

Consensus statement

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# Criteria for preclinical models of cholangio-carcinoma: scientific and medical relevance

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## **Criteria for preclinical models of cholangiocarcinoma: scientific and medical relevance**

Calvisi DF et al.

**Table S1. Members of the CCA model core team, steering committee and consortium.**

	<b>Name</b>	<b>Affiliation</b>	<b>Country</b>	<b>Role</b>	<b>Questionnaire</b>
<b>1</b>	Alejandro Forner	Hospital Clinic Barcelona	Spain	Steering	1, 2
<b>2</b>	Alexander Scheiter	University of Regensburg	Germany	Consortium	2
<b>3</b>	Anna Saborowski	Hannover Medical School	Germany	Steering	1, 2
<b>4</b>	Arndt Vogel	Hannover Medical School	Germany	Steering	1, 2
<b>5</b>	Benjamin Goeppert	University Hospital Heidelberg	Germany	Steering	1, 2
<b>6</b>	Cédric Coulouarn	INSERM, Université de Rennes 1	France	Steering	1, 2
<b>7</b>	Chiara Braconi	University of Glasgow	Scotland	Steering	1, 2
<b>8</b>	Chiara Raggi	University of Florence	Italy	Steering	1, 2
<b>9</b>	Diego F Calvisi	University of Regensburg	Germany	Core team	1, 2
<b>10</b>	Domenico Alvaro	University La Sapienza Rome	Italy	Steering	1, 2
<b>11</b>	Ester Gonzalez-Sanchez	University of Barcelona	Spain	Steering	1, 2
<b>12</b>	Florin M Selaru	Johns Hopkins University	USA	Consortium	2
<b>13</b>	Gregory J Gores	Mayo Clinic	USA	Steering	1, 2
<b>14</b>	Guido Carpino	University of Rome "Foro Italico"	Italy	Steering	1, 2
<b>15</b>	Javier Vaquero	Bellvitge Biomedical Research Institute	Spain	Steering	1, 2
<b>16</b>	Jesper B Andersen	University of Copenhagen	Denmark	Steering	1, 2
<b>17</b>	Jesus M Banales	Biodonostia Health Research Institute	Spain	Core team	1, 2
<b>18</b>	John Bridgewater	UCL Cancer Institute	UK	Steering	1, 2
<b>19</b>	Jose JG Marin	University of Salamanca	Spain	Steering	1, 2
<b>20</b>	Juan W Valle	University of Manchester	UK	Steering	1, 2
<b>21</b>	Katja Evert	University of Regensburg	Germany	Consortium	1
<b>22</b>	Kirsten Utpatel	University of Regensburg	Germany	Consortium	1
<b>23</b>	Lewis R Roberts	Mayo Clinic	USA	Steering	1, 2
<b>24</b>	Laura Fouassier	INSERM, Sorbonne Université	France	Core team	1, 2
<b>25</b>	Laura Broutier	INSERM, University Claude Bernard Lyon	France	Consortium	2
<b>26</b>	Luc JW van der Laan	Erasmus MC - University Medical Center Rotterdam	Netherlands	Core team	1, 2

27	Luca Fabris	University of Padua	Italy	Steering	1, 2
28	Luke Boulter	University of Edinburgh	Scotland	Core team	1, 2
29	Massimiliano Cadamuro	University of Padova	Italy	Consortium	2
30	Matthias Evert	University of Regensburg	Germany	Steering	1, 2
31	Merixell Huch	Max Planck Institute of Molecular Cell Biology and Genetics	Germany	Consortium	2
32	Mitesh J Borad	Mayo Clinic	USA	Steering	1
33	Monique MA Versteegen	Erasmus MC - University Medical Center Rotterdam	Netherlands	Core team	1, 2
34	Oreste Segatto	IRCCS Regina Elena National Cancer Institute Rome	Italy	Steering	1, 2
35	Pedro M Rodrigues	Biodonostia Health Research Institute	Spain	Steering	1, 2
36	Rocio IR Macias	University of Salamanca	Spain	Steering	1, 2
37	Robert Goldin	Imperial College London	UK	Consortium	2
38	Rui E Castro	University of Lisbon	Portugal	Core team	1, 2
39	Sergio Gradilone	University of Minnesota	USA	Consortium	2
40	Silvestre Vicent	CIMA - University of Navarra	Spain	Steering	1, 2
41	Stephanie Roessler	University Hospital Heidelberg	Germany	Steering	1, 2
42	Tim Kendall	University of Edinburgh	Scotland	Steering	1, 2
43	Vincenzo Cardinale	University of Sapienza Rome	Italy	Core team	1, 2
44	Xin Chen	University of California San Francisco	USA	Steering	1
45	Yoshimasa Saito	Keio University Tokyo	Japan	Consortium	2

The Core team includes the initiators and authors of this project (DC, JB, LF, LvdL, MV, RC, VC). Steering committee indicates all co-authors of the review, and Consortium members contributed to the consensus by filling in Delphi questionnaire 1 and/or 2. The consortium members are experts that were invited to fill the second questionnaire and are listed as one co-author «CCA model Consortium». Members represent institutes in Europe, USA and Japan.

**Suppl Table 2.** Summary of the immortalized cell lines derived from tumor cells and stromal cells that are used in cholangiocarcinoma preclinical studies

Cell type	Species	Name	Anatomic classification/ Origin	Source/animal model	Molecular studies	Observations	Original reference
Tumor cell lines	Human	CC-LP-1	Intrahepatic	Primary tumor			1
		CC-SW-1	Intrahepatic	Primary tumor			1
		CHGS	Intrahepatic	Primary tumor			2
		Cho-CK	Intrahepatic	Primary tumor			3
		Choi-CK	Intrahepatic	Primary tumor			3
		ETK-1	Intrahepatic	Ascites			4
		HCCC-9810	Intrahepatic	Primary tumor			5
		HChol-Y1	Intrahepatic	Primary tumor			6
		HKGZ-CC	Intrahepatic	Primary tumor			7
		HuCC-A1	Intrahepatic	Primary tumor			8
		HuCC-T1	Intrahepatic	Ascites	9, 10, 11		12
		HuH-28	Intrahepatic	Primary tumor	9, 10		13
		JCK	Intrahepatic	Primary tumor			3
		KKK-D049	Intrahepatic	PDX		Liver Fluke	14
		KKK-D068	Intrahepatic	PDX			14
		KKK-D131	Intrahepatic	PDX			14
		KKK-D138	Intrahepatic	PDX			14
		KKU-023	Intrahepatic	Primary tumor			15
		KKU-M055	Intrahepatic	Primary tumor	9	Liver Fluke	16
KKU-213A	Intrahepatic	Primary tumor	9	Liver fluke, formerly known as KKKU-213, <sup>17</sup>	16		
KKU-213B	Intrahepatic	Primary tumor		Liver fluke, formerly known as	16		

				KKU-214, <sup>17</sup>	
KKU-213C	Intrahepatic	Primary tumor		Liver fluke, formerly known as KKU-156, <sup>17</sup>	16
KKU-M139	Intrahepatic	Primary tumor		Liver Fluke	18
KKU-OCA17	Intrahepatic	Primary tumor		Liver Fluke	16
KMC-1	Intrahepatic	PDX			19
MT-CHC01	Intrahepatic	PDX			20
NCC-CC1	Intrahepatic	PDX			21
NCC-CC3-1	Intrahepatic	PDX			21
NCC-CC3-2	Intrahepatic	PDX			21
NCC-CC4-1	Intrahepatic	PDX			21
OZ	Intrahepatic	Ascites	9		22
TKKK	Intrahepatic	Primary tumor	9		23
RBE	Intrahepatic	Primary tumor			24
RPMI-7451	Intrahepatic	Primary tumor			25
SCK	Intrahepatic	Primary tumor			3
SG231	Intrahepatic	Primary tumor			26
SNU-1079	Intrahepatic	Primary tumor	9, 10		27
SSP-25	Intrahepatic	Primary tumor		Suspect of being a derivative from ETK-1	24
YSCCC	Intrahepatic	Ascites			28
ZJU-1125	Intrahepatic	Primary tumor			29
HBDC	Hilar	Ascites			30
KKU-100	Hilar	Primary tumor	10	Liver Fluke	31
KKU-452	Perihilar	Primary tumor			15
SNU-1196	Perihilar	Primaty tumor	9, 10		27

	ICBD-1	Extra-hepatic	Primary tumor			32
	KMBC	Extra-hepatic	PDX			33
	MEC	Extra-hepatic	Pleural effusion Metastasis			34
	QBC939	Extra-hepatic	Primary tumor			35
	RMCCA-1	Extra-hepatic	Primary tumor			36 <sup>1</sup>
	TBCN-6	Extra-hepatic	Primary tumor			37
	TGBC-47	Extra-hepatic	Primary tumor			38
	TK	Extra-hepatic	Ascites			39
	OCUCh- LM1	Extra-hepatic	Liver metastasis			40
	EGI-1	Distal	Primary tumor	9, 10, 11		41
	NCC-BD1	Distal	PDX			21
	NCC-BD2	Distal	PDX			21
	SK-ChA-1	Distal	Ascites	9		42
	SNU-245	Distal	Primary tumor	9, 10		27
	TFK-1	Distal	Primary tumor	9, 10, 11		43
	KMCH-1	Combined HCC-iCCA	Primary tumor			44
	KMCH-2	Combined HCC-iCCA	Primary tumor			45
Mouse	SB1-SB7	Intrahepatic	Male C57BL/6 mice, transposase- mediated transduction of Akt YAP in the biliary epithelium coupled with lobar obstruction and systemic IL-33			46

			administration mouse model			
	CGKP19	Intrahepatic	Male C57BL/6J mice, HTVI of KRASG12D and CRISPR/Cas9 sg-p19 mouse model			47
Rat	C611B	Intrahepatic	Fischer 344 male rats, furan treated rat model			48
	CGCCA	Intrahepatic	Male Sprague-Dawley rats, Thioacetamide (TAA)-induced CCA rat model			49
	REUSAL-C44	Intrahepatic	Male Wistar rats, Thioacetamide (TAA)-induced CCA rat model			50
	REUSAL-C49	Intrahepatic	Male Wistar rats, Thioacetamide (TAA)-induced CCA rat model			50
	BDE1neu	CCA	Fischer 344 rats, derived from BDE1 cholangyocytes by retroviral infection with the retrovirus Glu664-neu, containing the transforming rat			51



			erbB-2/neu oncogene.			
	BDEsp	CCA	Fisher 344 rats, derived from BDE1 cholangyocytes by spontaneous in vitro neoplastic transformation of BDE1 cell line			52
	LCSCs	Combined HCC-iCCA	2-acetylaminofluorene/ partial-hepatectomy followed by aflatoxin injection rat model			53
Hamster	HaLCCA-1	Intrahepatic	Syrian Golden Hamsters, liver fluke infection plus dymethylnitrosamine treatment hamster model			54
	HaTCCA-1	Intrahepatic	Syrian Golden Hamsters, liver fluke infection plus dymethylnitrosamine treatment hamster model			54
	Ham-1	Intrahepatic	Syrian Golden Hamsters, liver fluke infection plus dymethylnitrosamine treatment hamster model			55

		Ham-2	Intrahepatic	Syrian Golden Hamsters, liver fluke infection plus dymethylnitrosamine treatment hamster model			56
		CCA-OF	Intrahepatic	Syrian Golden Hamsters, liver fluke infection plus dymethylnitrosamine treatment hamster model			57
Stromal cell lines	Human	hTERT-HSC	Hepatic Stellate Cells	Healthy individual			58
		LX2-HSC	Hepatic Stellate Cells	Healthy individual			59
		LI90	Hepatic Stellate Cells	Epithelioid hemangioendotelium			60
	Rat	RGF	Portal fibroblasts	Male Sprague-Dawley rats			61
	Human	THP-1	Monocytes	Acute monocytic leukemia			62

**Suppl Table 2.** Summary of the immortalized cell lines derived from tumor cells and stromal cells that are used in cholangiocarcinoma preclinical studies

**Suppl Table 3.** Experimental model sheet.

Experimental model sheet	
1. <b>Type of model:</b> ( <i>in vitro</i> , <i>ex vivo</i> , <i>in vivo</i> )	
2. <b>Species:</b> (mouse, rat, hamster, human, etc.)	

3. <b>Sex:</b> (male, female, both)	
4. <b>Strain:</b>	
5. <b>Condition of the surrounding liver</b> (apparently healthy, cirrhosis, fibrosis, etc.) :	
6. <b>Method of generation:</b> (spontaneous, carcinogenic, chronic injury, infectious, transgenic, knockout, transposon-mediated, patient-derived xenograft, organoids, isolated from animal tumours, isolated from human tumours, etc.):	
7. <b>Tumour development:</b> (fast, slow)	
8. <b>Metastasis:</b> (yes, no, locations,...)	
9. <b>Anatomical location of the lesions (when applicable):</b> (intrahepatic, extrahepatic, both)	
10. <b>Cell of origin (if available):</b> (cholangiocyte, stem/progenitor cell, hepatocyte)	
11. <b>Types of samples and storage conditions for future analyses</b>	
12. <b>Presence of preneoplastic lesions:</b> (yes/no)	
13. <b>Type of preneoplastic lesions:</b> (IPNB, IPMN, BillIN, etc.)	
14. <b>Type of cholangiocarcinoma:</b> (iCCA, pCCA, dCCA, combined HCC/CCA)	
15. <b>Histology of tumours:</b> (large duct type, small duct type, CCA, lymphoepithelioma-like CCA, etc.)	

16. <b>Microenvironment features:</b> (presence of stroma/desmoplastic reaction, absence of stroma, immune infiltration yes/no)	
17. <b>Phenotype of the lesions:</b> (CK7, CK19, MUC1, MUC2, MUC5AC, MUC6, HNF4A, AFP, markers of stemness, markers of EMT, etc.)	
18. <b>Control samples used if applicable</b> (bile duct freshly isolated from liver or cell line)	

## Supplementary Data :

### Questionnaire 1 – Criteria for Preclinical Models of Cholangiocarcinoma : Scientific and Medical Relevance

Cholangiocarcinoma (CCA) constitutes a diverse group of malignancies emerging from the biliary tree. CCAs are divided into three subtypes depending on their anatomical site of origin: intrahepatic (iCCA), perihilar (pCCA), and distal (dCCA) CCA. iCCAs arise above the second-order bile ducts, whereas the point of anatomical distinction between pCCA and dCCA is the cystic duct. pCCA and dCCA may also be collectively referred to as "extrahepatic" (eCCA). pCCA is the single largest group, accounting for approximately 50–60% of all CCAs, followed by dCCA (20–30%) and iCCA (10-20%). CCA constitutes the second most common primary hepatic malignancy after hepatocellular carcinoma (HCC), comprising approximately 15% of all primary liver tumours and 3% of gastrointestinal cancers. CCAs are usually asymptomatic in early stages, thus being diagnosed when the disease is already in advanced phases. These features highly compromise the therapeutic options, resulting in a dismal prognosis. CCA is a rare cancer, but its incidence and mortality rates (1-6:100,000 inhabitants/year, globally) have been increasing in the last years worldwide, representing a global health problem. Indeed, despite all the advances in CCA awareness, knowledge, diagnosis, and therapies, the patients' prognosis has not improved significantly in the past decade, with 5-year survival (~7-20%) and tumour recurrence rates after resection still disappointing. Therefore, it is mandatory to conduct research studies on CCA with appropriate experimental models that constitute indispensable tools to understand CCA pathogenesis better, uncover diagnostic tools, and identify therapeutic drugs and/or strategies to cure patients.

