PROFYLEing Cancer for KiCS:
The Birth of Precision Pediatric Oncology

David Malkin, MD
Co-Director, SickKids Cancer Sequencing (KiCS) Program
Program Director, Terry Fox PROFYLE Initiative

Division of Hematology/Oncology
Genetics and Genome Biology Program
The Hospital for Sick Children
Department of Pediatrics
University of Toronto
The Problem: Survival at Relapse

10 years post relapse diagnosis

All-HR

All post-SCT

AML

NHL

Brain Tumors

Ewing Sarcoma

Osteosarcoma

Neuroblastoma

Stefan Pfister, DFKZ, Heidelberg
Pediatric “Precision Oncology”

- BASIC3 (TCH) (Parsons, et al *JAMA Onc* 2016)
  - 100/121 new cancer diagnoses consented to WES
  - 28% > 1 somatic Tier 1/2 mutation (proven/potential clinical relevance)
  - 10% diagnostic pathogenic germline mutations

- iCAT (DFCI) (Harris, et al *JAMA Onc* 2016)
  - 30% actionable alterations leading to individualized therapy (relapse/refractory disease)

- U Michigan (Mody, et al *JAMA Onc* 2016)
  - 45% actionable findings led to change in cancer management
  - incl. 10% germline mutation conferring cancer risk
PROFYLE’s Overall Objective

(PRecision Oncology For Young People)

aims 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.
PROFYLE Program Executive Committee

Chair:
Dr. David Malkin, Hospital for Sick Children

Members:
Dr. Jason Berman, Dalhousie University
Dr. Henrique Bittencourt, CHU Sainte-Justine
Dr. Jennifer Chan, University of Calgary
Dr. Rebecca Deyell, BC Children’s Hospital
Dr. David Eisenstat, University of Alberta
Dr. Conrad Fernandez, IWK Health Centre
Dr. Meredith Irwin, Hospital for Sick Children
Dr. Nada Jabado, McGill University
Dr. Rod Rassekh, BC Children’s Hospital
Dr. Adam Shlien, Hospital for Sick Children
Dr. Daniel Sinnett, CHU Sainte-Justine
Dr. Poul Sorensen, BC Cancer Agency
Mr. Patrick Sullivan, Team Finn Foundation
Dr. Michael Taylor, Hospital for Sick Children
The PROFYLE Precision Medicine Platform

POG

Vancouver

MUGQIC and TRICEPS

KiCS

Montreal

KiCS
PROFYLE’s $25M Total Budget

Year 1
- Sequencing
- Proteomics
- Ethics
- Data analysis core
- Biobanking
- Biomarker
- Model systems
- Program management

Year 2
- Sequencing
- Proteomics
- Ethics
- Data analysis core
- Biobanking
- Biomarker
- Model systems
- Program management

Year 3
- Sequencing
- Proteomics
- Ethics
- Data analysis core
- Biobanking
- Biomarker
- Model systems
- Program management

Year 4
- Sequencing
- Proteomics
- Ethics
- Data analysis core
- Biobanking
- Biomarker
- Model systems
- Program management

Year 5
- Sequencing
- Proteomics
- Ethics
- Data analysis core
- Biobanking
- Biomarker
- Model systems
- Program management
Children, Adolescents, and Young Adults (CAYA) with Hard-to-Treat Cancers

Models of CAYA Cancers

- Discover New Targets
- Develop Therapies
- Understand Biology

Biospecimen

Molecular Profiling & Precision Medicine Clinical Trials

Efficacy of Precision Medicine

IMPROVE OUTCOMES FOR HARD-TO-TREAT CAYA CANCERS

Canadian CAYA Cancer Biobank & Data Repository

- Biomarkers of Response
- New Profiling Tools
- Fuel Future Discoveries
Patient Enrollment Process

**Criteria for Enrollment**
Relapse/Refractory/Metastatic or Very Poor Prognosis at Diagnosis
Patient Enrollment Process (cont.)

**Program office**
- Assign unique PROFYLE case number
- Track pertinent case information

**Site lead**
- Coordinate consent procedure

**Profiling Site**
- Arrange sample shipment
- Generate molecular tumor profiles and prepare report for molecular tumor board
KiCS (SickKids Cancer Sequencing)

Study Objectives

• Establish the utility of NGS-based analyses, compared to conventional molecular diagnostics, for the routine management of oncology patients.
  – Patient Diagnostics and Monitoring:
    • Enable comprehensive diagnoses using somatic mutations
    • Identify genetically at-risk patients
    • Assess response to treatment
    • Enable disease monitoring
  – Guiding therapeutic decisions:
    • suggest new targets for therapeutic intervention based on tumor-specific genetic alterations.

