Liquid profiling (or liquid biopsy) as a new tool for clinical decision-making in oncology

Michael Neumaier MD PhD
Institute for Clinical Chemistry
Medical Faculty Mannheim
Heidelberg University

michael.neumaier@medma.uni-heidelberg.de
Declaration
Conflict of Interest

• Inostics (Sysmex)
Present & Future of Tumour Diagnostics: Implications for personalized Medicine

• **Past & Present**
  – hereditary/familial Tumours
    • Predisposition by Human Geneticist
  – primary Tumour Tissue
    • Histopathology/molecular Pathology
  – metastasized Disease and Follow-Up
    • Imaging
    • Lab Medicine (Serum Tumor Markers)
    • (detection of Tissue-specific mRNA Expression)
  – paraneoplastic Phenomena
    „non-specific“ Role for detecting Complications e.g. Anemia, Clotting Abnormalities, Hormones ...

• **Future**
  – primary Diagnosis & Follow-Up with liquid Profiling (aka Liquid Biopsy)
    • CTC and DTC
    • fcDNA (ctDNA, exosomes, µ-particles)
    • digital PCR
    • MPS (CaPPS; iDES-CaPPS)
classical serum tumor markers in humans

- **Schildrüse:** TG
- **Lunge:** CEA, NSE, SCC
- **Leber:** AFP, CA 19-9/CA 50
- **Prostata:** PAP, PSA
- **Hoden:** AFP, HCG
- **Brust:** CEA, CA 15-3
- **Magen:** CEA, CA 19-9/CA 50
- **Pankreas:** CA 19-9/CA 50
- **Dickdarm:** CEA, CA 19-9/CA 50
- **Ovar:** CA 125, CEA, AFP, HCG, SP1
- **Gebärmutter:** TPA, SCC, CA 50, CEA
## Table 1. Results of Markov Analysis With Respect to Mean Life Expectancy by Follow-up Evaluation With or Without CEA Monitoring, Using Data of Minton et al.

<table>
<thead>
<tr>
<th></th>
<th>Life expectancy (yr)</th>
<th>Difference (days)</th>
<th>Costs (dollars)</th>
<th>Marginal cost-effectiveness (K. CEA − K. SYM/L.E. CEA − L.E. SYM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEA</td>
<td>SYM</td>
<td>CEA − SYM</td>
<td>CEA</td>
</tr>
<tr>
<td>Total</td>
<td>6.4129</td>
<td>6.3951</td>
<td>+7</td>
<td>3377</td>
</tr>
<tr>
<td>Dukes' stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>21.4280</td>
<td>21.4307</td>
<td>-1</td>
<td>6686</td>
</tr>
<tr>
<td>B1</td>
<td>13.3735</td>
<td>13.3603</td>
<td>+5</td>
<td>5063</td>
</tr>
<tr>
<td>B2</td>
<td>11.0399</td>
<td>11.0249</td>
<td>+5</td>
<td>4501</td>
</tr>
<tr>
<td>C1</td>
<td>9.6401</td>
<td>9.6250</td>
<td>+6</td>
<td>4107</td>
</tr>
<tr>
<td>C2</td>
<td>2.4161</td>
<td>2.3933</td>
<td>+8</td>
<td>2901</td>
</tr>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.8881</td>
<td>7.8608</td>
<td>+10</td>
<td>3816</td>
</tr>
<tr>
<td>45</td>
<td>7.2961</td>
<td>7.2725</td>
<td>+9</td>
<td>3643</td>
</tr>
<tr>
<td>60</td>
<td>6.2233</td>
<td>6.2067</td>
<td>+6</td>
<td>3319</td>
</tr>
<tr>
<td>75</td>
<td>4.3195</td>
<td>4.3140</td>
<td>+2</td>
<td>2702</td>
</tr>
<tr>
<td>90</td>
<td>2.1280</td>
<td>2.1330</td>
<td>-2</td>
<td>1847</td>
</tr>
<tr>
<td>Ol. correction factor for incurability (uitcure)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>6.3834</td>
<td>6.3647</td>
<td>+7</td>
<td>3377</td>
</tr>
<tr>
<td>1.00</td>
<td>6.4424</td>
<td>6.4255</td>
<td>+6</td>
<td>3377</td>
</tr>
<tr>
<td>Overall operative mortality (opmort)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>6.4148</td>
<td>6.3965</td>
<td>+7</td>
<td>3379</td>
</tr>
<tr>
<td>0.04</td>
<td>6.4093</td>
<td>6.3923</td>
<td>+6</td>
<td>3374</td>
</tr>
<tr>
<td>0.06</td>
<td>6.4038</td>
<td>6.3881</td>
<td>+6</td>
<td>3370</td>
</tr>
<tr>
<td>0.08</td>
<td>6.3983</td>
<td>6.3840</td>
<td>+5</td>
<td>3366</td>
</tr>
<tr>
<td>Extreme patient variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tC2-30y</td>
<td>2.6123</td>
<td>2.5762</td>
<td>+13</td>
<td>2993</td>
</tr>
<tr>
<td>m A-90y</td>
<td>2.4937</td>
<td>2.4948</td>
<td>-0</td>
<td>1210</td>
</tr>
</tbody>
</table>

