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Patient-derived organoids from pancreatic cancer after pancreatectomy: Feasibility and organoid take rate in treatment-naïve periampullary tumors☆

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ABSTRACT

Background/objective: Patient-derived organoids (PDOs) have emerged as essential for ex vivo modelling for pancreatic cancer (PDAC) but reports on efficacy and organoid take rate are scarce. This study aimed to assess the feasibility of establishing PDOs from resected specimens in periampullary tumors.

Methods: Patients undergoing surgery for suspected periampullary cancer were included. PDO protocol amendments were tested, with organoid take rate as outcome measure. Samples from resected specimens were processed and expanded per protocol. Pooled estimate of take rates of PDOs in PDAC was derived from literature search.

Results: 23 specimens were available for PDO, of which 10 were PDAC. In 15 patients other histopathology was found: neuroendocrine tumors (NET; n = 2), neuroendocrine carcinoma (NEC; n = 1), intraductal papillary mucinous neoplasm (IPMN; n = 4), distal cholangiocarcinoma (dCCA; n = 1), ampullary carcinoma (n = 1), duodenal carcinoma (n = 1), intra-ampullary papillary tubular neoplasm (IAPN; n = 1), indeterminate PDAC/ampullary carcinoma(n = 1), and one patient with chronic inflammation/fibrosis. Organoid cultures were grown from 7 of 10 (70 %) PDAC, 1 dCCA, 1 NEC, 1 duodenal carcinoma, 1 indeterminate tumor type and 1 ampullary carcinoma (i.e. 12/18; 66.7 % across periampullary cancers). Overall take rate of PDOs was 12 of 23 (52.2 %) for all tumors. A pooled mean estimate PDO take rate of 62.3 % (95 % CI:54.8–69.3 %) was reported across available studies in the literature.

Conclusion: In the current study, we found that PDOs could be established from resected pancreatic tumors in over half of resected periampullary tumors, and highest in PDACs. As such, generating a pancreatic cancer PDO biobank for translational research was feasible after cryopreservation.

1. Introduction

Compared to several other solid organ cancers, pancreatic adenocarcinomas have shown only modest gains in survival over the last decades. Even among the patients receiving surgical treatment, the overall 5-year relative survival rate is reported at 8–13 % [1]. A deeper understanding of the specific tumor biology in pancreatic cancer is needed to improve treatments and obtain better systemic disease control.

One important asset to pancreatic cancer research is in vitro

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models [2]. Traditionally, cell culture used for cancer research have been far removed from the in vivo tumor environment, as they have been growing in two-dimensional monolayers [3] which may limit the transferability to the original in vivo tumor [4]. By growing cells in a three-dimensional (3D) configuration to form spherical aggregates, spheroid cultures more closely resemble tissue-like structures, potentially enhancing the replication of in vivo cellular functions and interactions. We have previously shown that the use of 3D models over monolayer cultures significantly impact on cellular functions, such as cancer cell metabolism and drug sensitivity [4,5]. While the more complex models allow for testing mechanisms and treatment response in a generic fashion, they may be less applicable to the individual patient.

Patient-derived organoids (PDOs) represents the opportunity to investigate individual cancer behavior in both translational research and for direct precision medicine [6]. PDOs from patients with pancreatic cancer was established less than a decade ago [7,8]. The benefits of using PDOs for basic science and clinical research are several [9,10]. PDOs have been shown to maintain the differentiation status, histoarchitecture and phenotypic heterogeneity of the primary tumor [8], hence making them representative for the individual patient [11]. PDOs also retain patient-specific physiological changes, including hypoxia, oxygen consumption, and epigenetic marks [8]. Moreover, PDOs may also be subject to genetic manipulation and for orthotopic transplantation into animal models [6,7,9,12]. Beyond the several meaningful roles for mechanistic cancer research, the feasibility to test PDOs to drug response in (pre-)clinical trials in pancreatic cancer has been demonstrated [13,14]. Hence, PDOs have an important role in the advancement of research in cancer biology and to tailor treatment in pancreatic cancer.

However, while the establishment of organoid models may advance translational research and bridge the gap between laboratory findings and clinical application, the methodology is not well-established outside a few pioneer and expert centers. Of note, the establishment of PDOs outside specialized laboratories is not known nor is the actual organoid take rate in a clinical setting, as it may vary with pre-sample treatment (chemotherapy naïve or pretreated samples), location of tumor (primary tumor, metastasis or ascites) [15] and sample size (resected specimen or biopsies) [16–18]. In one study [10], organoid success was achieved in 75 % (24 of 32) resection specimens, with an overall organoid take rate of 77 % from all tissues (either biopsies or resected specimens) procured in one expert organoid lab. Others have reported organoid take rate in 71–76 % of resected specimens and 53–56 % of biopsies from pancreatic ductal adenocarcinoma (PDAC) [19]. However, other report lower organoid take rates [20], with variation in growth rate and formation of organoids attributed to variations in growth conditions and sampling [21]. While this variation have led researchers to investigate ways of improving the overall output [22], the majority of these efforts continue to adhere to the foundational methodologies established by Boj et al. in their seminal 2015 paper [7].

Thus, the primary objective of this study was to evaluate the feasibility and take rate of establishing patient-derived organoids (PDOs) from patients resected for periampullary tumors, and to develop robust in-house protocols for tissue sampling, PDO generation, maintenance, and biobank procurement for translational research.

2. Materials and methods

2.1. Ethical approval and patient consent

The study protocol, encompassing patient recruitment and the utilization of clinical data and tissue samples, received approval from the Regional Committee for Medical and Health Research Ethics, Southeast Norway (REK Sør-Øst D, reference number #2022/494778) as part of a biobank project (GITAN project REK Vest#2013/1502), and was conducted in accordance with the Declaration of Helsinki.

