Genomic analysis of circulating tumour DNA: pushing the limits for cancer applications

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Disclosures:
Co-founder & CSO, Inivata Ltd.
Research funding, AstraZeneca
Earlier diagnosis → Treatment selection → Disease monitoring → Molecular profiling
Earlier diagnosis

Treatment selection

Disease monitoring

Molecular profiling

Diagram showing the progression from earlier diagnosis to molecular profiling.
Cancers are unique, and evolve in response to selective pressure of therapy.

ctDNA can be used:

• As a *quantitative marker*, of tumour burden or residual disease

• As a *genomic tool* for molecular characterisation, to inform choice of therapy

• Integrated analysis to study cancer evolution and resistance to therapy
ctDNA levels span a wide range of values. Applications need different types of information. Methods need to be fit-for-purpose.

Bettegowda, Diaz et al.  
Sci Transl Med 2014
Cell-free DNA can be analysed at different scales of resolution

- Single molecule analysis
- Targeted sequencing
- Whole genome sequencing

Increasing sensitivity for rare mutations

Increasing genomic coverage (and cost)

Images: Vogelstein and Kinzler 1999; Forshew, Murtaza et al. 2012; Chan, Jiang et al 2013
Why use targeted sequencing:

**Quantitative marker**
- Standardised panel for monitoring, avoiding patient-specific assays
- Increase sensitivity for detection, look for multiple needles in a haystack

**Genomic characterisation**
- Non-invasive molecular profiling (‘Liquid biopsy’)
- Track multiple clones, monitor evolution and selection
Targeted sequencing provides a range of working-points

**Tagged-Amplicon Sequencing of ctDNA**  
*(Forshew, Murtaza et al., Sci TM 2012)*

- Cell free DNA
- Amplify 100~200 loci by targeted primer pairs
- Add sequencing adaptors and barcodes
- Sequencing (10k~30k depth)
- Kilobases targeted

**WGS/Hybrid-capture sequencing of ctDNA**  
*(Murtaza, Dawson et al., Nature 2013)*

- cfDNA
- Add sequencing adaptors and barcodes
- Amplify all fragments (whole genome)
- Capture regions of interest (hybridisation baits)
- Sequencing (1k~10k depth)
- Megabases targeted
Tagged-Amplicon Sequencing:
Accurate quantification down to individual mutant molecules;
Mutation identification limited by PCR/sequencing noise

Forshew, Murtaza, et al.
Exome sequencing of plasma DNA before therapy and at relapse can be used to discover novel resistance mechanisms.

Muhammed Murtaza, Sarah-Jane Dawson, Dana Tsui, et al., Nature 2013
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Personalised monitoring of tumour burden:
Quantification of patient-specific sequence alterations

Identify patient-specific mutations

Quantification of defined mutations

e.g. Diehl et al., Nat Med 2008; Dawson et al., NEJM 2013
ctDNA levels are prognostic, and track dynamics of advanced cancer, identifying disease relapse ~6 months ahead of other markers/imaging.
An expanded targeted sequencing panel for metastatic breast cancer: 175 amplicons covering regions in 17 genes

<table>
<thead>
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Dana Tsui, Sarah-Jane Dawson, Francesco Marass, Carlos Caldas
(unpublished)

Forshaw, Murtaza, Brenton
(Sci Transl Med 2012)
Different mutations can show diverging patterns: … a bug or a feature?

Mutant allele fractions in primary breast tumour:
CDH1 – 77%
CDH1 – 74%
AKT1 – 54%
MAP3K1 – 14%

Mutant allele fractions in primary breast tumour:
(9 years before T=0)
TP53 splicing – 14%
PIK3CA – 10%
TP53 point mutation – 9%
Relative mutation levels in ctDNA demonstrate clonal dynamics in response to several lines of TKI/chemotherapy

Dana Tsui, Tan Min Chin
(unpublished)
Why track 1-2 mutations ...

Jonathan Wan, Pippa Corrie
(unpublished)
Why track 1-2 mutations ...
Patient-specific targeted sequencing panels allow us to track dozens or hundreds of mutations.
Multiplexed detection of a large number of mutations can improve detection of low-burden disease

Jonathan Wan, Pippa Corrie
(unpublished)
Residual ctDNA post-surgery is prognostic, indicating risk of relapse. How to make this clinically actionable?

Localised breast cancer
Garcia-Murillas, Turner et al.
Sci Transl Med 2015
N=37
By enhancing sensitivity, can we more accurately identify those patients that have been cured?