Kievit J & van de Velde CJH, Cancer (1990)
MRD Detection using CEA mRNA in Blood and BM

Specific Detection of Carcinoembryonic Antigen–Expressing Tumor Cells in Bone Marrow Aspirates by Polymerase Chain Reaction

By Markus Gerhard, Hartmut Juhl, Holger Kalthoff, Hans W. Schreiber, Christoph Wagener, and Michael Neumaier


Micrometastases and Survival in Stage II Colorectal Cancer

Gerrit-Jan Liefers, M.D., Anne-Marie Cleton-Jansen, Ph.D., Cornelis J.H. van de Velde, M.D., Ph.D., Jo Hermans, Ph.D., Johannes H.J.M. van Krieken, M.D., Ph.D., Cees J. Cornelisse, Ph.D., and Rob A.E.M. Tollenaar, M.D., Ph.D.

192 LN from 26 Patients
- N₀: 100% (26/26) by Histopathology
Limited Specificity of rtPCR of Tumor Marker CEA mRNA in Blood

- **single Tumor Cell**
  - strong Tissue-specific Expression
  - many mRNA Copies/Cell
  - single Cells
  - **positive** rtPCR Signal

- **Blood Leucocytes**
  - very weak Background Expression
  - very few mRNA Copies/Cell
  - great Majority of Cells
  - **positive** rtPCR Signal
Candidate mRNA Markers for colorectal Metastasis based on *in-silico*-Array Screening

Marker Gene List

Screening of cDNA sources (NCBI data bases)

281 positive in blood, PBL, BM etc.

92 negative in blood, PBL, BM etc.

1st RT-PCR
PBLs from healthy donors

25 negative (high specificity)

67 positive stop

RT-PCR or nested RT-PCR
PBLs from healthy donors (n=7)

4 negative (high specificity)

21 positive
qRT-PCR
protein detection by MS

patient cohort (intra-surgery blood draws)
diagnostic sensitivity

cell culture
spiking experiments
analytical sensitivity

Present & Future of Tumour Diagnostics: Implications for personalized Medicine

• Past & Present
  – hereditary/familial Tumours
    • Predisposition by Human Geneticist
  – primary Tumour Tissue
    • Histopathology/molecular Pathology
  – metastasized Disease and Follow-Up
    • Imaging
    • Lab Medicine (Serum Tumor Markers)
    • (detection of Tissue-specific mRNA Expression)
  – paraneoplastic Phenomena
    „non-specific“ Role for detecting Complications
    e.g. Anemia, Clotting Abnormalities, Hormones ...

• Future
  – primary Diagnosis & Follow-Up with liquid Profiling (aka Liquid Biopsy)
    • CTC and DTC
    • fcDNA (ctDNA, exosomes, µ-particles)
    • digital PCR
    • MPS (CaPPS; iDES-CaPPS)
the seminal Techniques: digital PCR & Emulsion PCR

Digital PCR

BERT VOGELSTEIN* AND KENNETH W. KINZLER

The Howard Hughes Medical Institute and the Johns Hopkins Oncology Center, Baltimore, MD 21231

Contributed by Bert Vogelstein, June 9, 1999

PNAS (1999)

limiting dilution of preamplicons (dPCR)

Emulsions-PCR (ddPCR)

high sensitivity

ASO

Genome sequencing in microfabricated high-density picolitre reactors


Applications of „circulating NA analytics“ in blood and body fluids

• Inflammation/Tissue damage (quantitative Analysis)
• NIPT in GYN/OB (quantitative Analysis)
• Transplantation Medicine (Chimerism; Rejection)
• Microbiology (pathogen detection)
• Oncology
  – molecular tumor characteristics
  – therapy stratification
  – MRD
  – early detection of resistance
Number of Publications listed in PubMed
(search terms: "circulating tumour dna" or "circulating tumor dna" or "circulating dna" or "cell-free dna" or "circulating nucleic acids")
circulating free Nucleic Acids as Biomarkers in Blood