In this Consensus Statement, we aim to provide a comprehensive and critical point of view regarding the current knowledge and what is envisioned on the horizon for the experimental models of CCA, focusing on pathology, genetic and epigenetic landscape, molecular perturbations, chemoresistance, and therapies.

The present review has three main scopes:

1. Defining minimal and advanced criteria of experimental models (based on a consensus): expert selection, tools for criteria, and information
2. In vivo models for CCA: Highlight strengths and weaknesses of experimental in vivo CCA models and how these models help to understand disease progression and treatment, including chemotherapy, personalized target therapy (mutation-based), and immunotherapy.
3. In vitro models for CCA: Experimental in vitro CCA models currently available and how these are required to progress disease comprehension and treatments.

#### The Delphi Method

Choices for definitions and nomenclature will be reached through the consensus of key experts in the field. In this way, the review will be more than just another review. It will be a true consensus document, its contents to be adopted widely.

To facilitate consensus, the Delphi method will be employed:

"The Delphi method is based on the principle that decisions from a structured group of individuals are more accurate than unstructured groups. The experts answer questionnaires in two or more rounds. After each round, a facilitator provides an anonymized summary of the experts' answers from the previous round and their reasons for their judgments. Thus,

experts are encouraged to revise their earlier answers in light of the replies of other members of their panel. It is believed that during this process, the range of the answers will decrease, and the group will converge towards the "correct" answer. Finally, the process is stopped after a consensus is reached."

-Wikipedia

After the first round, questions for which a  $\geq 90\%$  consensus has been reached will be removed from subsequent questionnaires. The following is the first questionnaire in the series. After 33 experts completed the questionnaire, the facilitator compiled and presented the answers. After experts in the field had time to analyze the answers, a second questionnaire was sent out.

### Contact information

Only the facilitator will have access to the contact information

Please fill in your contact information, including affiliation, below

What is your field of interest/expertise?

If you feel you don't have the right expertise to answer a question, just skip to the next one.

### Part 1. Defining minimal and advanced criteria for experimental models

#### **Question 1: What malignant features of a biliary tumour need always to be determined?**

- Invasion of the basement membrane
- Increased nucleus/cytoplasm ratio
- Dissemination/metastatic features
- Tumorigenic capacity of isolated cells after subcutaneous injection in immune-deficient mice
- Other

#### **Question 2: What type of histological investigation should be minimally done to characterize the tumour in a preclinical CCA model? (multiple answers can be ticked)**

- H&E
- Immunohistochemistry for at least one biliary cytokeratin (e.g., CK19, CK7, pan CK, etc.)
- Immunohistochemistry for two biliary cytokeratins (CK19 and CK7)
- Markers for inflammatory cells and CAFs
- PAS reaction for highlighting mucin
- A broad panel of markers for hepatobiliary malignancies and metastasis
- Other

#### **Question 3: Regarding the anatomical classification of human tumors, a preclinical model for CCA should specifically distinguish tumors in...(fill)**

- Intrahepatic CCA, perihilar CCA, and distal CCA
- Intrahepatic CCA and extrahepatic CCA
- No need for such classification
- Other

**Question 4: Regarding the histopathologic evaluation of human CCA, what aspects should be minimally mimicked by the tumors in a preclinical model and, therefore, should be characterized? (multiple answers can be ticked)**

- Intra-tumoral heterogeneity (high stroma, inflammatory response, epithelial phenotype)
- Presence of precursor lesions
- Inter-tumoral heterogeneity (large versus small bile duct tumour in iCCA)
- Pattern of growth (mass-forming, periductal infiltration, intraductal growth)
- Other

**Question 5: The anatomical and histomorphological subtypes of human CCA have been proposed to be clinically relevant in terms of patient outcome and carcinogenic mechanisms, i.e., IDH mutation predominantly in small duct type iCCA. Do you think that anatomical and histomorphological subtypes should also be accounted for in mouse models?**

- Agree
- Do not agree, because...

**Question 6: It has been proposed that iCCA may originate from several cells of origin. Do you agree that the following cell types may be cells-of-origin for iCCA? (multiple answers can be ticked)**

- Mature hepatocytes
- Cholangiocytes
- Hepatic progenitor/oval cells
- Peribiliary glands
- Other

**Question 7: How strongly do you agree that the following morphological and/or immunophenotypic features are required to classify a lesion in a preclinical model as CCA?**

*Please rank your answers from 1 (not required) to 9 (essential)*

A: Location within the liver or extrahepatic biliary tree: rank 1-9

B: An absence of a potential extrahepatic biliary primary lesion

C: Epithelial cytological features (cohesive groups or structures and/or pan-cytokeratin immunopositivity)

D: At least focal gland formation.

E: An absence of obvious hepatocellular differentiation (bile production and canalicular CD10 or BSEP).

F: At least focal mucin production.

G: Immunopositivity for CK7 or CK19

H: At least focal desmoplastic stroma.

## **Part 2. In vivo models for Cholangiocarcinoma**

**Question 8: What are the advantages of using ectopic (subcutaneous) xenograft models from either human (patient-derived) tumour cell lines or tissue (PDX)? (multiple answers can be ticked).**

- They recapitulate a tumour with histology that is similar to that of a tumour in humans

- They are easy to manage, and tumour size is easily measured by using a caliper
- They facilitate access to tumour tissue for testing physical-based local treatment
- Distinguishing tumour cells of human origin from microenvironment cells of murine origin for mRNA expression / RNA seq studies (example for pancreatic cancer: Nicolle *et al.*, 2017 [1])
- Other

**Question 9: What are the disadvantages of using ectopic (subcutaneous) xenograft models from either human cell lines or tissue (PDX)?**

- Poor immune components
- Incompatible for testing immune-based therapies
- Other

**Question 10: Should we systematically validate the ectopic xenograft model by an expert pathologist and show histology of the tumour in publications?**

1. Yes
2. No

**Question 11: Should the expert pathologist specify what type of CCA is found in the ectopic xenograft model?**

1. Yes
2. No

**Question 12: What are the main advantages of the ectopic xenograft models over orthotopic xenograft models?**

- Ectopic models have faster tumour development
- Ectopic models show higher reproducibility
- Ectopic models are easier to measure tumour growth using a caliper
- Other

**Question 13: What are the main advantages of the orthotopic xenograft models over ectopic xenograft models?**

- Orthotopic models have a correct anatomical localization of the tumor
- Orthotopic models show the development of native liver stroma
- Other

**Question 14: Does the genetic background of the mouse strain account for tumour development and growth kinetics?**

- Yes
- No
- There is no sufficient data to answer this

**Question 15: How representative for tumour development in human CCA is it to inject cells (either by xenografting or in syngeneic mouse models) into a healthy liver without a disease background?**

- Good
- Bad
- Moderate



**Question 16: Novel immunotherapies should be tested in ectopic or orthotopic syngeneic models since these models recapitulate the immunobiology of CCAs. Do you agree with this statement?**

1. Yes
2. No

**Question 17: All available CCA cell lines are known to have a tumorigenic capacity and generate tumors in ectopic or orthotopic models. Do you agree with this statement?**

1. Yes
2. No

**Question 18: Are syngenic orthotopic engrafted models the appropriate model to study CCA in preclinical rodent models?**

- Yes, I agree
- No, I do not agree (comment)

**Question 19: Should extensive molecular characterization be required to report engrafted models at the time of publication?**

1. Yes
2. No

**Question 20: Should immune profiling be reported when using engrafted models?**

1. Yes
2. No

**Question 21: Do you agree with the over-arching definition of genetically engineered mouse models: A genetically engineered mouse model (GEMM) is a mouse whose genome has been altered through genetic engineering techniques.**

- Agree
- Do not agree

**Question 22: What are the advantages of using genetically engineered mouse models? (multiple answers can be ticked)**

- They are genetically homogeneous, and carcinogenesis is highly reproducible
- Display the various phases of tumour development, starting from preneoplastic stages, which can be distinctively evaluated morphologically and molecularly
- Allow the study of cholangiocarcinogenesis in an immune-competent host
- Exhibit the complete cellular and extracellular features of the human disease (cancer cells and tumour microenvironment components)
- Permit reliable drug testing and therapy monitoring by physical and instrumental tools
- Possibility to study carcinogenesis in the presence of predisposing/concurrent conditions (hepatitis, cirrhosis, fibrosis)
- Other

**Question 23: What are the disadvantages of using genetically engineered mouse models?**

- The immune and microenvironment components are not of human nature

- In the transposon models, the mature hepatocyte is the cell of origin of the tumor
- Some conventional knockout and transgenic models are incompatible with life or are challenging to maintain due to reproductive defects (sterility, etc.)
- Conventional and conditional knockout and transgenic models require specific expertise and instrumentation
- Generally, carcinogenesis is rapid and does not allow the investigation of intratumourheterogeneity
- Costly compared to other models, and studies using these models require ethical approval
- Only rarely these models give rise to local and/or distant metastasis
- Other

**Question 24: What are the best methods to create GEMM for cholangiocarcinoma research? (multiple answers can be ticked)**

- Transgenic/knockout
- Transposon
- CRISPR/Cas9 gene editing
- Other

**Question 25: Which analyses should be necessary to ascertain the validity of a GEMM of cholangiocarcinoma for the human disease? (multiple answers can be ticked)**

- Histopathological evaluation
- Molecular and immunophenotypic characterization of the lesions
- Capacity to generate metastasis
- Genetic alterations
- Metabolic features
- Other

### **Part 3. In vitro models for Cholangiocarcinoma**

**Question 26: The culture medium (i.e., DMEM, MEM, RPMI, etc.) influences the results when performing in vitro experiments with cell lines or primary 2D cultures. Do you agree with this statement?**

- Yes, culture media formulation should be homogenized for each cell line and primary culture
- No, it does not matter. The outcome of in vitro experiments is not influenced by the type of culture medium
- Other

**Question 27: The plastic support (i.e., TPP, Falcon, Corning, +/- ECM layer) is important for in vitro experiments of cell lines or primary 2D cultures. Do you agree with this statement?**

- Yes, plastic support should be homogenized for each cell line and primary culture.
- No, it does not matter which plastic support is used for in vitro experiments
- Other

**Question 28: The percentage of confluency of 2D grown cell cultures influences the results of the experiments done with cell lines or primary 2D cultures. Do you agree with this statement?**

- Yes

- No

**Question 29: The mutational status should be known for each cell line or primary 2D cultures. Do you agree with this statement?**

- Yes, the mutational status should be known for each 2D culture
- No, the mutational status of the 2D grown cells is not important
- This depends on the aim of the study

**Question 30: The origin of the cells (i.e. intrahepatic, perihilar, distal) should be known and revised according to the new CCA classifications. Do you agree with this statement?**

- Yes, the origin of previously established cell lines should be revised according to the new CCA classification
- No, the origin of the cells is not important to translate the findings to the human setting.

**Question 31: The isolation protocol for culture of primary cells, influences the results of experiments done. Do you agree with this statement?**

- Yes, isolation protocols for primary cell culture should be homogenized for different cell types
- No, the isolation method is not important to translate the findings to the in vivo setting.

**Question 32: Passaging and sub-culturing of 2D cultured cell lines or primary cells using enzymatic or mechanical dissociation may influence the outcome of in vitro experiments. Do you agree with this statement?**

- Yes, this is an important issue
- No, not important

**Question 33: Cost/effectiveness and the convenient large-scale analysis (i.e., drug screening analysis) of 2D cultures are important to consider. Do you agree with this statement?**

- Yes, this is an important issue
- No, not important

**Question 34: What aspects of CCA should be minimally modelled in a 3D in vitro model? (multiple answers can be checked)**

- tumourmicroenvironment - stromal cells and ECM
- cell-cell and cell-matrix interactions
- tumour(stem) cells
- type of tumour (periductal, intraductal, mass forming)
- region of the tumour (intra- or extrahepatic perihilar/distal)
- cancer stages
- retention of mutational variance
- personal and precision medicine applicability
- Other

**Question 35: Not always do CCA biopsies grow successfully into organoids. What should the success rate to grow patient-specific organoids minimally be to be of value to the field?**

- 25%
- 50%
- 75%
- 100%

**Question 36: Spheroids, that are defined as "3D aggregates of cells (cell lines or primary cells), grown in the absence of a predefined culture substrate to adhere to.", as proposed by Fennema et al, 2013 [2] and are therefore distinct from organoids. Do you agree with this statement?**

- Yes, I agree
- No, I do not agree, because...

**Question 37: Contaminating non-tumour organoids often grow in the CCA organoid cultures, and might even overgrow the tumour organoids. What should be the conditions to positively select for the tumour organoids? (Multiple answers can be ticked)**

- Specific tumour "enrichment" medium (i.e., tumor-initiating medium as described by Broutier *et al.*, 2017 [3])
- Hand picking of organoids with a different phenotype / removing the 'normal-looking' organoids
- Xenotransplantation in mice to select for tumour clones
- Other

**Question 38: What type of characterization should be minimally done in order to confirm malignant origin in the established organoid lines. (multiple answers can be ticked).**

- Full genomic profiling
- Mutation analysis (targeted genomic profiling using a diagnostic panel)
- Phenotypic analysis
- Histological analysis (immunohistochemistry of EpCAM, KRT7)
- Xenotransplantation in mice
- Other

**Question 39: Should every organoid culture be characterized (as proposed in Q 38) before clinical applications such as drug screening?**

1. Yes
2. No

**Question 40: Personalized medicine applications such as drug screenings to find the best treatment for the patient will cost time. How much time is acceptable to initiate, grow and expand the organoids for these analyses? In other words, what is the maximum time acceptable to be of relevance to the clinic?**

- < 1 month
- < 3 months
- < 6 months
- Other

**Question 41: In engineering CCA tumour organoids from non-tumour liver tissue could be done by gene editing. What genetic alterations / mutations need to be minimally included to use this organoid line as a 'real' CCA model?**

Short answer text :

#### **Part 4. Preclinical models for Cholangiocarcinoma**

**Question 42: What type of preclinical model of CCA is likely to best reflect clinical practice?**

- Cell lines
- Syngeneic mice
- Knock out mice
- Established cell lines in nude mice
- PDX
- Organoids
- Other

**Question 43: What matters most to the clinician in a preclinical model?**

- Ease of establishing system
- Ease of maintenance
- Recapitulation of developmental biology
- Duration of experiments
- Genetic manipulation
- Genome-wide screening
- Physiological complexity
- Relative cost
- Recapitulation of human physiology
- Other

**Question 44: How should preclinical models best inform study design and practice?**

- Preliminary data to support study design
- Co-experiment (parallel experiments clinically and at the bench)
- Avatars
- Post-study bench exploration
- Other

**Question 45: What information from the research lab is most valuable to the clinician?**

- PK
- PD
- Genomic data to permit targeting
- Multi-omic data to permit exploration of novel approaches
- Other

**Question 46: How can clinicians best support the translation of lab research to the clinic?**

- By asking direct laboratory questions
- By providing serial tissue samples
- By providing serial whole blood samples
- By providing serial buffy coats
- By sharing clinical outcomes
- By sharing clinical toxicities
- Other

**Question 47: How can the laboratory researcher best support the development of clinical studies?**

- Getting the dose and schedule correct using PK/PD
- Elucidating why the treatment works
- Elucidating why the treatment does not work
- Better understanding the biology
- Better understanding the treatment
- Other

# Supplementary Results

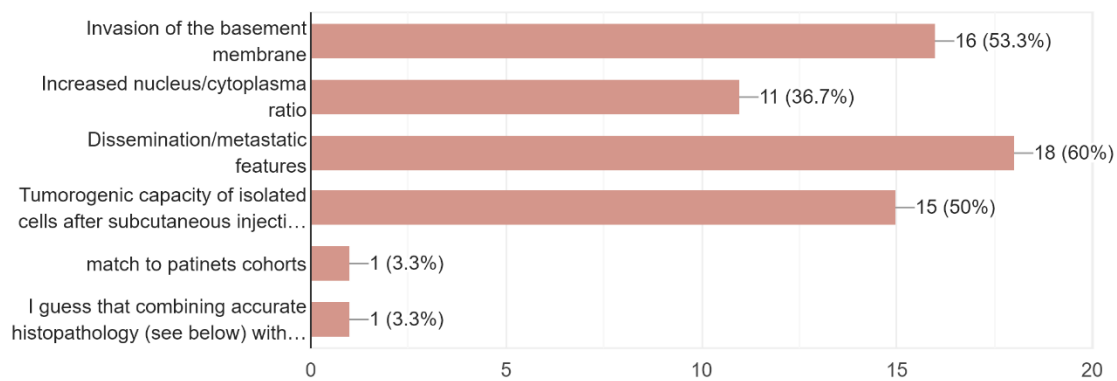
## Questionnaire 1. Summary

Here we list the results from the first questionnaire.

