

Letter of Intent, Ovarian Cancer Academy Award 2009

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Background. Although the majority of ovarian cancer patients have an initial positive response to surgery and chemotherapy, most will develop a recurrence and die from disease. Even in patients with an apparent complete response, with no visible or biomarker-detectable residual disease, most will recur. There is almost certainly a subpopulation of cells that either has inherent resistance, or develops resistance through exposure to chemotherapy, can lie dormant, and later be reactivated as chemoresistant disease. In many solid tumors, including ovarian, there is evidence that subpopulations of cancer cells with stem cell properties can be found. They have enhanced tumorigenicity, inherent chemoresistance, and the capacity to give rise to multi-lineage daughter cells. It is not known what the optimal markers to define such “cancer stem cells” (CSC’s) or “tumor initiating cells” (TIC’s) should be, and whether they can be targeted to improve outcomes.

Objective/Hypothesis. Based on preliminary data, we hypothesize that the population of cells with aldehyde dehydrogenase-1 activity (ALDH1) has properties of CSC’s in ovarian cancer. These properties include enhanced tumorigenicity, multipotentiality, and chemoresistance. We also hypothesize that targeting ALDH1 can decrease tumor growth and increase chemosensitivity in ovarian cancer. Furthermore, an additional protein required by multiple stem cell models, the Notch ligand Jagged1, appears important to ovarian cancer progression. Based on preliminary data, we hypothesize that Jagged1 has Notch-independent functions and can be targeted to improve killing in ovarian cancer.

Specific Aims. *Aim 1:* Identify a subpopulation of tumor initiating cells based on ALDH1 activity. Determine if these cells have properties of cancer stem cells by *in vitro* and *in vivo* functional assays, and if downregulation of ALDH1 decreases cancer growth and chemosensitivity. *Aim 2:* Target Jagged1 on tumor cells and the microenvironment vasculature by separately targeting human tumor cells and murine stromal cells with *in vivo* siRNA. Characterize the role of Jagged1 in ovarian cancer progression.

Study Design. In aim 1, the subpopulation of ovarian cancer cells with ALDH1 activity, as defined by the ALDEFLUOR assay, will be sorted by flow cytometry in ovarian cancer cell lines, cancer cells in ascites, and prospectively collected and dissociated ovarian tumors. Tumor initiating capacity will be tested by injection into immunodeficient mice, comparing ALDH-positive and ALDH-negative populations. Spheroid-forming capacity, a surrogate endpoint for progenitor cell potential, will be compared between these populations, with chemoresistance and expression of the Notch family assessed in these spheroids. Target ALDH1 using liposomal siRNA delivery *in vitro* and *in vivo* to determine if ALDH1 downregulation decreases tumor growth. In aim 2, the Jagged1 proteins, which have been implicated in ovarian cancer and stem cell biology, will be downregulated with siRNA to test *in vitro* effects on proliferation, spheroid-forming capacity, and chemosensitivity. Jagged1 will be overexpressed in cell lines negative for Jagged1 expression. Using retroviral transfections, the Notch pathway will be silenced by introduction of the Dominant Master Mind (DMM) protein, to allow examination of Notch-independent functions of Jagged1. Using siRNA *in vivo* delivery systems, we will then test effects of decreasing Jagged1 expression on murine endothelial cells with murine-directed siRNA, on tumor cells with human-directed siRNA, and both together, to determine their potential as therapeutic targets. Endpoints will include tumor growth, proliferation, induction of apoptosis, and anti-angiogenic effects compared to mice treated with non-targeting siRNA.

Summary. These studies will provide insight into ovarian carcinogenesis, mechanisms of chemoresistance, and communication between endothelial cells and tumor cells in the microenvironment. A better understanding of the role of two oncoproteins, ALDH1 and Jagged1, will be achieved. Furthermore, these two targets will be characterized in terms of their potential for therapeutic intervention, and both will afford opportunity for additional study as an independent investigator.