Patients aged >18 years scheduled for pancreatectomy (e.g., pancreatoduodenectomy, distal resection or total pancreatectomy) for suspected periampullary cancer at Stavanger University Hospital, Norway, were eligible for inclusion. As per the universal health care system and within a single-payer, governmental funding program, the routine work-up was done according to the national guidelines for pancreatic cancer, following the proposed diagnostic pathways endorsed by the Norwegian Directory of Health. The diagnostic work-up included standard diagnostic radiology contrast-enhanced computed tomography (CECT), clinical assessment and laboratory tests, including tumor biomarkers such as Ca19-9 and CEA, and multidisciplinary tumor board evaluation. For upfront resectable pancreatic head tumors neoadjuvant therapy was not the preferred standard of care and hence, endoscopic ultrasound (EUS) with biopsy was only entertained if diagnostic uncertainty indicated further evaluation prior to surgery. For jaundiced patients, pre-operative biliary stenting was avoided if patients could be taken directly to surgery after proper imaging studies, unless clinical symptoms indicated need for pre-operative biliary decompression (i.e. signs of cholangitis; poor clinical performance status; presence of renal failure etc). A thorough assessment ensured each patient's eligibility for inclusion in the study when operability and resectability was decided. Inclusion criteria included patients who provided informed written consent and retained the right to withdraw from the study at any time, with the assurance that their data and materials would immediately be deleted, should they wish to withdraw.

2.2. Initial sample collection and processing

The surgical specimen was sent for routine gross evaluation immediately after resection (Fig. 1A). Upon receipt, a certified pathologist with expertise in pancreatic pathology conducted a thorough macroscopic examination, ensuring accurate handling of the surgical specimen for routine diagnostics and biobanking of fresh frozen tissue. When the gross evaluation did not permit definitive tumor assessment, diagnostic accuracy was prioritized to safeguard the integrity of routine specimen sampling and staging.

Afterwards, when the tumor was identified and sectioned (Fig. 1), excision biopsies were systematically obtained from three distinct areas for organoid creation: two from the tumor's periphery and one from the core. Each sample, approximately 0.1 cm^3, was placed into individually labeled 50 mL tubes containing 45 mL of cold basal media and kept on wet ice to maintain optimal conditions. The biopsies were then promptly dispatched for transport to the organoid laboratory at the University of Stavanger to ensure minimal tumor ischemia times. After biobanking for fresh-frozen tissue and organoids, the gross surgical specimen was formalin fixated per clinical routine for at least 48 h before macroscopic examination with routine sectioning and histopathological evaluation per standard template.

2.3. Organoid culture

2.3.1. Cell media

Basal media comprised Advanced DMEM/F12 medium (Thermo Fisher Scientific), supplemented with HEPES 10 mM (Thermo Fisher Scientific), GlutaMAX 1X (Thermo Fisher Scientific), Penicillin-Streptomycin 1 % (Biowest).

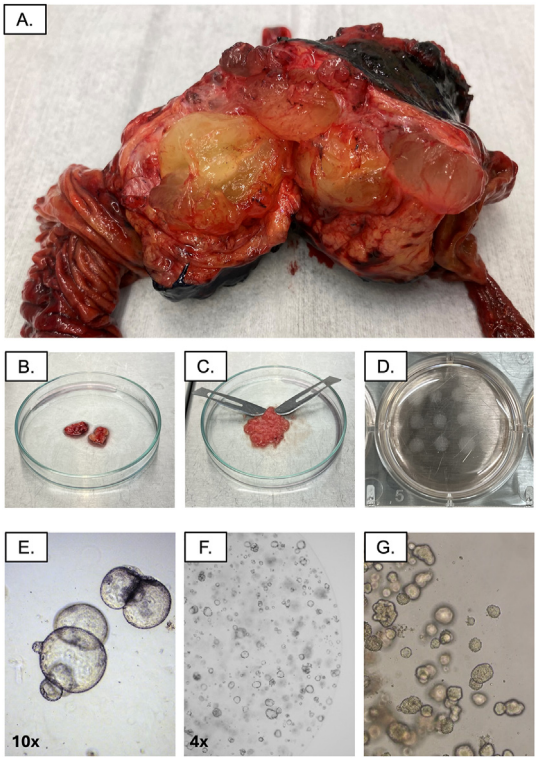

Fig. 1. Steps in specimen procurement and sample preparation. Demonstrated here is A) a pancreatoduodenectomy specimen, B) tissue harvest, C), tissue mincing, and D) organoid growth, with E, F and G representing organoids growing during different time phases.

Digestion media was prepared from basal media, with the added collagenase II 5 mg/mL (Gibco), DNASE I 10 μg/mL (Sigma-Aldrich) and Y-27632 Rho kinase inhibitor 10.5 μM (Sigma-Aldrich).

Conditioned media was derived from LWRN cell (ATCC CRL-3276) producing Wnt3a, R-spondin 3 and noggin. Briefly, CRL-3276 cells were expanded in selection media made of DMEM (Thermo Fischer Scientific) and FBS 10 % (Biowest), further containing G418 0.5 mg/mL (Gibco) and Hygromycin-B 0.5 mg/mL (Gibco) and subsequently cultured in ADMEM/F12 supplemented with FBS 10 % and Penicillin-Streptomycin 1 %. The conditioned media was harvested, centrifuged, and sterile filtered.

Complete organoid growth media consisted of equal parts conditioned media and basal media, supplemented further with B27 1X (Thermo Fisher Scientific), nicotinamide 10 mM (Sigma-Aldrich), N-acetylcystein 1.25 mM (Sigma-Aldrich), EGF 50 ng/mL (PeproTech), FGF-10 100 ng/mL (PeproTech), gastrin 10 nM (Tocris Bioscience), A83-01 500 nM (Tocris Bioscience), and if recently passaged Y-27632 10.5 μM.