Localised breast cancer
Garcia-Murillas, Turner et al.
Sci Transl Med 2015
N=37
Circulating Tumor DNA as an Early Indicator of Response to T-Cell Transfer Immunotherapy in Metastatic Melanoma

Dynamic patterns of BRAF-V600E in serum after initiating Tumor infiltrating lymphocyte (TIL) immunotherapy

Xi, Pham, Rosenberg, Raffeld et al., Clin Can Res 2016
Circulating Tumor DNA as an Early Indicator of Response to T-Cell Transfer Immunotherapy in Metastatic Melanoma

Xi, Pham Rosenberg, Raffeld et al., Clin Can Res 2016
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Next-generation sequencing panels to obtain molecular profiles of cancers directly from plasma, as a “liquid biopsy”

Forshew et al., Sci Transl Med 2012
To select patients for targeted therapy, we need to identify and quantify multiple “actionable” mutations with high fidelity.

Activating mutation
Resistance-conferring mutation
Other pathways

EGFR structure and mutations
(adapted from Clark et al. PLoS Med 2005)
Enhanced sensitivity for ‘actionable’ mutations in cancer genes: noise-reduced sequencing across a panel of 100s of amplicons

Lawson, Plagnol, Forshew, Gale, et al.
AACR 2015
A fit-for-purpose gene panel for clinical plasma DNA sequencing

Gale, Plagnol, Lawson, Forshew, et al.
AACR 2016
# A fit-for-purpose gene panel for clinical plasma DNA sequencing

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</table>

Exon tiling (88-100% coverage)  Hotspot regions

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Gale, Plagnol, Lawson, Forshew, et al.  
AACR 2016
In 174 NSCLC patients: alterations detected in 79% of cases. Treatment given based on cfDNA report in 17% of cases.

Treatment based on ctDNA profiling in 17% of patients: osimertinib (18, T790M), EGFR TKI (7), crizotinib (2, MET), afatinib (1, ERBB2).

*Gustave Roussy; @Inivata Ltd

Molecular Analysis for Personalised therapy (MAP)
London, September 2016
How good is a liquid biopsy? Compared to ...
ctDNA assays have been approved for use by the EMA and FDA as companion diagnostics for detection of EGFR mutations in plasma

FDA approval: (June 1st, 2016)
http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm

The agreement between the cobas EGFR Mutation Test v2 in plasma and the cobas EGFR Mutation Test v1 in tissue was evaluated for detection of EGFR mutations (Ex. 19del and L858R mutations) in NSCLC patients screened for participation in ENSURE. In 76.7% (70.5%, 81.9%) of tissue-positive specimens, plasma was also positive for an EGFR mutation.

The patients whose plasma results were positive for exon 19 deletion and/or an L858R mutations treated with erlotinib had improved progression-free survival (PFS) compared to those treated with chemotherapy.

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- More sensitive assays
- Other mutations as ”positive control” for EGFR-negative patients
- What is the expected level of concordance?
How good is a liquid biopsy?
Compared to …
day 5: extensive metastatic disease
(diffuse bone involvement, liver lesions,
pleural effusion, axillary and retroperitoneal lymph nodes, Figure S1)

day 700: progressive disease
(increase in size of liver lesion and new lesion within left ovary, Figure S4)

day 1077: progressive disease
(no change in liver lesion, new pulmonary nodules, Figure S6)
Murtaza, Dawson, Caldas et al. (Nat Comm. 2015)
Murtaza, Dawson, Caldas et al. (Nat Comm. 2015)
Murtaza, Dawson, Caldas et al. (Nat Comm. 2015)
Tumour heterogeneity may confound measurements of ctDNA sensitivity.
Plasma analysis tends to detect mutations shared between multiple tumour regions.

• Matched plasma analysis may aid interpretation of tumour mutation profiles from metastatic biopsies.
Stratify → profile → monitor → identify emerging resistance: adaptive therapy targeting the most prominent clone(s) in real time

What should we expect from ctDNA analysis going forward?
• Sensitive, quantitative, highly multiplexed
• Widely applied to a diverse range of applications in oncology
• Redefining the gold standard for oligo-metastatic disease?
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Rosenfeld lab and alumni, CRUK-CI

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- Chris Smith • Keval Patel • Jonathan Wan

Alumni: • Tim Forshew • Muhammed Murtaza •
Dana Tsui • Suzanne Murphy • Francesco Marass

Inivata Ltd.: • Michael Stocum • Clive Morris
• James Clark • John Beeler • Amanda Bettison
• Davina Gale • Tim Forshew • James Brenton
• Vincent Plagnol • Emma Green • Greg Jones
• Andrew Lawson • Sarah Smalley • et al.

James Brenton’s & Carlos Caldas’ labs, CRUK-CI, University of Cambridge

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