Cancer Genomics to Guide Therapy

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Beyond the Genetic Prescription Pad: Personalizing Cancer Medicine in 2014
Next-Generation Sequencing and Cancer Genomics

Technology and Computational Analyses Fuel a Revolution
By directed PCR and capillary sequencing, we determined that ~80% of Iressa responders have EGFR mutations in the tyrosine kinase domain.

W. Pao et al., PNAS 2004
Whole Genome Sequencing: Tumor vs. Normal

**Paired end NGS data from tumor (50-fold) and normal (30-fold) DNA isolates**

Align read pairs to reference genome

Detect Single-Nucleotide Variants and focused insertion/deletions

Detect anomalous read pair mapping, assemble reads and identify structural variations (inversions, translocations)

Use normalized read coverage levels and HMM-based algorithm to identify CNA and LOH regions
“AML1”: Cancer Genomics by Whole Genome Sequencing

- Caucasian female, mid-50s at diagnosis
- De novo M1 AML
- Family history of AML and lymphoma
- Informed consent for whole genome sequencing
- Solexa sequencer, 32 bp unpaired reads
- 10 somatic non-synonymous exonic mutations detected

Ley et al., Nature 2008
Tumor Sequencing is Driving Discovery

Total WGS samples: 2034
Pediatric and adult tumors with comprehensive clinical data to address clinically relevant questions

<table>
<thead>
<tr>
<th>Cancer Types</th>
<th>Number of Cases</th>
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<tbody>
<tr>
<td>AML</td>
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<tr>
<td>BRC</td>
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<tr>
<td>EMC</td>
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<tr>
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<td>GBM</td>
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<td>GI</td>
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<td>LUC</td>
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<td>MDS</td>
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<tr>
<td>SJTALL</td>
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AML: Acute Myeloid Leukemia
BRC: Breast
EMC: Endometrial
ESC: Esophageal
GBM: Glioblastoma Multiforme
GIA: Gastrointestinal
Adenocarcinoma
HCC: Hepatocellular Carcinoma
LUC: Lung
MDS: Myelodysplastic Syndrome
MEL: Melanoma
MMY: Multiple Myeloma
OVC: Ovarian
PCA: Pilocytic Astrocytoma
PNC: Pancreatic
PRC: Prostate
SJACT: Adrenocortical Tumor
SJALL: Acute Myeloid Leukemia M7
SJDBALL: B-cell precursor Acute Lymphoblastic Leukemia (ALL)
SJCBF: Core Binding Factor Acute Myeloid Leukemia
SJCP: Choroid Plexus Carcinoma
SJED2A: E2A-PBX (ALL)
SJEPD: Ependymoma
SJERG: ETS-Related Gene-Associated ALL
SJETV: ETV-associated ALL
SJES: Ewing's Sarcoma
SJHGG: High Grade Glioma
SJHYP: Hyperdiploid ALL
SJHypo: Hypodiploid ALL
SJHyp: Infant ALL
SJLGG: Low Grade Glioma
SJMB: Medulloblastoma
SJME: Melanoma
SJNL: Neuroblastoma
SJOS: Osteosarcoma
Pan-Cancer Analyses from TCGA

12 tumor types
- Leukemia (LAML)
- Lung adenocarcinoma (LUAD)
- Lung squamous (LUSC)
- Kidney (KIRC)
- Bladder (BLCA)
- Endometrial (UCEC)
- Glioblastoma (GBM)
- Head and neck (HNSC)
- Breast (BRCA)
- Ovarian (OV)
- Colon (COAD)
- Rectum (READ)

Thematic pathways

Oomics characterizations
- Mutation
- Copy number
- Gene expression
- DNA methylation
- MicroRNA
- RPPA
- Clinical data

Nature Genetics 45: 1113-1120 (2013)
Early Translation of Cancer Therapy Decision-making

NGS Aids a Difficult Diagnosis
Clinical case: atypical APL

37 y.o. female with de novo AML; M3 morphology

Chemo + ATRA

Complex cytogenetics, persistent leukemia

Chemo only

First remission, referred to WU for SCT. rBM: normal morphology, cytogenetics; negative for PML/RARA.

