Monitoring the cancer genome in plasma using circulating tumour DNA

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Earlier diagnosis → Treatment selection → Disease monitoring → Molecular profiling
In the past few years there has been considerable focus on the need for 'biomarkers'. These biomarkers should be surrogate indicators for a future event, such as disease recurrence, disease progression, or death, and should indicate if a specific treatment will reduce that risk. Despite extensive investigations, there are no currently approved applications for liquid biopsies in the clinical setting. Nevertheless, recently published data demonstrate that ongoing research holds considerable promise for the future of molecular testing of ctDNA—one of the most promising future tools for clinical application.

Assessment of prognosis

Prognosis

Assessing prognosis for an individual patient involves a combination of clinical observations, staging, and histopathological and biomolecular characterization of different tumour types. This information, derived from imaging and biopsy specimens, allows a clinician to appraise the tumour biology, precisely stage the patient's tumour and differentiate between those patients with more-aggressive or less-aggressive disease. In this context, liquid biopsies are unlikely to supersede current methods, but their use might be important in circumstances where a biopsy is not available or genetic analysis of archived tumour samples is not possible.

Assessment of disease stage is one of the most-reliable predictors of prognosis and the relationship between levels of tumour-specific genetic aberrations and stage requires thorough evaluation for all cancer types. Studies have revealed a statistically significant correlation between disease stage and the presence of tumour-associated genetic aberrations (including mutations in $TP53$, $KRAS$, and $APC$ and allelic imbalances) in the blood of patients with resectable breast, ovarian, pancreatic and colorectal cancer and oral squamous-cell carcinoma. Furthermore, following mastectomy as treatment for breast cancer, it was reported that patients with tumours with vascular invasion, more than three lymph-node metastases, and high histological grade at diagnosis had persistent tumour-associated microsatellite DNA alterations as detected by PCR post-surgery in plasma-extracted DNA. Moreover, the presence of tumour-associated genetic aberrations in the blood, including $TP53$ mutations and loss of heterozygosity, correlated with overall survival or disease-free survival as assessed.
Blood plasma contains fragments of DNA – including tumour DNA

Circulating cell-free tumour DNA (ctDNA)
The cancer genome can be tracked noninvasively via plasma DNA.
Cell-free DNA can be analysed at different scales of resolution

Increasing sensitivity for rare mutations

Single molecule analysis

Targeted sequencing

Whole genome sequencing

Increasing genomic coverage (and cost)

Images: Vogelstein and Kinzler 1999; Forshew, Murtaza et al. 2012; Chan, Jiang et al 2013
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 applications in cancer management and drug development

- Earlier diagnosis
- Treatment selection
- Disease monitoring
- Molecular profiling
Personalised monitoring of tumour burden: Quantification of patient-specific sequence alterations

Tumour biopsy

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.

Metastatic breast cancer
Sarah-Jane Dawson, Dana Tsui, Carlos Caldas et al., NEJM 2013
24 patients (62.5%) harbored at least one codon 61 mutation, and the 31 mutations in these 15 patients comprised 45% of the total (69) mutations observed. Forty-eight percent of the codon 61 mutations were in \textit{NRAS} and the remainder were in \textit{KRAS} (table S6 and Fig. 6).

**DISCUSSION**

Through the study of 640 patients, we learned that mutant DNA fragments are found at relatively high concentrations in the circulation of most patients with metastatic cancer and at lower but detectable concentrations in a substantial fraction of patients with localized cancers. These results have several translational implications and suggest important avenues of future research.

