The promises and pitfalls of liquid biopsy for the molecular portrait and monitoring of treatment response in NSCLC

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DISCLOSURE SLIDE

• Nothing to declare
THE AMBITION FOR PRECISION MEDICINE

monitoring of tumour clonal evolution

Krebs et al, Nature Reviews Clinical Oncology, 2014
THE AMBITION FOR PRECISION MEDICINE

monitoring of tumour clonal evolution

BIOPSY IS GOLD-STANDARD FOR MOLECULAR CHARACTERISATION BUT...

- Invasive and sometimes difficult to obtain. **NOT without risk**
- Insufficient quantity of tissue from small biopsies / cytology
- Archival tumour may not reflect current status of tumour
- Tumour heterogeneity of primary and metastases
- Serial biopsies to assess PD activity/ molecular evolution of tumour/ resistance mechanisms are challenging

Circulating tumour cells

Circulating tumour DNA
CTC ISOLATION TECHNOLOGIES

IMMUNOMAGNETIC CAPTURE - CELLSEARCH

Dilute with buffer
Incubate 10min
Filtration on ISET device 3min

10 spots = 1ml blood filtered per spot
Epithelial cells, tumour cells, tumour microemboli and ‘rare’ cells enriched on filter

On the filter:
Cytopathological analysis CTC counting per ml IHC, FISH, TUNEL

Mount filter on slide
Cytopathological analysis
Molecular analyses targeted to tumour cells after laser microdissectionCGH, DNA mutation; RT-PCR
Molecular analyses on enriched cells from the spots

ISOLATION BY SIZE - ISET

Tumour Cell
WBC
WBC
WBC
OTHER CTC TECHNOLOGIES

ISOFLUX

Parsortix

Clearbridge

CTC-iChip

Gilupi

CellCollector

Target cell

Size-based Sorting  Single File Alignment  Magnetic Separation

CTC labeled with magnetic beads (1-100/ml)

Red blood cell (8 X 10^6/ml)

White blood cell (5 X 10^6/ml)
CTCS AS A PROGNOSTIC BIOMARKER IN NSCLC PATIENTS

≥5 CTCs is prognostic

Overall Survival

Median OS 8.1 vs 4.3 months
HR 5.98 (95% CI 2.25-15.87), P<0.001

Independent prognostic factor (HR = 7.92) for OS in multivariate analysis

Krebs et al, Journal of Clinical Oncology, 2011
EXAMPLES OF CTCS AND CTM BY ISET IN NSCLC PATIENTS

CTM were observed in 15 (38%) of the 40 patients by ISET – not seen in matched CellSearch samples.

All images 40x magnification
CD45 immunostaining with haematoxylin counterstain
5/87 patients with ALK fusion disease. All 5 cases had strong ALK expression in CTCs by IHC and ALK gene rearrangement by FISH.

All ALK positive patients (n=18) had 4 or more ALK rearranged CTCs per ml of blood with unique 3’5’ split pattern seen in CTCs.
Questions
• Does the heterogeneity of CTCs reflect tumour heterogeneity
• Do the different populations of cells have unique driver mutations?
• Which of these survive and result in metastases?
• Single cell molecular analysis may help address these issues

Gerlinger et al, New England Journal of Medicine, 2012
FROM CTC ENRICHMENT TO SINGLE CELL ANALYSIS

1/10,000,000
Peripheral blood

Enrichment / Sample-Prep

1/1,000-10,000
Enriched CTCs

Veridex CellSearch®,
CTC Chips, Microfilters, etc.

