Understanding Cancer by Whole Genome Studies

Christof von Kalle
NCT Heidelberg
Oncology – A Leading Topic in Heidelberg

EXCELLENCE in

- Clinical Translation and Basic Research
- Clinical and Translational Cancer Programs
- Interdisciplinary Patient Care
NCT - Interdisciplinary Patient Care

Heidelberg University Medical School
61,210 Inpatients, 50,042 Day Hospital Cases
G-DRG Case-Mix 2012: 109,975  Index: 1,797
426,760 Outpatient cases / 1,029,920 Visits (2012)

NCT 2013
Newly Diagnosed Patients: 9,802
Out-Patient Visits: 55,235
Treatments: 19,300
Patients Enrolled in Clinical Trials: 20,4%
232 Clinical Trials, >70 HD IITs (2010/2012)

Clinical Care

Cancer Research Grants

DKTK
NCT 1.0 – Principles & Practice

All Translational Cancer Research Groups

EVERY TRIAL

All Clinical Departments with Oncological Activities

EVERY PATIENT

Creating Interdisciplinarity
NCT 2.0 – New Building & Structures

IDENTITY & VISIBILITY
NCT 3.0 – The Next Level

DELMIVER
Precision Oncology

EXCEL
Clinical and Translational Cancer Programs

GROW
Building Partners

SCIENTIFIC & CLINICAL EXCELLENCE
NCT 3.0 - Strategic Aims

• Position NCT as a Leading Comprehensive Cancer Center in Europe

• Expand NCT Precision Oncology Program

• Deliver at the Point of Care
  Translation – Personalization – Precision

• Expand Internationally Recognized Clinical Cancer Programs & Profile Areas

• Employ Novel, Innovative Treatment Concepts
NCT 3.0 – Programmatic Funding

Building Extension

NCT 3.0 Funding

NCT Base Funding

€5 Mio.

2015

NCT 3.0 Funding

€10 Mio.

2016

€15Mio.

2017

€20 Mio.

2018

€25Mio.

2019

NCT Base Funding
NCT Dresden

FUNDING
Up-to €15 Mio p.a. in 2019
New Research Building
CCC Building funded by DKH

PROFILE AREAS / RECRUITMENTS
- Innovative Medical Oncology
- Translational Research
- Radioimaging
- Theragnostics
- Link-up DKFZ HIPO, NCT POP & NCT MASTER
- Immunotherapy
- Systems Biology
- Multidisciplinary Profile Areas
GROW

- Partnerships
- Funding Programs
- Recruitments
- Talents
- Facilities

EXCEL

- Molecular Stratification
- Immunotherapy
- Profile Areas - Neurooncology, Pancreatic Cancer, Radiooncology, Lung Cancer, Prostate Cancer
- NCT OncoCheck-Programm
- Innovative Oncological Imaging
- Molecular Tumor Pathology
- Biomarker Platform
- NCT DataThereHouse & Cancer Registry
- New Programs – CardiOncology, Palliative Oncology, Hematology
- NCT Basic Research Pipeline

DELIVER

- NCT Precision Oncology
- Novel, Innovative Treatment Concepts
- Clinical Trials & Biobanking Infrastructure/Expertise

NCT 3.0 - Profile Areas to be Expanded
NCT Precision Oncology Program

EARLY DIAGNOSIS
RISK STRATIFICATION
PREVENTION

INDIVIDUALIZED THERAPY

NCT DataThereHouse Organized in SAP HANA

NCT OMICS

PATIENT ADMISSION – DOCUMENTATION - BANKING
The Paradigm Shift: Imaging each Individual Patient's Cancer At the Molecular Level
NCT Precision Oncology Program

- **Stratified Oncology**
  - Clinical Evaluation / Trials
  - Target Exploitation
  - Target Characterization
  - Target Identification
  - Patient Admission Documentation Banking

- **Early Diagnosis, Risk Stratification, Individualized Therapy & Prevention**
  - Researcher
  - Physician
  - Molecular Tumor Board
  - Patient

- **NCT DataThereHouse**
  - Metabolomics
  - Proteomics
  - Genomics
  - Immunomics
  - Radio/Imaging
  - Biomaterials
  - Clinical Data
DNA Sequencing Methods Applied in Oncology

- Whole Genome Sequencing
- Whole Exome Sequencing
- Gene Panel Sequencing
Cancer genome sequencing

Meyerson, Nat Rev Genet 2010
OneTouchPipeline

RNA sequences
- fastq QC
- alignment
- duplicates
- lane merging
- calculate coverage
- genotype matching

DNA sequences
- Pathogen integration
- Ploidy prediction
- INDELs
- SNVs
- Copy number variants
- Structural Variants
- Alternative Splicing

TERTIARY ANALYSIS: "INTERPRETATION"

