Functional Screening of ARID1A-Deficient Cholangiocarcinoma Using the MAPS Platform

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Abstract

Background & Objectives: Cholangiocarcinoma (CCA) is an aggressive malignancy characterized by a high frequency of chromatin-remodeling gene alterations and has limited therapeutic options. Approximately 47% of intrahepatic CCA cases present alterations in at least one gene associated with chromatin remodeling with loss-of-function mutations in ARID1A occurring in ~19-22% of cases. However, actionable vulnerabilities associated with ARID1A deficiency remain poorly defined. We hypothesized that ARID1A deficiency creates distinct, targetable cellular dependencies that can be systematically identified through functional screening.

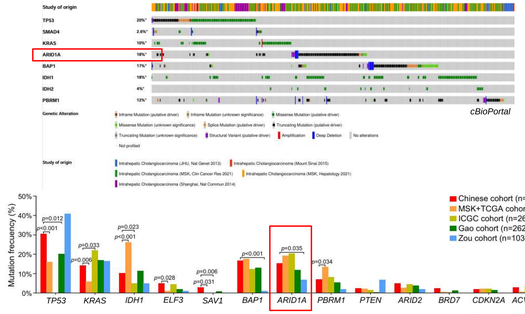
Method: To test this hypothesis, we utilized the Multifunctional Approach to Pharmacologic Screening (MAPS) platform to perform comparative functional screens in murine cholangiocarcinoma cell lines derived from genetically engineered mouse models that are Arid1a-null or Arid1a-wildtype. This platform enables rapid, high-throughput assessment of cellular responses to pharmacologic perturbations across hundreds of molecular pathways. The MAPS libraries contain compounds arrayed across ten dilution points, allowing for a robust understanding of differential drug sensitivities. Further, MAPS allows us to focus on compounds with established in vivo profiles, including FDA-approved drugs and compounds with known safety data, thereby accelerating the path from discovery to clinical implementation.

Results: Screening results revealed distinct sensitivity patterns between Arid1a-null and Arid1a-wildtype cells, supporting the hypothesis that ARID1A loss alters pathway reliance in cholangiocarcinoma. Notably, Arid1a-null cells displayed enhanced sensitivity to perturbations impacting stress response pathways, including ferroptosis.

Conclusion: This study establishes a functional screening framework to identify therapeutic targets associated with ARID1A loss and provides a foundation for downstream validation and translational studies aimed at improving treatment strategies for ARID1A-mutant CCA.

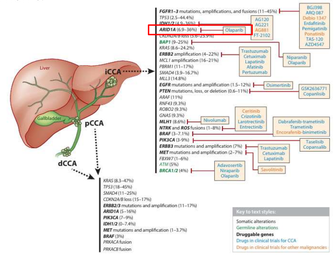
Background

- Frequently mutated genes in IHCC include *TP53*, *SMAD4*, *KRAS*, and *ARID1A*.



Zhou et al., 2024

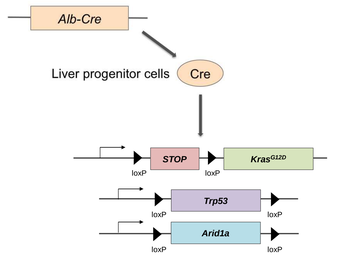
- Potential targeted therapies under investigation are limited for ARID1A-deficient cholangiocarcinoma



Rodrigues et al., 2021

Objective: To identify targetable cellular dependencies in ARID1A-deficient cholangiocarcinoma using the MAPS functional screening platform for further drug development

Experimental Strategy



AKPA = Alb-Cre; Kras^{G12D}; p53$^{L/+}$; Arid1a$^{L/L}$ = Arid1a-deficient
AKP = Alb-Cre; Kras^{G12D}; p53$^{L/+}$; Arid1a$^{+/+}$ = Arid1a-intact

Figure 1: Strategy to generate mutations in the mouse liver and subsequent derivation of cell lines for further studies. The albumin promoter, which is active in liver progenitor cells, drives expression of Cre recombinase to delete either the indicated floxed tumor suppressor or the stop codon for the oncogene *Kras^{G12D}*, thereby enabling constitutive expression of Kras^{G12D}.

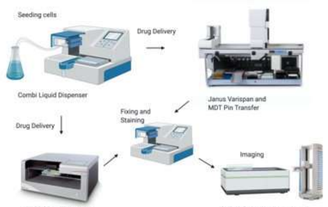


Figure 2: Multifunctional Approach to Pharmacologic Screening (MAPS) high throughput screening pipeline platform. Seeding cells using the Combi Liquid Dispenser, drug delivery next day via the Janus Varispan and MDT Pin Transfer (also D300 Digital Dispenser available for validation studies), followed by fixing and staining with Hoechst 72h later, and finally imaging using the CX5 High Content Imager and Orbitor RS2 Plate Loader.

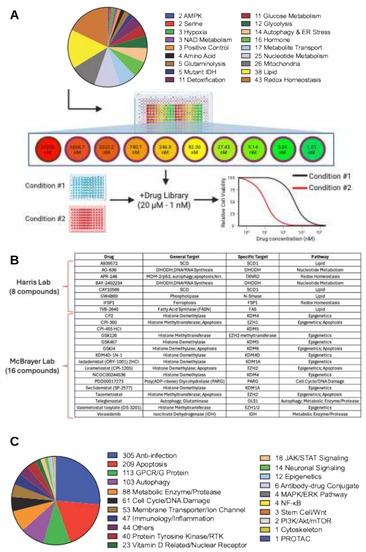


Figure 3: MAPS libraries screened that contain compounds arrayed across ten dilution points. A) Metabolic inhibitor library contains 240 compounds targeting 18 distinct metabolic pathways with 2-3 target proteins per pathway and 2-3 structurally distinct compounds per target. B) Epigenetic inhibitor library contains 24 compounds targeting key pathways of metabolic stress and epigenetic regulation. C) FDA-approved anti-cancer library contains 1,151 compounds across 21 pathways that have already undergone extensive safety testing and regulatory approval, allowing more rapid translation from bench to bedside.

