ALDH1 Isoform Landscape in Breast Cancer: Advancing Precision Therapies with a Potent ALDH1A3-Selective Inhibitor

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#60P

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Introduction

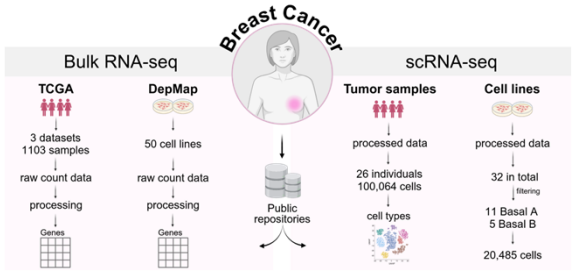
Triple-negative breast cancer (TNBC) is among the most aggressive and clinically challenging breast cancer subtypes, marked by high metastatic potential, poor prognosis and limited treatment options. This underscores the urgent need to identify novel molecular targets for effective therapies. Aldehyde dehydrogenase 1 (ALDH1) enzymatic activity is a hallmark of cancer stem cells (CSCs), strongly associated with drug resistance, tumor aggressiveness, and metastasis, making it an attractive therapeutic target. However, the distinct roles of ALDH1 isoforms hindering the rational design of selective inhibitors.

Materials and methods

This study integrates bulk RNA-seq data from 1,103 primary breast tumors, 50 breast cancer cell lines, and single-cell RNA-seq data from 26 patients to characterize ALDH1 isoform expression across molecular subtypes and the tumor microenvironment. Functional *in vitro* studies, including cell signaling and evaluation of metastatic potential, along with preclinical *in vivo* efficacy tests were performed to demonstrate the therapeutic potential of a novel ALDH1 inhibitor, ABD0171.

Results

Transcriptomic analyses



1. ALDH1A3 and 1A1 are highly expressed in breast cancer, with ALDH1A3 expression significantly higher in basal-like molecular subtypes.

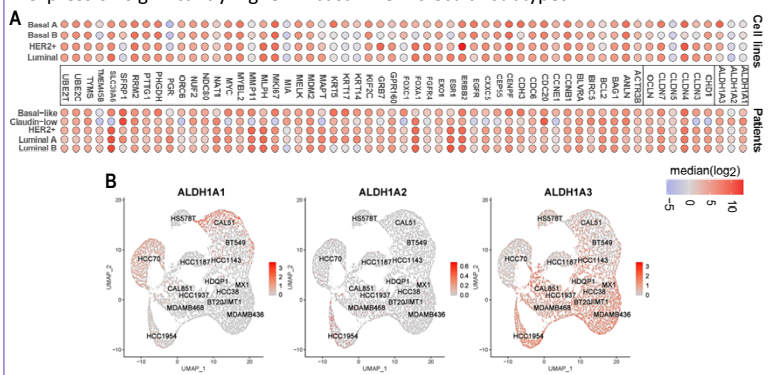


Fig 1. A. RNA-seq bulk gene expression of 59 genes, including genes for claudins, ALDH1A isoforms and the PAM50 signature, across breast cancer cell lines and patient samples, categorized by molecular classification. **B.** sc-RNA-seq expression of *ALDH1A1*, *ALDH1A2*, and *ALDH1A3* from 16 Basal-classified breast cancer cell lines.

2. ALDH1A3 and 1A1 are expressed in Myofibroblast-like cancer-associated fibroblasts (myCAFs) and ALDH1A3 is also highly expressed in epithelial cells.

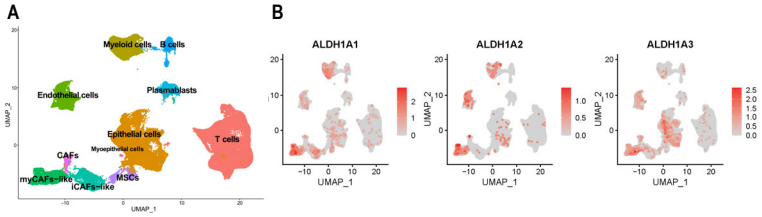


Fig 2. A. Single-cell analysis of 26 patient samples encompassing 100,064 cells detailing the ALDH1A expression landscape. **B.** Distribution of cell populations by molecular classification, illustrating the cellular heterogeneity within tumors.

