Speaker’s disclosures

- Dr. Pfister:
  - Nothing to disclose
  - Will not discuss non-approved use of drugs/devices
Pediatric Oncology – a success story
Based on the German Childhood Cancer Registry Mainz, 2011
Patient cohorts – Survival at relapse

ALL-HR  
ALL post-SCT  
AML  

Brain Tumors  
Ewing Sarcoma  
Neuroblastoma  

NHL  
Osteosarcoma  
Rhabdomyosarcoma
Fact 1: Pediatric tumor genomes are overall relatively simple

Driver versus passenger
...sometimes hard to tell!

...but at least much easier in children...

Alexandrov et al. Nature 2013
Fact 2: Pediatric tumor genomes display unique features

Single pathway disease

„Epigenetic“ disease
Agenda

1.) Two examples for single-pathway tumors: Pilocytic astrocytoma and SHH medulloblastoma

2.) „Where have all the drivers gone“: Epigenetic „hijacking“ in medulloblastoma

3.) H3.3mut glioblastoma in children – an epigenetic disease?

4.) INFORM – a nationwide registry trial for the individualized treatment of relapsed malignancies
Pilocytic astrocytoma

More hits in a single pathway disease
Genomic aberrations in pilocytic astrocytoma

Pfister and Remke et al., JCI 2008; Jones et al., Cancer Res. 2008

David Jones
PA: 100% MAPK hits – a single pathway disease

Jones et al. Nature Genetics 2013
SHH Medulloblastoma
Targeting a single pathway (?)
### Molecular classification of MB

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>WNT</th>
<th>SHH</th>
<th>Group 3</th>
<th>Group 4</th>
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<tbody>
<tr>
<td>Gender ratio</td>
<td><img src="image" alt="Gender Ratio WNT" /></td>
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Northcott et al. Nature Reviews Cancer 2012
The SHH pathway is activated only in SHH-MBs
...yet with driver mutations at different levels

<table>
<thead>
<tr>
<th></th>
<th>infants (≤3)</th>
<th>children (4 – 17)</th>
<th>adults (≥18)</th>
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<tbody>
<tr>
<td>SHH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTCH1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>SMO</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>SUFU</td>
<td>1</td>
<td></td>
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<tr>
<td>GLI2</td>
<td></td>
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</tr>
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<td>1</td>
<td></td>
<td></td>
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<td>TP53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic</td>
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<tr>
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<td></td>
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<tr>
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<td>0</td>
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SMO response?

- Infants: no, yes, ?
- Children: no, yes, ?
- Adults: no, yes

Histology

- Infants: MBEN, CMB, DMB
- Children: LCA, DMB
- Adults: CMB, LCA, DMB

Kool et al., Cancer Cell 2014
SMO inhibition in different SHH models

Kool et al., Cancer Cell 2014

Rob Wechsler-Reya

A
3 yr old female, desmoplastic
PTCH1 mutation

B
7 yr old male, anaplastic
TP53 mutation, MYCN amplification

C
0 yr old female, MBEN
SUFU mutation
Group 3 & 4 medulloblastoma

Oncogene activation by chromatin re-shuffling
# Molecular classification of MB

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Northcott et al. Nature Reviews Cancer 2012
Group 3 & 4 medulloblastoma: Where have all the drivers gone?
GFI1B activation in group 3 & 4 medulloblastoma

Northcott et al, Nature 2014
Chromatin re-shuffling leading to GFI oncogene activation in MB

Northcott et al, Nature 2014
GFI/GFI1B cooperate with MYC to induce MB

Northcott et al, Nature 2014
Glioblastoma in children
An Epigenetic Disease?
H3.3 (& HIST3H1B – H3.1) mutations in pediatric & young adult GBMs

Histone modifications
A combination of different molecules can attach to the histone tails and alter the activity of the DNA wrapped around them.

DNA methylation
Methyl marks added to certain DNA bases may repress gene activity.

Histone mut GBM have very distinct epigenomic patterns...

