Genome Sequencing in Cancer Research and Therapy

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Genome Sequencing in Cancer Research and Therapy

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IMBS Symposium: Science, Ethics and Society

Freiburg, August 28, 2015
Cancer Genome Sequencing
Pathogenetic Insights and Clinical Impact

Distinct mutations shared across multiple cancers
BRAF\textsuperscript{V600E/K} in melanoma; thyroid, lung, colorectal, ovarian, gastric, esophageal, head and neck cancer; gastrointestinal stromal tumor; glioma; hairy-cell leukemia; multiple myeloma; etc.

Common cancers as multiple rare diseases of the same organ, demanding unique therapies
Li et al. J Clin Oncol 2013

Improved clinical outcome through genotype-directed therapy
Verweij et al. Lancet 2004

Improved clinical outcome through genotype-directed therapy
Verweij et al. Lancet 2004
Cancer Genome Sequencing

“N = 1” Studies

Targetable FGFR fusions in diverse cancers

Wu et al. Cancer Discov 2013

Activating mTOR mutations in urothelial carcinoma

Wagle et al. Cancer Discov 2014

Inactivating TSC1 mutations in urothelial carcinoma

Iyer et al. Science 2012
Cancer Genome Sequencing
Importance of Rare Mutations

The Cancer Genome Atlas
Understanding genomics to improve cancer care

Majority of cancer genes mutated at frequencies of < 5% within any given histologic tumor subtype

“Long tail” pattern of actionable cancer gene alterations
TCGA Pan-Cancer Analysis
Lawrence et al. Nature 2013

Gene “mountains” and “hills”
Wood et al. Science 2007
NCT MASTER

**Molecularly Aided Stratification for Tumor Eradication Research**

- Molecular diversity and genetic taxonomy of cancer
- Individual, “private” patterns of molecular lesions
- Actionability of molecular lesions

**NCT MASTER Registry Trial**

- Prognostic / predictive markers
- Therapeutic targets

**Molecularly stratified clinical trial(s)**
Eligibility

- Patients younger than 51 years
- Patients with rare cancers
  - Incidence of less than 1/100,000 per year
- Measurable disease activity
- No curative treatment available
- Karnofsky Performance Status of at least 70%
- Life expectancy of at least 6 months

Objectives

- Prospective exome and transcriptome sequencing within clinical context
- Interdisciplinary evaluation and formulation of treatment recommendations
- Translation into individualized patient care

DKFZ-HIPO
Enrollment
NCT MASTER Protocol

Umbrella protocol for implementing precision oncology at NCT, consenting every patient for:

- Questionnaires – Health and Behavior
- Molecular Analysis
- Data Storage
- Clinical Data Analysis
- Recontact for Clinical Trials
SOP-Guided Preparation of Analytes

**Tasks**
- Sample registration and barcoding
- Sample preparation
- Nucleic acid extraction
- Quality assessment and identity check
- Sample submission to core facility
- Documentation
- Methods development and optimization
- Sample management and storage

**Submissions in 2014**
- Exome sequencing 958
- Whole-genome sequencing 183
- Transcriptome sequencing 298
- SNP array profiling 283
- Expression profiling 46
- Methylation profiling 164
- **Total** 1,932
High-Throughput Sequencing Unit

Tasks
• Automated library preparation
• Highly parallel sequencing using Illumina technology
• 14 HiSeq 2000, 2 HiSeq 2500 systems

Clinical Bioinformatics

Tasks
• Automated alignment
• Variant calling, selection of somatic mutations
• Gene expression analysis
• Annotation with COSMIC, Cancer Gene Census, potential drugs and pathways

Translational Oncology

Task
• Clinical interpretation of molecular data

Molecular Pathology

Task
• Validation of actionable genetic alterations
NCT MASTER
Precision Oncology Tumor Board

Clinical case
• Referring physicians

General characteristics
(number of alterations, mutation patterns etc.)
• Bioinformatics

Presentation of potentially actionable lesions
• Translational Oncology

Discussion of potential clinical implications
• All

Precision Oncology Outpatient Clinic
• Communication of results
• Initiation/monitoring of therapy

Referring Physicians
Translational Oncology
Pathology
Bioinformatics
Sample Processing Lab
Sequencing Facility

Friday, 2:30 PM
NCT, Room K4
Tumor Board Report

Validation Report

Universitätsklinikum Heidelberg

Ergebnis der Mutationsvalidierung im Rahmen des Personalisierten Onkologie Programms (POP)

