Molecular and Functional Characterization of CTCs in Non-Small Cell Lung Cancer

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Gustave Roussy, France
Introduction to CTCs

Predictive and resistance biomarkers

• ALK story

NSCLC CTC-derived xenografts
Clinical interest of CTCs (I)

- CTC counts, an independant pronostic factor
  - FDA approved in patients with metastatic breast, prostate, colorectal cancers as an aid to pronostic
  - Metastatic relapse prediction
  - Monitoring of treatment efficiency
  - Pharmacodynamic utility
  - Patient treatment stratification
Clinical interest of CTCs (II)

• A « Liquid Biopsy » for biomarker identification
  – Pressing needs:
    • Tumor biospies are invasive, impractical, associated with risk
    • Could be of poor quality
    • Serial biopsies are challenging
    • Not representative of tumor evolution and heterogeneity

– CTCs
  • Easily accessible, non invasive, repeatable
  • Serial sampling for real time monitoring of treatments
  • Detection of resistance biomarkers
  • May be analysed as single cells: a better representation of tumor heterogeneity than single site biopsies and ct DNA
CTCs: a window on the metastatic process

Massagué & Obenauf, Nature 2016
Main features of CTCs

- Very rare (1-10/mL, most often less)
- Issued from either the primary tumor or its metastasis
- Phenotypically and genetically heterogeneous
- Isolated or in clusters (collective cell-migration)
- Undergoing EMT (Epithelial to Mesenchymal Transition)
- Contain Cancer Stem Cells/Tumor Initiating Cells
- Complex and dedicated technologies are required for CTC counting, characterization and isolation
The epithelial to mesenchymal transition

Driver of tumor progression
Cause of intrinsic and acquired resistance
Linked to cancer stem cell properties

Nieto et al Cell 2016
A technological Challenge: Rarity and Heterogeneity

- Two steps: enrichment + detection
- No universal assay, more than 45 CTC detection technologies

Alix-Panabières & Pantel Nat Rev Cancer 2014
CTCs versus ctDNA

- Multiple DNA abnormalities
- RNA expression and fusion transcripts
- Protein expression and phosphorylation
- In vitro/in vivo culture
- CTC [cell number]
- Circulating tumor DNA [number of mutant molecules]
- Amplification and deletion
- Translocation
- Point mutations
- Chromosomal abnormalities

Haber & Velculescu Cancer Discovery 2014
Molecular Classification of NSCLC

- Adenocarcinoma: ~50%
- SCC
- LCC

Molecular Drivers:
- EGFR: 15%
- KRAS
- ALK: 4%
- HER2
- MET
- FGFR1: ~1-2%
- ROS1
- RET
- PI3KCA
- MEK1
- BRAF
- AKT1
- NRAS

Unknown: ~50%
A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas

CellSearch
FDA approved

ISET
Isolation by Size of Epithelial Tumor cells
A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas

Median CTC count: 0/ 7.5 mL – range 0 - 13500

Median CTC count: 5/ 7.5 mL – range 0 - 100

ISET
Isolation by Size of Epithelial Tumor cells
Selection of ISET for biomarker detection in NSCLC
KRAS | ALK | EGFR | WT

EMT % of total CTC

CTC numbers

0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80

Lindsay et al. *Annals Oncol* 2017
Clinical issues in ALK-rearranged patients

First line: Crizotinib

Biopsy

Diagnosis?

Response Prediction?

Resistance mutations?

2nd generation
Ceritinib
Afatinib
Brigatinib

3rd generation
Lorlatinib

Resistance mutations?

Resistance mutations?
Identification of ALK-rearranged patients

- Diagnosis of ALK positivity by FISH = 15% of ALK-rearranged tumor cells in tumor biopsies using the Vysis ALK Break Apart FISH test

- Can we use CTCs to detect ALK-rearrangement for diagnostic testing and monitoring response to crizotinib?
Development of combined methods – IF + FA-FISH

CTC enrichment: ISET filtration

Fluorescent staining: DAPI/CD45

Scanning Ariol (whole filter)

CD45 cell selection

FA-FISH: ALK, ROS1, RET, EGFR, c-MET, FGFR1, c-KIT, ERG, AR

Scanning Ariol (only selected CD45 cells)

Cell image relocation

Biomarker identification
Validation by a cytogeneticist

Pailler & Oulhen et al. BMC Cancer 2016
Semi-automated protocol for FISH analysis

- **Number of z-stacks**

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<th>CTC ID</th>
<th>5 z-stacks</th>
<th>10 z-stacks</th>
<th>15 z-stacks</th>
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- **Step between z-stacks**

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<tr>
<th>CTC ID</th>
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</table>

- **Multi-exposure protocol**

27 settings of exposure time
Detection of *ALK*-rearranged CTCs

- 4 *ALK*-rearranged CTC /1 mL
- $\kappa = 99.99\%$

Pailler *et al.* *J Clin Oncol* 2013
ALK-rearranged CTC harbor a unique ALK-rearrangement
Are CTC subsets with distinct ALK FISH patterns associated with clinical outcome under crizotinib treatment?

Baseline 2 months

N=39

✓ Baseline parameters
✓ PFS
✓ OS

Pailler et al. Cancer Research 2017
ALK-CNG CTCs are affected by crizotinib treatment.

**ALK-rearranged CTCs per 3 mL blood**

- **At baseline (Log10)**
  - Under crizotinib: $R^2 = 0.278$, $p = 0.003$

**ALK-CNG CTCs per 3 mL blood**

- **At baseline (Log10)**
  - Under crizotinib: $R^2 = 0.017$, $p = 0.503$

**Graphs showing changes in ALK-CNG CTC counts**

- Increase/Stable ($n = 16$)
- Decrease ($n = 13$)
Significant Association between the Dynamic Change of \textit{ALK}-CNG CTCs and PFS

- Multivariate analysis: strongest factor associated with PFS ($p=0.006$)
Acquired Resistance to crizotinib

Patterns of progression on crizotinib

- Isolated CNS progression
- Extracranial oligoprogression
- Multisite progression

Mechanisms of resistance to crizotinib

- Activation of bypass tracks (e.g. EGFR)
- Kinase domain mutations
- Copy number gain
- Epithelial-mesenchymal transition

Dagogo-Jack & Shaw, 2016
CONCLUSION

✓ Feasibility to reliably detect ALK-rearranged CTCs by combining filtration enrichment, IF+ FISH, and semi-automated scanning

✓ But, FISH is still time-consuming, meticulous, requiring expertise and the systematic validation of signals by a cytogeneticist. Not ready for clinical use

✓ The number of CTCs with a gain of ALK copies may predict PFS in ALK-rearranged patients treated with crizotinib.

✓ Although not dominant, the gain of ALK copies is one mechanism of acquired resistance to crizotinib in tumor biopsies.
Detection of ROS1-rearranged CTCs

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