AACR October 2nd, 2017

# OVATION Study

## *Immunological changes following intraperitoneal administration of a formulated IL-12 plasmid in combination with standard neoadjuvant chemotherapy in patients with newly diagnosed advanced stage ovarian cancer*

K. Anwer[1], J. Matsuzaki[2], W. Bshara[2], A. Lugade[2], A. Omilian[2], PH. Thaker[3], WH. Bradley[4], R. Arend[5], C. Gunderson[6], J. Fewell[1], N. Borys[1], L. Musso[1], K. Odunsi[2]

[1]Celsion Corp, Lawrenceville, NJ; [2]Roswell Park Cancer Institute, Buffalo, NY; [3]Washington University School of Medicine, St. Louis, MO; [4]Medical College of Wisconsin, Milwaukee, WI; [5]University of Alabama at Birmingham, Birmingham, AL; [6]University of Oklahoma Health Sciences Center, Oklahoma City, OK

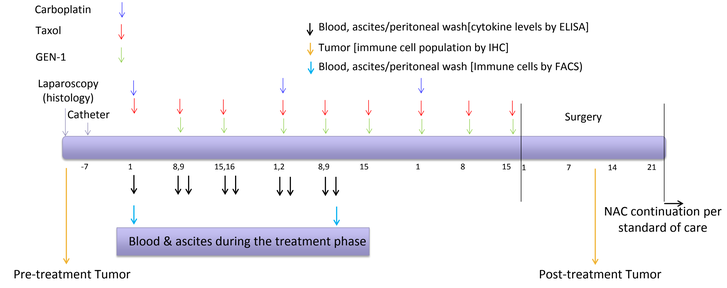
## BACKGROUND

- Most epithelial ovarian cancers (EOC) express high levels of antigens, which makes them a suitable target for immunotherapy. Treatment strategies involving activation of the immune system by antibodies, vaccines, immunocytokines or more recently the T-cell-based therapies offer potential advantages over conventional cytotoxic therapies. Intraperitoneally (IP) administered GEN-1 is an IL-12 plasma-based immunotherapy agent that provides persistent local levels of IL-12 facilitating anti-cancer immune response without systemic toxicities that are associated with the administration of recombinant IL-12. Treatment with GEN-1 in recurrent ovarian cancer patients is associated with increases in IL-12 and its downstream inflammatory cytokines, especially IFN-γ, in peritoneal fluid. The immunological effects of GEN-1 in newly diagnosed ovarian cancer patients have not been studied.
- Newly diagnosed patients undergoing neoadjuvant chemotherapy (NAC) are well suited for immunological studies due to accessibility of pre- and post-treatment primary tumor tissue. NAC alone has been shown to augment tumor infiltrating lymphocytes but fails to control immunosuppressive signals, suggesting that NAC combination approaches could relieve suppressive signal and ensure durable responses.
- Described here are the results of the completed translational research component of a Phase I study of weekly IP GEN-1 in combination with standard intravenous (IV) dose dense weekly taxane (T) and carboplatinum (C) every 3 weeks in epithelial ovarian, fallopian tube or primary peritoneal cancer patients undergoing NAC.