The freezing medium was prepared by combining 70 % complete organoid growth media (supplemented with Y-27632 at 10.5 μM) with 20 % FBS and 10 % DMSO (Apollo Scientific).

2.3.2. Extracellular matrix

Cells were encased in Engelbreth-Holm-Swarm (EHS) matrices, specifically Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2 (BioTechne). Cultrex solutions were aliquoted and stored at −80 °C and thawed prior to each use.

2.3.3. Tumor tissue processing

Sample processing, outgrowth and organoid culture protocols are adapted from previous reports [23,24]. Upon arrival of tumor tissue samples at the organoid lab, samples were placed in a 10 cm glass Petri dish and covered with 1–2 mL of basal media to prevent dehydration. Using two No. 10 scalpel blades, the tumor was dissected into 0.5–1 mm^3 fragments (Fig. 1C). These fragments were further immersed in 10 mL basal media and transferred to a 15 mL tube for centrifugation in a spin out rotor at 300×*g* for 5 min at 4 °C.

After resuspension in 2 mL digestion media, the cells were incubated at 37 °C in 5 % CO2, agitated manually every 5 min for 30–60 min, until dissociation into mostly single cells (>80 %) was observed under the microscope. The cell suspension was filtered through a 70 μm mesh filter to remove larger aggregates. Following two rounds of washing and centrifugation (300 g for 5 min at 4 °C) in pre-cooled basal media, the cell pellet was resuspended in ice-cold extracellular matrix (ECM), at a volume determined by cell count/estimation to 2000 cells/μL ECM, typically resulting in a 200 μL ECM-cell mixture. This mixture was plated in 10 μL domes (total 100 μL per well) in a 6-well plate. Immediately after seeding the ECM-cell mixture, the plate was inverted for 3 min in the laminar hood, following 20 min of incubation at 37 °C in 5 % CO2 to allow ECM polymerization. Lastly, 2 mL of pre-warmed (37 °C) complete organoid media with Y-27632 10.5 μM was added per well.

2.3.4. Passaging and maintenance

For passaging, media was removed from each well, and domes released from the bottom using a cell scraper. Organoids were released from the ECM using ice-cold Cell recovery solution (Corning) added to at least twice the volume of ECM. The domes were transferred to a 15 mL tubes and gently pipetted to help facilitate ECM breakup. After incubation on ice (30–45 min on a shaker) and quenching with 5 mL of cold basal media, the cells were centrifuged at 300 g for 5 min at 4 °C and washed with cold basal media. Subsequently, cells were counted using Muse Cell Analyzer (Cytek) and resuspended in cold ECM for replating at 2000 cell/μL. Domes where polymerized and covered in complete growth media and incubated at 37 °C, at 5 % CO_2.

Following two passages, organoids suitable for enzymatic dissociation were treated using TrypLE (Gibco) post-ECM breakdown using Cell Recovery Solution. The dissociation process, conducted in a 37 °C water bath and monitored under an inverted microscope, was quenched with basal media when approximately 80 % single cells were achieved. The cells were then washed twice using basal media and replated in cold ECM at a 1:4–6 ratio, as previously described above, for continued culture (Fig. 1E–G).

2.4. Aseptic conditions and quality control

All procedures were conducted under aseptic conditions in a controlled laboratory environment, routinely employing mycoplasma testing to ensure sample integrity using MycoStrip Mycoplasma Detection Kit (InvivoGen).

2.5. Biobanking procedures

2.5.1. Cryopreservation

Organoids, identified in a logarithmic growth phase (typically 3–5 days post-split), were harvested for cryopreservation. One well from a 6-well plate with organoids in 100 μL ECM served as a single aliquot. The ECM was disrupted using cold Cell Recovery Solution, followed by centrifugation at 300*g* for 5 min at 4 °C. After a single wash with cold basal media, the supernatant was aspirated, and cells were resuspended in 500 μL of freezing medium. The cell solution was aliquoted into cryotubes and placed in a Mr. Frosty freezing container kept at room temperature. It was immediately

stored at −80 °C overnight to achieve a controlled rate of cooling of 1 °C per minute. Finally, it was transferred to a liquid nitrogen tank for long-term storage.

2.5.2. Thawing procedure

Cryotubes containing the organoids were removed from the liquid nitrogen tank and immediately thawed in a 37 °C water bath. The contents were then carefully transferred dropwise to a 15 mL Falcon tube pre-filled with 5 mL of cold basal medium, followed by centrifugation at 300*g* for 5 min at 4 °C. After discarding the supernatant, the cells were replated in 100 μL of ECM, allowed to polymerize, and then covered with complete growth media containing Y-27632 (10.5 μM) to enhance survival after cryopreservation. The cultures were incubated at 37 °C and 5 % CO2. Post-thaw viability was assessed visually by monitoring organoid outgrowth under an inverted microscope during the first 1–2 days.

2.6. Quality control and genetic characterization

2.6.1. DNA analysis

DNA from biobanked patient-derived tumor organoids and matched tumor tissues from formalin-fixed, paraffin-embedded (FFPE) blocks with tumor cell content greater than 10 %, identified on H&E slides by a consultant pathologist, was characterized using the Ion Torrent Genexus System with the Oncomine Precision GX Assay (Thermo Fisher Scientific), following the manufacturer's protocol. DNA from blood samples and PDOs was isolated using DNeasy Blood and Tissue Kits (QIAGEN) according to the manufacturer's user manual. DNA from FFPE tumor blocks was deparaffinized using Deparaffinization Solution (QIAGEN), followed by isolation with the DNeasy Blood and Tissue Kits (QIAGEN).

