Genome-wide cfDNA Sequencing of Melanoma Progression

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Introduction: Cell free DNA is a promising solid cancer biomarker

- cfDNA allows minimally-invasive assessment of the current molecular features of a tumour
- Observed to be correlated with tumour size[1], stage[2], disease course[3], but varies across cancer type[2]
- The degree of information provided from cfDNA sequencing is not fully characterised
- We present whole genome and targeted sequencing of cfDNA from a metastatic melanoma patient over the course of treatment
- Developed custom *de novo* assembly and alignment based variant callers specifically for cfDNA WGS accounting for tumour haplotype complexity
- Used Frequentist and Bayesian methods to estimate strength of evidence for mutant alleles
- Identified ~40,000 somatic mutations across two WGS cfDNA samples (PPV = 0.985)

Abstract

Several projects are investigating the introduction of WGS into clinical cancer genomics (e.g. Genomics England).

Obtaining DNA from tumours is a major challenge. FFPE, fresh frozen, and liquid biopsy are all alternatives.

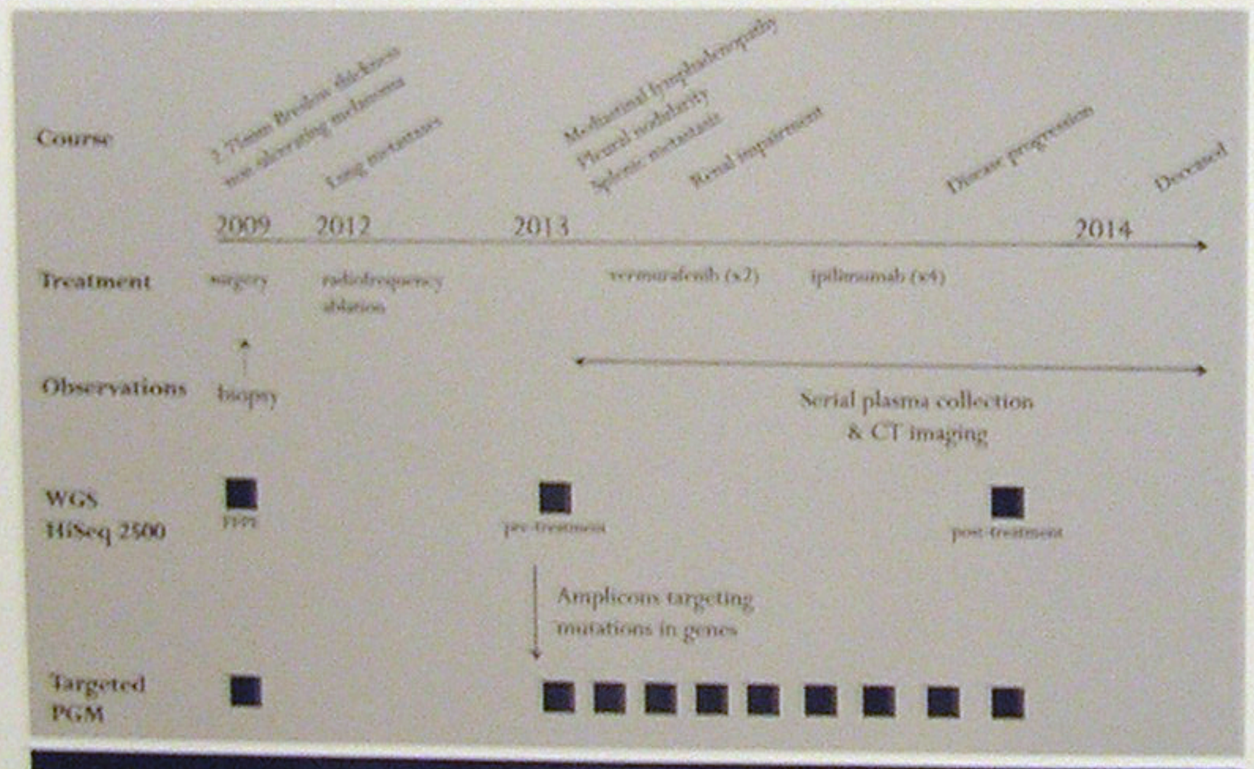
Tumour biopsy sequencing has severe limitations:

- Invasiveness limits follow up sampling
- Spatial bias limits the representation of sub-clonal heterogeneity
- Tissue preparation has a strong impact on sequencing library quality

cfDNA is minimally invasive, not spatially biased, and has robust protocols for the extraction from plasma.

