Supplementary Information

Supplementary Methods

Expert Invitation

Scientists (hereafter referred to as experts) were invited based on a PUBMED and Google Scholar database search for HPB organoids, regeneration and development. Experts were invited if they had made a significant contribution (either 1st, 2nd or senior authorship) to a peer-reviewed, published manuscript retrieved from the search. From the generated list of experts, select authors with extensive expertise in HPB organoids, regeneration or development were invited to join the steering committee (for members of the steering committee see supplementary table 1). Once invited, all experts had the option to suggest additional experts which would be evaluated by the core team initiating this effort (AM, FJMR, MMAV, LvdL and BS) before receiving an invitation. In a similar fashion, these experts had the option to suggest experts as well. After the first questionnaire round, no additional experts were eligible for invitation.

Building Consensus

To reach consensus, a modified Delphi method based on three successive questionnaires was employed. Consensus was defined as ≥90% agreement on a single question. Questions for which consensus was reached were removed from subsequent questionnaires. Additionally, if an option to a question received <15% of the votes from the expert panel, it was removed from the subsequent questionnaires. Experts had the option to suggest additional answers for each question during the first and second questionnaire. If a suggestion was mentioned by three or more experts, this suggestion was added as an option in the following questionnaire. Furthermore, experts had the option to make additional remarks in the questionnaire, as well as have an open discussion with the core team on their views regarding consensus statements. After each questionnaire, a summary document of the answers was created and sent out to the participating experts, along with an invitation to complete a new questionnaire. Importantly, as per the Delphi method the results of each questionnaire were anonymous. Topics for which consensus was not reached after the third and final questionnaire were deliberated by the steering committee through a round table discussion. Based on the discussion, a final proposition was made by the steering committee for these questions. All experts were given time to review the propositions made by the steering committee, along with the opportunity to discuss their views. In the end, every expert which completed all three questionnaires agreed with the final outcomes and participated in the consensus.

Questionnaires

Questionnaires were created in Google documents (Alphabet Inc., CA). The initial draft of each questionnaire was designed by the core team and evaluated by the steering committee before being sent out to invited experts. Each questionnaire was divided into four categories: (1) Definition of an organoid, (2) Nomenclature for Tissue-derived hepatic, pancreatic and biliary Organoids, (3) Fetal-derived organoids, and (4) Nomenclature for Tumor-derived hepatic, pancreatic and biliary organoids. Each questionnaire was designed to reflect the results of the previous questionnaire. Questionnaires can be found in supplementary questionnaires 1-3.

Supplementary Figures and Tables

Figure S1. Countries represented in the HPB Consortium.

Table S1. Members of the HPB Consortium

	Name	Affiliation	Country	Role
1	Ary Marsee	Utrecht University	The Netherlands	Core team
2	Floris Roos	Erasmus MC	The Netherlands	Core team
3	Monique Verstegen	Erasmus MC	The Netherlands	Core team
4	Hans Clevers	Hubrecht Institute, Princes Maxima Center, University Medical Center Utrecht	The Netherlands	Steering committee
5	Ludovic Vallier	University of Cambridge	United Kingdom	Steering committee
7	Takanori Takebe	Cincinnati Children's Hospital and Tokyo Medical Dental University	USA and Japan	Steering committee
6	Meritxell Huch	Max Planck Institute of Molecular Cell Biology and Genetics	Germany	Steering committee
8	Weng Chuan Peng	Princes Maxima Center	The Netherlands	Steering committee
9	Stuart Forbes	MRC Center for Regenerative Medicine	United Kingdom	Steering committee
10	Frédéric Lemaigre	Institut de Duve	Belgium	Steering committee
11	Eelco de Koning	Hubrecht Institute, Leiden University Medical Center, University Medical Center Utrecht	The Netherlands	Steering committee
12	Helmuth Gehart	Institute for Molecular Health Sciences	Switzerland	Steering committee
13	Luc van der Laan	Erasmus MC	The Netherlands	Core team
14	Bart Spee	Utrecht University	The Netherlands	Core team
15	Sylvia Boj	Hubrecht Organoid Technology (HUB)	The Netherlands	HPB Consortium
16	Pedro Baptista	Aragon Health Sciences Institute	Spain	HPB Consortium
17	Kerstin Schneeberger	Utrecht University	The Netherlands	HPB Consortium
18	Carol Soroka	Yale University	United States	HPB Consortium
19	Markus Heim	University of Basel	Switzerland	HPB Consortium
20	Sandro Nuciforo	University of Basel	Switzerland	HPB Consortium
21	Kenneth Zaret	University of Pennsylvania	United States	HPB Consortium
22	Yoshimasa Saito	Keio University School of Medicine	Japan	HPB Consortium
23	Matthias Lutolf	LSCB – Laboratory of Stem Cell Bioengineering - EPFL	Switzerland	HPB Consortium
24	Vincenzo Cardinale	Sapienza University of Rome	Italy	HPB Consortium
25	Ben Simons	University of Cambridge	United Kingdom	HPB Consortium
26	Sven van IJzendoorn	University of Groningen	The Netherlands	HPB Consortium
27	Akihide Kamiya	Tokai University	Japan	HPB Consortium
28	Hiromi Chikada	Tokai University	Japan	HPB Consortium

29	Shuyong Wang	The 8th Medical center of Chinese People's Liberation Army General Hospital	China	HPB Consortium
30	Seon Ju Mun	Korea Research Institute of Bioscience and Biotechnology	South Korea	HPB Consortium
31	Myung Jin Son	Korea Research Institute of Bioscience and Biotechnology	South Korea	HPB Consortium
32	Tamer Tevfik Onder	Koc University	Turkey	HPB Consortium
33	James Boyer	Yale University	United States	HPB Consortium
34	Toshiro Sato	Keio University School of Medicine	Japan	HPB Consortium
35	Nikitas Georgakopoulos	University of Cambridge	United Kingdom	HPB Consortium
36	Andre Meneses	Universidade Federal Rural da Amazônia	Brazil	HPB Consortium
37	Laura Broutier	Cancer Research Center of Lyon	France	HPB Consortium
38	Luke Boulter	University of Edinburgh	United Kingdom	HPB Consortium
39	Dominic Grün	Max Planck Institute of Immunology and Epigenetics	Germany	HPB Consortium
40	Jan IJzermans	Erasmus MC	The Netherlands	HPB Consortium
41	Benedetta Artegiani	Prinses Maxima Centrum	The Netherlands	HPB Consortium
42	Ruben van Boxtel	Prinses Maxima Centrum	The Netherlands	HPB Consortium
43	Ewart Kuijk	University of Utrecht	The Netherlands	HPB Consortium
44	Guido Carpino	Sapienza University of Rome	Italy	HPB Consortium
45	Gary Peltz	Stanford University School of Medicine	United States	HPB Consortium
46	Jesus Banales	Ikerbasque Basque Foundation for Science	Spain	HPB Consortium
47	Nancy Man	University of Hong Kong	Hong Kong, China	HPB Consortium
48	Luigi Aloia	LMCB – MRC Laboratory for Molecular Cell Biology	United Kingdom	HPB Consortium
49	Nicholas LaRusso	Mayo Clinic	United States	HPB Consortium
50	Gregory George	Mayo Clinic	United States	HPB Consortium
51	Casey Rimland	University of Cambridge	United Kingdom	HPB Consortium
52	George Yeoh	University of Western Australia Medical School	Australia	HPB Consortium
53	Anne Grappin-Botton	Max Planck Institute of Molecular Cell Biology and Genetics	Germany	HPB Consortium
54	Daniel Stange	Universitätsklinikum Carl Gustav Carus der TU Dresden	Germany	HPB Consortium
55	Nicole Prior	University of Southampton	United Kingdom	HPB Consortium
56	Janina E. E. Tirnitz-Parker	Curtin University	Australia	HPB Consortium
57	Emma Andersson	Karolinska Institutet	Sweden	HPB Consortium
58	Chiara Braconi	Glasgow University	United Kingdom	HPB Consortium
59	Nicholas Hannan	University of Nottingham	United Kingdom	HPB Consortium
60	Wei-Yu Lu	University of Birmingham	United Kingdom	HPB Consortium

61	Stephen Strom	Karolinska Institutet	Sweden	HPB Consortium
62	Pau Sancho-Bru	University of Barcelona	Spain	HPB Consortium
63	Shinichiro Ogawa	University Health Network	Canada	HPB Consortium
64	Vincenzo Corbo	University of Verona	Italy	HPB Consortium
65	Madeline Lancaster	MRC Laboratory of Molecular Science	United Kingdom	HPB Consortium
66	Huili Hu	Shandong University	China	HPB Consortium
67	Sabine Fuchs	University Medical Center Utrecht	The Netherlands	HPB Consortium
68	Delilah Hendriks	Hubrecht Institute	The Netherlands	HPB Consortium

Table S2. Nomenclature and key characteristics of adult human and mouse tissue-derived HPB organoids

Organoid Type	Reference	Nomenclature	Abbreviation	Key Characteristics			
				Organoid Structure	Cellular Features	Gene-Expression	Functionality
Cholangiocyte	(Huch *et al.*, 2013a; Huch *et al.*, 2015; Lugli *et al.*, 2016; Sampaziotis *et al.*, 2017)	Cholangiocyte organoids. Depending on the region: intrahepatic (I), extrahepatic (E) or gallbladder (G)	ICOs, ECOs or GCOs	Monolayer of polarized (basolateral-apical) epithelial cells surrounding a central lumen.	Cuboidal or columnar in shape, tight junctions near luminal side and luminal microvilli and primary cilium.	*EpCAM*$^{+}$, *KRT7*$^{+}$, *KRT19*$^{+}$, *CFTR*$^{+}$, *SCTR*$^{+}$, *ALB*$^{-/low}$	Ion and water transport via CFTR, AE2, ANO1 and AQP channels, Alkaline phosphatase and Gamma-GT activity.
Hepatocyte	(Hu *et al.*, 2018; Peng *et al.*, 2018)	Hepatocyte organoids	HOs	Polarized cells organized around bile canaliculi network.	Polygonal in shape, high nucleus to cytoplasm ratio, presence of very low-density lipoproteins, large amounts of smooth and rough ER.	*HNF4α*$^{+}$, *ASGR1*$^{+}$, *AHSG*$^{+}$, *ALB*^{high}	Serum protein, bile salt and cholesterol synthesis and secretion; Phase I and phase II-detoxification activity.
Pancreatic ductal	(Huch *et al.*, 2013b; Boj *et al.*, 2015; Loomans *et* al., 2018; Georgakopoulos *et al.*, 2020)	Pancreatic ductal organoids	PDOs	Monolayer of polarized (basolateral-apical) epithelial cells surrounding a central lumen and/or budding structures.	Cuboidal or columnar in shape, tight junctions near luminal side and luminal microvilli and primary cilium.	*EpCAM*$^{+}$, *CA2*$^{+}$, *KRT19*$^{+}$, *CFTR*$^{+}$, *SCTR*$^{+}$	Ion and water transport via CFTR and ANO1 and AQP channels, secretin signaling.
Pancreatic islet	(Wang *et al.*, 2020)	Pancreatic islet organoids	PIOs	Spheroidal with central pancreatic islet cells surrounded by endothelium.	Consisting of either one or multiple cell types from a pancreatic islet.	Depending on cells present. For instance: β-cells: *INS*$^{+}$, *SLC2A1*$^{+}$, *UCN3*$^{+}$	Metabolically regulated hormone release.

Table S3. Nomenclature and key characteristics of tumor-derived HPB organoids

Tumor Type	Reference	Nomenclature	Abbreviation	Key Characteristics
Hepatocellular carcinoma (HCC)	(Broutier *et al.*, 2017; Nuciforo *et al.*, 2018)	Hepatocellular carcinoma organoids	HCCOs	Derived from hepatocellular carcinoma specimens. Presents similar mutations, histoarchitecture as the original tumor and has tumorigenic potential *in vivo*.
Cholangiocarcinoma (CCA)	(Broutier *et al.*, 2017; Saito *et al.*, 2019)	Cholangiocarcinoma organoids. Depending on the region either intrahepatic (I), perihilar (ph) or distal (d)	iCCAOs, phCCAOs, dCCAOs	Derived from cholangiocarcinoma specimens. Presents similar mutations, histoarchitecture as the original tumor and has tumorigenic potential *in vivo*.
Gallbladder carcinoma (GBC)	(Saito *et al.*, 2019)	Gallbladder carcinoma organoids	GBCOs	Derived from gallbladder carcinoma specimens. Presents similar mutations, histoarchitecture as the original tumor and has tumorigenic potential *in vivo*.
Pancreatic ductal adenocarcinoma (PDAC)	(Boj *et al.*, 2015)	Pancreatic ductal adenocarcinoma organoids	PDACOs	Derived from pancreatic ductal adenocarcinoma specimens. Presents similar mutations, histoarchitecture as the original tumor and has tumorigenic potential *in vivo*.

Supplementary Data:

Questionnaires 1-3:

Questionnaire 1 - Building a Consensus on Definition and Nomenclature of Human Hepatic, Pancreatic and Biliary Organoids: a position paper

Over the last decade significant progress has been made in the culture of human Hepatic, pancreatic and biliary organoids. From the establishment of “liver epithelial organoids” from LGR5+ liver stem cells by Huch *et al.* to the generation of “liver bud organoids” by co-culturing iPSC-derived hepatic endoderm, endothelial progenitors, and mesenchymal progenitors by Takebe *et al.*, these complex 3D structures can be generated from a growing number of sources. Notably, hepatic-, biliary- and pancreatic organoids have also been established from primary cancer tissue of the liver, biliary tree and pancreas, respectively. To facilitate understanding between scientists we characterize each organoid subtype and develop nomenclature for referring to these systems through the consensus of experts in the field. We also provide the minimal criteria for classifying each organoid subtype based on gene expression and functional analysis. Importantly, we leave room for future organoid systems that will arise as the field advances. Finally, we provide a detailed overview of the recent progress in the culture of organoids derived from the liver, biliary tree and pancreas, as well as iPSC-derived organoids differentiated towards the Hepatic, pancreatic and biliary fate.

The present review has three main scopes:
1. Provide a clear definition of an "organoid," distinguishing this 3D cell model from others, i.e. spheroids, etc.

2. Introduce nomenclature for classifying tissue-derived Hepatic, pancreatic and biliary organoids. This is especially important regarding the former, as the term "liver organoid" has become too broad in recent years.

3. Provide an overview of the recent advances in the culture of Hepatic, pancreatic and biliary organoids, including current and future perspectives.

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 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 ≥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 and position?

Notice

If you feel you do not have the expertise to answer a question please skip it and proceed to the next.

Part 1 - Definition of an organoid

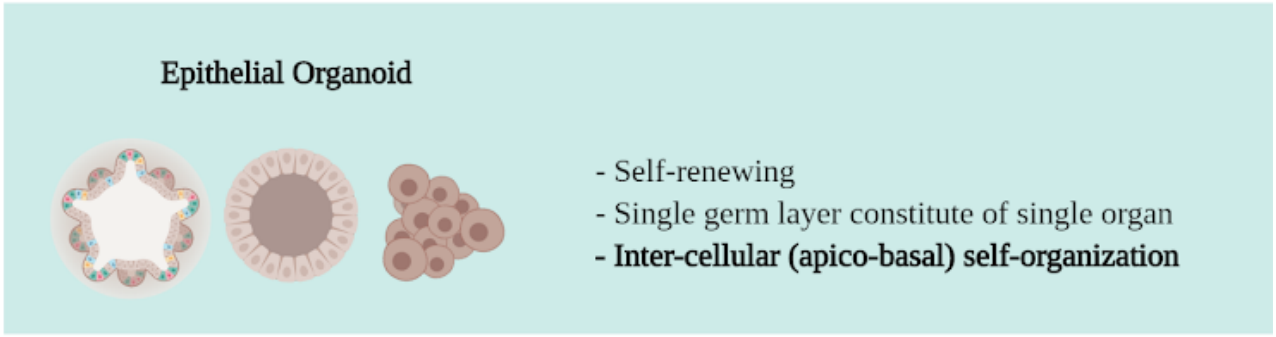
To accommodate systems which do not self-renew, for instance the "liver bud" developed by Takebe *et al.*, an over-arching definition of "organoid" could be applied along with three sub-classifications. Do you agree with the over-arching definition (found below)?

Organoid

Complex tissue derived from cell aggregates of (neoplastic) stem/progenitor cells, primed primary cells, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) that self-organize through cell-cell interactions and spatially restricted lineage commitments to generate 3D organ-like structures that recapitulate important aspects of the native tissue architecture and function.

[ ] I agree
[ ] I do not agree
Other: (please specify)

What are key characteristics of an epithelial organoid?
Sub-classification 1:



Examples: Huch *et al.*, 2015; Sampaziotis *et al.*, 2017; Hu *et al.*, 2018

[ ] Self-renewal (>3 months)
[ ] Single germ layer tissue
[ ] Self-organization that recapitulates key aspects of the original tissue architecture and function
[ ] Concomitant presence of progenitors and mature cells in the same structure
[ ] Always derived from self-renewing stem/progenitor cells
[ ] Can be derived from primed primary cells as well as self-renewing stem/progenitor cells

Other: (please specify)

What are key characteristics of a multi-tissue organoid?
Sub-classification 2:

Example: Takebe et al., 2017; Ouchi et al., 2018

[ ] Non self-renewing
[ ] Multi-germ layer tissue
[ ] Inter-tissue self-organization that recapitulates key aspects of the original tissue architecture and function
[ ] Concomitant presence of progenitors and mature cells in the same structure
[ ] Complex vascular and/or stromal networks
[ ] Mesenchymal-driven tissue condensation
Other: (please specify)

What are key characteristics of a multi-system organoid?
Sub-classification 3:

Example: Koike *et al.*, 2019

[ ] Non-self-renewing
[ ] Inter-organ self-organization that recapitulates key aspects of the original tissue architecture and function
[ ] Mult-germ layer tissue
[ ] Complex vascular and/or stromal networks
[ ] Pluripotent stem cell-derived
Other: (please specify)

Additional Comments for this part (please specify below):

Part 2 - Nomenclature for Hepatic, pancreatic and biliary Organoids

Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?

https://www.cell.com/cell/fulltext/S0092-8674(14)01566-9 https://www.nature.com/articles/s41596-019-0168-0 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Intrahepatic biliary organoids (IBOs)
[ ] Intrahepatic cholangiocyte organoids (ICOs)
[ ] Hepatic bile duct organoids (HBDOs)
[ ] Intrahepatic organoids (IOs)
Other: (please specify)

Do the intrahepatic bile duct-derived cells described by Huch *et al.* (Cell, 2015), Tysoe *et al.* (Nat. Protocols, 2019) and Rimland *et al.* (Hepatology, 2020) represent the same starting cell population (mature cholangiocytes)?

