Understanding Precision Medicine and Gene Sequencing for Neuroblastoma

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Meredith Irwin, MD
SickKids Sequencing Program

Things You Should Know

Precision Therapy
What is precision medicine?

• “personalized” vs. “individualized” vs. precision

• is an approach for disease treatment (and prevention) that takes into account individual variability in genes, environment, and lifestyle for each person.

• use of genes/ molecular characterization (+/- in conjunction with classic tumor clinical and biological features) to inform treatment
Early precision medicine: Low, intermediate and high risk neuroblastoma

Precision Cancer Therapy

1. Molecular Profiling
2. Prognostic Markers
   - Markers predictive of drug sensitivity/resistance
   - Markers predictive of adverse events
What causes cancer?

• What causes cancer?
  – Changes in genes that give cells the ability to grow fast, continuously divide, spread and form tumors

• GENETICS 101
Cancer Arises From DNA Mutations In Cells

Normal cell → DNA mutations → Uncontrolled proliferation

*Last DNA mutation from:*
- heredity
  - or
- radiation or chemicals
  - or
- spontaneous errors during DNA duplication
Mutations /alterations in genes cause cancer

(1) Mutation
(2) Extra copies
- oncogenes
(3) Missing copies
- suppressor genes
Flow of genetic information:

DNA → RNA → Protein

ATGGAGCCAACTATTGATGAA

AUGGAGCCAACUAUUUGAUGAA

Met - Glu - Pro - Thr - Ile - Asp - Glu
Flow of genetic information

DNA → RNA → Protein

Genes

ATGGAGCCAACTATTGATGAA

AUGGAGCCAACUAUUGAUUGAA

mRNA

Met - Glu - Pro - Thr - Ile - Asp - Glu

protein

X
Flow of genetic information

DNA → RNA → Protein

Genes

ATGGAGCCAACACTATTGATGAA

ATGGAGCCAACACTATTGATGAA

mRNA

AUGGAGCCAACAUUUGAUGAA

DNA

Genes

mRNA

DNA

Protein

MUTANT

WILD-TYPE

Met - Glu - Pro - Thr - Ile - Asp - Glu

Protein

Protein
Flow of genetic information

DNA → RNA → Protein

ATGGAGCCAACACTATTGATGAA

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MUTANT

WILD-TYPE

Met - Glu - Pro - Thr - Ile - Asp - Glu

Precision Therapy

protein
Roles for gene alterations in pediatric cancer?

– Unlike adult cancers it is rare to get pediatric cancer from an exposure to something toxic (cigarette smoke, sun)

– Although it can be inherited from parents most of the time it is not

– Changes in cancer cell genes lead to properties such as growth/survival, proliferation, invasion/metastasis......
Gene mutations can be inherited or new—what’s the difference?

Gametic mutations are inherited and occur in the testes of males and the ovaries of females.

Somatic mutations occur in body cells. They are not inherited but may affect the person during their lifetime.
How to sequence genes

• Sequencing = code of genes ACTG…..

• Previously- check one gene after another for alterations/ mutations

• Now can sequence all genes (DNA and RNA)
Breakthrough: next generation sequencing

- Can sequence all of the genes in a cancer cell rather than one at a time
The human genome stats

• 23 chromosome pairs
  – 1x/y, other 22
• Entire genome = over 3 billion basepairs
  – ACTG…..
• Approx 20,000 genes that code for proteins made in cells
  – Alot more important info in the other millions of basepairs
Genome wide sequencing is becoming less expensive than existing genetic tests

- ‘$ Cost’ of exome sequencing 1,000-2,000

Existing Genetic Dx Tests

<table>
<thead>
<tr>
<th>Year</th>
<th>Cost per Human Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>$30,000</td>
</tr>
<tr>
<td>2012</td>
<td>$25,000</td>
</tr>
<tr>
<td>2013</td>
<td>$20,000</td>
</tr>
</tbody>
</table>

- Sequencing ALK ~ $1500
Mutations in Neuroblastoma

- Mutation - changes in one or more DNA bases (eg A -> C) resulting in different amino acid that changes the protein
- Very heterogeneous
- In comparison to some cancers where there is a common mutation in most patients in NB variety of different gene mutations
  - ALK - most common mutation (10%)
  - Others - ATRX, Ras, NF1, PTPN11, .....

