Going Beyond the Standard of Care: Translation of Personalized Medicine into the Clinical Oncology Setting

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Objectives

- Discuss the current role of somatic genetic testing in clinical practice
- Explain the purpose and value of a molecular tumor board in terms of treatment recommendations
- Identify future challenges to the implementation of genetic-guided therapy into standard oncology clinical practice

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Guidelines are backward looking

With cancer, things change too rapidly for doctors to be able to rely on yesterday's guidelines for long.

Vincent T. DeVita, Jr, MD

The Death of Cancer

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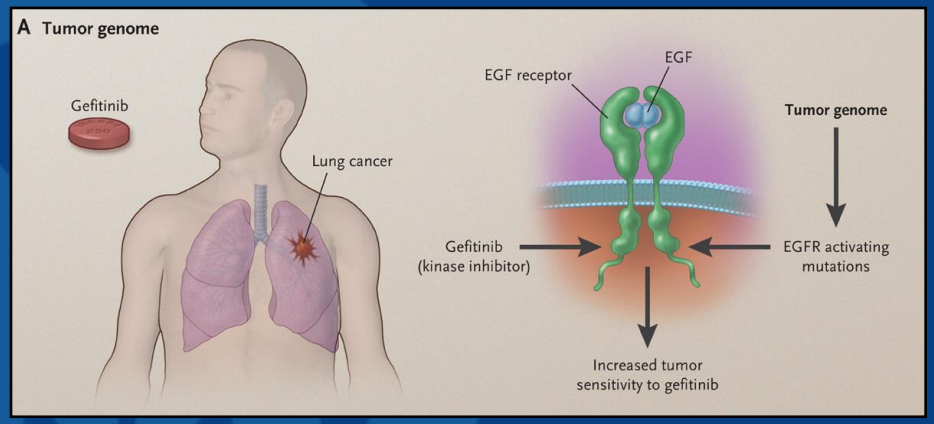
Tumor vs. Patient Genome

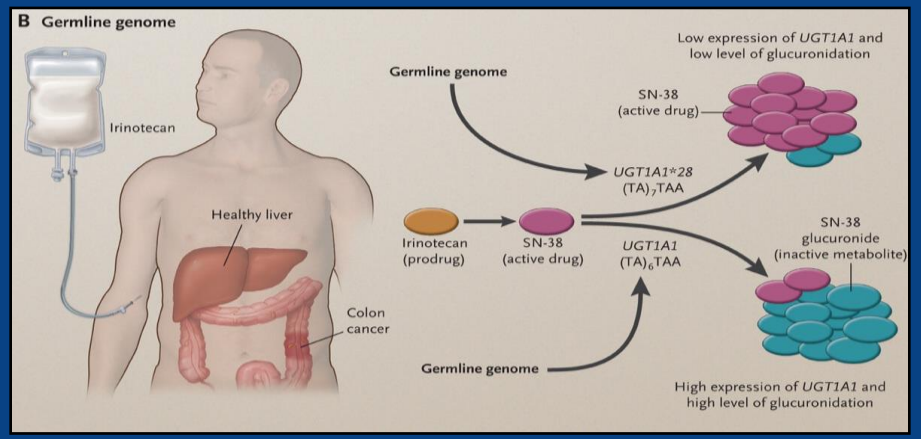
Tumor Genome

- Acquired genetic variation
- Predicts tumor response
  - HER2: trastuzumab
  - BCR-ABL: imatinib
  - BRAF V600E: vemurafenib
  - ALK+: crizotinib

Patient Genome

- Inherited genetic variation
- Predicts drug exposure
  - Enzymes
  - Transporters
- Predicts toxicity
  - Drug targets





Wang L et al. N Engl J Med 2011;364:1144-1153.

Targeting the Tumor Genome

- Genetic alterations in molecular pathways are involved in tumor development, survival, and progression/metastases
- We have the technology! We can profile it!
- Targeted anticancer drugs are available commercially or in clinical trials

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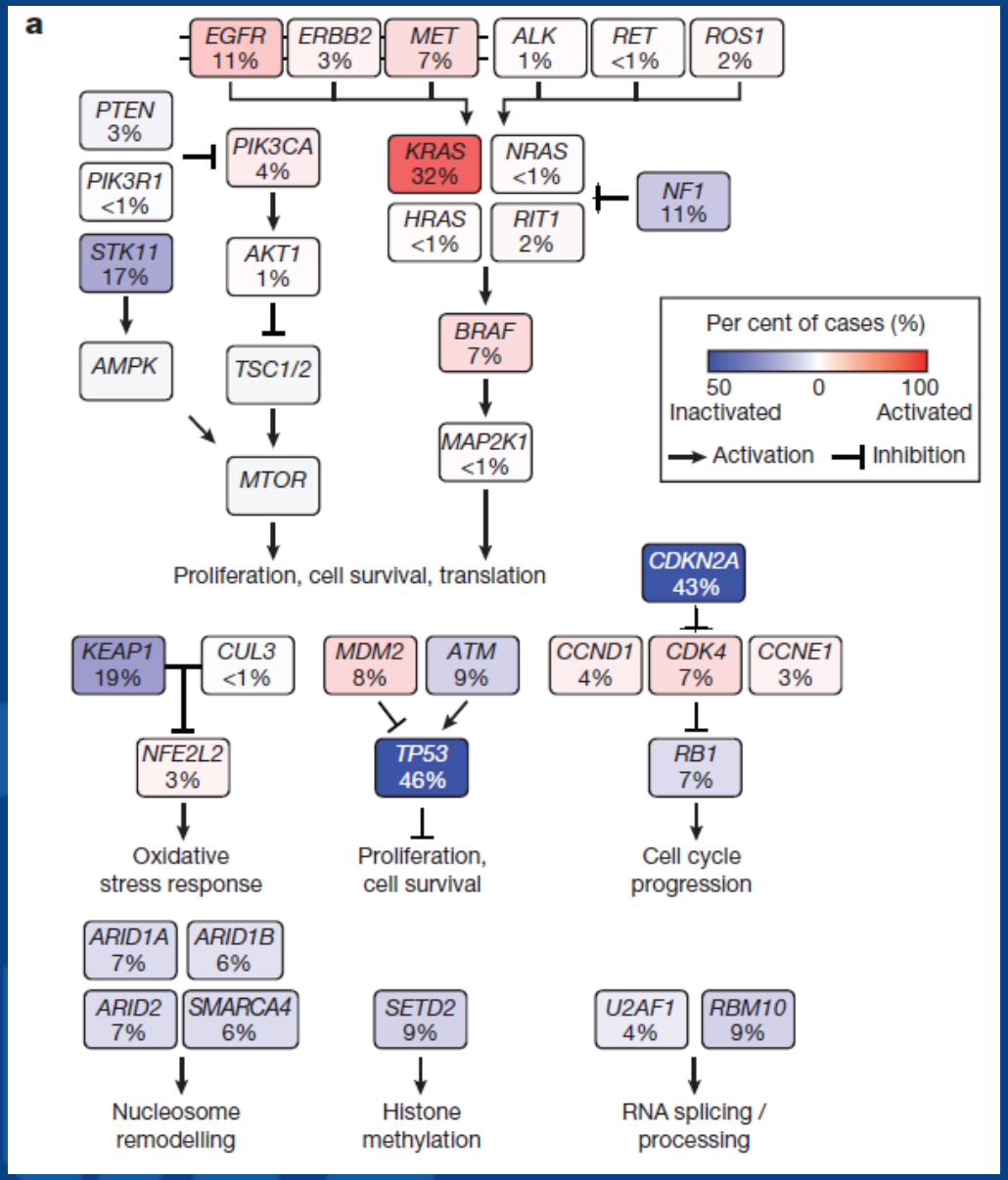
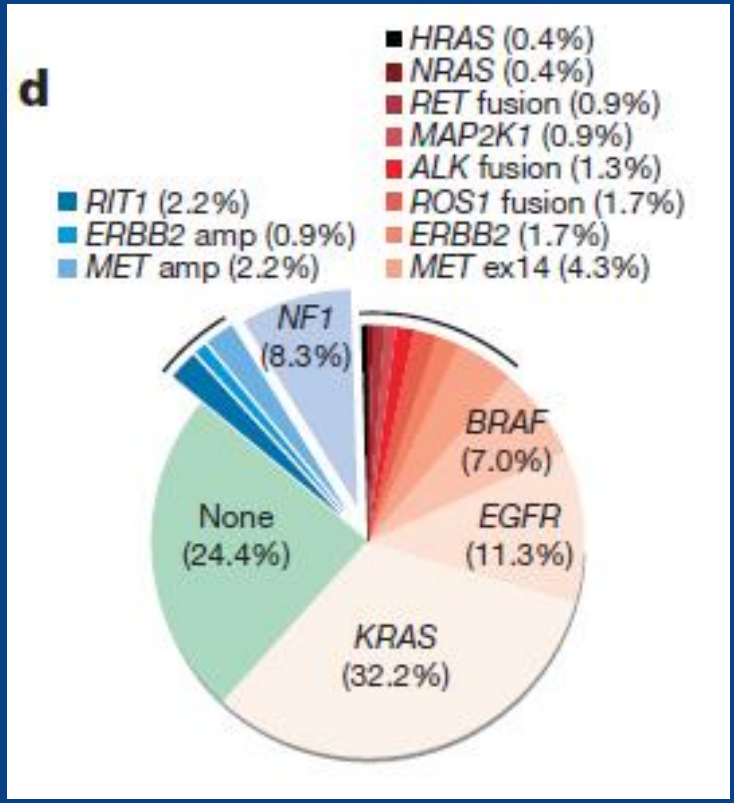
Garraway LA. J Clin Oncol. 2013;31:1806-1814.