Patienteninformation

Name:

Geburtsdatum:

Zu validierende Mutation

Chromosom

Position (bp19)

Genesymbol

Typ

SNP

Refrenz-Alt

Variante-Alt

Variante

p.K218E

Variante-Frequenz

35%

Ergebnis der Sanger-Sequenzierung:

☐ Die Mutation wurde bestätigt
☐ Die Mutation wurde nicht bestätigt

Bemerkung:

Anspruchsanteil (Pathologie):

V. Erdels (56-35989), R. Penzel (56-39507)

Die durchgeführte Analyse ist „research grade“, es handelt sich nicht um eine qualitativ gesicherte, diagnostisch belastbare Untersuchung im engeren Sinne.
**Level 1**

A: Drug is approved for the same tumor type harboring the specific biomarker.

B: Predictive value of the biomarker or clinical effectiveness of the corresponding drug in a molecularly stratified cohort was demonstrated in an adequately powered prospective study or a meta-analysis.

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**Level 2**

A: Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated in a prospective trial with biomarkers as a secondary objective or an adequately powered retrospective cohort or case-control study in the same tumor type.

B: Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated by clinical data in a different tumor type.

C: Case study or single unusual responder indicates the biomarker is associated with response to the drug, supported by scientific rationale.

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**Level 3**

Preclinical data (in vitro or in vivo models and functional genomics) demonstrate that the biomarker predicts response of cells to drug treatment.

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**Level 4**

Biological rationale exists that links the drug to the altered signaling pathway or relevant basket. No reported clinical or preclinical data on the response to the drug.

Adapted from: MD Anderson Cancer Center Institute for Personalized Cancer Therapy

[https://pct.mdanderson.org](https://pct.mdanderson.org)
July 2015
Registered  \( N = 384 \)
Eligible  \( N = 322 \)
Completed  \( N = 260 \)
Discussed  \( N = 212 \)
Recommendation  \( N = 125 \) (59%)
Treatment  \( N = 38 \) (18%)
# Findings With Established Clinical Implications by Histology

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mutation</th>
<th>Clinical Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>BRAF p.V600R</td>
<td>Vemurafenib, dabrafenib</td>
</tr>
<tr>
<td></td>
<td>ERBB4 p.P172F</td>
<td>Lapatinib</td>
</tr>
<tr>
<td>Uveal melanoma</td>
<td>GNAQ p.Q209L/P/R</td>
<td>Trametinib, selumetinib</td>
</tr>
<tr>
<td>Basal-cell carcinoma</td>
<td>PTCH1 p.648_650del</td>
<td>Vismodegib</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>BRAF p.V600E</td>
<td>Vemurafenib, dabrafenib</td>
</tr>
<tr>
<td>Myxoid liposarcoma</td>
<td>PIK3CA p.C420R/p.E545K</td>
<td>PI3K/AKT inhibitors, everolimus, temsirolimus</td>
</tr>
<tr>
<td></td>
<td>PTEN p.R130G</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor</td>
<td>KIT p.V560D</td>
<td>Imatinib</td>
</tr>
<tr>
<td></td>
<td>PDGFRA p.D842V</td>
<td>Crenolanib</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>TSC2 p.R505X</td>
<td>Everolimus, temsirolimus</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>PIK3CA p.E545K</td>
<td>PI3K/AKT inhibitors, everolimus, temsirolimus</td>
</tr>
<tr>
<td></td>
<td>TSC1/2del</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FGFR1amp</td>
<td>FGFR inhibitors</td>
</tr>
<tr>
<td>Pulmonary adenocarcinoma</td>
<td>EGFR p.T790M</td>
<td>AZD9291</td>
</tr>
<tr>
<td></td>
<td>TSC2mut</td>
<td>Everolimus, temsirolimus</td>
</tr>
<tr>
<td>Carcinoma of unknown primary</td>
<td>EGFR p.745_750del</td>
<td>Erlotinib</td>
</tr>
<tr>
<td></td>
<td>PIK3CA p.E545K</td>
<td>PI3K/AKT inhibitors, everolimus, temsirolimus</td>
</tr>
</tbody>
</table>
### Findings With Established Clinical Implications by Gene/Pathway

