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Abstract

Title of Study	Early diagnosis of pancreatic cancer by human whole genome sequencing testing of pancreatic cyst fluid (Early DiaPaC Study - hWGS test of PCyF)

Abstract and methodological description

Abstract:
The timely diagnosis of pancreatic cancer is challenging, due to a lack of disease-specific symptoms, resulting in the majority of patients presenting with advanced disease, and an associated dismal prognosis. Earlier detection of pancreatic cancer, at a stage where surgery is feasible, would prevent metastatic spread and greatly increase the 5-year survival rate. Detecting pancreatic cancer early is therefore a substantial unmet need.
Cysts found in the pancreas are often not cancerous, but a proportion of these have malignant potential. A type of pancreatic cyst known as main-duct intraductal papillary mucinous neoplasms (MD-IPMNs) have a 60-70% chance of becoming cancerous. Whereas another type, branch-duct IPMNs (BD-IPMNs) have a much lower risk at 15-20%. The standard treatment for all types of pancreatic cysts is surgery, but for some patients, it might not be needed. Current tests are invasive, uncomfortable and costly, and can be considered unnecessary in those cysts found to be benign, moreover they suffer from high rates of erroneous results (false positive or false negative). Existing biochemical tests (e.g., amylase, carcinoembryonic antigen and mucin) of pancreatic cystic fluids (PCyF) insufficiently differentiate pre-malignant pancreatic cysts from others. The ideal screening test would be capable of detecting pancreatic cancer accurately at these early stages to avoid unnecessary morbid surgery or initiation of inappropriate surveillance.
In this pump priming grant study, we propose to study genetic changes involved in pancreatic cancer development by using state-of-the-art platform technologies to perform whole genome sequencing of genomic and cell-free DNA obtained from PCyF. We will compare findings from patients in whom cancer has been detected or not to determine the potential of utilising these technologies for detecting early stages of pancreatic carcinogenesis.

Background:
Pancreatic ductal adenocarcinoma (PDAC) is a relatively rare type of cancer, with a reported crude incidence rate in the UK for 2017 of 15.7 per 100,000 population [1]. Despite this, it currently stands as the sixth leading cause of cancer deaths in the UK. While a marked improvement in survival over time has been observed with other cancer types, the 5-year survival rate for PDAC has only increased from 3-4% to 6-7% in recent years, with only moderate

improvement in over 40 years. Of particular note, progress is hampered by its remarkably high incidence-to-mortality rate. 2016-2018 figures acknowledged an average incidence of 10,300 newly diagnosed cases, which was almost matched by 9,400 deaths in the same period [1].

The dismal prognosis associated with PDAC can be largely attributed to the fact that disease-specific symptoms are rarely clinically apparent until the advanced stages of the disease [2] and consequently, of those patients with known stage at diagnosis, the majority (79%) present with stage III or IV disease [3]. At this point tumours have progressed beyond a stage when surgery is feasible. Patients diagnosed in time for surgery have a much more favourable prognosis and much improved chance of surviving beyond 5 years after diagnosis. It is therefore vital to develop an effective screening method that is capable of accurately detecting PDAC in its early stages (preinvasive or early invasive), as this could potentially dramatically improve prognosis for PDAC patients.

Recent advancements in imaging technology have led to the more frequent, often incidental detection of cystic pancreatic lesions [4]. These are identified in approximately 1% of all patients undergoing cross-sectional imaging of the abdomen for unrelated indications [5]. However, while some lesions may indeed represent significant precursors of invasive adenocarcinoma, many of the detected small, asymptomatic, lesions are benign or inconsequential. The key clinical goal is to identify and target all pancreatic cysts displaying high-grade dysplasia (without invasive, uncomfortable and costly histological analysis), whilst at the same time avoiding any unnecessary surgery and “overtreatment” of benign pancreatic cysts [6]. Existing biochemical tests (e.g., amylase, carcinoembryonic antigen and mucin) of pancreatic cystic fluids (PCyF) insufficiently differentiate pre-malignant pancreatic cysts from others.