Genetic Alterations in Lung Cancer





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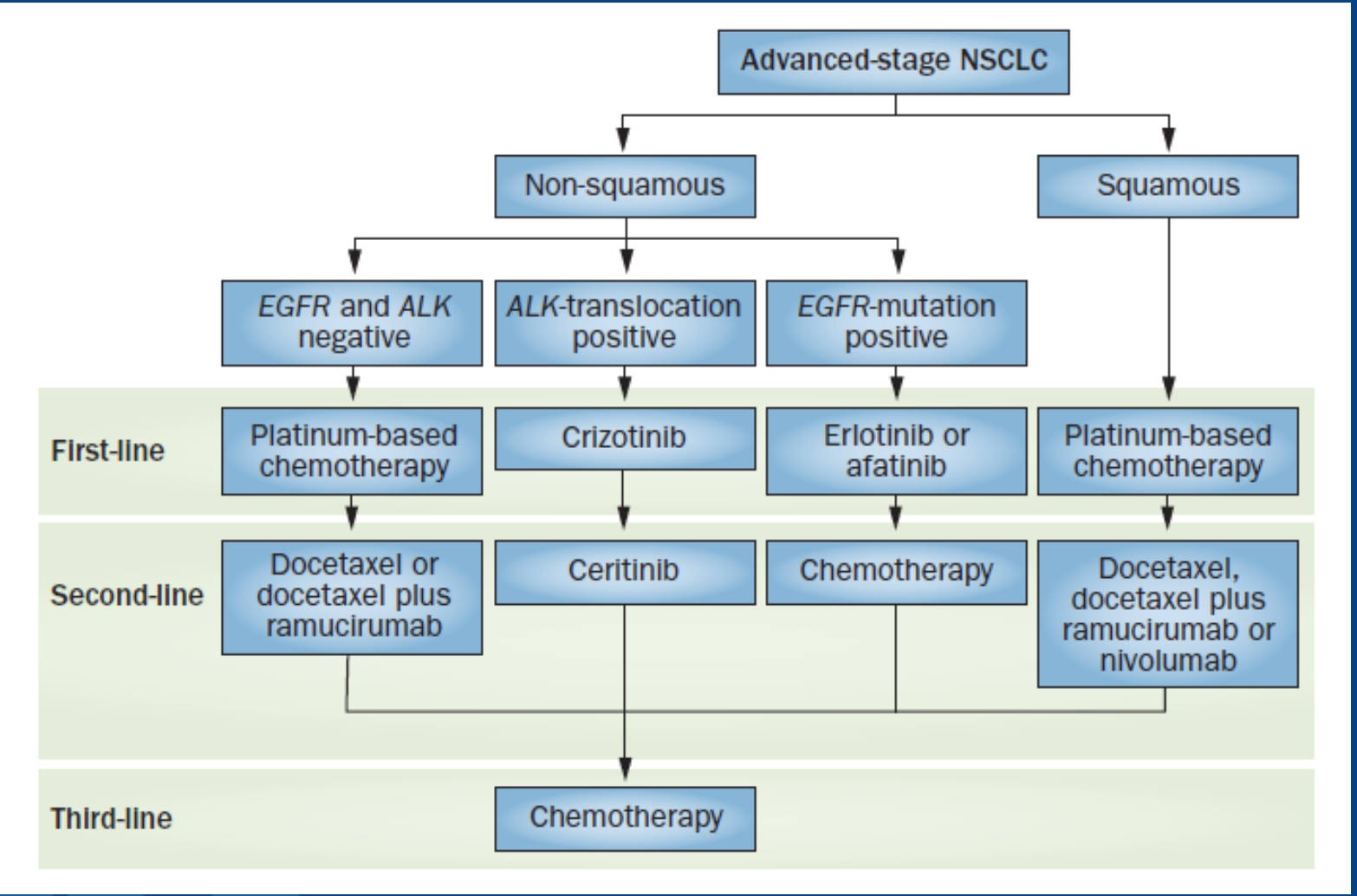
The Cancer Genome Atlas Research Network. Nature. 2014;511:543-549.

Current Treatment for NSCLC



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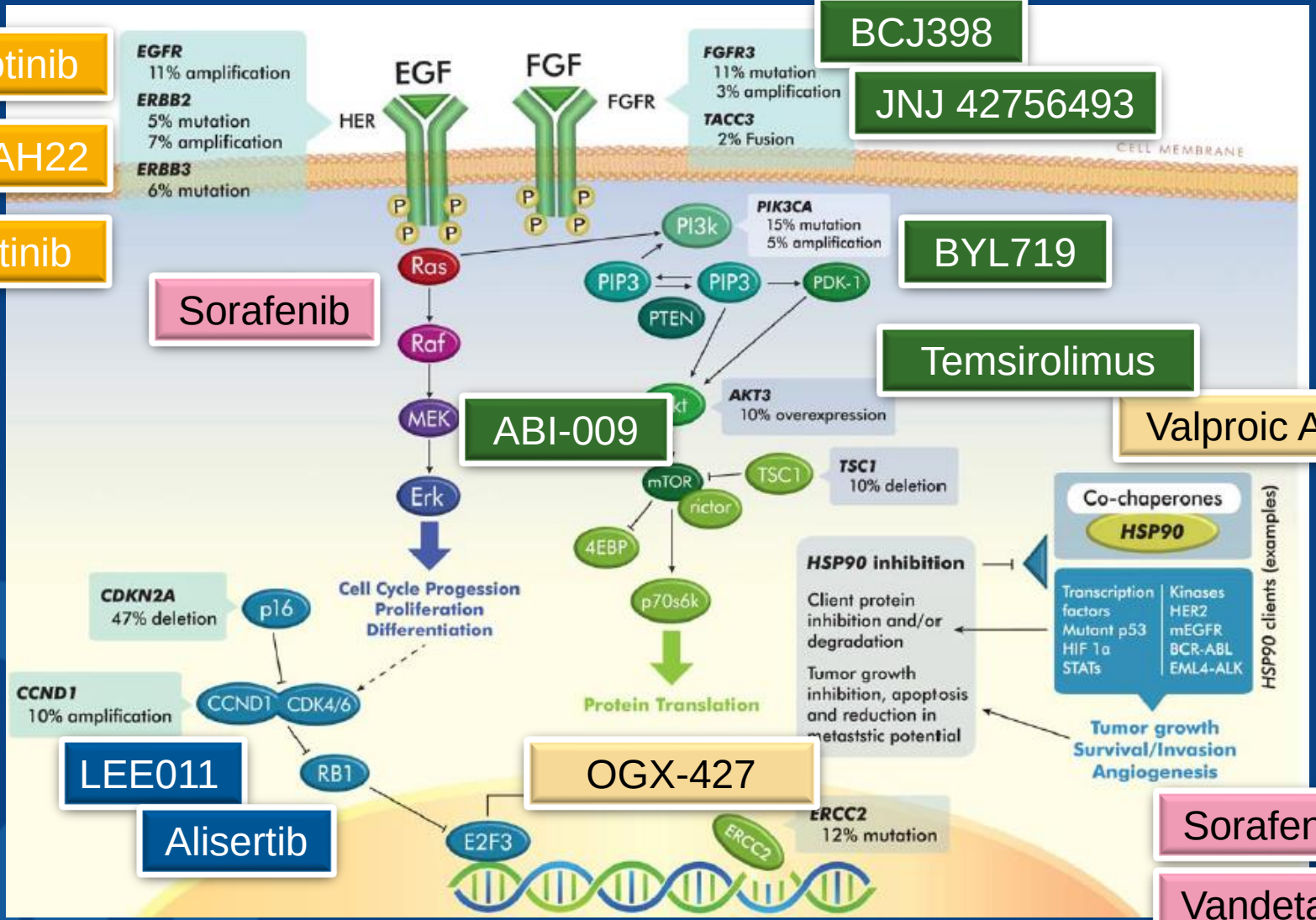
Nat Rev Clin Oncol. 2015;12:511-526

Targeted Therapy Options



Erlotinib

MGAH22

Afatinib

Sorafenib

BCJ398

JNJ 42756493

BYL719

Temsirolimus

Valproic Acid

ABI-009

LEE011

Alisertib

OGX-427

Sorafenib

Vandetanib

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Balar AV and Milowsky MI. Cancer. 2014;ahead of print.

