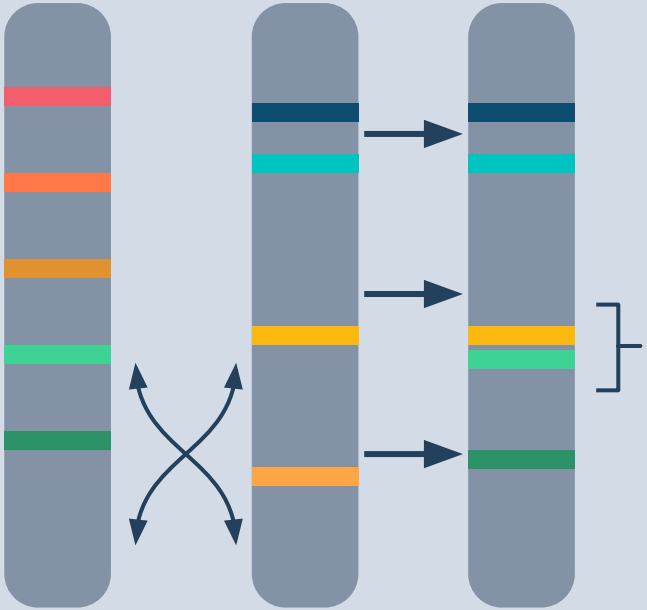


Analyzing Complex Genomic Variants in Somatic Cancer

A Practice-based NGS Webinar

Presenter: Amber Leigh
Clinical Application Scientist

Moderator: Josh Forsythe

Who We Are

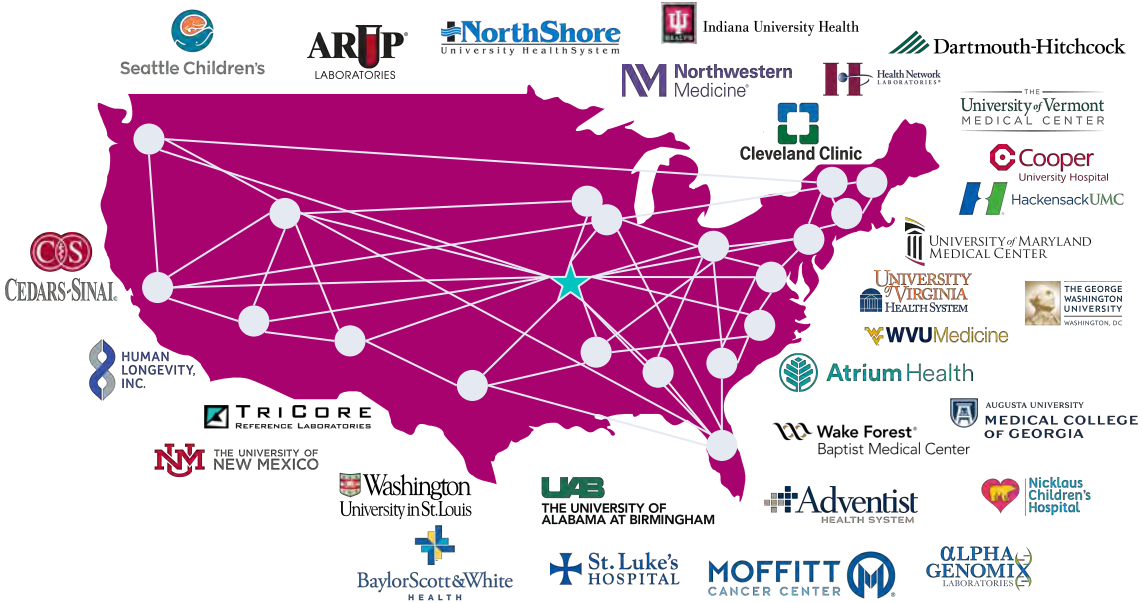
Keep cancer care in the community

By empowering local pathology and cancer centers to provide the same level of advanced testing and precision insights to their patients as major academic centers.

Rakesh Nagarajan, MD, PhD
Founder, Exec. Chairman

The Network Effect

Wisdom in Every Report



- **Today:** 45+ health systems and laboratories in partner sharing network
- **2019:** PierianDx signs multi-year deal with Illumina to support cancer research and diagnostics
- **2015:** PierianDx closes on $9.25 million in funding
- **2014:** PierianDx established; Moffitt Cancer Center 1st to go live
- **2011:** PierianDx technology developed at Washington University in St. Louis

PierianDx Solutions

Technology Enabled Services

Clinical Genomics Workspace

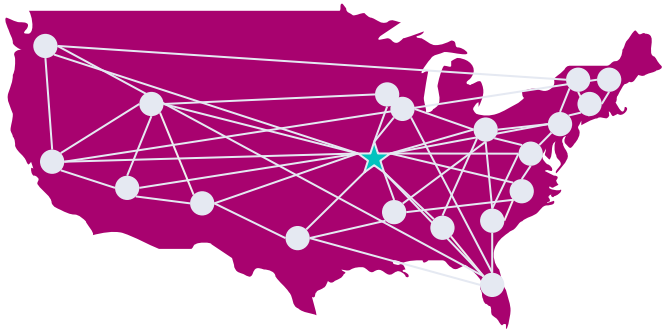
All-in-one informatics and reporting software

Validation & Interpretation Services

Experienced team to **fast-track growth**

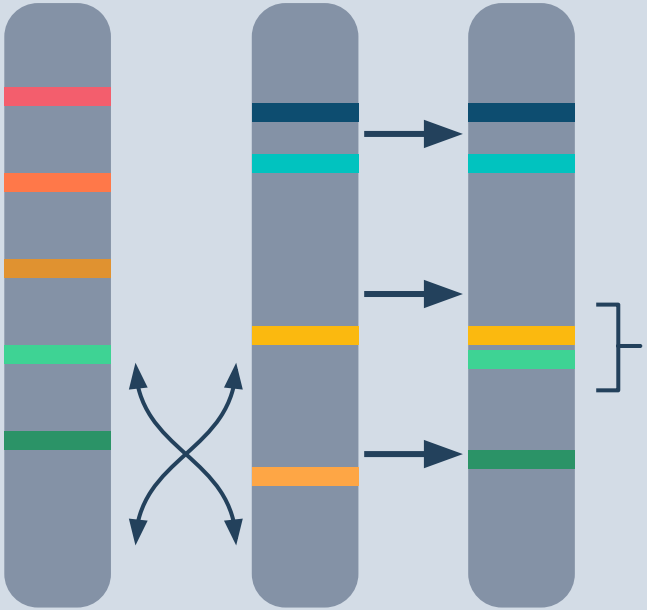
Medically Powered Knowledgebase

The largest opt-in content **sharing network**



Laboratory Services

Turnkey, validated assays and informatics



1. Co-occurring Variants
2. Splice Site, Exon Skipping
3. Gene Fusions
4. Copy Number Variants
5. Tumor Mutational Burden & Microsatellite Instability

Co-Occurring Variants

Background

Bioinformatics/Quality Control

Bioinformatics and quality checks for co-occurring variants will match typical SNV and INDEL processes.

Interpretation

Many NGS users do not have an easy way to identify and then interpret variants in the context of co-occurrence.

1. How do you quickly identify co-occurring variants (beyond lookup tables)?
2. How do you report on these variants?

Example: Non-small Cell Lung Cancer[1]

EGFR L858R confers increased sensitivity and response to tyrosine-kinase inhibitors such as erlotinib.[2]

EGFR T790M confers decreased sensitivity to tyrosine-kinase inhibitors such as erlotinib.[3]

Sources:
1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4114585/
2. https://www.mycancergenome.org/content/disease/lung-cancer/egfr/5
3. https://www.mycancergenome.org/content/disease/lung-cancer/egfr/4/

Conflicting Results Create Challenges

Result Summary - Non Small Cell Lung Cancer			
Variants Detected	**Tier 1**	**Tier 2**	**Clinical Trial Opportunity**
EGFR p.T790M	Responsive to **Osimertinib** Non Responsive to Afatinib, Erlotinib, Gefitinib	No	Yes
EGFR p.L858R	Responsive to Afatinib + Cetuximab, Afatinib, Erlotinib, Dacomitinib, **Osimertinib**, Gefitinib	No	Yes

As a Clinician

What does this mean for the patient?

As a Pathologist

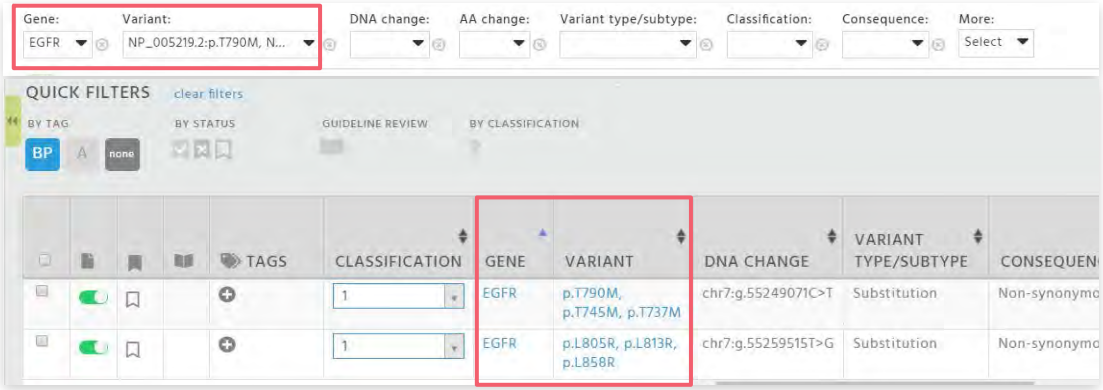
Have a duty to explain what is known about these conflicting results.

Identify Co-Occurring Variants

Figure 1. VCF File

#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	sample
chr1	154137492	TN_1	C	[1:156843543[C	.	.	SVTYPE=BND;ROWID=TN_1;	GT	0/1
chr1	156843543	TN_2	T	[1:154137492[T	.	.	SVTYPE=BND;ROWID=TN_2;	GT	0/1
chr1	154137492	INV_TN_1	C	<INV>	.	.	SVTYPE=intrachromosomal	GT	0/1
chr7	55259515	SNP1	T	G	.	.	percentMutated=0.4189;so	GT	0/1
chr8	128748315	CNV1	G	<CNV>	.	.	SVTYPE=copy_number_vari	GT	./.
chr9	5450503	CNV2	G	<CNV>	.	.	SVTYPE=copy_number_vari	GT	0/1
chr9	135772014	SNP2	C	T	.	.	percentMutated=0.68;sour	GT	0/1
chr7	55086678	CNV2	C	<CNV>	.	.	SVTYPE=copy_number_vari	GT	./.
chr2	42472827	EL_1_2	G	G]2:29446208]	.	.	SVTYPE=BND;ROWID=EL_1	GT	0/1
chr2	29446208	EL_2_2	C	C]2:42472827]	.	.	SVTYPE=BND;ROWID=EL_2	GT	0/1
chr7	55249071	SNP3	C	T	.	.	percentMutated=2.39;Entre	GT	0/1

Figure 2. PierianDx CGW Variant



Filters: IF/AND

Do systems automatically call this out?

Do you need them to have the same approximate variant allele frequency?

Easier

2 *EGFR* or 2 *KRAS* variants

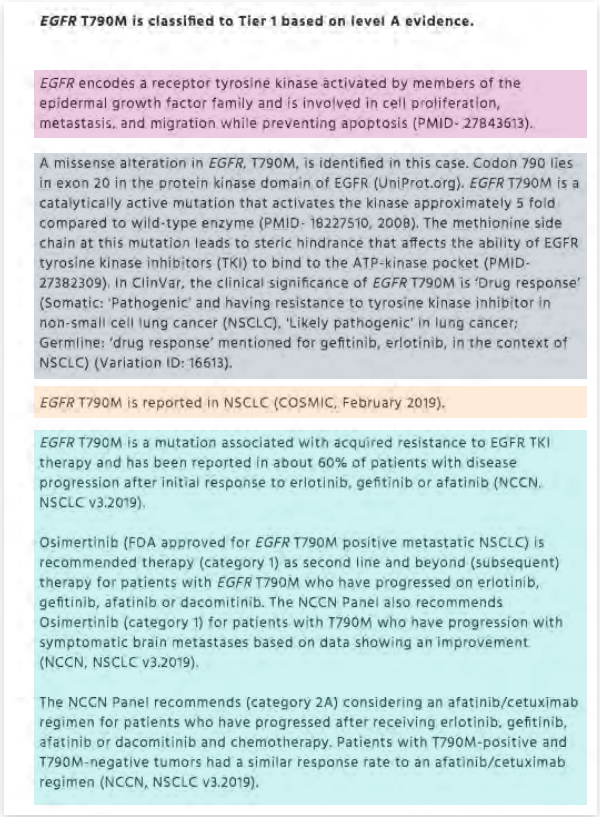
More Difficult

BRAF V600E and any *TERT* promoter

Co-occurring Variants

Interpretation in Isolation

EGFR T790M



***EGFR* T790M is classified to Tier 1 based on level A evidence.**

EGFR encodes a receptor tyrosine kinase activated by members of the epidermal growth factor family and is involved in cell proliferation, metastasis, and migration while preventing apoptosis (PMID- 27843613).

