Translating the Cancer Genome
Elaine R. Mardis, Ph.D.
Professor of Genetics
Co-director, The Genome Institute

Plant and Animal Genome Conference XXII

Next-Generation Sequencing and Cancer Genomics
Technology and Computational Analyses Fuel a Revolution

Beginnings: EGFR mutations in lung cancer

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

Whole Genome Sequencing and Deep Digital Validation

Paired end sequence 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

Use normalized read coverage levels and HMM-based algorithm to identify CNA and LOH regions

Design custom capture probes for each putative variant site

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

Deep sequencing at variant sites captured by hybridization

“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 mutations detected

Ley et al., Nature 2008
Tumor Heterogeneity by Bayesian Mixture Modeling

**Founder clone:** all cells carry the mutation

**Subclone:** founder plus novel mutations

- Total reads = 1000
- Mutant reads = 500
- Variant Allele Fraction (VAF) = 50%

- Total reads = 1000
- Mutant reads = 250
- Variant Allele Fraction (VAF) = 25%

VAF can change as a function of progression, metastasis, and other "evolutionary" forces such as therapy.

Genomics of Breast Cancer Metastasis

- **A1 Patient**
  - Stage II breast cancer
  - Non-buyer disease
  - 24 months after diagnosis

- **A7 Patient**
  - Stage II breast cancer
  - 24 months after diagnosis

Disease progression model for AML1

- **Diagnosis:** Multiple leukemic clones present
- **Clinical remission:** Loss of most leukemic clones
- **Relapse:** Acquisition of new mutations in a pre-existing clone

Tree Plot of the A7 Tumor Genomes

- **Kidney**
- **Lung**
- **Brain**
- **Liver**

Pan-Cancer Analyses from TCGA

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

Collaboration with Chuck Perou-UNC
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

Conclusion:
“...whole genome characterization will become a routine part of cancer pathology.”

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

Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors

Welch et al., JAMA 2011: 305(15): 1577-1584.

AML TCGA: Comprehensive genomics of 200 AMLs

Welch et al., JAMA 2011: 305(15): 1577-1584.
AML NGS into the Clinic

• For each newly diagnosed de novo AML patient in the AML clinic at Washington University:
  - Exome sequencing of marrow biopsy DNA and skin normal
  - Augmented with RMG probes (IDT) corresponding to the recurrently mutated genes in AML (259) for targeted capture
  - Transcriptome sequencing
  - This combined data set from diagnosis provides the following information for each patient:
    - High coverage at RMG sites and somatic mutations with variant allele fraction
    - Medium coverage exome data for novel mutation discovery and heterogeneity estimation at diagnosis
    - Evidence of highly expressed genes (not detectable from DNA), gene fusion evidence, corroborates expression of novel mutations
  - In addition to the Day 0 sequencing, a Day 30 marrow will be sequenced similarly and compared (MRD, relapse therapy).

RMG vs. Exome Coverage on a known AML

WUMS Genomics Tumor Board

• Cancer patients consented for genomic sequencing and return of information
• Cancer biopsies studied by WGS, exome and transcriptome integrated analysis
  - WGS drives comprehensive discovery
  - Exome contributes read depth for heterogeneity/clonality analysis
  - Transcriptome monitors aberrant gene expression and validates fusions
• 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

WUMS Genomics Tumor Board

• Cancer patients consented for genomic sequencing and return of information
• Cancer biopsies studied by WGS, exome and transcriptome integrated analysis
  - WGS drives comprehensive discovery
  - Exome contributes read depth for heterogeneity/clonality analysis
  - Transcriptome monitors aberrant gene expression and validates fusions
• 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

Annotating Somatic Alterations

- Tier 1: 1.3% (‘the exome’)
- Tier 2: 8.6% (conserved/regulatory)
- Tier 3: 51.4% (coding/consequence)
- Tier 4: 48.7% (repetitive)