???

Allogeneic SCT

Consolidation + ATRA
Use of Whole-Genome Sequencing to Diagnose a Cryptic Fusion Oncogene

Welch et al., JAMA 2011: 305(15): 1577-1584.
Cancer Genomics in the Clinic
Therapeutic Options by NGS
Integrated WGS/Exome/RNA-Seq

- **WGS** analysis yields:
  - SNVs (single nucleotide variants)
  - CNVs (amplification/deletion)
  - SVs (translocations, inversions)
  - Indels (focused insertions/deletions)

- **Exome**: validates WGS discoveries, integrated coverage depth allows clonality analysis

- **RNA-Seq**: over-expression metrics, expressed SNVs, gene fusions
WUMS Genomics Tumor Board

- Cancer patients consented for genomic sequencing and return of information
- Cancer biopsies studied by WGS, exome and transcriptome integrated analysis
- Interpretive analysis (DGIdb) will identify actionable targets, corresponding drugs, and available clinical trials
- All sequencing in a CLIA facility with pathology sign-out

The Genomics Tumor Board serves as a vehicle for education, decision-making, and patient monitoring

- Physicians work with junior faculty to develop and present case reports of each patient’s clinical history
- Oversight board of GTB reviews cases and determines 1-2 per month that are most likely to benefit from genomics (difficult diagnoses, late stage metastatic patients)
- Results of genomics communicated to the physician lead, then to GTB participants
- Physician lead presents their decision to treat, outcomes if available, difficulties encountered
Integrated Discovery: DNA and RNA

Whole genome sequencing

Exome sequencing

Whole transcriptome sequencing
Annotating Somatic Alterations

48.7% (repetitive)

1.3% ("the exome")

8.6% (conserved/regulatory)

1.4% other/unique
Malachi & Obi Griffith

Analysis & Therapeutic Interpretation

Somatic/Germline Cancer Events (DNA+RNA)

- Single Nucleotide Variants
- Insertion/deletions
- Structural Variants
- Copy Number Variations
- Expressed variants
- SV-predicted gene fusions
- Differentially Expressed Genes
- Differentially Expressed Isoforms

Clinical prioritization and reporting

- Functional annotation
- Filtered (activating/drivers)
- Candidate genes/pathways
- Clinically actionable events (aka “The Report”)

Drug Gene Interaction database (>50 database sources)

- Literature
- dGene
- DrugBank
- TTD
- clinicaltrials.gov
- PharmGKB
- TEND
- TALC
- MyCancerGenome
DGIdb: Drug Gene Interaction database

Predicted Therapeutic Targets from Integrated Analysis

Govindan et al., Cell 2012
Lukas Wartman, M.D. is Patient “ALL1”

The New York Times

In Treatment for Leukemia, Glimpses of the Future

Second Chance: Lukas Wartman, a leukemia doctor and researcher, developed the disease himself. As he faced death, his colleagues sequenced his cancer genome. The result was a totally unexpected treatment.

By GINA KOLATA
Published: July 7, 2012
Male patient, mid-30’s.

Initial presentation of acute lymphocytic leukemia (B-ALL) at age 25, induction & consolidation therapies to remission (patient’s biopsy sample was banked).

Relapse at age 30, salvage chemo to remission, BMT using HSPC from younger brother.

Severe 2nd relapse in July 2011 (age 33), CNS involvement. Salvage chemotherapy regimen.

