Molecular Testing of Solid Tumors

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Disclosure Information

- Advisory Board: Eli Lilly-ImClone
Learning Objectives

• After this presentation, you should be able to:

  – Describe the most common molecular diagnostics tests performed in the evaluation of malignant solid tumors

  – Have working knowledge of the Molecular markers recommended by the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for common solid tumors

  – Recognize some of emerging molecular markers
Molecular Biomarkers

- Potential therapeutic targets
- Provide further insight into the clinicopathologic features of cancer
- Guide to treatment decisions
  - Predictors of response to therapy
  - Prognostic indicators of risk
  - Monitor progression or response to treatment
Solid tumors where molecular diagnostics make the highest contribution

- Lung
- Colorectal
- Brain
- Breast
- Sarcomas
- Head and neck - Thyroid
- Melanoma
Lung Carcinoma
Lung cancer is the most common cause of cancer-related death in men and women. Responsible for over 1.3 million deaths annually worldwide.
A NEW WAY TO LOOK AT LUNG CANCER

“The Lung Adenocarcinoma Oncogenome”

Pie chart of mutually exclusive mutations (ca 2011)

Overlapping mutations: p53 (30%), LKB1 (15%), PIK3CA (2%)
THE EGFR ACTIVATION AND SIGNALING

PTEN → AKT → PI3-K

Survival

mTOR

Nucleus

EGFR (ErbB1, HER1)
- Receptor tyrosine kinase

EGF

CELL MEMBRANE

GRB2

SOS

NF1

RAS

RAF

MEK

MAPK

Proliferation
Biomarkers associated with

- Sensitivity to therapy (Strong evidence)
- Good prognosis (value still discussed)
  Sensitivity to therapy (value still discussed)
- Poor prognosis (value still discussed)
  Resistance to therapy (value still discussed)

Prognostic value

Predictive value

Targeted therapy
- EGFR mut exon 19del & L858R
- EML4-ALK fusion
- ROS fusions
- EGFR T790M
- KRAS mut
- MET amp

Cisplatin-based chemo
- High ERCC1 (IHC, mRNA)
- High BRCA1 (mRNA)
- High RRM1 (mRNA)
- High p53 (IHC)
- p27Kip1 (IHC)

High HER2 (IHC)
- High BRCA1 (IHC, mRNA)
- High RRM1 (IHC, mRNA)
- High P16INK4A loss
- High p53 (mut, IHC)

EGFR amp (FISH)
- KRAS mut
- MET amp
EGFR

- Receptor tyrosine kinase of the ERBB family

- 4 closely related receptors
  - ErbB1 – EGFR, HER1
  - ErbB2 – HER2/neu
  - ErbB3 – HER3
  - ErbB4 – HER4
Small Molecule EGFR Tyrosine Kinase Inhibitors (TKIs)

- Expression of EGFR by IHC in large proportion (50-60%) of lung CA provided initial rationale for trials
- Oral drugs with relatively low toxicity (rash, diarrhea)
- Pharmacokinetics – once-daily dosing

Erlotinib
Tarceva®
(Deutsche Genentech)

Gefitinib
Iressa®
(Astra-Zeneca)
Dramatic response to gefitinib

Clinical predictors
- Women
- East Asian
- Never smokers

Histologic predictors
- Adenocarcinomas with BAC features, TTF1+
- None completely reliable
**EGFR Mutations Associated with Sensitivity to EGFR-TKIs**

- **EGF ligand binding**
- **Tyrosine kinase**
- **autophos**

Exon:
- Exon 19: ELREA deletion
- Exon 20: V765A, T783A
- Exon 21: L858R, L861Q
- Exon 22: N826S, A839T, K846R, G863D

Mutations described in first reports:
- Lynch et al. '04; Paez et al. '04; Pao et al. '04

*Note: Details of mutations and their implications for EGFR-TKIs sensitivity are illustrated in the diagram.*
EGFR TK Domain Mutations

• Most reliable predictors of response to EGFR tyrosine kinase inhibitors
Iressa Pan Asian Study (iPASS)

Objective response rate in EGFR mutation positive and negative patients

- **Overall response rate (%)**
  - Gefitinib: 71.2%
  - Carboplatin / paclitaxel: 47.3%