A missense alteration in *EGFR*, T790M, is identified in this case. Codon 790 lies in exon 20 in the protein kinase domain of EGFR (UniProt.org). *EGFR* T790M is a catalytically active mutation that activates the kinase approximately 5 fold compared to wild-type enzyme (PMID- 18227510, 2008). The methionine side chain at this mutation leads to steric hindrance that affects the ability of EGFR tyrosine kinase inhibitors (TKI) to bind to the ATP-kinase pocket (PMID- 27382309). In ClinVar, the clinical significance of *EGFR* T790M is 'Drug response' (Somatic: 'Pathogenic' and having resistance to tyrosine kinase inhibitor in non-small cell lung cancer (NSCLC), 'Likely pathogenic' in lung cancer; Germline: 'drug response' mentioned for gefitinib, erlotinib, in the context of NSCLC) (Variation ID: 16613).

EGFR T790M is reported in NSCLC (COSMIC, February 2019).

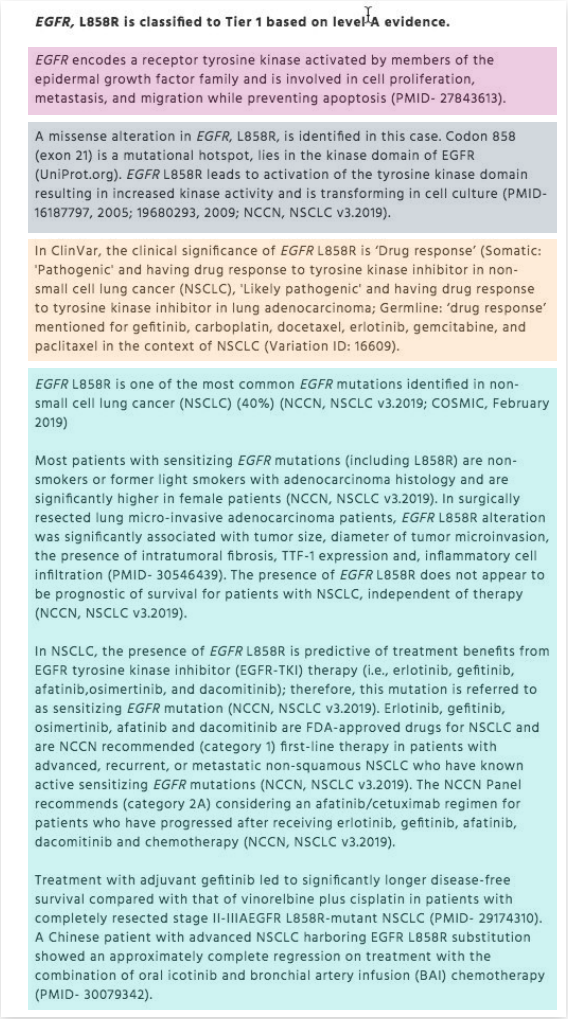
EGFR T790M is a mutation associated with acquired resistance to EGFR TKI therapy and has been reported in about 60% of patients with disease progression after initial response to erlotinib, gefitinib or afatinib (NCCN, NSCLC v3.2019).

Osimertinib (FDA approved for *EGFR* T790M positive metastatic NSCLC) is recommended therapy (category 1) as second line and beyond (subsequent) therapy for patients with *EGFR* T790M who have progressed on erlotinib, gefitinib, afatinib or dacomitinib. The NCCN Panel also recommends Osimertinib (category 1) for patients with T790M who have progression with symptomatic brain metastases based on data showing an improvement (NCCN, NSCLC v3.2019).

The NCCN Panel recommends (category 2A) considering an afatinib/cetuximab regimen for patients who have progressed after receiving erlotinib, gefitinib, afatinib or dacomitinib and chemotherapy. Patients with T790M-positive and T790M-negative tumors had a similar response rate to an afatinib/cetuximab regimen (NCCN, NSCLC v3.2019).

- Gene Level Info
- Variant Level Info
- Evidence in Databases
- Drug/Guideline Info

EGFR L858R



***EGFR*, L858R is classified to Tier 1 based on level A evidence.**

EGFR encodes a receptor tyrosine kinase activated by members of the epidermal growth factor family and is involved in cell proliferation, metastasis, and migration while preventing apoptosis (PMID- 27843613).

A missense alteration in *EGFR*, L858R, is identified in this case. Codon 858 (exon 21) is a mutational hotspot, lies in the kinase domain of EGFR (UniProt.org). *EGFR* L858R leads to activation of the tyrosine kinase domain resulting in increased kinase activity and is transforming in cell culture (PMID- 16187797, 2005; 19680293, 2009; NCCN, NSCLC v3.2019).

In ClinVar, the clinical significance of *EGFR* L858R is 'Drug response' (Somatic: 'Pathogenic' and having drug response to tyrosine kinase inhibitor in non-small cell lung cancer (NSCLC), 'Likely pathogenic' and having drug response to tyrosine kinase inhibitor in lung adenocarcinoma; Germline: 'drug response' mentioned for gefitinib, carboplatin, docetaxel, erlotinib, gemcitabine, and paclitaxel in the context of NSCLC (Variation ID: 16609).

EGFR L858R is one of the most common *EGFR* mutations identified in non-small cell lung cancer (NSCLC) (40%) (NCCN, NSCLC v3.2019; COSMIC, February 2019)

Most patients with sensitizing *EGFR* mutations (including L858R) are non-smokers or former light smokers with adenocarcinoma histology and are significantly higher in female patients (NCCN, NSCLC v3.2019). In surgically resected lung micro-invasive adenocarcinoma patients, *EGFR* L858R alteration was significantly associated with tumor size, diameter of tumor microinvasion, the presence of intratumoral fibrosis, TTF-1 expression and, inflammatory cell infiltration (PMID- 30546439). The presence of *EGFR* L858R does not appear to be prognostic of survival for patients with NSCLC, independent of therapy (NCCN, NSCLC v3.2019).

In NSCLC, the presence of *EGFR* L858R is predictive of treatment benefits from EGFR tyrosine kinase inhibitor (EGFR-TKI) therapy (i.e., erlotinib, gefitinib, afatinib,osimertinib, and dacomitinib); therefore, this mutation is referred to as sensitizing *EGFR* mutation (NCCN, NSCLC v3.2019). Erlotinib, gefitinib, osimertinib, afatinib and dacomitinib are FDA-approved drugs for NSCLC and are NCCN recommended (category 1) first-line therapy in patients with advanced, recurrent, or metastatic non-squamous NSCLC who have known active sensitizing *EGFR* mutations (NCCN, NSCLC v3.2019). The NCCN Panel recommends (category 2A) considering an afatinib/cetuximab regimen for patients who have progressed after receiving erlotinib, gefitinib, afatinib, dacomitinib and chemotherapy (NCCN, NSCLC v3.2019).

Treatment with adjuvant gefitinib led to significantly longer disease-free survival compared with that of vinorelbine plus cisplatin in patients with completely resected stage II-IIIAEGFR L858R-mutant NSCLC (PMID- 29174310). A Chinese patient with advanced NSCLC harboring EGFR L858R substitution showed an approximately complete regression on treatment with the combination of oral icotinib and bronchial artery infusion (BAI) chemotherapy (PMID- 30079342).

Co-occurring Variants
Combined Interp.

Gene Level Info

Variant Level Info

Evidence in Databases

Drug/Guideline Info

EGFR L858R

EGFR T790M is classified to Tier 1 based on level A evidence [AMP classification].

EGFR encodes a receptor tyrosine kinase activated by members of the epidermal growth factor family and is involved in cell proliferation, metastasis, and migration while preventing apoptosis (PMID- 27843613).

A missense alteration in *EGFR*, T790M, is identified in this case. Codon 790 lies in exon 20 in the protein kinase domain of EGFR (UniProt.org). *EGFR* T790M is a catalytically active mutation that activates the kinase approximately 5 fold compared to wild-type enzyme (PMID- 18227510, 2008). The methionine side chain at this mutation leads to steric hindrance that affects the ability of EGFR tyrosine kinase inhibitors (TKI) to bind to the ATP-kinase pocket (PMID- 27382309).

In ClinVar, the clinical significance of *EGFR* T790M is 'Drug response' (Somatic: 'Pathogenic' and having resistance to tyrosine kinase inhibitor in non-small cell lung cancer (NSCLC), 'Likely pathogenic' in lung cancer; Germline: 'drug response' mentioned for gefitinib, erlotinib, in the context of NSCLC) (Variation ID: 16613). *EGFR* T790M is reported in NSCLC (COSMIC, February 2019).

EGFR T790M is a mutation associated with acquired resistance to EGFR TKI therapy and has been reported in about 60% of patients with disease progression after initial response to erlotinib, gefitinib or afatinib (NCCN, NSCLC v3.2019).

This patient also harbors mutations in *EGFR* (L858R), *EGFR* amplification and *CD274* amplification. For patients with an underlying *EGFR* sensitizing mutation who have been treated with EGFR TKI, minimum appropriate testing includes high-sensitivity evaluation for p.T790M. The presence of p.T790M can direct patients to third-generation EGFR TKI therapy (NCCN, NSCLC v3.2019).. Regardless of PD-L1 expression levels, subsequent therapy with PD-1 or PD-L1 monotherapy appears to be less effective in tumors with *EGFR* mutations or *ALK* rearrangements based on data in the second-line setting. Data suggest that pembrolizumab is not effective as first-line therapy in patients with metastatic NSCLC and EGFR mutations, even those with PD-L1 levels more than 50% (NCCN, NSCLC v3.2019). For patients with sensitizing *EGFR* mutations who progress during or after first-line erlotinib, afatinib, gefitinib, dacomitinib, or osimertinib therapy, recommended subsequent therapy depends on whether the progression is asymptomatic or symptomatic and includes osimertinib if not previously given and T790M positive (NCCN, NSCLC v3.2019). A study in 86 patients who harbored *EGFR* activating mutations and *EGFR* amplification and received EGFR-TKI treatment reported longer PFS in patients with *EGFR* activating mutations and co-occurring gene amplification than those without (PMID:26141217).

The NCCN Panel also recommends Osimertinib (category 1) for patients with T790M who have progression with symptomatic brain metastases based on data showing an improvement (NCCN, NSCLC v3.2019). The NCCN Panel recommends (category 2A) considering an afatinib/cetuximab regimen for patients who have progressed after receiving erlotinib, gefitinib, afatinib or dacomitinib and chemotherapy.
Patients with T790M-positive and T790M-negative tumors had a similar response rate to an afatinib/cetuximab regimen (NCCN, NSCLC v3.2019).

Osimertinib alone or in combination with JAK1 inhibitor, INCB039110 (NCT02511106-phase III, NCT02917993-phase I/II); osimertinib + Bcl-2 family protein inhibitor, navitoclax (NCT02520778-phase I); osimertinib in combination with dacomitinib (NCT03810807-phase I); EGFR/HER2 Inhibitor, AP32788 (NCT02716116-phase I/II) are in clinical trials for NCLC patients with *EGFR* L858R and T790M mutations.

EGFR T790M

Significance of this variant with other variants

Notice a lack of FDA approved therapies specific to the co-occurring variant, but a clear indication of clinical trials suggesting Osimertinib

Variants Not in the Same Gene

Example: Thyroid Cancer | *BRAF/TERT*

National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2018
Thyroid Carcinoma

NCCN Guidelines Index
Table of Contents
Discussion

94. Basolo F, Torregrossa L, Giannini R, et al. Correlation between the BRAF V600E mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. J Clin Endocrinol Metab 2010;95:4197-4205. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20631031.

95. Liu R, Bishop J, Zhu G, et al. Mortality Risk Stratification by Combining BRAF V600E and TERT Promoter Mutations in Papillary Thyroid Cancer: Genetic Duet of BRAF and TERT Promoter Mutations in Thyroid Cancer Mortality. JAMA Oncol 2016. Available at: https://www.ncbi.nlm.nih.gov/pubmed/27581851.

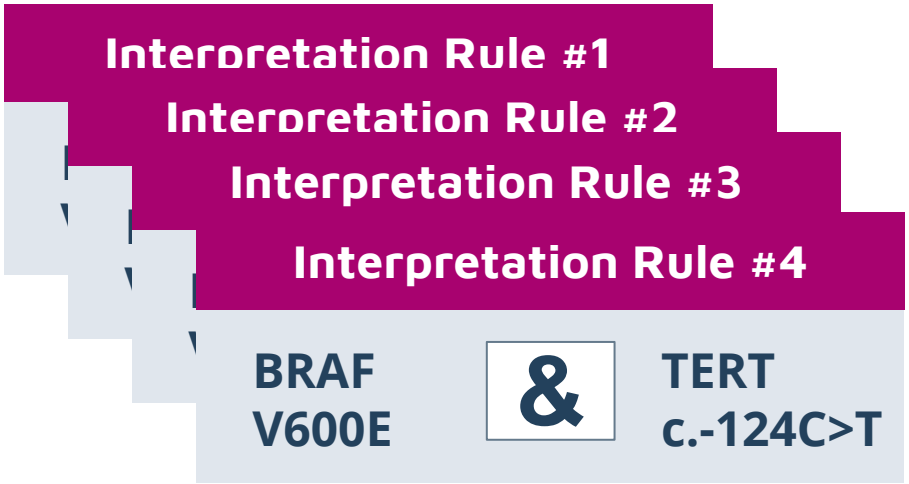
96. Vuong HG, Altibi AMA, Duong UNP, Hassell L. Prognostic implication of BRAF and TERT promoter mutation combination in papillary thyroid carcinoma-A meta-analysis. Clin Endocrinol (Oxf) 2017;87:411-417. Available at: https://www.ncbi.nlm.nih.gov/pubmed/28666074.

