Intra-tumoural heterogeneity in high grade serous ovarian cancer – what does it mean for designing clinical trials?

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Disclosure Information

• Accepted travel funding for clinical trials meetings [Clovis Oncology].

• I will not discuss off label use and/or investigational use in my presentation.
Heterogeneity in cancer

Population

Intra-patient
Spatial, temporal

Intra-tumor
Tissue

Intra-tumor
Genetic
Can genetic intratumoural heterogeneity explain resistance in HGSOC?

1. What determines severity and kinetics of divergent evolution?
2. How can we detect adverse phenotypes in the clinic?

Original Paper

Genetic intra-tumour heterogeneity in epithelial ovarian cancer and its implications for molecular diagnosis of tumours

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The clonal evolution of metastases from primary serous epithelial ovarian cancers

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4 Department of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, United Kingdom
5 Department of Pathology, St. Bartholomew’s and The Royal London, London, United Kingdom
Platinum-resistant cell lines show non-linear genetic change.

Can we quantitate heterogeneity?

Roland Schwartz, Charlotte Ng, Susie Cooke
Does relapsed HGSOC have a different mutational profile?

Multiple sampling at initial surgery

0 6 12 24 36

Months

Resistant
Sensitive
Refractory

↑ Biopsy of disease
BritROC-1

- The UK Translational Research in Ovarian Cancer Collaborative
  - 8 major ovarian cancer centres (Cambridge, Glasgow, Newcastle, Hammersmith, Barts, Edinburgh, Leeds, Manchester)
  - BritROC-1 study provides cost of biopsies and study coordinator
Circulating cell-free tumour DNA (ctDNA)
Circulating cell-free DNA is a promising biomarker

Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection

Y M Dennis Lo\textsuperscript{1,2,7}, Nancy B Y Tsui\textsuperscript{2,7}, Rossa W K Chiu\textsuperscript{1,2,7}, Tze K Lau\textsuperscript{3}, Tse N Leung\textsuperscript{3}, Macy M S Heung\textsuperscript{2}, Ageliki Gerovassili\textsuperscript{4}, Yongjie Jin\textsuperscript{5}, Kypros H Nicolaides\textsuperscript{4}, Charles R Cantor\textsuperscript{6} & Chunning Ding\textsuperscript{1,5,7}

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**Tumour DNA**

1991-1992 (Sidransky, Vogelstein)
Detection of cell-free tumour DNA

2005 (Diehl et al., JHU)
Quantification of ctDNA

**Fetal DNA**

1997 (YM Dennis Lo)
Fetal DNA in maternal plasma

2007 (YM Dennis Lo et al.)
Demonstration of T21 NIPD
Circulating cell-free DNA is a promising biomarker

Circulating mutant DNA to assess tumor dynamics

Frank Diehl\textsuperscript{1,5}, Kerstin Schmidt\textsuperscript{1,5}, Michael A Choti\textsuperscript{2}, Katharine Romans\textsuperscript{1}, Steven Goodman\textsuperscript{3}, Meng Li\textsuperscript{1}, Katherine Thornton\textsuperscript{1}, Nishant Agrawal\textsuperscript{1}, Lori Sokoll\textsuperscript{4}, Steve A Szabo\textsuperscript{1}, Kenneth W Kinzler\textsuperscript{1}, Bert Vogelstein\textsuperscript{1} & Luis A Diaz Jr\textsuperscript{1}

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**Tumour DNA**

1991-1992 (Sidransky, Vogelstein)
Detection of cell-free tumour DNA

2005 (Diehl et al., JHU)
Quantification of ctDNA

2008 (JHU group)
Monitoring ctDNA

**Fetal DNA**

1997 (YM Dennis Lo)
Fetal DNA in maternal plasma

2007 (YM Dennis Lo et al.)
Demonstration of T21 NIPD

2011 (Lo and others)
Clinical validation

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N\textasciitilde100

N\textasciitilde3000
Clinical Uses of ctDNA

1. Detection of minimal residual disease
2. Disease monitoring
3. Detection of new mutations
Assumptions about ctDNA
Obvious application in HGSOC

• Easily accessible analyte with attractive properties
  – 1800–13000 fragments of DNA per ml
  – Tumour fraction is 0.005–11.7%
  – Estimated half-life 114 min

• Circulating tumour cells are infrequent in HGSOC

• *TP53* mutations are ubiquitous

Different Analytical Approaches
Comparison of ctDNA quantification to CA125

HGSOC patients (n = 41)

Identification of TP53 mutations in FFPE tumour biopsy (n = 41/41)

Unique personalised assays (n = 32)

Digital PCR

Plasma collected during treatment (n = 323)

Christine Parkinson
Davina Gale
Anna Piskorcz
Nitzan Rosenfeld
Mutation specific analysis
Digital PCR using Fluidigm Arrays

a) Conventional PCR
Split sample by dilution

b) Digital PCR

Sample Inlets
Nanofluidic panels
Detection of unknown mutations?
Real time identification of arising actionable mutations: Sequencing multiple genes in parallel in fragmented DNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of primers</th>
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<td>TP53</td>
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<td>KRAS</td>
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<td>BRAF</td>
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Sensitive identification and accurate quantification of mutations

Forshew, Murtaza, Brenton, Rosenfeld (Sci Transl Med 2012)
Can we follow different or identify new tumour populations?
De-novo identification of an *EGFR* mutation by plasma sequencing

Forshew, Murtaza, Brenton

(Sci Transl Med 2012)
De-novo identification of an *EGFR* mutation by plasma sequencing
Resolving origin of recurrent cancer
Whole genome profiling?
Cancer genomes in plasma: a novel paradigm for noninvasive analysis of tumour evolution

Murtaza, Dawson, Tsui, Brenton, Caldas, Rosenfeld
(Nature 2013)
The cancer genome is represented in plasma as circulating DNA
Mutations found in plasma are representative of the tumour
Key Questions

• Does relapsed HGSOC have distinct mutation profiles? [**Biopsy at relapse now essential** in clinical trials, BRITROC1, OCTIPS]

• Must concentrate on targeting genomic drivers (TP53, BRCA1/2, PTEN) [**Fractionation/drug holidays?**]

• Can we detect very low allele frequency clones at diagnosis? [**Multiple sampling at surgery essential**]

• Does ctDNA have utility for prediction of response and treatment failure in clinical trials? [**Imaging and biopsy correlates**]
# Acknowledgements

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<th>Brenton Lab</th>
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