Next generation sequencing (NGS) is a state-of-the-art technology that has been successfully applied for the ultra-sensitive/efficient detection of different types of cancer using liquid biopsies [7]. NGS has been used on PCyF, although the progress with early detection of this type of cancer has been limited by detection of alteration in known oncogenes and tumour suppressors [8-10]. NGS approaches include whole genome sequencing (WGS), a comprehensive method for analysing entire genomes. WGS has been successfully used for analysis of many cancer types, including PDAC, at a

tissue biopsy level, e.g., from formalin fixed paraffin embedded samples [11-13]. However, to date the feasibility of conducting WGS using PCyF to detect genetic changes that would indicate early stages of development of PDAC, has not been tested. This warrants the need for exploring WGS in our novel study. We hypothesise that it would be feasible to analyse the entire genome using DNA (both genomic and cell-free; gDNA and cfDNA respectively) obtained from PCyF, as a liquid biopsy collected from the closest possible proximity to a suspected high-grade dysplasia or benign tissues, and identify genetic changes associated with early stages of malignant transformation in the pancreas. Overall, the study is intended to test the possibility to detect mutations associated with high-grade dysplasia in PCyF for early PDAC detection (pre-malignant changes), and thus to facilitate avoiding any unnecessary surgery and “overtreatment” of benign pancreatic cysts [6], gathering preliminary data to inform the design of a larger trial. Additionally, demonstration of the use of these assays in PDAC liquid biopsies will complement already tested cancer types and may have relevance to other cancer types with indeterminate lesions.
Platform Science and Bioinformatics is a priority research area/theme identified by NIHR that complements the NIHR Incubator in Health Data Science. The research conducted in the proposed project will support this theme by (1) contributing towards building the expertise in pancreatic cancer and (2) generating a platform that yields opportunity for clinical expansion through NIHR Academic Clinical Fellowships in Clinical Oncology/Pancreatic cancer by providing consumables for our current one (2020-2023), and (3) yield pump priming data for grant applications in 2021-2022 to Cancer Research UK (Early Detection and Diagnosis Primer Award; Deadline 8th September 2021; https://www.cancerresearchuk.org/funding-for-researchers/our-funding-schemes/early-detection-and-diagnosis-primer-award), NIHR (Public Health Research programme Rapid Funding Scheme; Deadline 31st December 2021; https://www.nihr.ac.uk/funding/public-health-research-programme-rapid-funding-scheme/20247 and a PhD scheme, 2022), Pancreatic Cancer Research Fund (2022; https://www.pcrf.org.uk/pancreatic-cancer-research/for-researchers/).

Study Design:

This is a sub-study of an ethically-approved and currently open to recruitment study: The Study of Tumour Regulatory Molecules as Markers of Malignancy in Pancreatic Cystic Lesions (TEM-PAC) [NCT03536793]. TEM-PAC is a prospective feasibility study based on the laboratory analysis of collected urine, blood and cystic fluid samples from patients with pancreatic cancer, pancreatic cystic lesions or with a number of benign hepato-pancreato-biliary conditions (control group). The main aim of TEM-PAC is to investigate potential markers for the early detection of PDAC using protein detection tests (e.g., ELISA). TEM-PAC has been recently amended, with ethical approval granted, to include the investigation of potential markers using platform-based technologies (e.g., NGS/WGS). As of March 2021, TEM-PAC has recruited 84 out of its target 180 patients of which 39 had pancreatic cysts. The volume of PCyF collected from these patients and stored, ready for the analysis, ranges from one to 25ml.

Aims and Objectives:
The key aim of this pump-priming study is to conduct WGS analysis of 4 PCyF samples from IPMNs that display (n=2; pre-malignant high-grade dysplasia IPMN) or not (n=2; non-malignant - serous cystadenoma and/or pseudo-cyst) high-grade dysplasia (as based on histological analysis of pancreatic cyst tissue biopsies), gathering preliminary/proof of concept data to inform the design of a larger trial incorporating the analysis of the full TEM-PAC cohort. The entire genome will be analysed using both gDNA and cfDNA obtained from PCyF samples, with a potential for identifying changes associated with early stages of malignant transformation and PDAC.