Targeting Therapy in Lung Cancer

BRAF

- Mutations seen in up to 7% of NSCLC with more than half being the V600E mutation which is associated with more aggressive disease
- **Dabrafenib** and **trametinib,** or **vemurafenib**

MET

- Exon 14 skipping seen in 3-4% of NSCLC
- Amplification in 2-4% untreated patients and 5-20% in EGFR-mutated tumors as acquired resistance
- **Crizotinib** or **Cabozantinib**

RET

- RET fusions seen in about 1% of NSCLC, but may be closer to 6-19% in select never-smokers
- **Cabozantinib, vandetinib, lenvatinib or ponatinib**

ERBB2

- Mutations seen in 2-4% of NSCLC with the majority being exon 20 insertion mutations
- **Trastuzumab**, **afatinib**, or investigational **neratinib**

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Nat. Rev. Clin Oncol. 2015;12:523

Goal of Precision Medicine

- Determine the optimal treatment or **sequence** of treatments for a patient
  - Which therapy will yield the best response?
  - How do we optimize the response?
  - How do we minimize toxicity?

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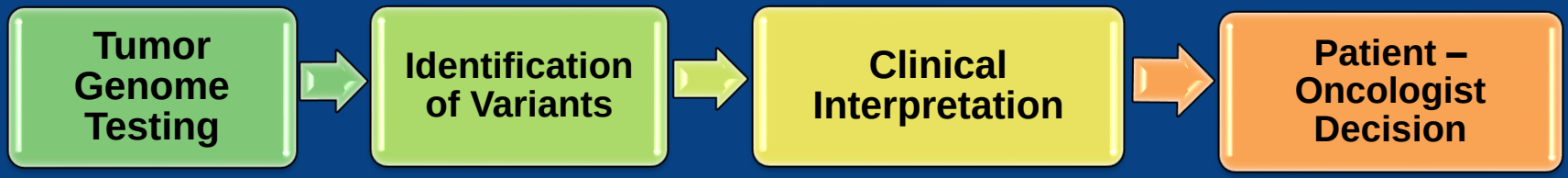
In which patients do we typically do tumor genetic testing?

- Prognostic questions
  - Hematology, especially CLL, AML, and MDS
- Predictive questions
  - Patients with standard of care targeted therapy
    - EGFR in metastatic NSCLC at diagnosis
  - Patients in whom there may not be standard of care options
    - Sarcoma, merkel cell, or cancer of unknown primary
  - Patients with advanced disease who may have limited options
  - Clinical trial enrollment
    - Basket trials

Tumor Genome Analysis Workflow



- What is the goal of the test?
- What test should be ordered?
- What tissue is available?

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Somatic Test Comparison

Test	Genes Analyzed	Tissue Analyzed	Comments
Foundation One®	**315** genes and introns of 28 genes involved in rearrangements	Tumor tissue	• Reports mutation burden (per MB) and MSI status
Foundation One® Heme	**406** genes (DNA), selected introns of 31 genes to assess rearrangements and 265 genes (RNA) to detect additional fusions	Tumor Tissue (may be blood or bone marrow for heme malignancies)	• Reports mutation burden (per MB) and MSI status • Used for sarcomas and other malignancies where a fusion may be expected
Genoptix® NexCourse Complete	**236** genes including select copy number alterations and rearrangements	Tumor Tissue (may be blood or bone marrow for heme malignancies)	• Does not include BCR-ABL
Foundation One® ACT	Complete exons of **27** genes, introns of 6 genes involved in rearrangements and select exons of **34** genes	**Blood for cell free DNA analysis**	• Correlation between volume of disease and concordance with cell-free DNA
Guardant360®	**73** cancer related genes including 6 select rearrangements	**Blood for cell free DNA analysis**	• Correlation between volume of disease and concordance with cell-free DNA

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Pathology Services - Moffitt Cancer Center
Phone: 1-813-745-1800
Website: moffitt.org

PATIENT INFORMATION

Name:	[redacted]	Accession Number:	[redacted]
Date of Birth:		MR#:	
Gender:		Ordering Physician:	
Disease:	Neoplasm of pleura		
Specimen Type:	Tissue specimen	Date Accessioned:	05/06/2015
Internal Related Case Number:	[redacted]	Date Collected:	05/04/2015
Percent Tumor Cell Nuclei in the Selected Areas:	40	Date Received:	05/06/2015
Indication:	adenocarcinoma,poorly differentiated		

Review Status Final

TEST PERFORMED

TSiT

TruSight Tumor Gene Set Targeted next-generation sequencing was performed on this sample of adenocarcinoma,poorly differentiated. See under Test Details for more information.

RESULT SUMMARY

Variants Detected	FDA Approved Therapies, Prognostic Indication, or Other Course of Action (in patient's tumor type)	FDA Approved Therapies, Prognostic Indication, or Other Course of Action (in another tumor type)
BRAF p.V600E	✓	✗

FoundationOne® / Heme / ACT

ABOUT THE TEST:
FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- 3 genomic alterations
- 1 therapy associated with potential clinical benefit
- 0 therapies associated with lack of response
- 4 clinical trials

TUMOR TYPE: LUNG ADENOCARCINOMA

Genomic Alterations Identified†
ROS1 EZR-ROS1 fusion
TP53 G245A
SETD2 L358fs*4

Additional Disease-relevant Genes with No Reportable Alterations Detected
ALK
KRAS
EGFR

†For a complete list of the genes assayed, please refer to the Appendix
‡See Appendix for details

THERAPEUTIC IMPLICATIONS

Genomic Alterations Detected	FDA Approved Therapies (in patient's tumor type)	FDA Approved Therapies (in another tumor type)	Potential Clinical Trials
ROS1 EZR-ROS1 fusion	Crizotinib	None	Yes, see clinical trials section
TP53 G245A	None	None	Yes, see clinical trials section
SETD2 L358fs*4	None	None	None

Note: Genomic alterations detected may be associated with activity of certain FDA approved drugs; however, the agents listed in this report may have varied clinical evidence in the patient's tumor type. Neither the therapeutic agents nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type.

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CLINICAL DATA
80-year-old female. Specimen [redacted]

Accompanying CBC report, dated 6/24/15, indicates WBC 24.20 K/uL, RBC 3.80 M/uL, Hgb 11.8 g/dL, HCT 38.0%, MCV 100.0 fL, MCH 31.1 pg, MCHC 31.1 g/dL, RDW 52.6 fL, platelets 78 K/uL with a differential count of neutrophils 60%, band 2%, lymphocytes 17%, monocytes 13%, eosinophils 1%, basophils 0%, blast 1%, myelocyte 1%, metamyelocyte 4%, 1+ polychromasia, poikilocytosis and tear drop cells.

Tumor Type	Unknown	Stage	Unknown	Specimen Site	Peripheral Blood

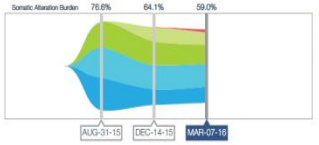
Results Summary

- 6 Genomic Alterations Identified
- 4 Genomic Alterations with Diagnostic and/or Prognostic Implications
- 0 Therapeutic Options Recommended in the NCCN Guidelines
- 2 Therapeutic Options Available for Consideration
- 0 Therapeutic Options with Potential Lack of Response
- 13 Potential Clinical Trials

Guardant360®

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the relative changes of observed cfDNA at different sample submission time points. The "Somatic Alteration Burden" value below refers to the maximum % cfDNA detected at each time point. Amplifications are not plotted and only the first and last four test dates are plotted. Please see the physician portal for the Tumor Response Map with all test dates.



13 Total Alteration(s) Detected
10 with Associated Therapy
2 Associated with Lack of Response
Multiple Clinical Trials Available

Summary of Alterations & Associated Treatment Options

The percentage, or allele frequency, of altered cell-free DNA (% cfDNA) circulating in blood is related to the unique tumor biology of this patient. Factors that may affect the amount/percentages of detected genomic alterations in circulating cell-free DNA in blood include tumor growth, turn-over, size, heterogeneity, vascularization, disease progression, or treatment.

Alteration		Mutation Trend	% cfDNA	cfDNA Amplification	FDA Approved in Indication	Available for Use in Other Indications	Clinical Drug Trials
EGFR	Exon 19 Deletion		59.0		Afatinib, Erlotinib, Gefitinib	None	Trials Available
	T790M		37.3		Osimertinib Lack of Response: Erlotinib, Gefitinib	Afatinib	Trials Available
	C797S		27.5		Erlotinib Lack of Response: Osimertinib	Afatinib, Gefitinib	Trials Available
	AMP			+++	None	Afatinib, Cetuximab, Erlotinib, Gefitinib, Necitumumab	Trials Available

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CLINICALLY RELEVANT RESULTS

	FDA Approved Therapies, Prognostic Indication, or Other Course of Action (in patient's tumor type)	FDA Approved Therapies, Prognostic Indication, or Other Course of Action (in another tumor type)
BRAF	p.V600E	

Interpretation: A *BRAF* p.V600E mutation was detected with an allele frequency of 46%. This activating mutation is seen in around 8% of colorectal cancer and is associated with poor prognosis (Roth AD et al.; J Clin Oncol 28; 466-74; 2010 Jan 20, Fariña-Sarasqueta A et al.; Ann Oncol 21; 2396-402; 2010 Dec). Similar findings were reported in patients with proximal colon adenocarcinomas or with recurrent colorectal cancer where the presence of mutated *BRAF* was associated with significantly poor overall survival and disease-free survival (Pai RK et al.; Am J Surg Pathol 36; 744-52; 2012 May; Yokota T et al.; Br J Cancer 104; 856-62; 2011 Mar 1), respectively. However, a recent study found that the combination of *BRAF* inhibitor (PLX4720) and *EGFR* inhibitors (cetuximab, gefitinib or erlotinib) produced pronounced response both in vitro and in vivo system (Prahallad A et al.; Nature 483; 100-3; 2012 Jan 26). Findings by another preclinical study suggest that administering vemurafenib in combination with standard-of-care or novel targeted therapies may lead to enhanced and sustained clinical antitumor efficacy in CRCs harboring the *BRAF*(V600E) mutation (Yang H et al.; Cancer Res 72; 779-89; 2012 Feb 1). Therefore, this variant may be actionable under certain clinical scenarios and under certain treatment regimens.

