Combined High-Resolution Single Cell Genome And Transcriptome Analysis For Clinical Samples

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Concept of personalized cancer therapy

Primary tumor → DTC or CTC → Micrometastasis → Metastasis → Molecular Analysis & Therapy Selection
Detection of lethal subclone in primary tumor

<table>
<thead>
<tr>
<th>Initial presentation</th>
<th>Tumor progression</th>
<th>Autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index genomic alterations</td>
<td>Lymph node metastasis (obtained at RRP)</td>
<td>PTEN SPOP TP53 AR</td>
</tr>
<tr>
<td>PTEN SPOP TP53</td>
<td>B1</td>
<td>PTEN SPOP TP53 AR</td>
</tr>
<tr>
<td>L1</td>
<td>Clonal selection</td>
<td>Liver</td>
</tr>
<tr>
<td>P1</td>
<td>Additional alterations</td>
<td>M5</td>
</tr>
<tr>
<td>Primary tumor</td>
<td>Lung metastasis (biopsy)</td>
<td>Lymph node</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M38</td>
</tr>
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<td></td>
<td></td>
<td>Liver</td>
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<tr>
<td></td>
<td></td>
<td>M40</td>
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- Ultradeep sequencing (40.000X) is needed to detect the lethal minority subclone in the primary tumor
- provided that the lethal subclone is already present in the primary tumor
Concept of personalized cancer therapy

Primary tumor → DTC or CTC → Micrometastasis → Metastasis

Molecular Analysis & Therapy Selection
Concept of personalized cancer therapy

Primary tumor → DTC / CTC → Micrometastasis → Metastasis

Surgery → DTC / CTC

Information on systemic cancer?

adjuvant disease

metastatic disease

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Single cell analysis in a diagnostic workflow

Whole Genome Amplification (WGA)
- robust and homogeneous amplification
- sufficient sample volume for test repetition
- sample archiving / biobanking
- compatible with downstream applications

Whole Transcriptome Amplification (WTA)
- insensitiveness against secondary structures
- robust and homogeneous amplification
- sufficient sample volume for test repetition
- sample archiving / biobanking
- compatible with downstream applications
## WGA technologies for single cell analysis

<table>
<thead>
<tr>
<th>WGA Technology</th>
<th>OmniPlex® GenomePlex®</th>
<th>Ampli1™ WGA GenomiPhi</th>
<th>Repli-G SurePlex™ EasyAmp™</th>
<th>PicoPlex™ MALBAC</th>
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<tr>
<td><strong>Mechanism</strong></td>
<td>PCR</td>
<td>PCR</td>
<td>MDA*</td>
<td>MDA* + PCR</td>
</tr>
<tr>
<td><strong>Primer design</strong></td>
<td>defined</td>
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<td>random</td>
<td>hybrid$</td>
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<td>up to 38.7% (Moller et al, 2013)</td>
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* Multiple Displacement Amplification

$ Primer in parts random
Whole Genome Sequencing with MALBAC

Genome coverage with fresh single cells from cell culture

Ni et al, PNAS 2013

93%

Genome coverage with single CTC from lung cancer patient

Zong et al, Science 2012

33%

High, random dropout problematic for diagnostic assays

Daley et al, Bioinformatics 2014
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# Single cell whole transcriptome amplification (WTA)

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<th>SMARTer®</th>
<th>Repli-G</th>
<th>RNA-Amp&lt;sup&gt;TM&lt;/sup&gt;</th>
<th>TransPlex®</th>
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<tr>
<td>cDNA synthesis</td>
<td>Oligo dT plus selective</td>
<td>Oligo dT plus template switch</td>
<td>Oligo dT</td>
<td>Oligo dT plus random</td>
<td>Oligo dT</td>
</tr>
<tr>
<td>Amplification</td>
<td>Linear</td>
<td>PCR</td>
<td>LM-MDA*</td>
<td>PCR</td>
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* Multiple Displacement Amplification

- No golden standard for reverse transcription of mRNA population
- No golden standard for mapping RNA-derived reads and isoform prediction
Selection criteria for clinical samples

Single Tumor Cell (DTC / CTC)

$\textit{Ampli1}^{\text{TM}}$ WGA → WTA

- **WGA-QC**: BioAnalyzer → QuBit
  - 6 – 9μg gDNA

- **WTA-QC**: BioAnalyzer → QuBit
  - 1 – 2.5μg cDNA

*Fraunhofer ITEM*
Sample selection from DTC / CTC collective

- Sample collective: ~ 4000 single DTC WGA samples
  (~ 750 samples for combined WGA & WTA)
  ~ 1200 single CTC WGA samples
- 19 putative M0 CaP DTCs (from 11 patients)
- 4 putative M1 CaP DTCs (from 1 patient)
- 1 validated M1 CaP DTC (from 1 patient)
- 2 peripheral blood lymphocytes (from 1 control)
- 6 cell pools from the VCaP cell line (1, 2, 10, 20, 40, 80 cells)
- RNAseq by Roche/454 pyrosequencing
Combined high-resolution analysis of single CaP DTCs
Combined mRNA and DNA analysis of a single cell

- Whole transcriptome amplification (WTA)
- Separation of nucleic acids
- Whole genome amplification (WGA) *Ampli1™*

- RNASeq analysis
  - 454 / Illumina
- Small RNA-Seq analysis
  - Illumina
- Expression profiling
  - Agilent 4 x 44K
- Expression profiling
  - Affymetrix HGU-133
- nCounter Single Cell Gene Expression Assay
- ArrayCGH
  - Agilent 4 x 180K
- ArrayCGH
  - Affymetrix Cytoscan HD
- Whole Genome Sequencing
- Whole Exome Sequencing
- Whole Methylome Sequencing

- In development
- In development
- In development on hold
- In development
- Optimization
- Optimization
- In development
Current lab members:

Stefanie Oesterberg
Julia Häring
Giancarlo Feliciello
Zbigniew Czyz
Urs Lahrmann

CEM lab members:

Isabell Blochberger
Manfred Meyer
Miodrag Guzvic

Collaborators:

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University of Regensburg, Urology

Michael Nerlich,
University of Regensburg, Trauma Surgery

Sebastian Winkler,
University of Regensburg, Orthopaedic Surgery

Head of CEM & ITEM-R:

Christoph Klein