[ ] Yes, these cells are the same. Differences arise from differing culture conditions.
[ ] No, these cells represent two distinct cell populations.
Other: (please specify)

Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?
https://www.embopress.org/doi/full/10.15252/embr.201642169 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Gallbladder biliary organoids (GBOs)
[ ] Gallbladder Cholangiocyte Organoids (GCOs)
[ ] Gallbladder organoids (GBOs)
[ ] Cholecyst organoids (COs)
Other: (please specify)

Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?
https://www.nature.com/articles/nm.4360 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Extrahepatic cholangiocyte organoids (ECOs)
[ ] Extrahepatic bile duct organoids (EBDOs)
[ ] Extrahepatic biliary organoids (EBOs)
Other: (please specify)

Nomenclature for hepatocyte-derived organoids described by Hu *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(18)31505-8

[ ] Hepatocyte organoids (HOs)
[ ] Primary hepatocyte organoids (PHOs)
Other: (please specify)

Nomenclature for pancreatic ductal-derived organoids described by Boj *et al.* and Rimland *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01592-X https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Pancreatic ductal organoids (PDOs)
[ ] Pancreatic organoids (POs)
Other: (please specify)

Nomenclature for hepatoblast-derived organoids?
https://www.enterprise.cam.ac.uk/wp-content/uploads/2019/04/HBO-inpart-Marketing-sheet-Final.pdf

[ ] Hepatoblast organoids (HBOs)
Other: (please fill in)

Additional Comments for this part (please specify below):

Part 3 - Fetal, Neonatal and Adult-derived Organoids

There are significant differences between fetal, neonatal and adult-derived organoids. How should this be addressed in the nomenclature?
Fetal extrahepatic bile duct-derived organoids used as an example:

[ ] Fetal extrahepatic cholangiocyte organoids (fetal ECOs)
[ ] Fetal extrahepatic cholangiocyte organoids (f-ECOs)
[ ] Fetal extrahepatic cholangiocyte organoids (fECOs)
[ ] Hepatoblast organoids (HBOs)
Other: (please specify)

Additional Comments for this part (please specify below):

Part 4 - Nomenclature for Cancer-derived Hepatic, pancreatic and biliary Organoids

Nomenclature for intrahepatic cholangiocarcinoma-derived organoids described by Broutier *et al.* and Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9 https://www.nature.com/articles/nm.4438

[ ] Intrahepatic cholangiocarcinoma organoids (Intrahepatic CCOs)
[ ] Intrahepatic cholangiocarcinoma organoids (Intrahepatic CCAOs)
[ ] Intrahepatic cholangiocarcinoma organoids (ICCOs)
[ ] Intrahepatic cholangiocarcinoma organoids (ICCAOs)
Other: (please specify)

Nomenclature for extrahepatic cholangiocarcinoma-derived organoids described by Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Extrahepatic cholangiocarcinoma organoids (Extrahepatic CCOs)
[ ] Extrahepatic cholangiocarcinoma organoids (Extrahepatic CCAOs)
[ ] Extrahepatic cholangiocarcinoma organoids (ECCOs)
[ ] Extrahepatic cholangiocarcinoma organoids (ECCAOs)

Other: (please specify)

Nomenclature for hepatocellular carcinoma-derived organoids described by Broutier *et al.* and Nuciforo *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(18)31078-7? https://www.nature.com/articles/nm.4438

[ ] Hepatocellular carcinoma organoids (HCCOs)
Other: (please specify)

Nomenclature for gallbladder cancer-derived organoids described by Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Gallbladder cancer organoids (GBCOs)
[ ] Gallbladder cancer organoids (GCOs)
[ ] Cholecystic cancer organoids (CCOs)
Other: (please specify)

Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01592-X

[ ] Pancreatic ductal adenocarcinoma organoids (PDACOs)
[ ] Pancreatic ductal adenocarcinoma organoids (PDAOs)
[ ] Pancreatic ductal cancer organoids (PDCOs)
[ ] Pancreatic cancer organoids (PCOs)
Other: (please specify)

Additional Comments for this part (please specify below):

Recommend Expert

Please include contact information and affiliation for recommend expert in the boxes below.

Questionnaire 2 - Building a Consensus on Definition and Nomenclature of Human Hepatic, Pancreatic and Biliary Organoids: a position paper

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Contact Information

Only the facilitator will have access to the contact information.

Name:

Your answer

Institute:

Your answer

Email address:

Notice

If you feel you do not have the expertise to answer a question please skip it and proceed to the next.

Part 1 - Definition of an organoid

To accommodate systems which do not self-renew, for instance the "liver bud" developed by Takebe *et al.*, an over-arching definition of "organoid" could be applied along with three sub-classifications. Do you agree with the over-arching definition (found below)?

> **Organoid**
>
> Complex 3D structure derived from (fetal/adult) primary cells, stem/progenitor cells or pluripotent stem cells (PSCs) that self-organize through cell-cell and cell-matrix interactions and spatially restricted lineage commitments to recapitulate important aspects of the native tissue architecture and function.

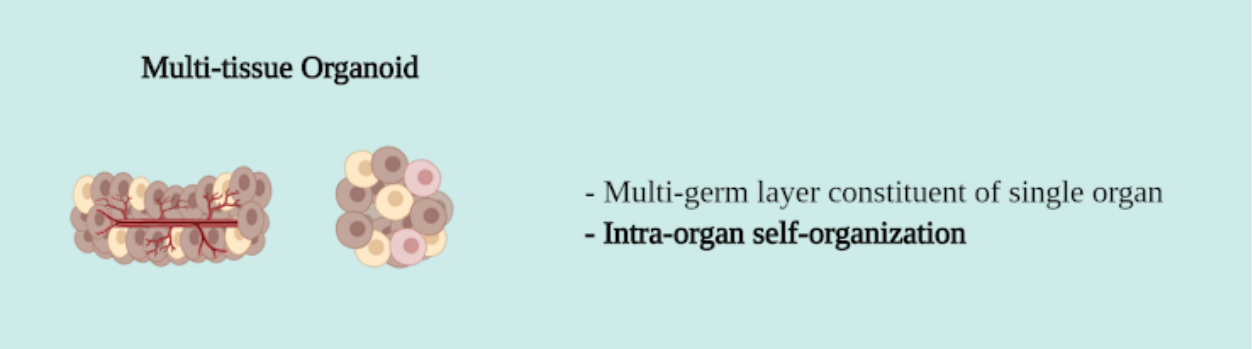
[ ] I agree
[ ] I do not agree
Other: (please specify)

Key characteristics of an epithelial organoid:
Sub-classification 1:

Examples: Huch *et al.*, 2015; Lugli *et al.*, 2016; Sampaziotis *et al.*, 2017; Hu *et al.*, 2018

[ ] I agree
[ ] I do not agree
Other: (please specify)

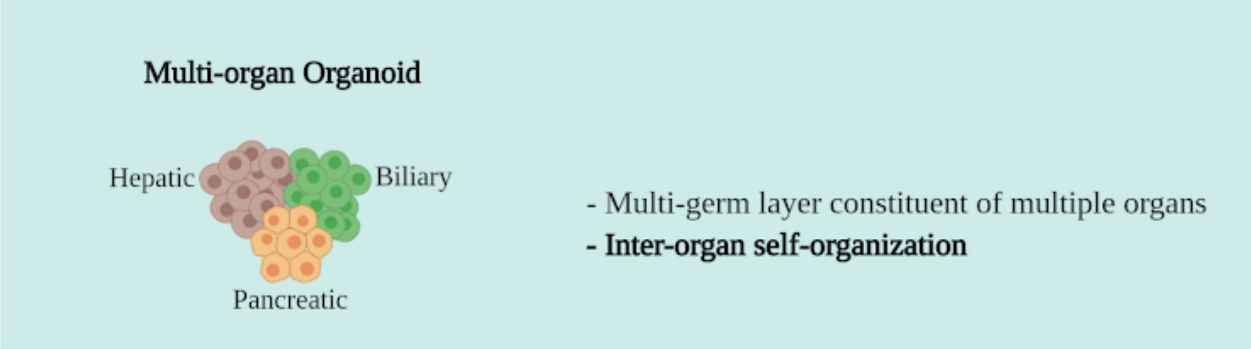
Key characteristics of a multi-tissue organoid:
Sub-classification 2:



Examples: Takebe *et al.*, 2017; Ouchi *et al.*, 2018

[ ] I agree
[ ] I do not agree
Other: (please specify)

Key characteristics of a multi-organ organoid:
Sub-classification 3:



Example: Koike *et al.*, 2019

[ ] I agree
[ ] I do not agree
Other: (please specify)
Additional Comments (please specify below):
Your answer

Part 2 - Nomenclature for Hepatic, pancreatic and biliary Organoids

What is the most important aspect the nomenclature for organoids should reflect?

[ ] Cell type of origin
[ ] Cell type of resemblance *in vitro*
[ ] Anatomical structure the organoid "partially" resembles
Other: (please specify)

Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01566-9 https://www.nature.com/articles/s41596-019-0168-0 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Intrahepatic biliary organoids (IBOs)
[ ] Intrahepatic cholangiocyte organoids (ICOs)
[ ] Intrahepatic bile duct organoids (IBDOs)
[ ] Intrahepatic cholangioids

Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?

https://www.nature.com/articles/nm.4360 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Extrahepatic cholangiocyte organoids (ECOs)
[ ] Extrahepatic biliary organoids (EBOs)
[ ] Extrahepatic bile duct organoids (EBDOs)

Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?

https://www.embopress.org/doi/full/10.15252/embr.201642169 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Gallbladder biliary organoids (GBOs)
[ ] Gallblader cholangiocyte Organoids (GCOs)
[ ] Gallbladder organoids (GBOs)

Nomenclature for hepatocyte-derived organoids described by Hu *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(18)31505-8

[ ] Hepatocyte organoids (HOs)

[ ] Primary hepatocyte organoids (PHOs)

Nomenclature for pancreatic ductal-derived organoids described by Boj *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01592-X

[ ] Pancreatic ductal organoids (PDOs)
[ ] Pancreatic organoids (POs)

Additional Comments (Please specify below):

Your answer

Part 3 - Fetal, Neonatal and Adult-derived Organoids

There are differences between fetal and adult-derived organoids. How should this be addressed in the nomenclature?
Fetal (primary) hepatocyte organoids used as an example: https://www.cell.com/cell/fulltext/S0092-8674(18)31505-8

[ ] Fetal (primary) hepatocyte organoids (fetal PHOs)
[ ] Fetal (primary) hepatocyte organoids (fPHOs)
Other: (please specify)

Additional Comments (Please specify below):

Your answer

Part 4 - Nomenclature for Tumor-derived Hepatic, pancreatic and biliary Organoids

How should organoids derived from primary tumors (benign/malignant) be referred to in general?

[ ] Tumor organoids
[ ] Organoids
[ ] Tumoroids
Other: (please specify)

Nomenclature for intrahepatic cholangiocarcinoma-derived organoids described by Broutier *et al.* and Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9 https://www.nature.com/articles/nm.4438

[ ] Intrahepatic cholangiocarcinoma organoids (Intrahepatic CCOs)
[ ] Intrahepatic cholangiocarcinoma organoids (ICCOs)
[ ] Primary liver cancer organoids (PLC organoids)

Nomenclature for extrahepatic cholangiocarcinoma-derived organoids described by Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Extrahepatic cholangiocarcinoma organoids (Extrahepatic CCOs)
[ ] Extrahepatic cholangiocarcinoma organoids (ECCOs)

Nomenclature for hepatocellular carcinoma-derived organoids described by Broutier *et al.* and Nuciforo *et al.*?

https://www.cell.com/cell-reports/fulltext/S2211-1247(18)31078-7 https://www.nature.com/articles/nm.4438

[ ] Hepatocellular carcinoma organoids (HCCOs)
[ ] Primary liver cancer organoids (PLC organoids)

Nomenclature for gallbladder carcinoma-organoids described by Saito *et al.*?
https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Gallbladder carcinoma organoids (GCOs)
[ ] Gallbladder carcinoma organoids (GBCOs)
[ ] Gallbladder cancer organoids (GCOs)
[ ] Gallbladder cancer organoids (GBCOs)

Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?

https://www.cell.com/cell/fulltext/S0092-8674(14)01592-X

[ ] Pancreatic ductal adenocarcinoma organoids (PDACOs)
[ ] Pancreatic ductal adenocarcinoma organoids (PDAOs)
[ ] Pancreatic carcinoma organoids (PCOs)

Additional Comments (Please specify below):
Your answer

Questionnaire 3 - Building a Consensus on Definition and Nomenclature of Human Hepatic, Pancreatic and Biliary Organoids: a position paper

Hepatic, pancreatic and biliary (HPB) organoids have proven to be powerful tools in the study of development, disease and regeneration, with applications ranging from basic research to regenerative medicine. Over the last decade, significant progress has been made in the culture of these complex 3D structures. HPB organoids can now be generated from multiple sources of fetal and adult primary tissue, as well as the directed differentiation of pluripotent stem cells (PSCs). Notably, organoids have also been established from primary and metastatic tumors of the liver, biliary tree and pancreas, respectively. As organoid research intensifies, with laboratories around the world culturing these diverse tissue-like structures, there is need for a clear definition and nomenclature to describe these systems. Here we review the recent progress in the culture of human Hepatic, pancreatic and biliary organoids, with special attention to tissue-derived epithelial organoids. To enable reproducibility and facilitate scientific communication between researchers, we revisit the concept of an organoid and introduce an intuitive classification system and nomenclature for referring to these 3D structures through the consensus of experts in the field. Importantly, we leave room for future organoid systems that will arise as the field advances.

The present review has three main scopes:

1. Provide a clear definition of an "organoid," distinguishing this 3D cell model from others.

2. Introduce nomenclature for classifying tissue-derived Hepatic, pancreatic and biliary organoids. This is especially important regarding the former, as the term "liver organoid" has

become too broad in recent years.

3. Provide an overview of the recent advances in the culture of Hepatic, pancreatic and biliary organoids, including current and future perspectives.

Final Questionnaire

The following represents the third and final questionnaire in the series. Thank you for your contribution to this collaborative project. We have already reached consensus on a number of topics, and hope to reach consensus on those that remain through this questionnaire. In the event that consensus is not reached for a particular matter, the steering committee will make a final proposition, which will be reflected in the manuscript. Invited experts that have completed all three questionnaires will receive a draft version of the manuscript before submission, along with the opportunity to discuss the decisions made by the steering committee.
Steering committee members (alphabetical order):

Hans Clevers - Hubrecht Institute
Stuart Forbes - The University of Edinburgh
Helmuth Gehart - ETH Zürich
Meritxell Huch - Max Planck Institute
Eelco de Koning - Leiden University
Luc van der Laan - Erasmus MC
Frédéric Lemaigre - Institut de Duve
Ary Marsee - Utrecht University
Weng Peng - Princess Máxima Center
Floris Roos - Erasmus MC
Bart Spee - Utrecht University
Takanori Takebe - Yokohama City University
Ludovic Vallier - Cambridge Stem Cell Institute
Monique Verstegen - Erasmus MC

Contact Information

Only the facilitator will have access to the contact information.

Name:

Your answer

Institute:

Your answer

Email address:

Your answer

Notice

If you feel you do not have the expertise to answer a question please skip it and proceed to the next.

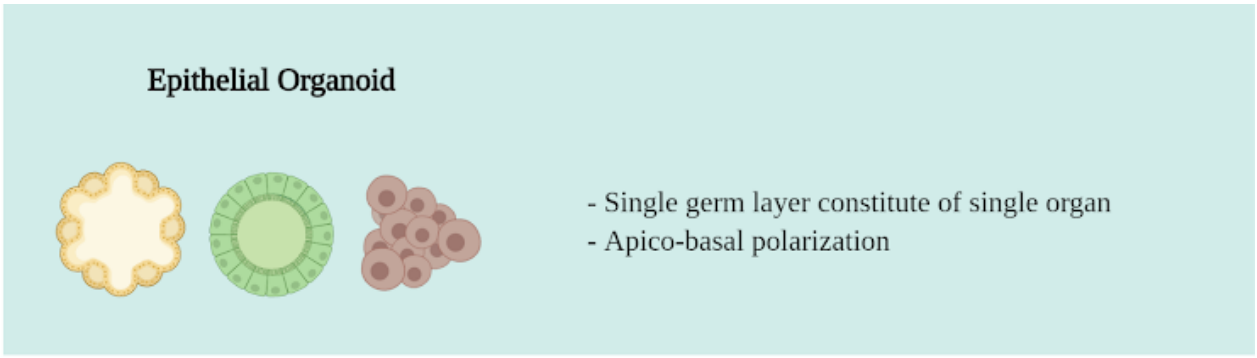
Part 1 - Definition of an organoid

To accommodate systems which do not self-renew, for instance the "liver bud" developed by Takebe *et al.*, an over-arching definition of "organoid" could be applied along with three sub-classifications. Do you agree with the over-arching definition (found below)?

> **Organoid**
>
> Three-dimensional structure derived from primary cells, stem/progenitor cells or pluripotent stem cells (PSCs) that self-organize through cell-cell and cell-matrix interactions to recapitulate aspects of the native tissue architecture and function.