Targeted therapies based on genetics

– Chemotherapies attack cancer and normal cells causing increased side effects

– “target” the different genes/proteins on the cancer cell that help it to grow and survive
SickKids Sequencing Program

Precision Therapy
KICS program

• 2016: sequenced – almost 200 completed
• Some led to identification of targeted therapies and other guidance for treatment decisions
  – ALK inhibitor drugs, others
• Discovery of new genes involved in NB
• Expanding to all Canadian sites (PROFYLE)
KiCS Study Objectives

• Establish the utility of sequencing for patient care (relapsed and refractory)
  – Patient Diagnostics and Monitoring Disease Response
  – Prognosis
  – Guiding therapeutic decisions:
    • suggest new targets for therapeutic intervention based on tumor-specific genetic alterations

• Establish the value of sequencing in identifying inherited genes
KiCS Consent - Overview

• Referral to KiCS → eligible → consent

• Consent
  – Entry Point 1 (tumour and germline/blood)
    • Two part consent available
      – Part A – samples
      – Part B – sequencing
  – Entry Point 2 (germline only)
  – Parent consent forms and assent

• Average consent: ~ 90 minutes
Why we do repeat biopsies at relapse?

- Evidence that there are more (and different) mutations at relapse (clonal evolution)
  - Most common targetable mutation in NB (ALK) increased at time of relapse vs. diagnosis

*Ramaswamy and Taylor, Nature Genetics 2015*
NGS Platforms and Analyses

Tumour DNA + RNA

Focused, deep sequencing

HiSeq 2500
NextSeq 500

800+ genes

Whole genome

HiSeq XTen

Broad, shallow sequencing

Non-tumour DNA

Library prep: 2 days
Sequencing: 1 day

Library prep: 2 days
Sequencing: 3 days

+ 6 to 30 hrs
SickKids Childhood Cancer Panel

18,950 exon annotations
Covering 3.9 million bases

Focused deep sequencing of 886 genes
SickKids Childhood Cancer Panel

18,950 exon annotations
Covering 3.9 million bases

MOLECULAR TUMOR BOARD
The KiCS Molecular Tumor Board

• Multi-disciplinary and weekly

• At Meeting:
  – data presented, interpreted and discussed:
    • “actionability” determined
  – link to potential agents or trials made
  – challenges or next steps reviewed
  – direct communication with primary oncologist → family

• Attendance:
  – Core KiCS team (oncologists, geneticists, pathologists, genome scientists, bioinformaticians, genetic counsellors, research associates, MRP, etc)
Driver vs. passenger mutations

- Many gene variants that can be considered
- Team including bioinformaticians need to identify possible “driver mutations” since many are just passengers

- DRIVER: mutation that gives the cancer cell a selective mutation (to grow faster, spread etc)= ENGINE

- PASSENGER: many other mutations or variants in cancer cells that are along for ride but have no functional effect
Research Reports from Cancer Panel

Ledia Brunga

121 patients enrolled
Incorporating genetics into treatment and care of patients

- Instead of pathology (neuroblastoma, sarcoma etc) treatment is based on a gene abnormality in tumor that can be targeted
- Phase I and II trials for one drug or combination that require a gene mutation/alteration to enroll
- Trials with many regimens that use biopsy results to assign patients to different arms of a the trial
Cancer Panel Research Report

KICS ID: XXXX
Primary Oncologist: X
Sex: X
Sample Type: Date: July 20, 2018
Prepared by: RESEARCH USE ONLY: The results contained in this report were generated in a lab that is not an accredited or licensed clinical laboratory. The results are intended for research purposes only. The underlying tests were not performed for the purposes of diagnosis, prophylaxis, or treatment.

