The feasibility of circulating tumour DNA as an alternative to biopsy for mutational characterization in Stage III melanoma patients

ASSC Scientific Meeting

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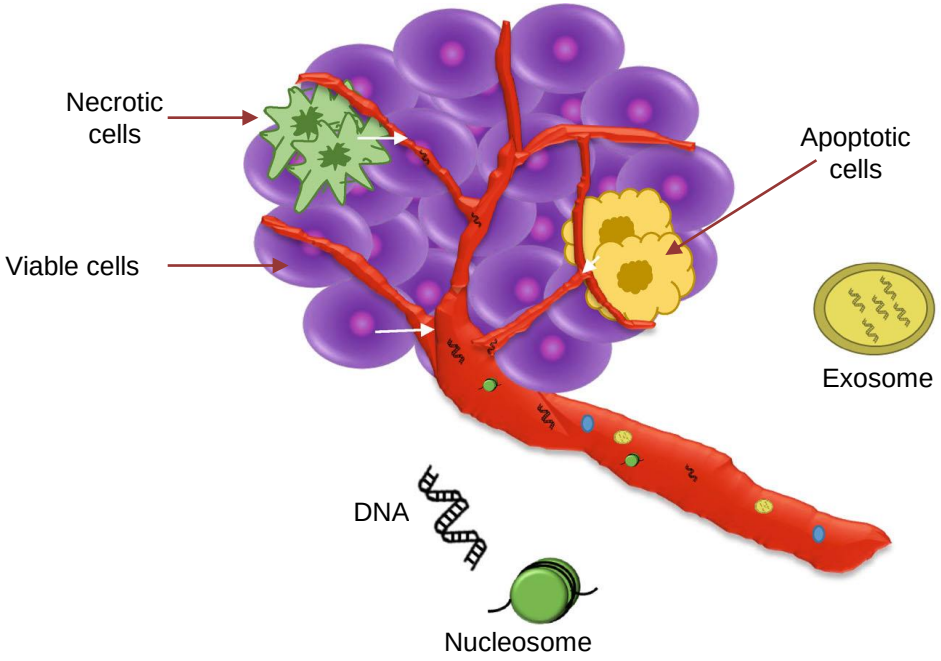
Cell-free DNA (cfDNA)

- Tumour DNA that is circulating in the bloodstream
  - ➢ higher concentrations in cancer patients than healthy individuals

- Released into the bloodstream by cells undergoing apoptosis, necrosis or viable cells

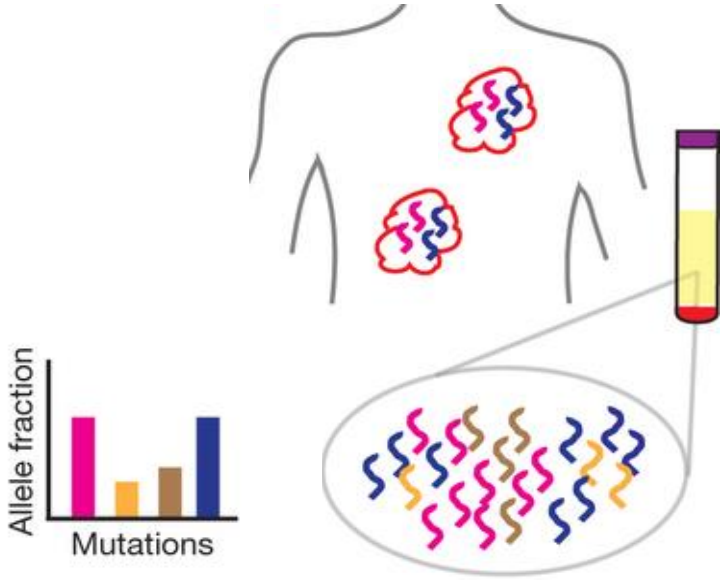


Aarthy et al. Mol Diagn Ther (2015)

