Moving beyond DNA sequence:

Genome-wide profiling of plasma DNA

Samantha Perakis
31st European Congress of Pathology
Nice, September 9, 2019
DISCLOSURES

• None
LIQUID BIOPSY

ctDNA reflects tumor-specific changes from different locations

Surrogate marker for the entire tumor genome

Easily accessible biofluids enable repeated sampling


NOT EVERY TUMOR TYPE EQUALLY SUITABLE FOR LIQUID BIOPSY

Bettegowda et al, STM 2014
ANALYSIS APPROACHES

El-Heliebi & Heitzer et al. Nucleic Acid Nanotheranostic 2019

Zhou et al. TCR 2017
Pre-screening with mFAST-SeqS  
Untargeted assessment of tumor DNA fraction

**Z-score > 5**  
Tumor fraction > 5-10%

**Z-score < 5**  
Tumor fraction < 5-10%
Z-score > 5
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Pre-screening with mFAST-SeqS
Untargeted assessment of tumor DNA fraction

Blood draw
Plasma extraction
Plasma DNA extraction

Z-score < 5
Tumor fraction < 5-10%

- Plasma-Seq
  - SCNA alone
  - SCNA & enrichment

ANALYSIS ALGORITHM
Blood draw

Plasma extraction

Plasma DNA extraction

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- High resolution profiling
  - Deep-Seq
  - NEBnext Direct Cancer Hotspot panel
  - dPCR
  - AVENIO ctDNA panels

ANALYSIS ALGORITHM
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1. **Blood draw**
2. **Plasma extraction**
3. **Plasma DNA extraction**

**Pre-screening with mFAST-SeqS**
- Untargeted assessment of tumor DNA fraction
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      - **High resolution profiling**
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**ICT study**
- Phase II study in order to identify actionable targets
  - **Plasma-Seq (SCNA)**
  - **Cancer hotspot panel**

**Analysis of ctDNA (Service)**
- Identification of actionable targets
  - Plasma-Seq + AVENIO ctDNA Expanded panel for clinical report generation

**Report to clinicians**
PATIENT COHORTS AND SAMPLES

Metastatic patients recruited: n=1537
Samples collected: n=4471

- CRC
  - Patients n=377
  - Samples n=1554
- Breast cancer
  - Patients n=234
  - Samples n=591
- Prostate cancer
  - Patients n=410
  - Samples n=1036
- Kidney cancer
  - Patients n=44
  - Samples n=254
- Lung cancer
  - Patients n=282
  - Samples n=515
- Other cancers
  - Patients n=132
  - Samples n=242

mFAST-SeqS z-score distribution

z-score = 5

healthy n=42
sarcoma n=158
renal n=242
breast n=206
lung n=158
colorectal n=657
prostate n=526
THERAPY MONITORING

DETECTION OF RESISTANCE
Suppan et al, Cancers 2019

CHANGING LEVELS OF CTDNA REFLECT RESPONSE TO TREATMENT | breast cancer
CTDNA AS A MONITORING TOOL | prostate cancer

Therapy response and PFS in relation to z-score

<table>
<thead>
<tr>
<th>Clinical covariates (log10 transformed)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate-specific antigen (PSA)</td>
<td>3.36 (1.58, 7.18)</td>
<td>0.002</td>
<td>2.754 (1.254, 6.005)</td>
<td>0.015</td>
</tr>
<tr>
<td>Lactate dehydrogenase (LDH)</td>
<td>4.83 (1.08, 11.76)</td>
<td>0.002</td>
<td>3.726 (1.471, 9.517)</td>
<td>0.008</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>0.687 (0.255, 1.703)</td>
<td>0.203</td>
<td>0.778 (0.382, 1.651)</td>
<td>0.471</td>
</tr>
<tr>
<td>Bone Metastases</td>
<td>10.11 (3.552, 30.47)</td>
<td>5.69E-05</td>
<td>6.092 (2.155, 20.65)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Belic et al, Int J Cancer 2018
CTDNA AS A MONITORING TOOL | prostate cancer

EMERGENCE OF AR AMPLIFICATION DURING TREATMENT WITH ABIRATERONE OR ENZALUTAMIDE

DE-DIFFERENTIATION INTO A NEUROENDOCRINE TUMOR

Belic et al, Int J Cancer 2018
EVOLUTION OF THE TUMOR GENOME
COLORECTAL CANCER | Characterization of the chr13q12.2 amplification

Fully included: \( \text{POLR1D, GSX1, PDX1, CDX2, PRHOXNB, ATP5EP2, FLT3} \)
- Partially included: \( \text{LNX2, PAN3} \)
- Non-coding gene: \( \text{LINC00543} \)
- Neighboring genes: \( \text{LNX2, PAN3} \)

FLT3 as a therapeutic target in CRC?

