A targeted NGS approach to detect activating and resistance mutations in the circulating tumour DNA of cancer patients

Julian.Cano-Flanagan@nbt.nhs.uk

Julian Cano-Flanagan[1], Claire Faulkner[1], Kirsty Russell[1], Paula Waits[1], Christopher Wragg[1]- [1]Bristol Genetics Laboratory, North Bristol NHS Trust

Introduction

- Circulating tumour DNA (ctDNA) is a component of cell-free DNA (cfDNA) that is derived from tumours which shed DNA into the bloodstream
- Liquid biopsy is a safer and less invasive alternative to a tissue biopsy
- Targeted treatment for cancer is a form of personalised medicine whereby drugs which target tumour-specific, activating mutations help to stop the cancer from growing and spreading.
- Non-small cell lung cancer (NSCLC) patients with tumours harbouring activating *EGFR* mutations can be treated with EGFR-tyrosine kinase inhibitors (TKIs)
- Melanoma patients with *BRAF* codon 600 mutations can be treated with BRAF and MEK inhibitors

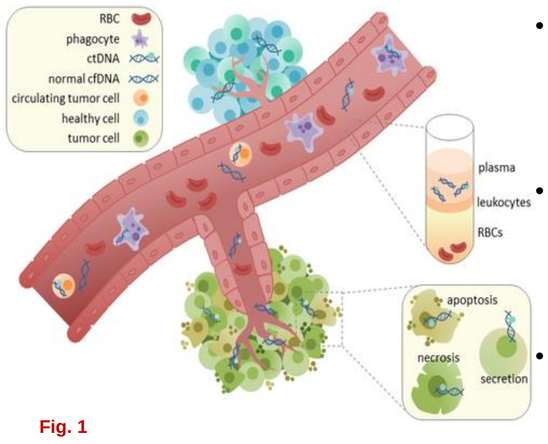


Fig. 1

Utility of ctDNA testing

- **Test for activating mutations to enable access to targeted therapy – less invasively than a tissue biopsy.**
- **Track the for emergence of resistance mutations to targeted therapy (e.g. *EGFR* c.2369C>T, p.(Thr790Met) T790M)**
- **Monitor tumour burden and response to treatment by measuring the level of ctDNA in the plasma**

Aims and Objectives

1. **To recruit eligible lung cancer and melanoma patients in liaison with colleagues in Clinical Oncology.**
2. **Evaluate and determine the extraction efficiency of the QIAamp Circulating Nucleic acid kit using positive controls in a synthetic matrix and a positive control spiked into plasma.**
3. **Verify the capabilities of ctDNA testing on an NGS platform on lung cancer and melanoma patients who have tested positive for an *EGFR* or *BRAF* activating mutation on tumour biopsy.**
4. **Evaluate the NGS assay's ability to detect resistance mutations and it's utility as a monitoring tool**
5. **Use a dilution series of positive control samples with known mutations to determine the limit of sensitivity of the NGS assay.**