Background

- Identified in patients with various cancer types and at different stages of disease
  - ➢ More frequently in advanced disease rather than localized
- Melanomas have intra- and inter-tumor heterogeneity
  - ➢ Biopsy does not provide a complete genomic picture as only a part of the tumor is tested

cfDNA captures primary and metastasis in a single sample



M. Murtaza et al, Nature (2013)

Clinical Utility

CRC

Used in stage II patients to select adjuvant chemotherapy[1]

- Patients with detectable cfDNA levels were more likely to have recurrence

Breast cancer

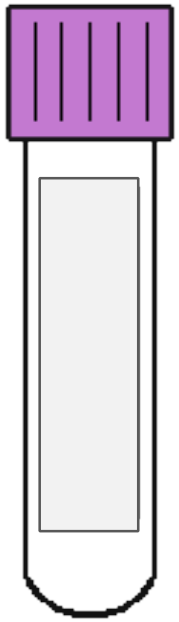
Predict relapse and response [2]

- Make predictions earlier than imaging (standard practice)

Mucosal melanoma

Used to track mutation KIT p. L576P in a patient undergoing immunotherapy and chemotherapy [3]

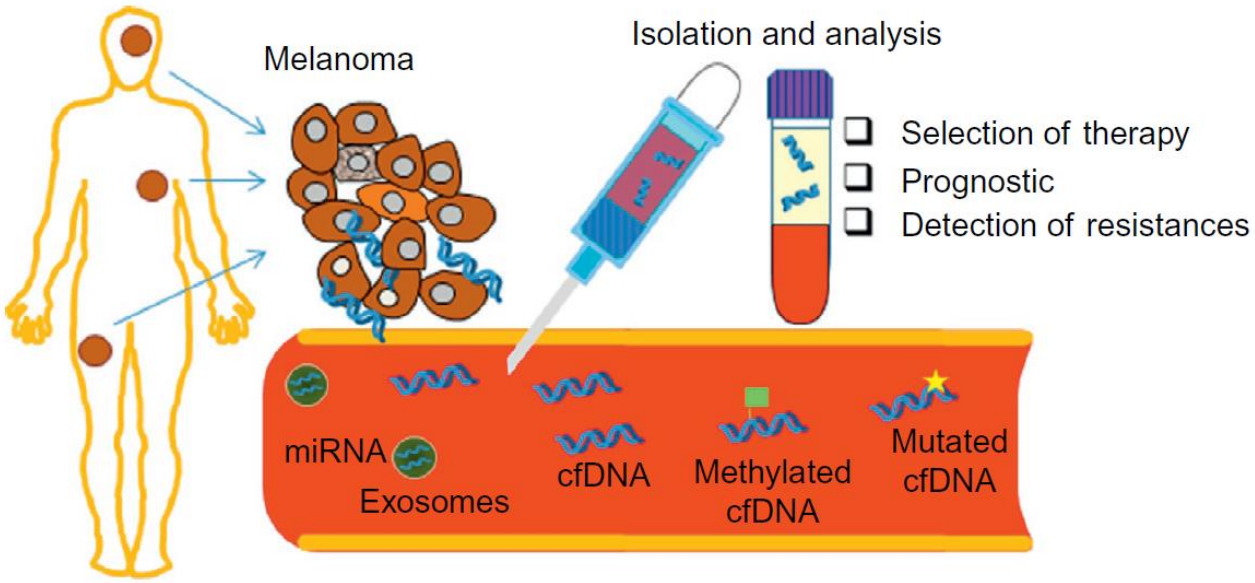
- Track genomic changes in tumour subclones



1. Garcia-Murillas et al. Sci Transl Med (2015)
2. Tie et al. Sci Transl Med (2016)
3. Gremel et al. Annal of Oncology (2016)

Significance

- cfDNA has immense potential as a cancer biomarker "**liquid biopsy**"
- **Non-invasive** way of assessing disease, monitoring treatment, response
- Detect mutations contributing to drug resistance
  - ➢ make therapeutic decisions accordingly



Alegre et al. Advances in Clinical Chemistry (2015)

Aims

To determine to what extent cell-free DNA can be used to elucidate the genomic profile of melanoma.

