16878 (2017); (8) Schmidt U. et al., International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), Granada, Spain, September 2018; (9) Thaker et al., Clin Cancer Res 2021.

CONTACT INFO

dfaller@imunon.com; kanwer@imunon.com