Schwarzenbach et al. (2011) Nature Reviews Cancer 11, 426-437
Concentration of biomolecules in human Plasma: Proteome, fcDNA and ctDNA

Mod. acc: N.L. Anderson & N.G. Anderson Mol Cell Proteomics 2002
Concentration of biomolecules in human Plasma: Proteome, fcDNA and ctDNA

free circulating DNA (fcDNA)
fcDNA: (110-170 bp)
ctDNA: ~ 0.01-10% of total

low concentrations of ctDNA
(entity/stage/clonal heterogeneity)

ctDNA – Preanalytics: preserve the tu/n ratio!

- reject hemolytic plasma
- tubes for stabilization of cfDNA and prevention/delay of leukocyte lysis

S.E. Norton et al., Clin Biochem 46, (2013)
Workflow of liquid Profiling for early detection of Tumor relapse in CRC patients

1. Patient with metastatic CRC
2. Physician’s indication to liquid Profiling
3. Blood collection
   - Transport service
4. Inquiry to Laboratory
5. spezialized blood sampling Tubes
6. ddPCR, OncoBEAM Analysis
7. Findings and Recommendations

http://w3.umm.de/7195.0.html
**BEAMing technology - Overview**

- **DNA Isolation**
  - Plasma sample

- **Pre-Amplification**
  - PCR amplification of target region

- **Emulsion PCR**
  - Water-in-oil emulsion
    - Pre-cycling
    - Post-cycling
  - DNA labeled with fluorescent probes

- **Hybridization**
  - DNA hybridized with probes specific for mutant or wildtype DNA

- **Flow Cytometry**
  - FACS Cytometry with quantification of beads bearing ctDNA amplicons

---

**Isolation of cfDNA from 3ml plasma**

**Multiplex PCR for codons 12,13,59,61,117 and 146 of kras and nras**

**Emulsion PCR:** Amplification of DNA fragments on magnetic beads in droplets

**Breaking of droplets**

**Hybridization with probes specific for mutant or wildtype DNA**

**FACS Cytometry** with quantification of beads bearing ctDNA amplicons
OncoBEAM – CRC Panel and Results

OncoBEAM™ RAS Mutation Panel for CRC

16 KRAS mutations covering Exons 2, 3 and 4

18 NRAS mutations covering Exons 2, 3 and 4

beads with ctDNA
Molecular Profiling of ctDNA for Companion Dx
(LLoD for different Methods)

Detection Capability
(mutant DNA / total DNA)

100 %
Sanger Sequencing

10 %
Pyrosequencing

1 %
NGS

0.1 %
Real-Time PCR

0.01 %
BEAMing

source: www.inostics.com


BRAF (1)
T1799A


AKT1 (1) G49T


Diehl F et al., PNAS (2005)
provisional conclusions

- Serum tumor markers are unspecific/ineffective – provide no actionable health information.
- Tumor-associated mRNA expression (by rtPCR) is plagued by illegitimate background expression in non-tumor cells (e.g. blood leukocytes).
- In-silico screening for new mRNA markers yields no enhanced specificity.
- Circulating nucleic acids (e.g. cfDNA) mirror molecular alterations in the tumor and are stable enough to be targeted.
- The digital PCR (1999) and the emulsion technique (2004) are applied for the sensitive detection of tumor-specific druggable mutations in blood – actionable!

- How does this translate to decision-making?
druggable Targets in mCRC

A Sartore-Bianchi et al., Annals Oncology (2016)
**kras status for Cetuximab eligibility in mCRC**

- 113 patients (mCRC, CTX-treated) typed for *kras* prior to Cetuximab
- OR in 26/66 *kras* WT; OR in 0/42 *kras* mut;
- median OS better in WT (p=0.020)
- initial tumor decrease in WT is prognostic (median OS 74.9 vs. 30.6 weeks, p=1.2E-8)

*DeRoock W et al., Ann Oncol 19, 508-515, (2008)*
**kras status for Cetuximab eligibility in mCRC**