• Establish the value of NGS in characterizing and managing metastatic and relapsed disease.

• Establish the value of NGS in identifying germline genetic alterations in individuals highly suspicious for having a cancer susceptibility syndrome.
KiCS Investigators

PRINCIPAL INVESTIGATORS:
• Dr. D. Malkin, Division of Haematology/Oncology
• Dr. A. Shlien, Division of Genome Diagnostics, Department of Paediatric Laboratory Medicine

CO-INVESTIGATORS:
• Dr. M. Abdelhaleem, Department of Paediatric Laboratory Medicine
• Ms. H. Druker, Division of Metabolics and Clinical Genetics
• Ms. B. Gallinger, Division of Metabolics and Clinical Genetics
• Dr. R. Hegele, Department of Paediatric Laboratory Medicine
• Dr. J. Hitzler, Division of Haematology/Oncology
• Dr. A. Huang, Division of Haematology/Oncology
• Dr. M. Irwin, Division of Haematology/Oncology
• Ms. Brittney Johnstone, Division of Metabolics and Clinical Genetics
• Dr. S. Meyn, Division of Clinical and Metabolic Genetics
• Dr. V. Ramaswamy, Division of Hematology/Oncology
• Dr. S. Scherer, The Centre for Applied Genomics
• Dr. M. Shago, Department of Paediatric Laboratory Medicine
• Dr. G. Somers, Department of Paediatric Laboratory Medicine
• Dr. J. Stavropoulos, Department of Paediatric Laboratory Medicine
• Dr. U. Tabori, Division of Haematology/Oncology
• Dr. M. Taylor, Division of Neurosurgery
• Dr. A. Villani, Division of Haematology/Oncology
• Dr. J. Wasserman, Division of Endocrinology
• Dr. J. Whitlock, Division of Hematology/Oncology
Patient Stratification

**Entry Point 1**

Any New or Relapsed Cancer Diagnosis

Identified by treating oncologist or surgeon

Clinical NGS-based test on *tumor and germline DNA*

- Patients with localized disease
  - Targeted genome and transcriptome sequencing
- Patients with metastatic or relapsed disease
  - Comprehensive whole genome and transcriptome sequencing

**Entry Point 2**

Suspected Cancer Susceptibility Syndrome (without cancer, or tumor tissue not available, or adult relative with cancer)

Identified by or referred to Cancer Genetics

Clinical NGS-based test on *germline DNA*

Whole Exome Sequencing
KiCS Program Workflow

Patient identification → Consent Part A → Consent Part B | Biologic sample collection → Quality Control → DNA/RNA Extraction → Library preparation → Tumor genome analysis and germline analysis for cancer susceptibility genes

Project Coordinator | Genetic Counselor

Initial Disclosure

Patient enrolment

Report generation

Results disclosure to primary team and patient/family

Sample processing → Informatics + Analysis

OR/IGT/Satellite Anesthesia

Phlebotomy

Dept of Pathology

Molecular Genetics

Sample & Analysis

Time

Patient

Project Coordinator

Genetic Counselor
# Preliminary Consent Data

## KiCS Enrollment

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<tbody>
<tr>
<td>Patients enrolled</td>
<td>~70 patients</td>
</tr>
<tr>
<td>Declined Participation</td>
<td>&lt;2% declined</td>
</tr>
<tr>
<td>Parent consent obtained</td>
<td>~40 parent sets</td>
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## Sample Type Decisions

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Opted In</th>
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<tbody>
<tr>
<td>Skin biopsy</td>
<td>~71%</td>
</tr>
<tr>
<td>Bone Marrow Aspirate</td>
<td>~84%</td>
</tr>
<tr>
<td>CSF</td>
<td>~84%</td>
</tr>
<tr>
<td>Adjacent Normal Tissue</td>
<td>~82%</td>
</tr>
<tr>
<td>Lymphoblastoid Cell Lines</td>
<td>~97%</td>
</tr>
<tr>
<td>On-going blood samples</td>
<td>~97%</td>
</tr>
</tbody>
</table>
# Preliminary Consent Data

## Re-Contact Decisions

<table>
<thead>
<tr>
<th>Reason for re-contact</th>
<th>Opted In</th>
<th><strong>Scollon et al. Genome Medicine 2014</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Actionable genetic variant on re-analysis</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Requesting additional samples</td>
<td>~97%</td>
<td>100%</td>
</tr>
<tr>
<td>Requesting additional clinical details</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Follow-up interview re: KiCS experience</td>
<td>~97%</td>
<td>-</td>
</tr>
</tbody>
</table>