Study Design and Set up

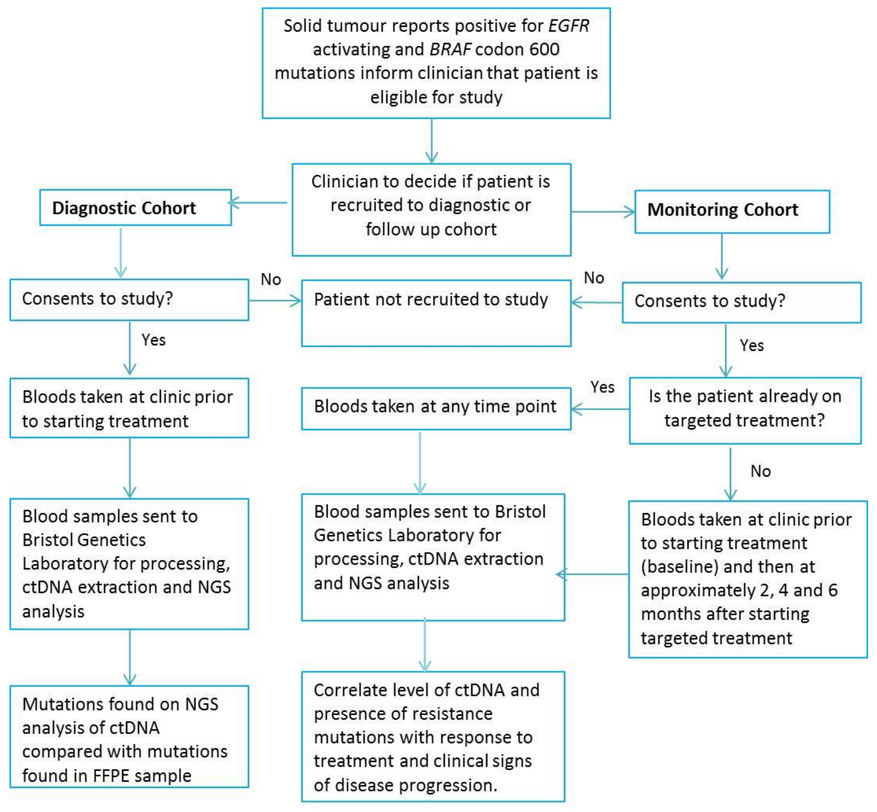
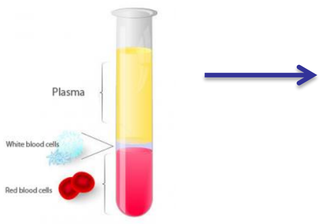


Fig. 2

Testing Workflow

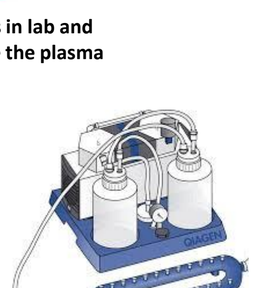
- NGS has the advantage of screening many genes simultaneously so can pick up a wide repertoire of actionable lesions
- Multiple cancer types can be tested on a single assay
- However, it's less sensitive compared with ddPCR

Sample taken into PAXgene or Streck cfDNA tube Transported same day or <96hrs



Sample arrives in lab and spun to isolate the plasma

Plasma stored in 1ml aliquots @ -80°C



ctDNA extracted from plasma using QIAamp CNA kit with QIAvac

QIAseq NGS Panel on MiSeq to test for clinically actionable mutations

Fig. 3

QIAseq Targeted DNA Panel

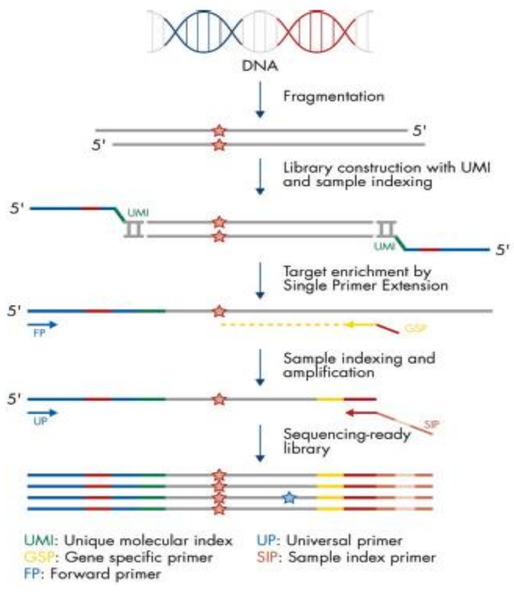


Fig. 4

- Qiagen's Human Actionable Solid Tumour Panel
- 22 genes found in various solid tumour types.
- 10-40ng of cfDNA input.
- PCR based target enrichment methodology for library preparation.
- The use of molecular barcodes, known as unique molecular indices (UMIs) helps distinguish true variants from sequencing artefacts, giving more confidence in calling low level variants.
- UMI reads are used for calling allele frequencies more accurately.

