Melanoma

Researchers

Primary Care

Laboratory Medicine and Pathology

Oncologists
Targeted Therapy
Population-Based vs. Personalized Cancer Treatment

• **Population-based:** Treatment based on pathology diagnosis and staging

• **Personalized:** Tailor therapy to molecular characteristics of the tumor
Outline

• *BRAF* mutations

• *KIT* mutations

• Methods and specimen considerations

• Future testing strategies
BRAF Mutations in Melanoma

• Predict response to BRAF inhibitors

• Activating mutations at codon 600

• Both V600E and V600K mutations associated with response

• Prognosis in metastatic melanoma
**Targeted Drugs**

Diagram showing the pathways of EGFR, RAS, BRAF, PI3K, AKT, and MAPK with inhibitors such as Erlotinib, Gefitinib (EGFR Tyrosine Kinase Inhibitors), Vemurafenib, Sorafenib (BRAF Inhibitors), BKM120, BGT226, XL147, GDC-0941 (PI3K inhibitors), Perifosine (AKT inhibitor), AZD6244, GSK1120212 (MEK Inhibitors).

Modified from Pritchard and Grady, *Gut* (2010)
Much More on the Horizon

<table>
<thead>
<tr>
<th>Target</th>
<th>Number of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>53</td>
</tr>
<tr>
<td>KIT</td>
<td>14</td>
</tr>
<tr>
<td>NRAS</td>
<td>9</td>
</tr>
<tr>
<td>MEK</td>
<td>24</td>
</tr>
<tr>
<td>MET</td>
<td>8</td>
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</table>

<table>
<thead>
<tr>
<th>Target</th>
<th>Number of Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>2</td>
</tr>
<tr>
<td>PDGFR</td>
<td>4</td>
</tr>
<tr>
<td>PI3K</td>
<td>7</td>
</tr>
<tr>
<td>CDK</td>
<td>6</td>
</tr>
<tr>
<td>NOTCH</td>
<td>5</td>
</tr>
</tbody>
</table>

www.clinicaltrials.gov search 5-09-2012; “Melanoma AND "XXX"
BRAF Mutations

- ~50% of all melanoma

BRAF Mutation Breakdown

- 78% V600E
- 18% Other
- 4% V600K

Analysis of 273 BRAF-mutant melanomas from 6 independent studies
# Features of *BRAF*+ Melanoma

<table>
<thead>
<tr>
<th>Feature</th>
<th><em>BRAF</em>-Mutant</th>
<th><em>BRAF</em>-Not Mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Younger (median 52)</td>
<td>Older (median 61)</td>
</tr>
<tr>
<td>Site</td>
<td>Chest and Abdomen</td>
<td>Head and Neck</td>
</tr>
<tr>
<td>Subtype</td>
<td>Superficial Spreading</td>
<td>Acral Lentiginous</td>
</tr>
<tr>
<td>Microscopic features</td>
<td>Heavy melanin; large epithelioid</td>
<td>Lighter melanin</td>
</tr>
<tr>
<td>Chronic sun-damaged skin</td>
<td>Less likely</td>
<td>More likely</td>
</tr>
<tr>
<td>Number of moles</td>
<td>Higher</td>
<td>Lower</td>
</tr>
</tbody>
</table>

Adapted from Long et al. (2011) *JCO*
BRAF Inhibitors In The News

Feb 22, 2010

(Target Cancer)
New Drugs Stir Debate on Rules of Clinical Trials

Sept 18, 2010
BRAF Inhibitors in Melanoma

*BRAF*-mutated melanoma treated with Vemurafenib

BRAF Status and Prognosis

Long et al. (2011) JCO
Why Not Try BRAF Inhibitors In All Melanoma Patients?
BRAF Inhibitors *Stimulate* Tumors Without BRAF Mutations

Modified from: Kwong and Chin Cell (2010).
Side Effects of BRAF Inhibition

Images Courtesy of Dr. Kim Margolin
BRAF Inhibitor Resistance

- Drug resistance often in <1 year

- NRAS Q61K

- "MAP Kinase" Activation
  - MEK1 C121S

Nazarian et al. 2010 *Nature*; Wagle et al. 2011 *JCO*
BRAF-Inhibitor Resistance

Before Treatment | Vemurafenib Treatment | Relapse at 6 months

Wagle et al. (2011) JCO
Outline

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• *KIT* mutations

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• Future Testing Strategies
Role of KIT Mutation Testing

• Imatinib therapy
  – 30-50% response in KIT-mutant melanoma
  – No response in KIT-WT

• Other related inhibitors?
## KIT by Melanoma Subtype

<table>
<thead>
<tr>
<th>Melanoma Type</th>
<th>KIT Mutant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acral</td>
<td>15%</td>
</tr>
<tr>
<td>Mucosal</td>
<td>20%</td>
</tr>
<tr>
<td>Chronic sun-damaged cutaneous</td>
<td>15%</td>
</tr>
<tr>
<td>Eye (ocular)</td>
<td>10%</td>
</tr>
<tr>
<td>All Others (Non-chronic sun-damaged cutaneous)</td>
<td>&lt;3%</td>
</tr>
</tbody>
</table>
KIT and BRAF Mutations Do Not Occur Together

