Molecular Profiling of Tumors

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Co-Director, Breast Health Center, Women & Infants Hospital
Warren Alpert Medical School of Brown University
The sub-classification of tumors has been improved through the identification of more cohesive biologic groups with the goal of improved outcomes through a personalized medicine approach.
63 year old male, engineer, 4 year history of smoking in the navy quitting 40 years ago

April 2017- Cough, yellow sputum, occasional blood streaked sputum
May 2017- Shortness of breath, weight loss. No response to antibiotics
July 14, 2017:

Metastatic Non-Small Cell Carcinoma, Adenocarcinoma with Neuroendocrine Features

EGFR Exon 19 Mutation:
NM_005228.3(EGFR):c.2236_2250delGAATTAAGAGAAGCA (p.Glu746_Ala750del)
EGFR tyrosine kinase domain mutations in NSCLC confer sensitivity to EGFR tyrosine kinase inhibitors (TKIs)

- Exon 19 deletion
- L858R
- G719 (S,C,A) (in-frame)
Response to First Generation EGFR Inhibitor Erlotinib

September 2, 2017 - begins Erlotinib

8/10/2017

11/2/2017
Response to Third Generation EGFR Inhibitor Osimertinib

March 2018- Profoundly short of breath, large pericardial effusion, tamponade physiology
Peripheral blood sent off for mutation analysis
Transferred to Yale from Westerly ER, hospitalized X 8 days, 1035cc fluid removed from pericardium.
NCCN Guidelines Version 4.2019
Non-Small Cell Lung Cancer
NCCN Evidence Blocks™

Clinical Presentation

Establish histologic subtype with adequate tissue for molecular testing (consider rebiopsy if appropriate)
• Smoking cessation counseling
• Integrate palliative care (See NCCN Guidelines for Palliative Care)

Histologic Subtype

• Adenocarcinoma
• Large cell NSCLC not otherwise specified (NOS)

Testing

Molecular testing

EGFR mutation testing (category 1)
• ALK testing (category 1)
• ROS1 testing
• BRAF testing

Testing should be conducted as part of broad molecular profiling

PD-L1 testing (category 1)

Testing Results

Sensitizing EGFR mutation positive (see NSCL-18)

ALK positive (see NSCL-21)

ROS1 positive (see NSCL-24)

BRAF V600E positive (see NSCL-25)

PD-L1 ≥1% and EGFR, ALK negative or unknown (see NSCL-27)

EGFR, ALK, ROS1, BRAF negative or unknown. PD-L1 <1% or unknown (see NSCL-28)

Sensitizing EGFR mutation positive (see NSCL-18)

ALK positive (see NSCL-21)

ROS1 positive (see NSCL-24)

BRAF V600E positive (see NSCL-25)

PD-L1 ≥1% and EGFR, ALK negative or unknown (see NSCL-27)

EGFR, ALK, ROS1, BRAF negative or unknown. PD-L1 <1% or unknown (see NSCL-29)

Testing should include the neurotrophic receptor tyrosine kinase (NTRK) gene fusion; if positive, see NSCL-28.

In patients with squamous cell carcinoma, the observed incidence of EGFR mutations is 2.7% with a confidence that the true incidence of mutations is less than 3.6%. This frequency of EGFR mutations does not justify routine testing of all tumor specimens. Forbes SA, Bhamra G, Bamford S, et al. The catalogue of somatic mutations in cancer (COSMIC). Curr Protoc Hum Genet. 2008;chapter 10: unit 10.11.


Note: For more information regarding the categories and definitions used for the NCCN Evidence Blocks, see page 881.

All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

NSCL-17
Target Therapy for Advanced or Metastatic Disease

Emerging Biomarkers to Identify Novel Therapies

**Sensitizing EGFR Mutation Positive**
- First-line therapy
  - Alatinib
  - Erlotinib
  - Dacomitinib
  - Gefitinib
  - Osimertinib
- Subsequent therapy
  - Osimertinib

**BRAF V600E Mutation Positive**
- First-line therapy
  - Dabrafenib/trametinib
- Subsequent therapy
  - Dabrafenib/trametinib

**NTRK Gene Fusion Positive**
- First-line/Subsequent therapy
  - Larotrectinib

**ALK Rearrangement Positive**
- First-line therapy
  - Alectinib
  - Brigatinib
  - Ceritinib
  - Crizotinib
- Subsequent therapy
  - Alectinib
  - Brigatinib
  - Ceritinib
  - Lorlatinib