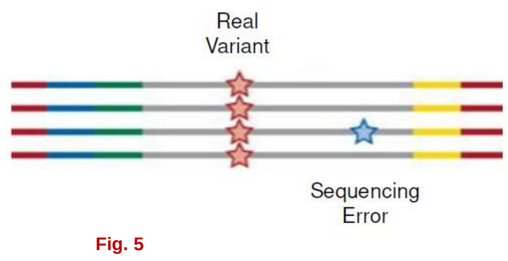


Fig. 5

Results: Evaluation of ctDNA Extraction Method

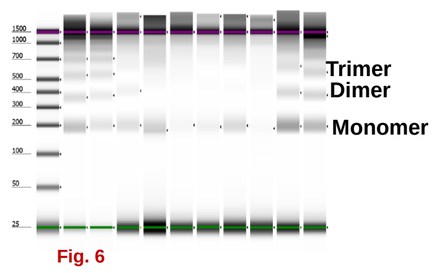


Fig. 6

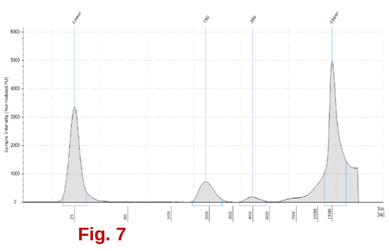
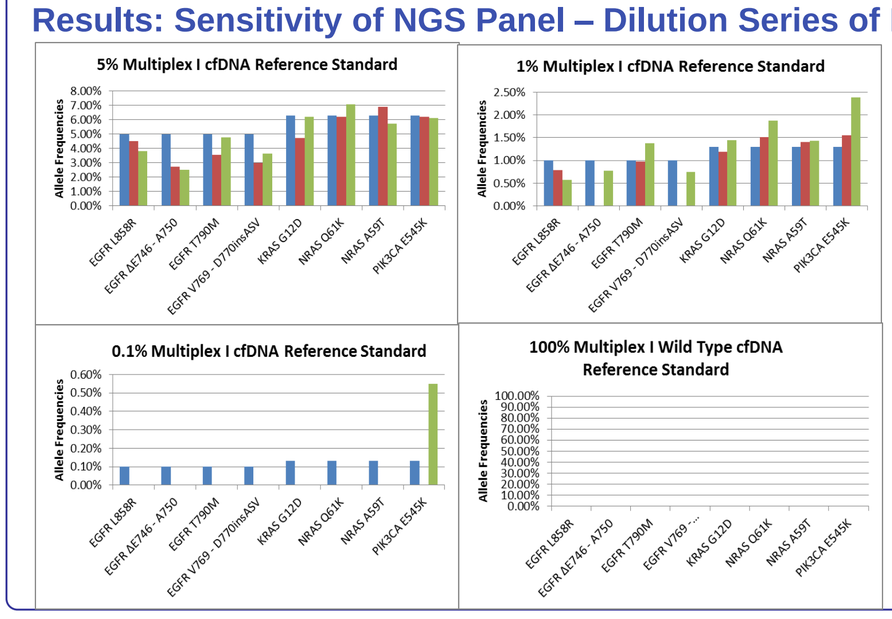


Fig. 7

- Fragments extracted from plasma samples are approx.160 – 200bp long – corresponds to DNA wrapped around a nucleosome
- Dimers and trimers of this length can also form.
- Extraction efficiency of Horizon reference standards set in a synthetic matrix which emulates plasma was **48.75% -** this a higher level than the expected 30% given by Horizon.