Tumour type: Neuroblastoma
Reason for Submission: Relapse
Notes: first relapse, bone sample

Genome Diagnostics ID: Pathology ID:
Normal: N/A
Tumour: XXXXXX XXXX

Overall Summary:
Oncogenic Mutation in ALK

High quality on target somatic substitutions
Total: 2. Coding (2), splicing [0], cancer gene [1]
Mutation Burden: 0.62 Mutations/Mb

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Type</th>
<th>AA</th>
<th>Chr</th>
<th>Pos</th>
<th>Ref</th>
<th>Alt</th>
<th>Cancer Gene</th>
<th>VAF</th>
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<tbody>
<tr>
<td>ALK</td>
<td>exonic</td>
<td>nonsynonymous SNV</td>
<td>p.R1275Q</td>
<td>2</td>
<td>29432664</td>
<td>C</td>
<td>T</td>
<td>TRUE</td>
<td>0.38</td>
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<td>NBL</td>
<td>exonic</td>
<td>nonsynonymous SNV</td>
<td>p.F1240L</td>
<td>8</td>
<td>48805816</td>
<td>A</td>
<td>G</td>
<td>FALSE</td>
<td>0.16</td>
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</table>

ALK – This gene encodes a receptor tyrosine kinase, which belongs to the insulin receptor superfamily. This protein comprises an extracellular domain, a hydrophobic stretch corresponding to a single pass transmembrane region, and an intracellular kinase domain. It plays an important role in the development of the brain and exerts its effects on specific neurons in the nervous system. This gene has been found to be rearranged, mutated, or amplified in a series of tumours including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer. The chromosomal rearrangements are the most common genetic alterations in this gene, which result in creation of multiple fusion genes in tumorigenesis, including ALK (chromosome 2)/EML4 (chromosome 2), ALK/RANBP2 (chromosome 2), ALK/ATIC (chromosome 2), ALK/TFG (chromosome 3), ALK/NPM1 (chromosome 5), ALK/SQSTM1 (chromosome 5), ALK/KIF5B (chromosome 10), ALK/CLTC (chromosome 17), ALK/TPM4 (chromosome 19), and ALK/MSN (chromosome X) (https://www.ncbi.nlm.nih.gov/gene/238). ALK is altered in 8.9% of neuroblastoma cases (1). Two of the four entries in ClinVar for this somatic mutation were found in neuroblastoma. Research treating cells with Crizotinib have shown efficacy against cells presenting the p.R1275Q mutation (2, 3).
Targeting ALK mutation with ALK inhibitor

1275 mutation

6 months later

ALK inhibitor

MUTANT WILD-TYPE protein
CLINICAL TRIALS
UMBRELLA and BASKET TRIALS

- One (or few tumor types)
- the mutation identified determines which treatment
- common in breast, lung

UMBRELLA and BASKET TRIALS

-One (or few tumor types)
-Common in breast, lung
-Multiple cancers eligible (histology agnostic)
-Mutation identified allows eligibility
-CAPTUR, MATCH, many

NCI Pediatric MATCH
Molecular Analysis for Therapy Choice

• Basket trial for relapsed solid tumors and lymphomas in US
  – 200-300/year; estimated 5-10% Match

• Biopsy and small % will have gene abnormality and can enroll on 1 of 8 trials of single agent that targets mutation
NCI Pediatric MATCH schema

MASTER PROTOCOL

Children with relapsed/refractory solid tumors and lymphomas

- Tumor biopsy
- Genetic sequencing
- Actionable mutation detected
- Matching study agent selected

Available MATCH study agents

- SD, CR or PR
- Continue until progression
- PD
- Another actionable mutation detected?

