Development of Ovarian Cancer Stem Cell Depleting ALDH Inhibitors

Brandt Huddle
Advisor: Dr. Scott Larsen
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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 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 isozymes 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 (IC_{50} = ~.2μM) with modest selectivity over ALDH2.(Fig. 1A) *In vitro,* **673A** selectively depletes $ALDH^+$ and $CD133^+$ cells ($CD133^+$ CC_{50} = 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)

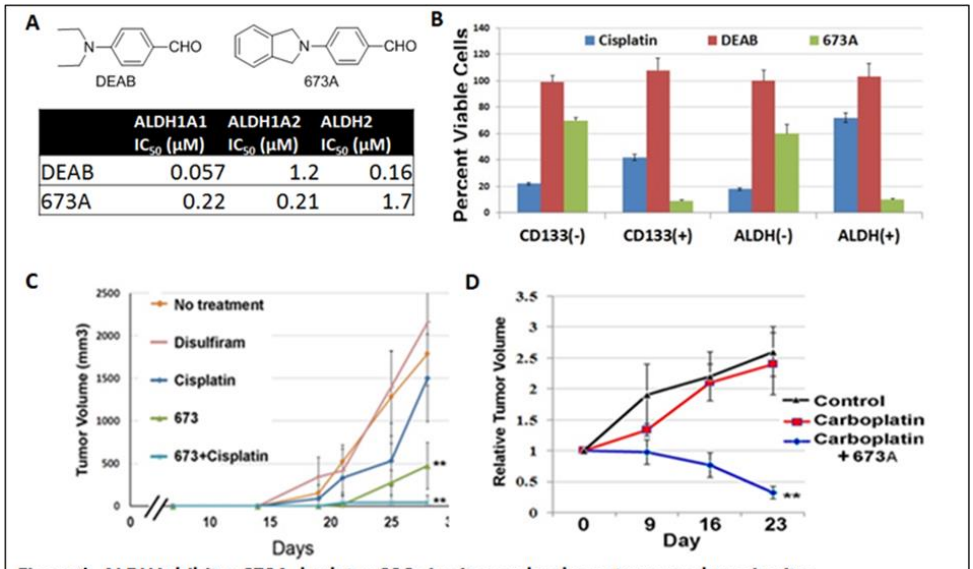

Figure 1. ALDH inhibitor 673A depletes CSCs *in vitro* and reduces tumor volume *in vitro*
(A) ALDH IC_{50} values for DEAB and 673A. **(B)** Viability plots of FACS isolated cell populations following administration of indicated compounds. **(C)** Tumor growth curves from 5000 live tumor cells following *in vivo* treatment with indicated compounds. **(D)** Tumor growth of a primary ovarian tumor explant treated with carboplatin alone or in combination with 673A.

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 (IC_{50} = 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 IC_{50} = 74 – 140 nM) as well as a potent 1A1 inhibitor (IC_{50} = 78 nM) with >20x selectivity against all other tested isozymes.

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 isozymes for CSC function.