Abstract #618: Ex vivo pharmacotyping of patient-derived tumor organoids identifies personalized therapeutic options for patients with biliary tract cancer

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Background

Biliary tract cancers (BTC), including cholangiocarcinomas and gallbladder adenocarcinomas, present significant therapeutic challenges due to limited treatment options and poor prognoses. We report findings from the CLIA-certified PARIS assay, evaluating drug sensitivities of patient-derived tumor organoids (PDTOs) to a panel of oncology drugs.

Methods

- Sample Collection: Fresh tissue samples were collected with informed patient consent in accordance with the Declaration of Helsinki and with Institutional Review Board (IRB) approval.
- Organoid Derivation Condition : organ-based serum free culturing media, low O_2 incubation, ultralow attachment plates (ULA) and Matrigel substrate depending on the tumor type.
- Functional Drug Screen: Organoids were seeded in 384 well plates, incubate at 37°C and exposed to compounds for 6 days
- Readout: Viability via ATP (Cell Titer Glow)
- Analysis (SEngine App): Drug responses were ranked using a novel score (SPM) ranging from 15 to 1, comprising absolute metrics of response and comparative analysis of each drug within an internal reference database. PDTOs were classified as sensitive versus resistant, with responses ranked: exceptional/good/moderate/low.

Results/Graphs/Data

PARIS® assay workflow and PDTO establishment success rates

PDTOs were successfully cultured from 27 out of 46 live tumor samples obtained from 43 BTC patients. The majority of patients (63%) presented with advanced metastatic disease (60% stage IV, 13% stage III, 10% stage II, 4.4% stage I, 10% unknown) and had exhausted standard therapeutic options. The clinical diagnosis included both intra and extrahepatic cholangiocarcinoma as well as gallbladder carcinoma.

The overall organoid establishment success rate was 58.7% (27/46). Failure to establish organoid cultures was most often due to insufficient tissue quantity or low tumor cell count. For samples which passed initial quality control steps, organoids were successfully established in 93.1% of cases. Of these, 96.3% (26/27), were successfully screened with a customized drug panel of an average of 50 drugs, encompassing chemotherapeutic agents and targeted therapies.

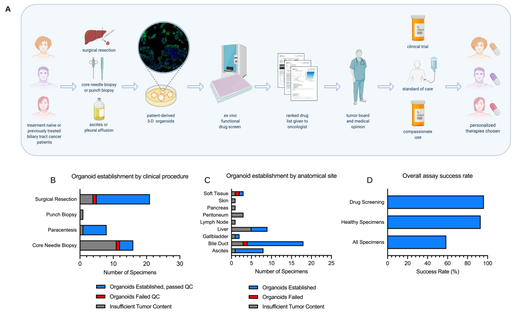


Figure 1: Description of PARIS® assay workflow and characterization of BTC cohort. A) Fresh tumor specimens from patients with biliary tract cancer were sent to SEngine Precision Medicine for the derivation of PDTO cultures. The PDTOs then underwent drug screening, and a ranked drug list was derived from the results (middle). This ranking includes information on targeted, endocrine, and chemotherapeutic agents and can be used by clinicians to guide treatment decisions (right). This illustration was created using BioRender software. B) Success rates of PDTO establishment according to clinical procedure used to obtain the specimen. C) Success rates of BTC PDTO establishment according to anatomical site of origin. D) Overall success rates of PDTO derivation and subsequent drug screening.

Combining genomic analysis with drug testing in tumor-derived PTDOs enables direct assessment of the association between genetic biomarkers and drug sensitivities, beneficial in guiding future treatment. Drug resistance in patients is functionally recapitulated in PTDOs, providing a model to study patient specific drug resistance.

Early integration of such assays in patient management, possibly at diagnosis or first recurrence holds promise for improving outcomes in this challenging disease.