Whole genome and transcriptome sequencing initiated on August 1, completed by August 30 2011.
ALL-1: Somatic copy number alterations
ALL-1: Biclonal Tumor Presentation

Tumor variant allele fraction

Proportion

Read coverage (X)

Tumor variant allele fraction
Interphase FISH: Deletion-carrying Cells at Diagnosis

2nd Relapse B-ALL

% positive

-5 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80

WGS
Salvage
Iphosamamide
Etoposide
Intrathecal therapy

Shashikant Kulkarni
By conventional pathology, the patient appears to be in remission following salvage chemotherapy. The more highly sensitive i-FISH analysis indicates presence of treatment refractory tumor cells in the marrow. The patient is not in CR and cannot receive a bone marrow transplant.
ALL-1: Somatic single nucleotide variations

- 100 somatic coding SNVs
- 42 with evidence of expression from RNA-seq analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ref.</th>
<th>Var.</th>
<th>AA</th>
<th>Type</th>
<th>WGS</th>
<th>Exome</th>
<th>RNA-seq</th>
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<tbody>
<tr>
<td>UBXN4</td>
<td>T</td>
<td>C</td>
<td>D → D</td>
<td>silent</td>
<td>51.25%</td>
<td>40.56%</td>
<td>53.40%</td>
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<td>OGT</td>
<td>G</td>
<td>T</td>
<td>C → F</td>
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<td>39.22%</td>
<td>38.7%</td>
<td>35.79%</td>
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<td>KIAA1033</td>
<td>C</td>
<td>T</td>
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<td>38.89%</td>
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<td>C15orf39</td>
<td>C</td>
<td>G</td>
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<td>47.37%</td>
<td>37.5%</td>
<td>48.74%</td>
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<tr>
<td>SPTAN1</td>
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<td>C</td>
<td>L → P</td>
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<td>42.39%</td>
<td>49.03%</td>
<td>50.17%</td>
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<td>DDX6</td>
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<td>G</td>
<td>L → P</td>
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<td>14.29%</td>
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<td>T</td>
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<td>NF1</td>
<td>C</td>
<td>T</td>
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<td>64.04%</td>
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<td>TNRC6B</td>
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<td>KIAA1462</td>
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<td>C</td>
<td>P → A</td>
<td>missense</td>
<td>70%</td>
<td>45.05%</td>
<td>41.14%</td>
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</table>

Of these candidates, none had a therapeutic match.
FLT3 Over-expression in ALL1

- FLT3 was within the top 1% of all expressed genes.
- Absent a normal comparator, the literature report from Marston identified FLT3 over-expression in pre-B-ALL.
- Based on wt FLT3 over-expression by the tumor cells, we predicted the cancer would be sensitive to the FLT3 inhibitor Sunitinib (Sutent) [DrugBank].

CR for the patient was confirmed by iFISH after 14 days of Sutent therapy.
A bone marrow transplant followed in September 2011.
The patient has maintained CR to-date.
Pediatric Brain Cancer Case: Low Grade Glioma

- Female (~10 y.o.) with low grade glioma progressing after chemotherapy with PET active node on the temporal lobe
- Sequencing performed using HiSeq 2500: WGS, exome & RNA
  - Whole genome: 66X for tumor, 34X for normal (PBL)
  - Exome: 135X for tumor, 88X for normal
  - RNA-Seq: tumor only
  - Gender & age-matched temporal lobe RNA was sequenced as a comparator
- A relatively small number (~500) of somatic single nucleotide variants (SNVs) were identified genome-wide
Pediatric LGG: Published Genomic Studies

- Themes
  - MAPK/ERK and PI3K pathways
  - Median of **one driver** per tumor genome
- Recurrent somatic events
  - SNVs and Indels
    - BRAF^{V600E}, NF1, IDH1, NTRK, RAF1, FGFR1, MYB, MYBL1, H3F3A, ATRX, FGFR1, EP300, WHSC1, CHD2
  - Fusions
    - NAV1-NTRK2, KIAA1549-BRAF, SRGAP3-RAF1, SRGAP3-RAF1, ST6GAL1-WHSC1, FGFR-TACC, FXR1-BRAF, BRAF-MACF1, QKI-RAF1, FAM131B-BRAF
  - Copy number aberrations
    - FGFR1 on chromosome 8
Somatic mutations in LGG1