Results: Sensitivity of NGS Panel – Dilution Series of Positive Controls



- Horizon Multiplex I cfDNA Reference Standards used as positive controls
- Each contain 8 mutations at 5%, 1%, 0.1% allele frequency or 100% wild type
- **Limit of detection for SNVs: 1% variant allele frequency (VAF)**
- **Limit of detection for indels (e.g. *EGFR* exon 19 deletions): 5% VAF**

Expected Allele Frequency

Measured Positive Control

Measured Positive Control (Extracted)

Fig. 8

Results: Evaluation of NGS assay – Melanoma and NSCLC Patient Samples

- 10 ctDNA samples from 5 patients tested on the panel so far (4 NSCLC and 1 melanoma)
- **Concordance in mutation detection between matched FFPE and ctDNA samples: 57%**
- NSCLC patient 4 – on EGFR TKI (gefitinib) for three years, had *EGFR* T790M resistance mutation on ctDNA which correlated with thickening of tumour on CT scan.
- NSCLC patient 1 – had *EGFR* T790M on tissue biopsy DNA but not detectable in ctDNA – highlights the need for confirmatory biopsy in case of negative result in suspected acquired resistance

Results: Monitoring Patients

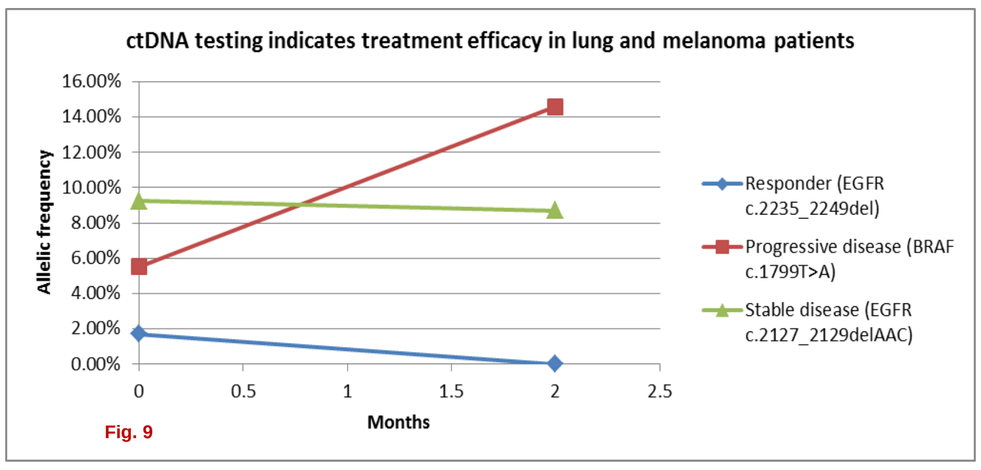


Fig. 9

Three patients on the monitoring cohort have shown correlation of ctDNA levels in plasma with either:

- **favourable response to treatment in a NSCLC (blue line)**
- **progression of disease in melanoma (red line)**
- **stable disease (green line) .**

Conclusions:

- **The utility of the NGS assay for detecting activating and resistance mutations in the ctDNA of lung cancer and melanoma patients was demonstrated.**
- **The QIAamp circulating nucleic acid kit demonstrated good extraction efficiency.**
- **Qiagen's NGS panel has a LOD of 1% for SNVs and 5% for indels**
- **Discordance between sample types suggests that a "liquid biopsy" should be considered complementary to the testing of tissue biopsy rather than a replacement**
- **Preliminary data has shown the potential for the NGS assay as a monitoring tool, with the level of ctDNA correlating to efficacy of treatment.**
- **The presence of a resistance mutation in plasma correlated with clinical signs of disease progression**
- **Future work will include testing subsequent patient samples recruited on to the study.**

Acknowledgements

Thank you to the Scientists and Technical Staff at BGL for their support with this STP project. Thanks also to Clinical Oncology at RUH Bath for recruiting patients and the Showering fund for funding the project. Thanks to the patients who consented to take part.

References: 1. Hoelder S, Clarke PA, Workman P. Discovery of small molecule cancer drugs: successes, challenges and opportunities. Molecular oncology. 2012;6(2):155-76 **2.**Peng Q, Satya RV, Lewis M, Randad P, Wang Y. Reducing amplification artifacts in high multiplex amplicon sequencing by using molecular barcodes. BMC genomics. 2015;16(1):589 **3.** Qiagen. QIAseq™ Targeted DNA Panel Handbook May 2017.