Results/Graphs/Data

Table 1. Clinical Characteristics of BTC cohort

Cohort characteristics	Number	Percentage
Total number of patients	43	-
Total number of samples	46	-
Total number of screens	49	-
Median age at diagnosis	52.2	-
Median age at screening	60.4	-
Gender		
Male	25	58.1
Female	18	41.9
Clinical diagnosis		
Intrahepatic cholangiocarcinoma	31	67.4
Extrahepatic cholangiocarcinoma	5	10.9
Gallbladder adenocarcinoma	6	13.0
Cholangiocarcinoma, NOS	3	6.5
Combined hepatocellular cholangiocarcinoma	1	2.2
Stage		
Stage I	2	4.4
Stage II	5	10.9
Stage III	6	13.0
Stage IV	28	60.9
Unknown	5	10.9
Specimen source		
Primary tumor	19	41.3
Distant metastasis	27	58.7
Specimen anatomical site		
Ascites	8	17.4
Bile duct	18	39.1
Gallbladder	2	4.4
Liver	9	19.6
Lymph node	1	2.2
Peritoneum	3	6.5
Pancreas	1	2.2
Skin	1	2.2
Soft tissue	3	6.5
Treatment exposure		
One or more treatment lines	29	63.0
Naive of treatment	15	32.6
Unknown	2	4.3

Table 1: Patient cohort detailing patient's age, gender, clinical diagnosis, stage, specimen source and anatomical site along with treatment exposure.

Table 2. Top drugs with exceptional to good responses

Drug	Target	Proportion of good – exceptional responses
Ulixertinib	ERK	66.7% (8/12)
HDM201	MDM2	63.6% (7/11)
Mivebresib	BET	60% (6/10)
Cobimetinib	MEK	58.3% (14/24)
Trametinib	MEK	55.6% (10/18)
Dasatinib	BCR-ABL, SRC	43.5% (10/23)
Neratinib	EGFR, ERBB2	42.1% (8/19)
Erlotinib	EGFR	40.9% (9/22)
Everolimus	mTOR	40% (10/25)
Navitoclax	BCL2	38.5% (5/13)
Ibrutinib	BTK	35.7% (5/14)
Carfilzomib	proteasome	33.3% (4/12)
Osimertinib	EGFR	33.3% (3/9)
Abemaciclib	CDK	30% (6/20)
Alpelisib	PI3K	27.3% (6/22)
Entrectinib	ROS1, TRK	25% (4/16)
Afatinib	EGFR	25% (2/8)
Dovitinib	FGFR	25% (2/8)

Table 2: Table of drugs that show good – exceptional drug responses in more than 25% PDTO cultures. Drugs that were tested in less than five PDTOs were excluded.

PARIS® assay identified exceptional and patient-specific sensitivities to targeted agents in 92.3% of cases

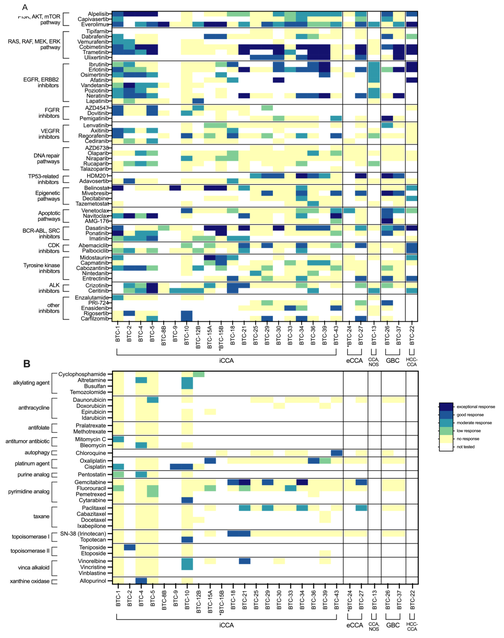


Figure 2: Drug sensitivity landscape of BTC organoids. A) Heatmap showing ex vivo responses to all targeted drugs tested in at least five organoid cultures. 92.30% (24/26) of organoid cultures exhibited a good to exceptional response (SPM 15-12) to at least one, and often more than one, FDA-approved targeted agent.
B) Heatmap showing ex vivo response to all chemotherapeutic agents tested in at least one organoid culture. In contrast, only 17 assays (7.3%) and 10 chemotherapies showed a good to exceptional responses, which include cisplatin and gemcitabine the standard of care BTC treatments
White rectangles indicate drugs that were not tested in that screen. Asterisks next to a patient number indicate that the drug screen failed some parameter of quality control but was still evaluable.