- **EGFR M+ odds ratio (95% CI)**: 2.75 (1.65, 4.60), p=0.0001
- **EGFR M- odds ratio (95% CI)**: 0.04 (0.01, 0.27), p=0.0013

Predictive and prognostic

Mok N Engl J Med 2009
Not all EGFR mutations are associated with response to TKI’s

• Well established sensitivity profile
  – G719 mutations in exon 18
  – L861 mutations in exon 21

• Well established resistance profile
  – Insertions is exon 20
  – S768I, L747S, D761Y and T854A

• Some mutations remain uncharacterized due to low prevalence
EGFR mutations associated with resistance to EGFR inhibitors

- **Secondary resistance**
  - Tumors initially sensitive to EGFR TKIs – become resistant after treatment
  - ~60% of patients with secondary resistance TKI’s develop the secondary mutation **T790M**
EGFR mutations associated with secondary resistance to EGFR TKI’s

EGF ligand binding

Tyrosine kinase

autophos

Exon: 18 19 20 21 22 23 24

G719A/C

deletion

T790M

L858R  L861Q

GXGXXG

K

DFG L L

sensitivity

resistance
EGFR MUTATIONS based on MSKCC experience 2009-2010
(1131 cases screened, 258 EGFR mutant, 23%)

Minor mutations

<table>
<thead>
<tr>
<th>Mutation</th>
<th>CASES</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L861Q</td>
<td>11</td>
<td>48</td>
</tr>
<tr>
<td>S768I</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>G719A</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>G709A</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>G719D</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>L861R</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>G719S*</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

* Concurrent with other minor mutations in all cases

Mutations in exon 20

Insertions – 23 cases (9%)
3-12 base pairs

T790M - 22 cases
20 acquired resistance
2 pre TKI
KRAS MUTATIONS

– Approximately 25-30% of adenocarcinomas
– Strong predictor of primary resistance to EGFR TKI’s
– Adverse prognostic factor
  • Specific KRAS mutations may have prognostic and predictive significance
  • Ex. G12C - reduced sensitivity to cisplatin but increased response to taxol and pemetrexed. Worse progression-free survival
Clinical and pathologic characteristics

• Smoking history more common
• Less common in Asians than non-Asians
  – 15-30% of American lung adenocarcinomas
  – <10% of Asian adenocarcinomas
• Histologic associations:
  – Usually less well-differentiated
  – Mucinous BAC
KRAS Mutations: A Negative Predictor for Response to EGFR TKIs in lung cancer

Table 1. Retrospective Analyses of EGFR Tyrosine Kinase Inhibitors in Lung Adenocarcinoma

<table>
<thead>
<tr>
<th>Author</th>
<th>Drugs</th>
<th>Patients tested for KRAS mutations (mutant/WT)</th>
<th>Response rate KRAS mutant</th>
<th>Response rate KRAS WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackman12</td>
<td>Erlotinib</td>
<td>41 (6/35)</td>
<td>0%</td>
<td>14%</td>
</tr>
<tr>
<td>Zhu13</td>
<td>Erlotinib</td>
<td>206 (30/176)</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>Miller9</td>
<td>Erlotinib</td>
<td>80 (18/62)</td>
<td>0%</td>
<td>30%</td>
</tr>
<tr>
<td>Massarelli14</td>
<td>Erlotinib/Gefitinib</td>
<td>70 (16/54)</td>
<td>0%</td>
<td>7%</td>
</tr>
<tr>
<td>Hirsch10</td>
<td>Gefitinib</td>
<td>138 (36/102)</td>
<td>1%</td>
<td>7%</td>
</tr>
<tr>
<td>Hirsch15</td>
<td>Gefitinib</td>
<td>152 (12/140)</td>
<td>0%</td>
<td>8%</td>
</tr>
<tr>
<td>Han16</td>
<td>Gefitinib</td>
<td>69 (9/60)</td>
<td>0%</td>
<td>27%</td>
</tr>
</tbody>
</table>

WT: wild type (non-mutated).

KRAS mutations work better as a negative predictor of response to EGFR TKIs than EGFR mutations do as a positive predictor.

