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Created: 16 Feb 2021, 10:34 a.m.

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Abstract

Title of Study	Equity of access to genomic testing for pancreatic cancer patients: Developing a cost-effective ctDNA-based platform
Abstract and methodological description	Abstract It is widely recognised that genomic profiling of actionable mutations will ultimately improve outcomes across multiple disease sites by identifying which treatments may benefit individual patients the most, and by providing earlier and more accurate diagnoses. To this end, the NHS' Genomic Medicine Service offers whole genome sequencing as part of the drive to improve cancer outcomes, but this is currently limited to haematological malignancies and sarcoma. Although capacity will ultimately expand, outside of clinical trials the majority of patients with solid tumours such as pancreatic cancer, do not yet have access to genomic testing and the benefits that it may bring. A number of alternate commercial platforms exist (and are increasingly being sought by patients privately), but remain too prohibitively expensive to implement within an NHS setting to guarantee equity of access. This will be a pilot study to undertake next generation sequencing in pancreatic cancer patients utilising an in-house delivered circulating tumour (ct) DNA platform which is currently being validated against Roche's Foundation

Medicine genomics platform. This will play a crucial part in our ambition to ensure that patients are directed to the most appropriate treatment strategies, have access to appropriate trials, and are able to undergo non-invasive serial monitoring to assess disease progression and temporal tumoural evolutionary changes that may affect treatment decisions.

Methodology

Plasma ctDNA detection and characterisation has great potential for utility as a non-invasive method to facilitate the early detection of pancreatic cancer, in addition to assessing important genomic indicators which may guide therapeutic strategies. A recent meta-analysis (Lee, 2019) suggested that ctDNA may be useful for stratifying risk of recurrence and death in pancreatic ductal adenocarcinoma, with a number of studies increasingly identifying specific mutation hotspots which may have future treatment implications (Adamo et al 2017, Macgregor-Das et al, 2020, Zvereva et al, 2020).

Leicester are world leaders in enhancing the understanding of ctDNA and its utility within the prognostic, diagnostic and treatment stratification settings, and have driven extensive methodological validation across a variety of genomic platforms. The ability to detect a range of changes in response to treatment in the breast cancer setting revealed dynamic temporal changes in both mutational spectra and gene amplifications, adding considerable value over monitoring treatment-induced changes by the standard blood-based marker CA15-3 (Page et al, 2017). Importantly, recent evidence suggests that ctDNA is detected prior to radiologically-detected relapse, which offers a greater window for intervention (Coombes et al, 2019), concurrent with the ability to significantly improve treatment burden, improving patient quality of life and longevity. A further important and novel question that we will consider in this study, is whether ctDNA can be detected at a higher frequency in the portal vein and if so, whether collection of blood samples in this way may have implications for detection of low frequency mutations that are important in prognostication and treatment strategies for pancreatic cancer. This is of particular interest in tumours with low ctDNA shedding rates, as this can hinder utility of this technology.

Expanding genomic testing capacity to pancreatic patients therefore has potential to benefit those patients that would otherwise not be able to access testing.

Recruitment

This pilot study will recruit patients from University Hospitals of Leicester NHS Trust who are awaiting surgical resection for pancreatic cancer. Patients will be consented to donate a pre-surgical blood sample, a peri-operative hepatic portal vein blood sample, and either a fresh tumour or FFPE tissue block sample. If sufficient FFPE or fresh frozen tissues are not available, then any archival tissue available from previous biopsies or surgeries may be used. Participants will be followed up for overall survival, further radiology and disease recurrence.

Blood (2 x 10 mL) will be drawn into K2 EDTA tubes and double spun to produce plasma and buffy coat within 2 hours of blood collection. Where possible, fresh frozen tissue (FF) will be taken at the time of surgery. Samples will be stored in a monitored -80°C in a HTA licenced facility. FFPE tumour tissue blocks will be retrieved from the pathology archive and H&E stained sections reviewed by a pathologist before 1 mm cores are taken from tumour areas.

Analyses

All analyses will be undertaken within the Leicester Molecular Diagnostics facility (LMD). LMD was set up specifically within LCRC to provide a service for local molecular oncology testing, incorporating targeted next generation sequencing and working to ISO 15189:2012 Medical Laboratory accredited standards in conjunction with their NHS Genomic Medicine counterparts.