The DEPArray™

Sequencing

Whole genome amplification
Reproducible copy number variation patterns among single circulating tumor cells of lung cancer patients

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PROS AND CONS OF CTC ANALYSIS

**PROS**

- An excellent research tool
- Powerful for investigating tumour heterogeneity and tumorigenicity (CTC derived xenografts)
- **Ability to assess morphology, protein expression and genetic aberrations all in the same cell**
- May help better understand mechanisms of metastasis and identify novel treatment targets

**CONS**

- Lack of validation/qualification for most technologies
- Expensive
- **Wide variations in CTC definition and technologies**
- Difficulty in reproducing assays across multiple sites limits ‘routine’ clinical use
- Need joined up approach across CTC community to validate assays
CIRCULATING TUMOUR DNA

Schwarzenbach et al, Nat Rev Cancer, 2011

Scorpion amplification refractory mutation assay (SARMS)

Sensitivity down to 1-2%
Determination of EGFR Status by ctDNA in Lung Cancer

- Phase IV study of gefitinib in EGFR mutation positive advanced NSCLC
- Compared EGFR status in tumour block and plasma using Scorpion ARMS assay

- 1033/1060 had evaluable tumour
- Mutation status determined for 859 patients
- 118/859 (13.7%) had EGFR mutation

- 803/1060 had 2 baseline plasma samples
- Mutation status determined for 784 patients
- 82/784 (10.5%) had EGFR mutation

Concordance = 94.3%
Sensitivity 66%
Specificity 100%

Douillard et al, Journal of Thoracic Oncology, Sep 2014
DETERMINATION OF EGFR STATUS BY ctDNA IN LUNG CANCER

- Phase IV study of gefitinib in EGFR mutation positive advanced NSCLC
- Compared EGFR status in tumour block and plasma using Scorpion ARMS assay

IRESSA receives CHMP positive opinion to include blood based diagnostic testing in European label

Fri, 26th Sep 2014

In cases where tumour material is not available, gefitinib may be started if EGFR mutation positive from ctDNA

Douillard et al, Journal of Thoracic Oncology, Sep 2014
DIGITAL PCR AND DISEASE MONITORING IN EGFR +VE DISEASE

- DIGITAL PCR increases sensitivity further and provides quantification of mutated alleles

↑ in mutated ctDNA occurred up to 16 weeks ahead of confirmed PD

T790M detectable ahead of clinical PD

Oxnard et al, Clinical Cancer Research, Mar 2014
FAILURE TO ERADICATE ctDNA = WORSE OS

N=62

Group A = no mutation identifiable in ctDNA at baseline
Group B = EGFR mutation eradicated by 10 weeks on EGFR inhibitor
Group C = EGFR mutation not eradicated by 10 weeks on EGFR inhibitor

QUANTIFICATION OF ctDNA IS COMPLEMENTARY TO IMAGING

Novel sensitive method for ctDNA quantification (CAPP-seq)

Newman et al, May 2014
REISTANCE MECHANISMS FROM ctDNA – WHOLE EXOME SEQUENCING

‘Genome-wide representation of tumour in plasma’

‘Increased representation of mutant alleles in association with therapy resistance’

‘A new paradigm for the study of clonal evolution’

Murtaza et al, May 2013
PROS AND CONS OF ctDNA ANALYSIS

PROS

• Various clinical applications of ctDNA – quantification and profiling
• DNA easy to extract and profiling assays are much more amenable to validation and qualification than CTCs
• Straight-forward blood test
• Already in clinic with EGFR mutation testing and gefitinib

CONS

• Different methods across different centres
• Quantification and whole exome sequencing are still in their infancy with reports including relatively small numbers of patients
• Technically challenging to look at whole exome from ctDNA
• May not be suitable for every patient – ? Stage IV/tumour burden; otherwise need biopsy
• Released from apoptosing cells – ? relevance
TUMOUR CHARACTERISATION TO GUIDE EXPERIMENTAL TARGETED THERAPY – THE TARGET TRIAL

Manchester Cancer Research Centre

TARGET

To molecularly profile ctDNA of all patients referred for consideration of phase 1 clinical trials
To select a relevant trial based on ctDNA ‘actionable aberrations’
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RECRUITING CLINICAL FELLOWS AND SENIOR LECTURERS TO ECMT IN MANCHESTER

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