Monitoring disease in advanced cancer patients

Genetic alterations could be identified in all 410 patients evaluated in this part of the study, making ctDNA a widely applicable biomarker for cancer patients. Moreover, >80% of patients with metastatic disease had detectable levels of ctDNA, higher than that reported for most conventional biomarkers (37). Unlike proteins such as CEA or CA19-9, which are expressed in normal cells as well as in neoplastic cells, genetic alterations of a clonal nature are only found in neoplasms. Our data indicate that measurements of ctDNA can also provide therapeutic, predictive, and prognostic information in patients with metastatic disease. As shown in Fig. 5, metastatic CRC patients with relatively low levels of ctDNA lived significantly longer than patients with higher levels, and there was a marked correlation between ctDNA concentration and survival. A similar association between survival and ctDNA concentration has recently been reported in patients with advanced breast cancers (16).

Although these advantages of ctDNA render it promising for monitoring patients, there are potential limitations. The specific mutations are defined by evaluation of the primary tumor, adding both time and expense to patient management. This may be so for no obstacles in the future because more cancer patients will have their tumors genetically analyzed to guide therapeutic decisions. The genetic alterations used to

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Bettegowda, Diaz et al.  
Sci Transl Med 2014

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ctDNA levels can be very high for advanced cancers. This allows ctDNA to be used as a “liquid biopsy” for molecular profiling.

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~10% mutant allele fraction
Personalised monitoring of tumour burden: Quantification of patient-specific sequence alterations

Tumour biopsy

Quantification of defined mutations

- e.g. Diehl et al., Nat Med 2008;
- Dawson et al., NEJM 2013
Hot-spot assays can detect individual ‘actionable’ mutations in blood. Available in various formats, including (since June 1\textsuperscript{st}) FDA-approved.

(adapted from Clark et al. PLoS Med 2005)
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)
Can we use next-generation sequencing to obtain molecular profiles of cancers directly from plasma, as a “liquid biopsy”?

Forshew et al., Sci Transl Med 2012
Mutation profiling by ultra-deep targeted sequencing of plasma
Mutation profiling by ultra-deep targeted sequencing of plasma

Forshew, Murtaza, Brenton
(Sci Transl Med 2012)
NGS-based liquid biopsy assays can help clinical decision making


AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, November 2015
How good is a liquid biopsy?
Compared to …
How good is a liquid biopsy?
Compared to …
How good is a liquid biopsy?
Compared to …
ctDNA levels can be very high for some advanced cancers. In those cases, genome-wide sequencing can be highly informative.
Exome sequencing of plasma DNA before therapy and at relapse can be used to discover novel resistance mechanisms

Muhammed Murtaza et al., Nature 2013
Exome-seq of plasma DNA at progression can be used to study novel resistance mechanisms.

Mutations identified in exome data:
- GAS6
- PDGFRA
- MED1

Murtaza, Dawson, Caldas, Rosenfeld
Nature 2013
Mutations identified in exome data:
- GAS6
- PDGFRA
- MED1

Exome-seq of plasma DNA at progression can be used to study novel resistance mechanisms

Mutant allele fraction

Days of follow up

Murtaza, Dawson, Caldas, Rosenfeld Nature 2013
Plasma DNA can be used to discover resistance mechanisms. This can supplement invasive analysis using repeat biopsies.

Murtaza, Dawson, Caldas, Rosenfeld
Nature 2013
Day of diagnosis (day 0)

Symptomatic brain metastasis resected (day 577)

Autopsy (patient died on day 1193)

Biopsy

Plasma

Docetaxel
Cyclophosphamide

Paclitaxel

Tamoxifen

Trastuzumab

Lapatinib
Capecitabine

 Imaging

T-1 (exome)
T-2 (exome)
T-3
T-4
T-5
T-6
T-7
T-8
T-9 (exome)

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)
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Biopsy

Plasma

Docetaxel

Cyclophosphamide

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Tamoxifen

Trastuzumab

Lapatinib

Capecitabine

Imaging

T-1 (exome) 77x

T-2 (exome) 80x

T-3

T-4

T-5

T-6

T-7

T-8

T-9 (exome) 140x

P-1, Breast (DCIS)

M-1, Brain Metastasis

Normal

P-2, Breast

M-2, Lung

M-3, Liver

M-4, Left Ovary

M-5, Vertebra

P-1, Breast (DCIS)

M-1, Brain Metastasis

Normal

P-2, Breast

M-2, Lung

M-3, Liver

M-4, Left Ovary

M-5, Vertebra

Figure 1: Timeline describing clinical course of the patient, treatments administered and samples collected.

