IMNN-001-mediated therapeutic delivery of IL-12 plasmid to tumor tissue and tumor microenvironment, promotes activation and persistence of T-cells which translates to a survival benefit in the clinic

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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 highly immunosuppressive tumor microenvironment (TME)[2].
- **Here we assess the immune changes in the TME generated by the experimental DNA immunotherapy IMNN-001 in patient biopsies from the OVATION-2 (NCT03393884) study.** OVATION-2 is a controlled Phase I/II study for stage III/IV newly diagnosed EOC patients where a total of 112 patients were randomized to IMNN-001+standard neo- and adjuvant chemotherapy (N/ACT) vs N/ACT alone (Figure 1).
- **The addition of intraperitoneal IMNN-001** (a DNA based IL-12 immunotherapy designed to promote localized IL-12 expression as well as its downstream effectors IFNγ and TNFα[3] (Figure 2) **to systemic N/ACT** was demonstrated to be **safe and manageable** (with barely detectable IL12 systemic exposure and lack of immune derived adverse events) and to **improve PFS and final OS by 3 and 14.7 months, respectively, over the SoC** control arm[4].
- Previously studied paired samples (pre-treatment and at debulking surgery) confirmed local tumor site immune activation and immunosuppressive marker decrease in the experimental arm, all considered contributing factors to the switch from "immune cold-to-hot" in the TME[5].

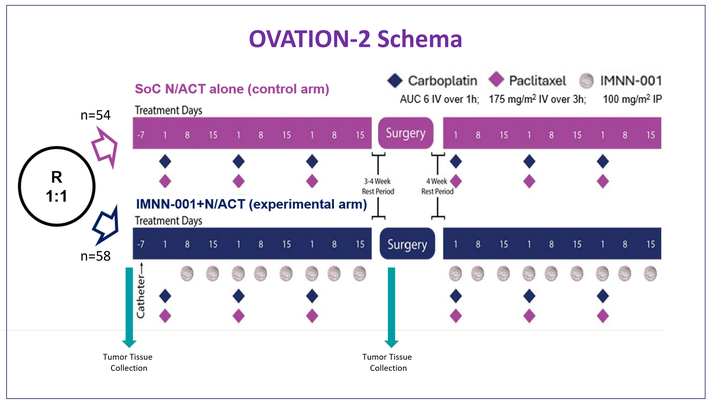


Figure 1: OVATION-2 schema and biological sampling timing for translational research

A: IMNN-001 Formulation and Delivery

B: IL-12 MoA

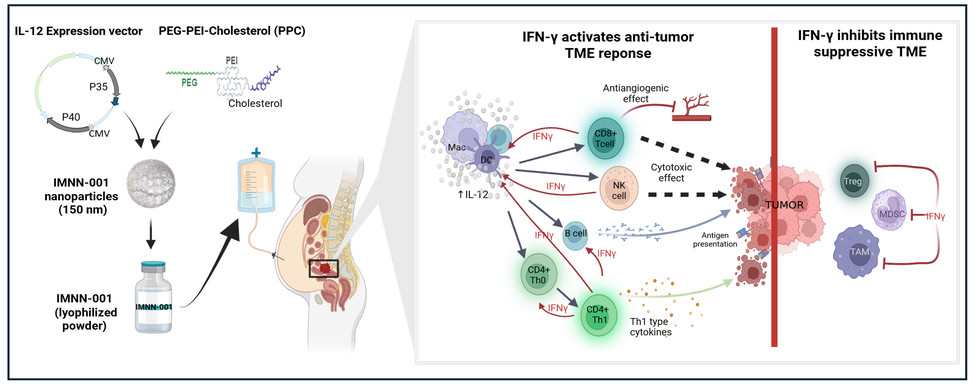


Figure 2 A: IMNN-001, comprises a DNA plasmid expressing the p35 and p45 subunits of hIL-12 encased in a synthetic lipopolymer delivery system composed of a polyethyleneimine (PE) backbone covalently linked to polyethylene glycol (PEG) and cholesterol, is delivered intraperitoneally in the clinic. **B:** 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.