To confirm the genetic identity of the organoids and assess potential contamination, short tandem repeat (STR) analysis was performed using the GenePrint 10 System (Promega). STR profiles of the organoids were compared to those of DNA derived from patients' corresponding blood samples and FFPE tumor blocks. The analysis was conducted according to the manufacturer's guidelines, with 9 STR loci (D5S818, D13S317, D7S820, D16S539, D21S11, vWA, TH01, TPOX, CSF1PO) and the amelogenin gender-determining marker. The STR fragments were run on the 3500 Dx Series Genetic Analyzer (Applied Biosystems) and analyzed using GeneMapper Software v6.1 (Applied Biosystems). The resulting STR profiles were evaluated for consistency, ensuring the genetic authenticity of the organoid cultures and identifying any discrepancies.

2.6.2. Pathologist assessment

The initial characterization of the tumor tissue was conducted by a certified pathologist as part of the standard clinical diagnostic and staging procedure at Stavanger University Hospital using a formal gross and microscopic template, according to national and international guidelines [25,26]. This included histopathological evaluation of the tumor subtype and grade and assessment of tumor stage.

In brief, organoids, still encased within extracellular matrix domes, were fixed using 10 % paraformaldehyde. Following fixation, they underwent dehydration, clearing, and then were embedded in paraffin. The embedded samples were subsequently sectioned into 5 μm thick slices, mounted on histological slides, and stained with hematoxylin and eosin (H&E).

2.7. Literature search

A systematic literature search was conducted to identify studies reporting on patient-derived organoids from resected pancreatic cancer, excluding those focused on metastatic disease. The databases PubMed, Scopus and Web of Science were searched in November 2024 using the search term: "organoid" AND "pancreatic cancer", limited to articles published in the last 10 years and restricted to studies published in English. Studies were included if they reported data on organoid take rate from resected specimens with sufficient detail for quantitative comparison. Exclusion criteria were studies focusing on metastatic pancreatic cancer, case reports, and non-human studies. Abstracts were screened to determine study eligibility. Data extraction was performed systematically, with an emphasis on organoid take rate, patient attempts, and success rates.

2.8. Statistical analyses

All statistical analyses were conducted using R (version 4.4.0). Data manipulation was performed with the *dplyr* package, while the *metafor* package facilitated the meta-analytical computations, including effect size estimation and heterogeneity assessment.

Descriptive statistics, including counts and percentages, were utilized to summarize patient demographics and organoid take-on rates across the included studies. For the meta-analysis, a random-effects model was employed to estimate the overall weighted mean organoid take-on rate among studies reporting on resected patients. The number of patients in each cohort, along with estimates of study variance, served as weights to account for variations in sample sized across studies. The pooled estimate organoid take-rate and 95 % confidence intervals (CIs) were calculated to assess precision. Heterogeneity among studies was assessed using Cochran's Q test, the I^2 statistic, and the tau^2 measure of between-study variance. The degrees of freedom for the heterogeneity test were determined based on the number of included cohorts.

3. Results

3.1. Organoid establishment and growth

A total of 16 patients were eligible and enrolled after informed consent, with patient demographics and clinical data presented in Table 1. In total, 23 resected specimens were available for processing of tissue for PDOs (Fig. 1). One patient was excluded due to an intraductal papillary mucinous neoplasm concurrent with PDAC (IPMN-PDAC), presenting extensively cystic disease macroscopically, but which precluded the identification of viable tumor tissue for organoid creation. All patients, except one, underwent pancreatoduodenectomy without neoadjuvant treatment. Total ischemia time until further processing was typically under 2 h, with the pathologist receiving the surgical sample within 15 min of the operative procedure.

Of the 23 specimens (Table 1), 10 were identified as PDAC (together with 1 indeterminate tumor type of PDAC and ampullary carcinoma), of which 7 successfully generated initial organoid cultures (PDAC specific organoid formation success rate 7/10 = 70 %), along with the indeterminate tumor type, one organoid derived from a distal cholangiocarcinoma (dCCA), one duodenal adenocarcinoma, one neuroendocrine carcinoma (NEC), and one ampullary carcinoma, totaling 12 successful organoid models (overall organoid formation rate 12/23 = 52.2 %, excluding non-malignant tumors 12/18 = 66.7 %). Of the 11 patients not resulting in successful organoid models, 3 were identified as PDAC, 4 as IPMN, 2 as pancreatic neuroendocrine tumor (PanNET), one ampullary carcinoma, one intra-ampullary papillary tubular neoplasm (IAPN), and one as chronic inflammation and fibrosis. Of the 12 generated organoids, 7 were successfully biobanked, totaling 4 PDACs, one duodenal adenocarcinoma, one dCCA and one NEC,

Table 1
Patient characteristics and clinical data.

PDO ID#	Sex	Age (years)	Origin	T-stage	N-stage	Patient Derived Organoids		
				pT	pN	Outgrowth	Biobank Status	Notes
1	F	65	PDAC	pT2	N1	Yes	Positive	–
2	M	70	IPMN	pTis	N0	No	–	Malignant cells absent in the specimen.
3	M	73	PDAC	pT2	N1	Yes	None	Failed to reestablish after the initial biobanking freeze
4	M	61	PDAC	pT2	N0	Yes	Positive	–
5	F	73	Duodenal adenocarcinoma	pT1a	N0	Yes	Positive	–
6	F	81	PDAC	pT3	N2	Yes	Positive	–
7	F	74	dCCA	pT3	N1	Yes	Positive	–
8	F	69	PDAC	pT3	N2	Yes	None	Failed to reestablish after the initial biobanking freeze
9	M	70	PDAC	ypT1c	N1	No	–	Few viable cells after neoadjuvant chemotherapy
10	F	78	PDAC	pT1c	N2	No	–	No tumor organoid formation detected
11	M	80	PDAC	pT1c	N0	No	–	Insufficient tumor specimen received
12	M	61	PanNET	pT2	N0	No	–	Specimen not of target tumor type
13	F	53	IPMN	pTis	N0	No	–	Malignant cells absent in the specimen
14	F	76	IPMN	pT0	N0	No	–	Malignant cells absent in the specimen
15	M	51	PDAC[a]	pT3b	N1	Yes	None	Biobanked cell line compromised by contamination
16	M	74	Ampullary carcinoma	pT3b	N1	Yes	None	Biobanked cell line compromised by contamination
17	M	57	IPMN	pT0	N0	No	–	Malignant cells absent in the specimen
18	M	48	Chronic inflammation/fibrosis	pT0	N0	No	–	Malignant cells absent in the specimen
19	F	74	PDAC	pT2	N0	Yes	None	Organoid culture failed after repeated passaging
20	M	78	PanNET	pT3	N0	No	–	Specimen not of target tumor type
21	F	67	IAPN	pTis	N0	No	–	Malignant cells absent in the specimen
22	M	62	NEC	pT3b	N1	Yes	Positive	–
23	F	79	PDAC	pT2	N2	Yes	Positive	–