EGFR and KRAS mutations: predictors of survival in resected lung adenocarcinoma

KRAS MUTATIONS based on MSKCC experience 2009-2010
1131 cases screened, 372 mutant, 33%)

KRAS: 372 MUTATIONS
Never smokers are significantly more likely to have G>A transition mutation as seen in G12D.

G>T transversion, a known tobacco smoke induced mutation, is the most common nucleotide change in former and current smokers, as seen in G12C.
ALK FUSIONS

• EML4-ALK fusions represent one of the newest molecular targets in NSCLC
• Strongly associated with never or light-smoking history
• Onset – younger age
• Most common in adenocarcinoma with solid-predominant histology and signet ring cells
2p

EML4 - echinoderm microtubule–associated protein–like 4
ALK - anaplastic lymphoma kinase
Detection

• Abbott LSI ALK dual color break apart probe assay now FDA approved for ALK gene testing for lung cancer therapy selection
  • >15% of 50 analyzed tumor cells is the cutoff point recommended by CAP and AMP
  • Should be verified by two independent personnel.

• RT-PCR requires multiple assays because of multiple possible isoforms

• IHC - Cell Signaling ALK clone D5F3 Rabbit mAb – not currently available
Tumor Responses to Crizotinib for Patients with \textit{ALK}-positive NSCLC

Best percent change in tumor size

*Partial response patients with 100% change have non-target disease present

Bang et al, ASCO 2010
BRAF MUTATIONS

- Identified in 2-3% of NSCLC
- No significant sex association
- Strong association with current/former smoker status
- Most common mutation is V600E, Exon 15
- Predicts resistance to EGFR inhibition
- Predicts benefit from MEK selective inhibition

1799 T>A
### BRAF MUTATIONS

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Description</th>
<th>DNA Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>G469</td>
<td>p.G469A</td>
<td>c.1406G&gt;C</td>
</tr>
<tr>
<td></td>
<td>p.G469E</td>
<td>c.1406G&gt;A</td>
</tr>
<tr>
<td></td>
<td>p.G469V</td>
<td>c.1406G&gt;T</td>
</tr>
<tr>
<td>D594</td>
<td>p.D594G</td>
<td>c.1781A&gt;G</td>
</tr>
<tr>
<td></td>
<td>p.D594V</td>
<td>c.1781A&gt;T</td>
</tr>
<tr>
<td>V600</td>
<td>p.V600M</td>
<td>c.1798G&gt;A</td>
</tr>
<tr>
<td></td>
<td>p.V600E</td>
<td>c.1799T&gt;A</td>
</tr>
</tbody>
</table>

- **G469A**: 31%
- **D594G**: 6%
- **V600E**: 53%
ROS1 fusions

• ROS1 is a receptor tyrosine kinase belonging to the insulin receptor family
• Fusion between ROS1 and several partner genes SLC34A2, CD74, TPM3, SD4, EZR, LRIG3
• Associated with sensitivity to crizotinib in preclinical models
Colorectal cancer

EGFR-targeted antibodies
– Erbitux/cetuximab, Vectibix/panitumumab show efficacy in 10-20% of mCRC patients; approx 80% show no response.
– IHC or FISH not predictive of response
– No *EGFR mutations*
Biomarkers associated with

- **Resistance to therapy** (Strong evidence)
- **Good prognosis** (value still discussed)
- **Sensitivity to therapy** (value still discussed)
- **Poor prognosis** (value still discussed)
  - Resistance to therapy (value still discussed)

**Prognostic value**

- **p53** (mut)
- **CIN** (Chr instability)
- **18q LOH** (DCC, SMAD2, SMAD4)
- **CIMP** (CpG island methylator phenotype)
- **PIK3CA**
- **BRAF V600E**
- **MSI-H**

**Predictive value**

- **5 FU based chemotherapy**
- **Irinotecan-based chemotherapy**

- **EGFR Monoclonal Ab**
  - **KRAS mutations**
  - **BRAF V600E**
  - **PIK3CA**
  - **MSI-H**
  - **18q LOH** (DCC, SMAD2, SMAD4)
## KRAS mutations