CSD = Chronic Sun Damaged

Curtin et al. 2006 JCO
Outline

• BRAF mutations

• KIT mutations

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Heterogeneity

- Specimen
- Tumor
Specimen Quality

• Tumors are usually fixed with formalin
  – Fixation time poorly controlled; affects DNA

• Other fixatives even worse
Specimen Quantity

• Limited amount of tissue

• Sometimes have to prioritize when multiple tests requested
Specimen Heterogeneity

- Tumor/non-tumor cells mixed
  - Too many non-tumor cells may affect test results
- Mutation heterogeneity within tumor
- Two different tumors in one sample
Manual Dissection to Enrich Tumor

This Tissue Was Used to Make DNA

Metastatic Colon CA 200X
Tumor Tests Must Account for Unique Specimen Issues

• Poor Quality DNA
  – Must detect mutations in fragmented DNA

• Limited Quantity
  – Highly robust conditions required

• Heterogeneity
  – Enrichment of tumor by manual dissection
Mutation Detection Strategies

• *BRAF*:
  – Melting Curve Analysis
  – Many Other Methods

• *KIT*:
  – DNA Sequencing
One Way To Detect *BRAF* Mutations

**PCR Product**

V600E Sensor  
Anchor  

*Mutation Absent*  
*(red trace)*  

**TEMPERATURE (C)**

**FLUORESCENCE –(dF/dT)**
One Way To Detect BRAF Mutations

V600E Sensor  Anchor

PCR Product

V600E Mutation

V600E Mutation (green trace)
**BRAF Assay**

![Graph showing comparison between Non-mutant Sample and V600E Mutant](image)

- **Non-mutant Sample**
- **V600E Mutant**

**TEMPERATURE (C)**
KIT Sequencing
Outline

• *BRAF* mutations

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‘Next-Gen’ Sequencing

- Technology maturing rapidly
- Theoretical high sensitivity
  - Detect low-level resistance?
- Analysis methods challenging
- Too much information?
  - Only a few actionable targets
Pilot Study Using Molecular Profiling of Patients’ Tumors to Find Potential Targets and Select Treatments for Their Refractory Cancers

Gene Panels

- Multiple genes tested at once
- Potential to detect mutations that can be targeted by experimental drugs
### OncoPlex™ v1

<table>
<thead>
<tr>
<th>Tier 1: Currently Actionable</th>
<th>e.g. BRAF, KIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 2: Actionable in the Near Future</td>
<td>e.g. MITF, MEK1</td>
</tr>
<tr>
<td>Tier 3: Frequently Mutated</td>
<td>e.g. GNAQ, GNA11</td>
</tr>
<tr>
<td>Germline Pharmacogenomics</td>
<td>e.g. UGT1A1, COMT</td>
</tr>
</tbody>
</table>

- **Genes Targeted:** 98
- **Total Exons:** 3,370
- **DNA Sequenced:** 494,848 bp
- **Average Read Depth:** 1,687
Each **RED** box represents a mutation

BRAF V600E
This mutation predicts response to Vemurafenib.
Next-Generation Sequencing on Tumor Tissue

Too Much Power?
"Actionable" means we already know how to use the test results; e.g. *BRAF*
Mutation Information

BRAF V600E (c.1799T>A) mutation in Melanoma

BRAF-Associated clinical trials

Great effort was made to include all clinical trials relevant for this mutation. However, the completeness of this information cannot be guaranteed.

United States By State (18)

<table>
<thead>
<tr>
<th>Protocol No.</th>
<th>Phase</th>
<th>Title</th>
<th>State</th>
</tr>
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<tbody>
<tr>
<td>NCT00049487</td>
<td>Phase I</td>
<td>Study of TAK-733 in Adult Patients With Advanced Nonhematologic Malignancies</td>
<td>Michigan, New York</td>
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<tr>
<td>NCT00859127</td>
<td>Phase I</td>
<td>A Study of ARRY-438162 (MEK162) in Patients With Advanced Cancer</td>
<td>California, Colorado, Maryland, Massachusetts, Michigan, Ohio, Tennessee, Texas</td>
</tr>
<tr>
<td>NCT01072175</td>
<td>Phase I</td>
<td>Investigate Safety, Pharmacokinetics and Pharmacodynamics of GSK2118430 &amp; GSK1120212</td>
<td>California, Connecticut</td>
</tr>
</tbody>
</table>

Author: Jeff Sosman, M.D.
Conclusions

- **BRAF** and **KIT** mutations in melanoma tissue predict response to targeted inhibitors
  - **BRAF**: Vemurafenib and other similar
  - **KIT**: Imatinib and other similar

- Specimen characteristics affect test accuracy

- Future testing is likely to analyze multiple genes and resistance mutations to determine optimal therapy