- 113 patients (mCRC, CTX-treated) typed for *kras* prior to Cetuximab
- OR in 26/66 *kras* WT; OR in 0/42 *kras* mut;
- median OS better in WT (p=0.020)
- initial tumor decrease in WT is prognostic (median OS 74.9 vs. 30.6 weeks, p=1.2E-8)

*DeRoock W et al., Ann Oncol 19, 508-515, (2008)*
FDA/EMA-approved Drugs recommended for a-priori Eligibility testing* (selection; 7/2015)

• Trastuzumab/Lapatinib → metastatic breast cancer, overexpression/amplification of HER-2
• Tamoxifen+/- chemo → ER+/HER2 - breast cancer, mutation pattern - multigene assays
• Cetuximab → metastatic colorectal cancer, overexpressing EGFR/wild-type KRAS
• Panitumumab → colorectal cancer with wild-type KRAS (mutations excluded)
• Nimotuzumab → metastatic colorectal cancer (still experimental)
• Gefitinib → non-small cell lung cancer with mutated EGFR
• Erlotinib → non-small cell lung cancer with mutated EGFR
• Crizotinib → non-small cell lung cancer with mutated EML4-ALK
• Vemurafenib (PX4032) → malignant melanoma with mutated B-RAF (wildtype excluded)
• Gemtuzumab-Ozogamicin → AML with CD33 ( > 60 yrs.)
• Imatinib → CML, bcr/abl–positive (activated PK),
• Imatinib → GIST with activated c-kit receptor tyrosine kinase/CD117, exon 9 mut
• Rituximab (+ CHOP), Y90-Ibritumomab, I131-Tositumomab → NH Lymphoma with CD20

*Strongly suggested by FDA's Drug-Diagnostic Co-Development Initiative
<table>
<thead>
<tr>
<th>Compound (n=70)</th>
<th>Field of application</th>
<th>target (n=36)</th>
<th>Indication and Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alemtuzumab</td>
<td>Oncology</td>
<td>ALK</td>
<td>Indications and Usage, Adverse Reactions, 4</td>
</tr>
<tr>
<td>Brentuximab Yedotin</td>
<td>Oncology</td>
<td>ALK</td>
<td>Clinical Studies, Indications and Usage, Adverse Reactions, 4</td>
</tr>
<tr>
<td>Brigatinib</td>
<td>Oncology</td>
<td>ALK</td>
<td>Indications and Usage, Adverse Reactions, 4</td>
</tr>
<tr>
<td>Ceritinib</td>
<td>Oncology</td>
<td>ALK</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Crizotinib (1)</td>
<td>Oncology</td>
<td>ALK</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Blinatumomab</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Buzufan</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Dasatinib</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Imatinib (2)</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Nilotinib (1)</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Omacetaxine</td>
<td>Oncology</td>
<td>BCR-ABL1</td>
<td>Clinical Studies, Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Bosutinib</td>
<td>Oncology</td>
<td>BCR-ABL1 (Philadelphia chromosome)</td>
<td>Indications and Usage, Adverse Reactions, 1</td>
</tr>
<tr>
<td>Ponatinib</td>
<td>Oncology</td>
<td>BCR-ABL1 (Philadelphia chromosome)</td>
<td>Indications and Usage, Warnings and Precautions</td>
</tr>
<tr>
<td>Cobimetinib</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Dabrafenib (1)</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Pembrolizumab (1)</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Trametinib (1)</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Venutafenib (1)</td>
<td>Oncology</td>
<td>BRAF</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Nilotinib</td>
<td>Oncology</td>
<td>BRCA</td>
<td>Clinical Studies</td>
</tr>
<tr>
<td>Olaparib</td>
<td>Oncology</td>
<td>BRCA</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Rucaparib (1)</td>
<td>Oncology</td>
<td>BRCA</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>Oncology</td>
<td>CD274</td>
<td>Clinical Pharmacology, Clinical Studies</td>
</tr>
<tr>
<td>Avelumab</td>
<td>Oncology</td>
<td>CD274</td>
<td>Clinical Pharmacology, Clinical Studies</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Oncology</td>
<td>CD274 (PD-1)</td>
<td>Adverse Reactions, Clinical Pharmacology, 1</td>
</tr>
<tr>
<td>Pembrolizumab (2)</td>
<td>Oncology</td>
<td>CD274 (PD-1)</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Ibrutinib (2)</td>
<td>Oncology</td>
<td>Chromosome 11q</td>
<td>Clinical Studies</td>
</tr>
<tr>
<td>Ibrutinib (1)</td>
<td>Oncology</td>
<td>Chromosome 17p</td>
<td>Clinical Studies, Boxed Warning, Contraindications, Warnings</td>
</tr>
<tr>
<td>Raclopride (2)</td>
<td>Oncology</td>
<td>CYP1A2</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>Rucaparib (3)</td>
<td>Oncology</td>
<td>CYP2D8</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>Rucaparib (2)</td>
<td>Oncology</td>
<td>CYP2D8</td>
<td>Clinical Pharmacology</td>
</tr>
<tr>
<td>Capcitabine</td>
<td>Oncology</td>
<td>DPYD</td>
<td>Warnings and Precautions, Patient Counseling</td>
</tr>
<tr>
<td>Fluorouracil (2)</td>
<td>Oncology</td>
<td>DPYD</td>
<td>Warnings and Precautions, Patient Counseling</td>
</tr>
<tr>
<td>Alitrisitib</td>
<td>Oncology</td>
<td>EGFR</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Cetuximab (1)</td>
<td>Oncology</td>
<td>EGFR</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Gefitinib</td>
<td>Oncology</td>
<td>EGFR</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Osimertinib</td>
<td>Oncology</td>
<td>EGFR</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Panitumumab (1)</td>
<td>Oncology</td>
<td>EGFR</td>
<td>Indications and Usage, Dosage and Admin</td>
</tr>
<tr>
<td>Everolimus (1)</td>
<td>Oncology</td>
<td>ERBB2</td>
<td>Indications and Usage, Adverse Reactions, 1</td>
</tr>
<tr>
<td>Fulvestrant (1)</td>
<td>Oncology</td>
<td>ERBB2</td>
<td>Indications and Usage, Adverse Reactions, 1</td>
</tr>
</tbody>
</table>
liquid profiling follow-up in stage II CRC in comparison to radiological recurrence