F denotes 'Female'; M denotes 'Male'; PDAC denotes 'pancreatic ductal adenocarcinoma'; dCCA denotes 'distal cholangiocarcinoma'; IPMN denotes 'intraductal papillary mucinous neoplasia'; PanNET denotes 'pncreatic neuroendocrine tumor'; IAPN denotes 'Intra-ampullary papillary-tubular neoplasm '; NEC denotes 'neuroendocrine carcinoma'.

[a] One patient had an indeterminate adenocarcinoma of the pancreatic head or ampullary, labeled here as PDAC pT2 to err on the conservative side.

resulting in a final biobanking success rate of malignant tumors of 7/18 = 38.9 % (PDAC specific 4/10 = 40 %). Of the four organoid cell lines not biobanked, two failed to reestablish after initial freeze, and two were deemed compromised after STR analysis due to cellular contamination of a commercial organoid cell line grown simultaneously.

Notably, two patient samples showed successful outgrowth from both the tumor core and periphery, highlighting potential intratumoral heterogeneity. All successful cultures demonstrated initial growth within the first 3 days, and all cultures were maintained for a total of 14 days before being evaluated for discard. Organoids were typically formed after 1 to 3 passages (Fig. 2), over the course of 7–21 days, with organoid formation generally beginning to appear within the first week of culture and mature organoids forming by day 21. The early stages typically presented a balloon-like morphology, most likely due to initial growth of normal pancreatic cells, transitioning into either cyst-like or solid structures in subsequent passages (Fig. 3), with the majority adopting the latter. This morphological transition suggests the selective proliferation of tumor cells over normal pancreatic cells, attributed to the culture conditions favoring tumor cell growth.

3.2. Characterization of PDOs

3.2.1. Histopathological evaluation and mutational profile

Morphological features were found to be preserved in the organoids compared to the parent tumor, as demonstrated by H&E-stained histology slides, exemplified in Fig. 3.

The mutational profile of the 12 patients with successful PDO is shown in Table 2. These results represent filtered genomic alterations, which have been processed to exclude variants of uncertain significance (VUS) and low-confidence findings. The variants shown are high-confidence alterations with clinical relevance for diagnosis, prognosis, or treatment. Variants without known clinical significance have been excluded for clarity.

3.2.2. Genetic identity and short tandem repeat (STR) analysis

To confirm the genetic identity of the organoids and assess potential contamination, STR analysis was performed on the biobanked organoids and compared to patient blood samples. STR profiles confirmed the genetic authenticity of 8 organoid cultures (the two PDOs no. 3 and 8 failed to reestablish after thawing and were discarded prior to DNA isolation), showing consistent matches with the patient's tumor tissues. However, two organoid cultures (referenced as PDO no. 15 and 16) were identified as contaminated during long-term expansion with the commercially available organoid cell line HCM-CSHL-0089-C25 (ATCC No. PDM-36) in all frozen passage numbers and were subsequently discarded. Detailed STR analysis data is available upon reasonable request.

3.2.3. Viability and biobanking efficacy

Ensuring long-term viability of organoids post-biobanking is critical, as their ability to withstand freeze-thaw cycles is essential for their utility as a research model. We assessed the success rate of our cryopreservation and thawing protocols for all organoid samples. Post-thaw, we observed a survival rate of 100 % of individual organoid samples, indicating a high level of viability, however one model exhibited slow growth post-cryopreservation.

3.3. Literature search, study collection and pooled analysis

The systematic literature search identified 774 results, of which 21 studies reported on patient-derived organoid yields from 23 cohorts of resected pancreatic cancer patients [7,8,13,15,18,19,21,23,24,27–42], together with the current study, as shown in Table 3. These studies were pooled in a meta-analysis to estimate the mean organoid yield and assess heterogeneity.

The pooled organoid take rate across the 21 studies was 60.2 % (95 % CI: 19.7 %–81.0 %). A random-effects model was employed to account for between-study variability, and heterogeneity analysis