### Part 1. Defining minimal and advanced criteria for experimental models

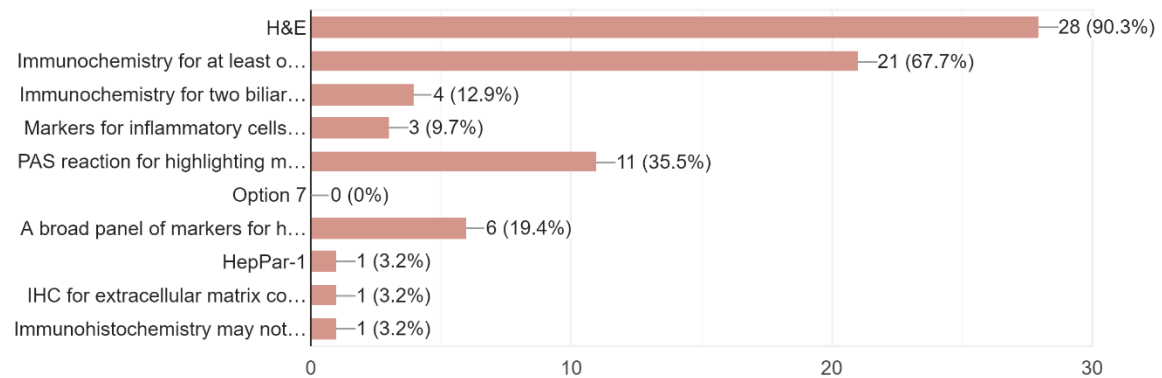
Question 1: What malignant features of a biliary tumor need always to be determined?

30 responses



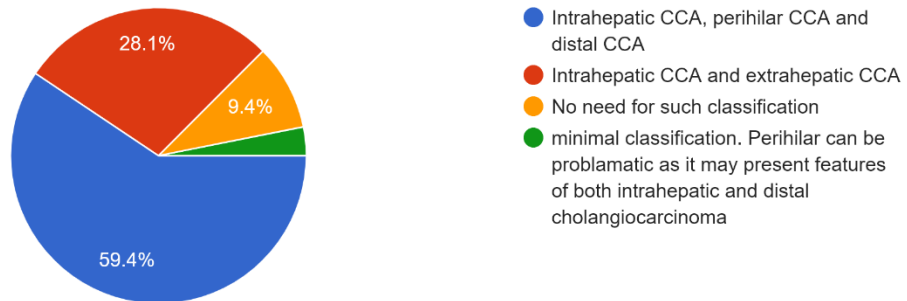
Question 2: What type of histological investigation should be minimally done to characterize the tumor in a preclinical CCA model? (multiple answers can be ticked)

31 responses



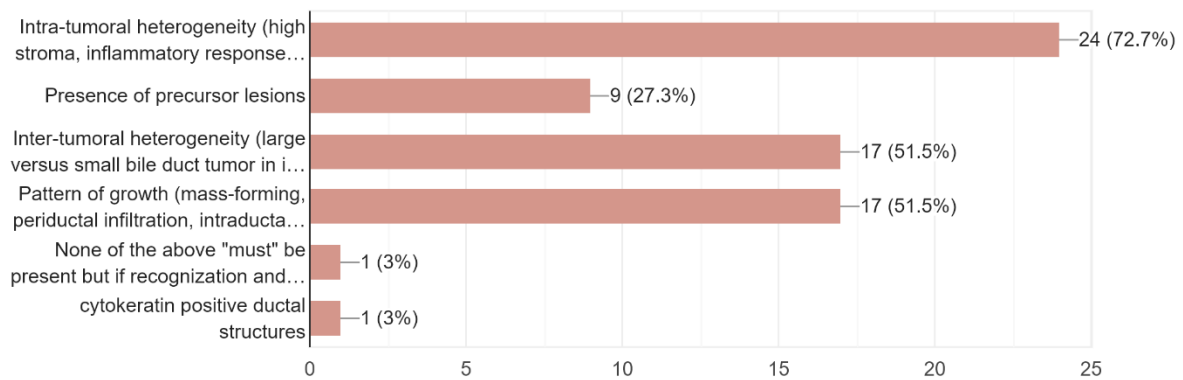
Question 3: Regarding the anatomical classification of human tumors, a preclinical model for CCA should specifically distinguish tumors in...(fill).

32 responses



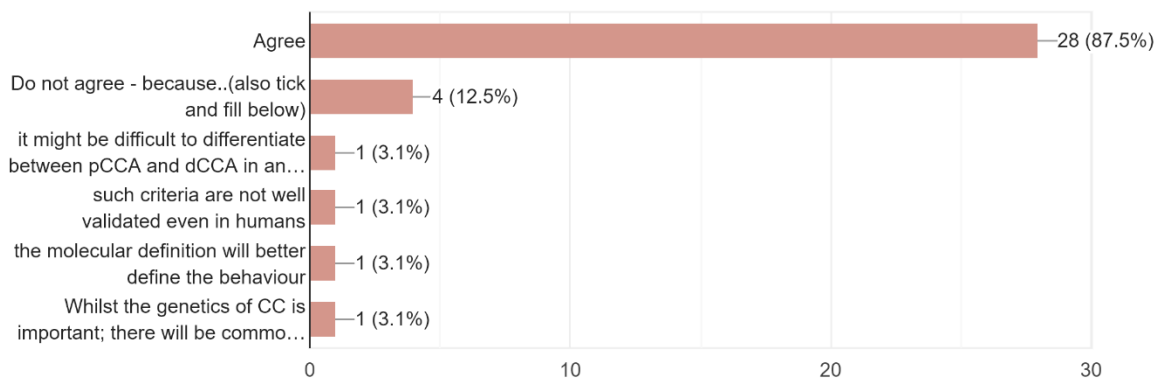
Question 4: Regarding histo-pathologic evaluation of human CCA, what aspects should be minimally mimicked by the tumors in a preclinical ...be characterized? (multiple answers can be ticked)

33 responses



Question 5: The anatomical and histomorphological sybtypes of human CCA have been proposed to be clinically relevant in terms of patient outc...ypes should also be accounted for in mouse models?

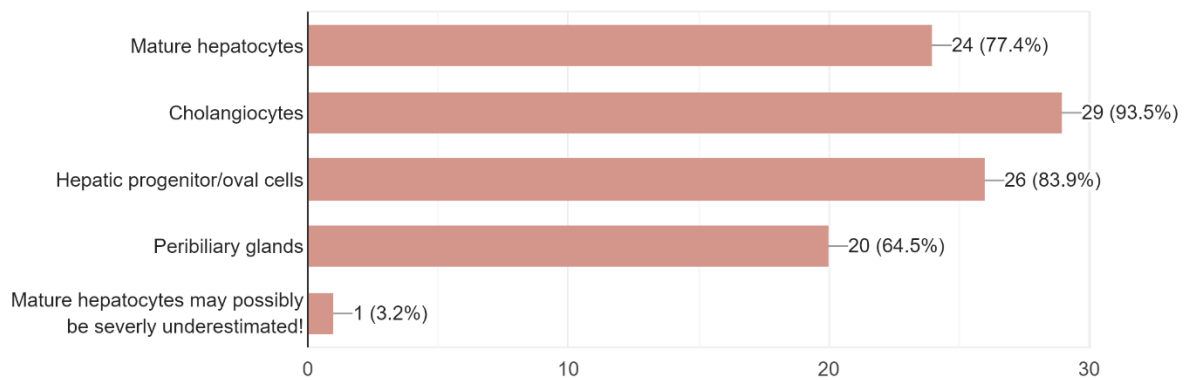
32 responses





Question 6: It has been proposed that iCCA may originate from several cells of origin. Do you agree that the following cell types may be cells-of-origin for iCCA? (multiple answers can be ticked)

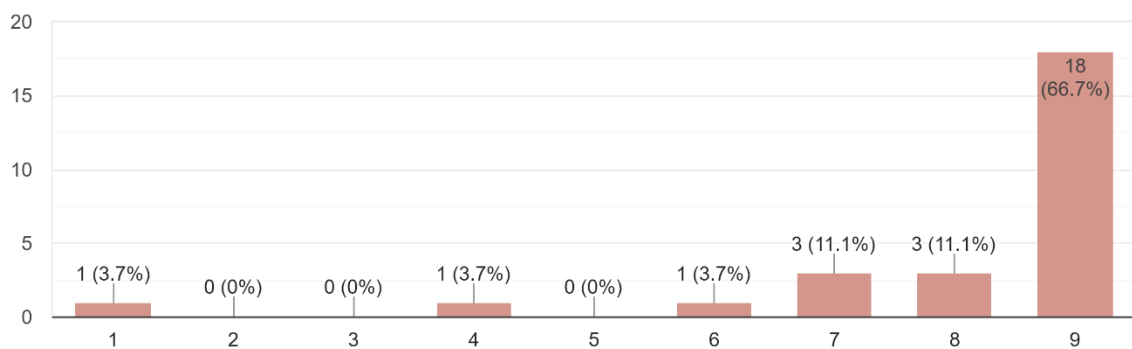
31 responses



Question 7: How strongly do you agree that the following morphological and/or immunophenotypic features are required to classify a lesion in a preclinical model as CCA ?

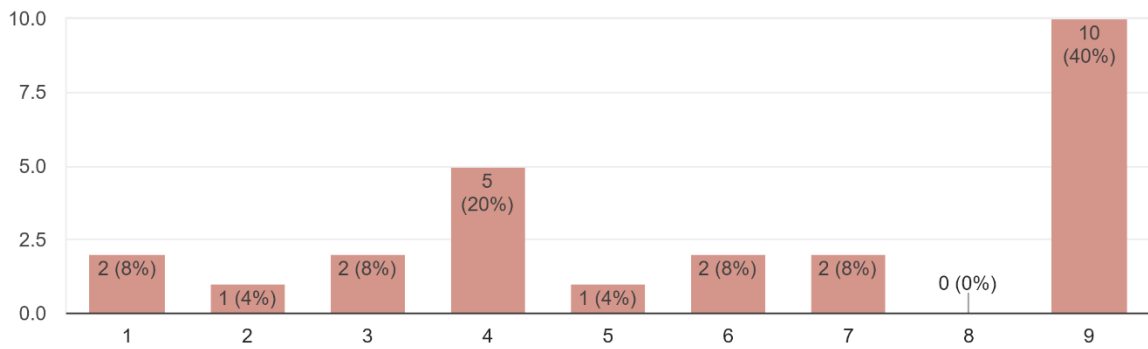
A: Location within the liver or extrahepatic biliary tree

27 responses



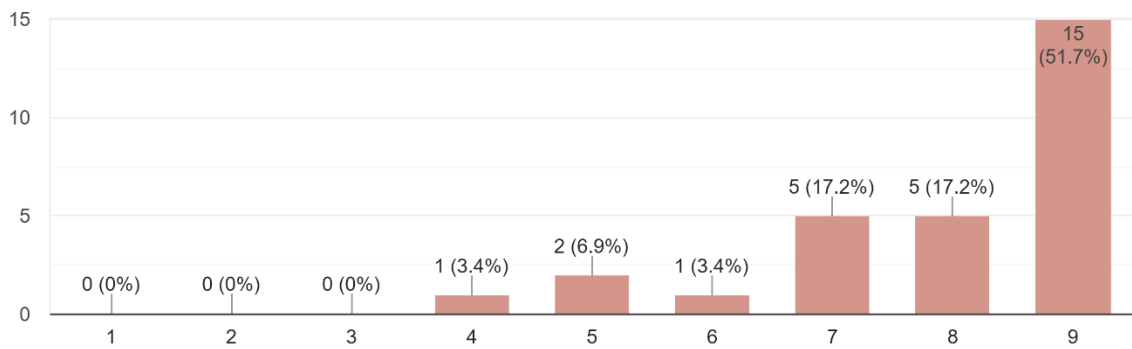
**B: An absence of a potential extrahepaticobiliary primary lesion**

25 responses



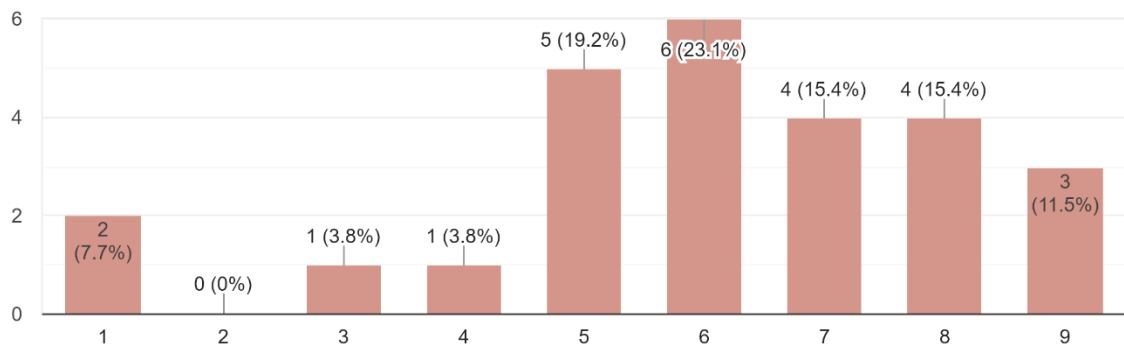
**C: Epithelial cytological features (cohesive groups or structures and/or pan-cytokeratin immunopositivity)**

29 responses



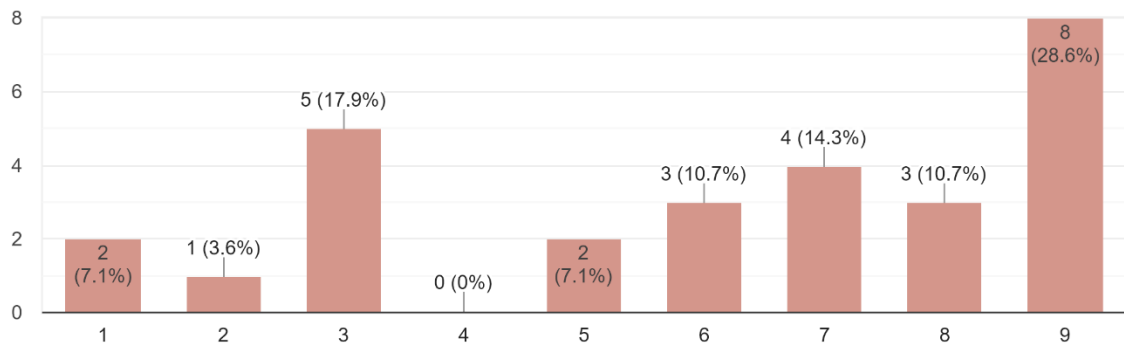
**D: At least focal gland formation.**

26 responses



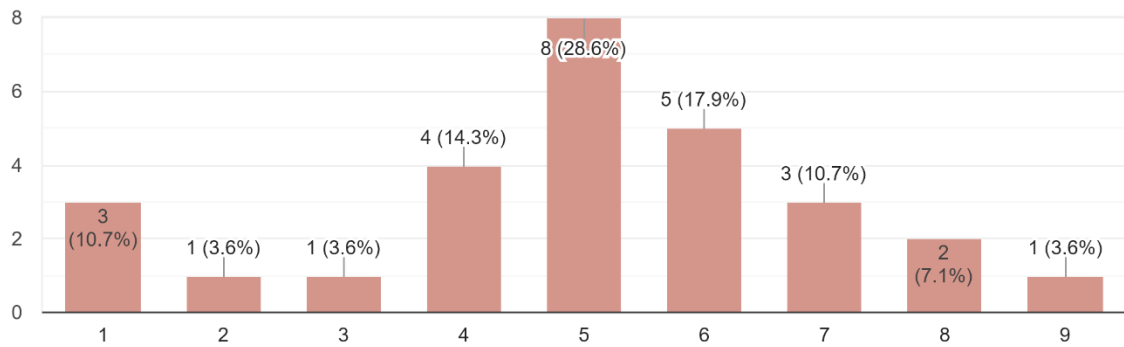
E: An absence of obvious hepatocellular differentiation (bile production, and canalicular CD10 or BSEP).