99. Canadas-Garre M, Becerra-Massare P, Lopez de la Torre-Casares M, et al. Reduction of false-negative papillary thyroid carcinomas by the routine analysis of BRAF(T1799A) mutation on fine-needle aspiration biopsy specimens: a prospective study of 814 thyroid FNAB patients. Ann Surg 2012;255:986-992. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22504197.

100. Lee ST, Kim SW, Ki CS, et al. Clinical implication of highly sensitive detection of the BRAF V600E mutation in fine-needle aspirations of thyroid nodules: a comparative analysis of three molecular assays in 4585 consecutive cases in a BRAF V600E mutation-prevalent area. J Clin Endocrinol Metab 2012;97:2299-2306. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22500044.

101. Kleiman DA, Sporn MJ, Beninato T, et al. Preoperative BRAF(V600E) mutation screening is unlikely to alter initial surgical treatment of patients with indeterminate thyroid nodules: a prospective case series of 960 patients. Cancer 2013;119:1495-1502. Available at:

Establishing Interpretation Rules



IF thyroid cancer **AND** *BRAF* V600E **AND** *TERT* Promoter mutation (c.-124C>T) **THEN** adjust interpretation to explain prognosis.

Example: Thyroid Cancer | *BRAF/TERT*

Tip #1

Establish rules for interpreting co-occurring variants.

Tip #2

Create dynamic filters to quickly identify co-occurring variants.

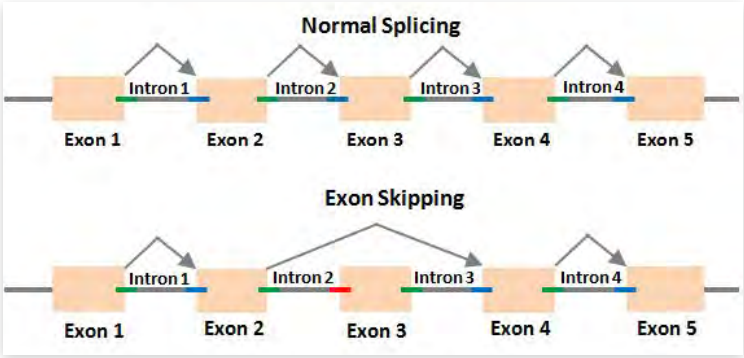
pieriandx

Splice Site Variants

Exon Skipping

Background

What is Exon Skipping?



Clinical testing at MGH via our next-generation sequencing (NGS) and NGS-rearrangement panels showed an additional 16 cases of *MET* exon 14 skipping, for an overall estimated frequency of 5.6%. A clinical case of a patient with *MET* exon 14 skipping treated with the MET inhibitor crizotinib is also described.

PUBMED:27022036

1 There are four important conserved cis-acting sequences necessary for the spliceosome to function: the 5' splice site (SS) GU, the branching site A, the polypyrimidine tract, and the 3' SS AG.

https://www.sciencedirect.com/science/article/pii/S1556086416304543

2 However, one of the challenges in establishing effective targeted treatments against cMET remains the accurate identification of biomarkers for the selection of responsive subsets of patients. Recently, splice site mutations have been discovered in cMET that lead to the skipping of exon 14, impairing the breakdown of the receptor. Patients with NSCLC who are carrying this splice variant typically overexpress the cMET receptor and show a response to small molecule inhibitors of cMET. Here, we review the main differences at the structural level between the wild-type and the splice variants of cMET and their influence on cMET signaling. We clarify the reason why this variant responds to small molecule inhibitors and their prognostic/predictive role.

3

https://www.sciencedirect.com/science/article/pii/S1556086416304543

Exon Skipping

Bioinformatics and QC

1. Does your BED file cover splice variants, and to what degree?

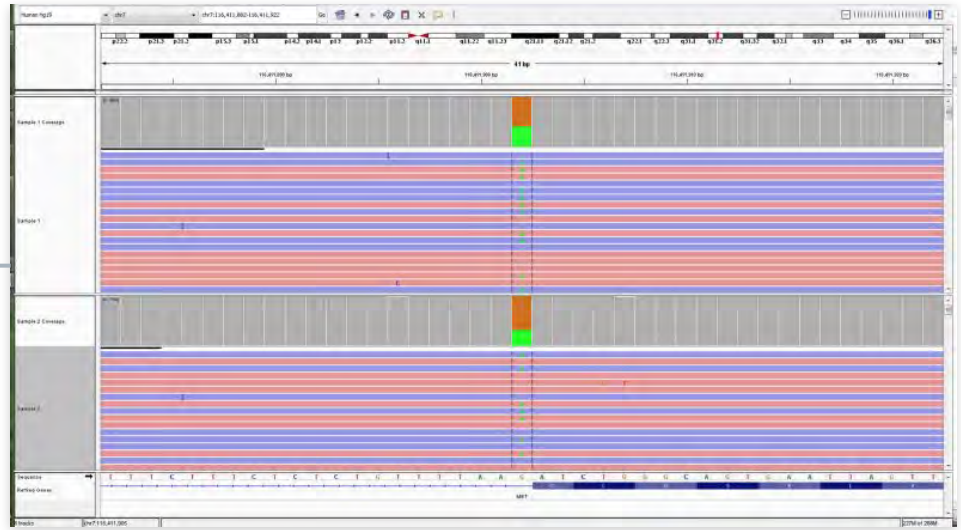
2. Does your caller identify splice variants?

- ✓ Illumina (specific pairings)
- ✓ Thermo Fisher (specific pairings)
- ✓ ArcherDx (no pairs, all variants)

3. Reviewing the BAM file: Is the variant real?

4. Setting up filters

Detection of the MET ex14 skipping mutation. A summary view from Archer Analysis illustrating the skipping of MET ex14 in the FusionPlex assay data.



Detection of the MET ex14 skipping mutation. IGV showing the G>A point mutation (c.2942-1G>A) identified by DNA sequencing.

Source:
http://www.imedpub.com/articles/met-exon-14-skipping-mutation-innonsmall-cell-lung-cancer-identifiedby-anchored-multiplex-pcr-and-nextgeneration-sequencing.php?aid=18004

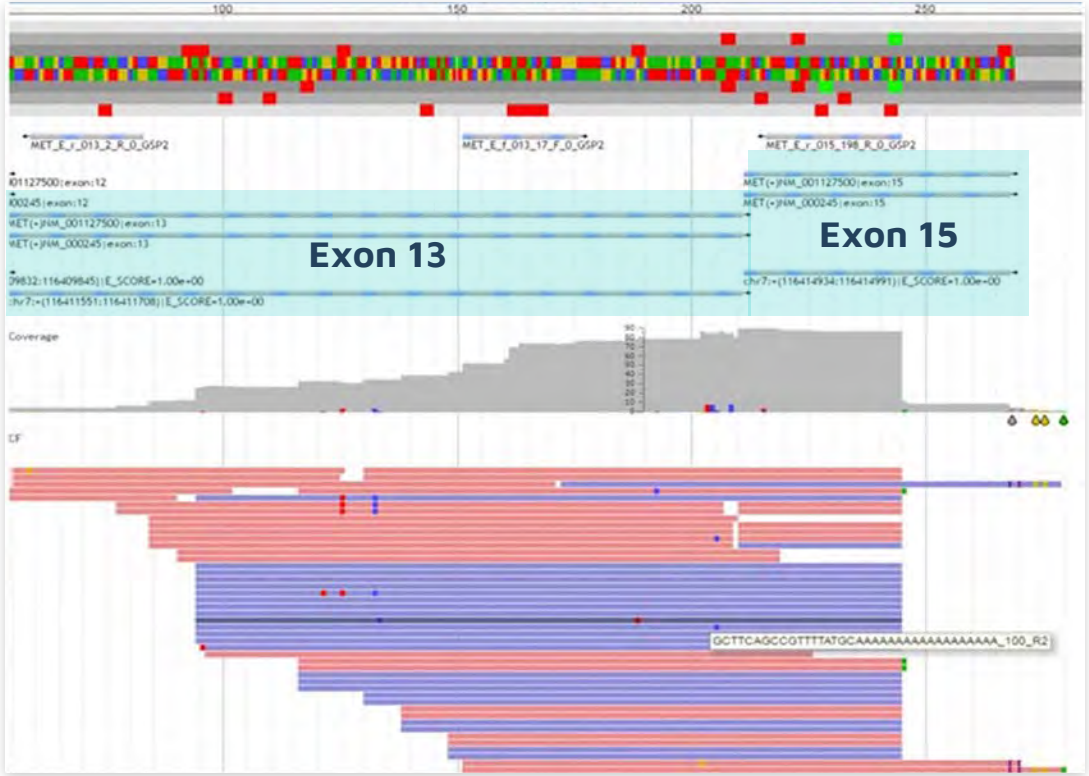
Exon Skipping

Sequencer Bioinformatics and QC

	Illumina	ArcherDx	Thermo Fisher
Additional Caller?	RNA Splice Variant Calling software	Traditional SNV/Indel	Traditional SNV/Indel
Inputs	BAM files and SJ.out.tab files from STAR	BAM	BAM
Outputs	VCF files ; additional tags for splice variants	VCF, additional tags for splice variants	VCF, additional tags for splice variants
QC Criteria	Meets validated read depth, check BAMs for isoforms,	Meets validated read depth, check bams for isoforms	Meets validated read depth, check bams for isoforms
Example Assays	TruSight™ Oncology 500*, TruSight™ Tumor 170	FusionPlex, VariantPlex Solid Tumor, and VariantPlex CTL	Ion Ampliseq RNA Fusion, RNA Exon Variant, Oncomine Focus

* PierianDx adding a tag in the VCF to differentiate splice variants from other fusions.

Reviewing the BAM Files & Filters

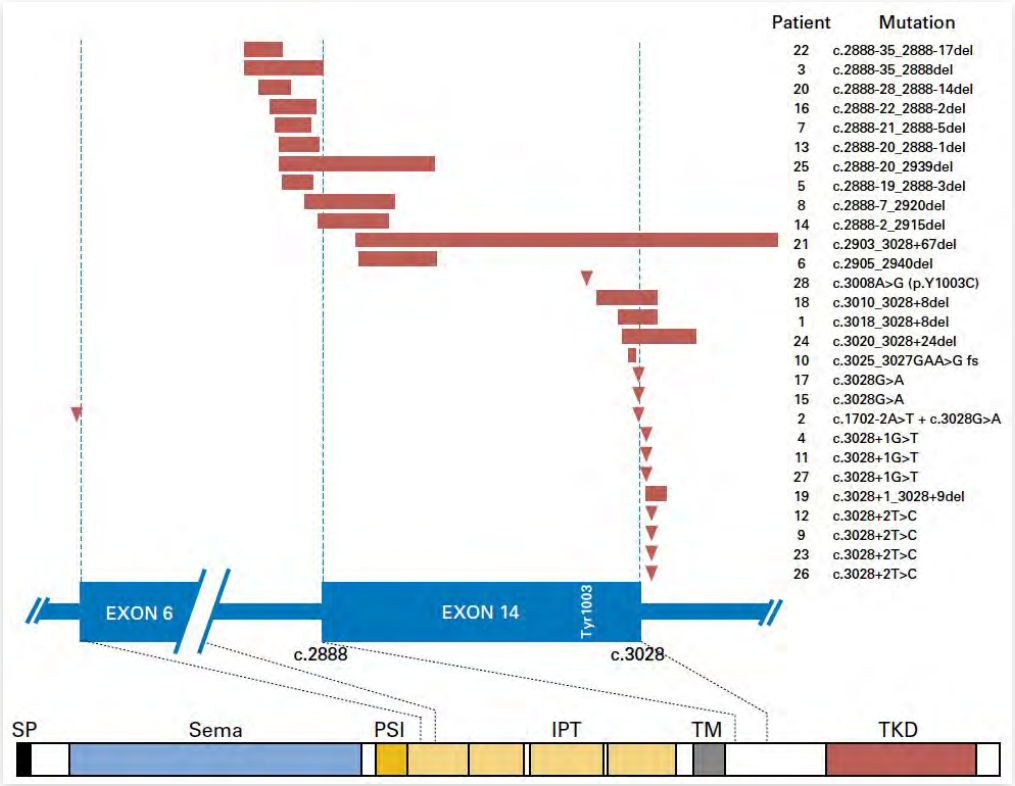


Sequencing data reads detecting a *MET* exon 14 skipping mutation.

Source: https://www.jto.org/article/S1556-0864(18)32745-X/fulltext

Exon Skipping

"Well Known" *MET* Exon 14 Skips



Locations of *MET* exon 14 genomic alterations found in 28 patients with non–small-cell lung cancer.

Source: https://dash.harvard.edu/bitstream/handle/1/32705575/jco.2015.63.4600.pdf

Exon Skipping
Interpretation

Example: MET Exon 14 Skipping Mutation

In 2015, interpretations were less robust.

Today, there are NCCN guidelines specifically dealing with *MET* Exon 14 skipping in lung cancer and the use of Crizotinib.