OTHER RESULTS

Other variants: See "All Identified Variants Detailed Information" section.

A non-synonymous single nucleotide variant was detected in *MET*, resulting in a predicted p.T992I protein change. This variant is described in the SNP database at a minor allele frequency of 1.2% (rs491913684). However, it has also been identified as a somatic change three times in lung cancer and four times in thyroid cancer. A recent study has shown that with concomitant *KRAS*, *PIK3CA*, *MET*, and non-sensitizing *EGFR* mutations, it is possible to detect up to 96.0% of patients with non-small-cell lung cancer that do not to respond to

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GENOMIC ALTERATIONS

GENE ALTERATION	INTERPRETATION
ROS1 EZR-ROS1 fusion	ROS1 encodes a receptor tyrosine kinase of the insulin receptor family that plays a role in cellular growth and differentiation. Ros1 activates several signaling pathways, including the mitogen-activated protein kinase ERK1/2, phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), Stat3, and Vav3 signaling pathways. ROS1 is considered to be an oncogene (Acquaviva et al., 2009; 18778756). The rearrangement in this tumor is expected to result in an in-frame fusion between the N-terminal portion of EZR and the C-terminal portion of ROS1, including the kinase domain of Ros1 (Jun et al., 2012; 22659450). This mutation is expected to result in constitutive activation of the Ros1 kinase. ROS1 mutations, including fusions, have been reported in 1-3% of lung adenocarcinomas (COSMIC, 2013) (Bergethon et al., 2012; 22215748, Davies et al., 2012; 22919003, Kim et al., 2013; 23788756). Approximately 19% of lung adenocarcinoma samples present with significantly elevated levels of Ros1 protein (Lee et al., 2013; 22915320). Ros1 expression has been reported to be a negative prognostic factor in stage I lung adenocarcinoma (Lee et al., 2013; 22915320). However, negative kinase fusion status (either ALK or ROS1) has been reported to be an indicator of poor prognosis in lung adenocarcinoma, indicating that patients harboring ROS1 fusions may have an improved prognosis (Takeuchi et al., 2012; 22327623). Lung adenocarcinoma patients harboring ROS1 rearrangements have been reported to be younger, more likely never-smokers, and have a tendency toward higher-grade tumors compared with other adenocarcinoma patients (Bergethon et al., 2012; 22215748). Tumors with Ros1 activation may benefit from receptor tyrosine kinase inhibitor (TKI) therapy. Crizotinib, a TKI approved for NSCLC, has demonstrated preclinical and early clinical evidence of activity in ROS1-rearranged lung adenocarcinoma (Bergethon et al., 2012; 22215748, Yasuda et al., 2012; 22617245, Davies et al., 2012; 22919003, Jun et al., 2012; 22659450). The MET tyrosine kinase inhibitor LY2801653, which has been found to have activity against ROS1, and anaplastic lymphoma kinase inhibitors are currently in clinical trials in patients with solid tumors (Yan et al., 2012; 23275061, Chin et al., 2012; 23070242). Lung adenocarcinoma patients with ROS1 rearrangements show increased sensitivity to pemetrexed and decreased sensitivity to Egfr TKIs (Kim et al., 2013; 23788756). A glycine-to-arginine substitution at codon 2032 in the ROS1 kinase domain has been reported to confer resitance to crizotinib in a patient with a CD74-ROS1 rearrangement (Zhu et al., 2013; 23769348).
TP53 G245A	Loss of tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers (Brown et al., 2009; 19935675). TP53 mutations that affect the p53 DNA-binding domain (DBD), such as observed in this tumor, are thought to result in loss of function via the loss of transactivation of p53-dependent genes (Joerger and Fersht, 2008; 18410249, Kato et al., 2003; 12826609). TP53 is one of the most commonly mutated genes in lung cancer; mutations are reported to be present in approximately 50% of NSCLC tumors (Mogi and Kuwano, 2011; 21331359). TP53 mutations have been reported in approximately 40-50% of lung adenocarcinoma tumors (Imielinski et al., 2012; 22980975, Ding et al., 2008; 18948947). TP53 mutations have been associated with lymph node metastasis in lung adenocarcinoma patients (Seo et al., 2012; 22975805). Germline mutations in TP53 are associated with Li-Fraumeni syndrome and the early onset of many cancers (Sorrell et al., 2013; 23355100, Nichols et al., 2001; 11219776). Therefore, in the appropriate clinical context, testing for the presence of germline mutations in TP53 is recommended. There are no approved therapies to address TP53 mutation.

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Interpretation: Key Genomic Alterations

Gene	Genomic Alteration	Mutation Effect	Allele Frequency	Pathogenic
ATM	c.7757A>G; p.N2586S	MISSENSE	7%	UNCERTAIN

Variant Assessment:

A genomic alteration in the ATM gene is detected (c.7757A>G; p.N2586S). This missense alteration has been previously reported (http://www.ncbi.nlm.nih.gov/clinvar/variation/133633/). However, based on the available evidence its clinical significance is uncertain.

Interpretation:

The effect of this aberration on ATM protein function is not known. Common aberrations in ATM are inactivating mutations.

Clinical Summary:

Biomarker overview: The dominant class of aberrations in ATM are inactivating mutations. ATM is commonly mutated in colorectal cancers, cervical cancers, bladder cancers, and lymphoid neoplasms (COSMIC). ATM is one of the causal genes underlying the rare, recessive disorder, ataxia-telangiectasia, which is characterized by progressive cerebellar ataxia, neuro-degeneration, radiosensitivity, cell-cycle checkpoint defects, genome instability, and a predisposition to cancer (PMID:20580718). The inactivation of both alleles of the ATM gene by deletion (as part of the common 11q22-q23 deletion) and deleterious point mutation is a hallmark of mantle cell lymphoma (MCL), indicating that ATM plays a key role in the initiation and/or progression of MCL (PMID:10706620). ATM mutations may be one of the key events in the pathogenesis of T-cell prolymphocytic leukemia (T-PLL). The NCCN guidelines suggest that detection of ATM mutations could be useful in certain circumstances in the diagnosis of T-cell prolymphocytic leukemia (NCCN guidelines, Non-Hodgkin's Lymphomas, version 2.2015, pages TPLL-1and MS-275). Additionally, it is recommended in the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian, that patients with ATM mutations receive MRI screening as they have a >20% risk of breast cancer (NCCN Guidelines, Genetic/Familial High-Risk Assessment: Breast and Ovarian, version 1.2015, page ADDIT-2). The presence of del(11q) in CLL patients is associated with short progression-free survival to chemotherapy and chemoimmunotherapy approaches and is therefore considered an unfavorable prognostic indicator (NCCN guidelines, Non-Hodgkin's Lymphomas, version 2.2015, page CSLL-A).

Guidelines: None

Clinical data: There are no clinical data to indicate N2586S in Chronic Myelomonocytic Leukemia is predictive of therapeutic response.

Pre-clinical data: There are no pre-clinical data to indicate N2586S in Chronic Myelomonocytic Leukemia is predictive of therapeutic response.