28 responses



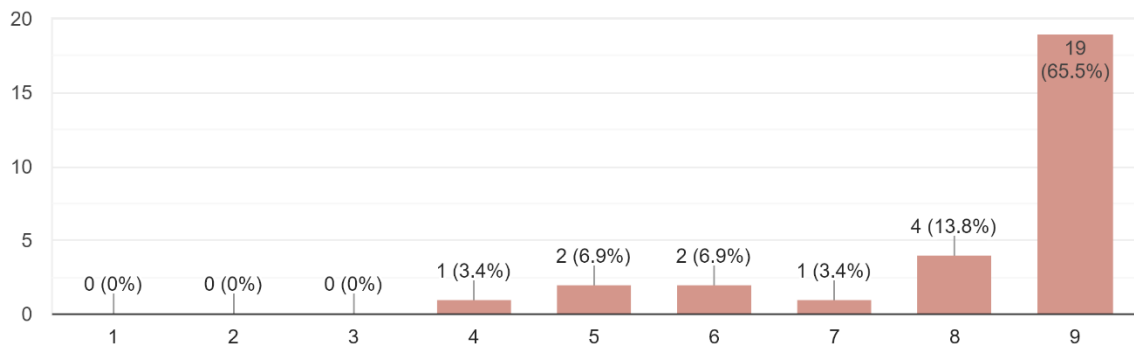
F: At least focal mucin production.

28 responses



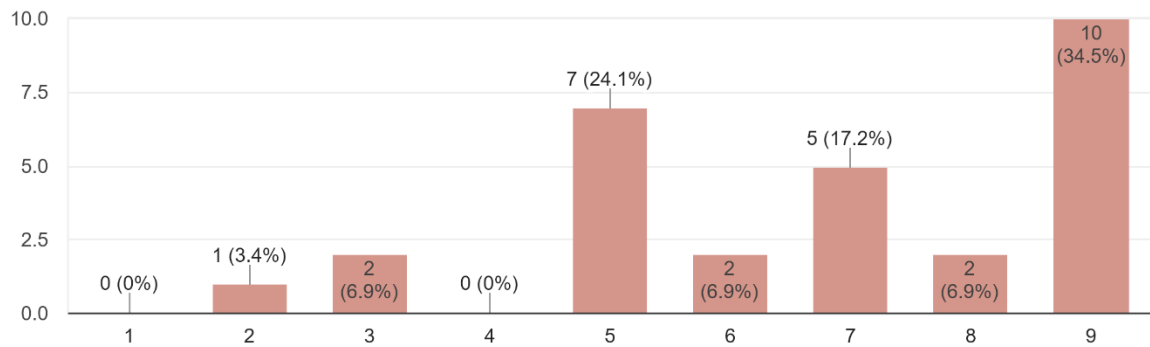
G: Immunopositivity for CK7 or CK19

29 responses



H: At least focal desmoplastic stroma.

29 responses



*Additional comments :*

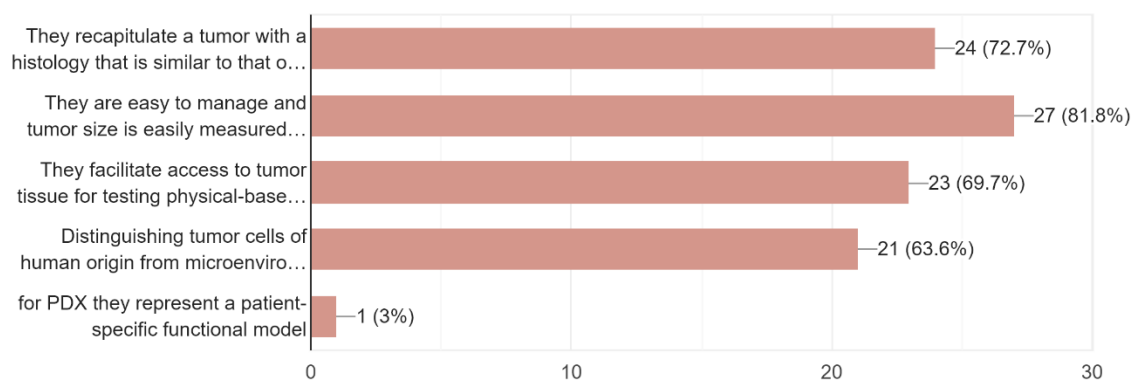
3 responses

1. Alone immunopositivity of CK7/19 is not indicative of CCA. There is a large group of HCC with CK19 positivity, no direct CCA features, and an overall poor prognosis.
2. CK7 and CK19 expression may be dependent on the tumour stage and grade of differentiation
3. Not sure how to answer A and B since preclinical models also include e.g., s.c. transplant

## Part 2. In vivo models for Cholangiocarcinoma

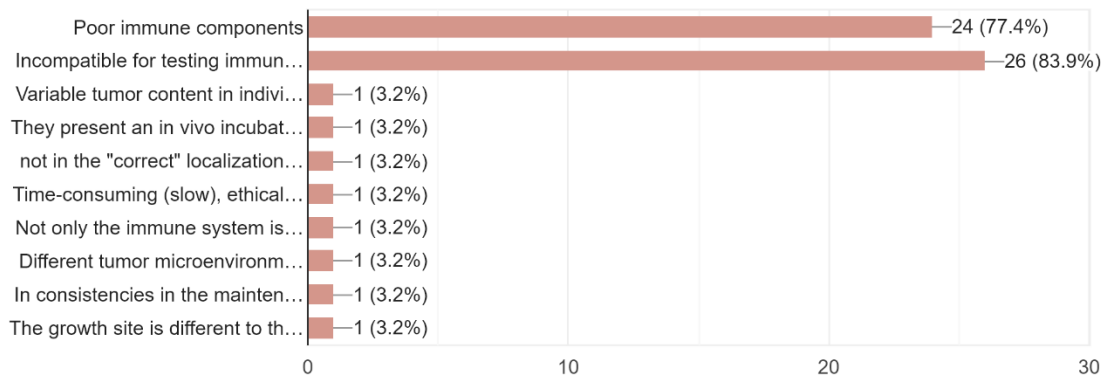
Question 8: What are the advantages of using ectopic (subcutaneous) xenograft models from either human (patient-derived) tumor cell lines or tissue (PDX)? (multiple answers can be ticked).

33 responses



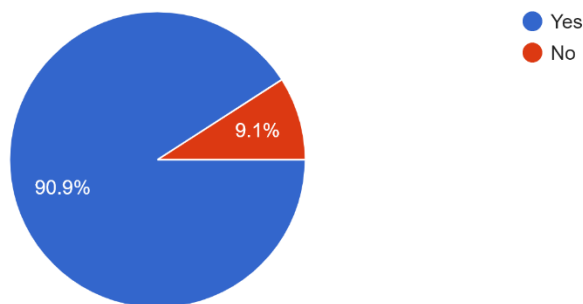
Question 9: What are the disadvantages of using ectopic (subcutaneous) xenograft models from either human cell lines or tissue (PDX)?

31 responses



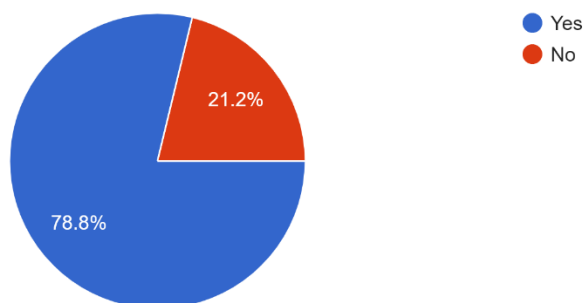
Question 10: Should we systematically validate the ectopic xenograft model by an expert pathologist and show histology of the tumor in publications?

33 responses



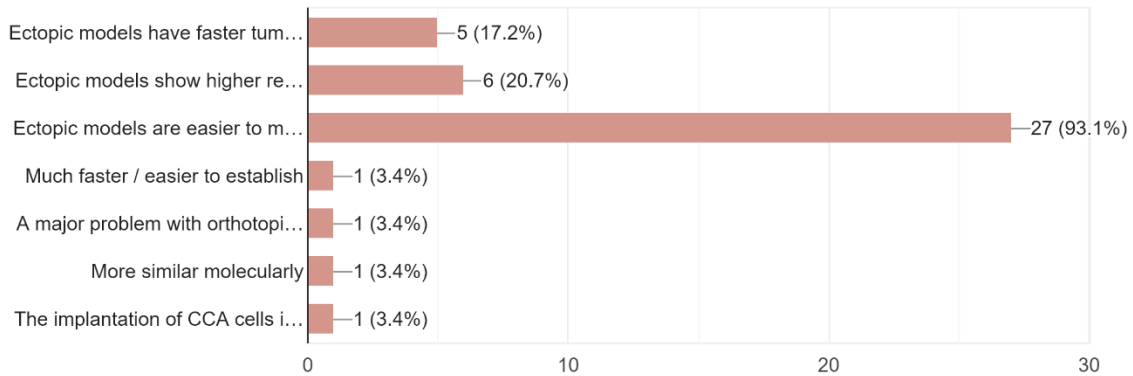
Question 11: Should the expert pathologist specify what type of CCA is found in the ectopic xenograft model?

33 responses



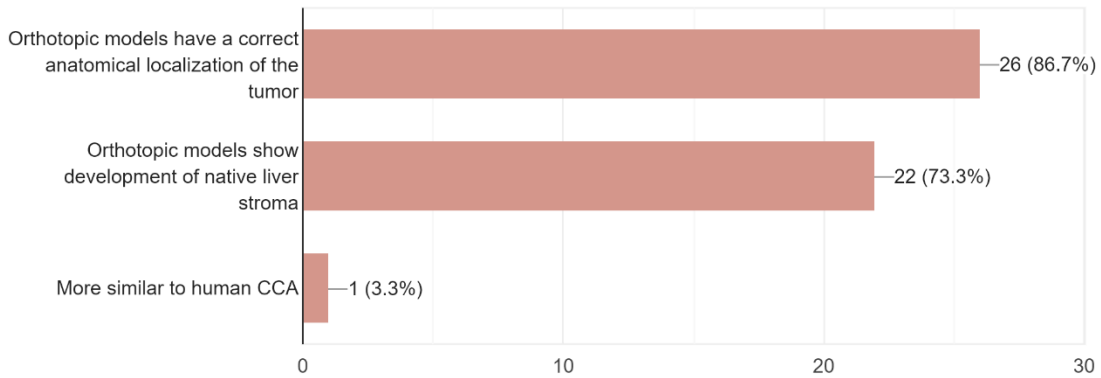
Question 12: What are the main advantages of the ectopic xenograft models over orthotopic xenograft models?

29 responses



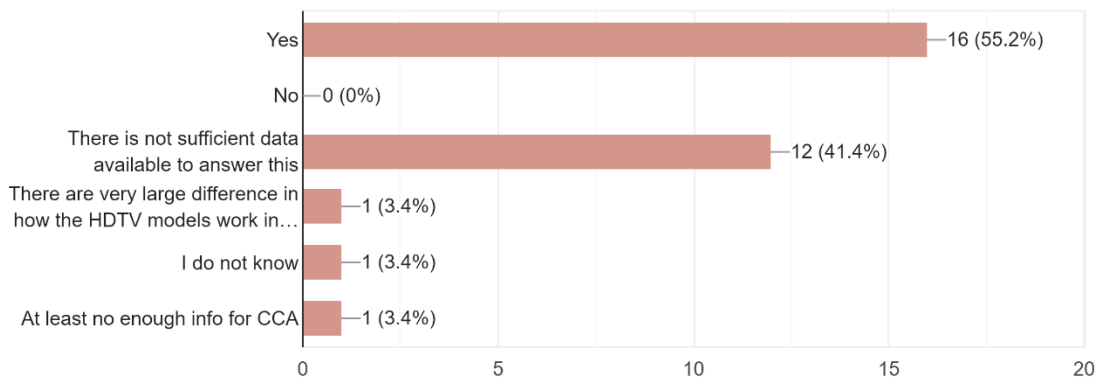
Question 13: What are the main advantages of the orthotopic xenograft models over ectopic xenograft models?

30 responses

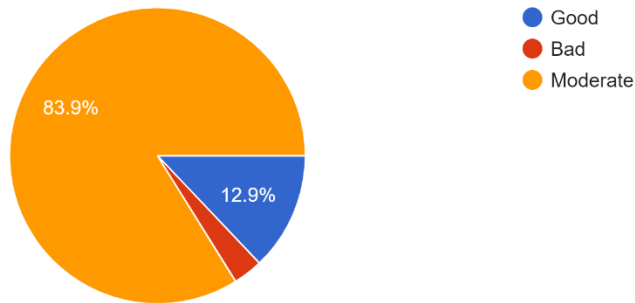


Question 14: Does the genetic background of the mouse strain account for tumor development and growth kinetics?