**ROS1 Rearrangement Positive**
- First-line therapy
  - Ceritinib
  - Crizotinib

<table>
<thead>
<tr>
<th>Genetic Alteration (ie, Driver event)</th>
<th>Available Targeted Agents with Activity Against Driver Event in Lung Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-level MET amplification or MET exon 14 skipping mutation</td>
<td>Crizotinib¹⁻⁵</td>
</tr>
<tr>
<td>RET rearrangements</td>
<td>Cabozantinib⁶,⁷ Vandetanib⁸</td>
</tr>
<tr>
<td>ERBB2 (HER2) mutations</td>
<td>Ado-trastuzumab emtansine⁹</td>
</tr>
<tr>
<td>Tumor mutational burden (TMB)*</td>
<td>Nivolumab + ipilimumab¹⁰ Nivolumab¹¹</td>
</tr>
</tbody>
</table>

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy. There is no consensus on how to measure TMB.
Reflex Testing for NSCLC Hotspot Driver Mutations – next-generation sequencing

50 gene panel = Ion AmpliSeq™ Cancer Hotspot Panel v2

### Appendix - Genes analyzed
This test only analyzes targeted regions ("hotspots") of the exons known to be frequently mutated in cancer.

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<td>NOTCH1</td>
<td>PTEN</td>
<td>SMARCB1</td>
<td>VHL</td>
</tr>
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</table>

Tumor DNA-only analysis (no matched normal DNA; no tumor RNA)

Alternative for specimens with very limited tumor tissue: TaqMan PCR-based genotyping assays
(for the most common mutations in EGFR, KRAS, ERBB2, BRAF and 3 other genes)
Extended Molecular Testing of NSCLC – Oncomine Comprehensive Assay

<table>
<thead>
<tr>
<th>Hotspot genes</th>
<th>Full-length genes</th>
<th>CNV genes (amplification)</th>
<th>Gene fusions (inter- &amp; intragenic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1, ERBB2, HNF1A, MED12, RAF1</td>
<td>ARID1A, MRE11A, RAD51C</td>
<td>AKT1, FGF3, PDGFRA</td>
<td>AKT2, JAK2, PRKACB</td>
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<tr>
<td>AKT2, ERBB3, HRAS, MET, RET</td>
<td>ATM, MSH2, RAD51D</td>
<td>AKT2, FGF1, PDGFRA</td>
<td>ALK, Kras, RAF1</td>
</tr>
<tr>
<td>AKT3, ERBB4, IDH1, MYC, RHEB</td>
<td>ATR, MSH6, RB1</td>
<td>AKT3, FGF2, PIK3CA</td>
<td>AR, MDM4, RELA</td>
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<tr>
<td>ALK, ERCC2, IDH2, MYC, RHOA</td>
<td>ATRX, NBN, RNF43</td>
<td>ALK, FGF3, PIK3CB</td>
<td>AXL, MET, RET</td>
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<tr>
<td>AR, ESR1, JAK1, MYCN, ROS1</td>
<td>BAP1, NF1, SETD2</td>
<td>AR, FGF4, PPARG</td>
<td>BRAF, MYB, ROS1</td>
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<tr>
<td>ARAF, EZH2, JAK2, MYD88, SFB1</td>
<td>BRCA1, NF2, SLX4</td>
<td>AXL, FLT3, RICTOR</td>
<td>EGFR, MYBL1, RSPO2</td>
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<tr>
<td>AXL, FGFR1, JAK3, NFE2L2, SMAD4</td>
<td>BRCA2, NOTCH1, SMARCA4</td>
<td>BRAF, IGF1R, TERT</td>
<td>ERBB2, NOTCH1, RSPO3</td>
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<tr>
<td>BRAF, FGFR2, KDR, Nras, SMO</td>
<td>CDK12, NOTCH2, SMARC8</td>
<td>CCND1, KIT</td>
<td>ERBB4, NOTCH4, TERT</td>
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<tr>
<td>BTK, FGFR3, KIT, NTRK1, SPOP</td>
<td>CDKN1B, NOTCH3, STK11</td>
<td>CCND2, KRAS</td>
<td>ERG, NRG1</td>
</tr>
<tr>
<td>CBL</td>
<td>CDKN2A, PALB2, TP53</td>
<td>CCND3, MDM2</td>
<td>ESR1, NTRK1</td>
</tr>
<tr>
<td>CCND1, FLT3, KRAS, NTRK2, SRC</td>
<td>CDKN2B, PIK3R1, TSC1</td>
<td>CCNE1, MDM4</td>
<td>ETV1, NTRK2</td>
</tr>
<tr>
<td>CDK4, FOXL2, MAGOH, PDGFRB</td>
<td>CREBBP, POLE</td>
<td>CDK2, MET</td>
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<td>CDK6, GATA2, MAP2K1, PDGFRB</td>
<td>FANCA, PCH1</td>
<td>CDK4, MYC</td>
<td>ETV5, NUTM1</td>
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<td>CHEK2, GNA11, MAP2K2, PIK3CA, U2AF1</td>
<td>FANC2, Pten</td>
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<td>FANCI, RAD50</td>
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<td>FBXW7, RAD51</td>
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<td>DDR2, H3F3A, MAX, PTPN11</td>
<td>MLH1, RAD51B</td>
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<td>EGFR, HIST1H3B, MDM4, RAC1</td>
<td>Tumor DNA and RNA are analyzed, as well as patient-matched normal DNA (germline).</td>
<td>FGF19, NTRK3</td>
<td>FLT3, PRKACA</td>
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What targetable alteration might be found by Oncomine in a NSCLC that is negative for drivers on reflex testing?