Functional *in vitro* studies

3. The novel ALDH1 inhibitor (ALDHi) ABD0171 induces extrinsic apoptosis and inhibits migration and invasion by suppressing STAT3 activation.

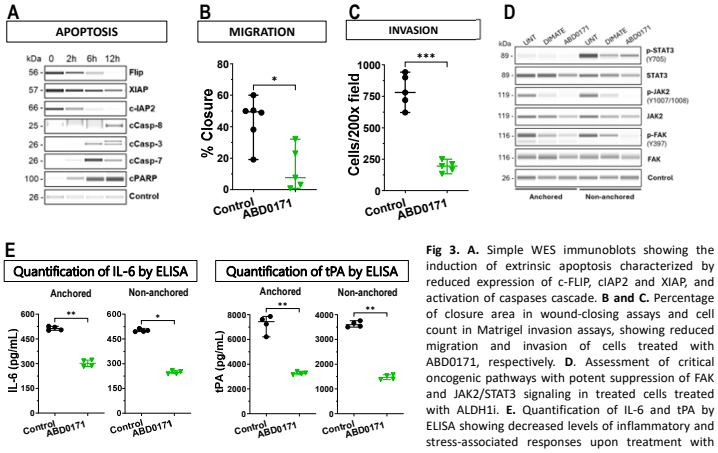


Fig 3. A. Simple WES immunoblots showing the induction of extrinsic apoptosis characterized by reduced expression of c-FLIP, cIAP2 and XIAP, and activation of caspases cascade. **B and C.** Percentage of closure area in wound-closing assays and cell count in Matrigel invasion assays, showing reduced migration and invasion of cells treated with ABD0171, respectively. **D.** Assessment of critical oncogenic pathways with potent suppression of FAK and JAK2/STAT3 signaling in treated cells treated with ALDH1i. **E.** Quantification of IL-6 and tPA by ELISA showing decreased levels of inflammatory and stress-associated responses upon treatment with ALDHi.

Functional *in vivo* studies

4. ALDHi ABD0171 inhibits tumor growth and has antimetastatic activity in TNBC preclinical models.

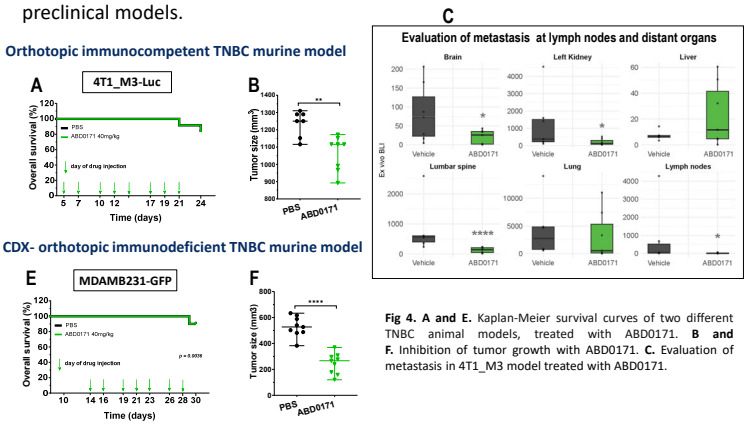


Fig 4. A and E. Kaplan-Meier survival curves of two different TNBC animal models, treated with ABD0171. **B and F.** Inhibition of tumor growth with ABD0171. **C.** Evaluation of metastasis in 4T1_M3 model treated with ABD0171.

Conclusion

1. ALDH1A3 is significantly overexpressed in basal-like breast cancer cells.
2. A novel compound, ABD0171, a potent inhibitor of ALDH1A3, exhibits potent anticancer and antimetastatic activity, inducing the extrinsic apoptotic pathway, induction of oxidative stress, inhibition of migration/invasion and disruption of IL-6/JAK2/STAT3, Src/FAK/STAT3 and tPA pathways.
3. ABD0171 shows tumor growth-inhibitory activity and antimetastatic activity in preclinical TNBC tumor models.

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Disclosure statement

I have no conflicts of interest to declare.

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