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Interleukin-12 Gene Therapy in Combination with Bevacizumab and PEGylated Liposomal Doxorubicin for Treatment of Disseminated Ovarian Cancer

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ABSTRACT

Despite recent improvements in treatment options for ovarian cancer patients, notably, the approval of using bevacizumab in combination with chemotherapies including pegylated liposomal doxorubicin (PLD), this disease is still the most deadly of all gynecological malignancies requiring new and novel therapeutics. Interleukin-12 (IL-12) is a highly active cytokine that can induce a potent anti-cancer immunity mediated through activation of cytotoxic T-lymphocytes, natural killer cell proliferation, and secretion of interferon-γ. We are developing an IL-12 based gene therapy for the treatment of gynecological malignancies that have spread into the peritoneal cavity. Our approach utilizes IL-12 plasmid (pIL-12) formulated with the PPC delivery system, which is comprised of a low molecular weight polyethylenimine covalently linked to polyethyleneglycol and cholesterol.

Previously we have shown in a mouse model of disseminated ovarian cancer efficacy of a treatment regimen of pIL-12/PPC used in combination with paclitaxel and carboplatin. The combination treatment significantly improved survival compared to either pIL-12/PPC alone or chemotherapy alone and demonstrated the feasibility of using an IL-12 immunotherapy in combination with cytotoxic chemotherapies to achieve additive therapeutic effects. Results from a Phase I clinical trial in platinum resistant patients have shown that intraperitoneal delivery of pIL-12/PPC in combination with PLD produced an overall clinical benefit of 57.1% (PR=21.4%; SD=35.7%) in patients with measurable disease. The highest percentage of PRs were found at the highest dose level (28.6%) along with highest percentage of patients achieving SD (57.1%).

Here we describe studies evaluating the combination of pIL-12/PPC with bevacizumab and PLD. For these studies 7,000,000 human SKOV-3 cells were implanted into the peritoneal cavity of immunocompromised Hsd:Athymic Nude-Foxn1nu mice. Treatment with pIL-12/PPC alone and bevacizumab alone resulted in a 50% and a 39% reduction of animals with visible tumors at the end of the study. Combining pIL-12/PPC + bevacizumab improved the response to 78% of animals with no visible tumors. Further, combining pIL-12/PPC + bevacizumab + PLD resulted in a >98% decrease in tumor burden in animals compared to controls and ~92% decrease in tumor burden compared to animals treated only with bevacizumab + PLD. All treatments were well tolerated and analysis of serum chemistries and hematology showed normal ranges of all parameters examined for all groups. There were no significant differences in animal weights between groups during the experiment. Together these results suggest synergistic efficacies can be achieved by combining a novel pIL-12/PPC immunotherapy with anti-angiogenesis therapies and cytotoxic chemotherapies in disseminated ovarian cancer.

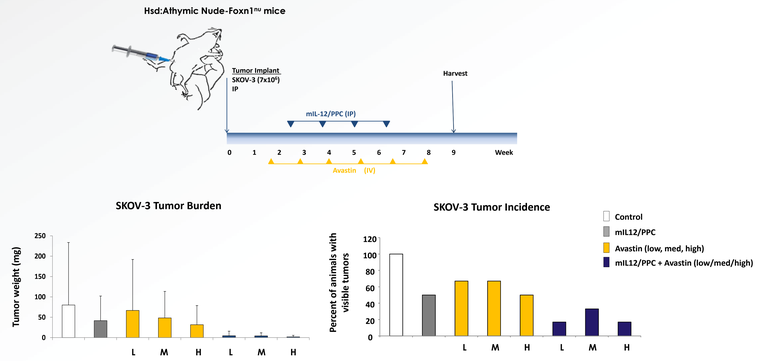
BACKGROUND

- GEN-1 is a novel immunotherapeutic agent that is comprised of a human IL-12 expressing plasmid formulated with a synthetic DNA delivery system of polyethyleneglycol-polyethylenimine-cholesterol (PPC) that is currently being developed as a treatment for ovarian cancer.
- Several clinical trials have been performed, the most recent being a Phase 1 clinical trial evaluating the combination of GEN-1 with pegylated liposomal doxorubicin (PLD) in recurrent platinum resistant patients.
- Weekly intraperitoneal administrations of GEN-1 produced encouraging results in this difficult to treat population and indicated an 85.7% overall response rate at the highest dose level (TABLE 1).
- The recent FDA approval of Avastin (bevacizumab) as a treatment for ovarian cancer with it's intended use to be in combination with chemotherapy has prompted our interest in combining IL-12 gene therapy with bevacizumab and in combination with standard of care chemotherapy.