Objectives:
1). To analyse current TEM-PAC sample database, select two PCyF samples which are in surplus (pilot samples) and conduct the pilot analysis for establishing conditions (e.g. detection limits etc.) for gDNA and cfDNA isolation from small amounts of these liquid biopsies, assuring quality control standards are met for human WGS (hWGS) by Novogene Company Limited, UK (https://en.novogene.com/); and including hWGS testing for one gDNA and one cfDNA sample (to be analysed separately), with basic bioinformatics of obtained data.

2). To perform hWGS of gDNA and cfDNA from PCyF from four pre-selected patients (two with and two without high-grade dysplasia) at Sequencing Depth of x50, as recommended for rare diseases and tumour tissues.
3). To catalogue (based on PCyF analysis by hWGS) a genetic constitution of these four patients, capturing all variants (single-nucleotide variations (SNVs), insertions and deletions (InDels), copy number variations (CNVs), and large structural variants (SV)) present in a single assay, providing complete and accurate characterisation of human genome and complementing missing sequencing reads, especially in highly polymorphic and highly repetitive regions from short reads sequencing.
4). To establish a pipeline for detailed bioinformatic analysis of generated data using high performance computer (HPC) cluster VIPER at the University of Hull (UoH), with a focus on somatic SNP/InDel/SV/CNV detection in tumour-normal un-paired samples (2 vs 2).
5). To perform confirmation analysis of a selected number of identified targets by PCR and quantitative PCR analysis using Applied Biosystems StepOnePlus system.
6). To disseminate data, prepare report and applications for further funding.

Patient Population:
Patients with pancreatic cystic lesions who have undergone follow-up for suspected pancreatic cancer.

Inclusion criteria:
1). Capable of giving written informed consent.
2). Age ?18 years.
3). Presence of cystic lesions that require further diagnostic intervention procedures (including fine-needle aspiration / endoscopic ultrasound) necessary OR Patient has resectable lesions suspicious for pancreatic malignancy and going to surgery.

Exclusion criteria:
1). Inability to provide written informed consent.
2). Other known malignant condition, either active or in complete remission ?5 years.

3). HIV, hepatitis C, or any other known communicable disease.

PCyF samples:
PCyF samples (n=39) have been collected at Castle Hill Hospital (Queens Centre for Oncology and Haematology, HUTH). For patients who had suspicious cystic lesions, cystic fluid has been obtained through sampling of the pancreatic cyst using endoscopic ultrasound. The group we propose to study will comprise of 1) patients with proven benign lesions; serous cystadenoma and/or pseudo-cyst (n=2), and 2) patients with lesions with high-grade dysplasia (within main duct IPMN; n=2). The total number of PCyF samples will be 6 (4 as above and two more from a pilot optimisation study; see Objective 1). The total number of samples to be analysed by hWGS will be 10 (cfDNA and gDNA samples separately for each PCyF sample, n=8; a cfDNA pilot sample, n=1; and a gDNA pilot sample, n=1).
The PCyF samples have been recorded and stored at -80°C in accordance with Human Tissue Authority, Hull University Teaching Hospitals NHS Trust and University of Hull regulations and are ready to be used for this study.

Research Team and Environment:
Our multidisciplinary team is composed of two clinicians with an extensive experience in the field of pancreatic cancer (PL, AM; [14-20]), an early career independent academic investigator (LN), an NIHR-funded Academic Clinical Fellow (ACF) in Clinical Oncology/Pancreatic cancer and collaborators (see below). The co-applicants have an established track record of collaboration, successfully utilising the infrastructure described above. They co-supervise three early career researchers (two PhD students and one ACF; two of whom are clinicians). LN is currently supported by the UoH PhD Scholarships Scheme, Castle Hill Charitable Melanoma Fund and also Department/Faculty. The project will be carried out in the research laboratory at the UoH Health Campus (Biomedical Sciences Department and HYMS; Allam Research Building) utilising PCyF samples collected and stored at the Castle Hill Hospital. A major strength of the application is integration of this laboratory within the newly built £28M Health Campus at Hull, that has excellent research support facilities and promotes translational opportunities principally via established connections to HUTH. LN’s research group has access and

