<table>
<thead>
<tr>
<th>Institution</th>
<th>Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP/UPenn</td>
<td>John Maris, MD    Edward Attiyeh, MD  Michael Hogarty, MD  Yael Mosse, MD  Sharon Diskin, PhD</td>
</tr>
<tr>
<td>COG</td>
<td>Wendy London, PhD (Statistics)  Julie Gastier-Foster, PhD (Pathology)</td>
</tr>
<tr>
<td>CHLA/USC</td>
<td>Robert Seeger, MD  Shahab Asgharzadeh, MD  Richard Sposto, PhD</td>
</tr>
<tr>
<td>NCI</td>
<td>Javed Khan, MD (Oncogenomics)  Daniela Gerhard, PhD (OCG)  Jinhui Zhang, PhD (CCR)  Subha Madhavan, PhD MS (caBIG)  Jim Jacobson, PhD (SPECS)  Malcolm Smith, MD PhD (CTEP)</td>
</tr>
</tbody>
</table>
Neurblastoma-TARGET: Motivation

• Important pediatric problem
  – 15% of childhood cancer mortality
  – 50% of cases metastatic and highly malignant at diagnosis
  – Cure rates stagnant over last two decades
    • Despite dramatic intensification of treatment intensity
    • Survivors with significant morbidity

• Neuroblastoma genomics highly predictive of clinical course
  – Recurrent amplification (MYCN) and deletions (1p36 and 11q23) used by COG to stratify therapy
  – But….no bona-fide and tractable molecular targets known
NBL-TARGET Workflow

Year 1

COG SDC
Sample Selection

Biopathology Center
Sample QC
Nucleic Acids Prep

416 High-risk NBLs
78 Low-risk NBLs

CHOP
DNA Copy Number
Illumina SNP

CHLA
RNA Copy Number
Affymetrix HuEx

NCI
Data Integration
Data Coordination
Data Analysis

Target Discovery
Resequencing 110 genes
188 high-risk NBLs

Biomarker Discovery
Molecular Classification
Outcome associations

Discovery and validation sets from most recent
COG Phase 3 trials

CHOP/CHLA/NCI Labs
Validation and Mechanism

Clinical Application
Using genomics to predict patient outcome

A model

1. Specific Trisomies
2. Numerical Aberrations
3. Favorable Signature
4. Unfavorable Signature

Risk for death

DNA copy number

Segmental Aberrations

RNA copy number
Using genomics to predict patient outcome

A model with supporting data

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<td>1, 2, and 5</td>
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Risk for death

Specific Trisomies

1

DNA copy number

RNA copy number

Stage 1 or 2; cured

Stage 4, MYCN normal

Stage 4, MYCN amplified

Unfavorable Signature

Favorable Signature

Risk for death
Discovering mutated targets
Gene resequencing selection criteria

- Genes within regions of copy number aberration
  - Homozygous deletion
  - Absolute loss and/or LOH
  - Relative gain (above the cell’s DNA index)
  - Amplification
- Genes with differential gene expression
- Genes supported by the literature
- Genes with known mutations in other cancers (COSMIC database)
- Most candidates supported by two or more criteria
**NBL-TARGET Resequeencing Summary**

- 188 samples
- 117 genes and microRNA sequenced
  - 1,066 exons
  - 1,591 amplicons
  - 1.11 Mb (0.037% of genome)
  - 679,862 traces generated so far

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<th>nonsense</th>
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<td>3.72%</td>
</tr>
</tbody>
</table>

*Several genes (eg ALK) still with poor coverage*
**ALK is an oncogenic kinase in neuroblastoma**

- Co-discovery of ALK as the familial neuroblastoma gene (Mosse, Nature 2008) and frequent somatic amplification and mutation (TARGET)
  - Amplification: 31/599 (5.2%)
  - Focal gain: 102/599 (17.0%)
  - Mutations in kinase domain: 43/552 (7.2%)
  - Mutations in extracellular domain: Present, frequency still be defined
Germline and Somatic ALK Kinase Region Mutations Fall in Regions Shown to be Major Targets of Cancer Mutations
**ALK is a tractable target for pharmacologic inhibition**

*(but sensitivity depends on mutation type)*

**PF'066 Dose Response Curves**

- NB1 (WT amplified)
- NB1643 (R1275Q)
- NBSD (F1174L)

**% Growth inhibition with PF066 at 333 nM**

- WT
- WT1275Q

**% growth inhibited versus control**

- WT
- F1174L
- R1275Q

**NB1643 (R1275Q)**

- NB1 (WT amplified)
- WT
- NBSD (F1174L)
Moving ALK inhibitors to the clinic
(Which drug, which mutations?)

Hepatotoxic in dogs

Kelly (F1174L): ALKi IC\textsubscript{50}

Adult Phase 1

- GSK085
- GSK765
- PF066
- GSK677
- Cep B
- Cep A
- GSK636

Vol (cm\textsuperscript{3})

- NB1643
  - R1275Q
    - (Most common mutation)

- NBSD
  - F1174L
    - (2nd most common mutation)

- NB-EBc1
  - WT
    - (but weak pALK)

- SKNAS
  - WT
    - (no pALK)
Getting around resistance mutations
ALK homology model with PF-02341066 bound

- Mutation NOT predicted to destabilize activation loop: No effect on PF-066 binding
- Highly conserved F; mutation predicted to destabilize hydrophobic pocket for PF-066 binding
Discovery, Validation and Implementation

**Therapeutic Validation**
- LMO1
- NOTCH1
- CASZ1
- KIF family
- Trk family
- others
- AURKA
  - AACR 2008

**Discovery**
- Germline
  - NBL-GWAS
    - R01-124709
- NBL-Hereditary
  - R01-078454, R01-140198
- Somatic
  - NBL-TARGET
    - U10-098543
    - R01-CA60104
    - R01-CA87847
    - NIH Intramural Program
- NBL-PPTP
  - N01-CM42216

**Biomarker/Diagnostic Validation**
- FLJ22536
  - NEJM 2008
- BARD1
  - Nat Gen 2009
- ALK
  - Nature 2008

**Clinical Evaluation**
- ALK Genetic Screening
  - CHOP
- ADVL0812 Phase 1/2
- ADVL0912 Phase 1/2
- DNA signature
  - ASCO 2007
- RNA signature
  - JNCI 2006; Genomics 2008
- COG and SIOPEN
  - eg COG ANBL0531

- ALK Genetic Screening
  - CHOP
- ADVL0812 Phase 1/2
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- DNA signature
  - ASCO 2007
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  - JNCI 2006; Genomics 2008
- COG and SIOPEN
  - eg COG ANBL0531
NBL-TARGET

Future goals

• Functional validation and translation of current leads ongoing
  – Focus on ALK and NOTCH1

• Consider year 1 results “proof-of-concept” with < 0.04% of genome sequenced

• Uniquely poised for a full genome sequencing effort
  – Due to size of regional aberrations, this should be done with a comprehensive epigenome profiling
    • Pilot studies on Illumina Infinium platform complete

• NBL-TARGET team has demonstrated ability to quickly validate and translate discoveries
  – Rapidly improving neuroblastoma patient care and outcome is a realistic and achievable goal of the NBL-TARGET project