Sturm, Witt and Hovestadt et al., Cancer Cell 2012
Possible Effects of *H3F3A* Mutations on the Glioblastoma Epigenome

adapted from Rheinbay et al. Cancer Cell 2012
K27M functionally inactivates EZH2/PRC2
→ How to re-establish EZH2 function?

long hydrophobic residue (methionin or norleucin) suffice to bind EZH2

Sebastian Bender
New concept: Individualizing therapies

INFORM – INdividualized Therapy FOr Relapsed Malignancies in Childhood

Angelika Eggert  Olaf Witt  Peter Lichter
INFORM: Overall concept

Feasibility-Registry Study (year 1+2)

- ALL-HR
- ALL post-SCT
- AML
- Medullo/Ependyma
- Ewing Sarcoma
- Neuroblastoma
- NHL
- Osteosarcoma
- Rhabdomyosarcoma
- Rhabdoid Tumors
- HGG (incl. DIPG)

Goals feasibility phase

Logistics
- molecular analysis, target identification, turnaround
- individualized risk management
- access mode compounds

Clinical data base
- molecular targets
- documentation of individualized treatments
- clinical courses, tox data

Molecular data
- genomic, epigenomic, transcriptomic profiles

Evaluation of data
- druggable target patterns within/across entities
- set of targeted compounds of interest “tool box” (BfArM)
- define number of eligible patients/year for AMG trial

Clinical Trial (year 3-5)

Phase II

Indication A
Indication B
Indication C

External review: Which of the study groups have fulfilled all predefined criteria to proceed to a clinical trial?
The INFORM Workflow

DKFZ Core Facility:

- RNA Seq
- Exome Seq
- Low-cov. WGS
- 450k methylation

Central Pathology Laboratory:
Quality Control & nucleic acid extraction

Molecular Tumor Board:
Target prediction

Central Pathology Laboratory:
Verification of
INFORM-pilot: current status

- 30 patients recruited for the pilot phase to date (from 10 centers)
- 3 had insufficient tumor material for further analysis (problems of stereotactic biopsy)
- 20 fully sequenced (20 DNA, 17 RNA)
- Where tumor DNA is minimal (<200ng), there is an option to do full 30x WGS plus ‘normal’ pipeline for blood DNA in 4 lanes rather than 2
- Average ~2 weeks from arrival of nucleic acids at core facility to first mutation results (range 7-24 days)
- Includes parallel processing of two cases at once (2-3 per week is achievable)
- >50% with an identified drug target with a priority of moderate or higher
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Summary

• 25% of pediatric oncology patients are not cured, and relapses are associated with dismal outcome => necessity to develop new therapeutic concepts (e.g. targeted therapies)

• It’s technically and economically feasible now to apply NGS in a clinical setting, appears to be possible in a nation-wide effort

• Mutation rate in pediatric tumors is low => closer to driver mutations, advantage for the development of individualized therapy. Still need for new discoveries (e.g., enhancer hijacking)

• Occurrence of single pathway disease => ideal for modeling of molecular interference (e.g., LGG)

• Pathway knowledge allows prediction of response to therapy => in vitro and in vivo models confirm predictions (e.g., SHH-MB)
ICGC PedBrain

ICGC-PedBrainTumor

WP1: Coordination (Lichter)
WP2: Banking (Korshunov, Witt, Pfister)
WP3: Ref. Pathology and QC (Reifenberger)
WP4: Isolation of Analytes (von Kalle)
WP5: Genomic Sequencing (Pfister, Lichter)
WP6: Paired-End Mapping (Korbel)
WP7: Methylome (Radlwimmer, Korn)
WP8: Transcriptome (Yaspo, Lehrach)
WP9: small RNAs (Landgraf, Borkhardt, Reifenberger)
WP10: Bioinformatics (Brors, Eils)
WP11: Data Management (Eils)
The INFORM Consortium

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Berlin / Charité: A. Eggert

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G. Fleischhack

Düsseldorf
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S. Wolf
F. Westermann
R. Eils/M. Schlesner
R. Witt

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J. Boos
H. Jürgens

Berlin
A. von Stackelberg

Hannover
D. Reinhardt

Göttingen
C. Kramm

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M. Frühwald

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M. Nathrath

Stuttgart/Tübingen
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Martin Schuhmann, Martin Ebinger (Tübingen)
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ICGC PedBrain: 

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Deutsche Krebshilfe 

INFORM: 

dkfz. German Cancer Consortium 

50 Years – Research for A Life Without Cancer 

+ hopefully soon: Kinderkrebsstiftung + Krebshilfe 

dkfz.