**Gene fusions:** NTRK1, NTRK2, NTRK3  
RET, NRG1  
EGFR, BRAF  
FGFR1, FGFR2, FGFR3

**Gene amplifications:** MET, EGFR, ERBB2  
PIK3CA, RICTOR

**MET exon 14 skipping:**
Liquid Biopsy and Genomics-Driven Oncology

Non-Invasive
More Comprehensive view of Tumor Genome

Serial Monitoring of Tumor Genotypes
-emergence of resistance
-VAF correlating with tumor volume
-new druggable targets

Artificial-Intelligence Based Bioinformatics Tools
Factors Influencing the Sensitivity of a Plasma Cell-Free DNA Test

Technical and physiological factors limit broad implementation of liquid biopsies in clinical practice. The achievable sensitivity of a circulating cell-free DNA (cfDNA) test on plasma is dependent on several factors, including sampling volume, the number of input molecules, the tumour fraction and the analytical sensitivity of the method being used. GE, genomic equivalents.
Amount of Input Cell-Free DNA Affects Ability to Detect Rare Variants
Tumor DNA Sequencing Report

Patient: John Doe
MR #: 1110000
DOB/Age/Sex: 12/30/1951 (Age: 65) M
Visit #: 123456789 (Referral)
Submitting Physician: Jane Smith, M.D.
Accession #: MP17-XX
Taken: 1/19/2017
Accessioned: 2/13/2017 16:11 YNHH
Adm-Disch Date: 1/19/2017 - 01/23/17
Reported: 02/24/2017

Results

CANCER MUTATION HOTSPOT (50 GENE) SEQUENCING PANEL

Specimen information: Colonic adenocarcinoma, omental nodule, excision (YNHH S17-XXXX, Part 1, Block 2 (50% estimated malignant cells).

DNA VARIANT DETECTED ALLELIC FRACTION

KRAS c.38G>T (p.Gly13Asp) 22%

INTERPRETATION:
KRAS, a member of the Ras family of small GTPases that mediate signal transduction downstream of growth factor receptors, plays a critical role in cell proliferation, survival and differentiation. KRAS variants are found in 30-40% of colorectal adenocarcinomas. According to NCCN guidelines, KRAS activating mutations predict a lack of response to therapy with antibodies targeted to the epidermal growth factor receptor (e.g., cetuximab, panitumumab). [NCCN Guidelines Colon Cancer Version 1.2017] The variant allelic fraction is consistent with heterozygosity within the malignant cells in this sample, and therefore this tumor is considered positive for this mutation.
ADDITIONAL DNA VARIANTS DETECTED

**SMAD** c.1245_1248delCAGA (p.Asp415GlufsTer20) 21%
**TP53** c.916 C>T(p.Arg306Ter) 20%

To date, the published evidence relating these additional genetic changes to cancer biology or treatment response may be insufficient to use this information for decisions regarding patient management outside the context of clinical research.

**Appendix - Genes analyzed**

This test only analyzes targeted regions ("hotspots") of the exons known to be frequently mutated in cancer.