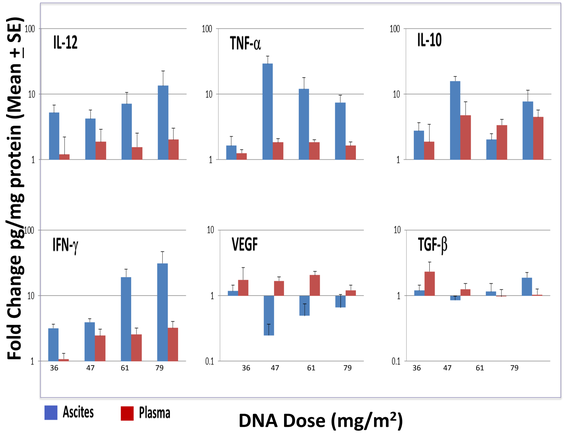
## STUDY DESIGN

- Sixteen patients newly diagnosed with epithelial ovarian cancer were eligible and enrolled for the study; patients who received prior radiotherapy or chemotherapy to any portion of the abdominal cavity and/or pelvis were excluded.
- A majority of the patients were Stage IIIC (10, 63%), followed by Stage IV (5, 31%) and one patient was Stage IIIB (1, 6%).
- All but one patient had high grade serous adenocarcinoma (15, 94%); the exception being clear cell adenocarcinoma (1, 6%).
- The median baseline CA-125 reported was 683 U/ml (78 – 4348 U/ml) across all 4 cohorts.
- Standard 3+3 design with approx. 30% GEN-1 dose increments between successive cohorts of patients. GEN-1 administered once every week for 8 weeks before the interval debulking; chemotherapy standard regimen. Tolerated dose is confirmed when 3-6 patients are treated at a dose level and <2 patients experience a dose-limiting toxicities (DLTs).
- Primary end point: safety/tolerability; Secondary end points: PFS, OS and translational parameters.

## TREATMENT SCHEDULE & TRANSLATIONAL RESEARCH SAMPLE COLLECTION

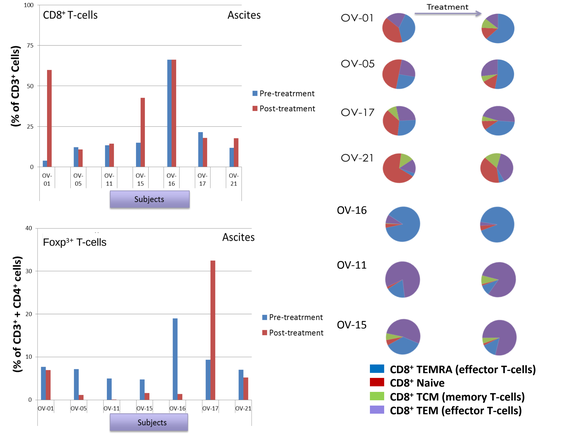



## CHANGES IN CYTOKINE LEVELS IN PLASMA & ASCITES



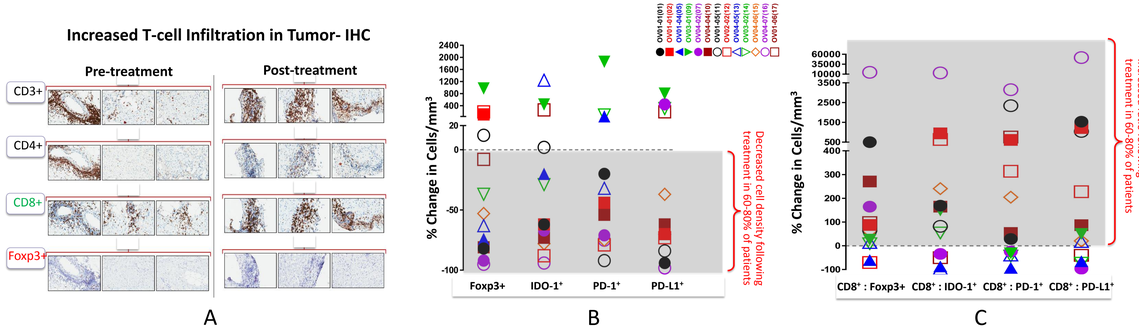

Blood, ascites or peritoneal wash samples were collected before and 24 hours after each of the first four weekly GEN-1 treatments and the accessible samples were assayed for cytokines by respective ELISA's. The results are expressed as Mean ± SE of fold changes in pg/mg protein levels between post-treatment and pre-treatment values.

## CD8⁺ AND CD3⁺ CD4⁺ FOXp3⁺ T-CELLS IN ASCITES




Ascites and peritoneal wash samples were collected before the start of NAC + GEN-1 treatment and 24 hours after the 4th GEN-1 treatment. Cell populations and phenotypes of ascites cells were analyzed using multicolor flow cytometer. The top left bar graph expresses CD8⁺ cells as % of CD3⁺ cells and Foxp3⁺ cells as % of CD3⁺CD4⁺ cells. The pie charts show the relative proportion of various CD8⁺ cell phenotypes before and after the treatment.

