Characterizing Small Molecule Inhibitors of ALDH1A1 by Establishing High Throughput Cell-based Assays

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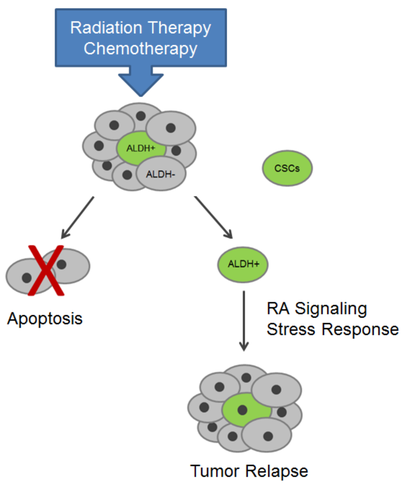
Introduction

Aldehyde dehydrogenases (ALDHs), a superfamily of NADP(+)-dependent enzymes, catalyze the oxidation of endogenous and exogenous aldehydes to their corresponding carboxylic acids.[1] ALDHs are involved in many cellular pathways, including retinoic acid signaling, which in turn activates cellular genetic programs that modulate cell differentiation, apoptosis, and growth.[2] In cancer, multiple ALDHs, particularly ALDH1A1, have been found overexpressed in a subpopulation of cancer cells known as cancer stem cells (CSCs).[1]

Epithelial ovarian cancer is the most lethal of all gynecologic malignancies[3] and the fifth most lethal type of cancer in women. ALDH1A1 levels serve as a prognostic marker associated with reduced response to treatment due to drug resistance and with poor clinical outcome.[4]

Because ALDH1A1 provides a potential target for ovarian cancer and CSC-directed therapeutics, as well as for multiple other diseases such as obesity and diabetes,[3] we have previously launched a small molecule screening campaign to identify ALDH1A1-specific inhibitors by high-throughput biochemical assays.[5] Here, our goal is to establish high-throughput cellular assays to support the characterization of these inhibitors.



ALDH1A1 in Ovarian Cancer Cell Lines

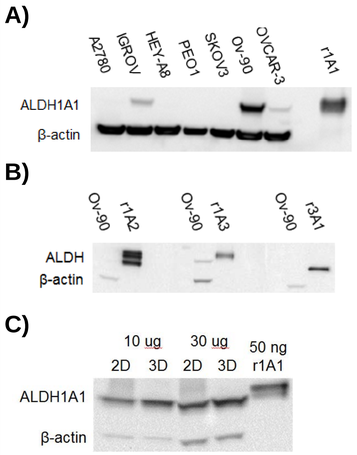


Figure 1. Characterizing ALDH1A1 expression in ovarian cancer cell lines. A) Ov-90 cells express high levels of ALDH1A1 protein. B) Ov-90 cells do not express other ALDH1A isoforms or ALDH3A1. C) Ov-90 cells cultured in 3D spheroids expressed higher levels (~2 fold) of ALDH1A1 than monolayer 2D cultures.

A High-throughput Imaging-based ALDEFLUOR™ Assay

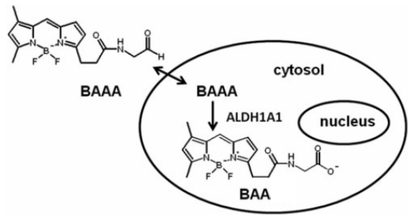


Figure 2. ALDEFLUOR™ Assay. This assay assay is a low-throughput, flow cytometry-based assay that identifies stem cells on the basis of ALDH activity. BAAA (BODIPY-aminoacetaldehyde) is converted into BAA (BODIPY-aminoacetatic acid) in the presence of ALDH. BAA is trapped inside viable cells.

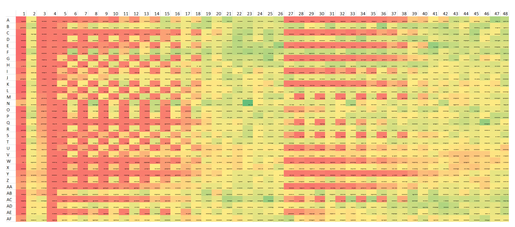


Figure 4. Heat Map. Representative heat map from a high-throughput imaging-based ALDEFLUOR™ assay in Ov-90 cells. Red shows inhibition and green shows no effect.

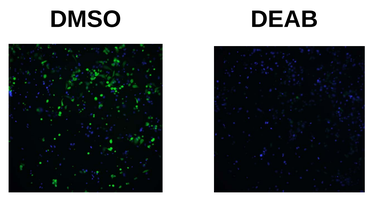
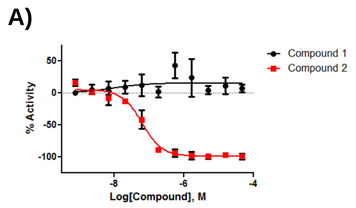


Figure 3. The ALDEFLUOR™ assay can be adapted to a imaging-based assay. We optimized and miniaturized this assay to a 1,536-well format. Representative images of DMSO and inhibitor DEAB treated cells. Green and blue are BAA and nuclear dye, respectively.



