Clinical decision making based on circulating (progenitor) cells:

Help or Struggle?

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• More and more anti angiogenic treatments developed; some already used in daily practice
  • RTKIs VEGFR
  • Monoclonal AB VEGF
  • VEGFR trap
  • VDA
  • mRNA antagonists/ ribozymes
• Difficult to define optimal dose and select benefiting patients
• Preferable to prevent overtreatment and unneeded toxicity

Biomarker development
Biomarkers in development

- Circulating cytokines
- Soluble VEGF receptors
- Circulating endothelial cells (CEC)
- Circulating endothelial progenitor cells (CEPC)
- Circulating progenitor cells (CPC)
- Plasma CD133 mRNA/DNA
- ...

Background (II) Potential implications

Circulating (Endothelial) Progenitor cells

- Reflection of angiogenic activity of tumor
- Correlation with disease status and response to therapy
- Target of therapy
Measuring endothelial cells

- Magnetic bead assay
- PCR based assays
- Flow cytometry analysis
Measuring endothelial cells

Magnetic bead assay
- Sensitive assay
- Possibility to sort cells and culture
- Time consuming
- Fresh material needed
- Selection based on 1 marker

PCR based assays

- Very sensitive assay
- Selection based on 1 marker
- No identification on individual cells
- Relative numbers (copies)

Survival according to CD133 positivity in patients with bone metastases

Flow cytometry analysis

- Selection based on multiple markers
- Frozen material
- Experience requiring method

Experience in more than 1000 samples, measured by one of the three modalities

Samples from:

• 450 patients in clinical trials
  • Flowcytometry analysis in 214 patients
    • Patients treated with chemotherapy
    • Patients treated with anti angiogenics
    • Patients treated with immunotherapy
    • Patients treated with surgery or RFA
  • 90 volunteers
# Important research questions

## Potential confounders

<table>
<thead>
<tr>
<th>Potential confounders</th>
<th>Research question</th>
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<tr>
<td>Low number of cells of interest</td>
<td>What is the <strong>intraprocedure variability</strong>?</td>
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<td>Experience requiring procedure</td>
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<tr>
<td>Natural variation within patients</td>
<td>What is the <strong>intrapatient variability</strong>?</td>
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<td>Storage procedure concerning fragile cells</td>
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<tr>
<td>Variation between patients</td>
<td>What is the <strong>interpatient variability</strong>?</td>
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<tr>
<td>Natural variation</td>
<td></td>
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<tr>
<td>Variation caused by disease/ therapy</td>
<td>What is the best <strong>representation</strong> of the data?</td>
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<tr>
<td>Variation caused by processing</td>
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</tbody>
</table>
What is the intraprocedure variability?

Comparison of duplo measurements (N=214)

Bland Altman analysis
Good repeatability
Spearman’s correlation
Good correlation

Spearman’s rho 0.937
P=0.001

Spearman’s rho 0.68
P=0.001
What is the intrapatient variability?

Comparison of baseline and screening sample in 1 patient
(samples taken no more than 7 days apart from each other; N=8)

Spearman’s rho 0.99
P=0.001

Spearman’s rho 0.86
P=0.001
What is the interpatient variability?

**Interpatient variability:**
**comparison of baseline samples**

**CEC**
- Median: $3.07 \times 10^5$ cells/l
- Interquartile range: $1.45 \times 10^5 - 1.52 \times 10^7$

**EPC**
- Median: $7.7 \times 10^5$ cells/l
- Interquartile range: $2.01 \times 10^5 - 1.69 \times 10^7$
Conclusions

Measurement of circulating endothelial progenitor cells by flowcytometry

- *is a preferable method*
  - enables specific phenotypic characterization of cells in stored samples
- *is a reliable method*
  - the intraprocedure and intrapatient variability is acceptable

Interpatient variability is substantial

For all three implementations and especially reflection of biological behavior of these cells, absolute numbers are required

Normalization of data with respect to the mononuclear cell count at the same timepoint is essential
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‘A Struggle with Perspective’
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