Efficacy of IMNN-001, an Interleukin-12 Immune Gene Therapy, at Different Dose Frequencies

Jean D. Boyer, Subeena Sood, Jessica Kim, Majed M. Matar, Olivia Signer, Jeff Sparks, Jennifer S. Rice,
Alanna M. Smith, Nicholas Borys, Corinne Le Goff, Khursheed Anwer
Imunon Inc., Lawrenceville, NJ

Abstract

The purpose of this study was to investigate the effect of dosing frequency on the antitumor activity of intraperitoneal IMNN-001, an interleukin-12 (IL-12) immune gene therapy in a mouse model of peritoneally disseminated ovarian cancer. IMNN-001 expressing murine IL-12 was designed for this study, mIMNN-001. Three dosing regimens were examined for efficacy in ID8 tumor-bearing mice either weekly, every 2 weeks or every 3 weeks (Table 1). Six animals from each group B, C, and D were harvested for translational research (TR) after 5 weekly (3 every 2-week and 2 every 3-week) treatments, respectively. The remaining 4 animals in each group were followed for weight change (tumor burden) and survival. Additionally, TR evaluated change in ascites T cell populations. There was a gradual rise in tumor burden and mortality in all treatment groups with comparable rate between once every week and once every 2-week regimens. Once every 3-week regimen had relatively higher mortality rate and higher tumor burden. There were similar or higher increases in T-cell and B cells with reduced treatment frequency with lesser increases in myeloid cell density with reduced treatment frequency. Once every 2-week dosing of IMNN-001 in human studies is warranted.

Introduction

Epithelial ovarian cancer (EOC) is the fifth deadliest malignancy among women in the United States (**1**). There are approximately 22,000 new cases of ovarian cancer every year and the majority, approximately 70% of cases, are diagnosed in advanced stages III and IV. EOC is characterized by dissemination of tumor in the peritoneal cavity with a high risk of recurrence (75%, stage III and IV) after seemingly successful surgery and chemotherapy (**2**). Immunotherapy interventions are considered promising candidates for the treatment of ovarian cancer considering the immunogenic nature of the malignancy (**3**). The evidence of immune activation in ovarian cancer has been demonstrated in the production of antibodies or antitumor T-cell lymphocytes in primary tumor, ascites, and blood (4-6). Presence of tumor-infiltrating cytotoxic T-cell lymphocytes has been linked to better prognosis while presence of immunosuppressive regulatory T cells (Tregs) has been associated with poor prognosis in ovarian cancer (**7-9**). The peritoneal cavity of patients with advanced ovarian cancer contains the primary tumor environment and is an attractive target for a regional approach to immune modulation.

Interleukin 12 (IL-12) is a pluripotent cytokine associated with stimulation of innate and adaptive immune response against cancer. Clinical responses to recombinant IL-12 have been observed in multiple cancers (**10-12**). IMNN-001 is a gene therapy that produces safe and durable local levels of IL-12 to stimulate innate and adaptive components of the immune system. The IMNN-001 nanoparticle comprises a DNA plasmid encoding IL-12 gene and a synthetic polymer facilitating plasmid delivery (**13**).

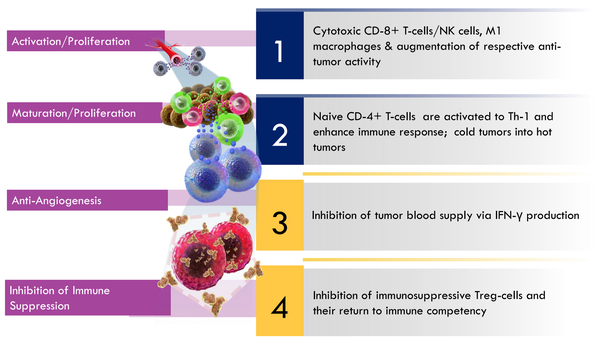

Figure 1. Mechanism of Action of IL-12

Methods

The preclinical study was conducted to compare once every week dosing with once every two weeks or once every three weeks dosing. 2.5 x 10^6 ID8 cells were injected intraperitoneal (IP) into 8-10 weeks C57BL/6 mice. ID8 is an aggressive tumor model with short life span therefore the time-dependence of the model was taken into the account in the study design. Treatment with mouse IL-12 was initiated at day 14. 250 mg DNA was delivered IP once every week, once every two weeks, or once every three weeks. Tumor burden (animal weight) was followed along with survival. Ascites was assessed by flow cytometry for immune infiltrating cells: $CD4^+$, $CD8^+$, $FoxP3^+$, CD8+/$CD4^+$, $CD8^+$/FoxP3.

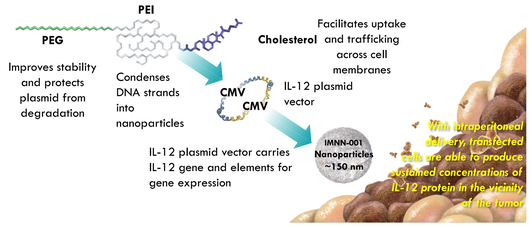

Figure 2. IMNN-001 Formulation. IMNN-001 is formulated with a three-component delivery system of polyethylene glycol (PEG) polyethyleneimine (PEI) cholesterol combined with IL-12 DNA plasmid

Table 1.