### Retrospective Analysis of Randomized Trials of EGFR Antibodies in Colorectal Cancer

<table>
<thead>
<tr>
<th>Author</th>
<th>Drugs</th>
<th>Patients tested for KRAS mutations (mutant/WT)</th>
<th>Response rate KRAS mutant</th>
<th>Response rate KRAS WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amado(^{22})</td>
<td>Panitumumab Supportive care only</td>
<td>208 (84/124) 214 (100/114)</td>
<td>0% 0%</td>
<td>17% 0%</td>
</tr>
<tr>
<td>Van Cutsem(^{23})</td>
<td>FOLFIRI + cetuximab</td>
<td>277 (105/172) 263 (87/176)</td>
<td>36% 40%</td>
<td>59% 43%</td>
</tr>
<tr>
<td>Bokemeyer(^{24})</td>
<td>FOLFOX + cetuximab</td>
<td>113 (52/61) 120 (47/73)</td>
<td>33% 49%</td>
<td>61% 37%</td>
</tr>
</tbody>
</table>

### Retrospective Analysis of Non-Randomized Trials of EGFR Antibodies in Colorectal Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population</th>
<th>Outcome measure</th>
<th>WT KRAS</th>
<th>Mutant KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-arm studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lievre et al. [12]</td>
<td>89 patients treated with C, C + I, or C + 5-FU + I</td>
<td>n</td>
<td>65</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR 40%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS 31.4 wks</td>
<td>10.1 wks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS 14.3 mos</td>
<td>10.1 mos</td>
<td></td>
</tr>
<tr>
<td>De Roock et al. [9]</td>
<td>113 patients treated with C or C + I</td>
<td>n</td>
<td>67</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR 41%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PFS 43 wks</td>
<td>27.3 wks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OS 74.9 wks</td>
<td>30.6 wks</td>
<td></td>
</tr>
<tr>
<td>Kambata-Ford et al.</td>
<td>110 patients treated with C alone</td>
<td>n</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>[11]</td>
<td></td>
<td>RR 10%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Di Fiore et al. [10]</td>
<td>59 patients treated with C + chemotherapy</td>
<td>n</td>
<td>43</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR 10%</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Benvenuti et al. [8]</td>
<td>48 patients treated with C, P, or C + chemotherapy</td>
<td>n</td>
<td>32</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RR (p = NS) 31%</td>
<td>6%</td>
<td></td>
</tr>
</tbody>
</table>
Comparison of *KRAS* G12 and G13 mutations in lung CA and colorectal CA

<table>
<thead>
<tr>
<th>KRAS</th>
<th>Lung</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>290</td>
<td>231</td>
</tr>
<tr>
<td>(%)</td>
<td>28</td>
<td>35</td>
</tr>
</tbody>
</table>

- G12D is the most frequent mutation in never smokers
- G12C is the most frequent mutation in former and current smokers
- Never smokers are significantly more likely to have G>A transition mutation \((p<0.0001)\) (G12D, G13D, G12S)
- G>T transversion = most common nucleotide change in former and current smokers (G12C, G12V, G13C)
Additional* Mutations Detected by Sequenom genotyping in 652 consecutive CRC cases
(*other than KRAS G12/G13, BRAF ex 15)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>A146T</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Q61H</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Q61R</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Q61L</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>K117N</td>
<td>1</td>
</tr>
<tr>
<td>NRAS</td>
<td>Q61K</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>G12D</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>G12C</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G13D</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>G13R</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Q61R</td>
<td>1</td>
</tr>
<tr>
<td>AKT1</td>
<td>E17K</td>
<td>4</td>
</tr>
<tr>
<td>MAP2K1</td>
<td>K57N</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Number</th>
<th>Concurrent</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>R88Q</td>
<td>1</td>
<td>2 KRAS G12D</td>
</tr>
<tr>
<td></td>
<td>C420R</td>
<td>1</td>
<td>3 KRAS G12V</td>
</tr>
<tr>
<td></td>
<td>E542K</td>
<td>12</td>
<td>2 KRAS G12D, 1 KRAS G12S</td>
</tr>
<tr>
<td></td>
<td>E545K</td>
<td>21</td>
<td>7 KRAS G12D</td>
</tr>
<tr>
<td></td>
<td>E545A</td>
<td>2</td>
<td>2 KRAS G12D</td>
</tr>
<tr>
<td></td>
<td>H1047R</td>
<td>12</td>
<td>4 KRAS G12D</td>
</tr>
<tr>
<td></td>
<td>H1047L</td>
<td>1</td>
<td>3 KRAS G13D</td>
</tr>
<tr>
<td></td>
<td>H1047Y</td>
<td>1</td>
<td>1 KRAS G12C</td>
</tr>
</tbody>
</table>