We therefore sought to assess the suitability of cfDNA whole genome sequencing as an alternative liquid biopsy.

Methods: Single metastatic melanoma patient



Results: Comparison between cfDNA and FFPE

Formalin-Fixed Paraffin-Embedded (FFPE) blocks are widely used, preserving tissue morphology but damaging nucleic acids.

The most common artefacts are

A. C>T base substitutions caused by deamination of cytosine bases

B. Strand breaks

A) Induces false signals of somatic point mutations, and B) increases variance in the genome-wide coverage of template molecules.

There has been recent progress in protocol development to guard against these errors.

Our analysis suggests that coverage uniformity is severely impacted by FFPE preparation.

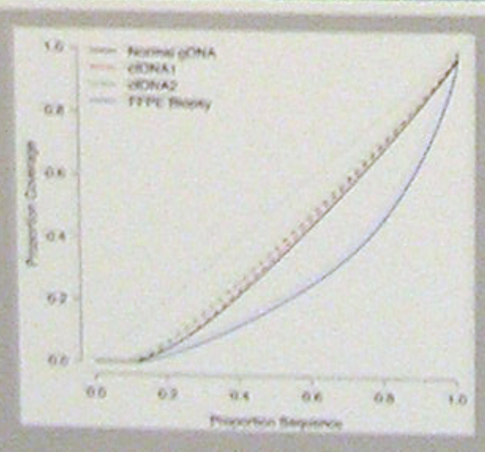


Figure 1: cfDNA WGS has outstanding sequencing uniformity. If coverage was perfectly uniform across the genome traces would track the diagonal. Deviation from the diagonal indicates non-uniformity.

Results: Disease biology

Figure 3: Transcription activating C>T mutation in the core promoter of telomerase reverse transcriptase (TERT)[4]. The mutation generates a consensus binding site for ETS transcription factors, resulting in a 2-4 fold increased transcription versus wild-type promoter status[5].