[ ] I agree
[ ] I do not agree

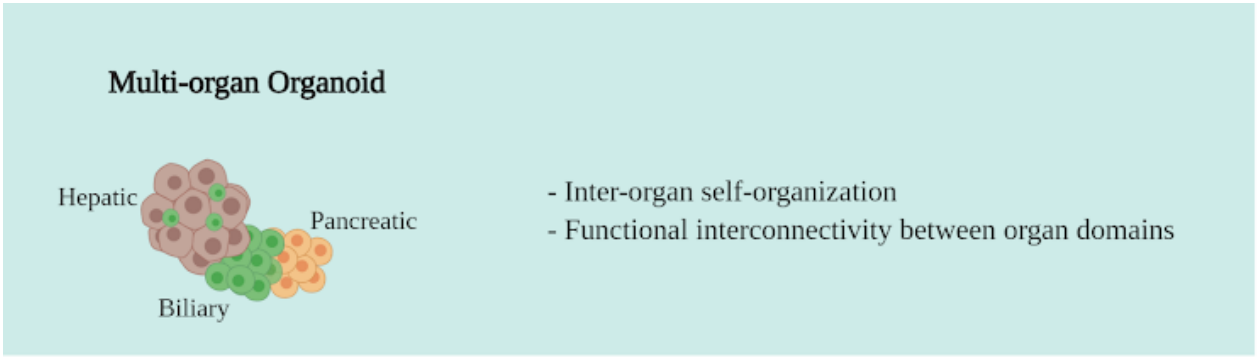
Key characteristics of an epithelial organoid:
Sub-classification 1:



Examples: Huch *et al.*, 2015; Lugli *et al.*, 2016; Sampaziotis *et al.*, 2017; Hu *et al.*, 2018

[ ] I agree
[ ] I do not agree

Key characteristics of a multi-organ organoid:
Sub-classification 3:



Example: Koike *et al.*, 2019

[ ] I agree
[ ] I do not agree

Additional Comments (please specify below):

Your answer

Part 2 - Nomenclature for Tissue-derived Hepatic, pancreatic and biliary Organoids

Aspect(s) the nomenclature for single cell type systems should reflect?

[ ] Cell type of origin + cell type of resemblance *in vitro*
[ ] Cell type of origin
[ ] Cell type of resemblance *in vitro*

Aspect(s) the nomenclature for multi-cell type systems should reflect?
Only for epithelial organoids with multiple cell types. Not to be confused with multi-tissue organoids. Examples include pancreatic islet organoids and intestinal organoids, which contain multiple epithelial cell types.

[ ] Cell type(s) of origin + anatomical structure of resemblance *in vitro*
[ ] Cell type(s) of origin
[ ] Anatomical structure of resemblance *in vitro*

Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01566-9
https://www.nature.com/articles/s41596-019-01680
https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Intrahepatic cholangiocyte organoids (ICOs)
[ ] Intrahepatic biliary organoids (IBOs)
[ ] Intrahepatic bile duct organoids (IBDOs)

Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?
https://www.nature.com/articles/nm.4360
https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Extrahepatic cholangiocyte organoids (ECOs)
[ ] Extrahepatic biliary organoids (EBOs)
[ ] Extrahepatic bile duct organoids (EBDOs)

Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?

https://www.embopress.org/doi/full/10.15252/embr.201642169 https://aasldpubs.onlinelibrary.wiley.com/doi/abs/10.1002/hep.31252

[ ] Gallblader cholangiocyte Organoids (GCOs)
[ ] Gallbladder biliary organoids (GBOs)

[ ] Gallbladder organoids (GBOs)

Nomenclature for mouse hepatocyte-derived organoids described by Hu *et al.* and Peng *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(18)31505-8
https://www.cell.com/cell/fulltext/S0092-8674(18)31504-6

[ ] Hepatocyte organoids (HOs)
[ ] Hepatocyte organoids (HCOs)
[ ] Primary hepatocyte organoids (PHOs)

Additional Comments (please specify below):
Your answer

Part 3 – Fetal-derived organoids

There are differences between fetal and adult-derived organoids. How should this be addressed in the nomenclature?

[ ] Spell out the word "fetal" (example: fetal PDOs)
[ ] Preface with a lower case "f" (example: fPDOs)

Additional Comments (Please specify below):

Your answer

Part 4 - Nomenclature for Tumor-derived Hepatic, pancreatic and biliary Organoids

How should organoids derived from primary or secondary tumors be referred to in general? Nomenclature for individual tumor-derived systems will be adapted to reflect the chosen answer to this question. Thus, to reflect both views subsequent questions on nomenclature for tumor-derived systems will have both organoids and tumoroids as an option. Depending on the consensus reached for this specific question, the nomenclature will be adjusted to end in either 'organoids' or 'tumoroids.'

[ ] Tumor organoids
[ ] Tumoroids

Cholangiocarcinoma (CCA) include a diverse group of malignancies of the biliary tree, which can be divided into three subtypes depending on their anatomical location. Nomenclature for CCA subtypes is well established: intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) CCA. To reflect this in the nomenclature for organoids we have adapted the questions for CCA derived organoid systems:

https://www.nature.com/articles/s41575-020-0310-z

[ ] I agree
[ ] I object

Nomenclature for intrahepatic cholangiocarcinoma-derived organoids?

https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9 https://www.nature.com/articles/nm.4438

Intrahepatic cholangiocarcinoma organoids/tumoroids (Intrahepatic CCOs/CCTs)
Intrahepatic cholangiocarcinoma organoids/tumoroids (Intrahepatic CCAOs/CCATs)

Nomenclature for perihillar cholangiocarcinoma-derived organoids?

https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Perihilar cholangiocarcinoma organoids/tumoroids (Perihilar CCOs/CCTs)
[ ] Perihilar cholangiocarcinoma organoids/tumoroids (Perihilar CCAOs/CCATs)

Nomenclature for distal cholangiocarcinoma-derived organoids?

[ ] Distal cholangiocarcinoma organoids/tumoroids (Distal CCOs/CCTs)
[ ] Distal cholangiocarcinoma organoids/tumoroids (Distal CCAOs/CCATs)

Nomenclature for gallbladder carcinoma-derived organoids?

https://www.cell.com/cell-reports/fulltext/S2211-1247(19)30427-9

[ ] Gallbladder carcinoma organoids (GBCOs)
[ ] Gallbladder carcinoma organoids (GCOs)

Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?
https://www.cell.com/cell/fulltext/S0092-8674(14)01592-X

[ ] Pancreatic ductal adenocarcinoma organoids (PDACOs)
[ ] Pancreatic ductal adenocarcinoma organoids (PDAOs)

Additional Comments (Please specify below):

Your answer

Supplementary Results

Questionnaire Responses

Based on the database search 61 authors were identified and subsequently invited to fill in the first questionnaire. An additional 39 authors were suggested by experts, making a total of 100 invitees. The first questionnaire was filled in by 74 experts (response of 74%). Experts which completed the first questionnaire were invited to fill in the second questionnaire. Out of the 74 eligible candidates, 69 (93%) completed the second questionnaire. Similar to the previous round, experts which filled in the second questionnaire were invited to complete the third and final questionnaire. In the end, 68 experts (see supplementary table 1) representing 16 countries around the world (Figure S1) participated in all three questionnaires.

Summary of Results to Questionnaires 1-3:

Questionnaire 1 Summary

Overview:

In this document you will find a summary of the results to the first questionnaire. The answers to each question are represented in a pie chart. Comments related to a particular section/question are grouped together.

The second questionnaire, *the link to which is included in the email*, was designed to reflect the results of the first. Modifications have been made to some questions based on the provided answers, and comments/suggestions made by several experts have been turned into questions/options.

Please take time to review the summary of the first questionnaire before proceeding to the second. We kindly ask that you complete the second questionnaire by **July 6th**.

General comments made by invited experts:

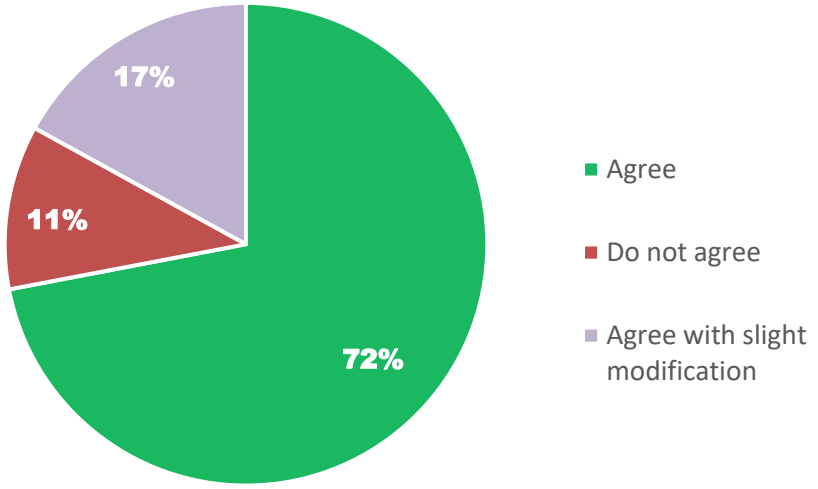
- Be consistent with nomenclature
- Nomenclature for organoid systems should be specific
- Nomenclature could be applied to other species
- Nomenclature should leave room for future, yet to be discovered organoid types

- Acronyms of three letters for non-neoplastic organoids and four for neoplastic organoids

Part 1 – Definition of an Organoid

To accommodate systems which do not self-renew, for instance the “liver bud” developed by Takebe *et al.*, an over-arching definition of "organoid" could be applied along with three sub-classifications. Do you agree with the over-arching definition (found below)?

> ***“Complex tissue derived from cell aggregates of (neoplastic) stem/progenitor cells, primed primary cells, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) that self-organize through cell-cell interactions and spatially restricted lineage commitments to generate 3D organ-like structures that recapitulate important aspects of the native tissue architecture and***

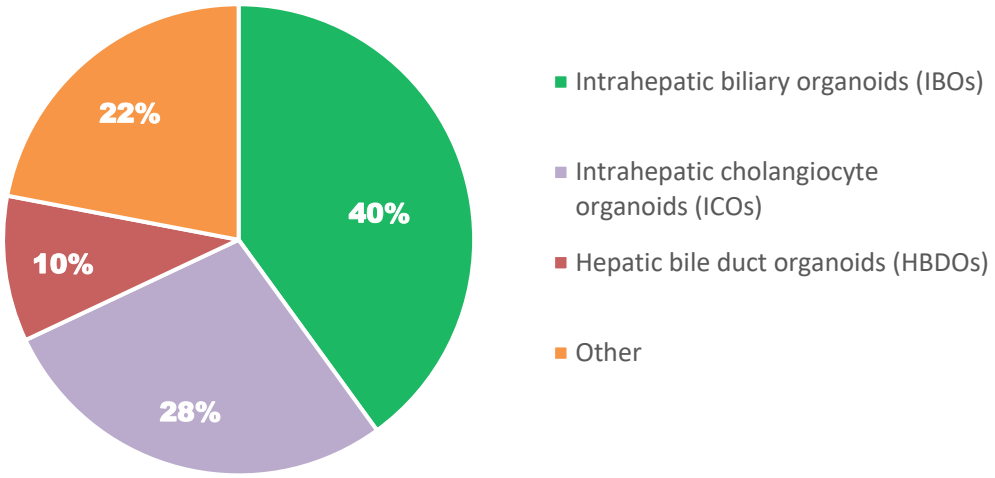


Comments:

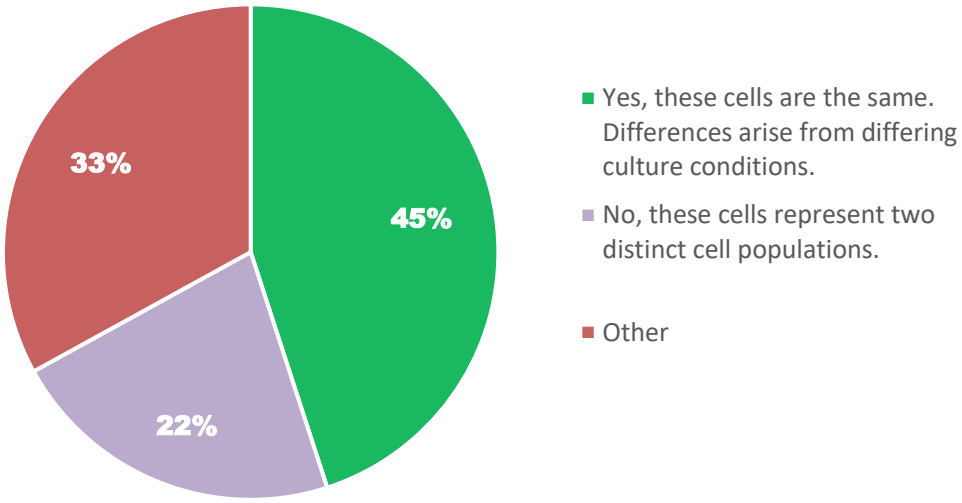
- The definition has been modified slightly; reflected in questionnaire 2.

Part 2 – Nomenclature for Tissue-derived HPB organoids

Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?



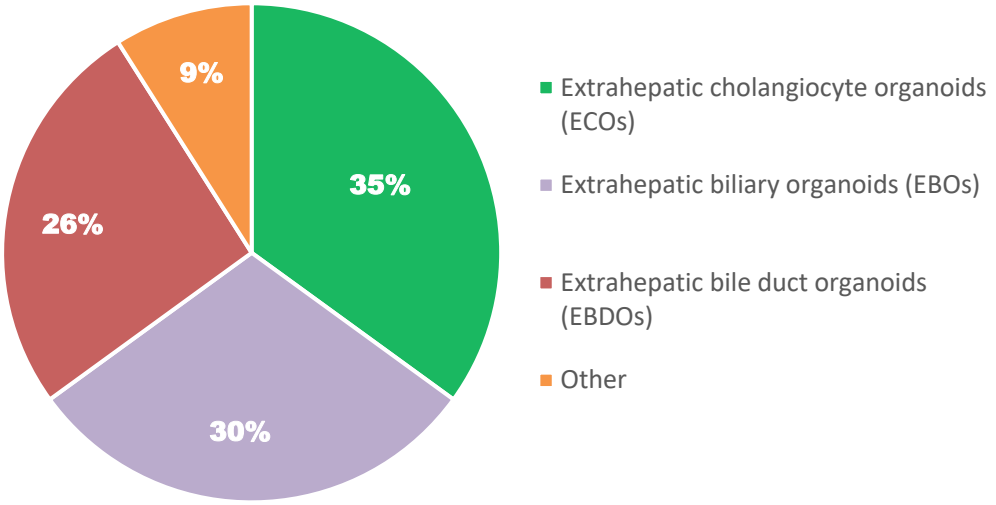
Do the intrahepatic bile duct-derived cells described by Huch *et al.* (Cell, 2015), Tysoe *et al.* (Nat. Protocols, 2019) and Rimland *et al.* (Hepatology, 2020) represent the same starting cell population (mature cholangiocytes)?



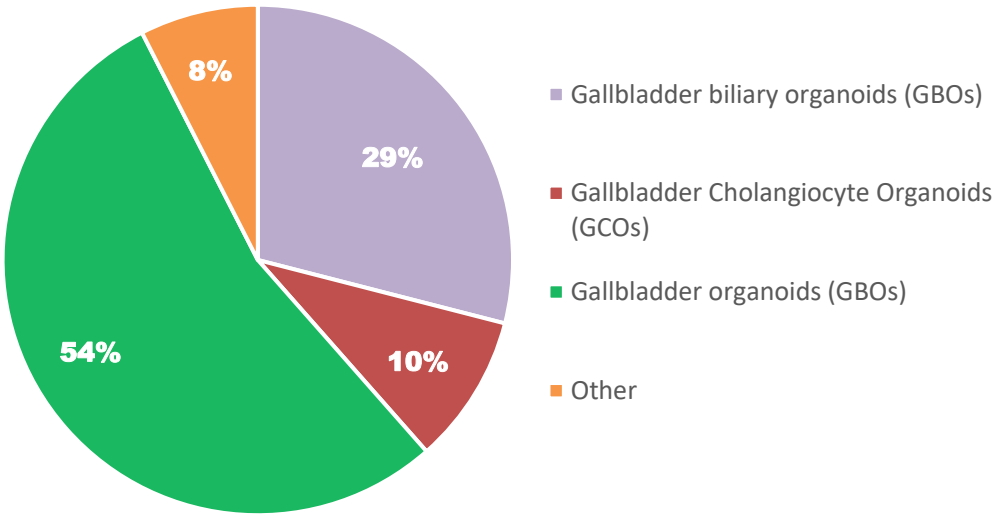
Comments:

- This question was designed for speculation and has been removed from the second questionnaire.

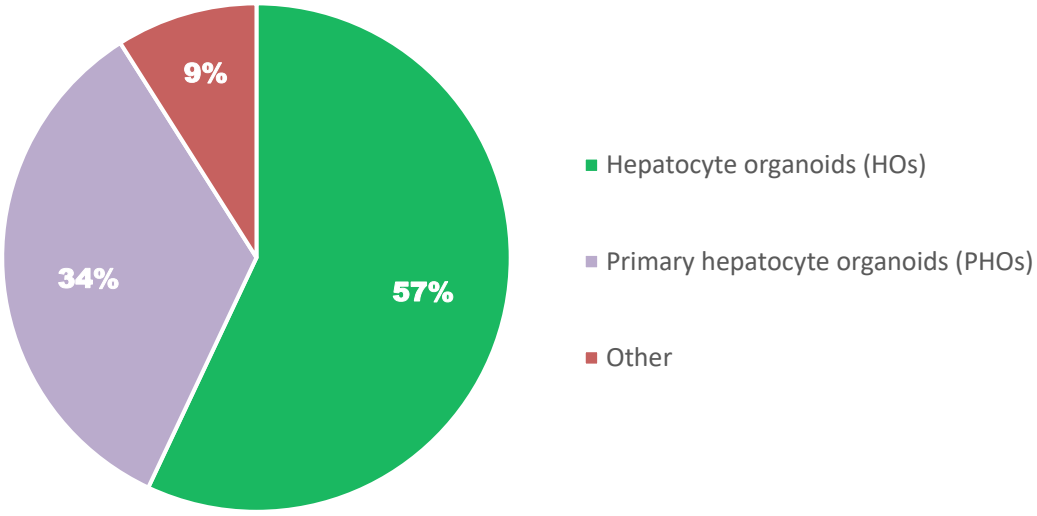
Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?



Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?



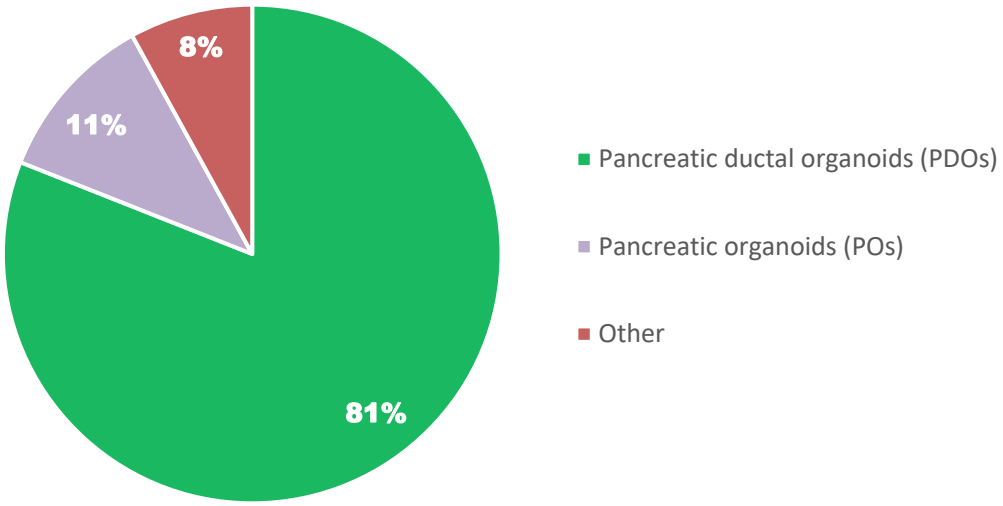
Nomenclature for hepatocyte-derived organoids described by Hu *et al.*?



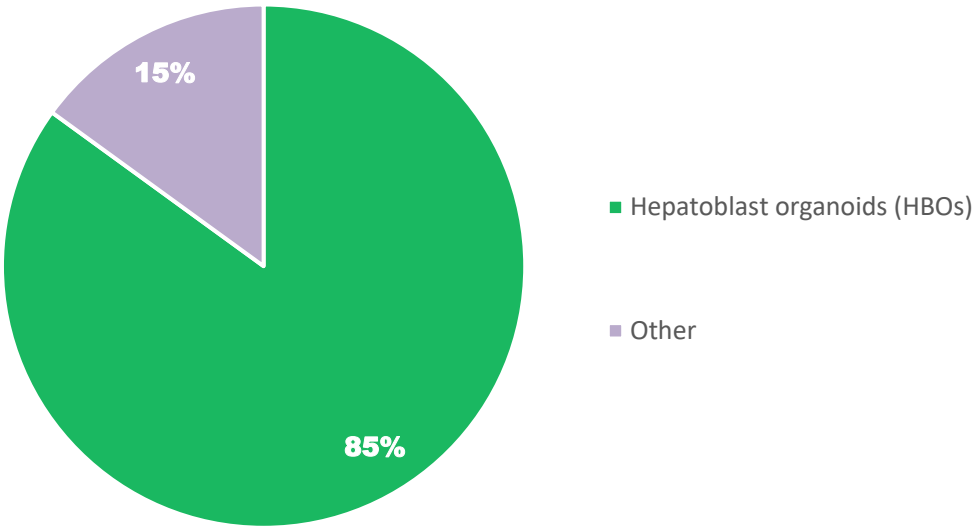
Comments:

- While it has been suggested that Hu *et al.* only established fetal hepatocyte organoids, the manuscript also states that pediatric and adult human hepatocytes were also cultured as organoids.
- HOs for hepatocyte organoids may not sound the best.

Nomenclature for pancreatic ductal-derived organoids described by Boj *et al.* and Rimland *et al.*?

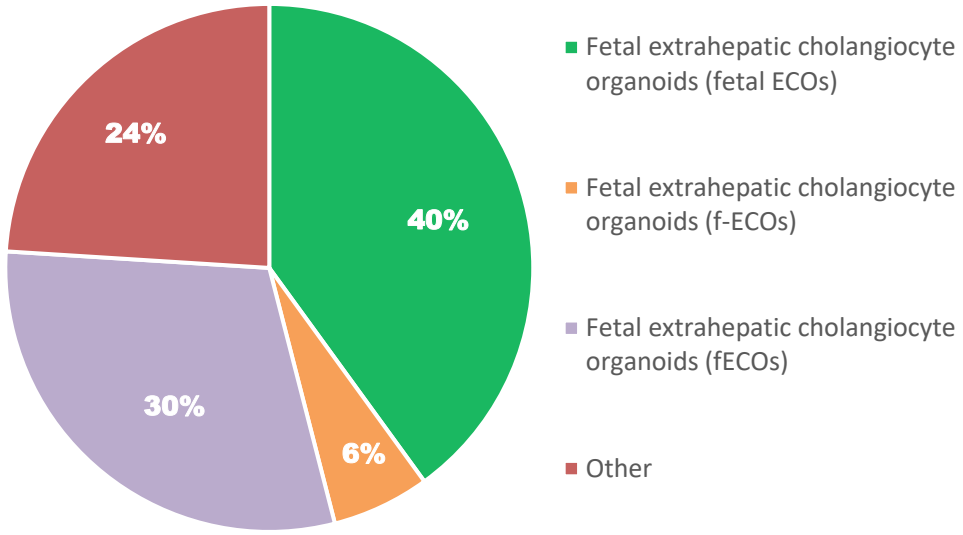


Nomenclature for hepatoblast-derived organoids?



Part 3 – Fetal, Neonatal and Adult-derived Organoids

There are significant differences between fetal, neonatal and adult-derived organoids. How should this be addressed in the nomenclature?

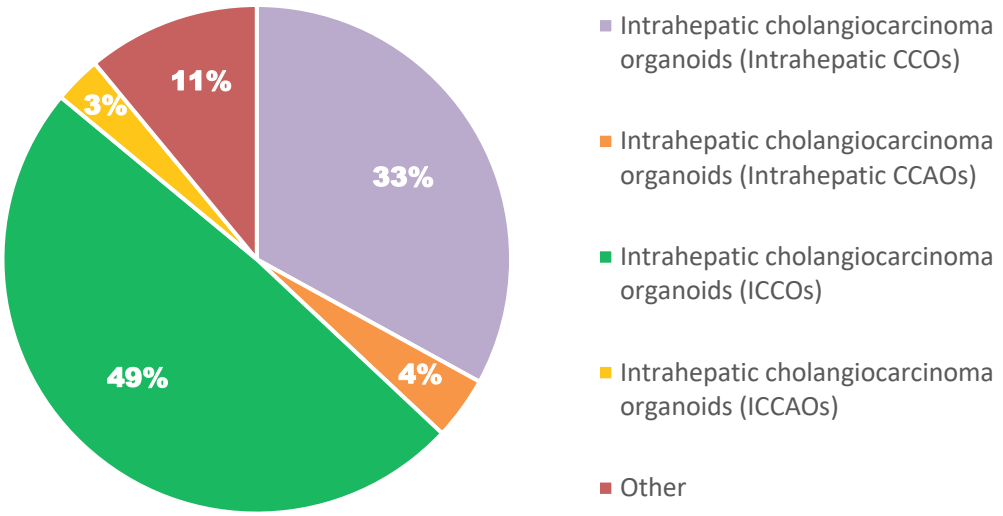


Comments:

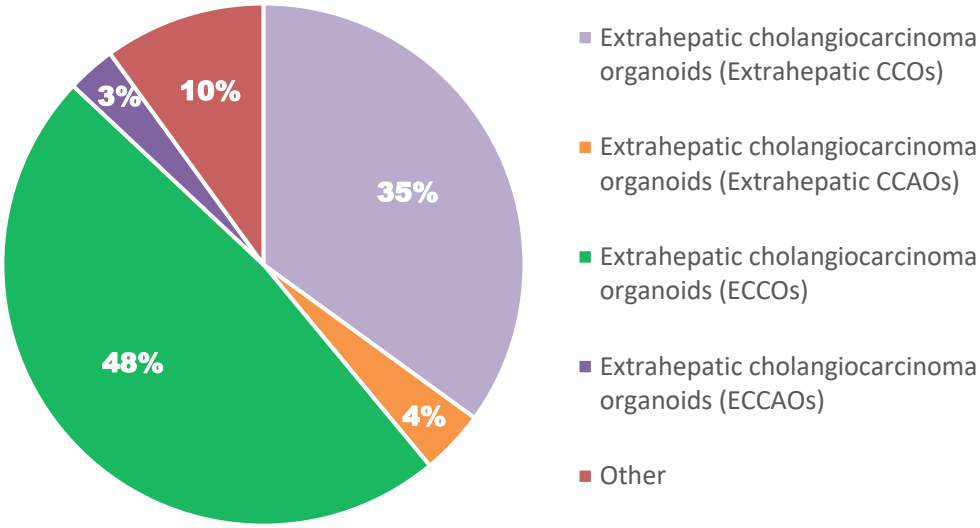
- The example for how to address the use of the word “fetal” within nomenclature has been changed to “fetal hepatocyte organoids”, as published by Hu *et al.*.

Part 4 – Nomenclature for Cancer-derived Hepatic, pancreatic and biliary Organoids

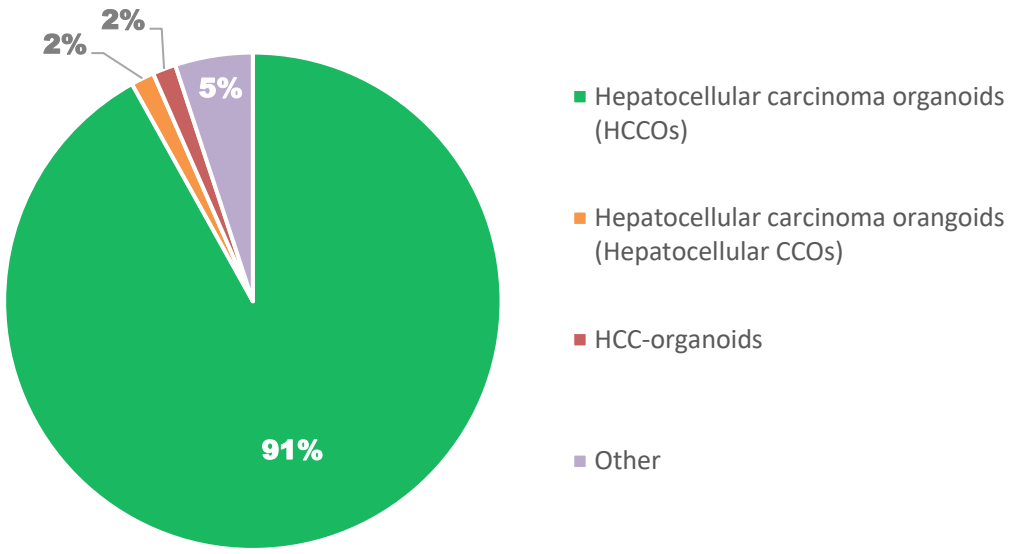
Nomenclature for intrahepatic cholangiocarcinoma-derived organoids described by Broutier *et al.* and Saito *et al.*?



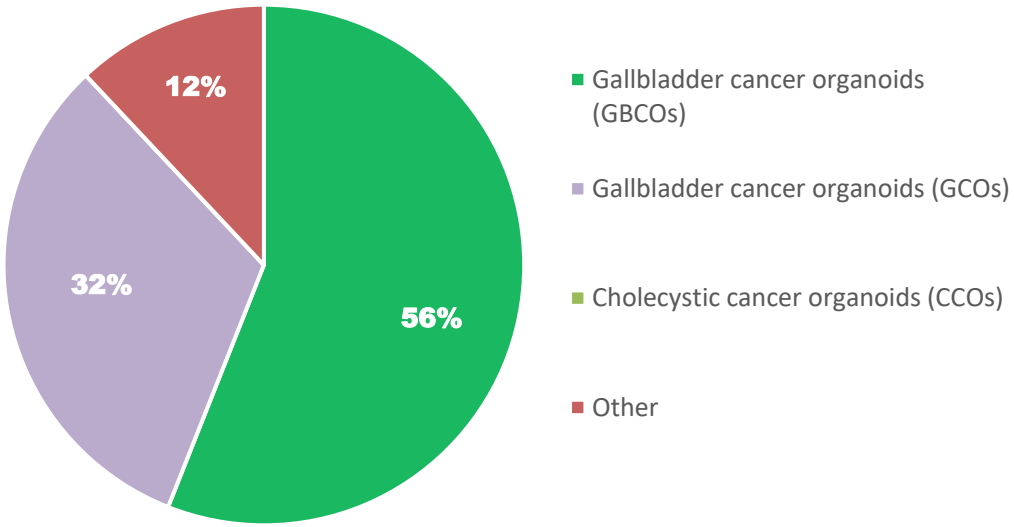
Nomenclature for extrahepatic cholangiocarcinoma-derived organoids described by Saito *et al.*?



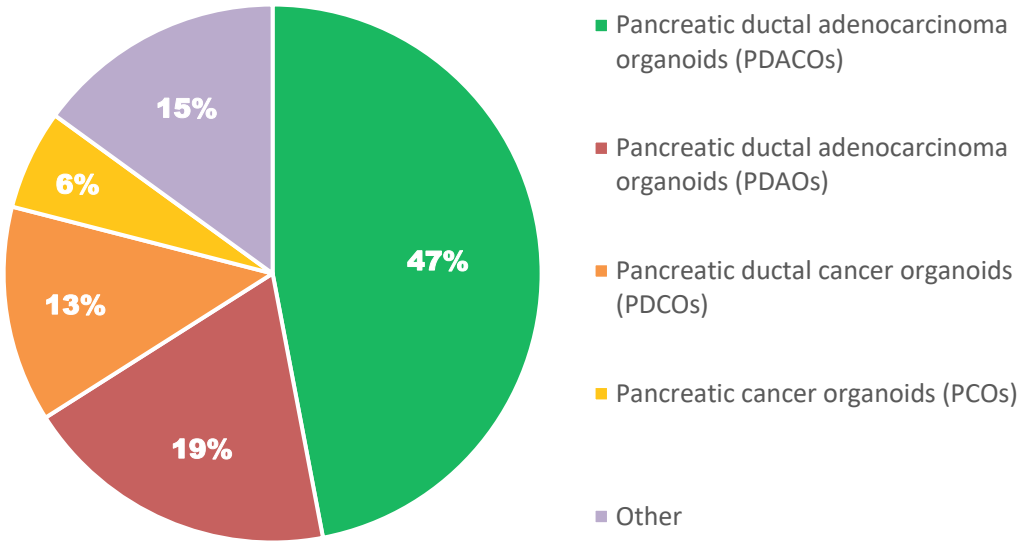
Nomenclature for hepatocellular carcinoma-derived organoids described by Broutier *et al.* and Nuciforo *et al.*?



Nomenclature for gallbladder cancer-derived organoids described by Saito *et al.*?



Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?



Questionnaire 2 Summary

Overview:

In this document you will find a summary of the results to the second questionnaire. The answers to each question are presented in a pie chart. Comments related to a particular section/question are grouped together.

The third and final questionnaire, the link to which is included in the email, was designed to reflect the results of the previous questionnaires. Modifications have been made to some questions based on the provided answers.

Please take time to review the summary of the second questionnaire before proceeding to the third. We kindly ask that you complete the third questionnaire by 28 September.

General comments made by invited experts:

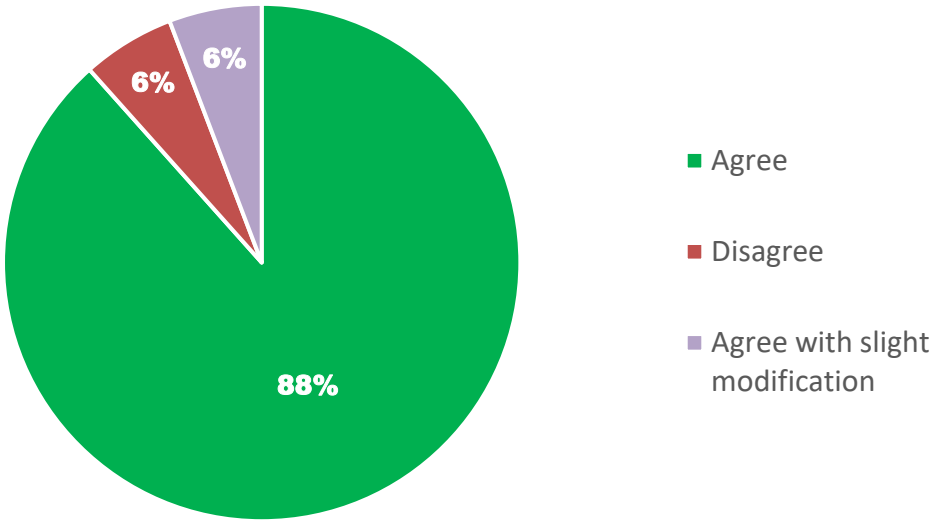
- Be consistent with nomenclature as well as precise in defining organoid systems.
- Nomenclature for tumor-derived systems should be consistent with the nomenclature of the tumors from which they are derived.
- Nomenclature could be applied to other species.

