Role of Circulating Tumor DNA Profiling in Cancer Management

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Changing Landscape of Drug Development

Increased Understanding of Cancer Biology

Advent of Targeted Therapies

High Attrition Rates/High Costs

Personalized Medicine
### Declining costs of sequencing: massively parallel next-generation sequencing and subsequent computational analysis

**Molecular Characterization for Patient Selection**

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COSMIC: Catalog of Somatic Mutations in Cancer

- COSMIC launched in 2004, detailed 4 cancer genes
- 2014: world's largest and most comprehensive resource
  - 2,002,811 coding point mutations in over one million tumor samples
  - 6 million noncoding mutations, 10,534 gene fusions, 61,299 genome rearrangements
  - 695,504 abnormal copy number segments and 60,119,787 abnormal expression variants

Transition From Histology → Genomic Driver Mutations

ALK inhibition in EML4-ALK + NSCLC

- High response rate in early phase trials (>50%)
- Around 5 years from filing of Investigational New Drug (IND) application and approval by the FDA in August 2011

Vemurafenib in BRAF V600E mutant melanoma

Pre-treatment

2 weeks vemurafenib
Exciting, dramatic responses, accelerated drug approvals

Phase I for Crizotinib – standard dose escalation in solid tumors, 2 pts responded → profiling showed ALK rearrangement → protocol amended to include an expansion cohort → 1500 patients screened from 2008-2010 to enroll 82 patients with FISH+ ALK rearrangement → 57% objective confirmed partial/complete response. 2011-Crizotinib approved by the FDA

Challenges:

Responses may be short-lived (e.g. vemurafenib)-development of resistance
Tumor heterogeneity
Defining a genetic aberration as ‘actionable’
Finding enough patients
Tumor heterogeneity

- Difference within regions of a given tumor and between primary and metastases

- Only 34% of all mutations detected by multiregion sequencing in the nephrectomy specimen were present in all regions

- So far, clinical decisions are based on data generated from one or 2 core biopsy specimens from any one lesion

- ‘Ongoing regional clonal evolution’

- Prognostic gene expression signatures shown to classify into good or bad prognostic categories for RCC varied by region


Is the sample representative of the disease being treated? Archival vs fresh tissue?
The number of mutations that would have been detected from each tumor by sampling one to $n$ biopsies (where $n$ was the number of biopsies sampled from that tumor)


Cell-free circulating tumor DNA (ctDNA)?
Circulating DNA

Can arise from various cell populations, could be nuclear or mitochondrial

Exist in number of structural forms: particulate structures (exosomes, microparticles, apoptotic bodies) or macromolecular structures (nucleosomes, virtosomes/proteolipid/nucleic acid complexes, DNA traps, links with serum proteins or to cell-free membrane parts

Timeline for important discoveries about circulating DNA

**CirDNA analysis applications**

- **1948**: Cell-free DNA molecules in the human blood compartment (Mandel et al)
- **1949**: cirDNA analysis (Kopreski et al)
- **1949**: RAS mutations detection by cirDNA analysis (Anker et al)
- **1950-60's**: Interest from Russian researchers for the Pangeness Theory (Michurin et al)
- **1963-1970**: Active cellular release of cirDNA as a messenger (Stroun, Anker and Gahan)
- **1977**: Higher concentration of cirDNA in the blood of cancer patients (Leon et al)
- **1979**: cirDNA derives from the tumor in cancer patients (Stroun and Anker)
- **1989**: Foetal DNA circulates in the blood of pregnant women (Lo et al)
- **1994**: Extracellular DNA from tumor cell line acts as oncogenic mediator (Anker et al)
- **1997**: Foetal DNA circulates in the blood of pregnant women (Lo et al)
- **1999**: cirRNA analysis (Kopreski et al)
- **1994**: Circulating nucleosomes (Holdenrieder et al)
- **2001**: Circulating nucleosomes (Holdenrieder et al)
- **2008**: Study of the tumor dynamics by cirDNA analysis (Diehl et al)
- **2001**: First clinical study on the detection of point mutations by cirDNA analysis (Diehl et al)
- **2004**: Identification of NETs (Brinkmann et al)
- **2010**: Genometastasis (Garcia Olmo et al)
- **2010**: Cir mitochondrial DNA is a DAMP (Zhang et al)
- **2011**: Cir DNA aneuploidy test for prenatal diagnostics commercially available (Lo)
- **2012**: Tracking tumor resistance by cirDNA analysis (Bardelli et al, Diaz et al)
- **2013**: cirDNA analysis to follow-up cancer (Dawson et al)
- **2014**: First clinical validation of cirDNA analysis (Thierry et al)
- **2015**: Involvement of NETs in oncogenesis (Cools-Lartigue et al)
- **2015**: cirDNA induces apoptosis and DNA damage into recipient cells (Mitra et al)

ctDNA

- Minimally invasive
- Longitudinal sampling
- Low cost
- Potentially represents multiple disease sites