Therapeutic Implications: None

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Clinical Relevance of Detected Alterations

Alteration	Role in Disease	Effect on Drug Sensitivity	Effect on Drug Resistance
EGFR T790M	The presence of an EGFR abnormality (mutation, amplification, or overexpression) can result in an overabundance or overactivity of Egfr protein, which can lead to excessive proliferation[1]. The EGFR T790M mutation has typically been reported as a secondary resistance mutation to the Egfr inhibitors erlotinib and gefitinib, but has been reported as a rare germline variant in de novo non-small cell lung cancer, particularly in lung adenocarcinoma[2]. Several studies have reported the presence of the T790M mutation in the germline in 0-1% of NSCLC cases, although one study did detect this mutation in 50% (5/10) of cases; in addition, T790M was associated with a 31% risk of lung cancer in never smokers in one study[3-6]. In NSCLC patients, EGFR mutation has been found to be more common in women, never-smokers, and in patients with adenocarcinoma histology[7-11]. EGFR mutations have been found to be mutually exclusive with ALK rearrangements and KRAS mutations in NSCLC[12-14]. However, case studies of NSCLC patients harboring ALK mutations or EML4-ALK fusions have reported the emergence of EGFR mutations upon acquired resistance to crizotinib, demonstrating a role for EGFR in crizotinib resistance in NSCLC[15-17].	The presence of a sensitizing EGFR mutation in a tumor is the strongest biological predictor of sensitivity to an Egfr tyrosine kinase inhibitor (TKI). Compared with conventional chemotherapy, Egfr TKIs have been shown to improve progression-free survival in non-small cell lung cancer patients whose tumors harbor EGFR mutations[18-21]. The Egfr TKIs erlotinib, afatinib, and gefitinib have been approved by the FDA for the treatment of EGFR mutant non-small cell lung cancer (NSCLC)[18,21-24]. Erlotinib, in combination with gemcitabine, has also been approved by the FDA for the treatment of locally advanced, unresectable, or metastatic pancreatic cancer[25,26]. However, some EGFR mutations, including T790M, have been reported to confer resistance to erlotinib and gefitinib[27]. Third generation irreversible Egfr TKIs that target the EGFR T790M mutation have shown efficacy in T790M-mutant NSCLC; osimertinib has received FDA-approval for the treatment of EGFR T790M-mutant metastatic NSCLC, and other therapies, including rociletinib, are under investigation[28-33]. Anti-Egfr monoclonal antibodies are also approved in some indications, including cetuximab, which is an approved therapy for HNSCC and colorectal cancer, panitumumab, which is approved in colorectal cancer, and necitumumab, which has received approval for the treatment of advanced squamous NSCLC[34-37]. For NSCLC patients with metastatic disease and tumors harboring a sensitizing EGFR mutation, the NCCN guidelines (v.2.2016) suggest treating with erlotinib, afatinib, or gefitinib if the alteration is discovered prior to first-line chemotherapy or interrupting/completing current therapy and treating with erlotinib, afatinib, or gefitinib if the alteration is discovered during first-line chemotherapy.	Some patients with EGFR-mutant NSCLC exhibit resistance to Egfr inhibition; resistance has been associated with insertions in EGFR exon 20, the T790M mutation in EGFR, and amplification of the gene MET[38-40]. Several studies have reported that resistance to Egfr TKIs in NSCLC is mediated by the transformation of NSCLC cell types to those of SCLC with neuroendocrine features[41-44]. A study in NSCLC cell lines with resistance to the Egfr inhibitors erlotinib, gefitinib, and afatinib reported increased expression of Smo, Gli1, and Ptc1 in resistant cell lines as compared with parental control cells; forced expression of Smo in a sensitive NSCLC cell line also resulted in resistance to gefitinib[45].

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Cell Free DNA (cfDNA) Assays

- Tissue biopsies are not always feasible
- Enables serial monitoring over time to assess for resistance mutations and changes in frequency
- May better represent tumor heterogeneity
- Value of cell free DNA (cfDNA) and serial sampling
  - Plasma derived assays
    - Best concordance when higher number of metastatic sites, lower albumin, higher number of prior therapies
    - Site of disease also showed correlation
  - Cerebral Spinal Fluid (CSF)
    - Somatic alterations found in 63% of CNS metastases from solid tumors and 50% of primary brain tumors

- J Clin Oncol. 2016;34:online 5/9/2016, Clin Cancer Res. 2016;22:2960-8

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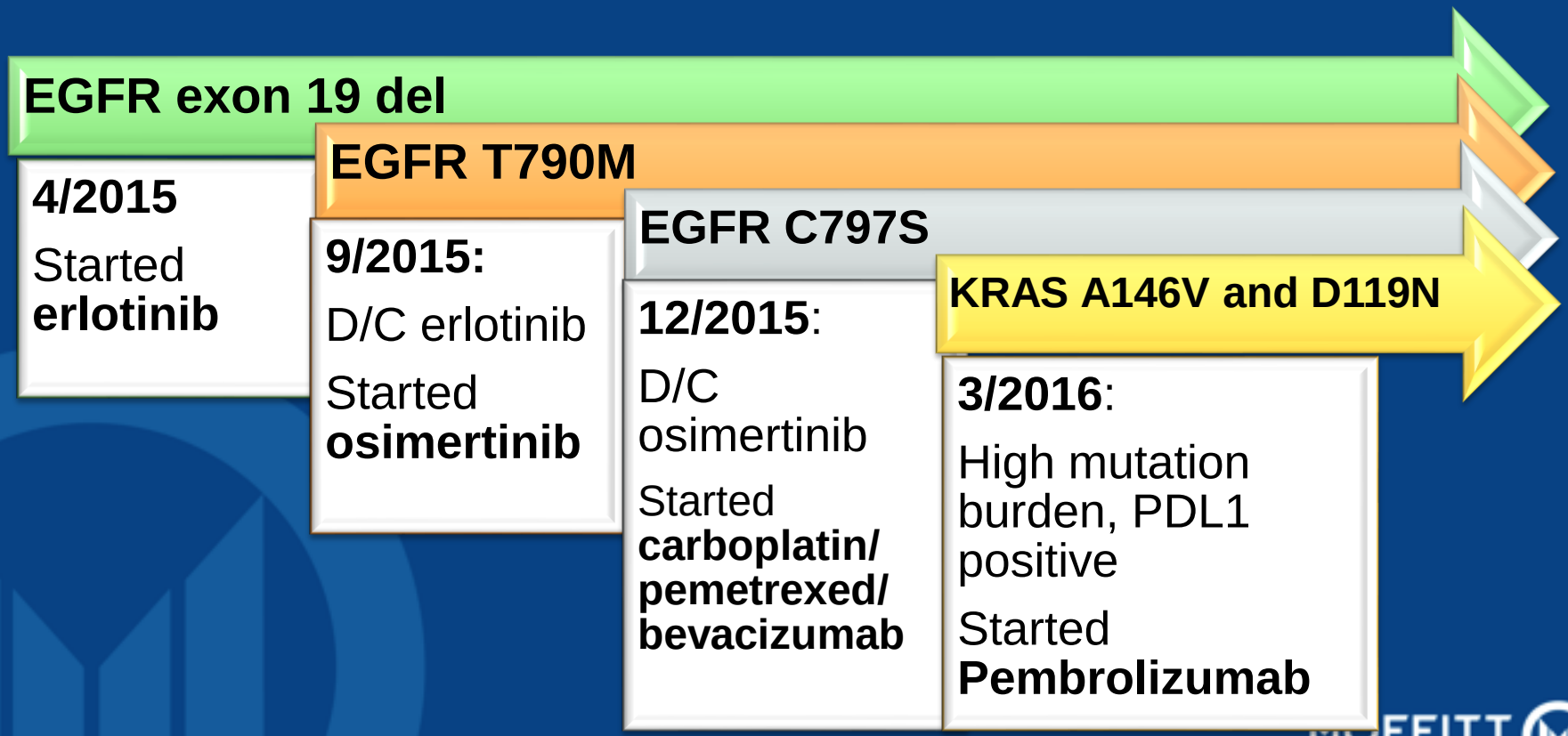
Mutation Landscape Changes over Time

- 40 yo non-smoking female diagnosed with Stage IV NSCLC, adenocarcinoma



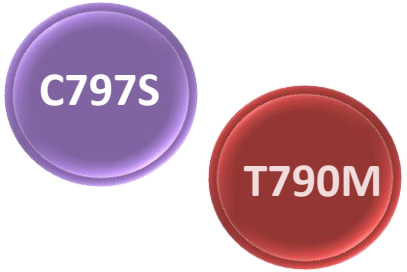
EGFR C797S and Resistance

- We are familiar with resistance mutations:
  - Erlotinib → T790M
  - Osimertinib → C797S → Retains activity to first generation agents
- EGFR C797S – acquired resistance mutation
  - Covalent binding site for 2nd and 3rd generation EGFR-inhibitors like afatinib and osimertinib

C797S mutation in **CIS** with **T790M**

Resistant to EGFR-inhibitors, use alternate therapy

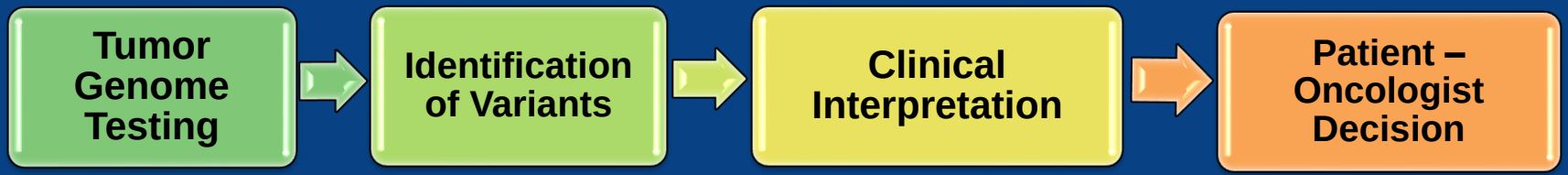
C797S mutation in **TRANS** with **T790M**



Combination of first- and third-line EGFR inhibitors

Clin Cancer Res. 2015;21:3924-33

Tumor Genome Analysis Workflow



- What is the goal of the test?
- What test should be ordered?
- What tissue is available?

- What type of variants will be assessed?
- Lower limit of quantitation, number of reads, etc

- How is actionability determined?
- Priority given to multiple actionable variants?
- How to handle variants of unknown or almost known significance?
- Germline variants?