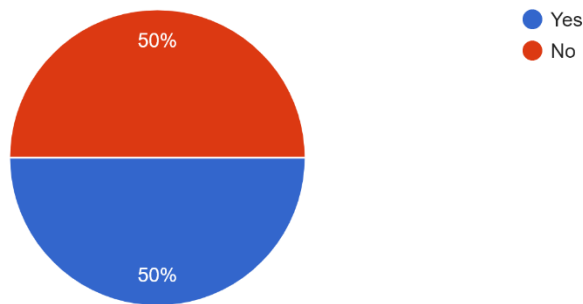
29 responses



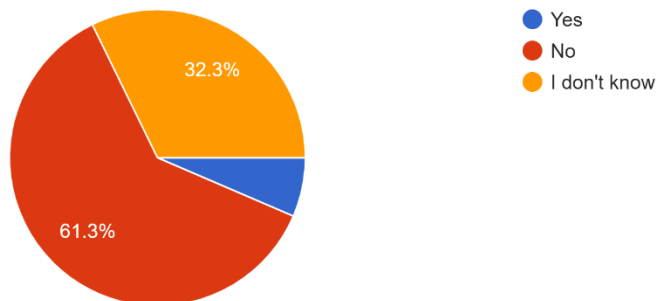
Question 15: How representative for tumor development in human CCA is it to inject cells (either by xenografting or in syngeneic mouse models) into a healthy liver without a disease background?  
31 responses



Question 16: Novel immunotherapies should be tested in ectopic or orthotopic syngeneic models, since these models recapitulate the immunobiology of CCAs. Do you agree with this statement?  
26 responses

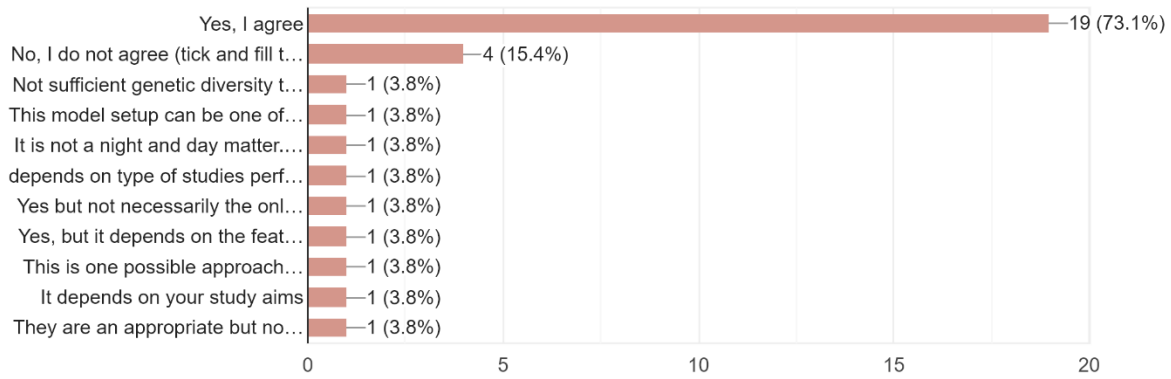


Question 17: All available CCA cell lines are known to have a tumorigenic capacity and generate tumors in ectopic or orthotopic models. Do you agree with this statement?  
31 responses



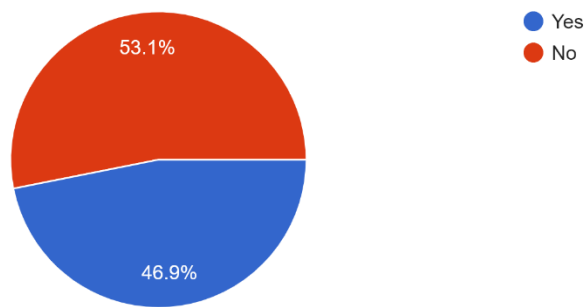
Question 18: Are syngenic orthotopic engrafted models the appropriate model to study CCA in preclinical rodent models?

26 responses



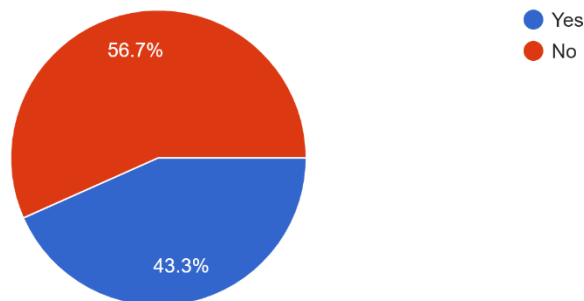
Question 19: Should extensive molecular characterization be required to report engrafted models at the time of publication?

32 responses



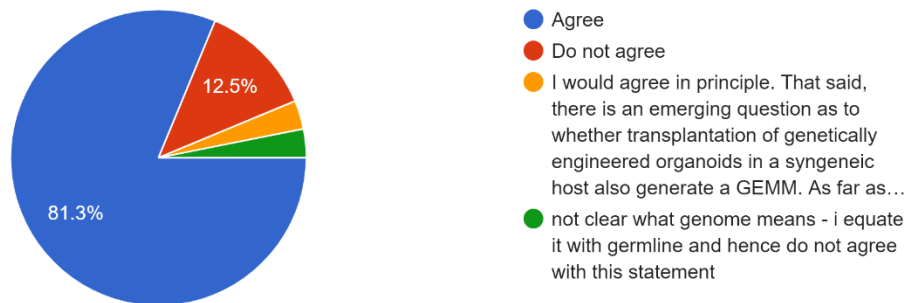
Question 20: Should immuno profiling be reported when using engrafted models?

30 responses

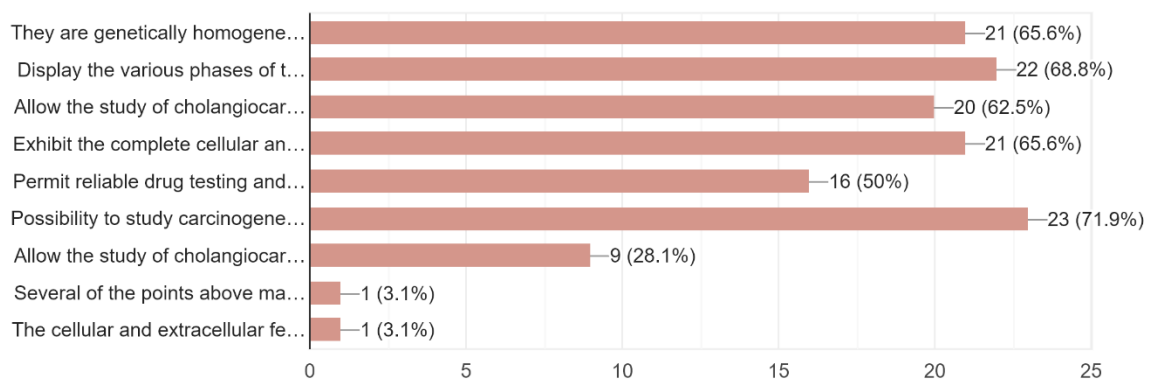




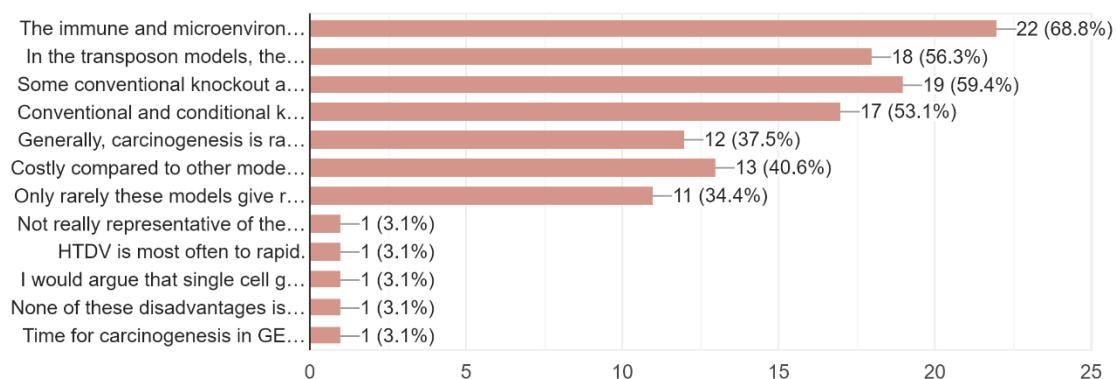
Question 21: Do you agree with the over-arching definition of genetically engineered mouse models: A genetically engineered mouse model (GE...altered through genetic engineering techniques.  
32 responses



Question 22: What are the advantages of using genetically engineered mouse models? (multiple answers can be ticked)  
32 responses

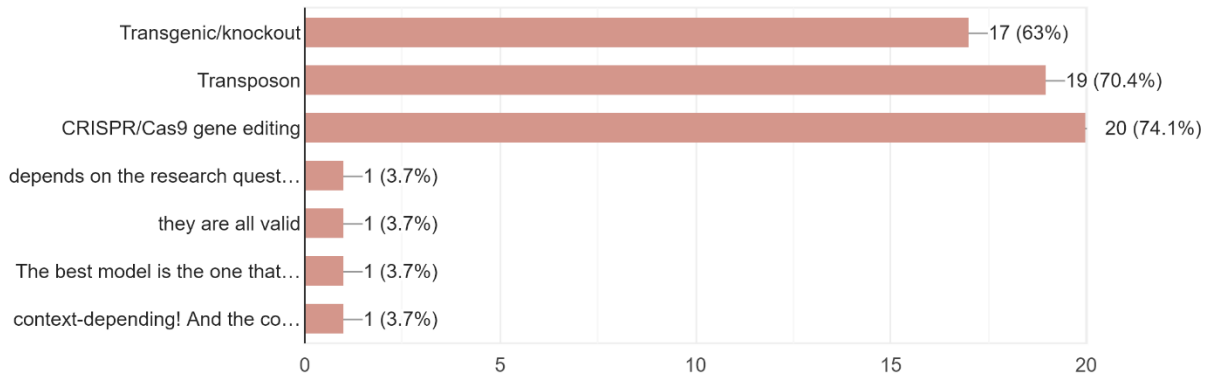


Question 23: What are the disadvantages of using genetically engineered mouse models?  
32 responses



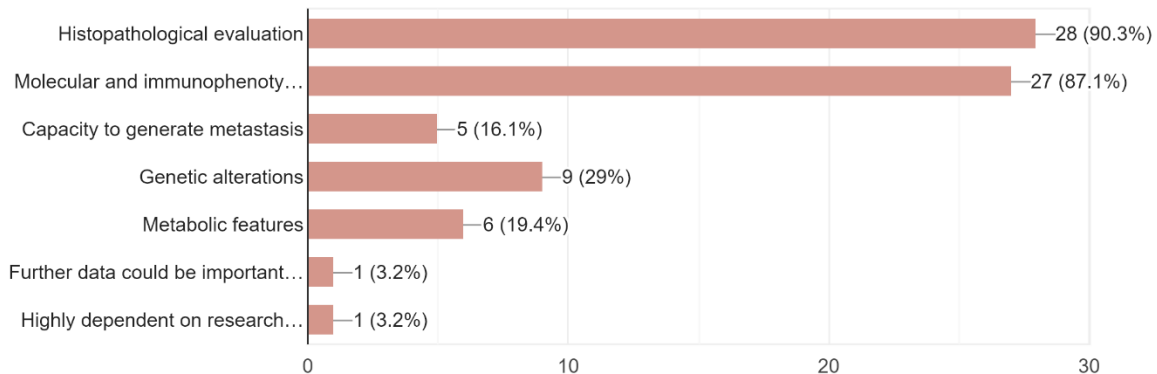
Question 24: What are the best methods to create GEMM for cholangiocarcinoma research?  
(multiple answers can be ticked)

27 responses



Question 25: Which analyses should be necessary to ascertain the validity of a GEMM of cholangiocarcinoma for the human disease? (multiple answers can be ticked)

31 responses



### Additional comments

3 responses

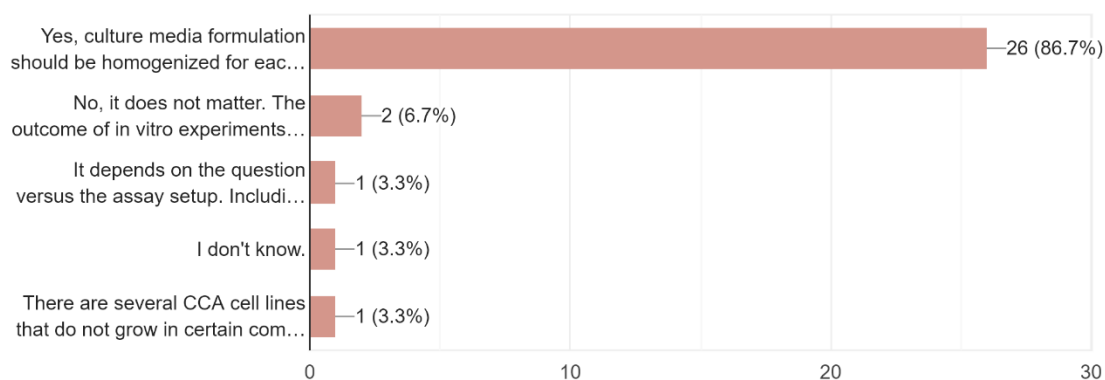
1. We have to be very careful not to set definitive rules/regulations that can hamper scientific freedom and thinking. Many questions/answers are truly dependent on the research question(s) raised in a specific study: to decide if the model/analysis is the valid /correct way forward.
2. Minimal requirements for preclinical studies could be: if a new model is presented or alterations to existing (well-characterized model), a minimum set of requirements could be histopathology evaluation at the basic level, transcriptome analysis of model tumours as well as cross-species integration (This require normal tissue data for each of the datasets!). CCA can be studied in healthy livers (% of cases arise in healthy livers or biliary system) or in livers or biliary system under injury (certain risk factors), depending on your study aims. Not all human CCA cell lines are well characterized (tumorigenic capacity and features)

3. The molecular and immune characterization of engrafted CCA models may be important or not depending on your aims. In Q 19 and 20, a better definition of "extensive molecular characterization" and immuno-profiling would be helpful.

### Part 3. In vitro models for Cholangiocarcinoma

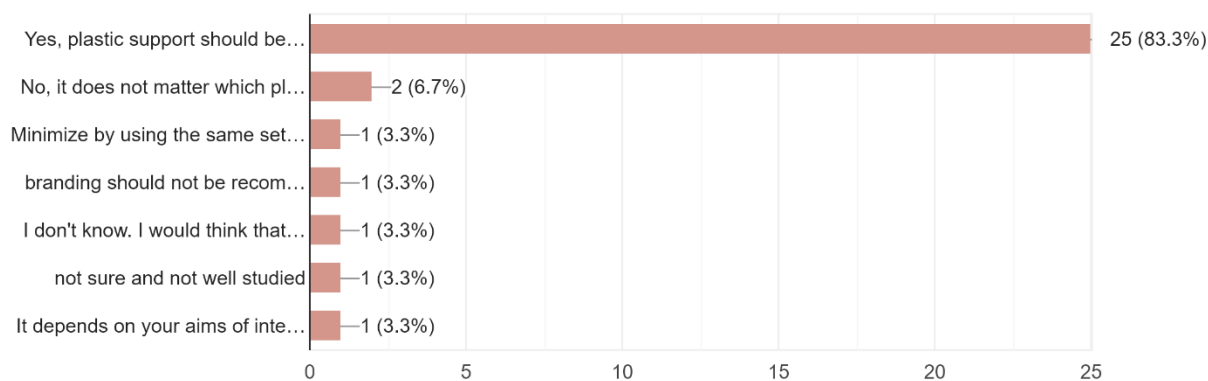
Question 26: The culture medium (i.e. DMEM, MEM, RPMI, etc) influences the results when performing in vitro experiments with cell lines or ...ary 2D cultures. Do you agree with this statement?

30 responses



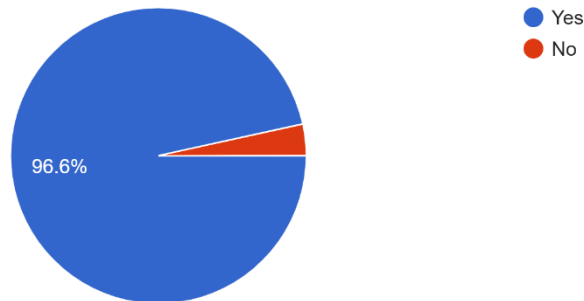
Question 27: The plastic support (i.e. TPP, Falcon, Corning, +/- ECM layer) is important for in vitro experiments of cell lines or primary 2D cultures. Do you agree with this statement?