OLD Published Clinical Genomics Workspace Interpretation

VIEW CLINICAL INTERPRETATION

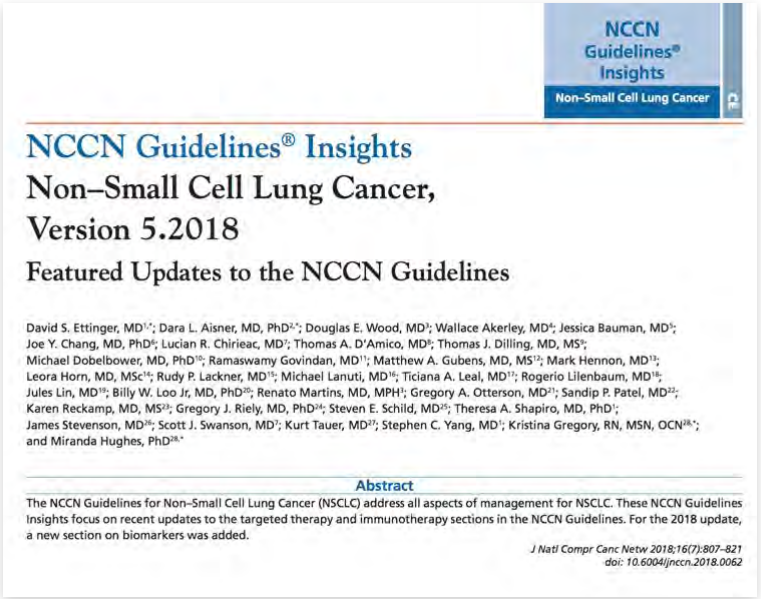
Variant text	c.3018_3028+14del
Date updated	09/16/2015 18:18
Variant(s)	Any
Chromosome	
Gene	MET
Start/Stop	
Min Copy Number	
Max Copy Number	
Disease	
Classification	4-Variant of uncertain significance
Status	PUBLISHED
Interpretation	A novel non-synonymous splice site variant caused by a 25 base pair deletion extending into exon 14 of *MET* was detected by our next generation sequencing and confirmed by Sanger sequencing. This deletion occurs in the tyrosine kinase domain. Missense mutations of *MET* are rarely found in patients with nonhereditary cancer, and most are found in the SEMA and juxtamembrane domain of *MET* (PMID:22128285). Some clinical trials using small molecular inhibitors of *MET* have shown some clinical response in tumors with *MET* activating mutations or *MET* gene amplifications (PMID:19666136). However, the functional consequence of this variant to this patient's lung cancer and its potential as an activating *MET* mutation are unknown.

NEW Published Clinical Genomics Workspace Interpretation

A *MET* mutation, c.2942-23_2942-15delinsC, was detected at the DNA level at a VAF of 95% in conjunction with MET exon 14 skipping at the RNA level (2,107 supporting reads). MET encodes a receptor tyrosine kinase that transduces signals from the extracellular matrix into the cytoplasm by binding to hepatocyte growth factor/HGF ligand. Binding of its ligand, hepatocyte growth factor, induces dimerization and activation of the receptor, which plays a role in cellular survival, embryogenesis, and cellular migration and invasion. This particular variant occurs in the polypyrimidine tract in intron 13 which is involved in splicing. MET mutations causing exon 14 skipping in this gene are associated with multiple human cancers, including about 5% of lung cancer cases (PMID: 25971939, 29139039, 19096300). NCCN recommends crizotinib (category 2A) in advanced stage lung cancer patients with high-level MET amplification or MET exon 14 skipping (NCCN, NSCLC v.4, 2018, NSCLC-H). Clinical investigation is underway to study the efficacy of drugs targeting MET, particularly in patients with MET exon 14 skipping or high MET amplification (NCT02414139, NCT02864992, NCT03088930; NCT03468985).

Interpretation

MET Exon 14 Skipping Mutation



NCCN Guidelines® Insights
Non–Small Cell Lung Cancer

NCCN Guidelines® Insights
Non–Small Cell Lung Cancer, Version 5.2018
Featured Updates to the NCCN Guidelines

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Abstract

The NCCN Guidelines for Non–Small Cell Lung Cancer (NSCLC) address all aspects of management for NSCLC. These NCCN Guidelines Insights focus on recent updates to the targeted therapy and immunotherapy sections in the NCCN Guidelines. For the 2018 update, a new section on biomarkers was added.

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Emerging Biomarkers

The NCCN Guidelines recommend broad-based molecular testing to assess for rare driver mutations for which effective drugs may be available (even if not yet FDA-approved for lung cancer), or to counsel patients regarding the availability of clinical trials in addition to assessing for established biomarkers. Emerging rare driver mutations include *HER2* mutations (ie, *ERBB2* mutations), *RET* rearrangements, high-level *MET* amplification, or *MET* exon 14 skipping mutations (see "Emerging Targeted Agents for Patients With Genetic Alterations" in the complete version of the NCCN Guidelines [NSCL-H]). Clinical trials are currently in progress for other emerging biomarkers; for example, new targeted agents are being assessed for effectiveness in patients with *NTRK* fusions.[74,75]

Immunotherapy

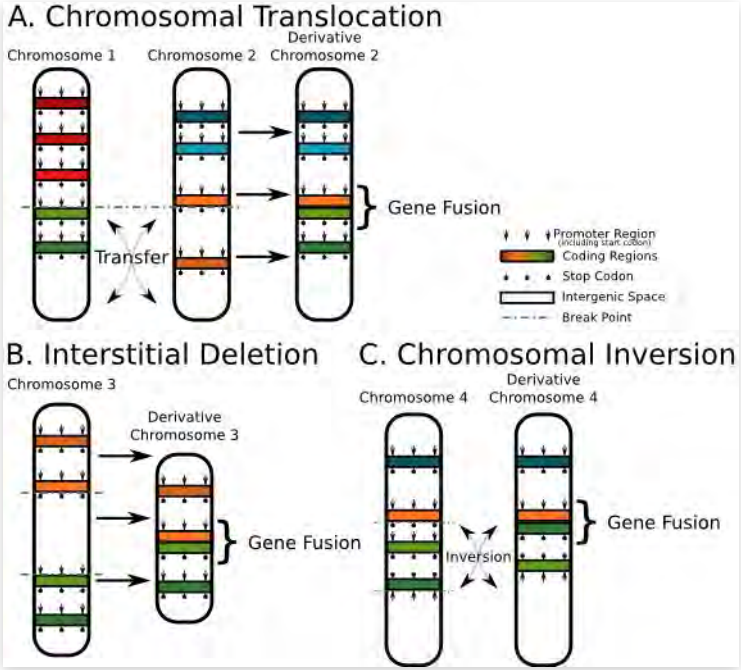
The NCCN panel recommends first-line pembrolizumab/carboplatin (or cisplatin)/pemetrexed (category 1) for patients with advanced nonsquamous NSCLC (ie, adenocarcinoma, large cell carcinoma) or NSCLC not otherwise specified based on data from phase II and III trials and on the FDA approval (pembrolizumab/carboplatin/pemetrexed).[76,77] Pembrolizumab/chemotherapy is recommended for patients without (or unknown) genetic alterations whose PD-L1 levels are <50% or unknown. Most patients received pembrolizumab/carboplatin/pemetrexed (72%; n=445), but some received pembrolizumab/cisplatin/pemetrexed (28%; n=171); patients did not have *EGFR* mutations or *ALK* rearrangements. The estimated OS rate at 1 year was 69.2% (95% CI, 64.1–73.8) for pembrolizumab/chemotherapy versus 49.4% (95% CI, 42.1–56.2) for chemotherapy alone (HR for death, 0.49; 95% CI, 0.38–0.64; $P<.001$) after a median follow-up of 10.5 months. OS was improved regardless of PD-L1 expression levels, although most patients (63%) had levels of ≥1%. Median PFS was 8.8 months (95% CI, 7.6–9.2 months) for pembrolizumab/chemotherapy

Gene Fusions

Background

Fusion Data can be absolutely critical to treatments:

1. Significant guidelines and FDA recommendations specific to *NTRK* fusions and larotrectinib treatments
2. *ROS1* fusions and crizotinib
3. Long known *EML4-ALK*



Source: https://en.wikipedia.org/wiki/Fusion_gene#/media/File:Gene_Fusion_Types.png

Fusion Types

Examples of FUSION:

5' breakpoint : Forward strand , Intron
3' breakpoint: Forward strand , Intron

```
chr4    57385021    bnd_1   T   T[chr4:62800557[    .   .   SVTYPE=BND;ROWID=bnd_1;MATEID=bnd_2;EVENT=fus90_FF89_brk_typ_1;VARTYPE=fusion   GT  ./.
chr4    62800557    bnd_2   G   ]chr4:57385021]G    .   .   SVTYPE=BND;ROWID=bnd_2;MATEID=bnd_1;EVENT=fus90_FF89_brk_typ_1;VARTYPE=fusion   GT  ./.
```

gSyntax: t(4;4)(q13.1;q12)(chr4:g.57385021::chr4:g.62800557)

cSyntax: t(4;4)(q13.1;q12)(chr4(NM_206919):c.192+2_chr4(LPHN3:NM_015236):c.1909-1)

pSyntax: (NP_996802:p.?)

Consequence : FUSION_INFRAME (as length of modified mRNA is multiple of 3)

Example 1
Inframe

5' breakpoint : Forward strand , Exon
3' breakpoint: Reverse strand , Intron

```
chr4    57385009    bnd_1   C   C]chr4:54876314]    .   .   SVTYPE=BND;ROWID=bnd_1;MATEID=bnd_2;EVENT=FR_EX_INT_FS;VARTYPE=fusion   GT  ./.
chr4    54876314    bnd_2   T   T]chr4:57385009]    .   .   SVTYPE=BND;ROWID=bnd_2;MATEID=bnd_1;EVENT=FR_EX_INT_FS;VARTYPE=fusion   GT  ./.
```

gSyntax: t(4;4)(q11;q12)(chr4:g.57385009::ochr4:g.54876314)

cSyntax: t(4;4)(q11;q12)(chr4(NM_206919):c.182_ochr4(CHIC2:NM_012110):c.448-2)

pSyntax: (NP_996802:p.?)

Consequence : FRAMESHIFT

Example 2
Frameshift

Fusion Types

5' breakpoint : Reverse strand , Intron
3' breakpoint: Reverse strand , Exon

```
chr7    140549962   bnd_1   G   G[chr7:141635612[   .   .   SVTYPE=BND;ROWID=bnd_1;MATEID=bnd_2;EVENT=RR_INT_EX_FUS;VARTYPE=fusion  GT  ./.
chr7    141635612   bnd_2   A   ]chr7:140549962]A   .   .   SVTYPE=BND;ROWID=bnd_2;MATEID=bnd_1;EVENT=RR_INT_EX_FUS;VARTYPE=fusion  GT  ./
```

gSyntax: t(7;7)(q34;q33)(chr7:g.140549962::chr7:g.141635612)

cSyntax: t(7;7)(q34;q33)(ochr7(NM_013252):c.345+2_ochr7(BRAF:NM_004333):c.189)

pSyntax: (NP_996802:p.?)

Consequence : FUSION_INFRAME

MicroHomolgy events :

CGW supports microhomology around breakpoints. Due to microhomology present around breakpoints Manta, tool that identifies fusion events, reports different breakpoints in two different rows of same event.

Example :

```
chr18   23612363    bnd_1   C   [chrX:52786931[C    42  MinQUAL SVTYPE=BND;ROWID=bnd_1;MATEID=bnd_2;EVENT=MH1_EX_EX_typ4;VARTYPE=fusion GT  ./.
chrX    52786929    bnd_2   A   [chr18:23612365[A   42  MinQUAL SVTYPE=BND;ROWID=bnd_2;MATEID=bnd_1;EVENT=MH1_EX_EX_typ4;VARTYPE=fusion GT  ./.
```

Consider,

1. row bnd_1 which represents exon-exon fusion,
2. row bnd_2 which represents intron- exon fusion,

Modified mRNA built in 1). is same as the one built in 2). ; hence the consequence will also be a match.

Bioinformatics and QC

Know your caller's challenges

(see Thermo Fisher recommendations for multiple fusions)

Check BAM

Some clients also run BLAST algorithms in NCBI to confirm

Filter

Note: Clinical Genomics Workspace new simplified syntax

Multiple fusions in the same gene

Occasionally, when a true positive fusion in a gene pair is detected, a second fusion in the same gene pair may also be reported. Generally, a single fusion has occurred in this pair of genes in this sample, and all the read evidence that covers any exon is reported, which may occasionally be shown as a second fusion, usually describing a different exon in one of the genes. This second fusion may be described as either a targeted fusion (i.e., the panel has a specific assay designed to detect this fusion, so it is designated Present in the Detection column) or a non-targeted fusion (detected, but the panel has no specific assay pair designed for this fusion, so it is designated Present-Non-targeted in the Detection column). Biologically, both of these calls are likely from the same underlying fusion in the gene pair.

Other information reported for fusions:

- The Locus column reports the start and end positions of the fusion transcript.
- The Read Counts column reports the number of reads that provide evidence for the fusion call. For ASSAYS_5P_3P, read counts are displayed in the order 5', 3'.
- The COSMIC/NCBI column provides links at which the fusion is described in these public web sites.
- The Variant ID column reports our internal short-hand for the fusion.