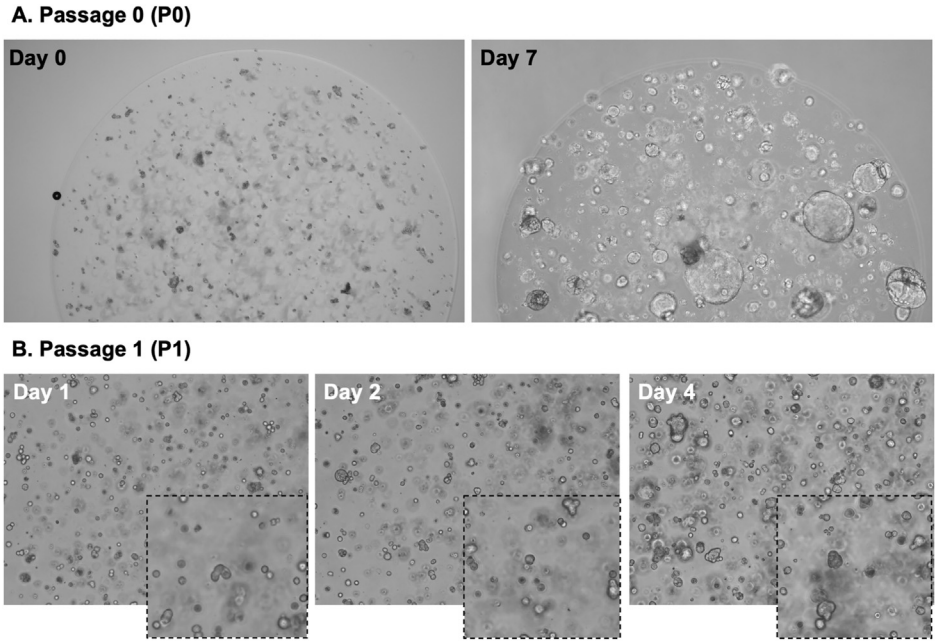

Fig. 2. Representative organoid culture after initial processing and seeding in an extracellular matrix dome A) Left panel: Representative organoid culture after initial processing and seeding in an extracellular matrix dome, designated as day 0, passage 0. Right panel: Typical presentation of tumor organoid formation on day 7, passage 0. The balloon-like morphology of normal pancreatic epithelium is also visible, which will subsequently die out in following passages. B) Days 1, 2, and 4 of tumor organoid growth after the first split using enzymatic digestion to form passage 1. The culture appears more homogeneous, comprising mostly tumor organoids.

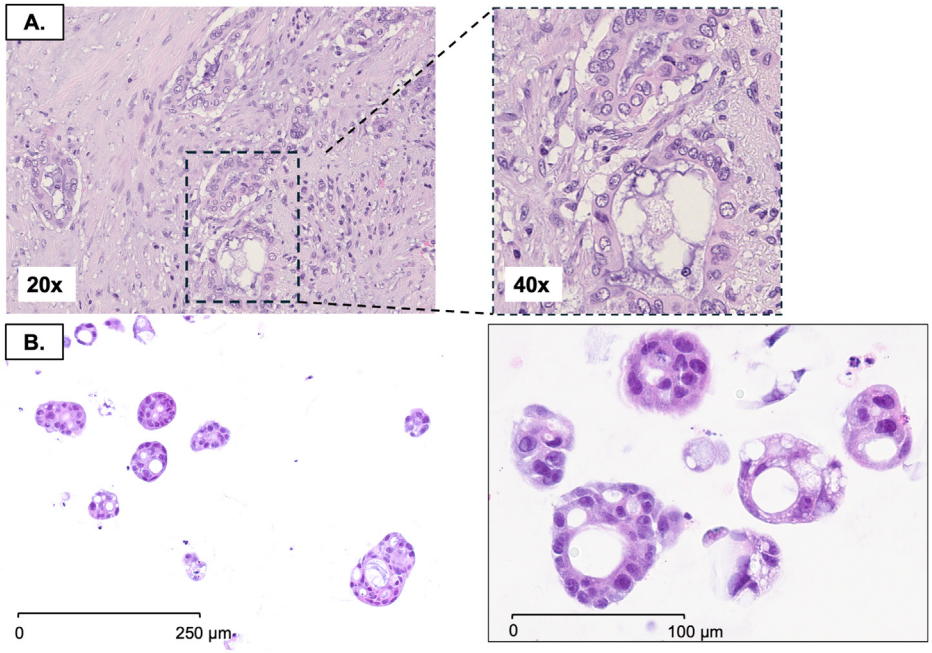

Fig. 3. Sample histopathology and organoid morphology
Legend: A) As an example is shown a case of distal cholangiocarcinoma, on A) histopathology of the surgical specimen in 20x and 40x resolution, Hematoxylin Eosin (HE) stain and B) the corresponding organoids grown from the same tumor sample at 40x resolution.

revealed moderate heterogeneity (I^2 = 57.45 %), indicating substantial variability among the included studies. Despite this variability, the pooled organoid take rate estimate was statistically significant (p = 0.001) based on the random-effects model. When including the current study, the pooled organoid take rate increased to 61.5 % (95 % CI: 18.8–77.1, I^2 = 58.84 %, p = 0.001).

4. Discussion

This study demonstrates the feasibility of establishing a biobank of patient-derived pancreatic tumor organoids from a series of consecutive resected specimens, with success in 7 out of 10 (70 %) patients with histologically verified PDAC. The total organoid take rate including all processed periampullary tumors was 12/23

Table 2
Detected mutations in 11 out of 12 cancers growing PDOs.

PDO ID	Tumor Type	Gene	Mutation	Type of Mutation	Allele Frequency (%)	Actionable Target	Potential Therapy
1	PDAC	*KRAS*	G12C	Missense	3	Yes	Sotorasib
3	PDAC	*KRAS*	G12V	Missense	15	No	–
		TP53	I195*	Nonsense	22	No	–
4	PDAC	*KRAS*	G12V	Missense	13	No	–
5	Duodenal adenocarcinoma	*BRAF*	F595L	Gain of function	47.3	No	–
		FGFR3	A391V	Gain of function	2.8	No	–
		KRAS	A146V	Gain of function	38.2	No	–
6	PDAC	*KRAS*	G12D	Missense	14	No	–
7	dCCA	*KRAS*	G12R	Missense	7.7	No	–
8	PDAC	*KRAS*	G12R	Missense	33	No	–
		TP53	I195T	Missense	12.3	No	–
15	PDAC	No mutations detected	N/A	N/A	N/A	N/A	–
16	Ampullary carcinoma	*KRAS*	G12V	Missense	35.8	No	–
		TP53	V272	Missense	19.7	No	–
19	PDAC	Not tested	–	–	–	–	–
22	NEC	*TP53*	R175H	Loss of function	92.6	No	–
23	PDAC	*KRAS*	G12D	Missense	2.6	No	–

PDAC denotes 'pancreatic ductal adenocarcinoma'; dCCA denotes 'distal cholangiocarcinoma'; IPMN denotes 'intraductal papillary mucinous neoplasia'; PanNET denotes 'pncreatic neuroendocrine tumor'; IAPN denotes 'Intra-ampullary papillary-tubular neoplasm '; NEC denotes 'neuroendocrine carcinoma'.