Part 1 – Definition of an Organoid

To accommodate systems which do not self-renew, for instance the “liver bud” developed by Takebe *et al.*, an over-arching definition of "organoid" could be applied along with sub-classifications. Do you agree with the over-arching definition (found below)?

Organoid

Complex 3D structure derived from (fetal/adult) primary cells, stem/progenitor cells or pluripotent stem cells (PSCs) that self-organize through cell-cell and cell-matrix interactions and spatially restricted lineage commitments to recapitulate important aspects of the native tissue architecture and function.



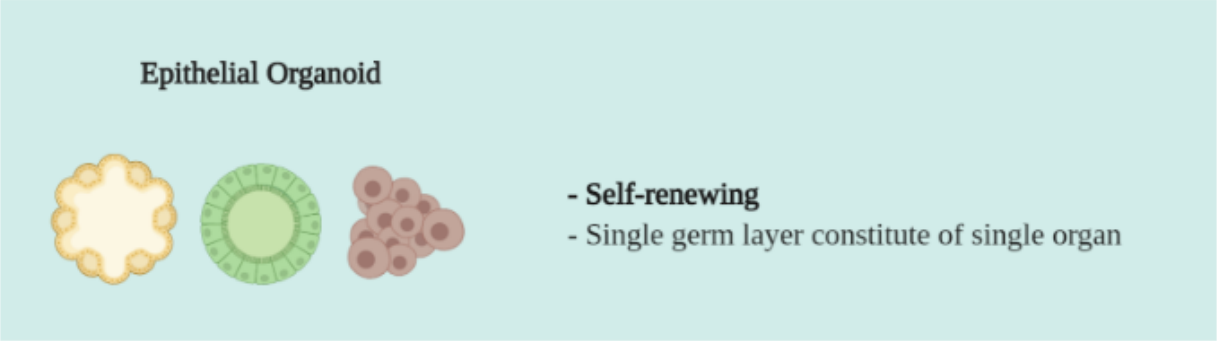
Comments:

- Avoid subjective terms, i.e. “important”

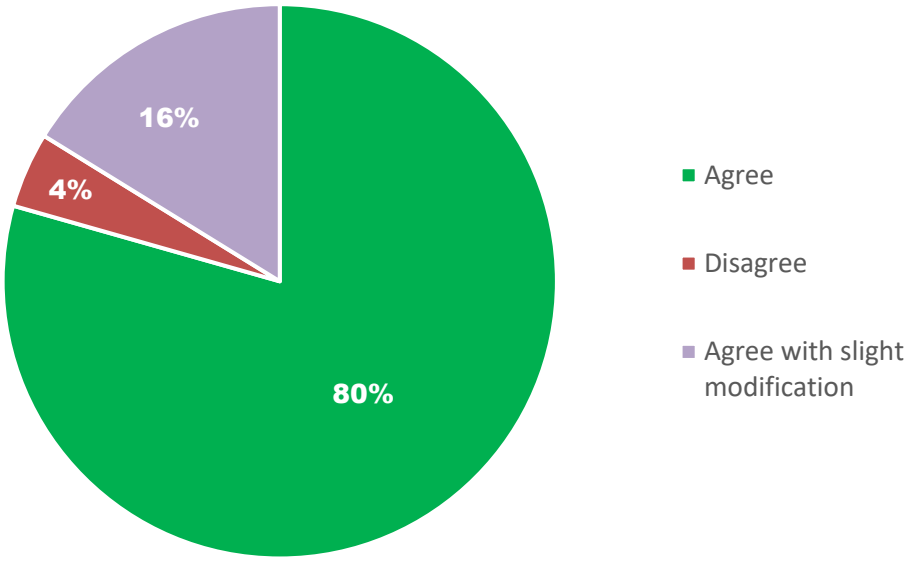
Sub-classification of Organoids

Key characteristics of an epithelial organoid:

Sub-classification 1:



Examples: Huch *et al.*, 2015; Lugli *et al.*, 2016; Sampaziotis *et al.*, 2017; Hu *et al.*, 2018



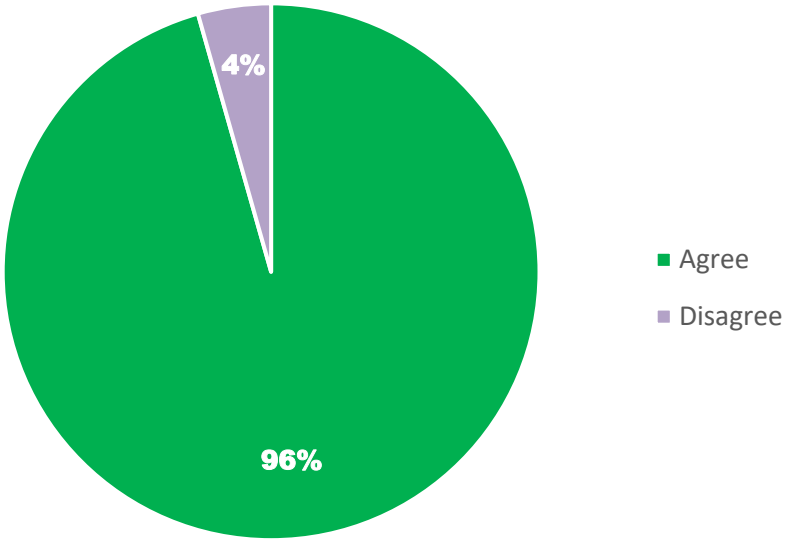
Comments:

- While self-renewal may be a dominant feature of most organoids, I would prefer to not restrict the field to this definition. Future improved culture methods may allow long-term culture of "complex 3D structures from primary cells" that are not self-renewing which, however, reliably "recapitulate important aspects of the native tissue architecture and function".
- There are both PSC-derived, ex. Akbari *et al.*, 2019 (https://www.cell.com/stem-cell-reports/fulltext/S2213-6711(19)30301-7), and tissue-derived epithelial organoids.

Key characteristics of a multi-tissue organoid:

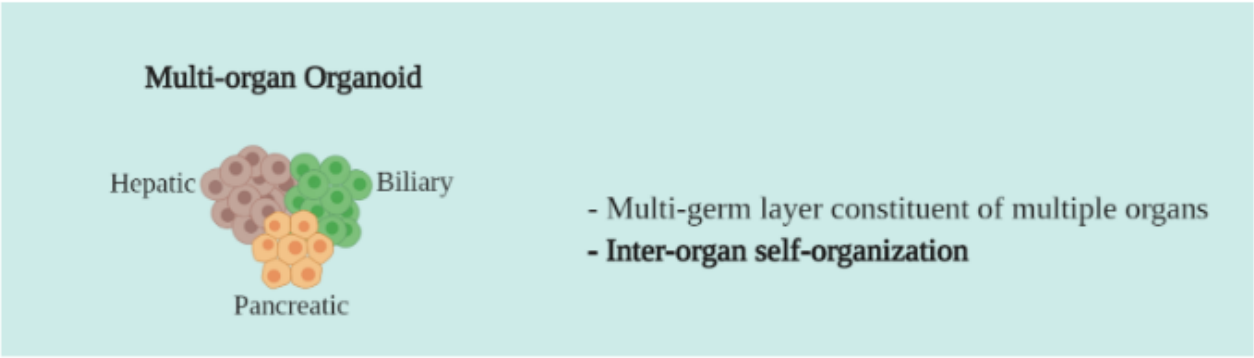
Sub-classification 2:

Examples: Takebe *et al.*, 2017; Ouchi *et al.*, 2018

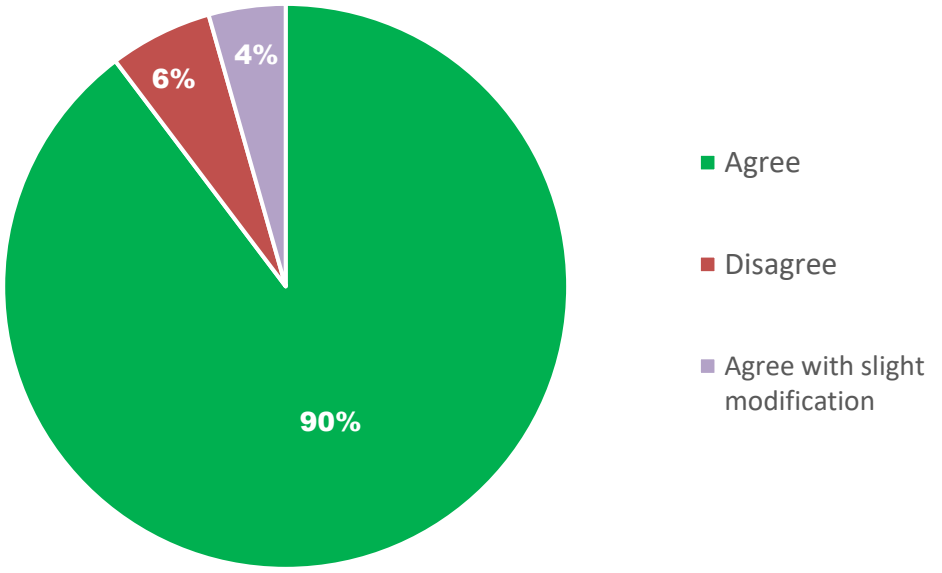


Key characteristics of a multi-organ organoid:

Sub-classification 3:



Example: Koike *et al.*, 2019

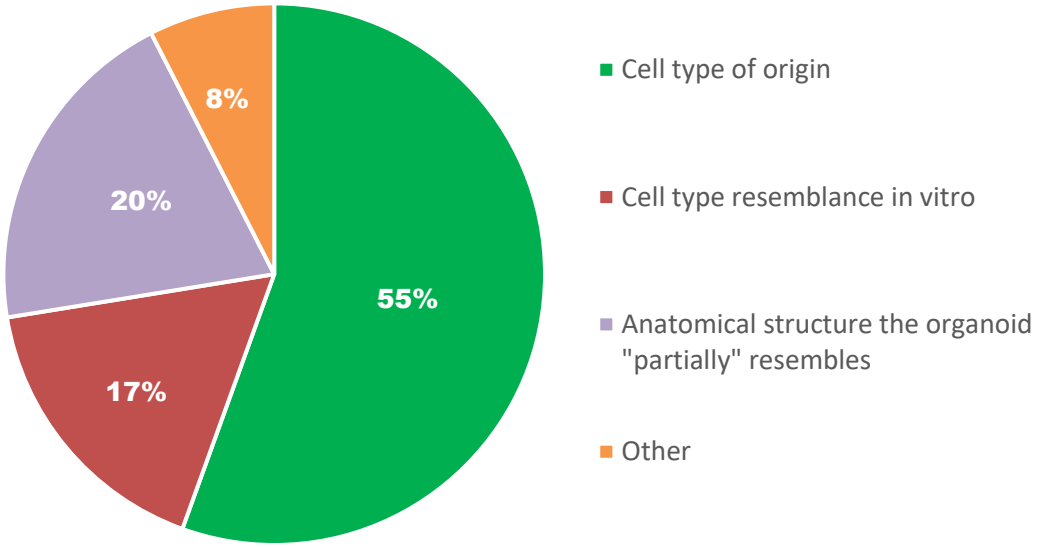


Comments:

- Does not need to be multi-germ layer per se, could also be single germ layer of multiple organs as well, i.e. epithelia from multiple organs.

Part 2 – Nomenclature for Tissue-derived HPB organoids

What is the most important aspect the nomenclature for organoids should reflect?



Comments:

- Both origin and "cell type of resemblance *in vitro*" will be equally important in future studies to appropriately interpret novel data (i.e., transdifferentiation-based organoids - pancreas-derived hepatic organoids etc.)
- A combination of *cell of origin* and *cell type of resemblance in vitro* should be used for systems capable of transdifferentiation, i.e., hepatocyte-derived cholangiocyte organoids as described in Hu *et al.*, 2018:

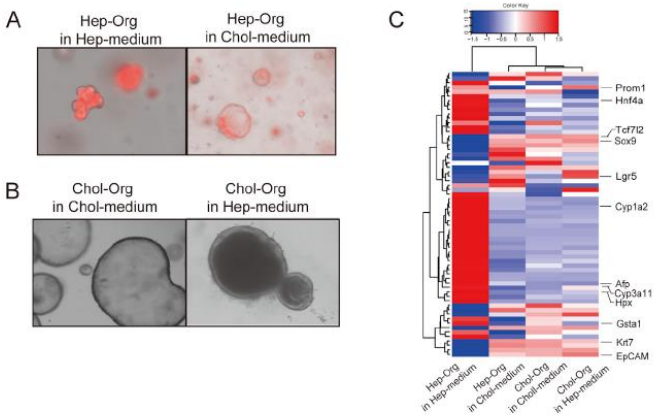
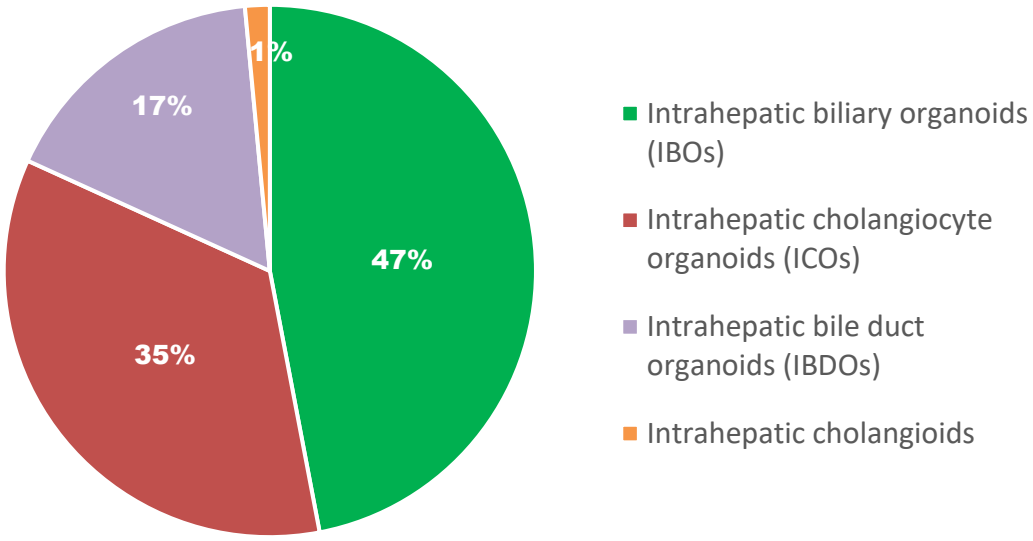
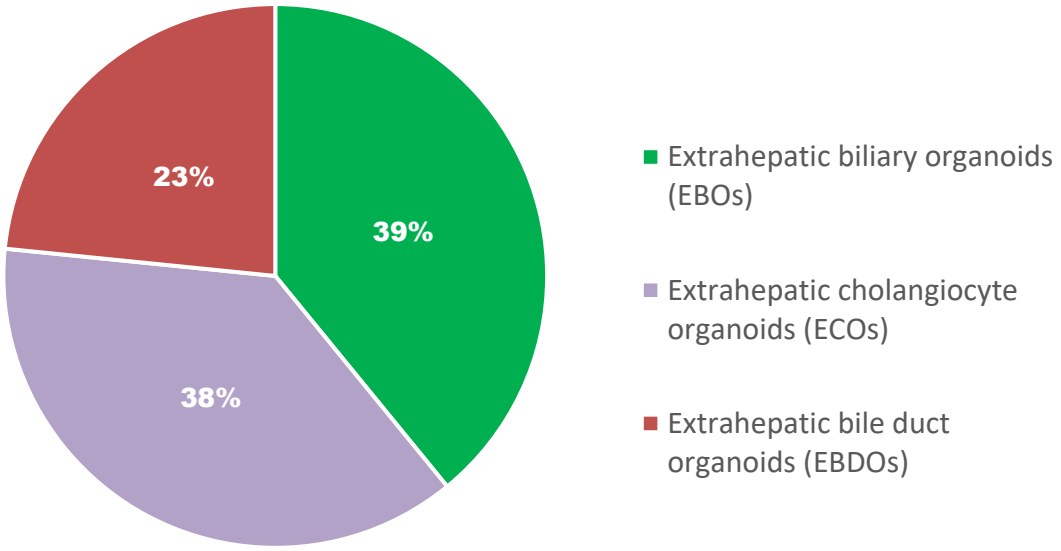


Figure S3. Hep-Orgs Transdifferentiate into Chol-Orgs in Chol-Org Medium, Related to Figure 3
(A) Fluorescent DIC images showing conversion of Tomato+ Hep-Orgs from Albumin-CreERT2; Rosa26-LSL-tdTomato into Chol-Orgs.
(B) DIC images showing typical change of Chol-Orgs in Hep-Medium.
(C) Heatmap of expression profile of RNA sequencing comparing Hep-Orgs with/without Chol-medium exposure and Chol-Orgs with/without Hep-Medium exposure for 10 days. Lane 1 and 3: Hep-Org 3 and Chol-Org 3 also appear in Figure 2I.

Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?



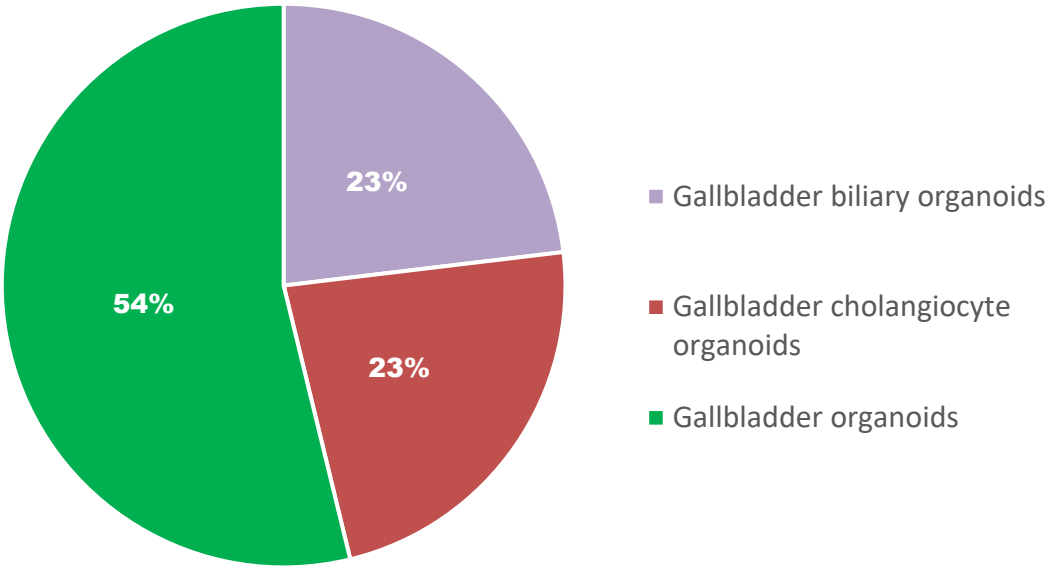
Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?



