

405

**A NON-CANONICAL ROLE FOR CCL2 IN T CELL
MIGRATION: IMPROVING DUAL-INTERFERON-TREATED
AUTOLOGOUS MONOCYTES AS A THERAPY FOR
OVARIAN CANCER**

Franklin Ning*, Thomas Meyer, Brittney Harrington, Christina Annunziata. *National Cancer Institute, Bethesda, MD, USA*

Background Ovarian cancer is the fifth leading cause of cancer-related death in women in the US and the most lethal gynecologic malignancy. Although many patients respond initially to platinum-based chemotherapy, over 70% of patients diagnosed with advanced stage ovarian cancer relapse within 24 months and have limited second-line treatment options. Our group pioneered an approach manipulating innate immunity where autologous monocytes, interferon-gamma, and interferon-alpha2b (AMIGA) were given intraperitoneally to women with recurrent, ovarian cancer in order to directly kill tumor cells and mitigate the immunosuppressive tumor microenvironment.

Methods 18 patients were enrolled in the Phase 1 clinical trial (NCT02948426). Peripheral blood mononuclear cells (PBMCs) were collected from patients pre- and post-treatment cycles. Malignant ascites was collected when present. PBMCs were used for bulk and single-cell RNA-sequencing. Downstream analysis was completed on the NIH's Integrated Data Analysis Platform. LegendPlex Essential Immune Response kit (Biolegend #740930) was used to quantify ascites' cytokines. For migration assays, conditioned media from 2D and 3D ovarian cancer cell cultures treated with AMIGA was used to assess T cell migration. Migration was assayed 24 hours after T cells were exposed to conditioned media by flow cytometry or spinning disk confocal microscopy.

Results Of the 18 enrolled patients, two had partial responses and four had stable disease. Of these, both responders and two of the four patients with stable disease maintained clinical benefit for five or more months, and were thus labelled as long-term responders (LTRs). Through bulk-cell RNA-sequencing, the PBMCs of these LTRs showed increased levels of T cell activation and migration genes compared to the 14 non-responders (NRs). In light of this, we assessed patient malignant ascites for relevant chemokines and found CCL2, a known monocyte, and less so T cell, trafficking molecule, had increased in response to treatment. We then validated *in vitro* that CCL2 is significantly increased in the supernatants of AMIGA-treated ovarian cancer cells, but that this increase correlated with increased T cell, and not monocyte, migration. Furthermore, by manipulating the CCL2-CCR2 signaling axis *in vitro*, we were able to significantly alter T cell migration.

Conclusions Overall, our findings suggest AMIGA aids T cell migration towards ovarian cancer by increasing the production of CCL2. By further understanding how AMIGA contributes to T cell migration and function in the ovarian cancer tumor microenvironment, we seek to combine innate- and adaptive-based immunotherapies to improve the treatment options and, ultimately, survival of women with ovarian cancer.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0405>