Mutation in RNAseq?
- Mutation annotation

Mutation type

Five prime base
- A
- C
- G
- T

Three prime base
- A
- C
- G
- T

Applied Bioinformatics

Benedikt Brors
NCT MASTER – Registry & Interventional Trial

Hanno Glimm  Stefan Fröhling

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

PI3K-AKT-mTOR  RAF-MEK-ERK  Tyrosine Kinases  Developmental Pathways  DDR Signaling  Hypermutated/Immuno-therapy

Stefan Fröhling, Christoph Heining, Hanno Glimm, Stefan Gröschel, Claudia Scholl (Functional Genomics) DKTK – LMU München, Frankfurt, Dresden, Essen/Düsseldorf, Freiburg, Berlin
Entities

- Bone and Soft Tissue
- Colorectal
- Pancreatic
- Hematologic
- Breast
- Dermatologic
- Lung
- Urogenital
- Neuroendocrine
- Head and Neck
- Hepatobiliary
- Renal
- Gastric
- Brain
- Unknown Primary
- Other

CURRENT STATUS

NCT MASTER – Registry & Interventional Trial
Metastatic gallbladder carcinoma
- Peritoneal and cutaneous metastasis during adjuvant chemotherapy with oxaliplatin/gemcitabine

Amplification of chromosome 17q12, including ERBB2
- Outlier ERBB2 mRNA expression
- ERBB2 protein expression by immunohistochemistry (3+ according to ASCO guidelines)

Dual ERBB2 blockade: trastuzumab/pertuzumab

Complete remission for >12 months

Czink et al. Z Gastroenterol 2016
35-year-old patient with bladder tumor, bilateral pulmonary masses, enlarged hilar lymph nodes, and disseminated bone lesions.

- Histology inconclusive
- Refractory to cyclophosphamide, doxorubicin, vincristine, and prednisone

Co-existence of HRAS p.Q61R and BRAF p.F595L within single clone

Mutational spectrum highly suggestive of non-Langerhans histiocytosis

Rationale for MAPK pathway blockade using sorafenib or MEK inhibitor

No rationale for vemurafenib or dabrafenib due to risk of paradoxical MEK/ERK activation

Kordes et al. Leukemia 2016
Loss of phosphorylated ERK expression in tumor cells after 3 weeks of sorafenib and interferon alpha treatment

Frequent coexistence of intermediate-activity BRAF mutations and oncogenic RAS in various cancers
Wan et al. Cell 2004
COSMIC database

Rationale for Experimental Targeted Treatment

Physiologic BRAF signaling
BRAF<sup>F595L</sup> signaling in the absence of RAS activity
Collaboration between mutant HRAS and BRAF<sup>F595L</sup>

Cooperative activity of BRAF p.F595L and HRAS p.Q61R in aggressive histiocytic sarcoma
Kordes et al. Leukemia 2016
DKTK MASTER
Critical Next Steps

Development of a common path for clinical cancer genome sequencing and molecular stratification within DKTK

Further development of strategies for clinical translation

Refinement and expansion of strategies for functional annotation

*Recommendation: 60% Treatment: 24%
Strategies for Clinical Translation
Continuous Reassessment with Flexible Extension – CRAFT

Study Arm Level
Responsible investigators, statistical planning/assessment, molecular pathology, prospective enrichment strategy, phase 1b/2a switch

Richard Schlenk (Ulm)
Meinhard Kieser (Heidelberg)
INFORM = INDividualized therapy FOR Relapsed Malignancies in childhood

Feasibility-Registry Study (Year 1+2)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>AML</td>
</tr>
<tr>
<td>HGG (incl. DIPG)</td>
<td>Medullo/Ependym.</td>
</tr>
<tr>
<td>Ewing Sarcoma</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>NHL</td>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>Rhabdoid Tumors</td>
</tr>
</tbody>
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Clinical Trial (Year 3-5)

Enrollment

Phase I/II

- Standard of Care: Backbone Chemotherapy
- Targeted Drug 1
- Targeted Drug 2
Somatic ‘drivers’ in medulloblastoma

Northcott et al., Nature Reviews Cancer 2012

n = 189

P. Northcott
D. Jones
N. Jäger
S. Pfister
What else is out in the genome

Enhanced...

Highly Recurrent TERT Promoter Mutations in Familial

MOBILE DNA IN CANCER

Extensive transduction of nonrepetitive DNA mediated by L1 retrotransposition in cancer genomes

Jose M. C. Tubio, Yilong Li, Young Seok Ju, Inigo Martincorenna, Susanna L. Cooke,
High abundance of fusion genes in breast cancer

Edgren et al., Genome Biology 2011

Stephens et al., Nature 2009
A Single Oncogenic Enhancer Rearrangement Causes Concomitant EVI1 and GATA2 Deregulation in Leukemia

Stefan Gröschel\textsuperscript{1,2,9}, Mathijs A. Sanders\textsuperscript{1,9}, Remco Hoogenboezem\textsuperscript{1}, Elzo de Wit\textsuperscript{3}, Britta A.M. Bouwman\textsuperscript{3}, Claudia Erpelink\textsuperscript{1}, Vincent H.J. van der Velden\textsuperscript{4}, Marije Havermans\textsuperscript{1}, Roberto Avellino\textsuperscript{1}, Kirsten van Lom\textsuperscript{1}, Elwin J. Rombouts\textsuperscript{1}, Mark van Duin\textsuperscript{1}, Konstanze Döhner\textsuperscript{2}, H. Berna Beverloo\textsuperscript{5,6}, James E. Bradner\textsuperscript{7,8}, Hartmut Döhner\textsuperscript{2}, Bob Löwenberg\textsuperscript{1}, Peter J.M. Valk\textsuperscript{1}, Eric M.J. Bindels\textsuperscript{1}, Wouter de Laat\textsuperscript{3}, Ruud Delwel\textsuperscript{1}.

