IMPROVING CARE AND KNOWLEDGE THROUGH TRANSLATIONAL RESEARCH IN BREAST CANCER

WELCOME TO IMPAKT

Brussels, Belgium 7-9 MAY 2015

IMPAKT Chairs: Nicholas Turner London, UK and Carsten Denkert, Berlin, Germany
DISCLOSURE SLIDE

No conflicts of interest
Exploring the intra-patient PIK3CA mutational heterogeneity of circulating tumour cells by massive parallel sequencing in patients with metastatic hormone receptor-positive breast cancer.

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Preclinical

Towards methodology of single cell PIK3CA mutational analysis by MPS

Quality Assurance & Assay Performance Characteristics
### Ex9 vs Ex 20

<table>
<thead>
<tr>
<th></th>
<th>Ex9</th>
<th>Ex 20</th>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>85%</td>
<td>71%</td>
</tr>
<tr>
<td>Specificity</td>
<td>90%</td>
<td>99%</td>
</tr>
<tr>
<td>PPV</td>
<td>94%</td>
<td>96%</td>
</tr>
<tr>
<td>NPV</td>
<td>78%</td>
<td>87%</td>
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Clinical

Exploring the intra-patient PIK3CA mutational heterogeneity of circulating tumour cells by massive parallel sequencing in patients with metastatic hormone receptor-positive breast cancer.

Study of mutational diversity between and within different compartments & early versus advanced
Patients with HR+ mBC before start of a new line systemic therapy (n=60 pts)

CellSearch Enumeration

Excluded: Patients with <5 CTCs/7.5mL (n=22 pts)

Patients with ≥5 CTCs/7.5mL (n=38 pts)

Excluded: n = 9 pts, of which CTC ≤ 10: n = 7

Disease Setting

**EARLY**

- PT (FFPE) (n=27 pts)
  - gDNA purification (n=27 pts)

- PLASMA (n=30 pts)
  - cfDNA purification (n=30 pts)

**ADVANCED**

- CTC cartridge ≥10 CTCs (n=29 pts)
  - WGA (n=29 pts)
    - Selection of (≥GII 1) CTC samples (n=26 pts)
      - Single CTC (n=146)
      - Group CTC (n=70)
      - Group WBC (n=30)

- Metastatic lesion (n=11 pts)
  - DTC cartridge ≥10 CTCs (n=2 pts)
  - DEPArray DTC purification (n=2 pts)
  - FFPE (n=9 pts)

Targeted sequencing of PIK3CA exon 9 and 20
PIK3CA mutational analysis of CTCs
Hotspot \textit{PIK3CA} mutational heterogeneity in CTC

A.

B.
Comparative *PIK3CA* mutational analysis: Early versus advanced disease

- Overall higher level of agreement between CTC and PT/META
- PT versus cfDNA versus CTC (n=18)
  - Plasma failed to detect 2 mutant patients (2/18, 11%)
    - Patients 889 and 3546
    - Mutation was present in PT and CTC or META
  - Disparity between early and advanced disease in 4 patient (4/25, 16%)
    - Patient 1529, 2139, 2648 and 3516
Conclusion

- PIK3CA status may change between primary (early) and metastatic (advanced) disease
  - Emphasizes the necessity of assessing the PIK3CA status in a real-time manner
    - CTC outperformed plasma analysis to assess PIK3CA mutational status non-invasively in women with advanced HR+ breast cancer
    - Discordance (gain/loss) in PIK3CA status was observed in 16% (4/25) of patients
      - Clinical utility of a real-time liquid biopsy
    - Intra-CTC analysis reveals the existence of intra-patient heterogeneity in the intravenous compartment at advanced disease
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