1. Perform genomic analyses on cell-free DNA extracted from the blood of melanoma patients presenting with stage III disease in order to identify somatic mutations.

2. Determine whether genomic mutations identified in the cfDNA \correlate with deep sequencing data from the corresponding tumours.

Tissue Bank

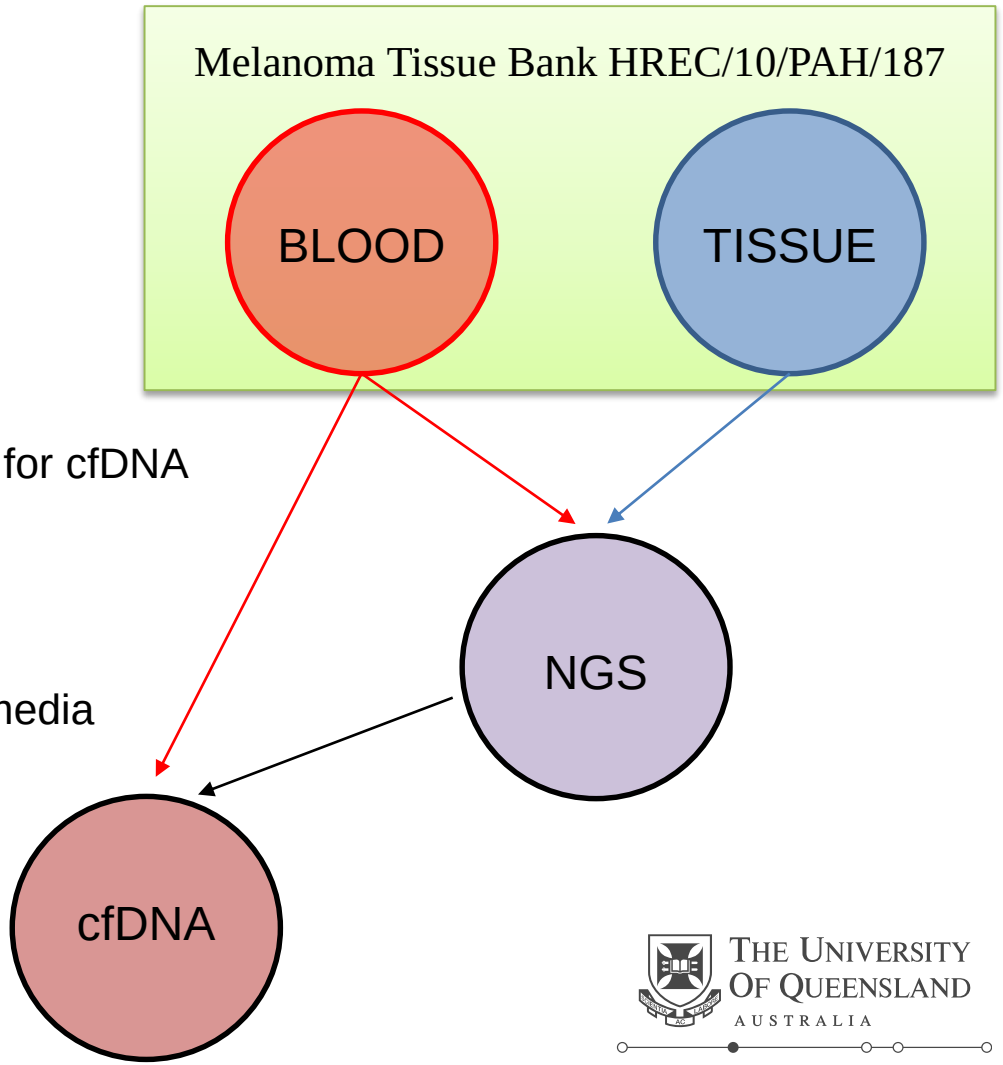
Stage III – IV melanoma (n=135)