Results: Mutational signatures from cfDNA WGS

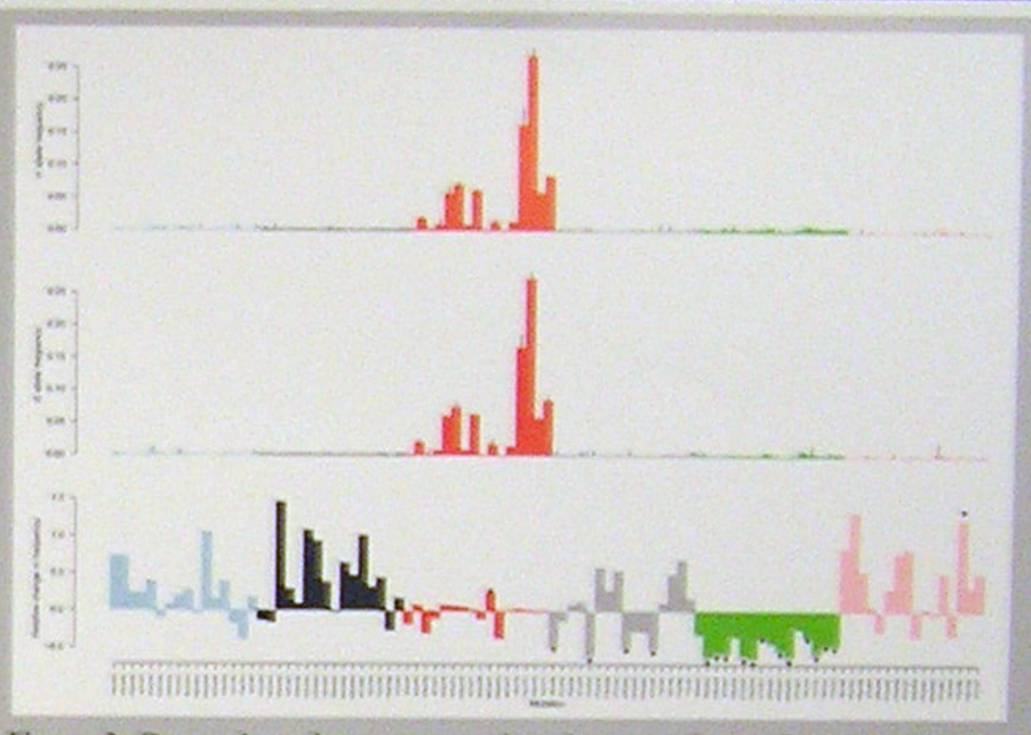


Figure 2: Dynamic melanoma mutation signature from cfDNA WGS. WGS-identified mutations stratified by triplet context ($N_{timepoint1}$=24377,$N_{timepoint2}$=35036). Observed profile concordant with Type 2 profile reported by Alexandrov et al (2013), compatible with UV-induced DNA damage (abundant C>T). First and second panels show mutational cfDNA WGS (bootstrapping, 95% CI). Third panel shows relative change in frequency between time points, stars represent significant changes ($p < 0.05$, FET).

Results: Frequency trajectories of WGS-identified mutations

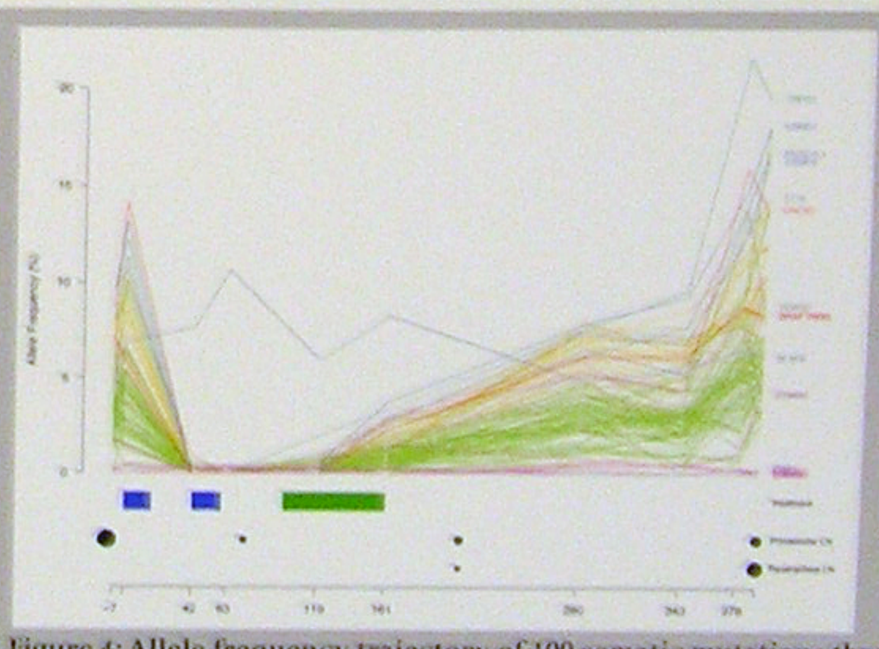


Figure 4: Allele frequency trajectory of 100 somatic mutations through treatment course. Variants tracked by amplicon-based sequencing of cfDNA samples on PGM (Life Tech). Loci assigned one of 8 colours based on hierarchical clustering (Euclidean distance). Treatment cycles of vermurafenib (blue) and ipilimumab (green) indicated as rectangles. Tumour diameter of prevasular lymph node (top), paratracheal lymph node (bottom) obtained using CT imaging.

Conclusions

- cfDNA WGS is readily applicable to patients with high systemic tumour burden.
- Enables comprehensive evaluation of clonal genomic evolution associated with treatment response and resistance.
- cfDNA results in more uniform WGS libraries than libraries from FFPE biopsy.
- We developed novel variant detection approaches for cfDNA WGS robust to misalignment and high haplotype diversity.
- We are developing enrichment-based targeted sequencing approaches and algorithms to improve sensitivity for focus areas (watch this space!).

References

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