Tie J et al., Sci Transl Med (2016)
follow-up in mCRC patients: all-ras liquid profiling using OncoBEAM

09/2013: 1st Dx: CRC C. descendens > hemicolecotomy
11/2013: resection of liver mets
10/2014: new liver mets

12/2013 – 06/2014
FOLFOX 4 (12 cycles)

11/2016
liquid profiling
no RAS mutation

03/2017
liquid profiling
no RAS mutation

05/2017
liquid profiling
k ras cd 12 mutation

04/2015 – 02/2016
FOLFIRI/Cetuximab
(20 administrations)

01-05/2017
Panitumumab
(9 cycles)

05-07/2017
FOLFIRI/Bevacizumab
(5 cycles)

Stop of anti-EGFR-Therapy
New Classification of CRC into 4 Subtypes
(Consensus Molecular Subtypes; CMS)

<table>
<thead>
<tr>
<th>CMS1</th>
<th>CMS2</th>
<th>CMS3</th>
<th>CMS4</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSI Immune</td>
<td>Canonical</td>
<td>Metabolic</td>
<td>Mesenchymal</td>
</tr>
</tbody>
</table>

- CMS1: MSI, CIMP high, hypermutation
- CMS2: SCNA high
- CMS3: Mixed MSI status, SCNA low, CIMP low
- CMS4: SCNA high

- **BRAF** mutations
- Immune infiltration and activation
- Worse survival after relapse

- **KRAS** mutations
- WNT and MYC activation
- Metabolic deregulation
- Worse relapse-free and overall survival

clinical course of BRAFi therapy in malignant Melanoma (MM)

Chapman et al 2011, NEJM
Hirth et al. 2012, Nat Drug Discov
Wagle et al., JCO 2011
Recurrence of MM under \textit{BRAFi} Therapy

(liquid profiling and tumor markers)

\textit{Haselmann V et al., Clin Chem (2018)}
Recurrence of MM under BRAFi Therapy (lead time bias by liquid profiling and tumor markers*)

Mean lead time bias: 110 days (up to 170 days)

Haselmann V et al., Clin Chem (2018)

*) compare also Cohen J et al., Science (2018)
2° resistance to EGFR therapy by selection: the hypothesis of therapy holiday/rechallenge

Santini et al., Ann Oncol (2012)
2° resistance to therapy by selection: the hypothesis of therapy holiday/rechellange

Chemoresistance evolution in TNBC
(single cell sequencing)

1. neoadjuvant chemotherapy
   - pre treatment
   - mid treatment
   - post treatment
   20 TNBC Patients