PD

Off study
to transform the care of CAYA patients across Canada by using next-generation molecular tools and cancer model systems to identify disease- and patient-specific biomarkers that are tractable targets for therapy to improve outcomes.
The PROFYLE Precision Medicine Platform
Building on the success of 3 regional programs

<table>
<thead>
<tr>
<th>Program</th>
<th>Dec 2015 Enrollment</th>
<th>Sept 2017 Enrollment</th>
</tr>
</thead>
<tbody>
<tr>
<td>POG (Vancouver)</td>
<td>32 (incl. 21 young adults)</td>
<td>98 (incl. 29 young adults)</td>
</tr>
<tr>
<td>TRICEPS (Montreal)</td>
<td>21 (up to 21 yrs.)</td>
<td>81 (up to 21 yrs.)</td>
</tr>
<tr>
<td>KiCS (Toronto)</td>
<td>33</td>
<td>158 (incl. 10 young adults)</td>
</tr>
</tbody>
</table>

157 new cases in 13 months
~ 12 per month

Adam Shlien
Goals:

• Enroll and molecularly profile 450 patients over 5 years

• Analyze the tumour genome, transcriptome, and proteome to look for treatment targets

• Develop strategies to access therapies for CAYAs with ‘hard to treat’ cancers
Children, Adolescents, and Young Adults (CAYA) with Hard-to-Treat Cancers

Models of CAYA Cancers

• Discover New Targets
• Develop Therapies
• Understand Biology

Molecular Profiling & Precision Medicine Clinical Trials

Efficacy of Precision Medicine

Biospecimen

Canadian CAYA Cancer Biobank & Data Repository

• Biomarkers of Response
• New Profiling Tools
• Fuel Future Discoveries

IMPROVE OUTCOMES FOR HARD-TO-TREAT CAYA CANCERS
The PROFYLE Precision Medicine Platform

To establish a national multi-institutional cooperative network for genomics, with the goal to ensure that ALL eligible patients in the country will have access to this resource.
PROFYLE CLINICAL NODE

Quality Assurance and Ethics Oversight

Identify, screen, consent /assent

Tissue collection, pathology review, Biobanking

Molecular Tumour Profiling, Data Analysis

Molecular Tumour Board

Actionable Genomic Finding(s)

No actionable Genomic Finding

CAPTUR clinical trial

≥18y

CAPTUR adult cohort

CAPTUR drugs

<18y

CAPTUR pediatric cohort

Pediatric phase I/II trials

Compassionate / SAP access

Health Canada n of 1 trial concept

PR, CR, SD → Continue therapy

ALK inhibitor
PDGFR inhibitor
mTOR inhibitor
BRAF inhibitor
MEK inhibitor
Checkpoint inhibitor
Sequencing also identifies germline mutations (inherited)

• May have some implications for treatment
• May have implications for other family members
  – Counselling
  – Screening/ surveillance
• New scientific discoveries
How to use genomics/sequencing to guide treatment

“OMICS” to find target
- genomics (DNA, RNA)
- proteomics
- other

1. Other trials
2. Special Access
3. N of 1
4. Inform therapy

Challenges to Precision Medicine in Children, AYA

- Pediatric cancers are rare (relatively)
  - Each subset – by histology and/or by genetic alteration is even rarer
  - Clinical trials challenges of small N, endpoints, controls
- Drug access for children <18 yo (<12)
  - Regulatory
    - US, EU some legislation to help facilitate
  - Pharma
  - Formulation
- Ethics
- (Gen)omics
  - Different sets of alterations that may not be easy to interrogate since most data is adult
  - Need research to understand new variants
The Future

• More basket trials and phase I/II trials in which eligibility is the genetic or other molecular alteration (histology agnostic)

• Use sequencing more at diagnosis, before relapse

• Cell Free DNA
  – DNA that tumors shed in the peripheral blood that can be isolated from a few ml of blood
    • Use to detect mutation and to quantify before/after treatment

• Ability to make models using patient samples and test drugs in real time .....
THANKS!

NEUROBLASTOMA RESEARCH PROGRAMS