Compound	IC_{50} (nM)
Compound 1	Inactive
Compound 2	66.07

Figure 5. Inactive and active inhibitor analogs are distinguished by the ALDEFLUOR™ assay. A) Dose-response curve of Compound 1 (inactive) and Compound 2 (active). B) Table of IC_{50} values.

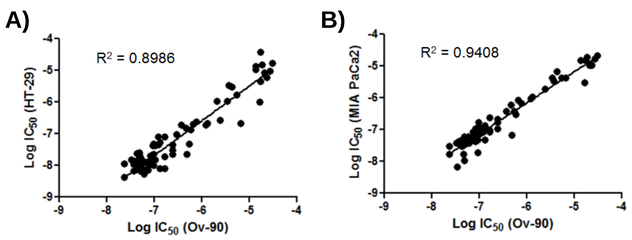
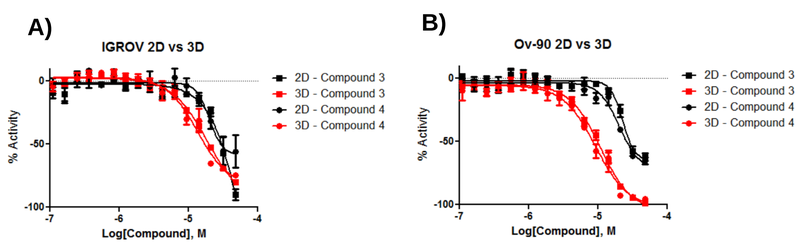


Figure 6. Compound activity in different cell lines. IC_{50} correlation plots for Ov-90 data with A) HT-29 and B) MIA PaCa2, two non-ovarian cancer lines with high ALDH1A1 expression.

A High-throughput 2D vs 3D Cell Viability Assay



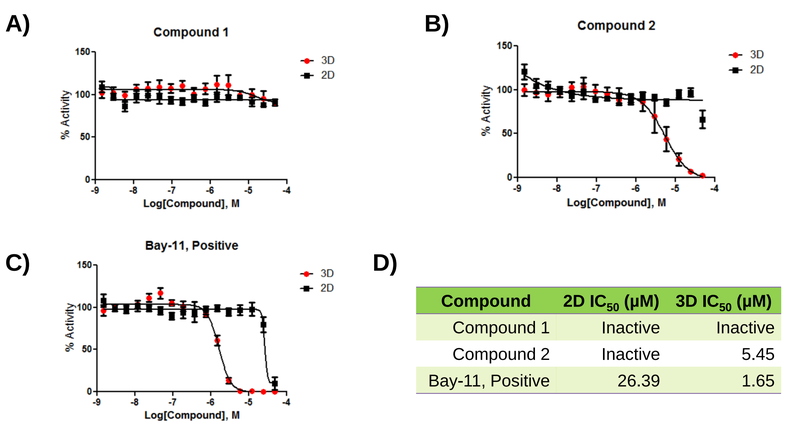
C) IGROV-1

Compound	2D IC_{50} (μM)	3D IC_{50} (μM)
Compound 3	69.2	18.43
Compound 4	20.07	13.62

D) Ov-90

Compound	2D IC_{50} (μM)	3D IC_{50} (μM)
Compound 3	22.73	10.91
Compound 4	20.25	9.26

Figure 7. Viability shift. Cell viability of A) IGROV-1 and B) Ov-90 cells in monolayer and spheroid cultures when treated with ALDH1A1 inhibitors. Ov-90 spheroids are more sensitive to ALDH1A1 inhibition. IC_{50} values for C) IGROV-1 and D) Ov-90 cells.



D)

Compound	2D IC_{50} (μM)	3D IC_{50} (μM)
Compound 1	Inactive	Inactive
Compound 2	Inactive	5.45
Bay-11, Positive	26.39	1.65

Figure 8. A 384-well viability assay. A) Compound 1 (inactive). B) Compound 2 (active). C) Bay-11, unspecific ALDH inhibitor. D) Table of IC_{50} values.

Conclusions and Future Directions

- Ov-90 is a good cellular model for assessing activity of ALDH1A1 inhibitors.
- We have characterized over 300 ALDH1A1 inhibitor analogs.
- We will obtain an Ov-90 drug resistant cell line for combination therapy studies with our inhibitors.
- We will perform CETSA (Cellular Thermal Shift Assay) on lead compounds to determine target engagement.

References

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2. Xu, Xia et al. Aldehyde dehydrogenases and cancer stem cells. Cancer letters 2015.
3. Condello S, Morgan et al. beta-Catenin-regulated ALDH1A1 is a target in ovarian cancer spheroids. Oncogene 2015.
4. Ayub, Tiyasha H. et al. Accumulation of ALDH1-positive cells after neoadjuvant chemotherapy predicts treatment resistance and prognosticates poor outcome in ovarian cancer. Oncotarget 2015.
5. Yang, Shyh-Ming et al. Discovery of NCT-501, a Potent and Selective Theophylline-Based Inhibitor of Aldehyde Dehydrogenase 1A1 (ALDH1A1). Journal of Medicinal Chemistry 2015