Comments:

- Biliary does not denote a cell type. The organoids come from biliary epithelial cells = cholangiocytes.
- Be consistent with nomenclature as well as precise in defining organoid systems.

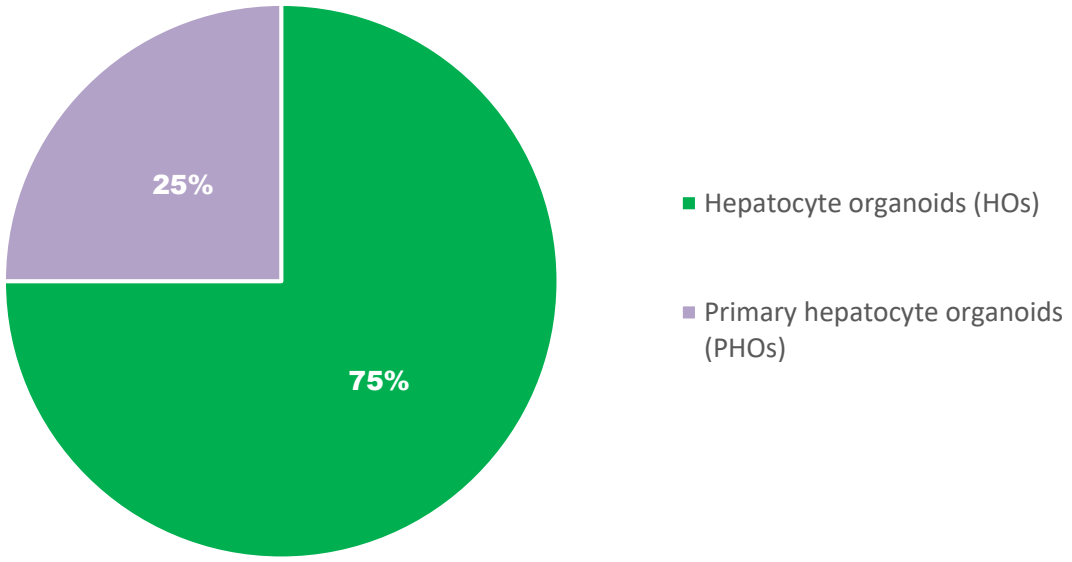
Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?



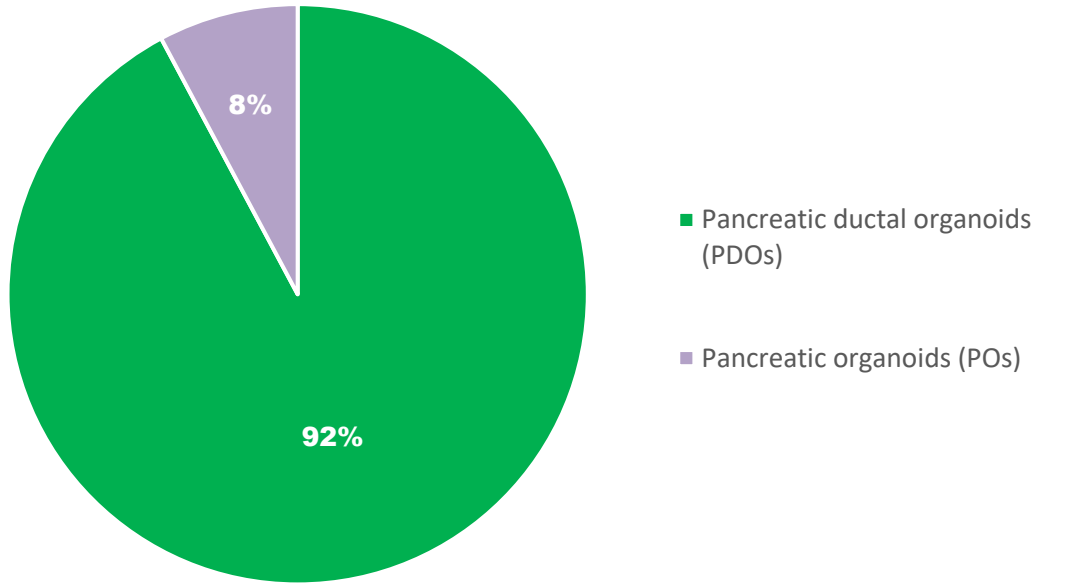
Comments:

- Gallbladder organoid does not reflect a cell type, but rather the complete organ, which is not the current *in vitro* situation. This system is derived from gallbladder cholangiocytes.

Nomenclature for hepatocyte-derived organoids described by Hu *et al.*?

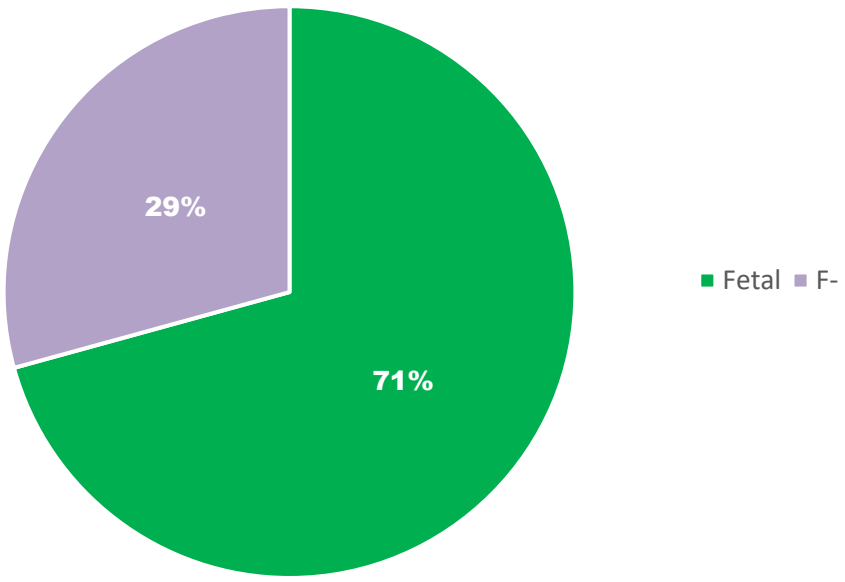


Nomenclature for pancreatic ductal-derived organoids described by Boj *et al.*?



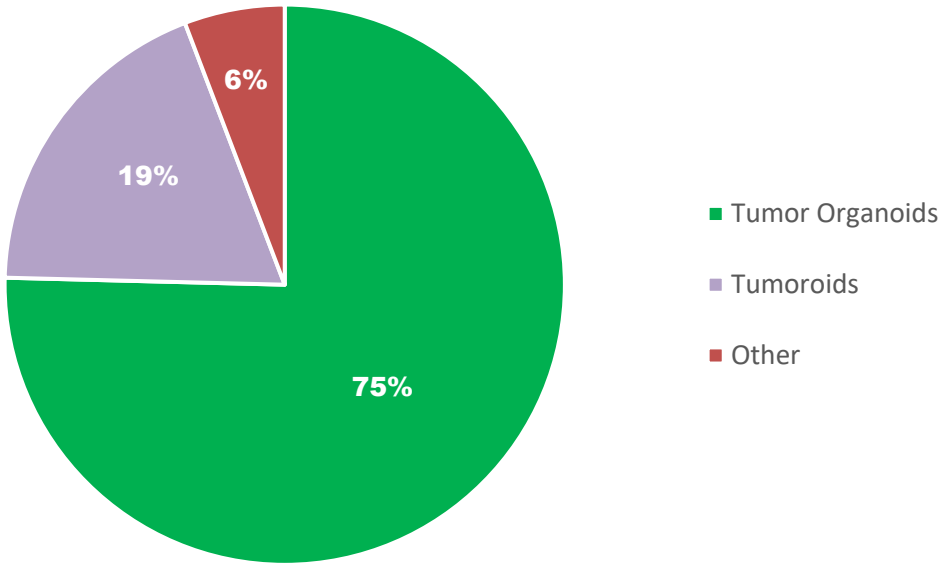
Part 3 – Fetal-derived Organoids

There are differences between fetal and adult-derived organoids. How should this be addressed in the nomenclature?



Part 4 – Tumor-derived Organoids

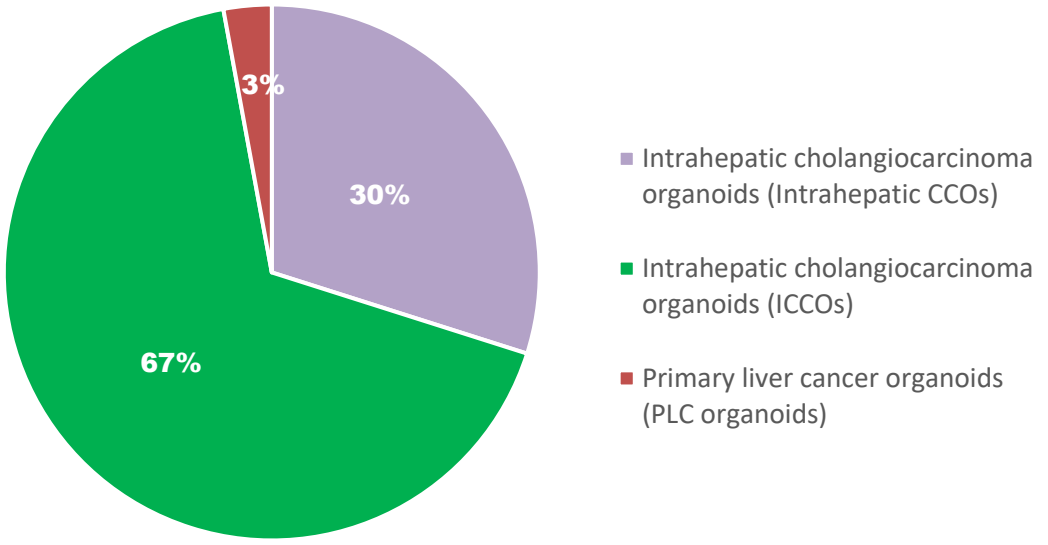
How should organoids derived from primary tumors (benign/malignant) be referred to in general?



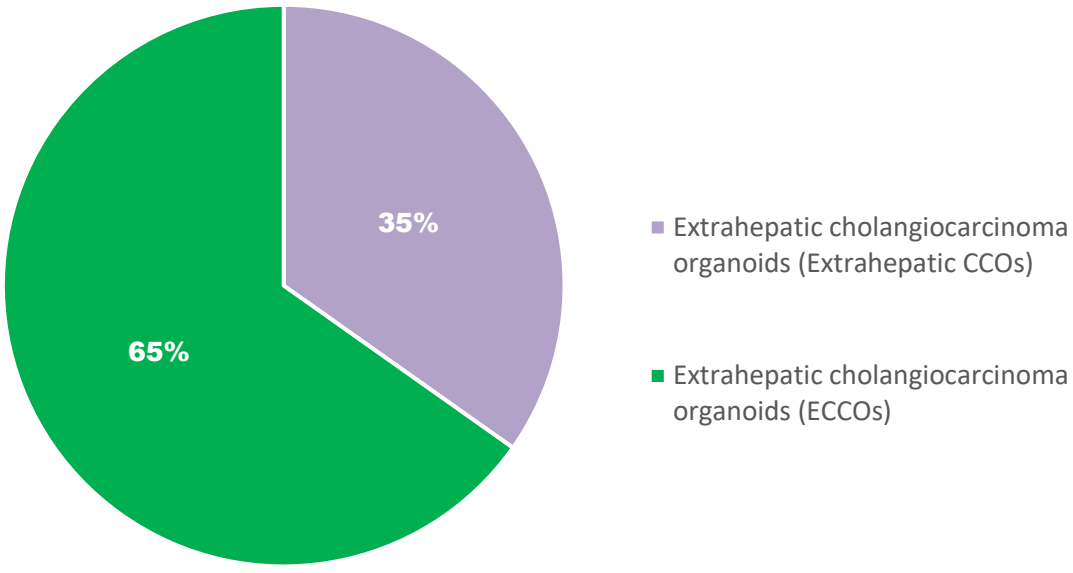
Comments:

- Broaden this subtype to include secondary tumors, which can also be cultured as organoids.
- Many cancer 3D cell models, such as cancer spheroids are referred to as tumoroids. To avoid confounding the systems avoid this term.

Nomenclature for intrahepatic cholangiocarcinoma-derived organoids described by Broutier *et al.* and Saito *et al.*?



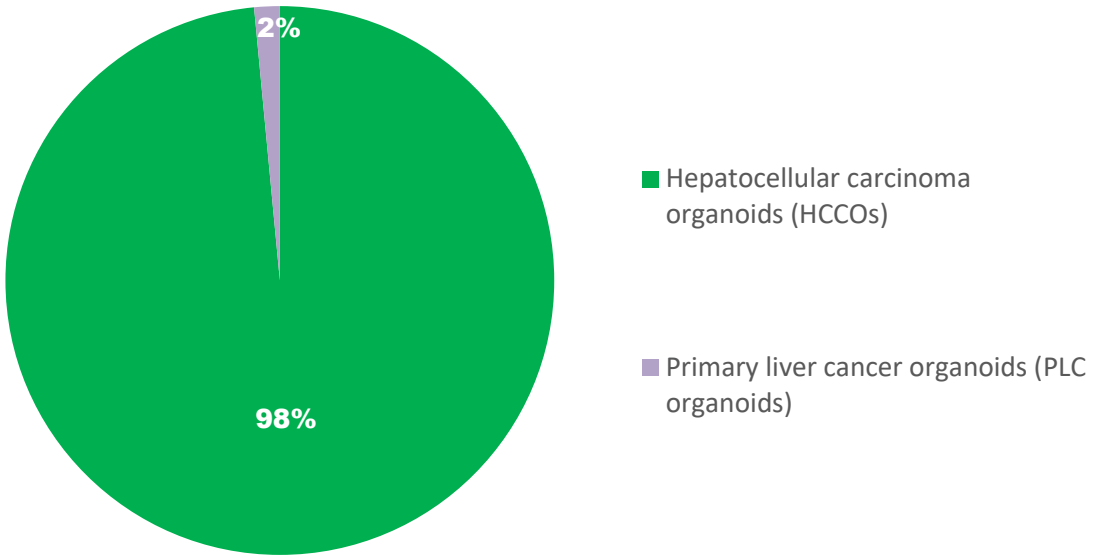
Nomenclature for extrahepatic cholangiocarcinoma-derived organoids described by Saito *et al.*?



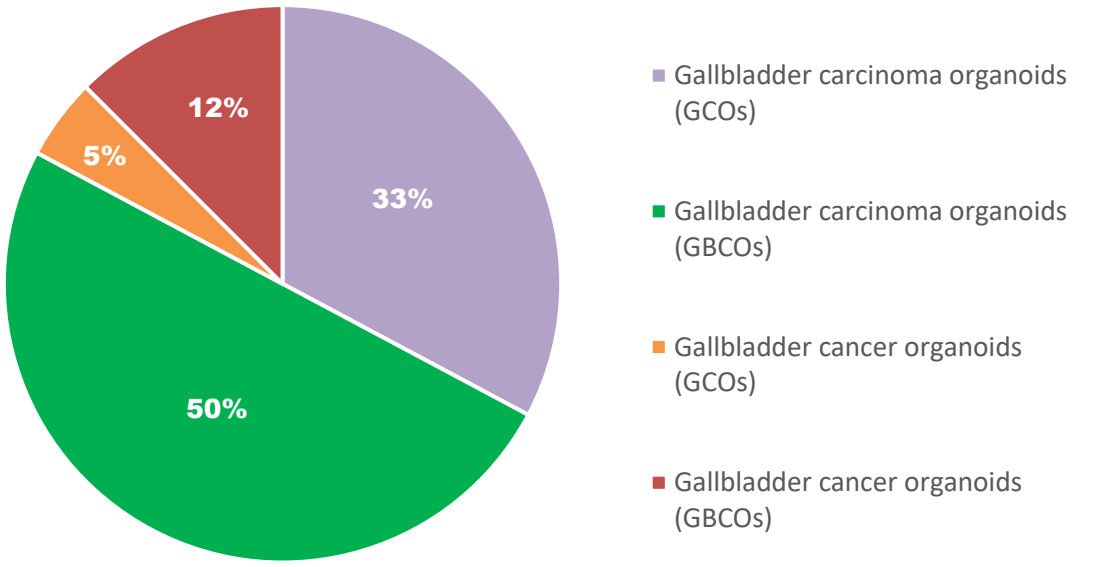
Comments:

- Cholangiocarcinoma (CCA) are divided into three subtypes depending on their anatomical location. Nomenclature for CCA subtypes is well established: intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) CCA (https://www.nature.com/articles/s41575-020-0310-z).