Recommended Reading from your vendor: (helpful hint, use pdf word finders for)

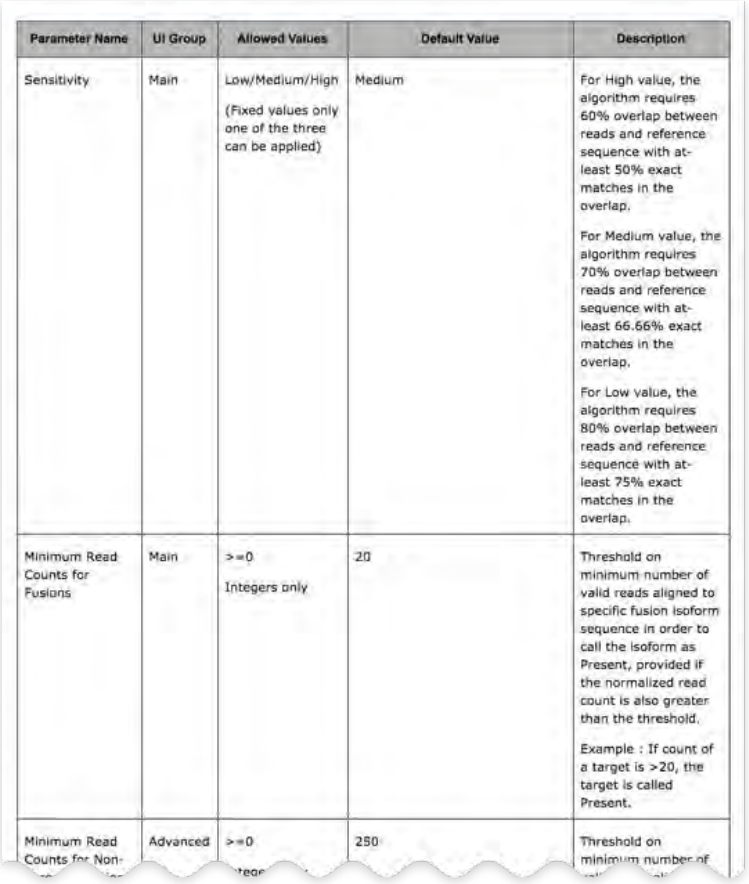
Thermo Fisher: https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0017605_IonReporter5_10_UG.pdf
Illumina: https://support.illumina.com/help/BS_App_TruSightTumor170_OLH_1000000028435/Content/Source/Informatics/Apps/FusionCallling_appT170.htm#
Manta specific: https://www.biorxiv.org/content/biorxiv/suppl/2015/08/10/024232.DC1/024232-1.pdf
ArcherDx: https://archerdx.com/support/faqs/software/archer-analysis/archer-analysis-troubleshooting-faqs/how-do-i-know-if-my-fusion-is-real
ArcherDx: https://archerdx.com/assets/documents/PN-MKT-0008-Fusion-detection-in-Archer-Analysis-Software.pdf

Fusion Callers

Thermo Fisher | Ion Torrent

Notice the caller will return a data point (in the vcf) with a value of low/medium/high.

You can pull your filter requirements directly from these caller information guides.



Parameter Name	UI Group	Allowed Values	Default Value	Description
Sensitivity	Main	Low/Medium/High (Fixed values only one of the three can be applied)	Medium	For High value, the algorithm requires 60% overlap between reads and reference sequence with at-least 50% exact matches in the overlap. For Medium value, the algorithm requires 70% overlap between reads and reference sequence with at-least 66.66% exact matches in the overlap. For Low value, the algorithm requires 80% overlap between reads and reference sequence with at-least 75% exact matches in the overlap.
Minimum Read Counts for Fusions	Main	>=0 Integers only	20	Threshold on minimum number of valid reads aligned to specific fusion isoform sequence in order to call the isoform as Present, provided if the normalized read count is also greater than the threshold. Example : If count of a target is >20, the target is called Present.
Minimum Read Counts for Non-	Advanced	>=0	250	Threshold on minimum number of