30 responses



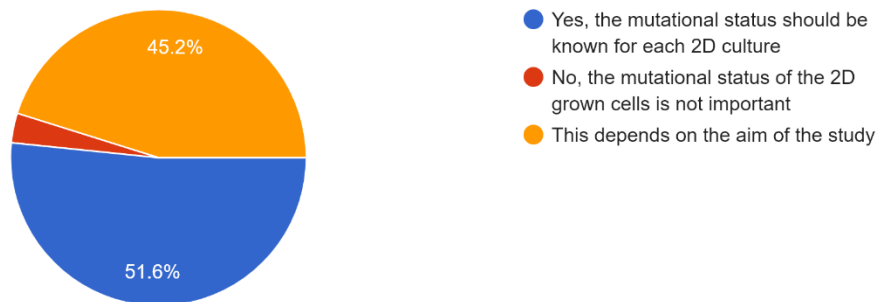
Question 28: The percentage of confluency of 2D grown cell cultures influences the results of the experiments done with cell lines or primary 2D cultures. Do you agree with this statement?

29 responses



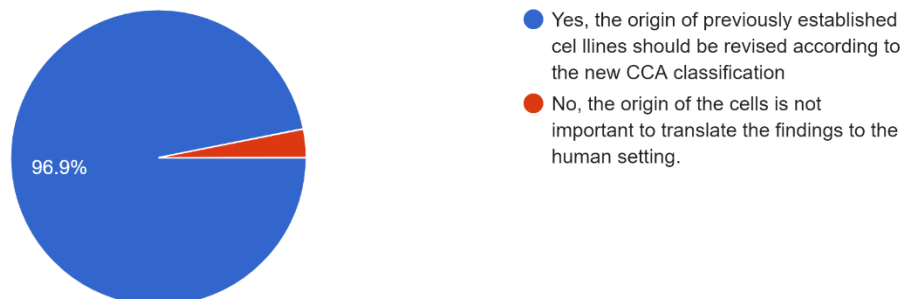
Question 29: The mutational status should be known for each cell line or primary 2D cultures. Do you agree with this statement?

31 responses



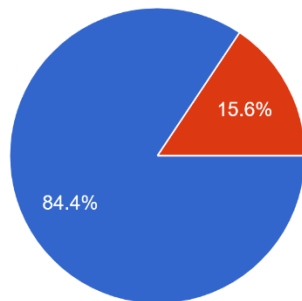
Question 30: The origin of the cells (i.e. intrahepatic, perihilar, distal) should be known and revised according to the new CCA classifications. Do you agree with this statement?

32 responses



Question 31: The isolation protocol for culture of primary cells, influences the results of experiments done. Do you agree with this statement?

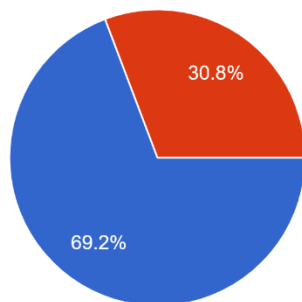
32 responses



- Yes, isolation protocols for primary cell culture should be homogenized for different cell types
- No, the isolation method is not important to translate the findings to the in vivo setting.

Question 32: Passaging and sub-culturing of 2D cultured cell lines or primary cells using enzymatic or mechanical dissociation may influence the outcome of experiments. Do you agree with this statement?

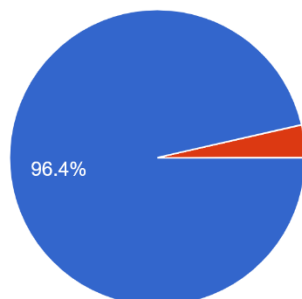
26 responses



- Yes, this is an important issue
- No, dissociation methods do not influence outcome

Question 33: Cost/effectiveness and the convenient large-scale analysis (i.e. drug screening analysis) of 2D cultures are important to consider. Do you agree with this statement?

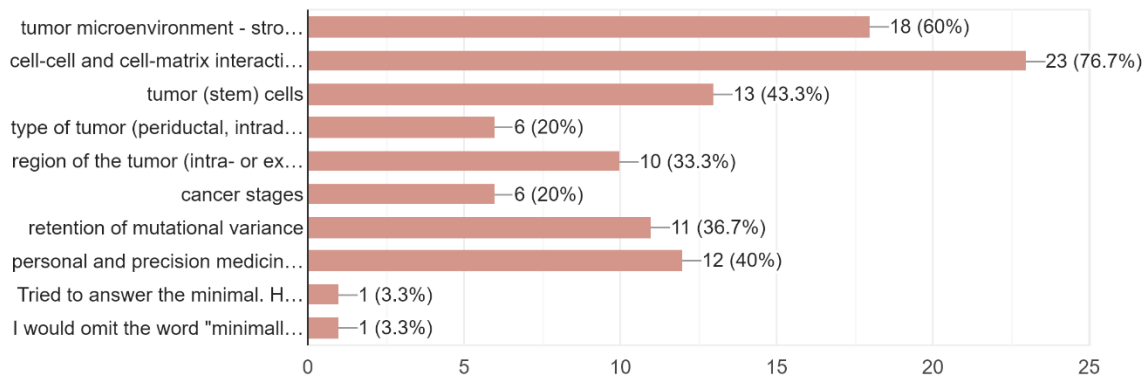
28 responses



- Yes, this is an important issue
- No, not important

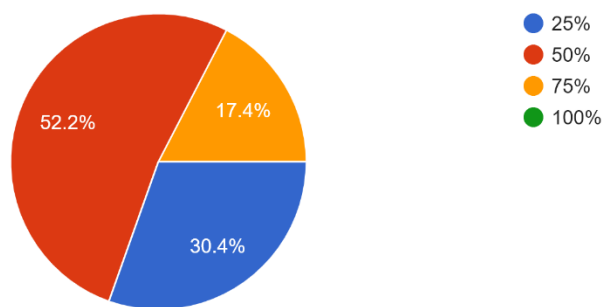
Question 34: What aspects of CCA should be minimally modelled in a 3D in vitro model? (multiple answers can be checked)

30 responses



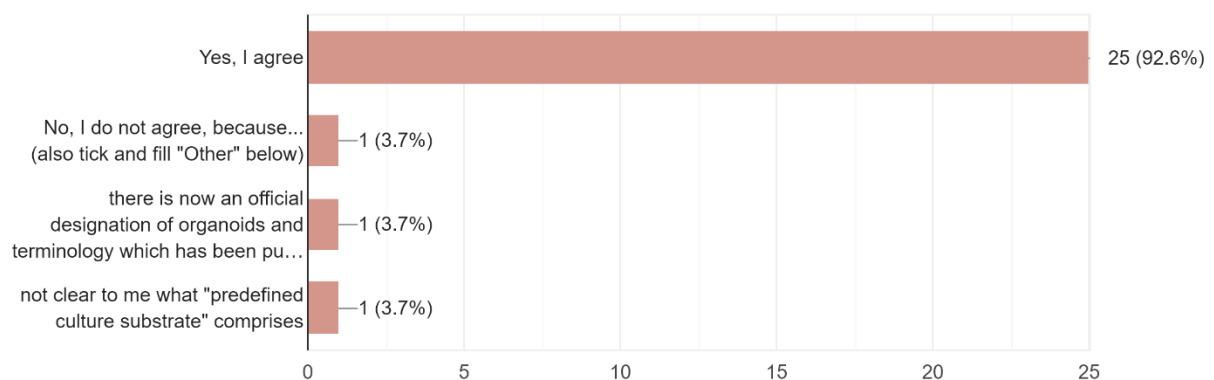
Question 35: Not always do CCA biopsies grow successfully into organoids. What should the success rate to grow patient-specific organoids minimally be to be of value to the field?

23 responses

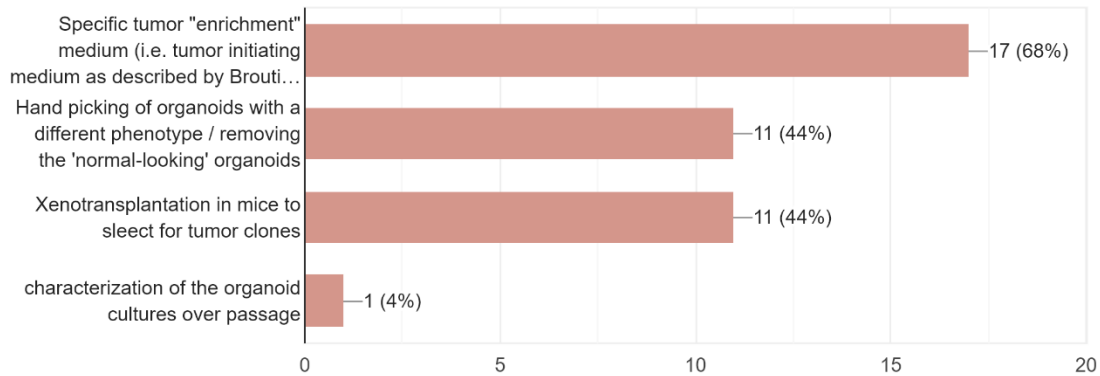


Question 36: Spheroids, that are defined as "3D aggregates of cells (cell lines or primary cells), grown in the absence of a predefined culture substrate...from organoids. Do you agree with this statement?

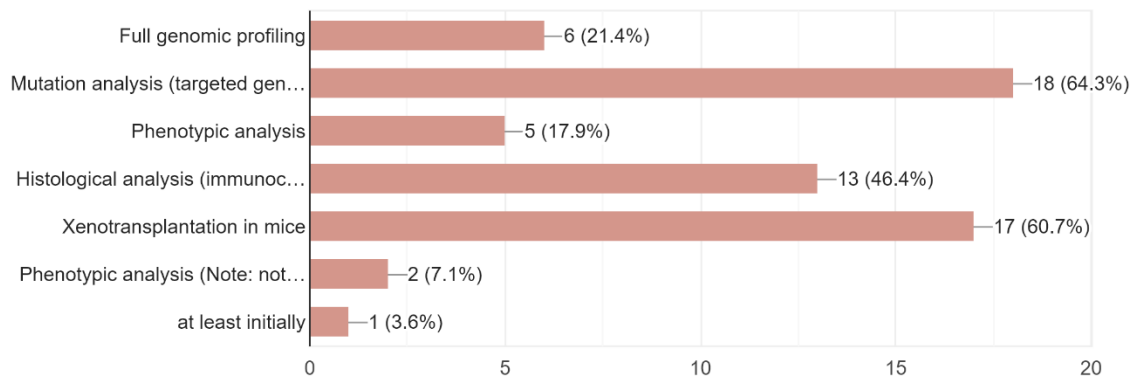
27 responses



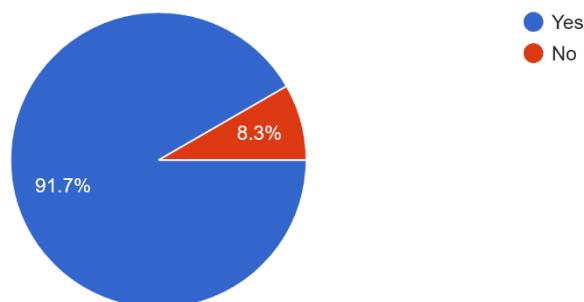
Question 37: Contaminating non-tumor organoids often grow in the CCA organoid cultures, and might even overgrow the tumor organoids. What should be done to reduce the number of non-tumor organoids? (Multiple answers can be ticked)  
25 responses



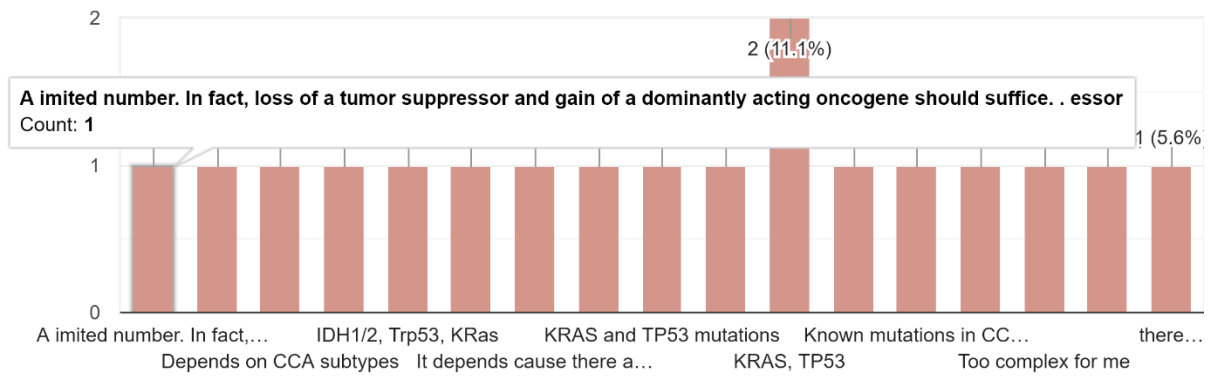
Question 38: What type of characterization should be minimally done in order to confirm malignant origin in the established organoid lines. (multiple answers can be ticked).  
28 responses



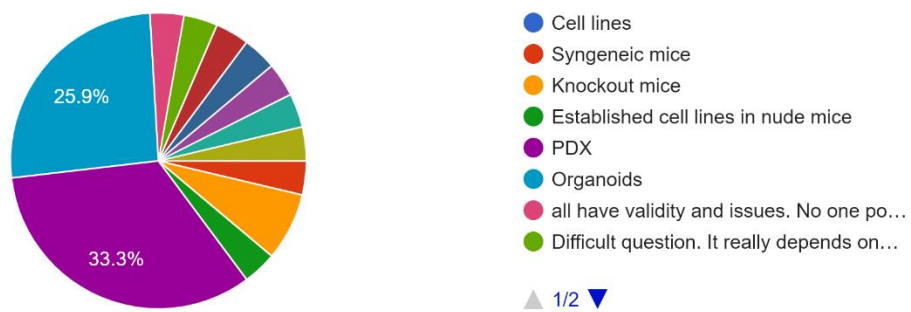
Question 39: Should every organoid culture be characterized (as proposed in Q 38), before clinical applications such as drug screening?  
24 responses



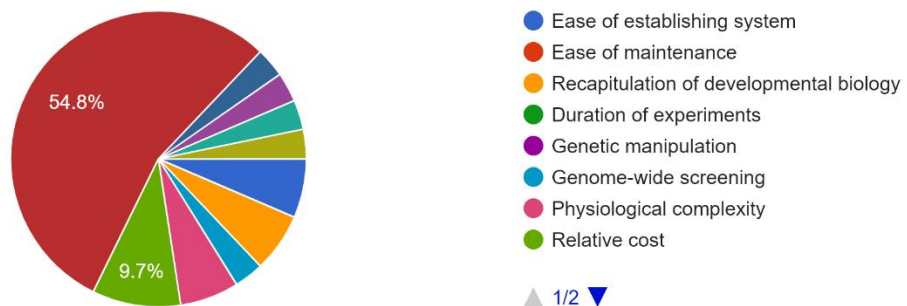
Question 41: In engineering CCA tumor organoids from non-tumor liver tissue could be done by gene editing. What genetic alterations / mutations ...er to use this organoid line as a 'real' CCA model?  
18 responses



Question 42: What type of preclinical model of CCA is likely to best reflect clinical practice?  
27 responses



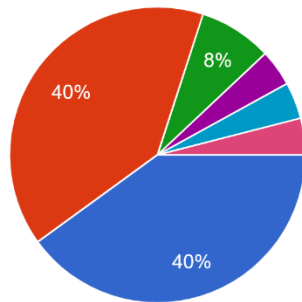
Question 43: What matters most to the clinician in a preclinical model?  
31 responses





Question 44: How should pre-clinical models best inform study design and practice?

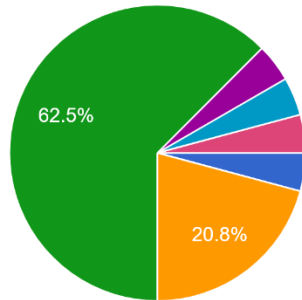
25 responses



- Preliminary data to support study design
- Co-experiment (parallel experiments clinically and at the bench)
- Avatars
- Post-study bench exploration
- Depends on the clinical questions. If defining a clinical trial (preliminary dat...
- all of above depending on setting
- both preliminary data and post-study bench exploration

Question 45: What information from the research lab is most valuable to the clinician?