Table 3
Pooled take rates of Patient-Derived Organoids (PDOs) after pancreatectomy for PDAC.

First author (reference)	Year	Organoid take rates	Percentage (%)
		n (PDOs/Resected Patients)	
Boj et al. [7]	2015	3/4 (cohort 1)	75
		5/6 (cohort 2)	83
Huang et al. [8]	2015	17/20	85
Tiriac et al. [28]	2018	61/78	78
Tsai et al. [27]	2018	21/37	57
Driehuis et al. [32]	2019	51/81	63
Hennig et al. [35]	2019	17/25	68
Seppälä et al. [18]	2020	24/32	75
Sharick et al. [37]	2020	14/22	64
Vaes et al. [29]	2020	8/8	100
Le Blanc et al. [30]	2021	44/73	60
Beutel et al. [34]	2022	2/2	100
Demyan et al. [19]	2022	39/51 (treatment naive)	76
		15/21 (neoadjuvant chemotherapy)	71
Grossman et al. [13]	2022	1/8	13
Hirt et al. [36]	2022	11/17	65
Hogenson et al. [33]	2022	26/66	40
Schuth et al. [40]	2022	5/10	50
Wang et al. [39]	2022	10/11	91
Watanabe et al. [15]	2022	8/19	42
Boilève et al. [42]	2024	3/4	75
Kim et al. [41]	2024	14/38	37
Kumano et al. [21]	2024	2/2	100
Wansch et al. [38]	2024	13/26	50
Current study	2024	12/23	52
Pooled weighted mean		*61.5 % (95 % CI: 18.8-77.1)*	

(52.2 %). The sampling and growth of resected pancreatic tumors to establish patient-derived organoids seen is consistent with outcomes reported in prior studies [7,8,18,19,23,24,27–32]. The take rate is important for future research into pancreatic cancer mechanisms and biological understanding, allowing for experiments that may further our understanding of cancer biology and treatment resistance in PDAC([43]). Furthermore, the availability of PDOs may allow for drug screening and eventually tailored treatment to the individual patient [44].

As our results point out, it should be achievable to obtain PDOs from a relatively high number of PDACs, at 70 % in our series and with a weighted mean of 62.3 % across other series (Table 3). Consequently, while the success rate is good, it is influenced by the limited sample size and may likely deviate towards the pooled estimate as more tumor samples are processed with time. However, this is noted across other labs and research groups and may be more related to the pancreatic cancer per se, i.e. more aggressive tumors are more likely to grow and hence have a higher take rate, than premalignant or less aggressive tumors. This is also reflected in the growth of one neuroendocrine cancer (NEC), a highly aggressive and difficult to treat malignancy for which there are but few reports on patient-derived organoids so far [45]. Furthermore, this study also demonstrates success in other periampullary tumors, such as duodenal cancer and distal cholangiocarcinoma, which appears to thrive under the same organoid protocol. However, other entities such as low-grade pancreatic NET and precursor lesions such as IPMN did not grow in our series, which is as expected, as various tumor entities require different supplemented growth factors to mimic their specific tumor niche [33,46]. Hence, PDO protocols need to be tailored towards the tumor entity under

investigation, as was done with the aim for pancreatic ductal adenocarcinoma in the current study. Protocol amendments are necessary to achieve a high chance of success for the tissue type under investigation.

Several factors are critical in order to establish pancreatic tumor organoid models in broader laboratory setting [44,47]. Skilled personnel are crucial throughout the entire process, including multidisciplinary tumor boards for accurate diagnostic work-up; research nurses for patient inclusion; surgeons for tumor retrieval; pathologists for accurate tumor sampling and later characterization; laboratory technicians managing biobanking and logistical aspects; and molecular biologists for the creation of tumor organoids and subsequent experimental analysis (Fig. 4). In our study, a trained research pathologist processed each resected specimen, identified, and sampled each tumor, which likely increased take rates, as pancreatic tumors are known to be rich in stroma and sparse in actual tumor cells and may be difficult to evaluate on gross inspection. Additionally, the logistics flow has been refined over years through ongoing biobanking projects of resected gastrointestinal tumors [48], and in the laboratory, there has been a gradual shift from 2D cell cultures to 3D spheroid models [4,5], with organoids being the latest advancement, thus building on prior knowledge.

As part of the quality control measures, we conducted short tandem repeat (STR) analysis to confirm the genetic identity of the organoids and to detect potential contamination, particularly after multiple models exhibited similar mutational profiles during the initial genetic characterization. This prompted further investigation to rule out cross-contamination and ensure the distinct genetic identity of each organoid model. STR profiling confirmed the genetic consistency between each frozen passage of the organoid models and their original patient tumors, except for two models, which were found to be contaminated. This ensured the fidelity of all other organoid models to the original tumors. The two contaminated organoid cultures were grown alongside a fast-growing commercially available tumor organoid model (used for control in other experiments), which subsequently infiltrated and outgrew the initial cultures. This finding underscores the critical importance of stringent quality control protocols to prevent inclusion of organoid growth that do not qualify as unique PDOs. Consequently, the contaminated cultures were excluded from subsequent analyses to preserve the integrity of the study. To enhance quality assurance, all future tumor models are subject to STR-analysis before biobanking, thereby improving the reliability and reproducibility of the biobank. This may further be repeated in the case of extended time series, to ensure cross-contamination is not an issue. To our knowledge, this is infrequently done in most reports and across series and per personal communication with other labs.