29 Fusion Parameters

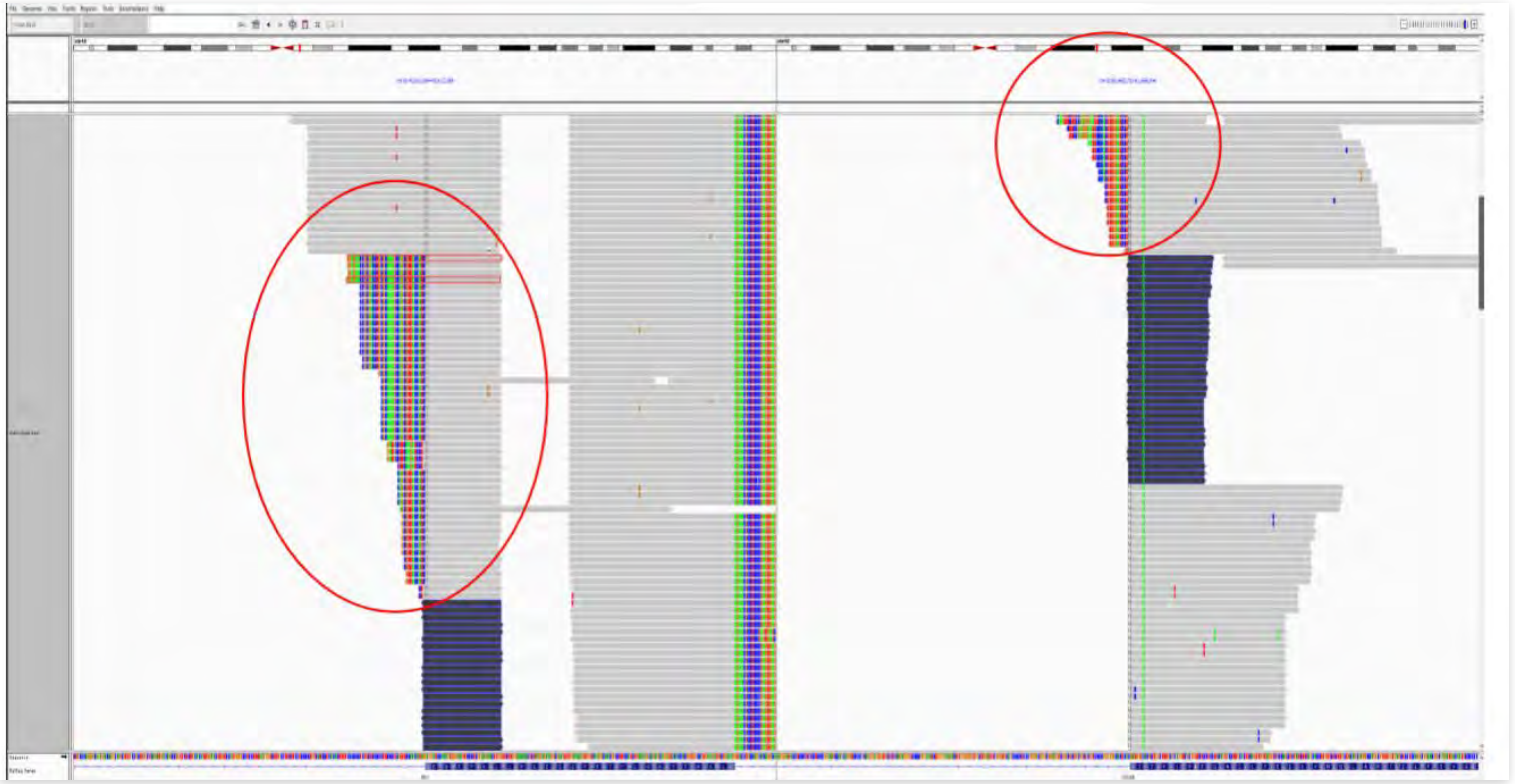
Gene Fusions

Reviewing BAM Files

KIF5B, RET Fusion

Reviewing BAM Files

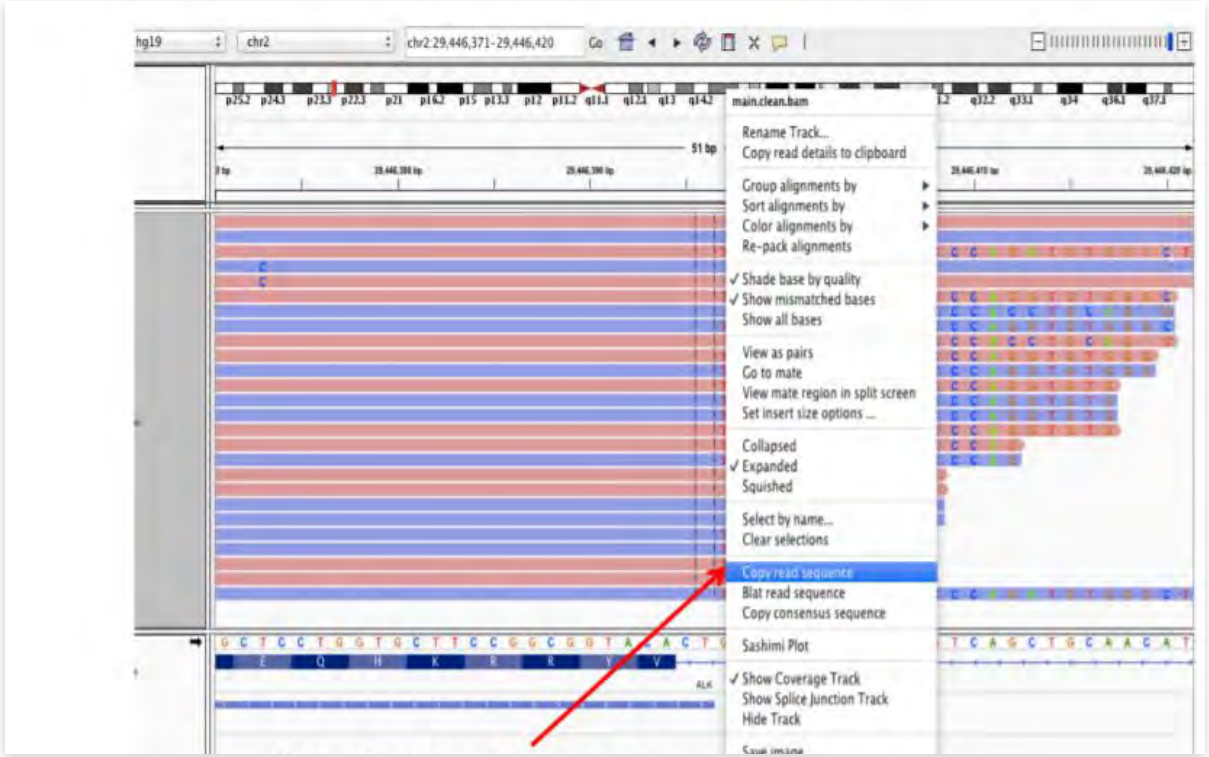
RET-CCDC6 Fusion



Check BLASTn

EML4-ALK Fusion

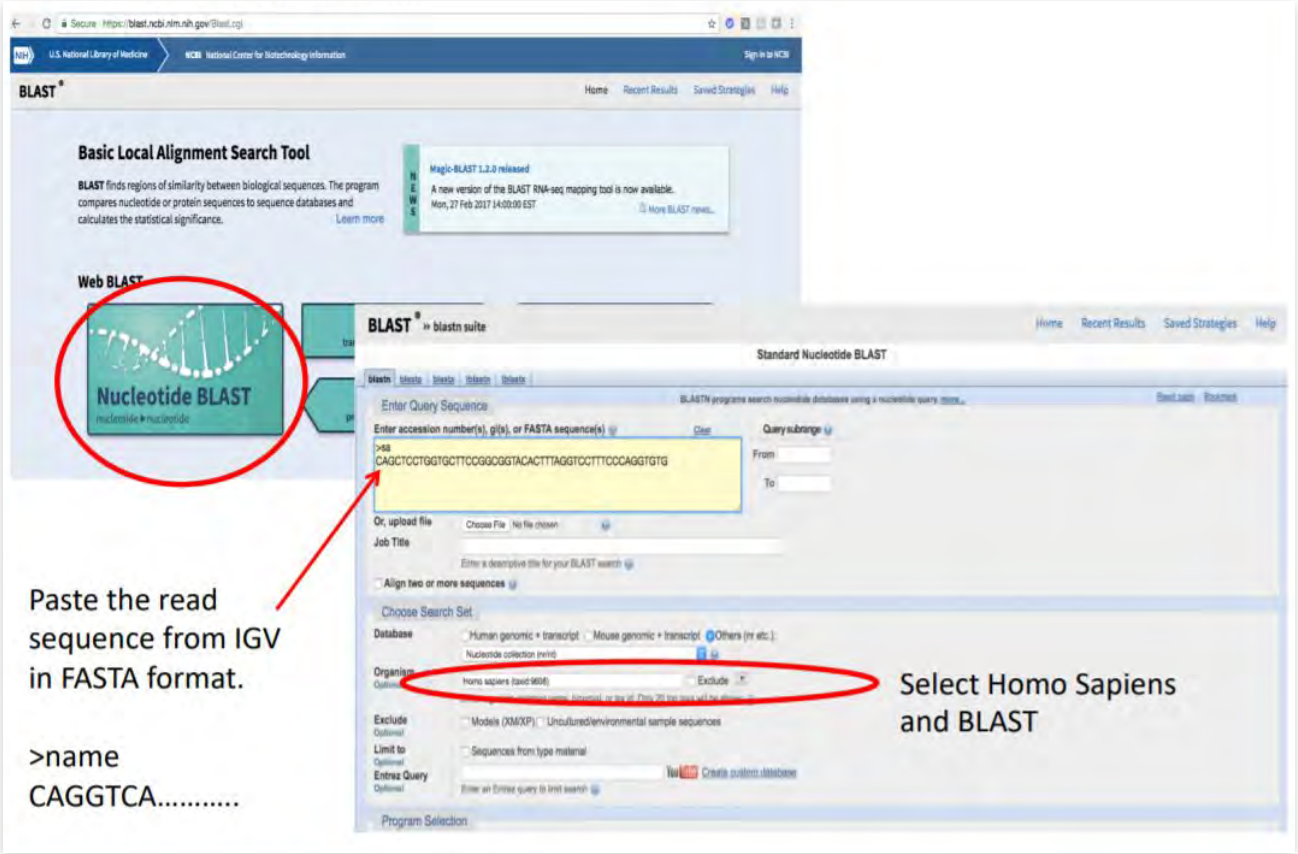
Step 1
Copy read sequence



Check BLASTn

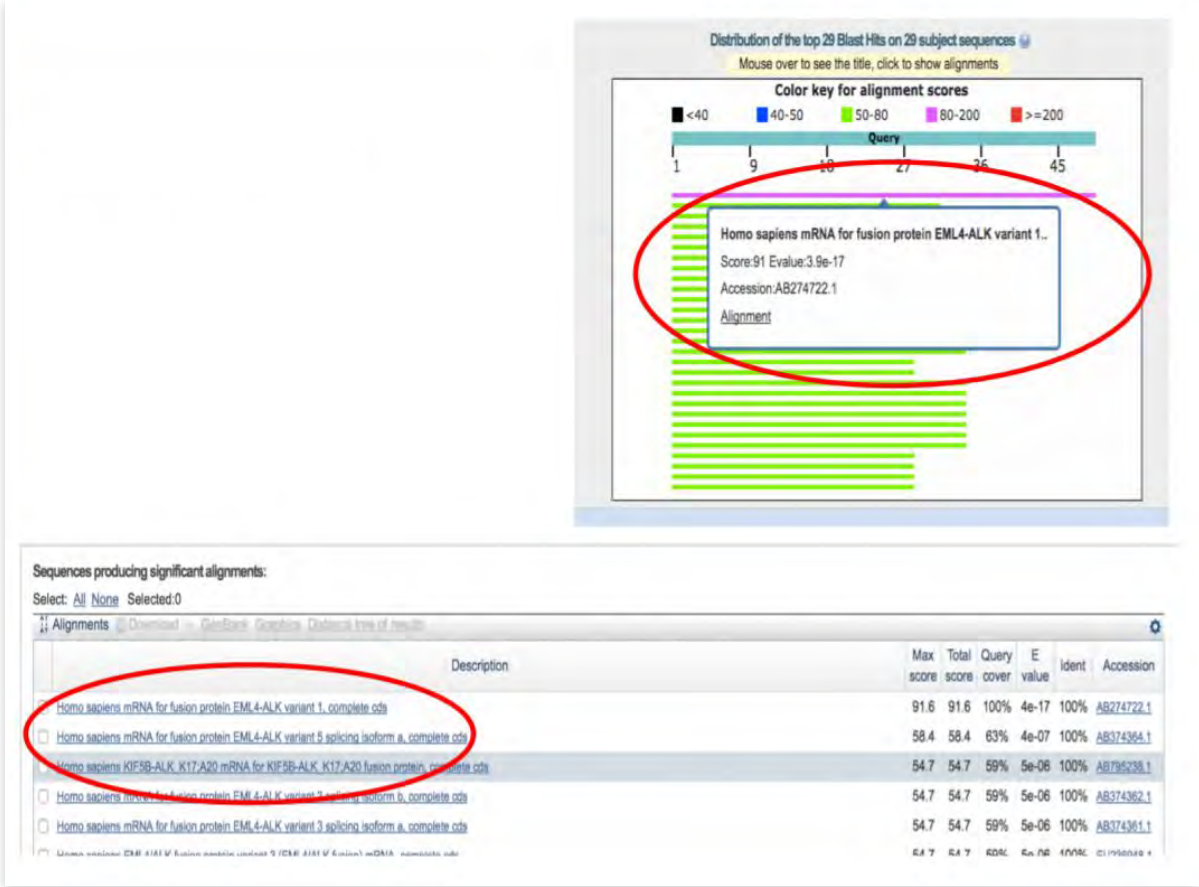
Step 2

Blast (Compare)



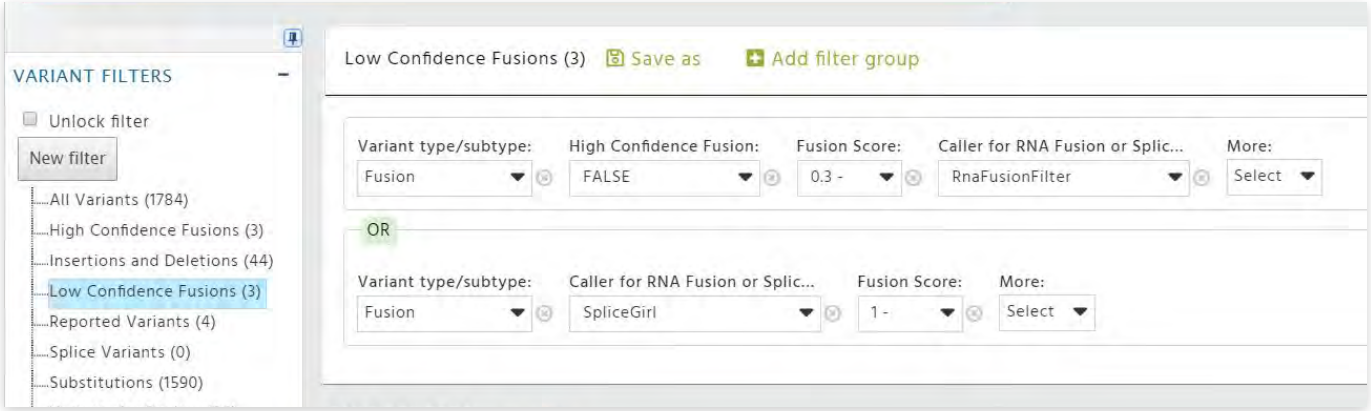
Gene Fusions
BLASTn

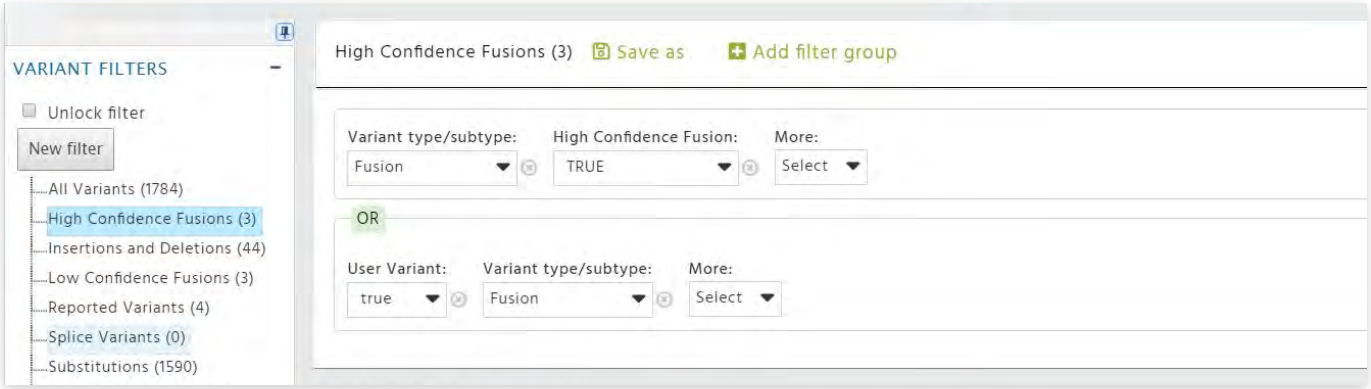
Step 3
Review Results



Gene Fusions

Setting Up Filters





Interpretations

RESULT SUMMARY

Variants Detected	Therapies or Prognostic Indication (in patient's malignancy)	Therapies or Prognostic Indication (in another malignancy)	Clinical Trial Opportunity
EML4, ALK t(2;2)(p23;p21) Fusion (chr2:g.29446208::ochr2:g.42472827)	✓ Responsive to Brigatinib, Crizotinib, Alectinib, Lorlatinib, Ceritinib	✓ Responsive to Crizotinib, Ceritinib	Yes
TPM3, NTRK1 t(1;1)(q21-q22;q21.2) Fusion (ochr1:g.154142876::chr1:g.156844363)	✓ Responsive to Larotrectinib	✓ Responsive to Larotrectinib	Yes

Nomenclature Difference

TAGS	CLASSIFICATION	GENE	VARIANT	DNA CHANGE	VARIANT TYPE/SUBTYPE	CONSEQUENCE	KNOWLEDGEBASE SOURCES	CALLER FOR RNA FUSION OR SPLICE VARIANT	FUS ALT SPL REA
	III	ALK, TRMT6...	*ALK-TRMT61B* fusion transcript	t(2;2) (p23.2;p23) (ch...	Fusion	Frameshift		RnaFusionFilter	40
	III	EML4, ALK	*EML4-ALK* fusion transcript	t(2;2) (p23;p21) (chr2...	Fusion	Frameshift		RnaFusionFilter	154
	III	PTPN3, ALK	*PTPN3-ALK* fusion transcript	t(2;9) (p23;q31) (chr2...	Fusion	Frameshift		RnaFusionFilter	133

Interpretations

EML4-ALK Fusion: Therapy Information

Interpretation: An ALK rearrangement (2p23) was detected. ALK mutations have been reported in numerous cancers including neuroblastomas, large cell lymphoma, colorectal carcinomas, and non-small cell lung carcinomas (NSCLC) (PMID 18923524, 8122112, 22327622, 18593892). Multiple EML4-ALK fusion variants have been reported in NSCLC, including the EML4-ALK E13;A20 variant. ALK is a receptor tyrosine kinase that plays a role in neurogenesis, as well as regulation of cell growth, differentiation and transformation (PMID 19737948, 27573755). EML4-ALK fusion variants have been found to promote cell migration and invasion in NSCLC, but have not been found to have significant effect on cell proliferation (PMID 30236141). These fusions are found in approximately 5% of patients with NSCLC, and are associated with younger age and light-to-never smoking history. Among all stage IV NSCLC patients, those with EML4-ALK fusions experience the longest survival, with median survival exceeding 5 years, when treated with local ablative therapy and at least two consecutive ALK inhibitors, or with crizotinib followed by a second, next-generation ALK inhibitor and pemetrexed (PMID 29363116, 30599201). There are multiple ALK tyrosine kinase inhibitors with FDA approval, including crizotinib, ceritinib, alectinib, and brigatinib. A phase 2 clinical trial is recruiting patients to study the safety and effectiveness of the ALK and ROS1 tyrosine kinase inhibitor Lorlatinib in patients with ALK positive Stage IV NSCLC with central nervous system metastasis in the absence of measurable extracranial lesions (NCT02927340). Additionally a phase 2 clinical trial is recruiting patients to evaluate the activity of targeted therapy with the ALK inhibitor Ceritinib and stereotactic ablative body radiation in patients with ALK positive NSCLC who have either received therapy with one prior ALK inhibitor or are ALK-inhibitor naïve (NCT02513667). Finally a phase 2 clinical trial is recruiting patients to evaluate the efficacy and safety of Ceritinib in patients with ALK positive NSCLC with metastasis to the brain and/or leptomeninges (NCT02336451).

EML4-ALK Fusion: NO Therapy Information

EML4 encodes a member of the echinoderm microtubule-associated protein-like family which is involved in microtubule formation (RefSeq, Jan 2015).

ALK (Anaplastic Lymphoma Kinase) encodes a receptor tyrosine kinase, which belongs to the insulin receptor superfamily. *ALK* is found to be rearranged, mutated, or amplified in a series of tumors including anaplastic large cell lymphomas, neuroblastoma, and non-small cell lung cancer (RefSeq, Jan 2011).

Abnormal fusion of parts of *EML4* gene with portions of the *ALK* gene generates *EML4-ALK* fusion transcripts, which is one of the primary mutations associated with non-small cell lung cancer (RefSeq, Jan 2015).

An *EML4-ALK* fusion is identifiedt. Exons 1 - 13 of the *EML4* gene get fused with exons 20-29 (encompassing the cytoplasmic portion of *ALK* with the tyrosine kinase domain) of *ALK*. This fusion is expected to result in a chimeric protein with N terminal *EML4* fused with *ALK*. Fusion *EML4-ALK* contains the N-terminal half of *EML4* and the intracellular catalytic domain of *ALK* and translocation occurs through a paracentric inversion (PMID- 26579422).

EML4-ALK gene rearrangement is most widely reported in non-small cell lung cancers (NSCLCs) (COSMIC, December 2018; NCCN, NSCLC, v2.2019).

About 5% of NSCLC patients have *ALK* rearrangements and have similar characteristics to those with EGFR mutations i.e (adenocarcinoma histology, never smokers, light smokers)

NTRK Fusion, "Tissue Agnostic"

FDA approves an oncology drug that targets a key genetic driver of cancer, rather than a specific type of tumor

New drug Vitrakvi targets specific receptor kinase that promotes tumors

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For Immediate Release

November 26, 2018

Release

Español

The U.S. Food and Drug Administration today granted accelerated approval to Vitrakvi (larotrectinib), a treatment for adult and pediatric patients whose cancers have a specific genetic feature (biomarker).

This is the second time the agency has approved a cancer treatment based on a common biomarker across different types of tumors rather than the location in the body where the tumor originated. The approval marks a new paradigm in the development of cancer drugs that are "tissue agnostic." It follows the policies that the FDA developed in a guidance document released earlier this year.

Vitrakvi is indicated for the treatment of adult and pediatric patients with solid tumors that have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity and have no satisfactory alternative treatments or that have progressed following treatment.

LOXO

VITRAKVI (larotrectinib) 25-mg/100-mg CAPSULES 20-mg/mL ORAL SOLUTION

Listen to the FDA D.I.S.C.O. podcast about this approval

On November 26, 2018, the Food and Drug Administration granted accelerated approval to larotrectinib (VITRAKVI, Loxo Oncology Inc. and Bayer) for adult and pediatric patients with solid tumors that have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion without a known acquired resistance mutation, that are either metastatic or where surgical resection is likely to result in severe morbidity, and who have no satisfactory alternative treatments or whose cancer has progressed following treatment.