Total cfTNA (circulating free total nucleic acids) will be isolated from blood plasma using the MagMAX Cell-Free Total Nucleic Acid Isolation kit (ThermoFisher) and DNA and RNA from FFPE tumour cores using the MagMAX FFPE DNA/RNA Ultra kit (ThermoFisher) on the automated

Kingfisher Flex according to manufacturer's instructions. DNA and RNA will be extracted from fresh frozen tissue using the QIAamp DNA Mini kit (Qiagen) and the RNeasy Mini kit (Qiagen) respectively. Germline DNA will be extracted from buffy coat using the DNA Blood Mini kit (Qiagen). DNA and RNA will be quantified and quality checked using the relevant assay on the TapeStation 4200 (Agilent).

In-house genomic assessment will be via library preparation using the Oncomine Comprehensive panel v3 (ThermoFisher) for tissue, and via the Oncomine PanCancer cfTNA panel (ThermoFisher) for bloods on the Ion Torrent S5 sequencer according to manufacturer's instructions. Both panels are able to assess DNA and RNA, allowing single nucleotide variations (SNV), CNVs and fusions to be detected and have already been robustly tested in-house with appropriate controls. Whilst these panels are not as comprehensive as some of the commercial tests i.e. Foundation Medicine, they exhibit considerable cross-over and will maximise information without compromising sensitivity as well as offering a significant cost saving. The assays to be performed on each sample will be as follows:

• ctDNA analysis on plasma samples (pre-surgery and peri-operatively) via Oncomine PanCancer cfTNA panel analysis
• FF or FFPE tumour sample analysis via Oncomine Comprehensive v3 panel analysis
• Matched germline control analysis obtained from the pre-surgery blood sample via Oncomine Comprehensive v3 panel analysis

The alignment of raw sequencing data to the human genome (hg19) will be performed by the Torrent Suite Software (ThermoFisher) and further analysis will be conducted using the preconfigured workflows for each assay in the Ion Reporter software (ThermoFisher) to identify SNPs, insertions/deletions, CNVs and gene fusions. Each cfTNA and FF/FFPE tissue sample will be compared with the matched lymphocyte DNA as a germline reference. Any discrepancies can be reviewed manually using the Integrated Genomics Viewer (IGV) software. Preliminary analyses of total cfDNA levels, ctDNA and comparison with clinical variables will be performed using basic tabulations and association

measures, prior to building appropriate statistical models where necessary. We will examine any differences between the cfDNA from the standard pre-operative blood sample and the hepatic portal vein sample to establish if one has a higher frequency and compare the ctDNA analysis between the two samples to determine their prognostic or predictive value.

The output of this analysis can then be further examined using the Oncomine Knowledgebase Reporter genomic analysis tool (ThermoFisher). This enables the sample-specific variants to be investigated to understand their therapeutic relevance to on-market oncology drugs, published evidence and current global clinical trials. Monitoring of tumour and ctDNA profiles will provide information regarding tumour mutational status, over and above mutations that are routinely tested for. This may open up the possibility in future, of being able to match patients with a clinical trial that is targeting therapy towards these mutations, using the national Experimental Cancer Medicine Centre trial tracker.

References

Lee et al. Circulating tumour DNA as a prognostic indicator in resectable pancreatic ductal adenocarcinoma: A systematic review and meta-analysis. Scientific reports. 2019.9:16971

Macgregor-Das et al. Detection of Circulating Tumor DNA in Patients with Pancreatic Cancer Using Digital Next-Generation Sequencing. J Mol Diagn. 2020.22(6):748-756

Zvereva et al. Circulating tumour-derived KRAS mutations in pancreatic cancer cases are predominantly carried by very short fragments of cell-free DNA. Circulating tumour-derived KRASmutations in pancreatic cancer cases are predominantly carried by very short fragments of cell-free DNA. EBioMedicine. 55:102462

Page et al; Next generation sequencing of circulating cell-free DNA evaluating mutations and gene amplification in metastatic breast cancer. Clin Chem. 2017 February; 63(2): 532–541.

	Coombes et al. Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. Clinical Cancer Research. 2019. 25:4255–63

Timetable

Name	Sample collections to be completed by
Date	31/06/2021

Name	Sample processing and DNA extractions to be completed by
Date	31/07/2021

Name	NGS and informatics to be completed by
Date	31/11/2021

Funding

Name	Oncomine Comprehensive Manual v3 kit
Amount	7290.0

Name	Ion Xpress™ Barcode Adapter kit
Amount	1816.0

Name	Acrometrix controls
Amount	900.0

Details of ethical approval

Development and Application of Ex Vivo Assays to Assess Efficacy Biomarkers in the Prevention and Treatment of Cancer (ExPAT).
REC Ref: 14/WA/1166
This is an ongoing tissue collection study. Date of initial favourable REC opinion: 23/10/14

Institutional approval information

Institutional approval received 2014 following favourable REC opinion

Declaration

Confirm Declaration: Yes

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