Results

A

Rank	Compound	% AUCDiff	Rank	Compound	% AUCDiff
1	Cytarabine	74.9	13	Tamoxifen	50.3
2	Elesclomol	69.4	14	Disulfiram	50.1
3	Cobimetinib (racemate)	60.5	15	Palbociclib (isethionate)	49.9
4	Ingenol Mebutate	59.4	16	Fludarabine (phosphate)	48.7
5	Tucatinib (hemiethanolate)	56.4	17	ML-162	48.6
6	Arglabin	54.5	18	Clofarabine	47.6
7	Midostaurin	53.7	19	Fostamatinib Disodium	46.5
8	Mobocertinib	52.4	20	Simotinib	45.2
9	Alectinib	51.5	21	Avapritinib	44.5
10	ML-210	51.5	22	Docetaxel	44.3
11	Gallic acid (hydrate)	50.9	23	APR-246	41.3
12	1S,3R-RSL3	50.5	24	Cladribine	41.1

Redox Homeostasis
Purine Synthesis
Protein Tyrosine Kinase

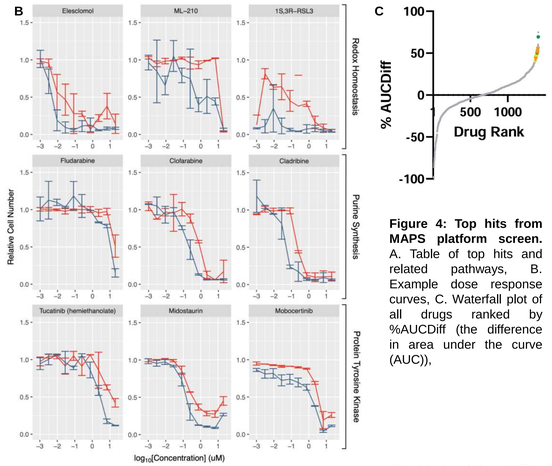
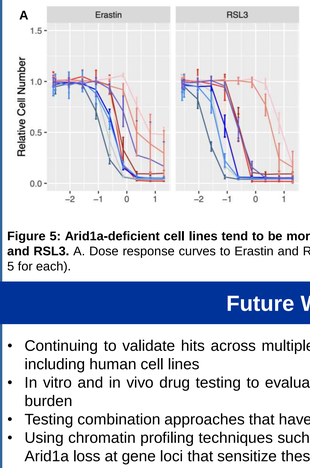


Figure 4: Top hits from MAPS platform screen. A. Table of top hits and related pathways, B. Example dose response curves, C. Waterfall plot of all drugs ranked by %AUCDiff (the difference in area under the curve (AUC)),

Blue/shades of blue = AKPA
Red/shades of red = AKP



B

	Erastin IC50 (µM)		RSL3 IC50 (µM)	
	AKPA	AKP	AKPA	AKP
1	0.13	0.503	0.019	0.275
2	0.21	0.546	0.051	0.095
3	0.327	0.544	0.085	0.253
4	0.285	2.285	0.049	6.887
5	0.934	1.466	0.251	3.004

Figure 5: Arid1a-deficient cell lines tend to be more sensitive to ferroptosis activators Erastin and RSL3. A. Dose response curves to Erastin and RSL3. B. Table of IC50 for tested cell lines (n = 5 for each).

Future Work

- Continuing to validate hits across multiple Arid1a-null and Arid1a-WT cell lines, including human cell lines
- In vitro and in vivo drug testing to evaluate impact on growth, cell death, tumor burden
- Testing combination approaches that have potential synergistic effects
- Using chromatin profiling techniques such as CUT&Tag to evaluate the impact of Arid1a loss at gene loci that sensitize these cells to the compound(s) of interest

Patient Friendly Lay Summary Panel

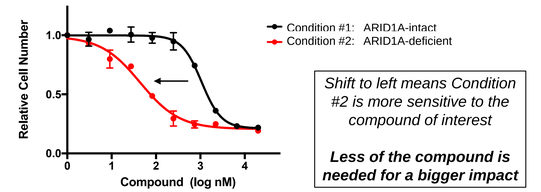
- Cholangiocarcinoma (CCA): type of liver cancer
- Treatment options are limited and prognosis is poor
- Chromatin remodeling proteins are frequently mutated
  - Modifies DNA structure to alter gene expression
  - Example: *ARID1A*; part of a large chromatin remodeling complex
  - Mutations lead to loss of ARID1A protein

Loss of ARID1A-deficient mutations make CCA treatment harder

BUT

creates new weaknesses that can be exploited—these can be explored to find new treatments

- Screening platform: tested hundreds of compounds to identify specific ARID1A-deficient weaknesses



Shift to left means Condition #2 is more sensitive to the compound of interest

Less of the compound is needed for a bigger impact

- Calculated %AUCDiff: the difference in area under the curve (AUC) between Condition 1 and Condition 2
  - Larger %AUCDiff = larger difference in sensitivity
  - Smaller %AUCDiff = smaller difference in sensitivity

Larger, positive %AUCDiff means greater impact of the compound of interest on ARID1A-deficient cell lines

- Pathways and compounds with the greatest impact on ARID1A-deficient cell lines include ferroptosis, a type of cell death, those that interfere with DNA formation, and those involved in cell signaling
- Further studies will be aimed at understanding how the pathway is impacted by the compound(s) of interest

References

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Figures 2 and 3 courtesy of Sara K. Blick-Nitko