<table>
<thead>
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<th>Mutation</th>
<th>Disease</th>
<th>Clinical Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF p.V600R/p.V600E</td>
<td>Multiple myeloma, Melanoma</td>
<td>Vemurafenib, dabrafenib</td>
</tr>
<tr>
<td>PIK3CA p.C420R/p.E545K</td>
<td>Breast cancer, Myxoid liposarcoma, Carcinoma of unknown primary</td>
<td>PI3K/AKT inhibitors, everolimus, temsirolimus</td>
</tr>
</tbody>
</table>
| TSC1\text{mut}/TSC1\text{del}  
TSC2\text{mut}/TSC2\text{del} | Esophageal adenocarcinoma, Gastric cancer, Breast cancer, Ovarian cancer, Pulmonary adenocarcinoma | Everolimus, temsirolimus                             |
| FGFR1\text{amp} | Breast cancer, Leiomyosarcoma, T-cell prolymphocytic leukemia | FGFR inhibitors                                      |
| ERBB2\text{amp}/p.D769Y | Colorectal cancer, Cholangiocarcinoma, Gallbladder carcinoma, Plexiform schwannoma | Trastuzumab, pertuzumab, lapatinib, neratinib        |
25-year-old patient with metastatic retroperitoneal leiomyosarcoma
- No local therapy options
- Refractory to doxorubicin/ifosfamide
- Refractory to trabectedin

Focal 8p amplification involving FGFR1, validated by FISH

Treatment with FGFR inhibitor within phase 1 clinical trial for 2 months
- Discontinuation due to adverse events

Ongoing stable disease for >12 months with minimal metabolic activity of metastatic lesions
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Personalized Oncology at NCT – NCT MASTER

Rationale for Experimental Targeted Treatment (1)

Whole-exome sequencing

Fluorescence in-situ hybridization
46-year-old patient with metastatic triple-negative breast cancer
- Refractory to multiple lines of chemotherapy

NRG1 c.1053-1G>A (splice site mutation)
NRG1 expression by immunohistochemistry

Aberrant NRG1 expression and ERBB3 activation, enhanced dimerization with other ERBB family members, and constitutive signal transduction as rationale for pan-ERBB blockade in “ERBB2-low” breast cancer

Treatment with pertuzumab, eribulin, and bevacizumab

Stable disease for >6 months
ERBB2 amplification and overexpression in gastrointestinal cancers
- KRAS<sup>WT</sup> colorectal cancer (n=3)
- Gallbladder carcinoma (n=1)
- Cholangiocarcinoma (n=1)

Aberrant ERBB2 expression and constitutive signal transduction as rationale for ERBB2 blockade with trastuzumab, pertuzumab, or lapatinib

*Ongoing partial remission in a 37-year-old patient with metastatic gallbladder carcinoma treated with trastuzumab, pertuzumab, and nab-paclitaxel since 11/2014

HERACLES Trial
Trastuzumab/lapatinib in patients with heavily pretreated, ERBB2<sup>amp</sup>, KRAS<sup>WT</sup> colorectal cancer; DCR: 78%; median TTP: 5.5 months
*Siena et al. ASCO Annual Meeting 2015
66-year-old patient with glioblastoma
- Status post incomplete resection
- Adjuvant chemotherapy with temozolomide

**PIK3R1 p.G376R**
- Activation of PI3K, low transforming potential

**TTYH3-BRAF fusion**
- Novel, structurally distinct fusion involving near-full-length BRAF
- BRAF activation and transforming potential

**BRAF p.47_438del**
- Loss of N-terminal regulatory domain and constitutive BRAF activation
- Described as *splicing alteration* associated with constitutive BRAF dimerization and resistance to vemurafenib in melanoma

Rationale for Experimental Targeted Treatment (6)

Rationale for MAPK pathway blockade using sorafenib or MEK inhibitor
Rationale for Experimental Targeted Treatment (6)

Sun et al.  
*PNAS 2010*

Poulikakos et al.  
*Nature 2011*

Quayle et al.  
*PLoS One 2012*
Rationale for Experimental Targeted Treatment (6)

Sun et al.  
*PNAS* 2010

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*Nature* 2011

Quayle et al.  
*PLoS One* 2012

NCT MOLECULAR DIAGNOSTICS PROGRAM

*Personalized Oncology at NCT – NCT MASTER*

Tilman Brummer
University of Freiburg
NCT MOLECULAR DIAGNOSTICS PROGRAM
Personalized Oncology at NCT – NCT MASTER

$N = 322$  $N = 260$  $N = 212$

Patient Sample Asservation  Sequencing  Bioinformatic Analysis  Clinical Evaluation  Validation  Molecular Tumor Board*