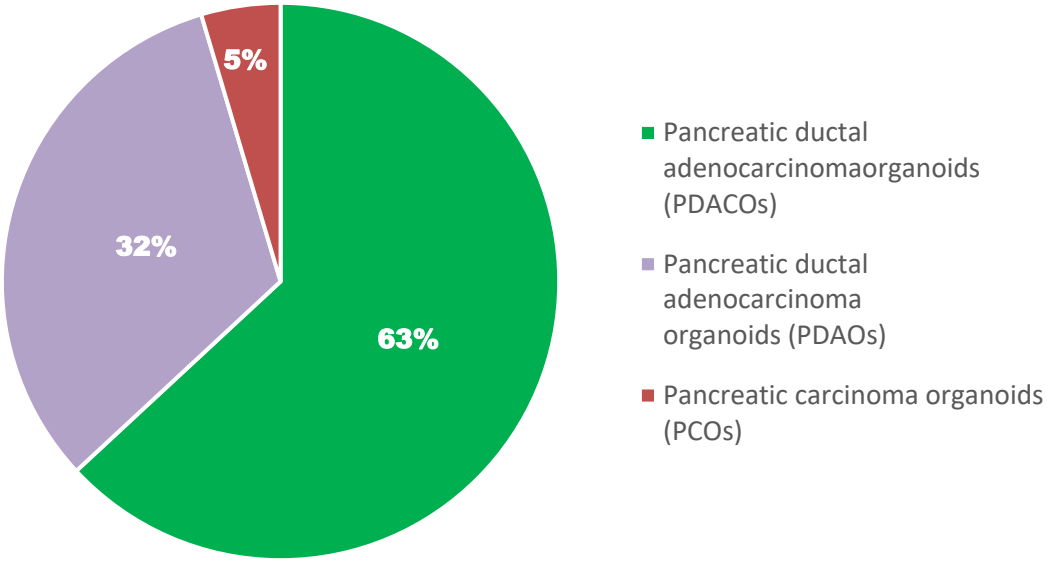
Nomenclature for hepatocellular carcinoma-derived organoids described by Broutier *et al.* and Nuciforo *et al.*?



Nomenclature for gallbladder carcinoma-organoids described by Saito *et al.*?



Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?



Once you have adequately reviewed the results of the second questionnaire please proceed to the third: https://forms.gle/C22K9N7AxM4LsVbJ6

Questionnaire 3 Summary

Overview:

Overview: In this document you will find a summary of the results to the third and final questionnaire. The answers to each question are presented in a pie chart. Comments related to a particular section/question are grouped together.

Through the three questionnaire we have reached consensus on numerous topics, including the definition and subclassifications of organoids. Topics for which consensus was not reached through the three questionnaires were deliberated by the steering committee during a round table discussion. The outcomes of this discussion are reflected in the attached manuscript.

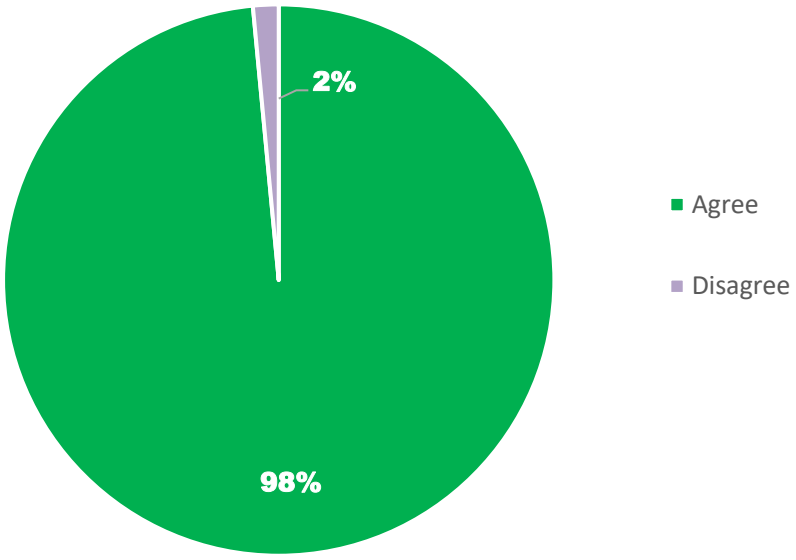
Please take time to review the results of the third questionnaire.

Part 1 – Definition of an Organoid

To accommodate systems which do not self-renew, for instance the “liver bud” developed by Takebe *et al.* as well as to make sure we create a future proof-definition for organoids, an over-arching definition of "organoid" could be applied along with sub-classifications. Do you agree with the over-arching definition (found below)?

Organoid

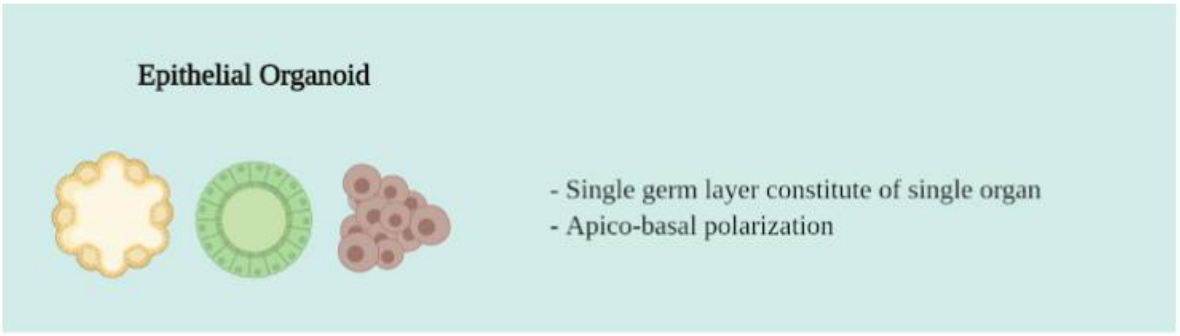
Three-dimensional structure derived from primary cells, stem/progenitor cells or pluripotent stem cells (PSCs) that self-organize through cell-cell and cell-matrix interactions to recapitulate aspects of the native tissue architecture and function.



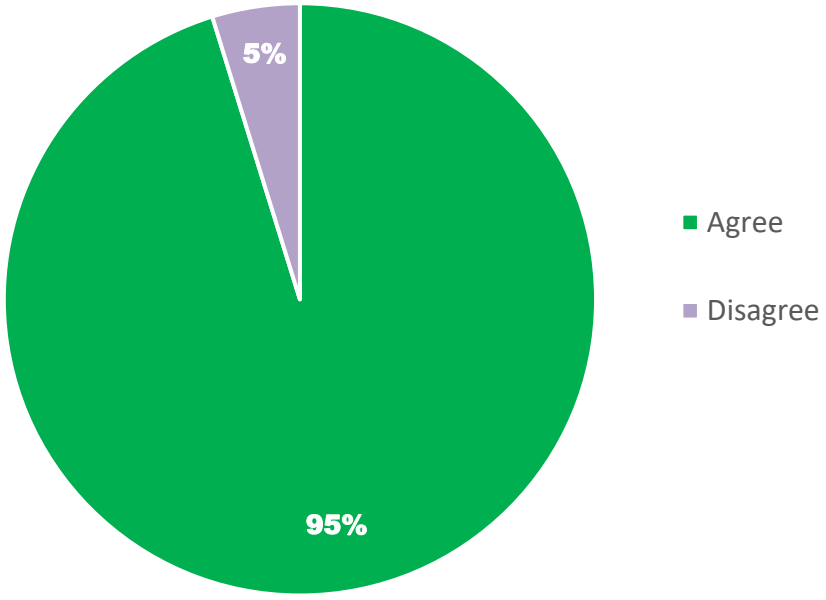
Sub-classification of Organoids

Key characteristics of an epithelial organoid:

Sub-classification 1:

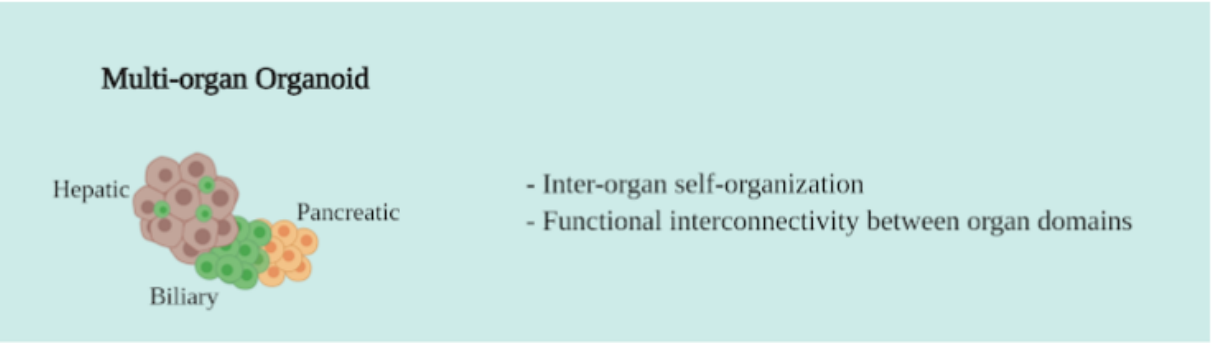


Examples: Huch *et al.*, 2015; Lugli *et al.*, 2016; Sampaziotis *et al.*, 2017; Hu *et al.*, 2018

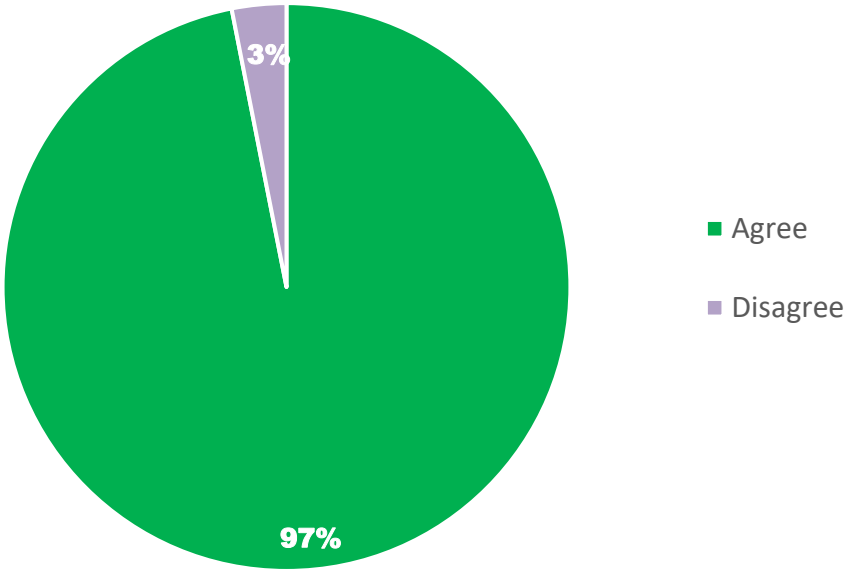


Key characteristics of a multi-organ organoid:

Sub-classification 3:



Example: Koike *et al.*, 2019

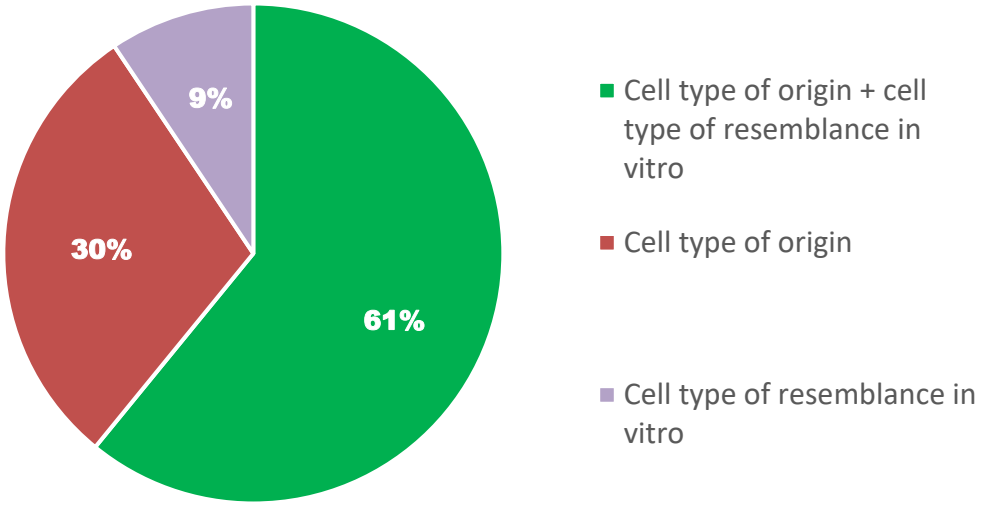


Comments:

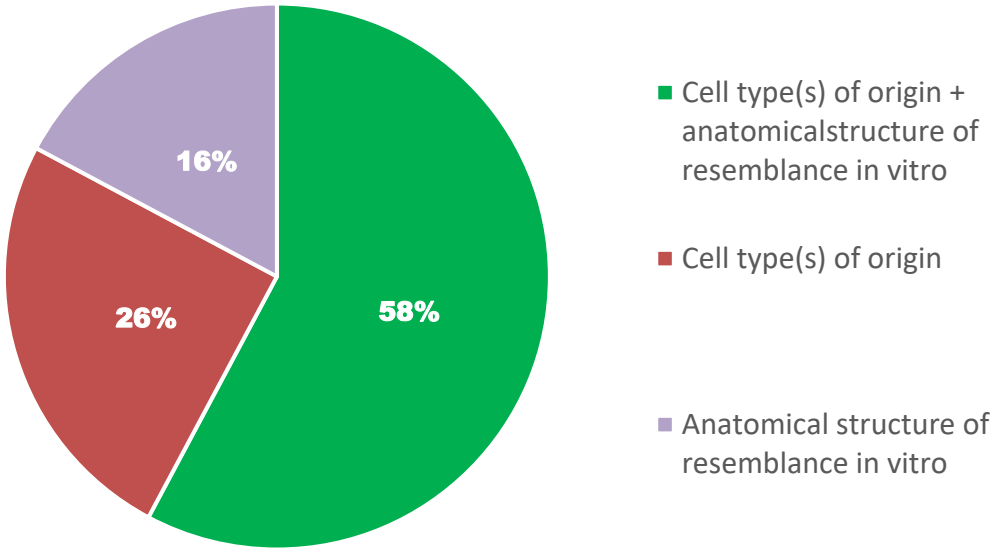
- Epithelial organoids self-renew under certain conditions
- Do organoids only exist *in vitro*?
- Apical-basal polarization might not exist in PSC-derived organoids
- Replace functional with self-organization in multi-organ organoids

Part 2 – Nomenclature for Tissue-derived HPB-organoids

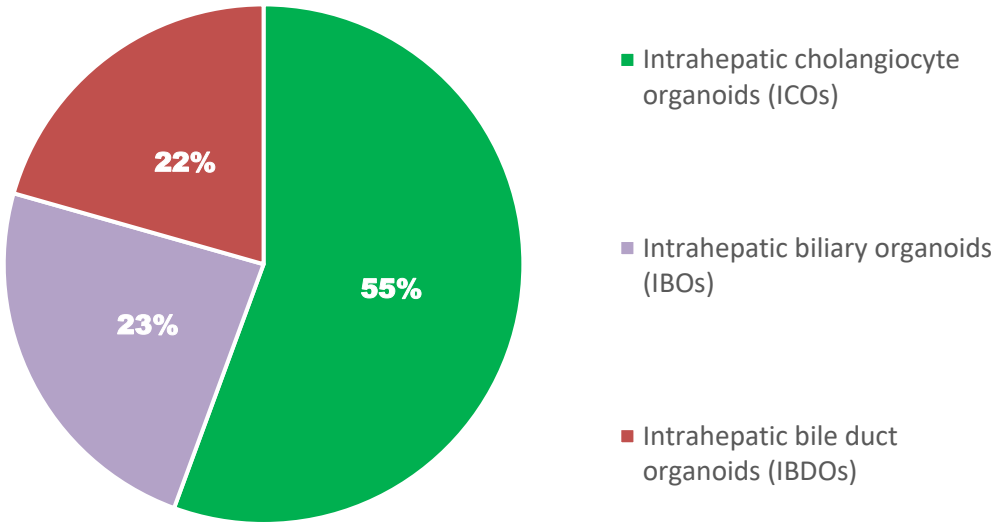
What is the most important aspect the nomenclature for single cell type systems should reflect?



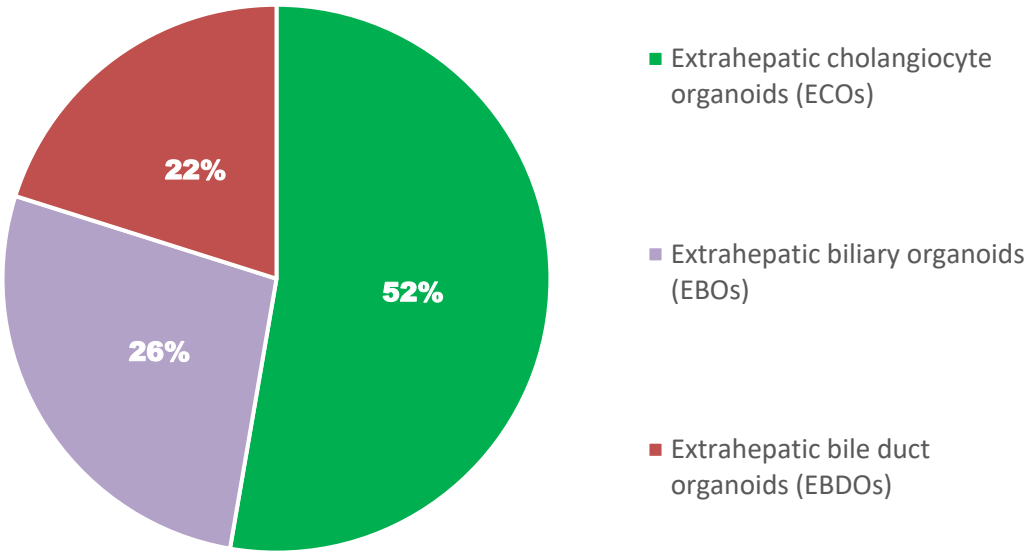
What is the most important aspect the nomenclature for multi-cell type systems should reflect?