*Additional Mutations Detected by Sequenom genotyping in 652 consecutive CRC cases (*other than KRAS G12/G13, BRAF ex 15)
Comparison of NRAS and additional KRAS (non-G12/G13) mutations in lung and colorectal CA

More common in CRC than in lung CA

<table>
<thead>
<tr>
<th>Additional</th>
<th>Lung</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>NRAS</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>1.74%</td>
<td>4.44%</td>
</tr>
</tbody>
</table>

Slide provided by Marc Ladanyi
Overall Distribution of Mutations in Colorectal Carcinoma
N = 652

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS Exon 2</td>
<td>35%</td>
</tr>
<tr>
<td>BRAF V600E</td>
<td>4%</td>
</tr>
<tr>
<td>Pending</td>
<td>61%</td>
</tr>
</tbody>
</table>

Routine clinical testing for KRAS G12/G13 mutations and BRAF V600E mutations

Sequenom detects mutations in an additional 10% of cases

Highly multiplexed Sequenom assays detect additional mutations in metastatic colorectal cancer.
Major recurrent, non-overlapping, activating mutations in signaling pathways in metastatic CRC

Best current estimate: approx. 50% of metastatic CRC contain one of the following driver oncogenes, all mutually exclusive:

- KRAS 40-45%
- BRAF 4-5%
- PIK3CA alone 3%
- NRAS 2%
- Other rare mutations (MEK1, AKT) 0.5%

Major undiscovered driver gene?
KRAS codon 61 and 146 mutations

- Mutations lead to activated RAS/RAF MAPK pathways
- Like patients with the common KRAS G12/G13 mutations, metastatic CRC patients with these mutations do not respond to anti-EGFR monoclonal antibodies

Median PFS 3.8 mo vs 5.1 mo WT

Wild-Type *BRAF* Is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer

Federica Di Nicolantonio, Miriam Martini, Francesca Molinari, Andrea Sartore-Bianchi, Sabrina Arena, Piercarlo Saletti, Sara De Dosso, Luca Mazzucchelli, Milo Frattini, Salvatore Siena, and Alberto Bardelli
Objective response of mCRC patients treated with EGFR-targeted monoclonal antibodies according to KRAS and PIK3CA/PTEN analysis

Predictive genotyping of mCRC for EGFR-targeted therapies

• Recommended mutations for routine genotyping (>1% prevalence):
  – KRAS: codons G12, G13 (could be first step in algorithm approach)
  – KRAS: minor codons Q61, A146
  – NRAS: codon Q61
  – BRAF: codon V600
  – PIK3CA: codons E542, E545, H1047

• Additional markers:
  – Additional negative predictors: PTEN mutation/loss, AKT1 E17K mutation, HER2 amplification (?)
  – Positive predictor: Expression of EGFR ligands EREG and AREG

• Recommended assay sensitivity: 1-10% range
  – May be reserved for difficult samples only
Brain neoplasms
Oligodendrogiomas

- Second most common primary brain tumor after astrocytomas
- Diffusely infiltrating glioma – often not fully removed surgically
- More likely to respond to PCV chemotherapy (procarbazine, lomustine, and vincristine)
- No clinical or pathologic features that allow accurate prediction of response
# Oligodendroglioma vs Astrocytoma