Blood (at various time points)

- Buffy coat
- serum/plasma
- additional centrifugation of plasma for cfDNA

Tissue (at time of surgery)

- LN dissection
- RNAlater, formalin, tissue culture media
- DNA/RNA for NGS studies



Patients

Stage III patients as these have a poor prognosis compared to stage I and II

- More likely to isolate cfDNA from their plasma

Stage IV patients do not usually have their tumours resected

- tumour material is rarely available for genomic profiling

Blood samples have been collected **prior or at time of surgery**.

Aim 1

GENOMIC ANALYSIS OF CELL-FREE DNA

Samples:

- 25 plasma and tumour pairs from melanoma tissue bank

Generation of Genomic Data:

- cfDNA will be extracted from 2 ml plasma (minimum)
- QIAamp Circulating Nucleic Acid Kit
  - **minimum 10ng** of cfDNA
- Whole-exome sequencing (Macrogen)
  - Additional PCR to increase sample input
  - 200x coverage

cfDNA

MelR059

2 x primary SSM (R arm, lower extremity)

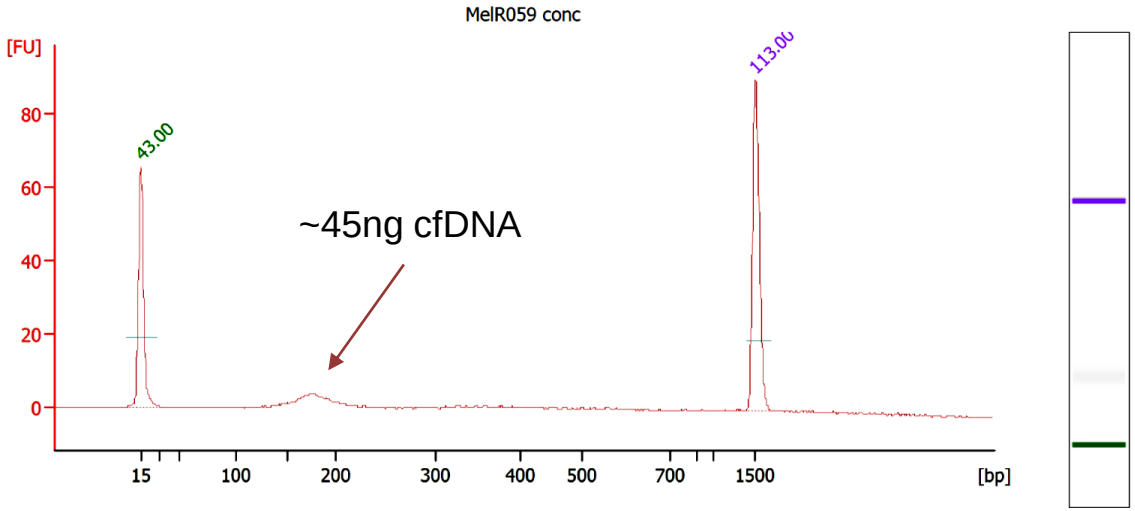
Stage 3c melanoma (October 2015)

Resection of axillary node

4/35 +ve nodes

Progressed 11 months after surgery

2.2 ml plasma



Expecting cfDNA fragments ~166bp

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Aim 1

Nic Waddell – QIMR Berghofer

Analysis of Genomic Data from cfDNA:

- Somatic mutations detected using an in-house developed tool qSNP and GATK
  - Single base substitutions identified by both tools have an accuracy of >95%
- gene and protein annotated using ENSEMBL

These methods have been used to explore the genomes of other cancer types

- Use somatic mutations to predict intra-tumour heterogeneity
- Annotate actionable mutations ie. BRAF p.V600
- Mutation load will be estimated by mutations/Mb of genome

  - **is this predictive of treatment response or survival?**

Aim 2

CORRELATION BETWEEN cfDNA AND TUMOURS

Generation of Genomic Data from Tumours:

SNP arrays (2.5 M Illumina) will be performed prior to sequencing

- tumour content
- copy number changes
- LOH

Deep whole-exome sequencing

- 500X tumour, 100X germline
- 100bp paired reads
- Illumina Hiseq4000
- deep tumour coverage to perform clonal and heterogeneity analysis

Genomic Data Analysis: performed as in Aim 1

Aim 2

Comparison of tumour mutations to those detected in cfDNA:

Directly compare sequence output cfDNA to tumour

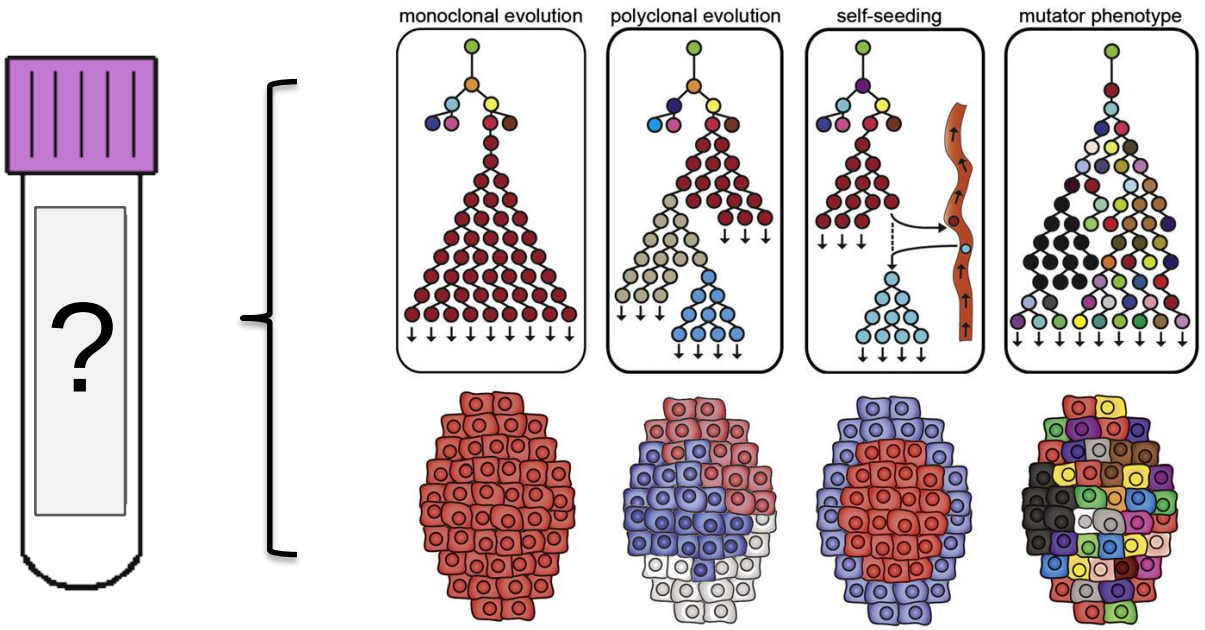
- Does it reflect the genomic landscape present in tumours?

Use a “pileup” approach to look for mutations in cfDNA

- Look at sequence reads which cover mutated positions in deep WES
- Identify mutations occurring at low allele frequency in cfDNA by mutation calling

How representative is cfDNA of tumour?

I. actionable mutations
II. overall tumour burden
III. tumour heterogeneity and sub clonal changes



Navin et al. 2010

Outcomes

Can cfDNA can be used to elucidate the genomic profile of **stage III** melanoma patients?

- treatment plan
- implemented as soon as transition occurs to stage IV
- no delay in therapy

Can this be applied to **stage IV** patients where it is not possible to sequence the tumour tissue directly?

- Can patients with **unresectable, high-risk disease** to be treated with a precision medicine approach in an appropriate time frame?
- window for treatment may be as little as a few months

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