## T-CELL POPULATIONS IN PRIMARY TUMOR BEFORE AND AFTER GEN-1 + NAC TREATMENT




Tumor tissue was collected before treatment (laparoscopy) and at debulking surgery and processed for immunohistochemistry to determine the density of various immune cell biomarkers (CD8⁺, Foxp3+, IDO-1⁺, PD-1⁺, PD-L1⁺) in the tumor microenvironment (A). Percent change in the immunosuppressive biomarker cell density from pre-treatment to post-treatment tissue is plotted for each evaluable patient (B). The ratio of the density of CD8⁺ tumor killing T-cells to cells expressing immunosuppressive biomarkers (Foxp3+, IDO-1⁺, PD-1⁺, and PD-L1⁺) (C). The decrease in immune suppressive biomarkers and an increase in the ratio of CD8⁺ immune stimulatory cells to immune suppressive cells after treatment in a majority of patients indicates an immune-favoring shift in the tumor microenvironment.

## EFFICACY (RESPONSE RATE, RESECTION SCORE)

| *RECIST Response* | Cohort 1 (n=3) | Cohort 2 (n=3) | Cohort 3 (n=3) | Cohort 4 (n=5) | Total (n=14) |
|---|---|---|---|---|---|
| Complete Response | 1, 33.3% | 0, 0% | 0, 0% | 1, 20% | 2, 14% |
| Partial Response | 0, 0% | 3, 100% | 3, 100% | 4, 80% | 10, 72% |
| Stable Disease | 2, 66.6% | 0, 0% | 0, 0% | 0, 0% | 2, 14% |
| *Interval Debulking Status* | Cohort 1 (n=3) | Cohort 2 (n=3) | Cohort 3 (n=3) | Cohort 4 (n=5) | Total (n=14) |
| R0 | 2, 66.6% | 0, 0% | 2, 66.6% | 5, 100% | 9, 64.3% |
| R1 | 1, 33.3% | 2, 66.6% | 0, 0% | 0, 0% | 3, 21.4% |
| R2 | 0, 0% | 1, 33.3% | 1, 33.3% | 0, 0% | 2, 14.3% |
| *Pathological Response* | Cohort 1 (n=3) | Cohort 2 (n=3) | Cohort 3 (n=3) | Cohort 4 (n=5) | Total (n=14) |
| cPR | 1, 33.3% | 0, 0% | 0, 0% | 0, 0% | 1, 7% |
| micoPR | 1, 33.3% | 2, 66.6% | 1, 33.3% | 3, 60% | 7, 50% |
| macroPR | 1, 33.3% | 1, 33.3% | 2, 66.6% | 2, 40% | 6, 43% |

## CONCLUSIONS

- A combination of GEN-1 IP and neoadjuvant chemotherapy regimen produced immunological changes in tumor tissue and peritoneal cavity that are consistent with the IL-12 stimulation of the immune system.
- Evidence of IL-12 gene transfer and increases in the downstream cytokine IFN-γ is dependent on GEN-1 dose and is localized primarily in the peritoneal cavity with relatively little changes in the systemic circulation.
- Analysis of tumor tissue by IHC showed increased T-cell infiltration in several patients; in ascites the CD8⁺ T-cell population was shifted from predominantly naïve T-cells to cytotoxic effector T-cells .
- The density of immunosuppressive Treg cell marker (Foxp3) in tumor tissue was reduced in 60-80% of patients, consistent with a decrease in CD25⁺Foxp3⁺ Treg population in the ascites.
- Increases in the CD8⁺/Foxp3⁺CD25⁺ cell ratio, a prognostic indicator of improved survival, was increased in 70% of the patients. Similar increases in CD8⁺ cell ratios against other immunosuppressive T-cell signals (IDO-1, PD-1, PDL-1) were observed.
- Based on the known attributes of IL-12 function the observed immunological changes can be attributed to GEN-1 treatment, however, the contribution of chemotherapy to these changes cannot be ruled out.
- Overall, GEN-1 + NAC treatment resulted in activation of the immunostimulatory signals and inhibition of immunosuppressive signals. These immunological changes are consistent with the encouraging clinical responses and surgical outcome, and could potentially translate into better survival outcome as the PFS/OS data matures.

## CONTACT INFORMATION

- NCT02480374 on https://clinicaltrials.gov
- For questions, please contact Lauren Musso at lmusso@celsion.com