No significant correlation identified between FLT3 copy number and expression

Colony formation assay showed no significant changes of proliferation after overexpression of FLT3 in Oxco-2

Zhou et al, under review
Chr13q amplification is associated with:
- distant metastasis
- tumor stage
- lymph node metastasis
- tumor location

Zhou et al, under review
COLORECTAL CANCER | Characterization of the chr13q12.2 amplification

Positive correlation between copy number and expression in 5 genes (i.e. \textit{CDX2}, \textit{LNX2}, \textit{POLR1D}, \textit{PDX1} and \textit{PAN3}) in TCGA cohort

Silencing of \textit{POLR1D} in both cell lines showed reduction in cell viability over 15%
COLORECTAL CANCER | Characterization of the chr13q12.2 amplification

Amplified cohort showed increased expression of VEGFA and EREG

Zhou et al, under review
13q12.2 amplification correlated with the development of progressive disease and resistance to bevacizumab

Zhou et al, under review
13q12.2 amplification in first plasma sample, but disappeared after anti-EGFR treatment → novel focal amplification on 17q12

Resistance to anti-EGFR treatment within 9 months

After switch to anti-VEGF treatment 13q12.2 amplification reappeared
IDENTIFICATION OF ACTIONABLE TARGETS
LIQUID BIOPSY FOR PRECISION MEDICINE | reality or hype

Median age = 56 years (range 38-74)

INDIVIDUALIZED CANCER TREATMENT STUDY | study design and rationale

- Prospective, non-randomized, monocentric, open two-stage clinical phase II study
- Non-invasive molecular profiling of ctDNA from peripheral blood could eliminate ineffective therapy attempts
- For how many patients can an anti-tumor therapy based on a molecular, biological tumor profile be defined?
- Success of a targeted drug therapy based on a molecular and biological tumor profiling (1.2x)

EUDRACT NUMBER: 2014-005341-44

1. PRIMARY:
   \[
   \text{PFS ratio} = \frac{\text{PFS on targeted therapy}}{\text{PFS on the last evidence-based drug therapy}}
   \]

2. SECONDARY:
   - Progression–free survival (PFS)
   - Overall survival (OS)
   - Overall response rate (ORR)
   - Safety

Unpublished data

Number of Cases Per Tumor Entity (n=24)

Median age = 56 years (range 38-74)
INDIVIDUALIZED CANCER TREATMENT (ICT)

Plasma DNA extraction
Optional tissue biopsy
Interdisciplinary Board (Human Genetics, Oncology, Pathology)
Interpretation & search for actionable target (My cancer genome, DGIdb - Mining the Druggable Genome, CIViC)

Plasma-Seq
Hotspot panel

Study initiated by Hellmut Samonigg/Armin Gerger
ICT STUDY | results

LIMIT OF DETECTION AT 5% TUMOR FRACTION

INFORMATIVE RESULTS ACHIEVED IN 18 PATIENTS (75%)

MEDIAN TUMOR-SPECIFIC DNA FRACTION OF 22.7% (RANGE 5.2-40.3)

9 (37%) PATIENTS RECEIVED TREATMENT BASED ON MOLECULAR PROFILE

1 (5.6%) PATIENT MET ENDPOINT
MATCHING GENOMIC ALTERATIONS TO SPECIFIC THERAPY
CUREMATCH | retrospective analysis

PreciGENE™
Personalized Combination Therapy

OVERVIEW

Below is an overview of the results of the CureMatch analysis. The graphs at the bottom provide a snapshot of all possible combinations of 1, 2 or 3 drugs that were considered in the analysis, ordered by descending PreciGENE Score. Definitions of these terms and the details of the treatment options are provided on subsequent pages of the report.