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Clinical Actionability

- Genetic alteration predicts response to a particular therapy
  - Benefit or resistance to a particular therapy
  - FDA approved therapy in the patient's tumor or another type of tumor
  - Clinical trial for the particular alteration or reasonable based on molecular biology
- Genetic alteration provides diagnostic or prognostic information
- Clinically relevant germline alteration that informs disease risk or pharmacokinetic or pharmacodynamics

Actionability and Levels of Evidence

Supporting Data

- Comparative trial with biomarker selection/stratification (patient's tumor type or different tumor type)
- Retrospective cohort or case-control trials
- Biomarker association with response less robust (secondary endpoint)
- Case study or case series
- Preclinical data only (in vitro or in vivo models)

Clinical Actionability

- FDA approved therapy in **patient's** tumor type
- FDA approved therapy in **different** tumor type
- Clinical trial based on specific mutation
- Clinical trial based on application of pathway biology
- Prognostic information
- Not clinically actionable at this time

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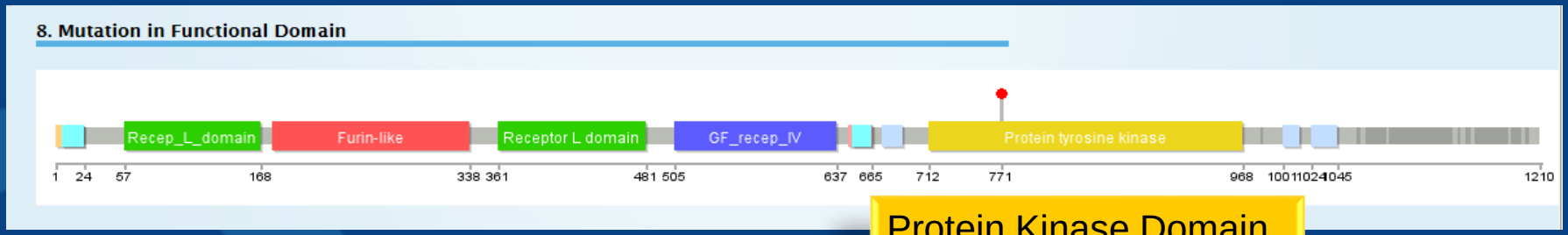
Variants of Almost Known Significance

- Variation found in clinically significant gene in area of known tyrosine kinase binding or other known relevant area
  - **Specific alteration itself is unknown**
  - Example**: EGFR N771Y**
    - Located in the EGFR tyrosine kinase domain in exon 20 but has not been previously reported in COSMIC or other sources



- Value of functional based assays
- Importance of data sharing, especially regarding relevant clinical outcomes

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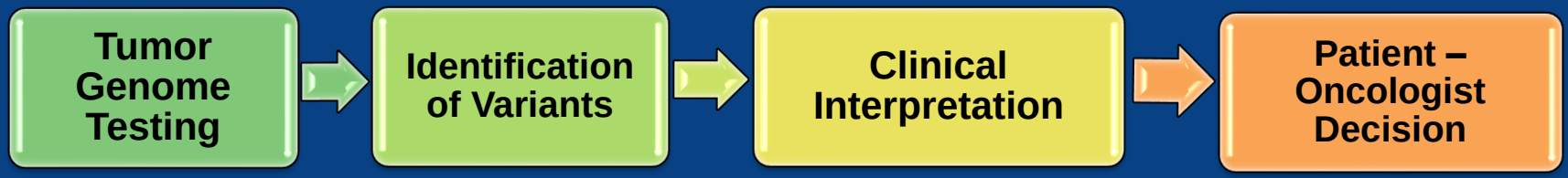
Tumor Genome Analysis Workflow



- What is the goal of the test?
- What test should be ordered?
- What tissue is available?

- What type of variants will be assessed?
- Lower limit of quantitation, number of reads, etc

- How is actionability determined?
- Priority given to multiple actionable variants?
- How to handle variants of unknown or almost known significance?
- Germline variants?

- Ability to qualify and travel for a clinical trial?
- Ability to acquire off label therapy?
- Other patient factors to consider?

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Translating Recommendations into Clinical Decision Making

- Researching and presenting available data to facilitate the decision making process
- Considering the interaction of all the mutations together
  - Cyclin D pathway alteration + RB1 loss
- Consideration of each patient's unique characteristics
  - Desire for a clinical trial and ability to travel
  - Availability and ability to qualify for a clinical trial
  - Sequencing of treatment options
  - Insurance coverage and ability to afford off label therapy
  - Patient preference on treatment options
  - Where patient is in his/her treatment course

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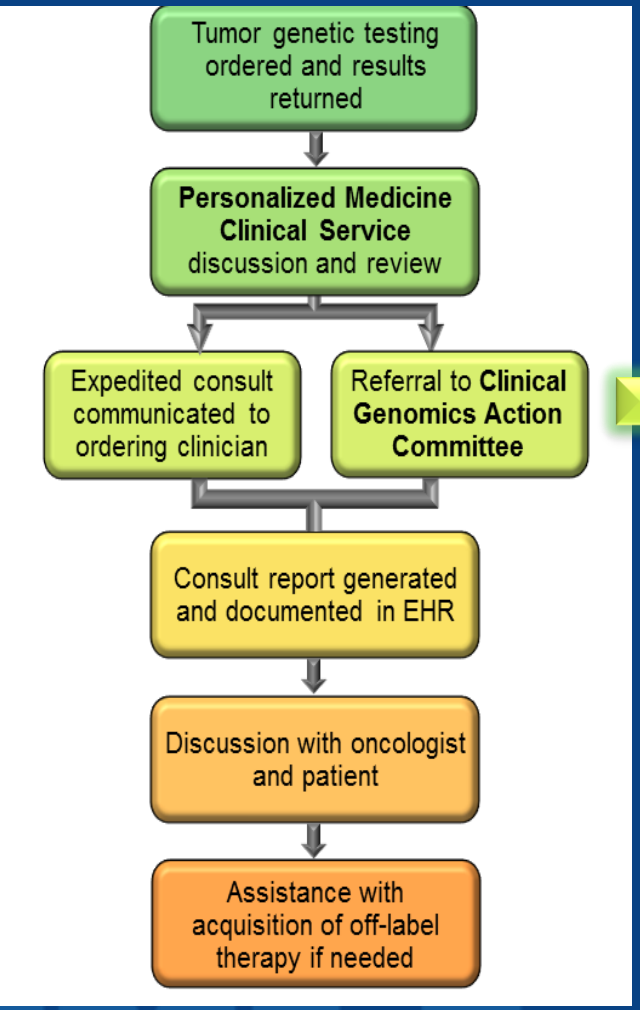
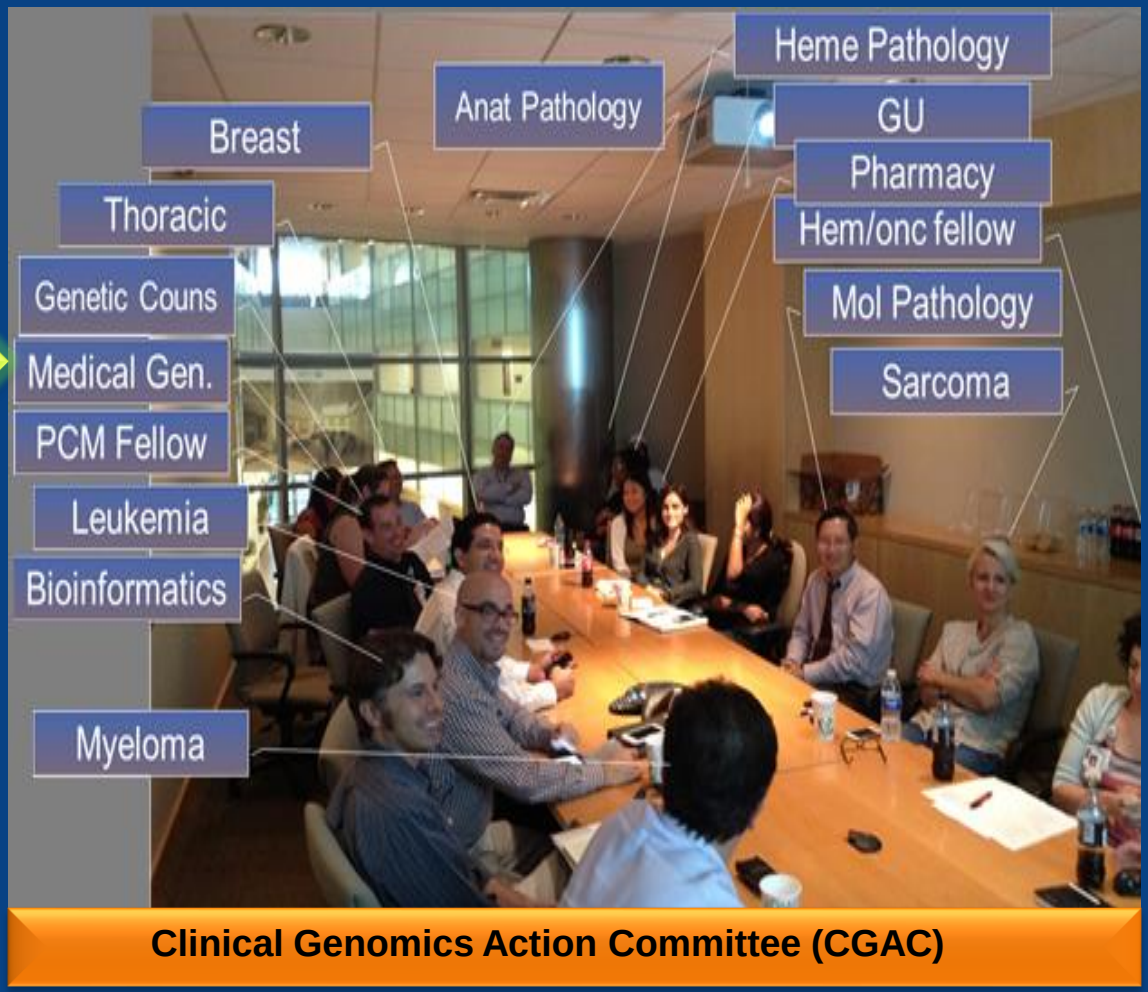
Personalized Medicine Clinical Service (PMCS) and Clinical Genomics Action Committee (CGAC)