Unbiased Functional Testing  Focused Functional Testing

Feed Back Results  Adapt Treatment/Functional Testing

*Recommendation: 59%  Treatment: 18%

Stefan Fröhling, Stefan Gröschel, Christoph Heining, Hanno Glimm, Claudia Scholl (Functional Genomics)
DKTK: Frankfurt/Mainz, Munich, Dresden (Berlin, Essen, Freiburg)

(1) BAY1125976 (“Allo-AKT”): PI3K/AKT$^{\text{mut}}$
(2) BRF117019 (“ROAR”): BRAF$^{V600E}$
(3) EORTC 90101 (“CREATE”): ALK/MET$^{\text{abn}}$
<table>
<thead>
<tr>
<th>Program</th>
<th>Analyses</th>
<th>Patients</th>
<th>Actionable&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Action</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mi-Oncoseq</td>
<td>WES RNA-seq</td>
<td>369</td>
<td>59%</td>
<td>23%</td>
<td>Response</td>
</tr>
<tr>
<td>UCSD PREDICT</td>
<td>Panel-seq (236 genes)</td>
<td>347</td>
<td>–</td>
<td>25%</td>
<td>Response, PFS, OS&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>NCT MASTER</td>
<td>WES RNA-seq</td>
<td>212</td>
<td>59%</td>
<td>18%</td>
<td>Response</td>
</tr>
</tbody>
</table>

<sup>1</sup>Rationale for drug in development or off-label use of approved drug; prediction of response to treatment; predisposing germline alteration

<sup>2</sup>SD/PR/CR: 35% vs. 16% (P=0.02); PFS: 4 vs. 3 months (P=0.04); PFS/\text{PFS1}\ge1,3: 45% vs. 19% (P=0.004 and P=0.06); trend for superior PFS following treatment „matched directly“ vs. „matched indirectly“ (P=0.1); OS: 14 vs. 11 months (NS)
LONG-TERM OUTCOME OF STRATIFIED THERAPY
Meta-Analysis of Targeted Treatment Strategies

- UC San Diego, MD Anderson Cancer Center, Institut Gustave Roussy, WIN Consortium, ASCO
- 570 phase 2 trials; 32,149 patients; targeted agents as monotherapy; 641 treatment arms
- Therapy according to biomarker vs. unselected treatment
- Superior outcome compared to conventional chemotherapy through targeted therapy according to biomarker (“matched directly” and “matched indirectly”)
  - Response, PFS, OS (each \(P<0.0001\))
  - Toxicity (\(P<0.001\))
- Inferior outcome compared to conventional chemotherapy through unselected “targeted” therapy
  - Response, PFS (each \(P<0.0001\))
  - OS (\(P=0.048\))
- Better outcome with targeted approaches based on genomic alterations (mutations) vs. aberrant expression (RNA, protein) as biomarker
  - Response, PFS, OS (each \(P<0.05\))

Schwaederle et al. ASCO Annual Meeting 2015
**NCT MOLECULAR DIAGNOSTICS PROGRAM**
*Personalized Oncology at NCT – NCT MASTER*

\[ N = 322 \quad N = 260 \quad N = 212 \]

- Patient Sample Asservation
- Sequencing
- Bioinformatic Analysis
- Clinical Evaluation
- Validation
- Molecular Tumor Board*

*Unbiased Functional Testing*

*Focused Functional Testing*

\*Recommendation: 59%
Treatment: 18%

**Potential Strategies for Interventional Clinical Trial(s)**

- Fill baskets and collect information on treatment outcome
- Identify successful baskets (OR or SD for \( \geq 6 \) months in \( \geq 2/10 \) patients)
- Define trial design, for example:
  - “Randomization” between patients receiving genomics-guided treatment and patients receiving standard of care (due to logistical or regulatory reasons etc.)

*In collaboration with Annette Kopp-Schneider and Axel Benner, Division of Biostatistics, DKFZ*
NCT / DKFZ
Translational Oncology
Stefan Fröhling, Christoph Heining, Stefan Gröschel, Hanno Glimm
Medical Oncology
Dirk Jäger and Team

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Board of Directors
Peter Lichter, Roland Eils, Christof von Kalle

DKFZ
Sequencing Core Facility
Stephan Wolf and Team
Clinical Bioinformatics
Barbara Hutter, Benedikt Brors

Heidelberg University
Molecular Pathology
Volker Endris, Roland Penzel, Albrecht Stenzinger, Wilko Weichert, Peter Schirmacher