TABLE 1: Objective tumor response in ovarian cancer patients with measurable disease following administration of GEN-1 in combination with PLD.

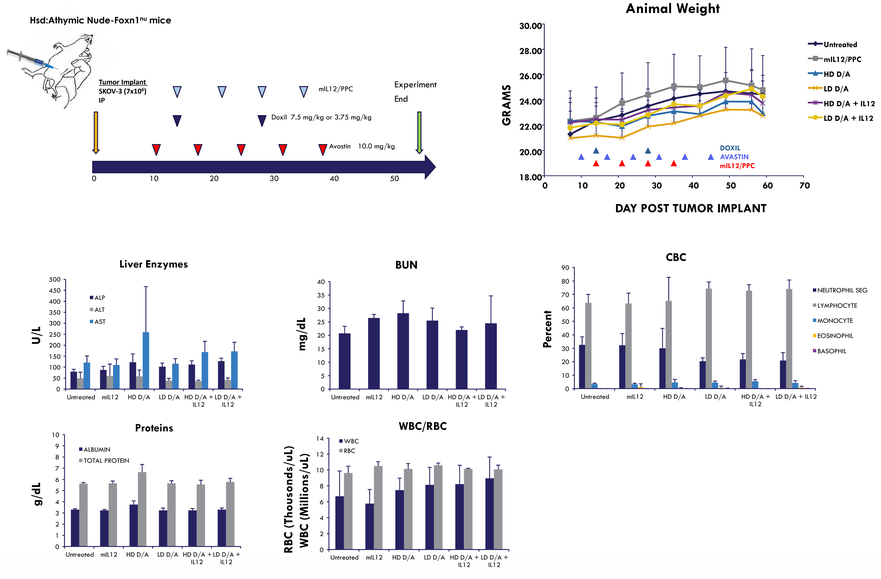
	Dose Level			
Total	n (%) 14	GEN-1 24 mg/m² + PLD 40mg/mL	GEN-1 36 mg/m² + PLD 40mg/mL	GEN-1 36 mg/m² + PLD 50mg/mL
Objective Tumor Response				
Partial Response	3 (21%)	0 (0%)	1 (20%)	2 (29%)
Stable	5 (36%)	0 (0%)	1 (20%)	4 (57%)
Increasing Disease	3 (21%)	2 (100%)	1 (20%)	0 (0%)
Indeterminate	3 (21%)	0 (0%)	2 (40%)	1 (14%)

mIL12/PPC + BEVACIZUMAB (Avastin)

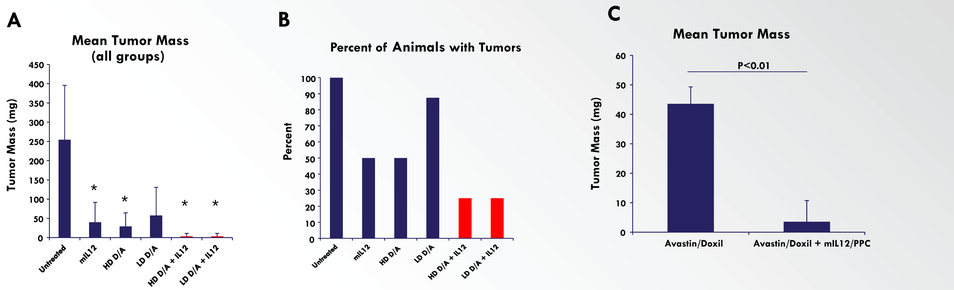


- Mice were administered 7x10⁶ SKOV-3 cells IP followed by weekly administration of the murine homolog of GEN-1 (mIL12/PPC; 5 mg/Kg) and/or Avastin at 5 mg/Kg (L), 10 mg/Kg (M), 20 mg/Kg (H). Animals were euthanized 59 days after tumor implant.
- Treatment with mIL12/PPC in combination with Avastin resulted in a reduction in total tumor mass (~80%) compared to untreated animals and a significant decrease in the percent of animals with visible tumors relative to all other groups.