<table>
<thead>
<tr>
<th>Diagnostic Method</th>
<th>Oligodendroglioma</th>
<th>Astrocytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Imaging</strong></td>
<td>Cortical, well-demarcated, calcified</td>
<td>Central (subcortical), infiltrative</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Round, regular nuclei, Paucity of glial processes, Perineural satellitosis, Microcysts filled with mucin</td>
<td>Elongated, irregular nuclei, Abundant glial processes</td>
</tr>
<tr>
<td><strong>IHC</strong></td>
<td>Not generally useful, astrocytomas may express strong GFAP and p53, overlap.</td>
<td></td>
</tr>
<tr>
<td><strong>Genetics (by LOH or FISH)</strong></td>
<td>Loss of 1p in 80%, Loss of 19q in 80%, <strong>Loss of 1p and 19q in 60–80%</strong>, Losses in 9p and 10q increase with grade, No EGFR amplification</td>
<td>Loss of 1p in 30%–40%, Loss of 19q in 10%–15%, <strong>Loss of 1p and 19q in 5%</strong>, Losses in 9p and 10q increase with grade, EGFR amp in high grade astros (esp GBM)</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Chemosensitivity if combined loss of 1p and 19q, Better prognosis</td>
<td>Worse, with unclear significance of combined loss of 1p and 19q cases</td>
</tr>
</tbody>
</table>
LOH - Indications for Testing

- To assist in the diagnosis of oligodendroglial differentiation in brain tumors
- To identify patients with oligodendroglial tumors more likely to respond to PCV chemotherapy

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Markers</th>
<th>Back up marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p</td>
<td>D1S548, D1S1592, D1S552</td>
<td>D1S468, D1S1612, D1S496</td>
</tr>
<tr>
<td>19q</td>
<td>D19S219, D19S412, and PLA2G4C</td>
<td>D19S606, D19S1182</td>
</tr>
</tbody>
</table>
Microsatellite (LOH) vs FISH

- Requires normal tissue for comparison
- Dilution of tumor cells by non-tumor cells can reduce ability to detect 1p or 19q loss

FISH pictures provided by Dr. Lu Wang
IDH1/IDH2 mutations

- Somatic mutations in the isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) genes have been described in high frequency in gliomas, glioblastomas, and secondary glioblastomas.

- IDH1 is mutated in up to 75% of grade II and grade III diffuse gliomas.

*IDH1 is* mutated in up to 75% of grade II and grade III diffuse gliomas.
Indications for testing and clinical relevance

- **Diagnostic value:**
  - When the traditional diagnostic methods are inconclusive
  - Tumor vs. no tumor
  - Distinguishing pilocytic astrocytoma diffuse astrocytoma or oligodendroglioma

- **Prognostic:**
  - Associated with favorable prognosis

*Graph generated from compiled data
<table>
<thead>
<tr>
<th>Gene Exon #</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDH1 Exon 4</td>
<td>V71I</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132C</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132G</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132S</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132L</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132H</td>
</tr>
<tr>
<td>IDH1 Exon 4</td>
<td>R132H</td>
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<tr>
<td>IDH1 Exon 4</td>
<td>R140W</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R140Q</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R140L</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R172G</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R172M</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R172K</td>
</tr>
<tr>
<td>IDH2 Exon 4</td>
<td>R172S</td>
</tr>
</tbody>
</table>

IDH1 R132H is the most common mutation

![Gene expression profile](image.png)
EGFR vIII

- EGFR is amplified/over-expressed and/or mutated in 40-50% of high grade gliomas
- EGFRvIII is the most common EGFR mutation seen in high grade gliomas
- Favorable clinical response to EGFR targeted inhibitors gefitinib and erlotinib
EGFRvIII

- Truncated EGFR
- 801bp deletion involving exons 2 to 7 of the extracellular ligand-binding domain
- Leads to ligand-independent tyrosine kinase activity and downstream pathway activation.
Breast cancer

• First histologic type of carcinoma to incorporate robust biomarkers in daily practice

• Classified in various ways
  – Stage
  – Grade
  – ER/PR status
  – HER2 status
  – Gene Signatures
HER2 testing

• Prognostic:
  – HER2+ associated with higher grade tumors and more likely to metastasize

• Predictive:
  – Benefit from targeted therapy (trastuzumab and lapatinib)

• Testing based on a ratio of HER2 to CEP17 signals
  – >2 amplified
  – 1.8-2.1 equivocal range requires recounting of wider areas
## Gene expression profiles