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**Methodology**

The clinical tumor sample was enriched for malignant cells by manual microdissection of formalin-fixed, paraffin-embedded (FFPE) tissue sections. DNA was extracted using a commercially available kit (Qiagen, Inc.) and quantitated on a Qubit 2.0 fluorimeter. The isolated DNA was amplified using the Ion AmpliSeq Cancer Hotspot Panel v2 multiplex PCR primer set. These primers amplify 207 amplicons covering exonic regions of 50 cancer-related genes in which mutations have been reported in various types of cancer. In aggregate, ~30 kilobases of DNA sequence were amplified for subsequent nucleotide sequence analysis, which was performed on either an Ion Torrent PGM™ or an S5™ XL next generation sequencer (Thermo Fisher Scientific). The raw data generated were pre-processed within Torrent Suite, with the alignment of sequencing reads performed by the Torrent Mapping (TMAP) algorithm. Variants identified by the Ion Reporter (IR), MuTect2 and Strelka variant callers were passed through Ensembl Variant Effect Predictor (VEP) to derive annotations from multiple genomic databases. Chromosomal positions of variants refer to the human reference sequence assembly released by the Genome Reference Consortium in February 2009 (GRCh37). Unless otherwise indicated, the relative abundances (allelic fractions) of variants in tumor DNA were calculated on the basis of at least 100 sequencing reads spanning the region of DNA containing the variation. Variants corresponding to common germline polymorphisms (minor allele frequency >0.001) are not reported. The reported allelic fractions should be regarded as approximate and considered within the context of the estimated concentration of malignant cells in the material analyzed. The analytic sensitivity of this test for single nucleotide variants (SNVs) is estimated to be 5%. This assay examines tumor tissue only and does not examine normal (non-tumor) tissue. The test was designed to find gene mutations within tumors (somatic variants). It was not designed to find germline (or hereditary) mutations.
Results

Targeted NGS Cancer Gene Panel (Oncomine Assay)

Tumor specimen analyzed: YNHH S17-7221, Part 1, Block 1
Site / Organ: Lung, left upper lobe, endobronchial bx
Histology: Favor adenocarcinoma (large and spindle cells)
Percent malignant cells (after manual microdissection): 60%
Normal (germline) control specimen: buccal swab

This assay examines tumor DNA for mutations and/or amplifications in 134 cancer-related genes. This assay also examines tumor RNA for the presence of 271 fusion transcripts involving 24 oncogetic driver genes.

Somatic variant detected in the tumor:

Variant Allelic Fraction
TP53 Splicing 29%

Variants are reported as their predicted amino acid changes. Allelic fraction indicates the percentage of DNA sequencing reads containing the variant and has not been corrected for the estimated fraction of tumor cells in the sample or for gene copy number within tumor cells. Double asterisks (**) indicate variants that may be amenable to targeted therapy; see interpretation.

No gene amplifications (copy number ≥5) were detected in the tumor.
The following gene fusion was detected in the tumor:

**MET(13)**-**MET(15)**

Double asterisks (**) indicate oncogenic driver genes that may be amenable to targeted therapy; see Interpretation

**Gene Fusion:**

**Fusion name and database ID:** MET(13)-MET(15)

**Oncogenic driver gene:** MET

**Additional notes:** The MET gene encodes a receptor tyrosine kinase that is activated by hepatocyte growth factor and transduces signals through the RAS-RAF-MAPK, PI3K-AKT-mTOR, and PLCgamma-PKC pathways. Mutations and deletions that affect MET exon 14 splicing have been recently shown to cause oncogenic MET activation by a mechanism called "exon 14 skipping", in which the loss of part of the juxtamembrane domain (encoded by exon 14) generates a form of the protein that cannot be down-regulated by Cbl. [Awad MM. Impaired c-Met receptor degradation mediated by MET exon 14 mutations in non-small-cell lung cancer. J Clin Oncol. 2016; 34:879-81] Somatic mutations that result in MET exon 14 skipping occur most frequently in lung cancer of various types, especially pulmonary sarcomatoid carcinoma (5%) [Liu X, et al. Next-generation sequencing of pulmonary sarcomatoid carcinoma reveals high frequency of actionable MET gene mutations. J Clin Oncol. 2016; 34:794-802] and have also been identified in other tumor types, including carcinomas of unknown origin (0.4%). [Frampton GM, et al. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. Cancer Discov. 2015; 5:850-9] Importantly, tumors driven by this activated, oncogenic form of MET have shown clinical sensitivity to tyrosine kinase inhibitors such as crizotinib and cabozantinib. [Paik PK, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. Cancer Discov. 2015; 5:842-9] In a significant fraction of MET exon 14 skipping variants, there is no MET splice site mutation that causes the exon skipping, but rather a deletion involving one of the splice sites. The Oncomine sequencing assay cannot detect these deletions so the variant is only detected by the RNA fusion component.
Targeted NGS Cancer Gene Panel (Oncomine Assay)

Tumor specimen analyzed: Mercy Hospital, 14-8484, Part 1, Block 4
Site / Organ: Pelvic lymph node.
Histology: Metastatic prostatic adenocarcinoma.
Percent malignant cells (after manual microdissection): 60%
Normal (germline) control specimen: Buccal swab.