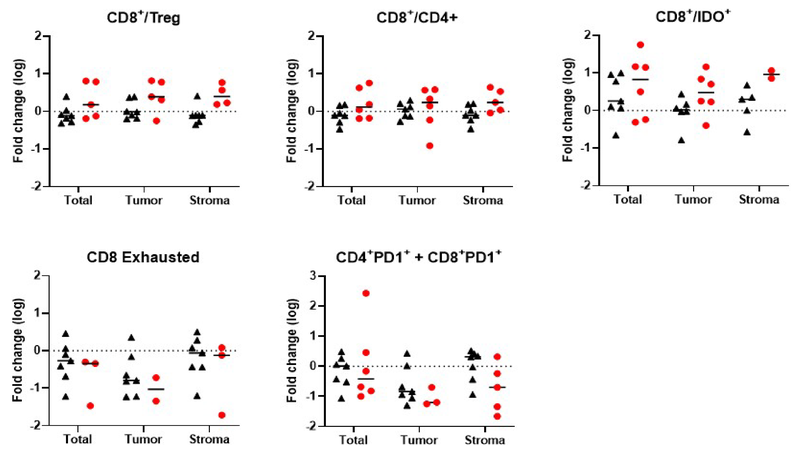
CONCLUSIONS

- The addition of IMNN-001 to the SoC N/ACT for the treatment of patients with advanced EOC is primarily responsible for the increased anti-tumoral activity in T cells, as shown by the increase in beneficial ratios: CD8+/Tregs, CD8+/CD4+ cells, - both associated with improved patient outcomes[8,9] - as well as CD8+/IDO+ ratio, in both tumor and stroma. Additionally, IMNN-001 causes a decrease in PD1+ T cells in the stroma, all favoring the anti-tumor activation of the immune system.
- The chemotherapy effect supports proinflammatory macrophage polarization in all compartments and decreases myeloid infiltration, particularly in the tumor.
- This data corroborates the translational results of the previous Phase 1 OVATION-1 study[10] and provides a mechanism for the efficacy seen in the clinic in the OVATION-2 study[4]. The complementary effects of IMNN-001 on both the activation and persistence of a T-cell immune response, together with the suppression of protumor myeloid cells by the chemotherapy component of the treatment, confirms the hypothesis that this IMNN-001 + N/ACT treatment favors the switch from a "cold" to an immunologically "hot" TME.
- A Phase III trial (OVATION-3, NCT06915025) is currently enrolling, which aims to confirm the clinical results and add to the immunological translational research.

RESULTS

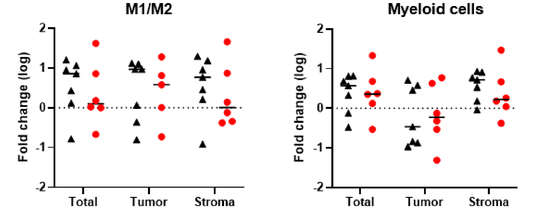
Analysis of T cells

▲ Control arm: Treated with SoC neo + adjuvant chemotherapy
● Experimental arm: Treated with SoC neo + adjuvant chemotherapy + IMNN-001



Analysis of Myeloid cells

▲ Control arm: Treated with SoC neo + adjuvant chemotherapy
● Experimental arm: Treated with SoC neo + adjuvant chemotherapy + IMNN-001



CD8⁺ cells (CD3⁺/CD4⁻/CD8⁺)
T_{reg} (CD3⁺/CD8⁻/CD4⁺FoxP3⁺)
CD4⁺ (CD3⁺/CD8-/CD4⁺)
CD8 Exhausted (CD3⁺/CD4⁻/CD8⁺/PD1⁺)
Myeloid (CD45⁺/CD3⁻/CD20⁻/PANCK⁻)
M1 MACS (CD3⁻/CD20⁻/CD68⁺/ HLA-A⁺/HLADR⁺/CD163⁺)
M2 MACS (CD3⁻/CD20⁻/CD68⁺/ HLA-A⁺/HLADR⁻/CD163⁺)

Figure 3. Immune cell densities (per mm³) in tumor tissue collected at screening laparoscopy and debulking surgery from control and experimental arms were determined as described in methods. To evaluate the treatment effect, the cell density post treatment vs pre-treatment was calculated in both arms. The fold change is expressed in logarithmic scale in the scatter plots for treatment effect comparisons between the experimental (red circles) and control arm (black triangles) both in total tissue and separately in each (tumor and stroma) compartment.

METHODS

To distinguish the immune effect contribution of IMNN-001 from the CT treatment, available paired tumor samples from the experimental arm (n=6) and control arm (n=7) were analyzed using 21 relevant immune markers in tumor and stroma by cyclic immunofluorescence analysis (Phenocycler-fusion). Cells in specimens segmented by a pathologist were quantified and normalized by tissue area to total cells/mm². Relevant immune cell populations' density values identified in post-treatment tissue were divided by pre-treatment values to obtain fold change upon treatment in total tissue, tumor and stroma.

The whole slide Phenocycler-fusion images were analyzed using Qupath Version 5.1. StarDist package -DAPI channel- was used 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 were then combined into composite classifiers to determine the full phenotypic profile of each cell. Pixel classifiers were then generated (PAN-CK channel) 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 [2 6,7].

REFERENCES

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