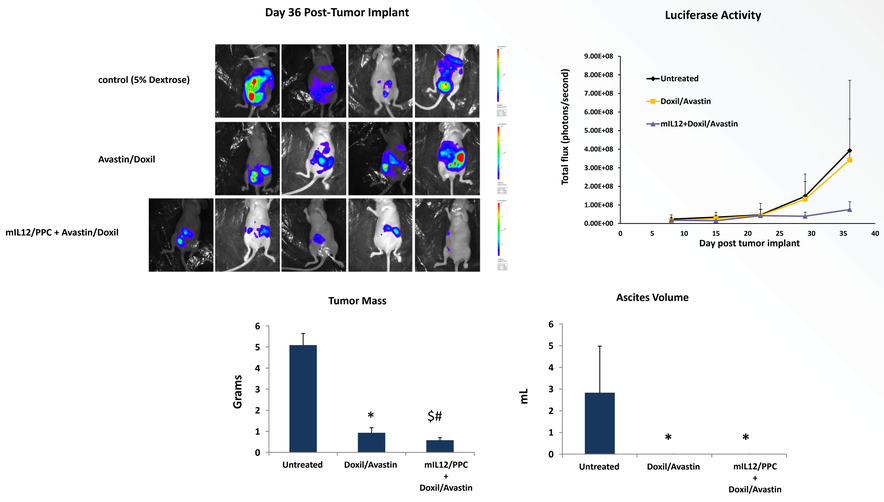
mIL12/PPC + BEVACIZUMAB/PEGyLATED LIPOSOMAL DOXORUBICIN (Doxil)



- Mice were administered 7x10⁶ SKOV-3 cells IP followed by weekly administration of mIL12/PPC (5 mg/Kg) and/or Avastin + Doxil treatment regimen. Avastin was administered 10 mg/Kg and Doxil was administered at a dose of either 3.75 mg/Kg or 7.5 mg/Kg. Animals were euthanized 59 days after tumor implant.
- Treatment resulted in little indication of acute or sub-chronic systemic toxicities as indicated by unremarkable changes in animal weights during the course of the study or changes in serum chemistries and other hematological parameters measured at the termination of the study



- Tumor Burden in mice treated with mIL12/PPC + Avastin/Doxil. (A) Combination treatment resulted in an overall >98% decrease in tumor mass compared to untreated animals. Mean values (± SD) for all experimental groups. * = p<0.05 vs. untreated animals. B: Tumor incidence C: Tumor mass comparison between Avastin/Doxil and Avastin/Doxil + mIL12/PPC treated animals independent of Doxil dose (both high dose and low dose included/combined). Addition of mIL12/PPC decreased tumor burden by 92%.



- Parallel study performed using a SKOV-3-Luc cell line. Luciferase expression and measured tumor mass significantly reduced in mIL12/PPC + Doxil/Avastin treated group. * = p<0.05 compared to Untreated; $ = p<0.01 vs Untreated; # = p<0.05 vs Doxil/Avastin.
- Tumor growth of this cell line resulted in ascites accumulation that we completely abrogated in mice treated with Doxil/Avastin alone or with mIL12/PPC + Doxil/Avastin.

SUMMARY/CONCLUSIONS

- The combined treatment regimen consisting of mIL12/PPC with Avastin and Doxil was evaluated in a murine model of disseminated ovarian cancer. Combining mIL12/PPC with Avastin alone or Avastin and Doxil resulted in improved treatment efficacy (determined by decreased tumor burden) compared to any single treatment.
- The addition of of mIL12/PPC to Avastin/Doxil resulted in a 92% decrease in tumor burden compared to Avastin/Doxil treatment only and a 98% reduction in tumor burden compared to untreated controls.
- The addition of mIL12/PPC to either treatment was well tolerated and did not result in systemic toxicities.
- Phase I/II Clinical testing of GEN-1 (human IL12 plasmid formulated with PPC) in recurrent ovarian cancer patients is anticipated to start in Q4 2016.