This assay examines tumor DNA for mutations and/or amplifications in 134 cancer-related genes. This assay also examines tumor RNA for the presence of 183 fusion transcripts involving 22 oncogenic driver genes.

Variants detected in both the tumor and normal (germline) tissue:

<table>
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<tr>
<th>Variant</th>
<th>Frequency (Tumor)</th>
<th>Frequency (Normal)</th>
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<tr>
<td>BRCA2^{*} S3147fs</td>
<td>51%</td>
<td>47% (Pathogenic)</td>
</tr>
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Variants are reported as their predicted amino acid changes. Frequency indicates the percentage of DNA sequencing reads containing the variant and has not been corrected for the estimated fraction of tumor cells in the sample or for gene copy number within tumor cells. Double asterisks (**) indicate variants that may be amenable to targeted therapy; see Interpretation.

No somatic single nucleotide or multi-nucleotide variants were detected in the tumor.
No gene amplifications (copy number ≥5) were detected in the tumor (See Note).
No gene fusions were detected in the tumor.

Note: The ability of this assay to detect copy number gains (≥5) may be reduced in DNA isolated from this tumor, because the sample was estimated to contain only 60% malignant cells.
**Interpretation (Tumor DNA Sequencing):**

**Gene name:** BRCA2 (breast cancer 2, early onset)

**Variant (protein):** S3147fs (p.Ser3147CysfsTer2)

**Variant (coding DNA):** c.9435_9436delGT (ENST00000544455)

**Previously reported:** No

**Predicted effects:** Damaging; loss of tumor suppressor function

**Additional notes:**
- BRCA1 and BRCA2 are tumor suppressor proteins involved in maintenance of genome stability, specifically the homologous recombination pathway for double-strand DNA repair.
- Germline pathogenic variants in BRCA1 or BRCA2 cause Hereditary Breast and Ovarian Cancer Syndrome, which is characterized by an increased risk for breast cancer, ovarian cancer, prostate cancer, and pancreatic cancer. The lifetime risk of prostate cancer in a carrier of a pathogenic BRCA1 or BRCA2 mutation has been estimated to be as high as 39%. The BRCA2 dinucleotide deletion variant in this case (p.Ser3147CysfsTer2) is predicted to result in a frameshift at amino acid 3147 (of 3418) and thus is expected to have a damaging effect on the protein function. This BRCA2 frameshift variant was detected at a frequency of 47% in DNA isolated from the patient's buccal swab and at a frequency of 51% in DNA isolated from the tumor. These findings suggest that the BRCA2 p.Ser3147CysfsTer2 variant is a heterozygous germline mutation that is also present as a heterozygous mutation in the tumor. The frequency of this variant in DNA isolated from the tumor does not suggest that the BRCA2 gene has undergone loss of heterozygosity in the tumor through deletion of the wild-type allele; however, it is possible that the second BRCA2 allele in this tumor might be inactivated by an epigenetic or genetic mechanism that is not detected by this assay. Tumor cells harboring defects in DNA repair have been shown to be highly sensitive to inhibition of Poly (ADP-ribose) polymerases (PARPs), a family of enzymes involved in repair of single-strand DNA breaks. [Ricks TK, et al. Successes and Challenges of PARP Inhibitors in Cancer Therapy. Front Oncol. 2015 Oct 14;5:222. doi: 10.3389/fonc.2015.00222. eCollection 2015.] In a clinical trial conducted in patients with metastatic, castration-resistant prostate cancer, those patients whose tumors harbored defects in DNA-repair genes, including BRCA2, showed a high response rate to the PARP inhibitor olaparib. This included patients with biallelic somatic loss of BRCA2 as well as patients with germline BRCA2 mutation. [Mateo J, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med. 2015 Oct 29;373(18):1697-708.] Given that the BRCA2 pathogenic variant was detected in DNA from the patient's non-tumor tissue, genetic counseling is strongly recommended for this patient.
Thank You

Janina Longtine, MD
Zenta Walther, MD, PhD
Ira Sitko, MD