<table>
<thead>
<tr>
<th></th>
<th>Oncotype Dx</th>
<th>MammaPrint</th>
<th>Theros</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provider</strong></td>
<td>Genomic Health</td>
<td>Agendia</td>
<td>Biotheranostics</td>
</tr>
<tr>
<td><strong>Type of assay</strong></td>
<td>21 gene recurrence score</td>
<td>70 gene assay</td>
<td>2 gene ratio of HOXB13 to IL17R and molecular grade index</td>
</tr>
<tr>
<td><strong>Type of tissue</strong></td>
<td>FFPE</td>
<td>Fresh frozen</td>
<td>FFPE</td>
</tr>
<tr>
<td><strong>Q-RT-PCR</strong></td>
<td>Q-RT-PCR</td>
<td>DNA microarray</td>
<td>Q-RT-PCR</td>
</tr>
<tr>
<td><strong>Indication</strong></td>
<td>Predicts <strong>risk of distant recurrence in ER positive</strong>, node negative disease treated with tamoxifen. Identify pts at low risk of recurrence who may not need adjuvant chemo</td>
<td>Prognostic prediction in pts &lt;61yrs stage I or II, node negative disease, tumor &lt; 5cm</td>
<td><strong>Stratify ER-positive patients</strong> into groups with a predicted low risk or high risk of recurrence and a predicted good or poor response to endocrine therapy</td>
</tr>
<tr>
<td><strong>FDA clearance</strong></td>
<td>NO</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Availability</strong></td>
<td>Europe and US</td>
<td>Europe and US</td>
<td>US</td>
</tr>
</tbody>
</table>

- **Gene expression profiles**

- **Oncotype Dx**: Predicts risk of distant recurrence in ER positive, node negative disease treated with tamoxifen. Identify pts at low risk of recurrence who may not need adjuvant chemo.

- **MammaPrint**: Prognostic prediction in pts <61yrs stage I or II, node negative disease, tumor <5cm.

- **Theros**: Stratify ER-positive patients into groups with a predicted low risk or high risk of recurrence and a predicted good or poor response to endocrine therapy.
Oncotype Dx

• Measures
  – Proliferation (Ki67, STK15, survivin, cyclin B1, MYBL2)
  – Invasion (Stromolysin 3, cathepsin L2)
  – HER2 (GRB7, HER2)
  – Estrogen (ER, PGR, BCL2, SCUBE2)
  – Other (GTSTM1, CD68, BAG1)
  – Reference genes (Delta-actin, GAPDH, RPLPO, GUS, TFRC)
Gastrointestinal stromal tumors

- Most common soft tissue tumor of the GI tract (gastric 60%, small intestine 35%)
- IHC + for KIT (CD117) and CD34
- Prognostic - tumor size and mitotic activity
- Unresponsive to standard chemotherapy but responsive to TKI therapy (Gleevec)
- Specific mutations predict response to therapy.
KIT and PDGFR

- Nearly all cases have a TK mutation
- KIT (95%), PDGFR (5%) - Mutually exclusive
- Morphologic correlate:
  - KIT+ spindled morphology
  - PDGFR+ epithelioid morphology
The *KIT* and *PDGFR* genes encode highly homologous TK’s.

Both genes located on 4q12
<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>frequency</th>
<th>Mutation</th>
<th>Clinical correlate</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
<td>9</td>
<td>10-15%</td>
<td>Dup ins 501-502</td>
<td>Malignant behavior, Small intestine Intermediate response</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td><strong>20-60%</strong></td>
<td>Del ins 550-561, ITD’s or pt mutations</td>
<td>Deletion – poor prognosis, good response to TKI</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>&lt;5%</td>
<td>Pt mutation 642</td>
<td>Poor response to TKI</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>&lt;1%</td>
<td>Pt mutation 820</td>
<td>Poor response to TKI</td>
</tr>
<tr>
<td>PDGFR</td>
<td>12</td>
<td>1%</td>
<td>Pt mutation 561, Indel 560-571</td>
<td>Good response to TKI</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>&lt;0.5%</td>
<td>Ins 582-586</td>
<td>response to TKI (in vitro)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>2-3%</td>
<td>Pt mutation 659</td>
<td>D842V resistant to imatinib, Other - good response to TKI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pt mutation 842</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Del-sub 842-847</td>
<td></td>
</tr>
</tbody>
</table>

- **Secondary resistance mutations**
  - *KIT* exon 13 *K642E*, *KIT* exon 17 *Y823D*
Questions