In the U.S. 22,000 women are diagnosed with epithelial ovarian cancer (EOC) each year. More than half will die within 5 years of diagnosis. The current first line therapy for EOC is surgical debulking of the tumor and a combination of cisplatin and paclitaxel. Most EOC patients relapse after completing chemotherapy and are often unresponsive to additional treatment, potentially due to a subpopulation of highly tumorigenic, chemoresistant Cancer Stem-like Cells (CSCs) within the tumor.

A variety of mechanisms are believed to account for the chemoresistance of CSCs including quiescence, drug inactivation, and drug efflux. Ovarian CSCs can be identified by increased Aldehyde Dehydrogenase (ALDH) activity, either alone, or in combination with the expression of the surface protein CD133. The presence of ALDH+/CD133+ cells within a tumor is associated with poorer patient outcome. Although many ALDH isozymes have been implicated in various cancers, the strongest evidence supports the role of the ALDH1 family. ALDH1A1 knockdown increases sensitivity to docetaxel and cisplatin in chemoresistant cell lines in vitro and in vivo. ALDH1A1 may enable CSCs to bypass cell cycle checkpoints and avoid apoptosis following exposure to chemotherapeutics.

As of yet it is unknown which isozyme selectivity profile of ALDH inhibition is best able to deplete the ALDH+/CD133+ cells within a tumor. ALDH1A1 knockout mice are viable without significant defect; however, the various ALDH isoforms are widely distributed throughout the body. Selectivity against unrelated isoforms is therefore an important consideration for minimizing potential off target effects.

To gain insight into the ALDH isoforms most relevant to the CSC phenotype, we have performed siRNA knockdown of several ALDH isozymes in FACS purified CD133+ A2780 cells. By measuring the live cell number following transfection with siRNA targeting ALDH1A1, 1A2, 1A3, 1B1, 3A1 in this EOC CSC model, we have shown that simultaneous inhibition of ALDH1A1 and 1A2 offers the greatest potential to deplete CSCs.

Encouraged by these results, we assessed CSC depletion following treatment with several analogs of known ALDH1A1 inhibitor 4-Diethylamino benzaldehyde (DEAB). This led to 673A, an inhibitor of ALDH1A1 and 1A2 (IC50 = ~0.2µM) with modest selectivity over ALDH2. (Fig. 1A) In vitro, 673A selectively depletes ALDH+ and CD133+ cells (CD133+ CC50 = 2µM). (Fig. 1B) In vivo it prevents tumor growth when combined with cisplatin. (Fig. 1C) In a murine patient derived xenograft study, 673A combined with carboplatin led to a 50% reduction in tumor volume while tumors treated with carboplatin alone doubled in volume following 23 days of treatment. (Fig. 1D)

Unfortunately 673A and its analogs are slow substrates for several ALDH isoforms leading to complicated SAR and limited drug exposure in vivo. A high throughput screen for ALDH1A1 inhibitors led to the discovery of A39 a novel, non-substrate ALDH1A1 inhibitor (IC50 = 0.4 µM). A39 did not selectively deplete CSCs as 673A did, potentially due to a suboptimal ALDH selectivity profile or poor cell permeability. Early optimization of A39 has led to a potent pan-inhibitor of the ALDH1A family (ALDH1A1, 1A2, 1A3 IC50 = 74 – 140 nM) as well as a potent 1A1 inhibitor (IC50 = 78 nM) with >20x selectivity against all other tested isoforms.

Future work will focus on generating A39 analogs in search of varied isozyme selectivity profiles and improved PK properties. We hope to recapitulate the compelling activity of 673A with a more drug-like molecule. These analogs will help elucidate the most important ALDH isoforms for CSC function.