Group	N	Route	ID8	
A	10@	IP	2.5X10⁶ ID8/mIMNN-001	Weekly (6 Rx)
B	10@	IP	2.5X10⁶ ID8/mIMNN-001	Every 2 weeks (3 Rx)
C	10@	IP	2.5X10⁶ ID8/mIMNN-001	Every 3 weeks (2 Rx)
D	15*	IP	2.5X10⁶ ID8	No mIMNN-001 Rx

*Extra animals for untreated controls as they may start to die earlier
@if the animals survive longer than 6 weeks (12/09/2021) the IMNN-001 treatment frequency will continue per design and the harvest will be done in two stages- First harvest on 12/09/2021, and second harvest thereafter per survival status.

Results

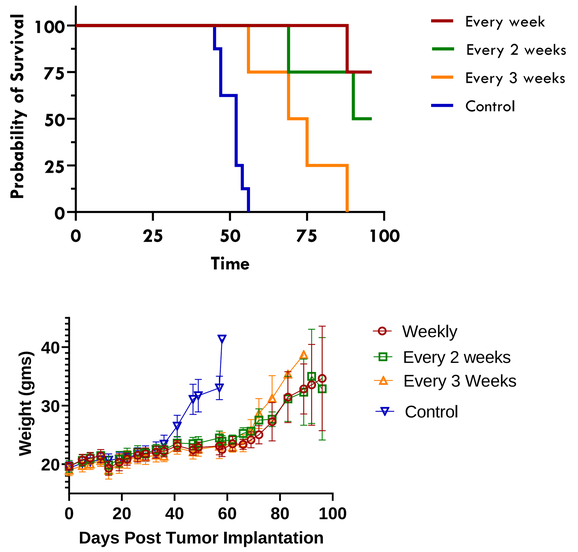

Figure 3. Survival and Tumor Growth. Mice were challenged with 2.5X10^6 ID8 Cells. Following establishment of tumor, mice were treated weekly, every two weeks or every three weeks with mIMNN-001.

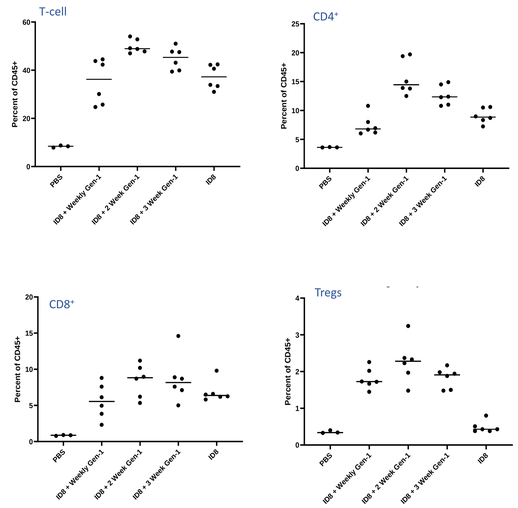

Figure 4. Day 49: Increases in T-cell Populations in Ascites after mIMNN-001 Treatment Every 2-week and every 3-week treatments yield similar or higher T-cell response

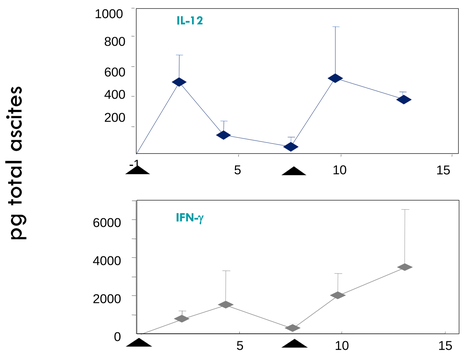

Figure 5. IL-12 and IFN-γ levels in ascites following two weekly IP infusions (at arrows) of mIMNN-001 in peritoneal disseminated ID-8 ovarian tumor bearing mice (Fewell, 2009)

Conclusion

mIMNN-001 demonstrated stimulation of the immune response in the ID8 ovarian tumor model. Of the 3 dosing regimens tested, the once every 2-week regimen demonstrated comparability to the weekly regimen while showing superiority to the once every 3-week regimen, particularly with respect to mortality and tumor burden (**Figure 3**). Thus, exploring once every 2-week dosing of IMNN-001 in human studies is warranted.

REFERENCES 1. Howlader N, *SEER Cancer Statistics Review, 1975–2017*, Bethesda, MD: NCI. Available from: https://seer.cancer.gov/csr/1975_2017; 2. Du Bois A, *Cancer,* 2009; 3. Ghisoni E, *Int J Mol Sci* 2019; 4. Fortner RT, *Gynecol Oncol* 2017; 5. Singh M, *Immunol Invest,* 2016, *Ann Oncol,* 2017; 6. Odunsi K., Ann Oncol 2017; 7. Toker A, *Clin Cancer Res,* 2018; 8. Curiel TJ. *Nat Med* 2004; 9. Polcher M. *Cancer Immunol Immunother,* 2010; 10. Lu X. *Curr Cancer Drug Targets,* 2017; 11. Tugues, S, *Cell Death Differ,* 2015; 12. Lenzi R, *Clin Cancer Res,* 2002; 13. Fewell JG, *J Control Release* 2005.