	actively uses state-of-the-art facilities including the HPC VIPER and NGS equipment. The access to other specific expertise and facilities will be via established partnerships and collaborative strands developed by the applicants within UoH and externally (genomics - with Novogene Co., Ltd., Dr David Buck, Wellcome Trust Centre for Human Genetics, University of Oxford; hWGS bioinformatics – with Dr Nischalan Pillay, Cancer Institute, University College London) [21, 22]. Importance and Potential: Determining a feasibility of obtaining high quality cfDNA and gDNA from PCyF and analysing the entire genome by hWGS is essential for this project (as it would gather preliminary/pump priming data to inform the design of a larger trial and lead to larger grant applications to Cancer Research UK, NIHR and Pancreatic Cancer Research Fund) and, ultimately, for the field (as it might change the clinical practice in pancreatic oncology). Testing PCyF, as a liquid biopsy collected from the closest possible proximity to a suspected high-grade dysplasia or benign tissues and conducting hWGS at the currently highest (and also a value for money) Sequencing Depth of x50, are key novel factors that have the potential to facilitate identification of genetic changes associated with early stages of malignant transformation in the pancreas. The project also provides the opportunity for NIHR-funded ACF to contribute to this project, obtain laboratory training and collect data leading to a PhD study. Detecting mutations associated with high-grade dysplasia in PCyF for early PDAC detection would facilitate avoiding any unnecessary surgery and “overtreatment” of benign pancreatic cysts.

Timetable

Name	Analysis of current database and selection of two PCyF samples which are surplus to TEM-PAC (pilot samples).
Date	30 June 2021

Name	Isolation of gDNA and cfDNA from two pilot PCyF samples and quality control; hWGS data of one gDNA and one cfDNA sample (to be analysed separately), including basic bioinformatics of obtained data.
Date	30 June 2021

Name	Analysis of the rest of the PCyF samples (n=4, main study), including isolation of gDNA and cfDNA, hWGS of 4 gDNA and 4 cfDNA samples, including basic bioinformatics of obtained data.
Date	30 August 2021

Name	Preparing application for a larger funding. Cancer Research UK (Early Detection and Diagnosis Primer Award). https://www.cancerresearchuk.org/funding-for-researchers/our-funding-schemes/early-detectio
Date	8 September 2021

Name	Establishment of a pipeline for extensive/detailed bioinformatic analysis of generated data using HPC cluster VIPER at the University of Hull.
Date	30 September 2021

Name	PCR and quantitative PCR analysis of identified targets.
Date	31 October 2021

Name	Report preparation and submission to the funding body.
Date	30 November 2021

Name	Preparing application for other larger funding. NIHR (Public Health Research Programme Rapid Funding Scheme; https://www.nihr.ac.uk/funding/public-health-research-programme-rapid-funding-scheme/20247)
Date	31 December 2021

Name	Preparing application for another larger funding. Pancreatic Cancer Research Fund (2022; https://www.pcrf.org.uk/pancreatic-cancer-research/for-researchers/).
Date	2022

Funding

Name	MagMAX™ Cell-Free DNA Isolation Kit, ThermoFisher Scientific, Catalog number: A29319.
Amount	320.0

Name	MagMAX™ DNA Multi-Sample Kit (for gDNA isolation) ThermoFisher Scientific, Catalog number: 4413020.
Amount	108.0

Name	IlluminaNovaSeq6000 system PE150 hWGS (WBI, DNA sample QC; whole genome library preparation, 350bp; >x50 coverage; 152G raw data; standard bioinformatics analysis; hard disk delivery, 2T), x10 samples
Amount	10972.8

Name	Shipment costs.
Amount	40.0

Name	Confirmation of findings by PCR and qPCR.
Amount	500.0

Details of ethical approval

The TEM-PAC Study gained Research Ethics Committee (REC) Favourable Opinion on 27.04.2018, Health Research Authority (HRA) approval on 30.04.2018 and Hull University Teaching Hospitals NHS Trust (HUTH) Confirmation of Capacity and Capability (C&C) on 18.10.2018. The study end date is 29.02.2024.

Institutional approval information

HUTH continue to provide Sponsorship and C&C for the TEM-PAC study. There is a current research contract between HUTH and the University of Hull. Excess costs (£1,940.80) will be covered from alternative sources by the applicants through their associated institutions.

Declaration

Confirm Declaration: Yes

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