Some limitations of this study must be acknowledged. Its preliminary scope may not fully represent the clinical spectrum of the disease, as it only includes patients eligible for resection, excluding those with advanced disease, which could be amended through biopsy of metastases. Additionally, not all resected tumors might be inherently suitable for organoid generation, leading to variable success rates. This variability may be due to factors such as media composition favoring certain genetic backgrounds [32] or tumor type. For example, intraductal papillary mucinous neoplasms (IPMNs) and poorly to moderately differentiated tumors were previously reported to fail organoid formation [8]. In our cohort 11 patients out of 23 (47 %) did not lead to organoid outgrowth. Of these, 3 were identified as PDAC, where either the amount of available tumor material was the likely culprit, either due to a highly enriched stromal compartment resulting in relatively few tumor cells, the genetic background of the tumor inhibiting ex vivo growth or, due to cellular necrosis secondary to neoadjuvant chemotherapy [7], which may impact success rates of PDO development. The latter represent a possible explanation for one patient in our cohort that failed to grow. Bar the one patient with chronic inflammation, 4 patients had IPMN, and 4 constituted other periampullary tumors, which typically may require different media compositions to ensure organoid growth and thus were not grown

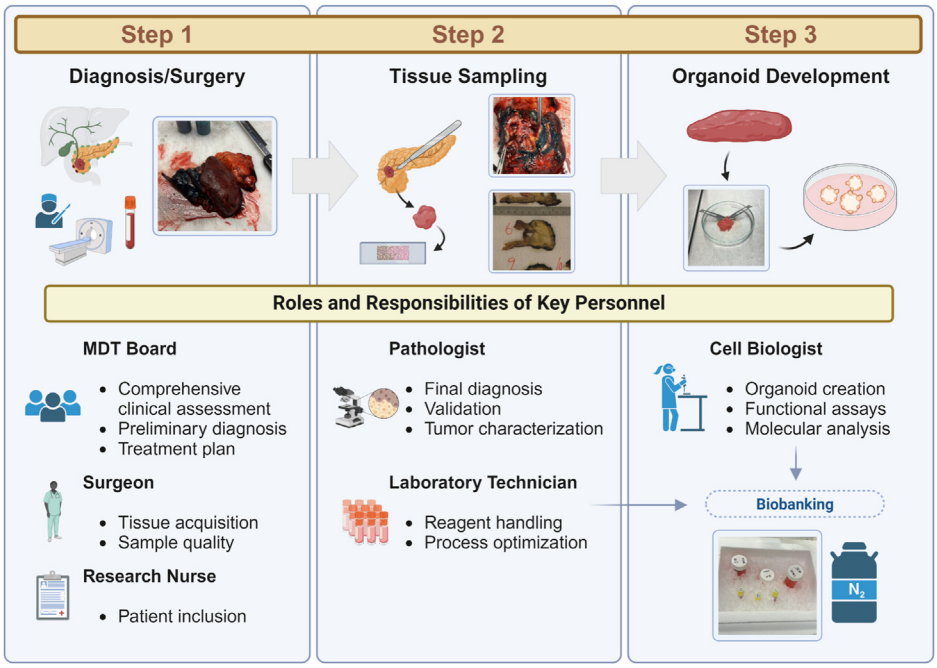

Fig. 4. Workflow of the pipeline from operation to organoid procurement
Legend: Workflow for Pancreatic Tumor Organoid Biobanking. This outlines the sequential steps and associated roles, from clinical assessment and tumor resection to organoid creation and preservation, emphasizing the interdisciplinary tasks across the biobanking pipeline.

under optimal conditions likely limiting growth [49–51]. Moreover, capturing the full cellular and molecular heterogeneity of the original tumor is challenging, potentially leading to an over-simplified model detailing only parts of the tumor. This concern extends to the culturing conditions, including media constituents and ECM, which lack standardization, complicating comparisons between organoid models. Additionally, the expenses associated with equipment and personnel makes large-scale organoid generation and high-throughput screening cost-prohibitive for the time being, challenges that are not easily overcome in every research setting. Despite these challenges, the organoid technology represents an asset in pancreatic cancer research. It holds the promise of not only advancing our understanding of the disease but also opening new avenues for future studies, particularly in the realm of personalized treatment approaches by multi-drug testing ex vivo tailored to patients' treatment and in-depth tumor analysis for individual patients and at different time during treatment.

5. Conclusion

In this study, we confirmed a good take rate in over half of all periampullary tumors (and up to 70 % specifically for PDAC) for establishing patient-derived pancreatic tumor organoids from resected pancreatic specimens, with important impact for utilization in future translational cancer research. Highest take rate was noted from PDAC, with an expected lower take rate from precancerous lesions (IPMN) and other tumor entities.

Ethical statement

Approved by the Regional Committee for Medical and Health Research Ethics, Southeast Norway (REK Sør-Øst D, reference number #2022/494778) as part of a biobank project (GITAN project REK Vest#2013/1502), and was conducted in accordance with the Declaration of Helsinki.

Author contributions

MR (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing, Final approval), MA (Investigation, Methodology, Visualization, Writing – review & editing, Final approval), MW (Investigation, Writing – review & editing, Final approval), CO (Investigation, Methodology, Visualization, Writing – review & editing, Final approval), (DL (Investigation, Visualization, Writing – review & editing, Final approval), CZ (Investigation, Writing – review & editing, Final approval), HRH (Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing, Final approval), KS (Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Visualization, Writing – review & editing; Final approval).

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Conflict of interest

None of the authors have any conflicts of interest to report.

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