Interleukin-12 Gene Therapy in Combination with Bevacizumab and PEGylated Liposomal Doxorubicin for Treatment of Disseminated Ovarian Cancer
Jason G. Fewell, Majed M. Matar, Jennifer K. Rice, Diane McClure, Elaine Brunhoever, Jeff Sparks, Stefanie Greenleaf, Kelley Smith, Khursheed Anwer
Celsius Corporation, 601 Genome Way, Ste 3100 Huntsville, AL 35806

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 on a 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 polysaccharide covalently linked to polyethylene glycol and chitosan.

Previously we have shown in a mouse model of disseminated ovarian cancer efficacy of a treatment regimen of pIL-12PPC used in combination with parenteral and carboplatin. The combination treatment significantly improved survival compared to either pIL-12PPC alone or chemotherapy alone and demonstrated the feasibility of using an aminoglycoside chemotherapy in combination with genetically-encoded cytokine therapy. We have recently shown that intraperitoneal delivery of pIL-12PPC is combined with PLD produced a significant clinical benefit of 10% (P=0.46, 90% CI=7.9%) in patients with miscreatic disease. The highest percentage of PFS were found at the highest dose level (28.6%) along with highest percentage of patients achieving SD (21.1%). Here we describe studies evaluating the combination of pIL-12PPC with bevacizumab and PLD. For these studies, 1,080-1,200 human SKOV-3 cells were implanted into the peritoneal cavity of immunocompromised nude/thymus nude mice. Treatment with pIL-12PPC alone and bevacizumab alone resulted in a 50% and a 30% reduction in tumors with visible tumors at the end of the study. Combining pIL-12PPC + bevacizumab improved the response to 70% of animals with no visible tumors. Further, combining pIL-12PPC + bevacizumab + PLD resulted in an +68% decrease in tumor burden in animals compared to controls and a ≥98% 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 effects can be achieved by combining a novel pIL-12 based immunotherapy with anti-angiogenesis therapies and cytokine 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 polyethylene glycol-polyethylene-polyamine-chitosan (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 87.5% overall response rate at the highest dose level (Table 1).
• The current FDA approval of Avastin (bevacizumab) as a treatment for ovarian cancer with 3% intent used to be in combination with chemotherapy has prompted our interest in combining IL-12 gene therapy with bevacizumab 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.

<table>
<thead>
<tr>
<th>Total</th>
<th>n=11</th>
<th>n=1</th>
<th>n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>50%</td>
<td>0%</td>
<td>50%</td>
</tr>
<tr>
<td>Progression Free Survival</td>
<td>14.3%</td>
<td>12.5%</td>
<td>16.6%</td>
</tr>
<tr>
<td>Progression Rate</td>
<td>25.0%</td>
<td>25.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Median Time to Progression</td>
<td>4.9</td>
<td>4.9</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Objective Tumor Response
Efficacy (%)
Progression Free Survival (%)
Progression Rate (%)
Median Time to Progression (months)

• Mice were administered 7×106 SKOV-3 cells IP followed by weekly administration of mIL-12 (ppIL-12PPC) (5 mg/kg) and/or Avastin (200 mg/kg). Animals were euthanized 56 days after tumor implantation.
• Treatment with mIL-12PPC in combination with Avastin resulted in a reduction in total tumor mass (%M) compared to untreated animals and a significant decrease in the percent of animals with visible tumors relative to all other groups.

SUMMARY/CONCLUSIONS
• The combined treatment regimen consisting of mIL-12PPC with Avastin and Doxil was evaluated in a mouse model of disseminated ovarian cancer. Combining mIL-12PPC 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 mIL-12PPC to Doxil/Avastin resulted in a 98% decrease in tumor burden compared to Doxil/Avastin treatment only and a 98% reduction in tumor burden compared to untreated controls.
• Phase II clinical testing of GEN-1 (human IL-12) planned formulated with PPC in recurrent ovarian cancer patients is anticipated to start in Q4 2016.