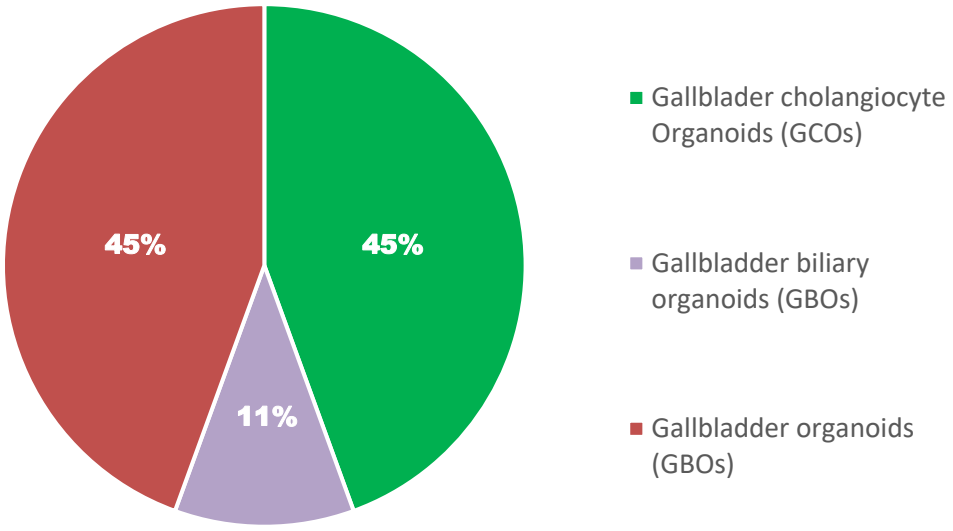
Nomenclature for intrahepatic bile duct-derived organoids described by Huch *et al.*, Tysoe *et al.* and Rimland *et al.*?



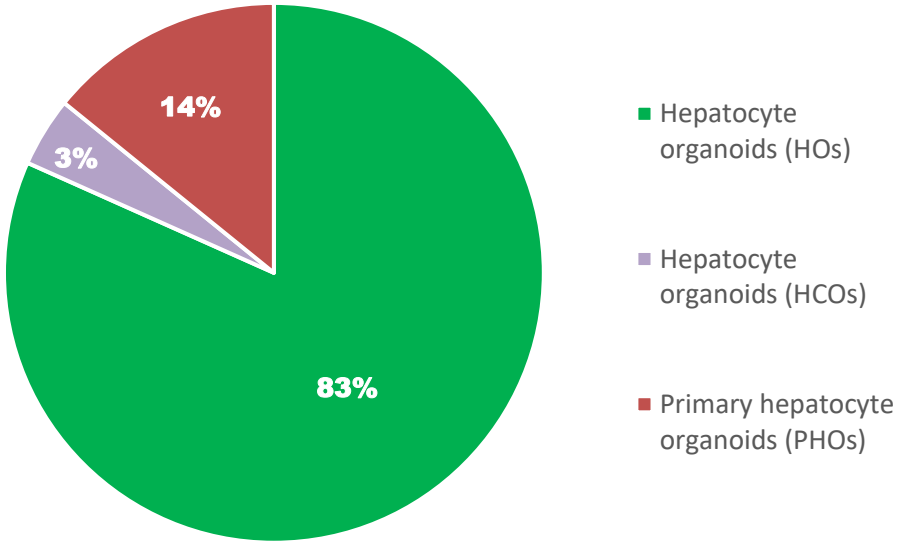
Nomenclature for extrahepatic bile duct-derived organoids described by Sampaziotis *et al.* and Rimland *et al.*?



Nomenclature for gallbladder-derived organoids described by Lugli *et al.* and Rimland *et al.*?



Nomenclature for mouse hepatocyte-derived organoids described by Hu *et al.* and Peng *et al.*?

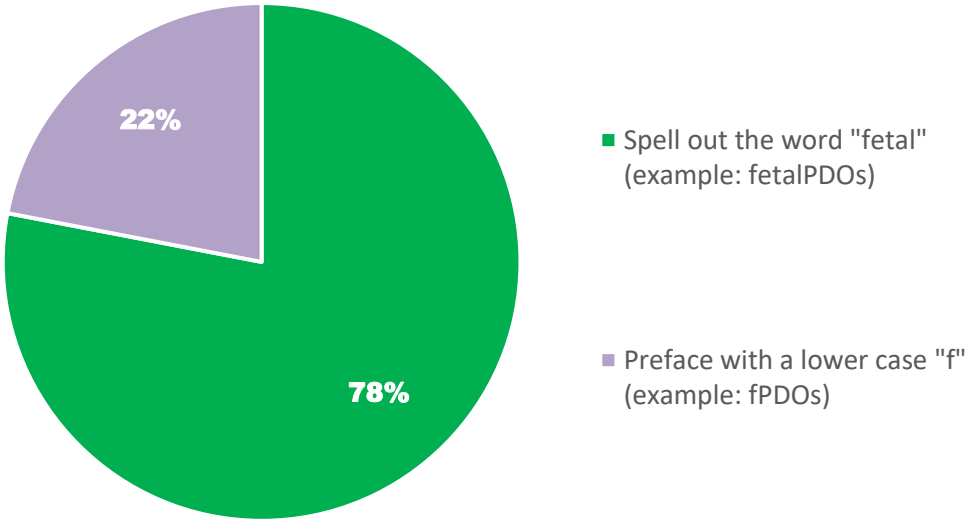


Comments:

- For gallbladder-derived organoids cell-of-origin has never been determined
- It is essential that we should define criteria for certain-organoid types
- For human hepatocyte organoids cell-of-origin has never been determined

Part 3 – Fetal-derived Organoids

There are differences between fetal and adult-derived organoids. How should this be addressed in the nomenclature?

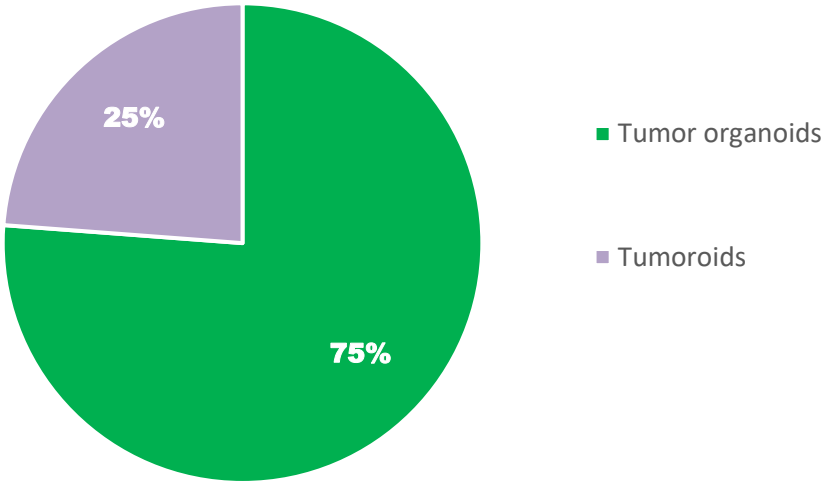


Comments:

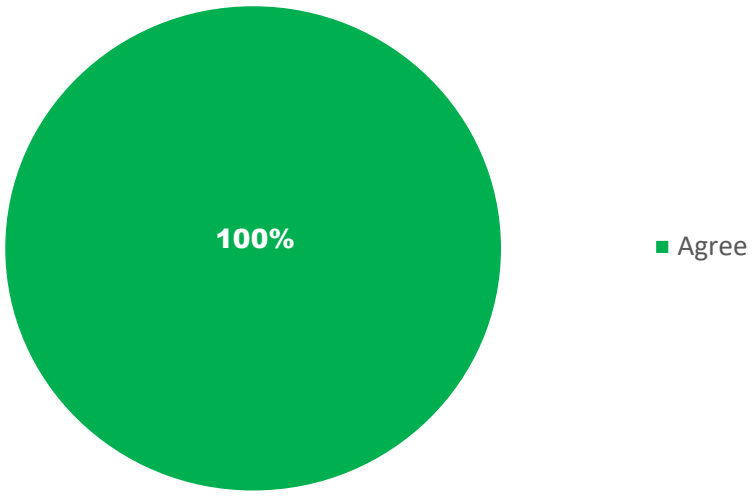
- Spelling out avoids confusion

Part 4 – Nomenclature for Tumor-derived Hepatic, Pancreatic and Biliary Organoids

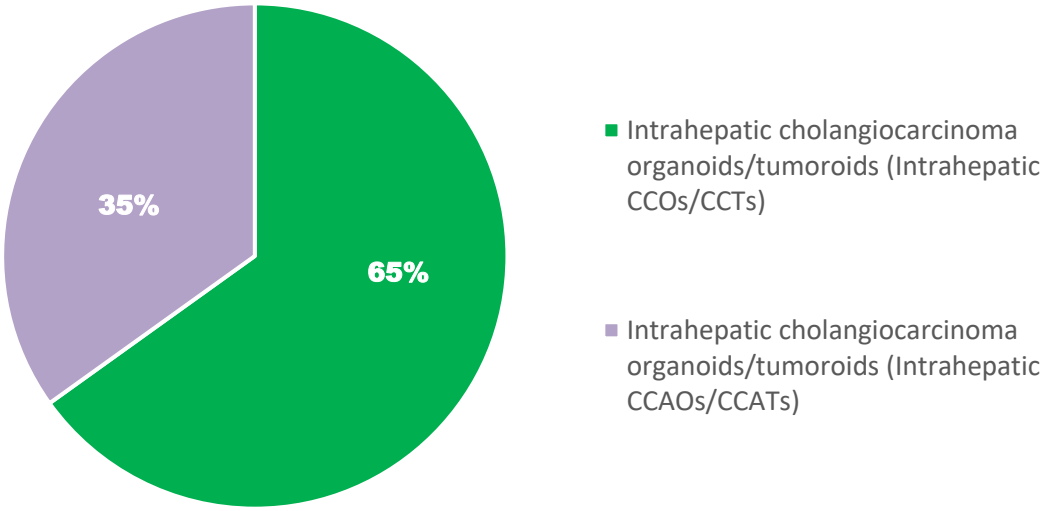
How should organoids derived from primary or secondary tumors be referred to in general?



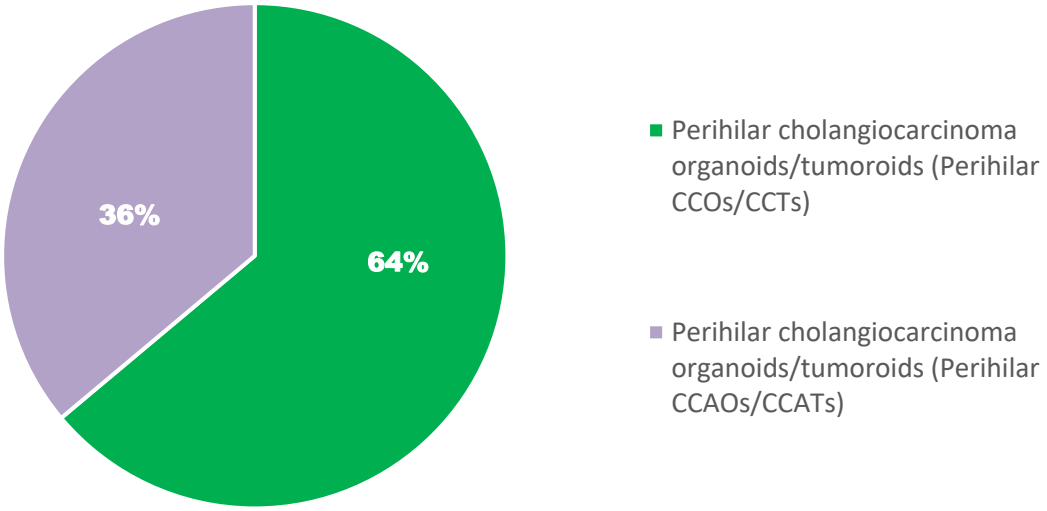
Cholangiocarcinoma (CCA) include a diverse group of malignancies of the biliary tree, which can be divided into three subtypes depending on their anatomical location. Nomenclature for CCA subtypes is well established: intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) CCA. To reflect this in the nomenclature for organoids we have adapted the questions for CCA derived organoid systems:



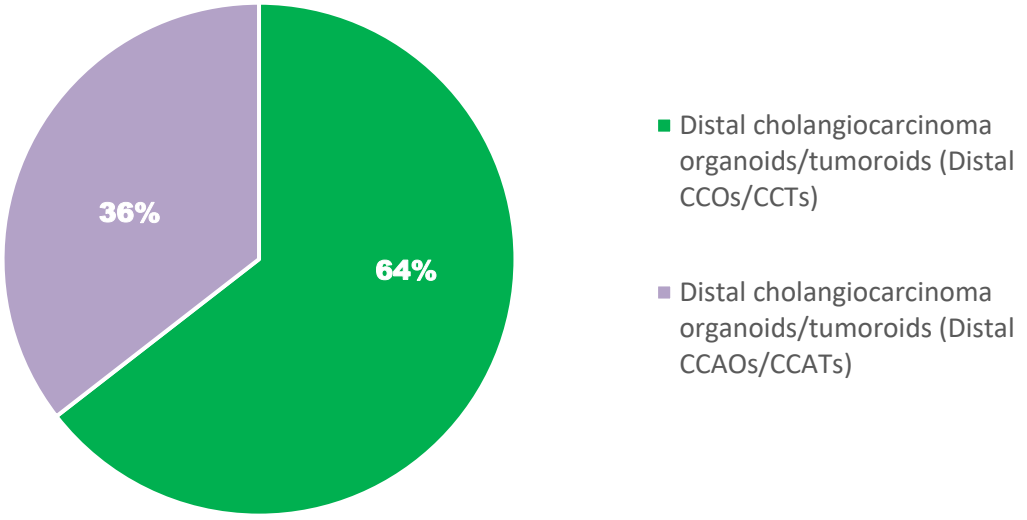
Nomenclature for intrahepatic cholangiocarcinoma-derived organoids?



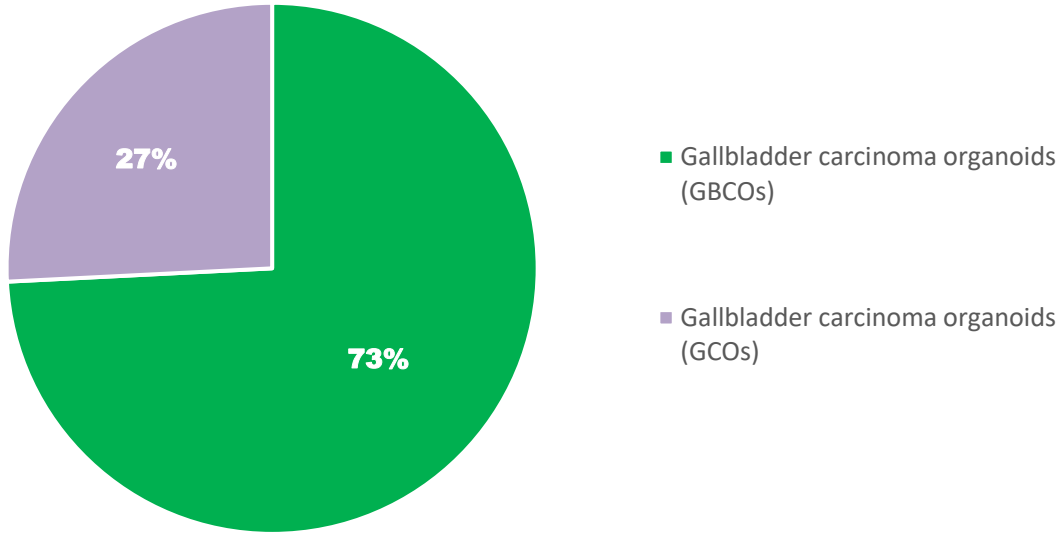
Nomenclature for perihilar cholangiocarcinoma-derived organoids?



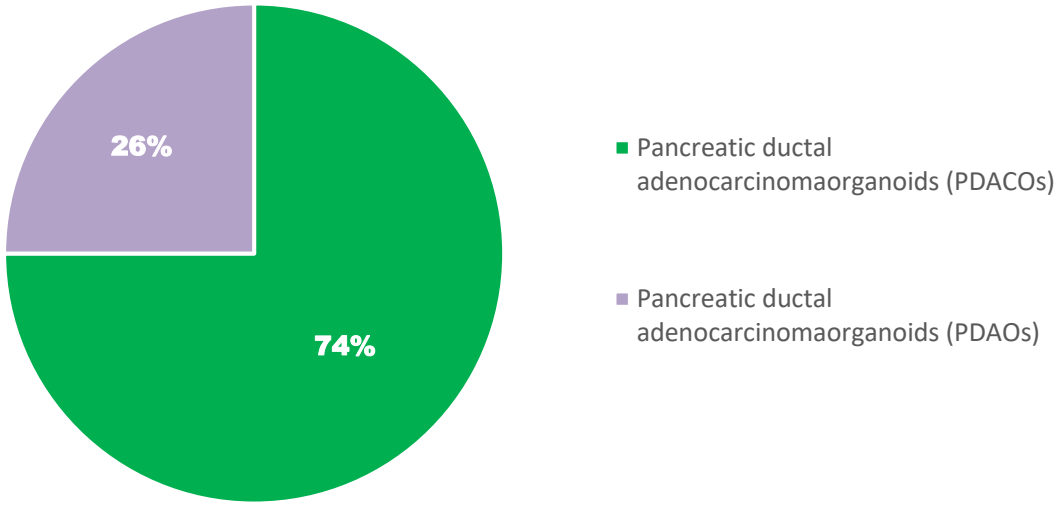
Nomenclature for distal cholangiocarcinoma-derived organoids?



Nomenclature for gallbladder carcinoma-derived organoids?



Nomenclature for pancreatic ductal adenocarcinoma organoids described by Boj *et al.*?



Comments:

- What are functions of the native tumor tissue that could be recapitulate *in vitro*?