This is the second tissue-agnostic FDA approval for the treatment of cancer.

Approval was based on data from three multicenter, open-label, single-arm clinical trials: LOXO-TRK-14001 (NCT02122913), SCOUT (NCT02637687), and NAVIGATE (NCT02576431). Identification of positive NTRK gene fusion status was prospectively determined in local laboratories using next generation sequencing (NGS) or fluorescence *in situ* hybridization (FISH). NTRK gene fusions were inferred in three pediatric patients with infantile fibrosarcoma who had a documented ETV6 translocation by FISH. The major efficacy outcome measures were overall response rate (ORR) and response duration, as determined by a blinded independent review committee according to RECIST 1.1.

Efficacy was evaluated in the first 55 patients with unresectable or metastatic solid tumors harboring an NTRK gene fusion enrolled across the three trials. All patients were required to have progressed following systemic therapy for their disease, if available, or would have required surgery with significant morbidity for locally advanced disease. Twelve patients were less than 18 years of age. A total of 12 cancer types were represented, with the most common being salivary gland tumors (22%), soft tissue sarcoma (20%), infantile fibrosarcoma (13%), and thyroid cancer (9%).

ORR was 75% (95% CI: 61%, 85%), including 22% complete responses and 53% partial responses. At the time of database lock, median duration of response had not been reached. Response duration was 6 months or longer for 73%, 9 months or longer for 63%, and 12 months or longer for 39% of patients.

Interpretation

Date updated	02/10/2019 14:16
Variant(s)	chr1:g.154137492_156843543inv
Chromosome	
Gene	NTRK1
Start/Stop	
Min Copy Number	
Max Copy Number	
Disease	Non-small cell lung cancer
Classification	1-Predictive or prognostic in tumor type
Status	PUBLISHED
Interpretation	**Interpretation:**

***TPM3-NTRK1* inversion is classified to Tier 1 based on level A evidence [AMP classification].**

NTRK1 encodes receptor tyrosine kinase which is involved in the development and the maturation of the central and peripheral nervous systems through regulation of proliferation, differentiation, and survival of sympathetic and nervous neurons (UniProt.org).

TPM3 binds to actin filaments in muscle and non-muscle cells and plays a central role, in association with the troponin complex, in the calcium dependent regulation of vertebrate striated muscle contraction (UniProt.org).

NTRK1 Fusion

An intrachromosomal inversion resulting in *TPM3-NTRK1* fusion is identified in this case. The exons 1-10 of *TPM3* are fusing with exon 8-17 of *NTRK1*. *TPM3–NTRK1* fusion encodes a functional and oncogenic chimeric protein containing the coiled-coil domain of the *TPM3* and transmembrane and kinase domains of *NTRK1* (UniProt.org; PMID- 26001971; 24962792). *NTRK* gene fusions encode tropomyosin receptor kinase (TRK) fusion proteins (eg, TRKA, TRKB, TRKC) that act as oncogenic drivers for solid tumors including lung (NCCN, NSCLC v3.2019)

TPM3-NTRK1 fusion has been reported in non-small cell lung cancer (NSCLC) (PMID- 30215037; 26881293).

NTRK fusions occur in 0.2% of patients with NSCLC (NCCN, NSCLC v3.2019).

Larotrectinib is a FDA approved for the treatment of adult and pediatric patients with solid tumors that have a *NTRK*gene fusion. The NCCN panel recommends larotrectinib (category 2A) as a first-line therapy option for patients with *NTRK* gene fusion–positive metastatic NSCLC (NCCN, NSCLC v3.2019).

Merestinib (MET inhibitor) (NCT02920996- phase II), TPX-0005 (ALK/ROS1/TRK inhibitor) (NCT03093116- phase I/II), Larotrectinib LOXO-101 (Trk inhibitor) (NCT02576431, phase II; NCT03215511, phase I/II) and Entrectinib (Pan-TRK/ALK/ROS tyrosine kinase inhibitor) (NCT02568267, phase II) are in clinical trials for NSCLC/advanced solid tumor patients harboring *NTRK1* fusion or rearrangements.

Interpretation

CD74-ROS1

Interpretation: A *CD74-ROS1* fusion is detected in this squamous cell carcinoma specimen. Targeted DNA and mRNA sequencing demonstrated that *CD74* exon 6 is fused in-frame with *ROS1* exon 34 with retention of the ROS1 kinase domain. *CD74* is a commonly observed fusion partner (MyCancerGenome, 12/2018), and the major fusion transcript is the same as has been previously described (PMID: 23724914). *ROS1* encodes a transmembrane protein with intracellular tyrosine kinase activity (PMID: 18778756). *ROS1* rearrangements where the kinase domain is retained are implicated in a range of human epithelial cancers, most commonly non-small cell lung cancer (PMID: 22215748). Such *ROS1* rearrangements are believed to be oncogenic through dysregulated kinase expression and activation (PMID: 18083107), but are infrequent in lung squamous cell carcinoma (PMID: 27635639, 26973202). *ROS1* fusions in NSCLC predict response to crizotinib or ceritinib and portend improved outcomes (PMID: 25264305, NCCN guidelines NSCLC v2.2019). If progression due to development of resistance occurs, loratinib is recommended by NCCN guidelines (NSCLC v2.2019). A patient with biphasic pulmonary blastoma harboring a *CD74-ROS1* fusion responded to crizotinib (PMID: 29343973). In one series, a higher rate of brain metastases, and a shorter progression-free survival on crizotinib (of borderline significance), were observed among patients with *CD74-ROS1* fusions compared with *ROS1*-fusions with other genes (PMID: 29704675); others have reported no independent prognostic significance of a *ROS1* fusion in non-adenocarcinoma NSCLC patients (PMID: 27635639).

Copy Number Variants

Background

Bioinformatics/Quality Control

- Different callers, different visualizations
- Relative copy number (min/max)

Interpretation

Copy number variant interpretations

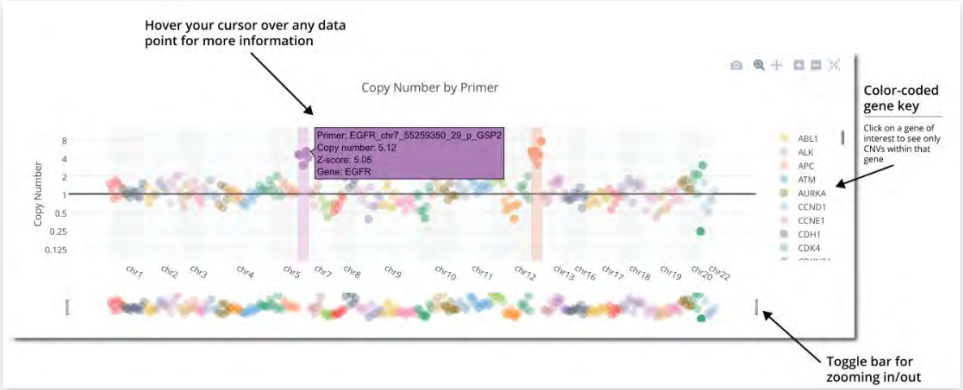
- A CNV result is not the same as expression result
- Filters: set for MinMax
- How do you report on these variants?
- As with co-occurring variants, you may need a table to demonstrate copy min/max values

Example

Non-small Cell Lung Cancer

CD274 Amplification (PDL-1)
CCND1 Amplification
MYC Amplification
EGFR Amplification (Level 2)

Bioinformatics and Quality Control

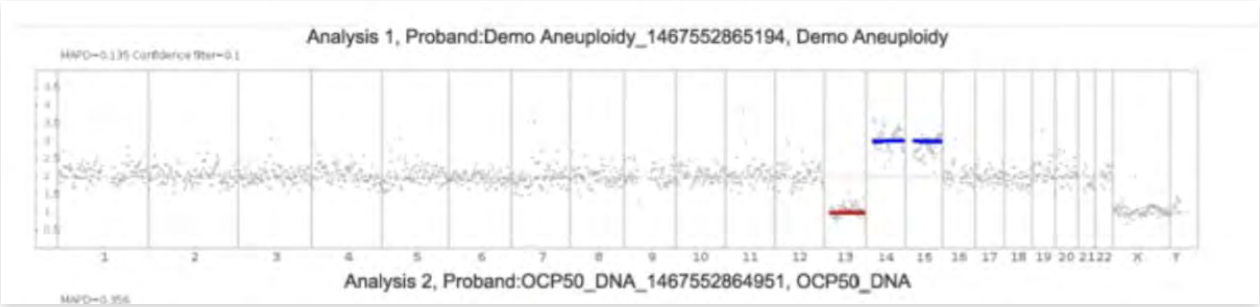


What does your system provide?

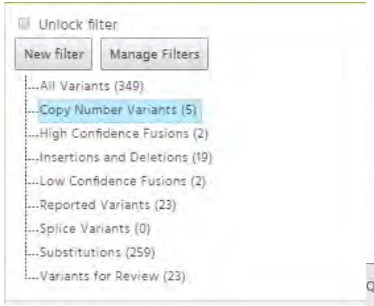
Copy number?

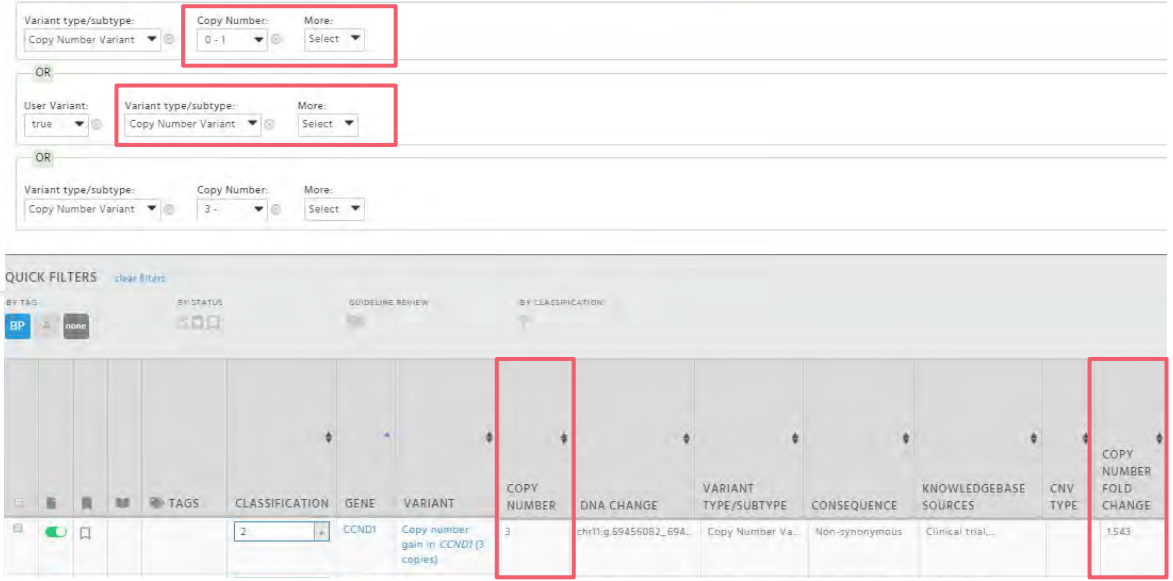
Copy number fold change?

Copy number min/max?



Filters and Rules





Tip #1

Create dynamic filters to quickly identify copy number variants

Tip #2

Establish rules for interpreting copy number variants

Rather than identifying an interpretation for ALL copy number variants (which would capture copies of 0,1,2), we identify variants within a range or above a specific number.

Interpretation Rule #5

ERBB2 **&** **Copy Number <3**

Interpretation

CD274 (PDL-1) Amplification

Interpretation:

***CD274* amplification (5 copies) is classified to Tier 1 based on level A evidence.**

CD274 (knowns as PD-L1) gene encodes an immune inhibitory receptor ligand that is expressed by T cells and B cells and various types of tumor cells. In tumor microenvironments, the interaction of this ligand with its receptor provides an immune escape for tumor cells through cytotoxic T-cell inactivation (RefSeq, Sep 2015).

A whole gene amplification of the *CD274* gene (5 copies) is identified in this case. Studies have reported that tumors with *CD274*/PD-L1 gene amplification displayed a significantly higher frequency of PD-L1 expression and protein levels and harbored significantly higher mutational load compared to non-amplified cases (PMID- 26918453, 27106868). However, pooled analysis in more than 100 type of solid tumors show that PDL1 amplification did not always correlate with high-positive PD-L1 expression by immunohistochemical analysis (PMID- 29902298).
CD274 amplification has been reported in lung adenocarcinoma (COSMIC, February 2019).

In NSCLC patients, PD-L1 copy number gains were significantly associated with PD-L1 expression, patients with PD-L1 amplification tended to have a poorer survival outcome when the analysis was restricted to PD-L1-positive cases (PMID - 27050074). NCCN Panel recommends (category 2A) IHC testing for PD-L1 expression before first-line treatment in patients with metastatic NSCLC with negative or unknown test results for *EGFR* mutations, *BRAF* V600E mutations, *ALK* rearrangements, and *ROS1* rearrangements (NCCN, NSCLC v3.2019).

Pembrolizumab is FDA approved for patients with NSCLC tumors with PD-L1 expression levels ≥1% (NCCN, NSCLC v3.2019). For patients with metastatic nonsquamous NSCLC whose PD-L1 levels are less than 50% or unknown, pembrolizumab/carboplatin (or cisplatin)/pemetrexed is a preferred category 1 option (NCCN, NSCLC v3.2019).