Clinical Genomics Action Committee (CGAC)

Tumor Genome Analysis Workflow

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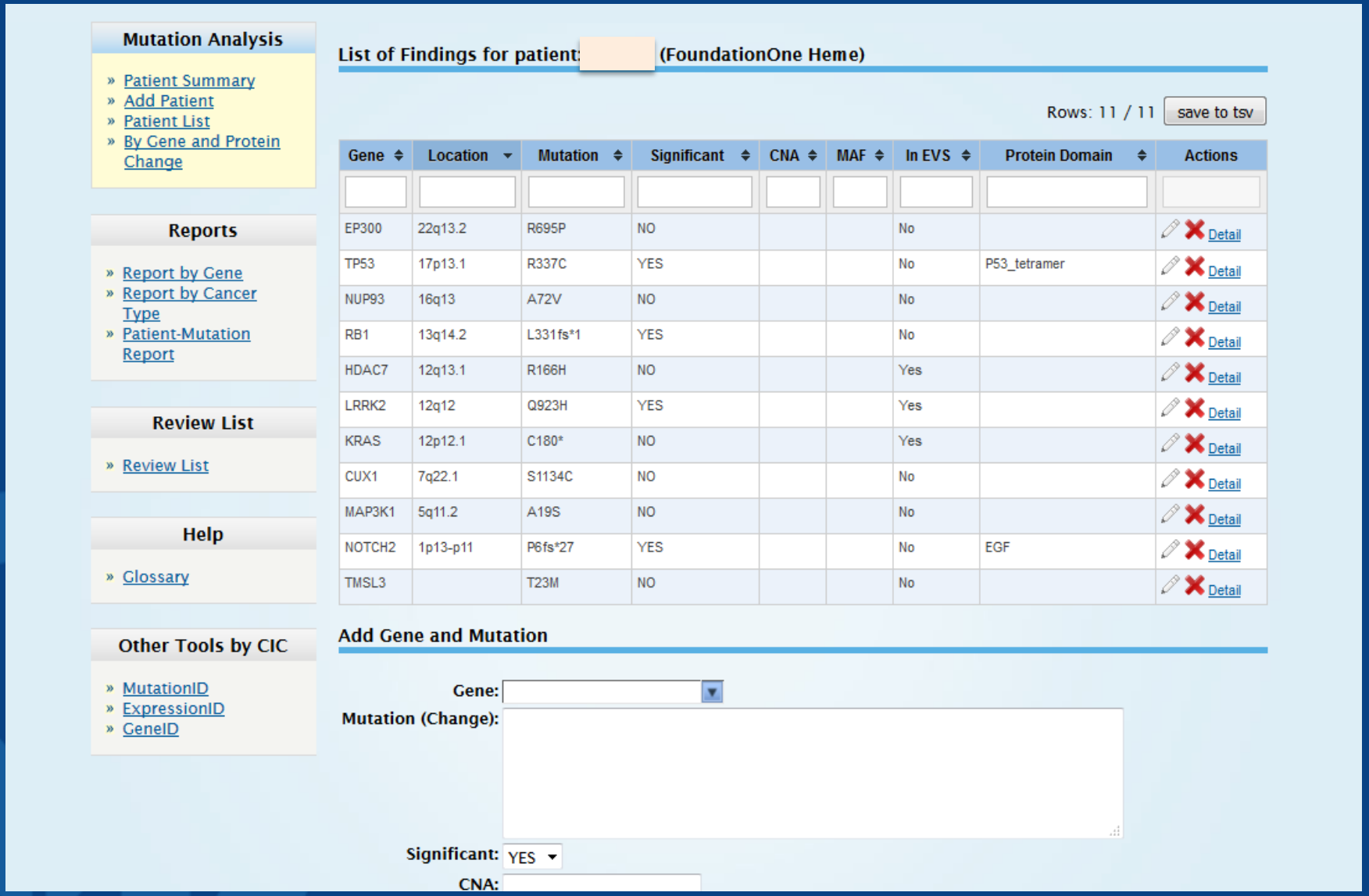
CGAC Clinical Database



Mutation Analysis

- Patient Summary
- Add Patient
- Patient List
- By Gene and Protein Change

Reports

- Report by Gene
- Report by Cancer Type
- Patient-Mutation Report

Review List

- Review List

Help

- Glossary

Other Tools by CIC

- MutationID
- ExpressionID
- GeneID

List of Findings for patient: (FoundationOne Heme)

Rows: 11 / 11 save to tsv

Gene	Location	Mutation	Significant	CNA	MAF	In EVS	Protein Domain	Actions
EP300	22q13.2	R695P	NO			No		Detail
TP53	17p13.1	R337C	YES			No	P53_tetramer	Detail
NUP93	16q13	A72V	NO			No		Detail
RB1	13q14.2	L331fs*1	YES			No		Detail
HDAC7	12q13.1	R166H	NO			Yes		Detail
LRRK2	12q12	Q923H	YES			Yes		Detail
KRAS	12p12.1	C180*	NO			Yes		Detail
CUX1	7q22.1	S1134C	NO			No		Detail
MAP3K1	5q11.2	A19S	NO			No		Detail
NOTCH2	1p13-p11	P6fs*27	YES			No	EGF	Detail
TMSL3		T23M	NO			No		Detail

Add Gene and Mutation

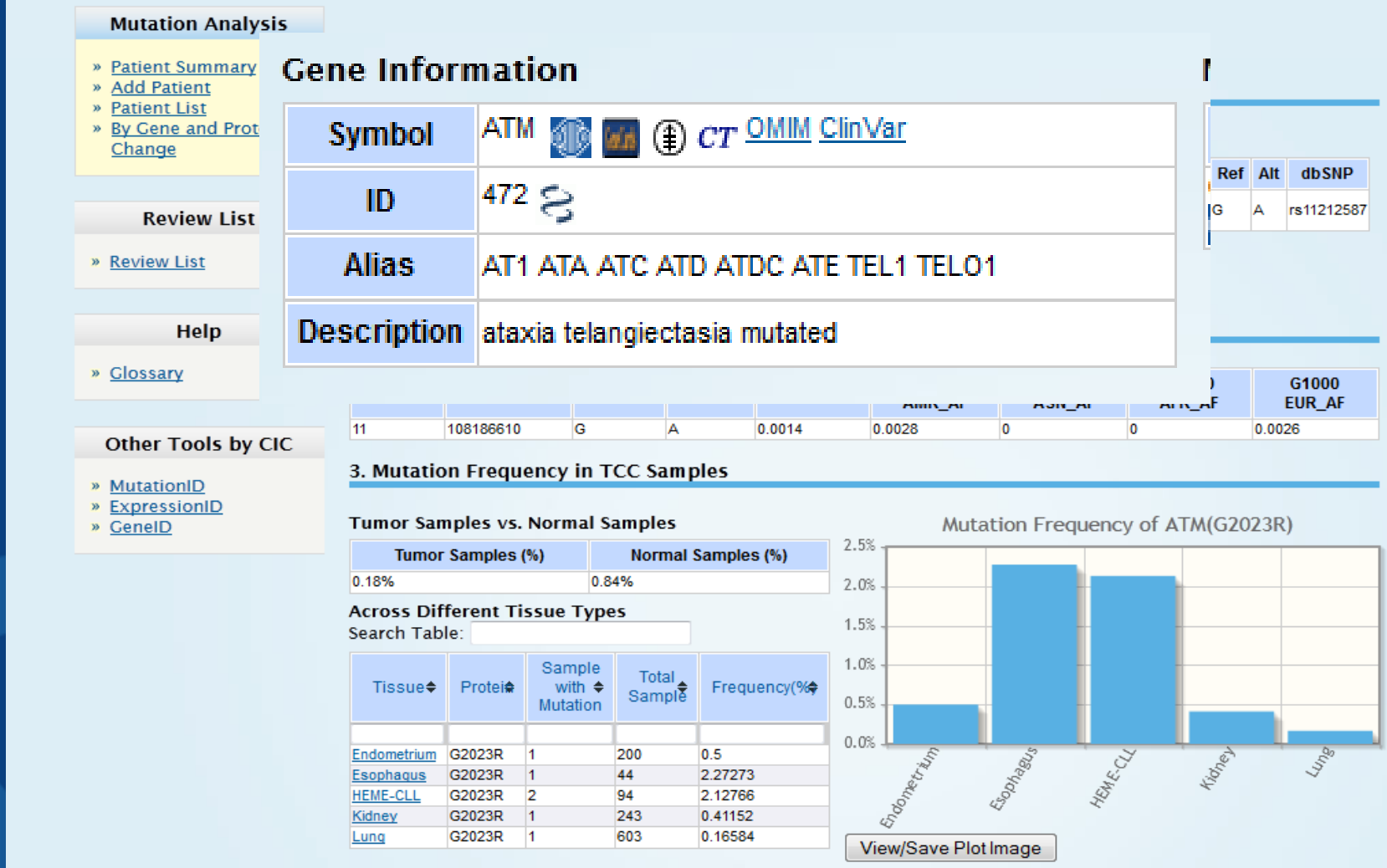
Gene:

Mutation (Change):

Significant: YES

CNA:

CGAC Database



Clinically Important Genetic Resources

Category	Resource	Utility
Variants of Unknown Significance	1000 Genomes Project (http://www.1000genomes.org/)	Provide a probability of the variant being germline
	Exome Variant Server (http://evs.gs.washington.edu/EVS/)	Provide a probability of the variant being germline
Inherited Cancer Risk	International Agency for Research on Cancer (IARC) (http://p53.iarc.fr/)	Frequency of a TP53 mutation in germline and tumor samples
	HCI Breast Cancer Gene Prior Probabilities (http://priors.hci.utah.edu/PRIORS)	Data on all possible single nucleotide substitutions in BRCA1/2
	ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/)	Association of a variant with an inherited disease
	American College for Clinical Genetics (ACMG)	Association of a variant with an inherited disease

- Knepper, T, et al. The Oncologist. 2016: in press

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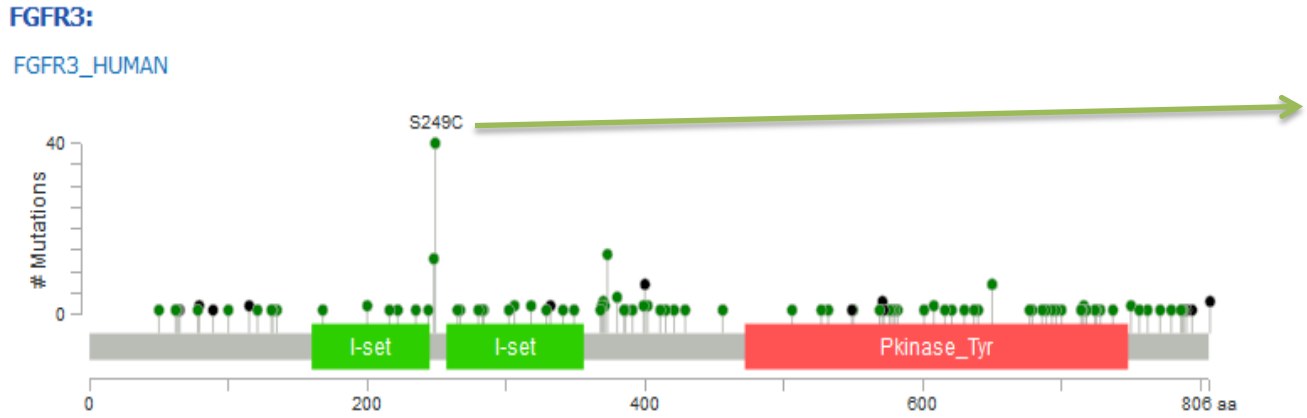
Clinically Important Genetic Resources

Category	Resource	Utility
Variants from across Cancer Types	cBioPortal (http://www.cbioportal.org/)	The frequency of a variant across cancer types and the location of the variant in the functional domains of the gene
	Catalogue of Somatic Mutations in Cancer (COSMIC) (http://cancer.sanger.ac.uk/cosmic)	The frequency of a variant across cancer types
Therapeutic Association	MyCancerGenome (http://www.mycancergenome.org/)	Association of mutation with tumorigenesis, related therapeutic implications and available clinical trials
	PharmGKB (https://www.pharmgkb.org/)	Interactive tool for researchers investigating how genetic variation effects drug response
	Personalized Cancer Therapy Knowledge Base for Precision Oncology (https://pct.mdanderson.org)	Knowledge base resource for the implementation of personalized cancer therapy and integrating information about tumor DNA, RNA, protein and metabolomics profiles with predicted therapy response

- Knepper, T, et al. The Oncologist. 2016: in press

CRCA Patient with FGFR3 S249C



40 mutations
AA Change: S249C

Cancer Type	Count
Bladder Urothelial Carcinoma	36
Lung Squamous Cell Carcinoma	2
Head and Neck Squamous Cell Carcinoma	1
Papillary Renal Cell Carcinoma	1

- In vitro bladder cancer cell data supports this mutation induced phosphorylation of PLCg1, FRS2 and ERK1/2. Differences were seen between different FGFR3 mutations and different cell types
- Pazopanib was shown in vitro to inhibit FGFR3 activating mutations at an IC50 of 100nM-1uM and one SqCC head and neck cancer patient with an FGFR2 P253R mutation had a response to pazopanib
- 67 yo woman with metastatic papillary urothelial carcinoma s/p several chemotherapy agents found to have FGFR3 amp and **S249C** (58%), treated with **pazopanib** and had a PR > 6 months.
- **AZD4547** is part of the NCI-MATCH trial expanded arms
  - Subprotocol W (FGFR1-3 amplifications, mutations or translocations)

cBioPortal July 2016, Oncogene 2009;28:4306-16
European Urology. 2015;68:167-170

Germline Challenges

- If tumor is analyzed with matched normal tissue, can subtract out alterations found in the normal tissue
  - If normal tissue not analyzed, more difficult to separate
  - Allele frequency of 50% or 100% may indicate germline alterations in some assays
- Available databases
  - Exome variant server
  - ClinVar
- ACMG recommendations regarding incidental findings for suspected germline mutations in tumor tissue

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Mutation Load and Immunotherapy

- **Exciting therapy, but not everyone has a response**
  - Durable responses to anti-PD1 therapy were seen in:
    - 31-44% of melanoma
    - 19-20% of lung cancer
    - 22-25% of renal cell carcinoma
  - Potential biomarkers:
    - Density of CD8+ T cells in tumors
    - Expression of PDL1 on tumors
    - **Mutation burden and microsatellite instability:** now being reported by some molecular testing companies for individual patients

Example: MSI: Stable
Mutation Burden: **High**, 25 mutations per megabase

- Nat Rev Cancer. 2016;16:275-287

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Mutation Load and Immunotherapy

Number of Mutations

- Improved **overall survival** with CTLA4-inhibitors in melanoma patients with > 100 mutations (p=0.04)
  - 64 patients treated with ipiliumumab or tremelimumab
  - Neoantigen response signature developed
- Improved **mPFS** in lung cancer patients treated with pembrolizumab with high mutation burden
  - Patients with durable responses had a median of 302 mutations vs. 148 in those without a durable response (p=0.02)

Microsatellite Instability

- 41 patients with MMR-deficient colorectal cancer, 9 patients with other MMR-deficient cancer and 21 MMR-intact colorectal cancer patients
  - All treated with pembrolizumab
- Whole exome sequencing mean number of somatic mutations per tumor
  - MMR-deficient: 1782 mutations
  - MMR-intact: 73 mutations
  - Higher somatic tumor burden = improved mPFS

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N Eng J Med. 2015;372:2509-20

Future of Somatic Genomics

- What are the optimal mutational profiling approaches?
- How do we translate these findings into clinical practice for the average oncologist?
  - Defining “clinically actionable”
  - Handling “variants of unknown significance”
  - Facilitating patient discussions
  - Ethics on germline findings
- What clinical trials should we be doing?
  - Novel trial design like “Basket Studies”

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J Clin Oncol. 2013;31:1806-1814.

Ongoing ~~Challenges~~ OPPORTUNITIES

- Identify, interrogate and validate the correct biomarkers for targeted and immunotherapies
- Utilize novel clinical trial designs to assess outcomes across tumor types and mutations
  - Basket trials
  - Genetic-guided Registry trials
    - **T**argeted **A**gent and **P**rofiling **U**tilization **R**egistry (TAPUR)
      - Goal: To learn from the real world practice of prescribing targeted therapies to patients with advanced cancer whose tumor harbors a genomic variant known to be a drug target or to predict sensitivity to a drug
      - Currently open at 4 sites with many more planned, 15 arms
      - NCT02693535

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Optimizing Targeted Therapy

- Translate our understanding of cancer biology crosstalk and feedback signaling into rationale drug combinations
- Modify the immune environment to improve tumor identification and destruction
- Improve biomarker identification and validation to target the right genetic drivers

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Nat Rev Drug Discov. 2014;13:140-156

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