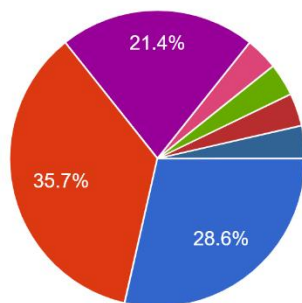
24 responses



- PK
- PD
- Genomic data to permit targeting
- Multi-omic data to permit exploration of novel approaches
- it depends on the setting. PK and PD are valid for phase I, genomics may b...
- I don't think that there is a single "most" valuable parameter. PK and PD value...
- What is PK? PD? I would choose mult...

Question 46: How can clinicians best support translation of lab research to the clinic?

28 responses

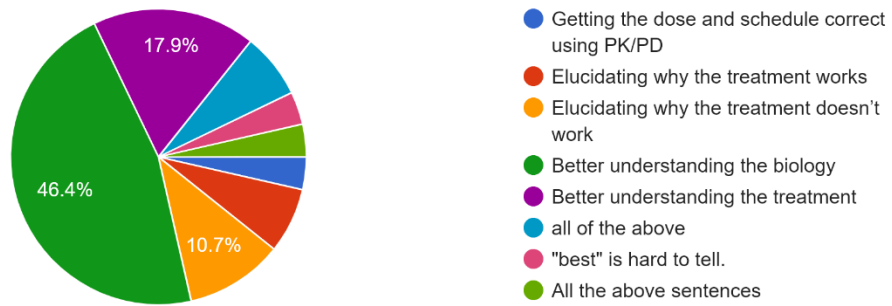


- By asking direct laboratory questions
- By providing serial tissue samples
- By providing serial whole blood samples
- By providing serial buffy coats
- By sharing clinical outcomes
- By sharing clinical toxicities
- Interaction btween researchers and clin...
- all of the above

▲ 1/2 ▼

Question 47: How can the laboratory researcher best support the development of clinical studies?

28 responses



## Questionnaire 2. Summary

Based on the answers in Questionnaire 1, a new questionnaire was designed, consisting of 13 new questions to reach consensus. This questionnaire was sent out to the 38 experts that filled the first questionnaire and an additional XX experts in the field of Cholangiocarcinoma that were introduced by the first (Table S1).

### Criteria for preclinical models of Cholangiocarcinoma: scientific and medical relevance.

In this Consensus Statement, we aim to provide a comprehensive and critical point of view regarding the current knowledge and what is envisioned in the horizon for the experimental models of CCA, focusing on pathology, genetic and epigenetic landscape, molecular perturbations, chemoresistance, and therapies.

A Consensus is sought on minimal and advanced criteria for experimental models (both in vitro and in vivo), focusing on which minimum criteria should always be experimentally validated and included in publications.

### The Delphi Method

Choices for definitions will be reached through the consensus of key experts in the field. In this way the review will be more than just another review. It will be a true consensus document, its contents to be adopted field wide.

To facilitate consensus, the Delphi method will be employed:

"The Delphi method is based on the principle that decisions from a structured group of individuals are more accurate than those from unstructured groups. The experts answer questionnaires in two or more rounds. After each round, a facilitator provides an anonymized summary of the experts' answers from the previous round as well as the reasons they provided for their judgments. Thus, experts are encouraged to revise their earlier answers in light of the replies of other members of their panel. It is believed that during this process the range of the answers will decrease, and the group will converge towards the "correct" answer. Finally, the process is stopped after a consensus is reached."  
-Wikipedia

Here we employ a modified Delphi method. After each round, questions for which a  $\geq 90\%$  consensus has been reached will be removed from subsequent questionnaires. Additionally, answers with  $< 15\%$  consensus will also be removed from subsequent questionnaires. The following is the first anonymous questionnaire in the series. After experts have completed the questionnaire the facilitator will compile and present the answers. After experts in the field have had time to analyze the answers, a second anonymous questionnaire will be sent out. This process will repeat until a consensus is reached for all questions.

### Contact information

Only the facilitator will have access to the contact information

Please fill in your contact information, including affiliation, below

What is your field of interest/expertise?

If you feel you don't have the right expertise to answer a question, just skip to the next one.

## **Defining minimal and advanced criteria for experimental models**

### **Histological assessment**

#### **Question 1: Which of the following ones are malignant features of biliary tumours?**

Answer with Yes / No

1. Invasion of the basement membrane
2. Increased nucleus/cytoplasm ratio
3. Distant metastasis
4. Tumorigenic capacity of isolated cells after subcutaneous injection in immune deficient mice

#### **Question 2: What type of histological investigation(s) should always be done to characterize an early-stage tumour in a preclinical CCA model?**

Answer with Yes / no

1. Morphological examination of H&E
2. Immunohistochemistry for at least one biliary cytokeratin (e.g. CK19, CK7, pan CK, etc)
3. Immunohistochemistry for two biliary cytokeratins (CK19 and CK7)
4. Markers for inflammatory cells and CAFs
5. PAS reaction for highlighting mucin
6. A broad panel of markers for hepatobiliary malignancies and metastasis

#### **Question 3: To allow correlation with the anatomical classification of human tumours, a preclinical model of CCA should specifically classify tumours induced as:**

Answer with Yes / No

1. Intrahepatic CCA, perihilar CCA and distal CCA
2. Intrahepatic CCA and extrahepatic CCA
3. No need for such classification

#### **Question 4: Which of the following morphological and/or immunophenotypic features must be present in order to classify a lesion as CCA in a preclinical model?**

Answer with Yes / No

1. Location within the liver or extrahepatic biliary tree
2. Absence of an extrahepaticobiliary primary lesion
3. Epithelial cytological features (cohesive groups or structures and/or pan-cytokeratin immunopositivity)
4. At least focal gland formation
5. Absent hepatocellular differentiation (bile production, and canalicular CD10 or BSEP)
6. Immunopositivity for CK7 or CK19
7. Focal desmoplastic stroma
8. Presence of precursor lesions
9. Primary origin within the intra- or extrahepatic biliary tree
10. Absence of hepatobiliary primary lesion

#### **Question 5: What histopathological features of human CCA must be assessed in a preclinical model of CCA?**

Answer with Yes / No

1. Intra-tumoral heterogeneity (high stroma, inflammatory response, epithelial phenotype)
2. Inter-tumoral heterogeneity (large versus small bile duct tumor in iCCA)
3. Pattern of growth (mass-forming, periductal infiltration, intraductal growth)
4. Proportion of tumour showing gland formation
5. Immunopositivity for CK7 or CK19
6. Focal desmoplastic stroma
7. Presence of precursor lesions

**Question 6: It has been proposed that iCCA may originate from several cells of origin. Which of the following cell types may be cells-of-origin for iCCA?**

Answer with Yes / No

1. Mature hepatocytes
2. Mature cholangiocytes
3. Hepatic progenitor/oval cells
4. Peribiliary glands

### **In vivo and in vitro models for Cholangiocarcinoma**

#### **Xenograft models, Genetically Engineered Mouse Models (GEMM)**

**Question 7: Concerning newly developed patient-derived xenograft models**

Answer with Yes / No

1. Should the model(s) be validated by an expert pathologist and histology of the tumor shown in publications?
2. Should immuno profiling also be reported?
3. Should the model(s) be validated in more than one mouse strain?
4. Should the expert pathologist specify what type of CCA is found in the model?
5. Do orthotopic xenograft models represent the most disease-relevant tumor environment in which to test a drug, comparing to ectopic xenograft models?
6. Should a drug be tested in more than one model?

#### **2D culture models for cholangiocarcinoma**

**Question 8: Which cell culture procedures should be standardised in experiments with cell lines or primary 2D cultures and be reported in publications?**

Answer with Yes / No

1. Question 8: Which cell culture procedures should be standardised in experiments with cell lines or primary 2D cultures and be reported in publications?
2. Choice of plastic support (i.e. TPP, Falcon, Corning, +/- ECM layer, etc)
3. Level of confluence when performing the experiments
4. Isolation protocol for culture of primary cells
5. Passaging and sub-culturing methods (i.e. enzymatic vs. mechanical dissociation, etc)

**Question 9: The origin of any cell line (previously established or new) should be stated for publication according to the new CCA classification (i.e. intrahepatic, perihilar, distal)**

YES

NO

### 3D cultures for Cholangiocarcinoma

**Question 10: Contaminating non-tumor organoids often grow in CCA organoid cultures. How should selection for tumor organoids be performed?**

Answer with Yes / No

1. Specific tumor "enrichment" medium (i.e. tumor initiating medium (as described by Broutier *et al.*, 2017, DOI: 10.1038/nm.4438)
2. Hand picking of organoids with a different phenotype / removing the 'normal-looking' organoids
3. Xenotransplantation in mice to select for tumor clones

**Question 11: Which analyses should be done to confirm the malignant origin of established organoid lines and be reported in publications?**

Answer with Yes / No

1. Full genomic profiling
2. Mutation analysis (targeted genomic profiling using a diagnostic panel)
3. Phenotypic analysis
4. Histological analysis (immunohistochemistry of EpCAM, KRT7)
5. Xenotransplantation in mice

**Question 12: Should every organoid culture be characterized (as proposed in Q 11), before clinical applications such as drug screening?**

YES

NO

**Question 13: Personalized medicine applications such as drug screenings to find the best treatment for the patient, will cost time. How much time is acceptable to initiate, grow and expand the organoids for these analysis? In other words, what is the maximum time acceptable to be of relevance to the clinic?**

- < 1month  
 < 3 months  
 < 6 months  
 Other

#### **Recommend an expert**

Please include contact information and affiliation of an expert we might have missed

## Supplementary Results

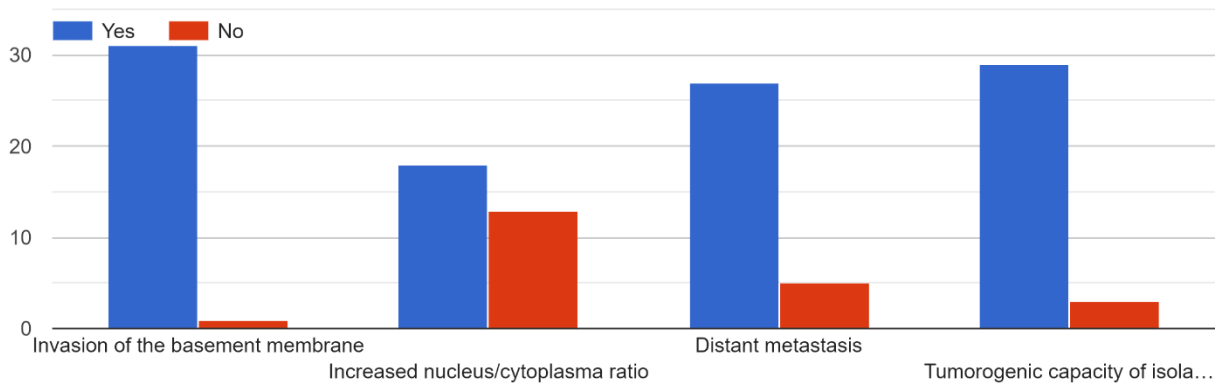
### Questionnaire 2. Summary

Here we list the results from the second questionnaire.

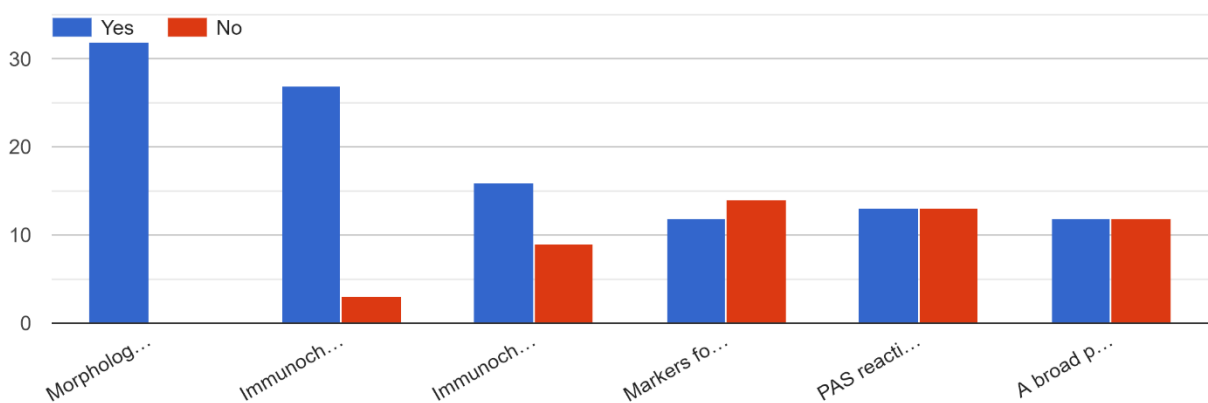
#### Defining minimal and advanced criteria for experimental models

##### Histological assessment

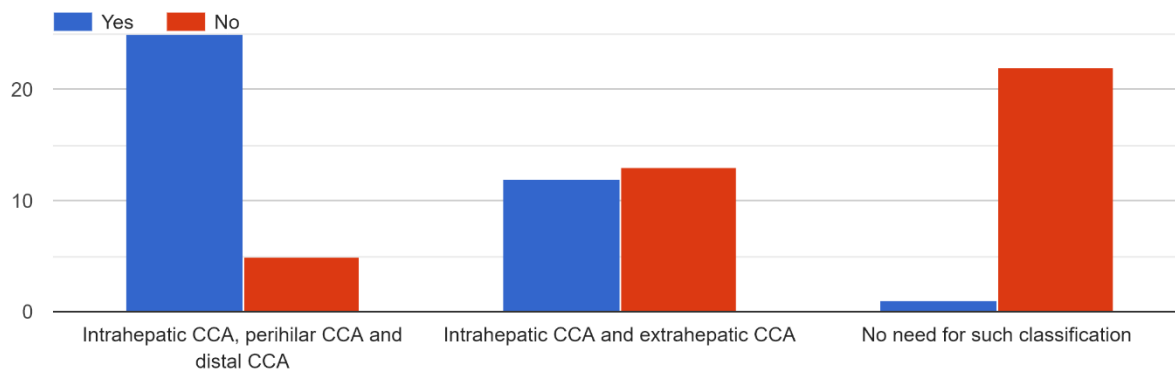
Question 1: Which of the following ones are malignant features of biliary tumours?



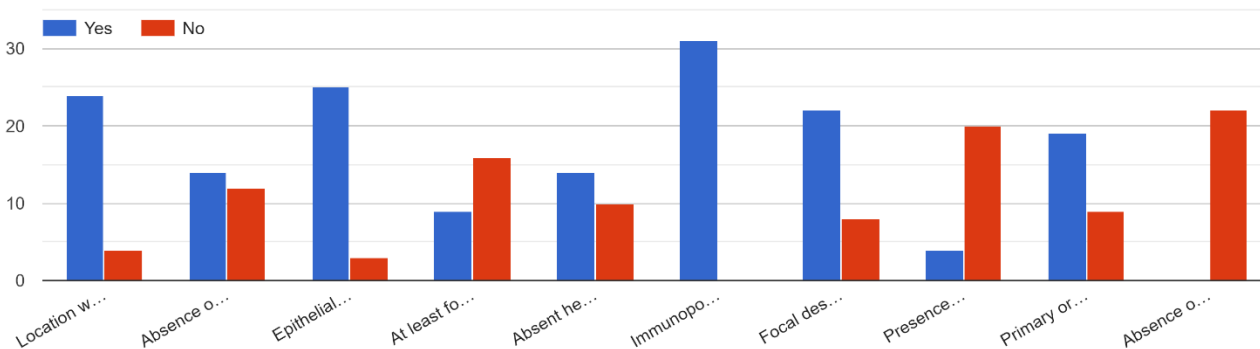
Question 2: What type of histological investigation(s) should always be done to characterize an early-stage tumour in a preclinical CCA model?