## Future Research Decisions

<table>
<thead>
<tr>
<th>Sharing samples/data with:</th>
<th>Opted In</th>
<th><strong>Scollon et al. Genome Medicine 2014</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Future research studies</td>
<td>~81%</td>
<td>90%</td>
</tr>
<tr>
<td>Scientific databases</td>
<td>~81%</td>
<td>87%</td>
</tr>
</tbody>
</table>
NGS Platforms and Analyses

Tumour DNA + RNA

Non tumour DNA

800 genes
Focused, deep sequencing

Whole genome
Broad, shallow sequencing

HiSeq 2500
NextSeq 500

Library prep: 2 days
Sequencing: 1 day

HiSeq XTen

Library prep: 2 days
Sequencing: 3 day

+ 6 to 30 hrs

Sample ID: 262196 Post Processing ID: 1477

Sample Variants

Gene Name | Protein Change | Effect | ClinVarSig | HGMD Disease | Alt Allele Coverage | Ref Allele Coverage | ACMG Gene
---|---|---|---|---|---|---|---
TP53 | p.Arg246Cys | missense_variant | pathogenic | Sarcoma | 217 | 0 | yes
The KiCS Molecular Tumor Board

- Multi-disciplinary
  - Neuro-onc/solid tumor/leuk-lymphoma; NAIT; bioinformatics; genomics; pathology; hematopathology; cytogenetics; genetic counseling; program coordinator; treating oncologist(s)
  - Weekly

- Detailed report pre-circulated
Research Reports from Cancer Panel

Tumour Types Run on Cancer Panel

70 patients enrolled (as of Oct 12, 2016)

Ledia Brunga
Primary Tumor Site: Research Reports from Cancer Panel

- Brain: 36.1%
- Colorectal: 16.4%
- Other: 13.1%
- Blood: 8.2%
- Breast: 7.1%
- Ovary: 5.8%
- Nervous System: 5.4%
- Mesenchymal: 5.3%
- Bone: 5.2%
- Bladder: 4.7%
- Kidney: 4.3%
- Liver: 2.9%
- Head and Neck: 2.6%
- Lung: 1.8%
Examples of Actionable Findings

**Mutations found in established drug targets or that helped diagnosis**

- FGFR4 missense
- TFG-MET fusion
- ALK missense
- BRAF missense
- BRAF fusion
- PDGFRA missense
- EWS-ETV1 fusion
- C110rf95-NCOA2 fusion
- DICER1 missense (germline)
- hyper-mutation (multiple patients)
- SMARCA4 stop gain
- NRAS missense

**Mutation profile that helped explain clinical history**

- Clonal relationship between late relapse and primary

**Mutation profile that suggested more aggressive treatment**

- Profile inconsistent with supratentorial ependymoma

**Potentially actionable mutations**

- MET non-canonical missense
- RAD21 truncating
12 mo with multi-focal/metastatic NB
- Initial biology all favorable
- Progressed - 3 chemotherapy regimens
- IGT biopsies of two masses
  - Adrenal, skull nodule
  - Path/biology and KiCS

**Biopsy Results:**
1. Adrenal:
   - panel: \( ALK \ F1245V \)
   - hotspot TK activating
2. skull:
   - panel: \( BRAF \ V600E \)
Matched to ALK inhibitor

- Validate variant
  - DPLM assay for ALK: Sanger sequencing
- “match” target to available drug
  - Open clinical trial for agent or basket/umbrella
  - Special access
  - HC/FDA approved drug (rare)
  - Inform therapy decision (sensitivity/resistance)

- LDK378 (ceritinib)- Phase I
  - Eligibility: ALK fusion, ALK amplification (>9 copies), ALK missense mutation (TK domain)
12 mo with multi-focal/metastatic NB
- Initial biology all favorable
- Progressed; 3 chemotherapy regimens
- IGT biopsies of two masses
  - Adrenal, skull nodule
  - Path/biology and KICS

**Biopsy Results:**
1. Adrenal:
   - panel: *ALK F1245V*
   - hotspot TK activating
2. Skull:
   - panel: *BRAF V600E*
12 mo with multi-focal/metastatic NB

- Initial biology all favorable
- **Progressed**; 3 chemotherapy regimens
- IGT biopsies of two masses
  - Adrenal, skull nodule
  - Path/biology and KICS

*Biopsy Results:*
1. Adrenal:
   - panel: *ALK* F1245V
   - hotspot TK activating
2. skull:
   - panel: *BRAF* V600E

*Outcome*
- Outpatient oral med (liquid)
- 2 hospitalizations
- Improved QoL, development
- Partial response (target lesion)
- Decreased urine catechols

6 months later
Metastatic Rhabdomyosarcoma and NF1

TP53 - This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome (7157). As with other splicing variants, it is difficult to ascertain the repercussion of this splice donor mutation, although it is predicted to be harmful by variant allele predictor.