**Dual gene deregulation in inv(3) AML**

**Monocyte/macrophage differentiation upon enhancer deletion**

Gröschel et al., Cell, 2014
Examples of other NonCodingMutations:


- Weinhold et al., 2014, Nat. Genetics 46, 1160: Genome-wide analysis of noncoding regulatory mutations in cancer. (PLEKHS1, WDR74) (C. Sander) (Bladder Ca.)

- Denisova et al., 2015, Oncotarget 6, 35922: Frequent DPH3 promoter mutations in skin cancers (R. Kumar) (skin cancer: SCC, BCC)
Assessing heterogeneity/clonality/evolution

- highly variable subclonal diversification
- no strict temporal order
- alterations in driver genes occurring early in some tumors and late in others
- resistance to therapy and invasive potential arose from subclones that were detectable in early lesions

=> Necessity to include subclonal and longitudinal analysis in clinical trials of primary breast cancer

Yates et al. 2015
LIQUID BIOPSIES

Analysis of body fluids: Blood, Plasma, Sputum, Urine, Spinal Fluid

Focus on circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA), for

• Prognosis
• Prediction
• Molecular Heterogeneity
• Identification of drug targets
• Monitoring of therapy response
• Monitoring of resistance mechanisms

“non”-invasive approach to generate genetic tumor profiles

Haber et al. Cancer Discov 2014
Development of experimental systems for functional validation

Immortalized non-transformed cell lines

Introduction/modification of genetic alterations by lentiviral gene or shRNA transfer or CRISPR/Cas9 technology

Isogenic cell lines

Cancer cell lines

Functional annotation of molecular lesions

1. Viability/proliferation
2. Apoptosis/cell cycle
3. Anchorage independence
4. Invasion/migration
5. Pathway activation
6. Others

Tumorigenicity (mouse, CAM)

Unbiased identification of functional dependencies

2. Genome-wide or targeted shRNA screens
3. Genome-wide or targeted CRISPR/Cas9 screens

Stefan Fröhling, Claudia Scholl, Michael Boutros (NCT Heidelberg/DKFZ) and Frank Buchholz (NCT Dresden)
NCT DataThereHouse

Display Data from Various Sources for a Comprehensive Overview of Information Relevant for Personalized Treatment
**Current Scenario**

- **PATIENT**
  - Sprechstunde/Ambulanz
  - Begleitende Dienste
  - Therapie am NCT oder extern

- **CASE MANAGER**
  - KIS, PACS, REGISTER

- **REPORTER**
  - Report

- **BOARD**
  - Board

- **PHYSICIAN SCIENTIST**
  - Physicist
  - Forscher

- **KIS, PUBMED, UPTODE, BIOBANK, INTRANET, REGISTER**

- **EINSCHLUSS & AUSSCHLUSS KRITERIEN**

- **NEUE STUDIE**
  - Studienprotokoll

- **STUDY NURSE**
  - Study Nurse

- **AUFLÄRUN/SCREENING**

- **BEGLEITENDE DIENSTE**

- **THERAPIE**

- **ARZT**
  - Arzt

- **FORSCHER**
  - Forscher

- **DOKUMENTAR**
  - Documentar

- **HYPOTHESE**

- **LOI**

- **KIS, PACS, REGISTER**

- **KONFERENZ**

- **Datenfluß**

- **Datenbank**

- **Patientenakte**

- **Ablauf**

- **Patientenpfad**

- **Arztbrief**

- **Artefakte**

- **Current Scenario**

- **NCT DataThereHouse**
NCT DataThereHouse

NCT Clinical Development Strategy

Organized in SAP HANA

IS-H, PUBMED, UPTODATE, BIOBANK, INTRANET, IS-H, PACS
Translational Oncology…

- needs to RETHINK the old & the new
- needs SMART Data
- is a TEAM Effort
NCT Partnerships

Academic Collaboration

DKTK

EMBL

DKFZ-ZMBH ALLIANCE

Joint Industry-Academia Collaboration

Roche

Bayer

SAP

Cancer Core Europe Consortium Centers

dkfz.

NCT

Gustave Roussy

NKI-AVL

Karolinska Institutet

VHIO
NATIONAL CENTER FOR TUMOR DISEASES

NCT HEIDELBERG

THANK YOU