Question 3: To allow correlation with the anatomical classification of human tumours, a preclinical model of CCA should specifically classify tumours induced as:

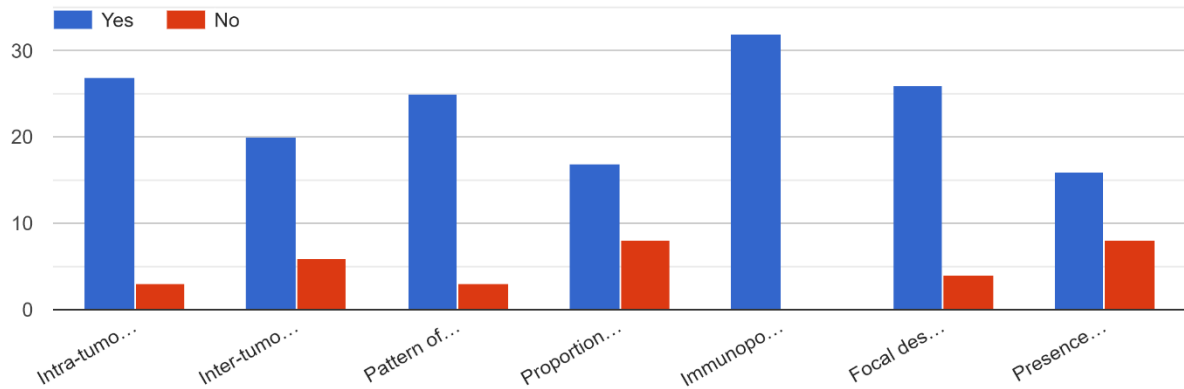


Question 4: Which of the following morphological and/or immunophenotypic features must be present in order to classify a lesion as CCA in a preclinical model?

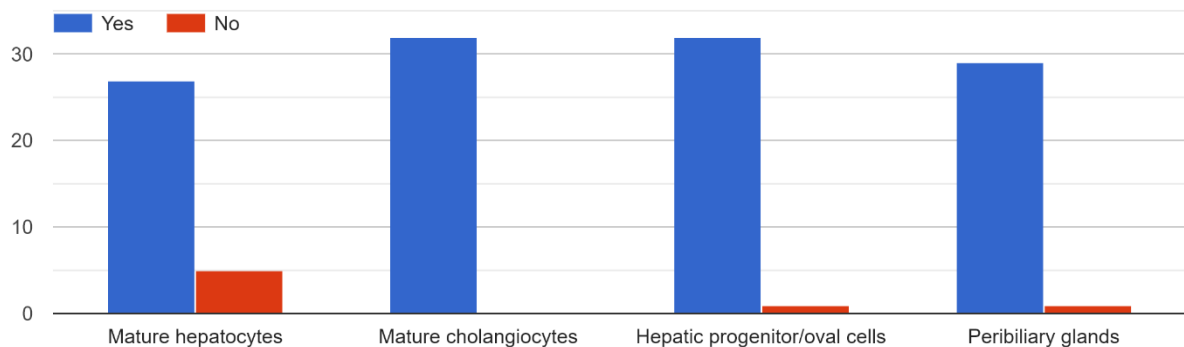




Question 5: What histopathological features of human CCA must be assessed in a preclinical model of CCA?



Question 6: It has been proposed that iCCA may originate from several cells of origin. Which of the following cell types may be cells-of-origin for iCCA?



## Comments for this section

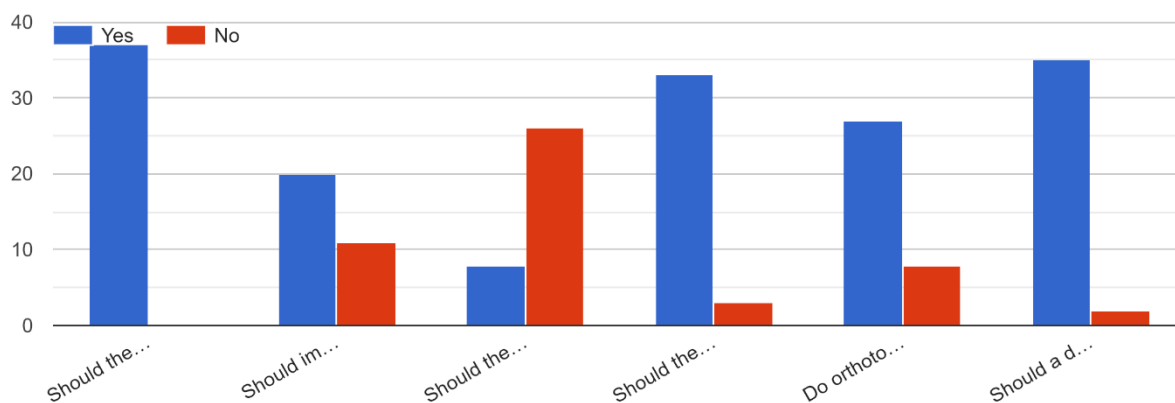
4 responses

1. An intermediate category would have been helpful. e.g. "should always be done" in Q2 should in my view not lead to ask for CK 7 AND CK19 ("always") but can be helpful in many models. Similarly "focal desmoplastic stroma" is not necessary for diagnosing CCA but appreciable. etc.
2. not sure is always possible to differentiate origin in perihilar or distal ducts in animal models
3. I am not an expert pathologist and therefore I prefer not to answer the questions above
4. Very informative, critical points are all well taken

## In vivo and in vitro models for Cholangiocarcinoma

### Xenograft models, Genetically Engineered Mouse Models (GEMM)

Question 7: Concerning newly developed patient-derived xenograft models:



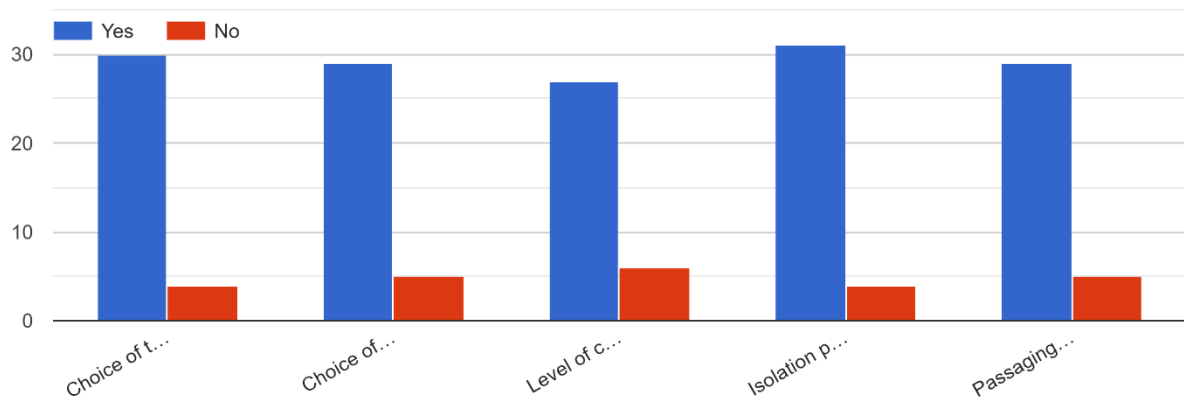
#### Comments for this section

4 responses

1. Q4: Sometimes another type of CCA may arise that does not fit to the human disease, i.e. the spectrum of experimental tumors in mice may be even bigger than in humans.  
Q6: Not necessarily in animals but at least in additional cell lines
2. Regarding last question (should a drug be tested in more than one model), I would change should for "recommended"
3. Well comprehensive
4. Many of the previous answer would depend on the stage of the investigations.

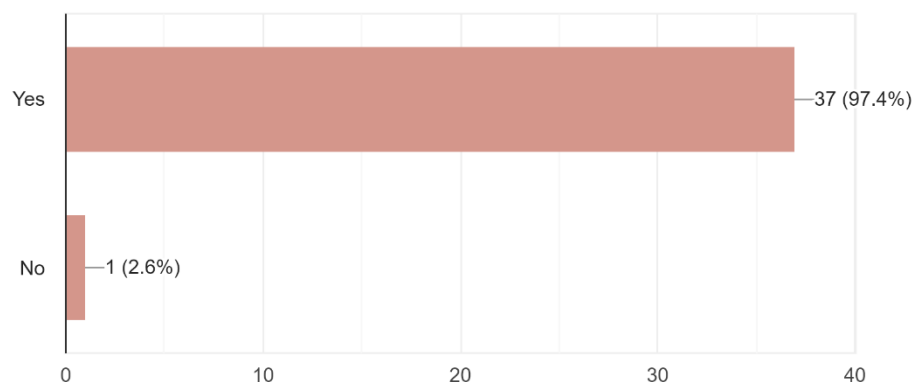
## 2D culture models for cholangiocarcinoma

Question 8: Which cell culture procedures should be standardised in experiments with cell lines or primary 2D cultures and be reported in publications?



Question 9: The origin of any cell line (previously established or new) should be stated for publication according to the new CCA classification (i.e. intrahepatic, perihilar, distal)

38 responses



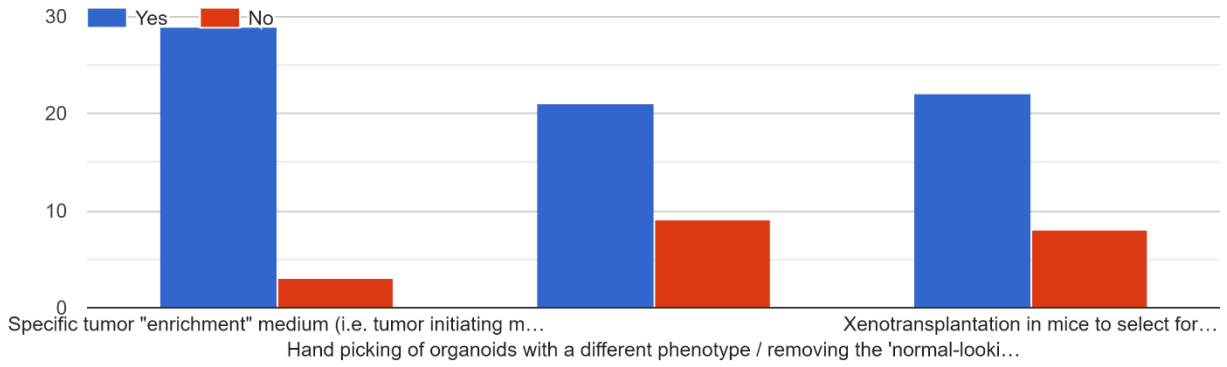
### Comments for this section

4 responses

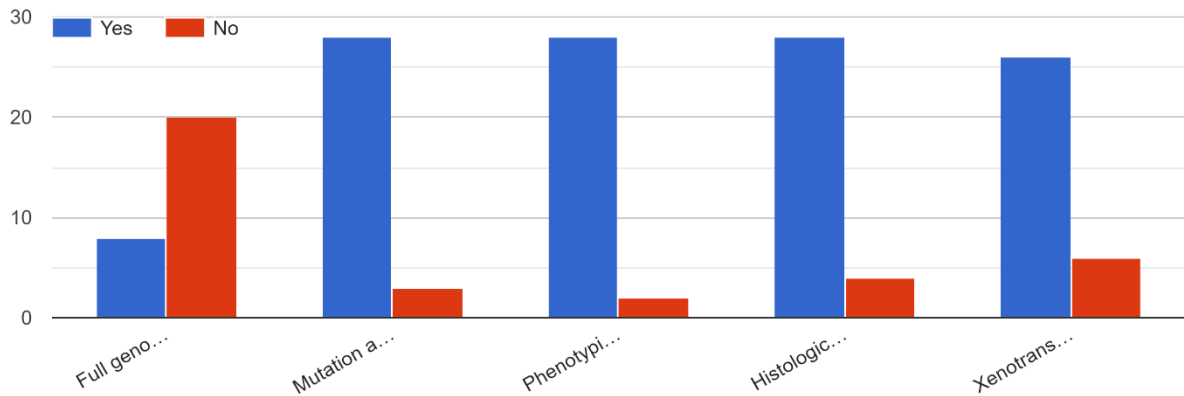
1. Plastic, methodology (confluence, passaging etc.) are parameters up to the assay evaluated. It should of course be recorded in the M&M section.
2. Q9: if not accessible, mutational analysis can be performed to get more information.
3. Precise, previous issues on the subject have all been addressed
4. Classification into i, p or dCCA of cell lines established a long time ago may not be possible anymore but new cell lines should be classified.

### 3D cultures for Cholangiocarcinoma

Question 10: Contaminating non-tumor organoids often grow in CCA organoid cultures. How should selection for tumor organoids be performed?

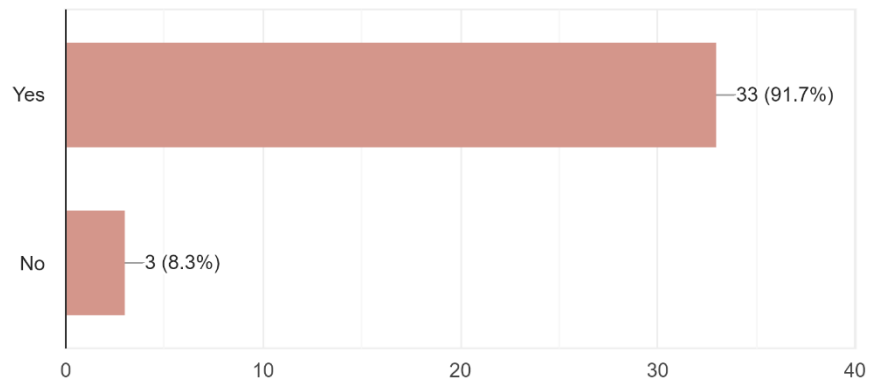


Question 11: Which analyses should be done to confirm the malignant origin of established organoid lines and be reported in publications?



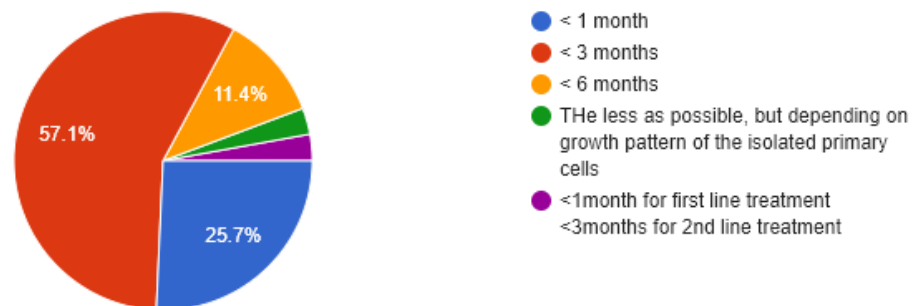
Question 12: Should every organoid culture be characterized (as proposed in Q 11), before clinical applications such as drug screening?

36 responses



Question 13: Personalized medicine applications such as drug screenings to find the best treatment for the patient, will cost time. How much... time acceptable to be of relevance to the clinic?

35 responses



### Comments for this section

3 responses

1. Most phase 1 departments are attempting to do precision medicine testing in avatars within 1 month
2. Well prepared, no suggested changes
3. Drug screening could be undertaken during first-line therapy, in readiness for 2nd- or 3rd-line treatment

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