Treatment Options

3 Drug Combinations

2 Drug Combinations

1 Drug Combinations

TOP RANKED COMBINATION THERAPIES USING A MAXIMUM OF 3 DRUGS

<table>
<thead>
<tr>
<th>PRECIGENE SCORE</th>
<th>DRUGS</th>
<th>TARGETING DESCRIPTION</th>
<th>INDICATIONS &amp; RECOMMENDATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>36%</td>
<td>bevacizumab, trametinib</td>
<td>TP53 via FLIT1 pathway, TP53 via KDR pathway, KRAS via MAP2K1 pathway, KRAS via MAP2K2 pathway, SMAD4 via MAP2K1 pathway, SMAD4 via MAP2K2 pathway</td>
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<td>afatinib</td>
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Satellite (RAAD) or colorectal (RRAAD) may be added to this combination.

PSA doubling warning: 
- bevacizumab: gastrointestinal perforations, surgery and wound healing complications, and hemorrhage

Drug-drug interactions:
- There may be drug-drug interactions that are not listed here.
- Administering drug combinations is an active discussion in the physician.

Example of ongoing clinical trials using similar drugregimen in the prostate type:
- While clinical trials using these drugs alone or in combination with other drugs exist, no clinical trial testing the exact association of drugs can be found.
BEYOND MUTATIONS AND COPY NUMBER
FOCAL ALTERATIONS ARE A DRIVING FORCE IN CANCER

Novel focal alterations occur in ~20-40% of metastatic patients

Can other information than mutations and copy number alterations be retrieved from plasma DNA?

RNA-Seq of plasma is challenging

Correlation of focal amplifications and expression status in plasma is unknown
NUCLEOSOME OCCUPANCY MAPPING

NDR: Nucleosome-depleted region

TSS

Preferential digestion

Snyder et al, Cell 2016
Are blood samples from patients with cancer informative for expressed cancer driver genes?
COVERAGE AT TRANSCRIPTION START SITE INFORMS ABOUT EXPRESSION STATUS

Ulz et al, Nat Genet 2016

2000bp around TSS
200bp around TSS
86.1% and 88.1%, respectively, agreement between gene expression in B7 and B13 according to RNA-seq of the primary tumors and plasma DNA data for overrepresented regions.
CFDNA ACCURATELY REFLECTS TF-NUCLEOSOME INTERACTIONS

- Comprehensive TF-nucleosome interaction maps (n=676)
- Using “accessibility score” to measure different activity

Coverage profiles at TFBS of healthy controls

Ulz et al, under review
CTCF > 55,000 binding sites, associated with TADs

PU.1 hematopoietic

Spi-B hematopoietic

GRH-L2 epithelial

Lyl-1 hematopoietic

FOXA1 cellular differentiation prostate cancer associated

Ulz et al, under review
ZNF644 is associated with transcriptional repression

REST key mediator of neuroendocrine differentiation

Ulz et al, under review
USING TFBSs FOR EARLY CANCER DETECTION

Comparisons of accessibilities for selected TFs

Logistic regression with 504 TFs for samples from the colon cancer cohort

Ulz et al, under review
Different tumor entities/clinical scenarios require different methodologies.

High levels of ctDNA are associated with bad prognosis in a variety of tumor types.

Changing levels of ctDNA are predictors of response.

Untargeted methods can monitor tumor evolution.

WGS can derive functional information from cfDNA.

Nucleosome occupancy patterns might indicate changing activities of genes/pathways (CAVEAT: only for high tumor levels).
Special thanks to all patients, their families and our collaborators

<table>
<thead>
<tr>
<th>Liquid biopsy group</th>
<th>MUG internal departments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michael R. Speicher</td>
<td>Gynecology: E. Petru, G. Pristauz</td>
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<tr>
<td>Ellen Heitzer</td>
<td>Oncology: M. Balic, T.</td>
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<tr>
<td>Jochen B. Geigl</td>
<td>Bauernhofer, N. Dandachi, A.</td>
</tr>
<tr>
<td>Peter Ulz</td>
<td>Gerger, M. Pichler</td>
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<tr>
<td>Qing Zhou</td>
<td>Orthopedics: A. Leithner</td>
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<tr>
<td>Tina Moser</td>
<td>Pathology: G. Höfler, S. Jahn, K.</td>
</tr>
<tr>
<td>Ricarda Graf</td>
<td>Kashofer, B. Liegl-Atzwanger, K.</td>
</tr>
<tr>
<td>Sabrina Weber</td>
<td>Urology: G. Pummer, K.</td>
</tr>
<tr>
<td>Isaac Lazzeri</td>
<td>Augustin, M. Seles</td>
</tr>
<tr>
<td>Benjamin Spiegler</td>
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