Analysis & Therapeutic Interpretation

<table>
<thead>
<tr>
<th>Somatic/Germline Cancer Events (DNA+RNA)</th>
<th>Drug Gene Interaction database (&gt;50 database sources)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Nucleotide Variants</td>
<td>Literature</td>
</tr>
<tr>
<td>Insertions/Deletions</td>
<td>eGene</td>
</tr>
<tr>
<td>Structural Variants</td>
<td>DrugBank</td>
</tr>
<tr>
<td>Copy Number Variations</td>
<td>TTD</td>
</tr>
<tr>
<td>Expressed variants</td>
<td>clinicaltrials.gov</td>
</tr>
<tr>
<td>SV-predicted gene fusions</td>
<td>PharmGKB</td>
</tr>
<tr>
<td>Differentially Expressed Genes</td>
<td>TEND</td>
</tr>
<tr>
<td>Differentially Expressed Isoforms</td>
<td>TALC</td>
</tr>
</tbody>
</table>

Clinical prioritization and reporting

Functional vs annotation

Filtered vs activating/driver

Candidate gene/pathways

Clinically actionable events (aka “The Report”)

Malachi & Obi Griffith
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.

- 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].
An encouraging message

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.

Personalized Immunotherapy

Expressed variants Inform vaccine design

Many other challenges remain...

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

Patient-specific mutation directed DC vaccine

- Identifying the most highly expressed tumor-specific neo-antigens in each patient’s tumor requires an algorithmic evaluation of their missense mutations with the HLA-Class I subtype using netMHC.
- This approach generates a prioritized list of neo-antigens for further testing of immune response.
- We are establishing a workflow in a tractable clinical timeframe for neo-antigen identification, vaccine production and in vitro functional evaluation.

Identifying the most highly expressed tumor-specific neo-antigens in each patient’s tumor requires an algorithmic evaluation of their missense mutations with the HLA-Class I subtype using netMHC.

Sequncing Pediatric Brain Cancers

- July 2013: two pediatric brain cancer cases studied
- Patient #1 (<5 y.o.) with an indeterminate diagnosis from pathology of surgically resected tumor had no detectable driver mutations.
  - Decision to pursue low dose, pinpoint radiation and minimal chemotherapy following surgery
  - Imaging-based monitoring at periodic intervals
- Patient #2 (>10 y.o.) with low grade glioma diagnosed at 2 y.o. A novel BRAF insertion adjacent to V600 (3 bp insert adds Threonine) was identified; unknown response to Vemurafenib.
  - MEK inhibitor peds clinical trial enrollment
  - Regression reported at first MRI
Dendritic Cell Vaccine Platform

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 two doses of vaccine (of 3), this patient has measurable disease and will be monitored for progression, stability or regression
- Patient 3 has completed sequencing-based analysis, peptide analysis and we await GMP peptides to condition the dendritic cells. This patient also has measurable disease
- Patients 4 and 5 have been identified

Conclusions

- NGS has accelerated cancer discovery and now is being used in clinical translation to predict targeted therapy for individuals
- 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

Acknowledgements

The Genome Institute
Malachi Griffith, Ph.D.
Obi Griffith, Ph.D.
Ben Ainscough
Zach Skidmore
Allison Regier
Lee Trani
Jassmeet Hundal, M.S.
Vincent Magrini, Ph.D.
Sean McGrath
Ryan Demeter
Jason Walker
David Larson, Ph.D.
Lucinda Fulton
Robert Fulton
Chris Mahler, Ph.D.
Li Ding, Ph.D.
Richard K. Wilson, Ph.D.

WUSM/Siteman Cancer Center
Timothy Ley, M.D.
Matthew Ellis, M.B., Ph.D.
John DiPersio, M.D., Ph.D.
Timothy Graubert, M.D.
Matthew Walker, M.D.
John Walt, M.D., Ph.D.
Shashikant Kulkarni, Ph.D.
Peter Westervelt, M.D., Ph.D.
Lukas Wartman, M.D.
Robert Schreber, Ph.D.
William Gillanders, M.D., Ph.D.
Gerry Linette, M.D., Ph.D.
Beatriz Carreno, M.D., Ph.D.

Our patients
NHGRI
NCI
WUSM