LRRK2 - This gene is a member of the leucine-rich repeat kinase family and encodes a protein with an ankyrin repeat region, a leucine-rich repeat (LRR) domain, a kinase domain, a DFG-like motif, a RAS domain, a GTPase domain, a MLK-like domain, and a WD40 domain. The protein is present largely in the cytoplasm but also associates with the mitochondrial outer membrane. Mutations in this gene have been associated with Parkinson disease-8 (120892). This variant does not occur within a protein subdomain and is not listed within COSMIC as a known somatic variant.

FGFR4 - The protein encoded by this gene is a member of the fibroblast growth factor receptor family, where amino acid sequence is highly conserved between members and throughout evolution. FGFR family members differ from one another in their ligand affinities and tissue distribution. A full-length representative protein would consist of an extracellular region, composed of three immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain. The extracellular portion of the protein interacts with fibroblast growth factors, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation. The genomic organization of this gene, compared to members 1-3, encompasses 18 exons rather than 19 or 20. This particular family member preferentially binds acidic fibroblast growth factor and, although its specific function is unknown, it is overexpressed in gynecological tumor samples, suggesting a role in breast and ovarian tumorigenesis (2264). p.V550E is a gain of function variant occurring within the protein tyrosine kinase subdomain of FGFR4 (25219510). This is a known alteration within rhabdomyosarcoma (19809159, 24436047). Expression analysis from RNA-seq of the tumour places expression of FGFR4 in the top 0.5% of genes. The following drugs are listed as inhibitors of FGFR4 in the DGlub: ponatinib (23468082), lenvatinib (17943726), dovatinib (25219510), ENMD-2076, and NINTEDANIB. Clinicaltrials.gov currently lists 5 clinical trials with specific requirement for genetic alterations of FGFR genes: (FGF401: NCT02325739), (Ponatinib: NCT02272998), (Blu-554: NCT02508467), (ARQ 087: NCT01752920), and (U3-1784: NCT02690350), the trial on Blu-554 is specifically focussed on hepatocellular carcinomas.
Karyotype:
46,XY,t(3;7)(q13~21;q31~32)[1]/53,sl,+2,+11,+16,+16,+18,+20,+21[7]/
46,XY[12]

Mary Shago
Overall Summary: TFG-MET in-frame fusion found between chr3 and chr 7.

A. Fusion transcripts

Table 4: Fusion transcripts discovered by focussed analysis of Fusion validator output

<table>
<thead>
<tr>
<th>Read Support</th>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Break Point 1</th>
<th>Break Point 2</th>
</tr>
</thead>
</table>
| 29 Primary Alignments | TFG    | MET    | Chr3:100455548      | Chr7:116414935      

**TFG** - There are several documented fusion oncoproteins encoded partially by this gene. This gene also participates in several oncogenic rearrangements resulting in anaplastic lymphoma and mixed chondrosarcoma, and may play a role in the NF-kappaB pathway (10342).

**MET** - This gene encodes a member of the receptor tyrosine kinase family of proteins and the product of the proto-oncogene MET. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that are linked via disulfide bonds to form the mature receptor. Further processing of the beta subunit results in the formation of the M10 peptide, which has been shown to reduce lung fibrosis. Binding of its ligand, hepatocyte growth factor, induces dimerization and activation of the receptor, which plays a role in cellular survival, embryogenesis, and cellular migration and invasion. Mutations in this gene are associated with papillary renal cell carcinoma, hepatocellular carcinoma, and various head and neck cancers. Amplification and overexpression of this gene are also associated with multiple human cancers (4233). Crizotinib is an inhibitor of the MET and ALK tyrosine kinase domains (21154129), which is actively being studied for use in pediatric cancer (NCT02034981, ASCO abstract).

**Conclusion**: The fusion product brings together the 5' of TFG with its protein binding PB domain at exon 6 meeting exon 15 of MET with the tyrosine kinase domain intact.
Optimizing the Process

1. Monitoring the workflow: ~ 300 data points per patient
2. Optimized extractions of DNA and RNA from small needle core biopsies
3. Real-time data sharing across country
4. Speeding up clinical interpretation
Summary and Future Steps (I)

• Incorporation of NGS into clinical management of children with refractory/metastatic/relapsed cancer is feasible
• Implementation of PROFYLE/KiCS progressing well
• Education of all stakeholders is ongoing
Summary and Future Steps (II)

• Expand patient enrollment to include ALL newly diagnosed children with cancer
• Expand cohort to include all children across Ontario (part of Provincial Pediatric Oncology Plan (PPOP))/Canada (PROFYLE)
• Evaluate psychosocial, clinical outcome and economic impact
Thank You!