2. frozen tissue → single nucleus dissociation
   - single cell DNA sequencing
   - single cell RNA sequencing
   - exome deep sequencing

3. clonal extinction (10 patients)
   - pre-tx
   - post-tx

4. clonal persistence (10 patients)
   - pre-tx
   - post-tx

4. adaptive genome evolution & transcriptional reprogramming
   - phenotypes
     △ resistant
     ○ sensitive
   - genotypes
     - clone A
     - clone B
     - clone C

Kim C et al., Cell (2018)
current Diagnostic Workflow in Tumor Profiling

1st line
Tumor Tissue (FFPE)

Targeted re-sequencing
(Cancer Panel)

individual Tumor
mutational Profile

MRD Follow-up
(precision or personalized)
Assays*

improved lead Time

2nd line
Plasma (ctDNA)

*) e.g. mAF ≤0.01%
Changing the molecular diagnostic Strategies
detecting unknown somatic alterations in plasma

future Laboratory Diagnostics in Oncology: Improvement of NGS Data Interpretation*

*) integrated Digital Error Suppression (iDES) in CaPP-Seq

Newman A et al., Nature Biotech., supplement (2016)
future Laboratory Diagnostics in Oncology:
Improvement of NGS Data Interpretation*

*) integrated Digital Error Suppression (iDES) in CaPP-Seq

Newman A et al., Nature Biotech., supplement (2016)
Changing molecular diagnostic Strategies: improved Performance of liquid Profiling of ctDNA in peripheral Blood


A Newman et al., Nature Biotech (2016)
Present & Future of Tumour Diagnostics: Implications for personalized Medicine

**Past & Present**
- hereditary/familial Tumours
  - Predisposition by Human Geneticist
- primary Tumour Tissue
  - Histopathology/molecular Pathology
- metastasized Disease and Follow-Up
  - Imaging
  - Lab Medicine (Serum Tumor Markers)
  - (detection of Tissue-specific mRNA Expression)
- paraneoplastic Phenomena
  - “non-specific” Role for detecting Complications e.g. Anemia, Clotting Abnormalities, Hormones ...

**Future**
- primary Diagnosis & Follow-Up with liquid Profiling (aka Liquid Biopsy)
  - CTC and DTC
  - fcDNA (ctDNA, exosomes, µ-particles)
  - digital PCR
  - MPS (CaPPS; iDES-CaPPS)
**MRD in CRC: Detection through CTC**

- **CTC are strong Predictors of clinical Outcome.**
- **The Dynamics of CTC is prognostically relevant.**
- **Are CTC appropriate for Therapy Stratification?**

*Miller MC et al., Int. J. Oncol. 617421 (2010)*
10 x Genomics data set in blood cells
(single cell 5’ transcriptome)
Conclusions
Modern Laboratory Diagnostics in Oncology......

• detects minimal residual Disease (MRD) from blood samples (using digital droplet PCR methods - NGS is getting there, too) – unknown mutations.
• will reduce the need for tissue sampling - genetic footprint will be available in the blood by liquid profiling.
• detects the „biologic Achilles Heel“ of Tumours (driver mutations), thus recommending Therapy Regimens actionable health information
• identifies therapy resistance with Lead-Time over imaging (up to 10 Months reported) actionable health information
• change/abandon inefficient therapies - rechallenge
• shall analyse exosome/microparticles as carriers for intercellular NA transfer (mRNA, µRNA, exosome proteomics)
• will combine molecular and phenotypic information for comprehensive tumor targeting.
Acknowledgment

Institut für Klinische Chemie, UMM
Dr. Verena Haselmann
Dr. Maren Hedtke
Ingrid Brechtel
Angelika Duda
Maximilian Kittel

Sysmex, Hamburg
Prof. Dr. Hartmut Juhl
Dr. Frank Diehl
Dr. Barbara Behrens
Dr. Frederick S. Jones

Klinik für Dermatologie, UMM
DFKZ, Heidelberg
Prof. Dr. Jochen Utikal
Dr. Christoffer Gebhardt
Dr. Tim Holland-Letz

Klinik für Dermatologie, UK-Essen
Prof. Dr. Dirk Schadendorf
Dr. Antje Sucker

Deutsches Krebsforschungszentrum
Zertifiziertes Hauttumorzentrum
Universitätsklinikum Essen
Thank you for your kind Attention!

michael.neumaier@umm.de