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)
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- T-1 (exome)
- T-2 (exome)
- T-3
- T-4
- T-5
- T-6
- T-7
- T-8
- T-9 (exome)

Biopsy
Plasma

- Imaging
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)

- Paclitaxel
- Cyclophosphamide
- Trastuzumab
- Lapatinib
- Capecitabine

Murtaza, Dawson, Caldas et al. (Nat Comm. 2015)
Day of diagnosis (day 0)
Symptomatic brain metastasis resected (day 577)
Autopsy (patient died on day 1193)

Biopsy
Plasma

Brain Metastasis
Lung Autopsy
Vertebral Autopsy
Ovarian Autopsy
Liver Autopsy

Normal Tissue
DCIS

Breast Autopsy
Lung Autopsy

Murtaza, Dawson, Caldas et al. (Nat Comm. 2015)
Day of diagnosis (day 0)

Symptomatic brain metastasis resected (day 577)

Autopsy (patient died on day 1193)

T-1 (exome)

T-2 (exome)

T-3

T-4

T-5

T-6

T-7

T-8

T-9

Biopsy

Plasma

Imaging

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

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Symptomatic brain metastasis resected (day 577)

Autopsy (patient died on day 1193)

T-1 (exome)

T-2 (exome)

T-3

T-4

T-5

T-6

T-7

T-8

T-9 (exome)

Biopsy

Plasma

Tamirol

Docetaxel

Cyclophosphamide

Trastuzumab

Paclitaxel

Capecitabine

Imaging
day 5: extensive metastatic disease (diffuse bone involvement, liver lesions, pleural effusion, axillary and retroperitoneal lymph nodes, Figure S1)
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Figure 1: Timeline describing clinical course of the patient, treatments administered and samples collected.

Figure 2

P-1

P-3

P-2

M-1

M-3

M-4

M-5

M-2

M-1, Breast (DCIS)

M-2, Lung

M-3, Liver

M-4, Left Ovary

M-5, Vertebra

Normal Tissue

P-3 (Lymph node)

P-1 & P-2 private mutations

Metastatic clade mutations

Private mutations

Stem mutations

Plasma mutations

Mean AF across all mutations in group

Relative Plasma Abundance

Days of follow-up
Analysis of a single metastatic lesion picks up trunk driver mutations; but many of the mutations identified are subclonal mutations private to this lesion.
Analysis of plasma “enriches” for mutations that are prevalent across all lesions.
ctDNA can provide a basis for adaptive/reactive therapy: targeting the most prominent clone(s) in real time

- ctDNA can be used to track tumour burden over time
- ctDNA analysis can be used to study cancer evolution
- ctDNA can be used to guide targeted therapy in real time
ctDNA applications in cancer management and drug development

Earlier diagnosis  Treatment selection  Prognosis & monitoring  Molecular profiling !
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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
Needle in a haystack?? Look for multiple needles → By multiplexed analysis of multiple patient-specific mutations.
Residual ctDNA post-surgery is prognostic, indicating risk of relapse. More sensitive detection will identify further at-risk patients.

Localised breast cancer
Garcia-Murillas, Turner et al.
Sci Transl Med 2015
N=37
Can we identify those patients who have been cured?

Localised breast cancer
Garcia-Murillas, Turner et al.
Sci Transl Med 2015
N=37
When will our methods be sensitive enough for earlier diagnosis? Will performance be good enough at sustainable costs?

Few copies of tumour DNA in stage I-II

Which genomic alterations? How many can we test?
ctDNA applications in cancer management and drug development

Earlier diagnosis?  
Treatment selection  
Prognosis & monitoring …  
Molecular profiling!
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