Tumor biopsy

- Invasive, associated risks
- Limited sampling
- Expensive, resource intense
- Limited sample from one site
Role of ctDNA in Cancer Management

- Known driver mutations; known aberrations in that disease
  - Disease burden
  - As an early response marker
  - Monitor for recurrence
  - Tumor heterogeneity—does it provide a more complete picture of the presence of various clones?
  - Emergence of resistant clones
  - Diagnostic
    - Presence of actionable mutations
    - Differentiate between benign and malignant disease

- Broad profiling to look for genetic aberrations
Assessing tumor burden, marker of response

Percentage of ctDNA to total cirDNA is a measure for tumor burden

Cancer Personalized Profiling by deep Sequencing [CAPP-Seq] in lung cancer

Hypothesis: Monitoring of tumor-specific chromosomal rearrangements in ctDNA can detect occult metastatic disease and serve as a sensitive, specific, and thus potentially clinically useful noninvasive biomarker in the early stage disease setting.
55 women with early stage breast cancer who received neoadjuvant chemo \( \rightarrow \) definitive t/t
Mutation tracking with serial ctDNA samples was predictive of relapse (median of 7.9 mos lead time over clinical relapse)
In the 3 pts with CNS only relapse, no ctDNA was detected before or at relapse

ctDNA: Is it better able to define tumor heterogeneity?

- Analysis of tumor biopsies from patients with NSCLC progressing on EGFR TKIs demonstrated presence of potential additional resistance mechanisms in ~15% of cases.

- In 41 pts with T790M mutation+ NSCLC progressing on EGFR TKIs, additional putative resistance mutations were detected in 46% (19 pts) in pre-treatment plasma.
  
  14 pts had increased copy number in MET or ERBB2
  3 pts had SNVs in EGFR, PIK3CA or RB1
  2 had both an increased copy number in MET and SNVs in PIK3CA or RB1

- The ability to detect additional abnormalities at baseline may impact choice of subsequent therapy and better define innate resistance.

- Not a direct comparison of tumor biopsy vs ctDNA in the same patient

Defining resistance in pts with EGFR+ NSCLC

Rociletinib selectively targets T790M containing subclones
43 pts with T790M EGFR + NSCLC-samples baseline and at progression
At progression, 28/43 pts (65%) had one or more putative resistance mechanism

Red, blue, purple: SCNA affecting MET, ERBB2, EGFR resp.

SNV: single nucleotide variants
SCNA: somatic copy-number alterations
ctDNA Analysis as a Companion Diagnostic

- June 2016: FDA approved cobas® EGFR Mutation Test v2 using plasma specimens as a companion diagnostic test for the detection of exon 19 deletions or exon 21 (L858R) substitution mutations in EGFR gene to identify patients with metastatic NSCLC eligible for treatment with Tarceva® (erlotinib).

- Ph III ENSURE trial: Efficacy and safety of Tarceva versus gemcitabine plus cisplatin as first-line treatment for stage IIIB/IV NSCLC pts. T/t was assigned based on tumor tissue results, 98.6% of pts also had plasma samples available.
  
  In 76.7% (70.5%, 81.9%) of tissue-positive specimens, plasma was also positive for an EGFR mutation.
  
  Plasma was negative for EGFR mutation in 98.2% (95.4%, 99.3%) of tissue-negative cases.

- Test approved for pts too ill or otherwise difficult to get tumor tissue

- If ctDNA result is positive then proceed with EGFR inhibitor, if negative then obtain tumor tissue.
Good concordance between melanoma ctDNA and primary tumors when samples were collected before treatment or less than one year apart.

http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0162809
Somatic variants in lung cancer tumor (diagnostic biopsy) and plasma DNA


http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0162809
Definitions

• **Analytical performance (analytical validity):** how accurately the test detects the analyte(s) of interest

• **Clinical Validity:** How well does the assay result correlate with outcome?

• **Clinical Utility:** How does use of the assay improve outcome?