Exome sequencing peri-hilar cholangiocarcinoma

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Agenda

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1 Introduction and background

2 Study aims

3 Methodology

4 Results

5 Ongoing validation

Next Generation Sequencing

DNA SEQUENCING

Whole Exome Sequencing

Exons – Protein coding

- 85% disease associated variations.
- Single nucleotide variants.
- Somatic mutations (non-germline).
- Non-synonymous, frameshifts.

Sequencing Disparity

INTRA-HEPATIC	EXTRA-HEPATIC
N ≥1750	N = 251
Intra-hepatic only	pCCA and distal disease
International	Eastern predominance
Multi-omics platforms	Targeted studies in Western cohorts
Targeted clinical trials	Unmet need

Peri-hilar Cholangiocarcinoma

EXOME REMAINS ILL DEFINED

- Nakamura et al, Nature Genetics, 2015.
- Jusakul et al, Cancer Discovery, 2017.
- Wardell CP et al, J Hepatology, 2018.
- Western cohorts targeted panels only.

Limited fluke negative pCCA

?

Relevance to Western populations….

Absence of peri-hilar cholangiocarcinoma
tailored chemotherapy?

Aims

1. Characterize mutational landscape of UK pCCA
2. Validate Eastern mutations
3. Identify novel pCCA mutations
4. Elicit actionable targets
5. Functional analyses

TISSUE PREPARATION

- 40 x pCCA FFPE
- Tumor and duct
- Consultant review
- Microdissection
- Qiagen DNEasy

Fragment Analysis

25 X TUMOR
FOR SEQUENCING

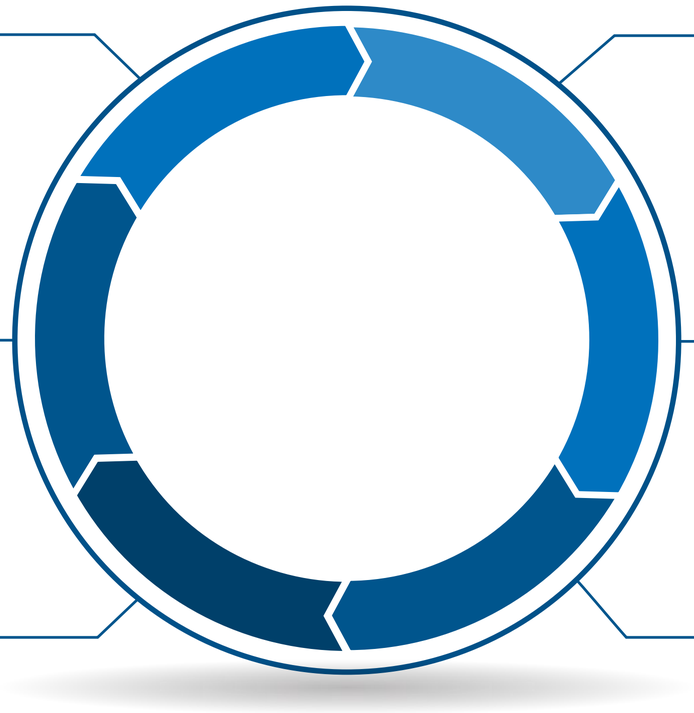
Liverpool Centre for Genomic Research

FRAGMENT ANALYZER

TUMOR X 25
260:280 AND 260:230

DNA LIBRARY PREPARATION

AGILENT SURESELECT V6
EXON CAPTURE
MAGNETIC BEAD PROBES
AMPLIFICATION



SANGER VALIDATION

SIGNIFICANT DIFFERENTIALLY
EXPRESSED MUTATIONS

ILLUMINA HISEQ 4000

2 X 150 BP
PAIRED END SEQUENCING

>100X COVERAGE

AVERAGE NUMBER OF TIMES
BASE IS READ

BASE CALL ACCURACY

TRIM Q<20 (1/100)
REDUCE FALSE POSITIVES

Bioinformatics Pathway

- Mapping
- Duplicate Removal
- Annotation

BWA hg38 (80% mapping).

Reads <10% mapping quality removed.

Picard duplicate filtering.

Local realignment around small insertions and deletions (GATK).

Somatic SNV detection (STRELKA).

Significant difference between tumor/normal classified somatic (Fisher’s exact – 5% cutoff).

Spectrum of Inter-tumoral Heterogeneity

I. Tumor Mutational Burden (median) = 18/Mb

II. Total mutations (median) = 1307 (IQR 1206 – 1653)

III. Moderate impact (median) = 334 (IQR 213 – 454)

IV. High impact (median) = 53 (IQR 46 – 97)

Comparative Extra-hepatic Landscape

Published		Liverpool
50%	TP53	47%
40%	KRAS	10%
15%	SMAD4	38%
10%	ERBB2	15%
5%	BRAF	22%

Validation ongoing

INSTITUTE OF TRANSLATIONAL MEDICINE

Higher Frequency

Established

Peri-hilar CCA Mutations

- 4 x known actionable cancer mutations
- Low frequency (published datasets)
- High frequency Liverpool (coding)
- Sanger sequencing (n=70)
  and IHC (n=98)

Validation ongoing

INSTITUTE OF TRANSLATIONAL MEDICINE

High Frequency

Novel mutations

- 6 x novel pCCA mutations
- Coding/promoter
- Cell cycle arrest/regulation
- Immune response regulation

Summary

UNMET NEED IN PERI-HILAR DISEASE

Significant inter-tumoral heterogeneity

Intra-tumoral heterogeneity?

Differing biological landscape

Need for new target based trials

Higher Western mutational burden

Progressive technology

Mutations – Old and new

Pathway analysis

Thank you

AMMF

Mr Hassan Malik
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Professor Daniel Palmer