Drug sensitivities & known pathogenic alterations

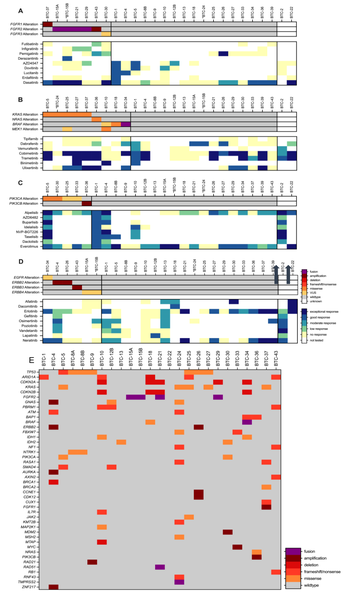


Figure 3. Correlation of *ex vivo* drug response and genetic biomarkers. A)-D) Heatmap of drug response against druggable genomic alterations were evaluated for multiple genes such as FGFR, KRAS/NRNAS, BRAF, MEK, PIK3CA and EGFR/ERBB2. Of note: 75% of BTC PTDOs that were pre-treated were generally resistant to previous drugs the patients were exposed to. For example: If the patient has been treated with FGFRi earlier and acquired resistance those were reflected in PDTO response and bypass pathways activation was noticed.

E) Oncoprint of all structural rearrangements, pathogenic mutations, copy gains, and copy losses of genes taken from clinical histories and third-party NGS sequencing for the BTC cohort are noted. Among the structural variants in this current BTC cohort most prominent were FGFR alterations (15.4%) along with copy no. alterations in CDKN2A/B (19.2%, 15.4%). Variants of unknown significance (VUS's) are not included.

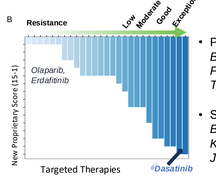
Clinical utility of PTDO based drug testing

- Five patients were able to obtain off-label use of drugs guided by assay results and two patients remained on treatment over 5 weeks.
- Notably, these two patients showed clinical benefit with everolimus (10 weeks) and dasatinib (11 weeks), experiencing symptom's relief and reduced ascites. Overall survival was about 3 months and 8.5 months respectively.
- All patients had several drugs that scored exceptional, though all had advanced disease and had progressed through at least two treatment lines.
- Below the PARIS test report results for the Dasatinib case is highlighted.

A

	Compound	Target	Cmax (M)	IC50 (M)	AUC	SPM Score
Sensitive	**Dasatinib**	**BCR-ABL**	**4.17E-07**	**3.21E-08**	**0.12**	**15**
	Belinostat	HDAC	1.83E-04	2.57E-07	0.26	15
	Entinostat	HDAC	3.00E-07	2.31E-07	0.33	14
	Everolimus	mTOR	3.86E-08	N/A	0.30	14
	Crenolanib	FLT3, PDGFR	1.00E-03	1.41E-07	0.33	14
	Midostaurin	FLT3, PKC	1.40E-06	1.55E-07	0.33	14
	Dabrafenib	BRAF	2.84E-06	6.15E-07	0.50	13
Insensitive	*Oxaliplatin*	*platinum agent*	*8.10E-06*	*1.60E-06*	*0.63*	*13*
	Gemcitabine	*pyrimidine analog*	*3.20E-06*	*5.41E-07*	*0.51*	*5*
	Olaparib	PARP	3.27E-05	N/A	1.10	1
	AZD4547	FGFR	N/A	N/A	0.93	indeterminate
	Erdafitinib	FGFR	6.02E-06	N/A	1.03	indeterminate
	Fluorouracil	pyrimidine analog	4.26E-04	N/A	0.89	indeterminate

B



- Patient had pathogenic *FGR2-BICC1* fusion, equivocal *FGFR2* amplification, and *TP53* mutation
- Somatic VUS's in *ALK, BRCA2, ERBB4, FANCE, KDM6A, FGF10, FLYWCH1, JDM6A, SGK1, SPEN, TRAF2*

Figure 4. PARIS Test indicated future treatment option for an advanced metastatic case. A) and B) Drug sensitivity assay indicates Dasatinib as top scoring drug and lack of response to previous treatments.

Acknowledgments: We thank Matthew Kase for poster development, Dr. Sameek Roychowdhury for pt sample, and Karo Majauskaite for experimental help.

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