- 33 tier 1 (coding) somatic point mutations
- An interesting SNV that could have been critical to tumor initiation is a mutation in FOXO3
  - FOXO3 (P295L, P75L)
  - CASP9 (V404M)
- 4 somatic indel mutations
  - BRAF (600in_frame_insT)
  - CACNA1A (QQ2313in_frame_del)
  - SNORA36 (RNA gene)
  - RP11-830F9 (V197fs)
A 3 bp insertion in *BRAF* adds a threonine codon
GET analysis summary: LGG1

- The candidate driver events in this tumor are protein-altering mutations in BRAF, FOXO3, & EPB41L4A
- LGG1 appears to be triploid for chromosomes 5, 6 & 11
- EPB41L4A and FOXO3 mutations were likely acquired prior to duplication of chromosomes 5 and 6 respectively (i.e. two mutant copies of each of these chromosomes and one wild type copy)
- No obvious focal or large deletions were observed
- 5 DNA (SV) fusions & 41 RNA chimeras were identified, none involves a known cancer driver or druggable gene
- Some potentially druggable genes appear to be highly expressed (e.g. FGFR1 and PDE4B)
- Based on the BRAF insertion, the patient was enrolled onto a pediatric clinical trial of MEK inhibitor therapy
Dear Elaine,

We received the best news today!! Our daughter’s tumor is responding to the MEK inhibitor and for the very first time in almost 11 years of her battling cancer, her tumor has shown some regression!!!

We are so thrilled and this comes one week before her birthday.

I feel that you and your group’s efforts have been so critical in our recent journey and we wouldn't be here without you.

There will be more treatments to come but this gives more hope than ever that there is a real chance for us to beat this disease.

With tremendous gratitude.
Many other challenges remain...

tumor heterogeneity  who pays?
sample purity    DNA/RNA quantity
DNA/RNA quality aneuploidy
politics          return of results
proper consent    physician education
access to drugs   FFPE samples
RNA quality       data sharing

MAP Kinase Pathway Alterations in BRAF-Mutant Melanoma Patients with Acquired Resistance to Combined RAF/MEK Inhibition
Cancer Discov January 2014 4:61-68; Published OnlineFirst November 21, 2013; doi:10.1158/2159-
Personalized Immunotherapy
Expressed variants inform vaccine design
Patient biopsied metastatic melanoma lesions

Tumor and germline DNA sequenced, somatic mutations identified; RNA capture verifies expressed mutations and expression level; netMHC algorithm identifies immunoepitopes

Sequencing to identify tumor-specific immunoepitopes:

Mardis, Schreiber et al., Nature 2012

Apheresis samples from patient used to verify the algorithmically-identified immunoepitopes that elicit T cell memory
A dendritic cell-based approach is currently being tested in an FDA approved protocol for metastatic melanoma patients:

- Patient 1 has received all three doses of vaccine, and is being monitored.
- Patient 2 has received three doses of vaccine, this patient has measurable disease and will be monitored for progression, stability or regression.
- Patient 3 has completed sequencing-based analysis, in vitro analysis and a dendritic cell vaccine is in preparation. This patient also has measurable disease.
- Patients 4 and 5 have been identified, and genomic analysis is underway.
Conclusions

• NGS has accelerated cancer discovery and now is being used in clinical translation to predict targeted therapy for individuals, and as a means for stratification in clinical trials
• Our efforts are producing decision support tools and an educational base to aid cancer care specialists in this new era of genomics-based medicine
• Integration of RNA data aids our interpretation of DNA analyses, and provides additional evidence for therapeutic decision making
• Genomics can also inform personalized immunotherapy design, with several studies underway
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Our patients
NHGRI
NCI
WUSM