Pembrolizumab/carboplatin/paclitaxel (or albumin-bound paclitaxel) is a category 1 recommended option for patients with squamous cell NSCLC regardless of PD-L1 expression levels; this regimen is preferred for those with whose PD-L1 levels are less than 50% or unknown. Pembrolizumab/cisplatin/paclitaxel (or albumin-bound paclitaxel) is a category 2A recommended option in this setting (NCCN, NSCLC v3.2019)

This patient harbors mutations in *EGFR* (L858R, T790M) and *ALK* rearrangement.

Data suggest that pembrolizumab (category 1) is not effective as first-line therapy in patients with metastatic NSCLC and *EGFR* mutations, even those with PD-L1 levels more than 50% (NCCN, NSCLC v3.2019). Regardless of PD-L1 expression levels, subsequent therapy with PD-1 or PD-L1 monotherapy appears to be less effective in tumors with *EGFR* mutations or *ALK* rearrangements based on data in the second-line setting (NCCN, NSCLC v3.2019).

KA2507 (HDAC6 inhibitor) is currently in phase I clinical trial recruiting patients with PD-L1 expressing solid tumors (NCT03008018). ARRY-382 (cFMS tyrosine kinase inhibitor) in combination with pembrolizumab is in phase I/II clinical trial recruiting advanced/metastatic PD-L1-positive NSCLC patients (NCT02880371).

Note: Clinical correlation, further assessment of PD-L1 protein expression by IHC is recommended to appropriately determine clinical significance of PD-L1 amplification in patient's disease.

FDA guideline for expression levels

NCCN guidelines for sub-division of disease, requires review)

Request IHC confirmation of expression levels to corroborate CNV

Interpretation

MYC Amplification

Interpretation:

***MYC* amplification is classified to Tier 2 based on level C evidence [AMP classification].**

MYC is a proto-oncogene and encodes a nuclear phosphoprotein that plays a role in cell-cycle progression, apoptosis, and cellular transformation (RefSeq, Aug 2017).

A whole gene amplification of the *MYC* gene (7 copies) is identified in this case. *MYC* copy-number gains showed elevated average expression in vitro (PMID- 25407018). High c-Myc expression is expected to result in aberrant expression of target genes leading to tumor cell growth and proliferation (PMID- 23021215, 2012; 29313490, 28466200).

MYC amplification has been reported in lung adenocarcinoma (ADCs) (COSMIC, February 2019).

In patients with lung ADCs, c-MYC amplification was reported as an independent poor prognostic factor for overall survival in univariate analysis; and was associated with lymphatic invasion and recurrence (PMID- 24809777). A study in patients with early-stage lung ADCs, *MYC* amplification was associated with poor prognosis in terms of relapse-free survival (RFS) (PMID-21148746, 2011). In retrospective analysis of NSCLC patients, increased *MYC* gene copy number was reported to be a strong predictor of worse survival (PMID-25806711).

A checkpoint kinase inhibitor prexasertib (LY2606368) is in phase II clinical trial in patients with advanced solid tumors exhibiting *MYC* amplification (NCT02873975). ATR kinase inhibitor M6620 (VX-970) is also in phase II clinical trial in patients with solid tumors harboring *MYC* amplification (NCT03718091)

Interpretation

EGFR Amplification

Interpretation:*EGFR* amplification is classified to Tier 2 based on level C evidence [AMP classification].

EGFR encodes a receptor tyrosine kinase activated by members of the epidermal growth factor family and is involved in cell proliferation, metastasis, and migration while preventing apoptosis (PMID- 27843613).

A whole gene amplification of the *EGFR* gene (16 copies) is identified in this case. *EGFR* amplification is reported to result in overexpression of *EGFR* (PMID- 25053711). *EGFR* amplifications have been associated with EGFR expression and activation leading to tumor invasion and growth (PMID- 23429996, 2013).

EGFR amplification has been reported in lung adenocarcinoma (ADCs) and squamous cell carcinoma (COSMIC, February 2019).

EGFR copy number gain is a commonly observed alteration and has been reported in up to 50% cases of NSCLC (PMID- 22263017, 2010).

EGFR gene amplification in NSCLC patients was related to lymphatic metastasis and TNM stage (PMID- 26400330). Lung ADCs patients with *EGFR* amplification had a significantly worse outcome in univariate analysis (PMID- 19826035, 2010).

A recent meta-analysis of patients treated with EGFR-tyrosine kinase inhibitor (EGFR-TKI) has found that increase in *EGFR* gene copy number was associated with higher overall response rate (ORR), overall survival (OS) and progression-free survival (PFS) compared with patients without a high *EGFR* gene copy number (PMID- 27664271). A similar analysis among EGFR-TKI treated patients reported that increased *EGFR* gene copy number appears to be associated with improved survival outcomes; the effect on OS appears to be limited to patients of non-Asian descent(PMID- 20826716, 2011).

Results of SQUIRE trial state that, NSCLC patients harboring *EGFR* amplifications treated with necitumumab (monoclonal EGFR antibody) showed an improved overall survival (PMID- 29158193). Data from phase III clinical trials indicated that patients with advanced NSCLC, whose tumors express high levels of EGFR protein and/or gene copy numbers of *EGFR* (≥4 *EGFR* copies) derive greater therapeutic benefits from *EGFR*-directed mAbs used in combination with immunotherapy and are absent when used in combination with antiangiogenic agents (PMID- 29905778).

Neratinib, a pan -ERBB2 inhibitor alone or in combination with palbociclib, trametinib or everolimus is also currently being investigated in clinical trials in advanced or metastatic solid tumors patients with *EGFR* amplification (NCT03065387-phase I; NCT01953926-phase II). FATE-NK100 (allogeneic CD3- CD19- CD57+ NKG2C+ NK cells) in combination with cetuximab are being studied in phase I clinical trial in EGFR+ solid tumor patients (NCT03319459).

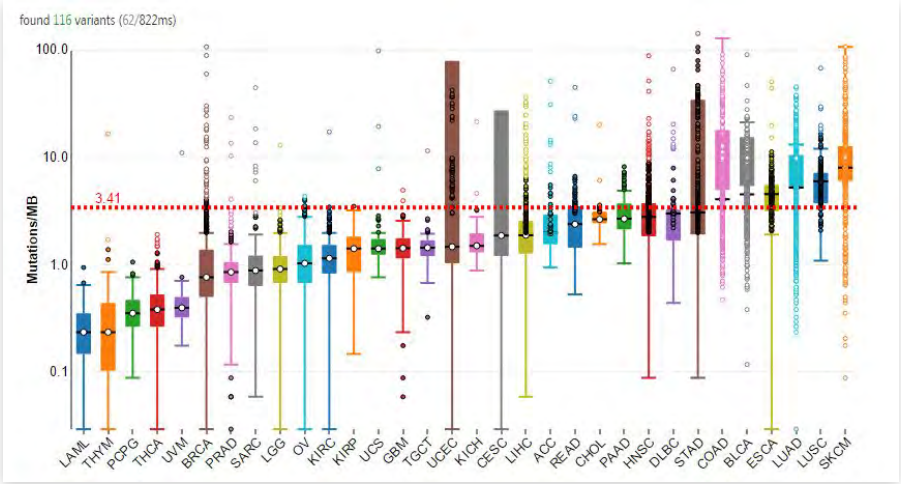
TMB/MSI

Background

TMB and MSI are not Variants

- They don't show up in VCF.
- They are calculated by algorithms.
- They don't follow classification rules (Tier 1, pathogenic, for example).
- There are drugs and clinical trials that are approved and applicable.
- Determine candidacy for immunotherapy

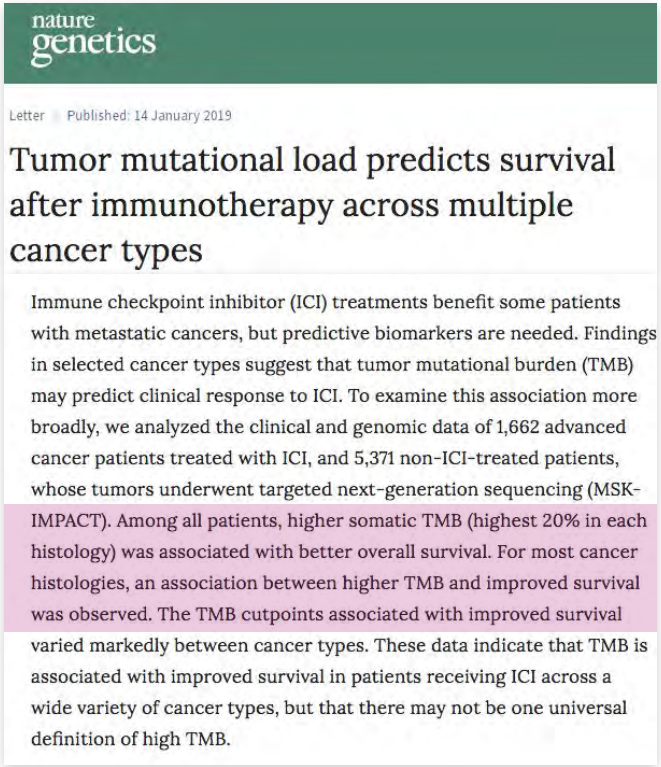
Mutational load by cancer type



IMMUNOTHERAPY CONSIDERATIONS

TUMOR MUTATIONAL BURDEN (TMB): TMB is an emerging marker associated with greater sensitivity to immunotherapeutic agents (Rizvi et al. 2015, Rosenberg et al. 2016, Snyder et al. 2014, Le et al. 2015). This tumor exhibits a mutational burden of **2 Muts/Mb** which is low relative to the TMB distribution observed in patients that show clinical benefit with anti-PD1 therapy in patients with NSCLC (Rizvi et al. 2015).
MICROSATELLITE INSTABILITY (MSI) STATUS: Low This tumor does not exhibit evidence of Microsatellite Instability (MSI). MSI instability and mismatch repair deficiency may predict sensitivity to immune checkpoint inhibitors across a number of solid tumors.

Background



nature genetics

Letter Published: 14 January 2019

Tumor mutational load predicts survival after immunotherapy across multiple cancer types

Immune checkpoint inhibitor (ICI) treatments benefit some patients with metastatic cancers, but predictive biomarkers are needed. Findings in selected cancer types suggest that tumor mutational burden (TMB) may predict clinical response to ICI. To examine this association more broadly, we analyzed the clinical and genomic data of 1,662 advanced cancer patients treated with ICI, and 5,371 non-ICI-treated patients, whose tumors underwent targeted next-generation sequencing (MSK-IMPACT). Among all patients, higher somatic TMB (highest 20% in each histology) was associated with better overall survival. For most cancer histologies, an association between higher TMB and improved survival was observed. The TMB cutpoints associated with improved survival varied markedly between cancer types. These data indicate that TMB is associated with improved survival in patients receiving ICI across a wide variety of cancer types, but that there may not be one universal definition of high TMB.

Abstract

Immunotherapy has shown promising results in various types of cancers. Checkpoint inhibitor drugs developed for cancer immunotherapy have been approved by the US Food and Drug Administration (FDA) for patients with advanced melanoma, non-small cell lung cancer, renal cell carcinoma, bladder cancers, and refractory Hodgkin lymphoma. In the latest announcement, the FDA has granted accelerated approval to pembrolizumab for pediatric and adult patients with microsatellite instability-high (MSI-H) or mismatch repair-deficient solid tumors. This is the first time the agency has approved a cancer treatment based on a common biomarker rather than organ-based approach. MSI-H, either due to inherited germline mutations of mismatch repair genes or epigenetic inactivation of these genes, is found in a subset of colorectal and noncolorectal carcinomas. It is known that MSI-H causes a build up of somatic mutations in tumor cells and leads to a spectrum of molecular and biological changes including high tumor mutational burden, increased expression of neoantigens and abundant tumor-infiltrating lymphocytes. These changes have been linked to increased sensitivity to checkpoint inhibitor drugs. In this mini review, we provide an update on MSI-related solid tumors with special focus on the predictive role of MSI for checkpoint immunotherapy.

https://www.ncbi.nlm.nih.gov/pubmed/28877075

More Recent Interpretations

Example: Colon Cancer

TMB Qualitative: High; TMB Quantitative: 41.24 per Mb **TMB-High is classified to Tier 2 based on level C evidence.**

Interpretation: The Cancer Genome Atlas (TCGA) showed that 16% of colorectal cancers (CRCs) display high TMB (PMID29184690). In a recent study of colorectal cancer (CRC) patients, tumors with high TMB occurred in younger patients (≤45 years old) (PMID- 30018131).

In CRC patients, patients with high TMB had a longer overall survival (OS) than those with low TMB (Ref-http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.4_suppl.572).

In a study of stage II/III CRC patients treated with adjuvant chemotherapy, high TMB was identified as an independent marker of good prognosis (PMID- 30042065). However, in another study of chemotherapy-treated CRC patients, patients with high TMB tended to have worse outcomes, with decreased progression-free survival (PFS), although statistically not significant. In the same study, no difference in time to recurrence was observed in the TMBLow and TMB-Intermediate/High arms in patients treated with oxaliplatin-based therapy in perioperative setting (PMID-29184690).

In metastatic CRC patients treated with bevacizumab (anti-VEGF monoclonal antibody), patients with mutation load >5 had longer overall survival than patients with mutation load ≤5 (Ref-http://ascopubs.org/doi/abs/10.1200/JCO. 2017.35.15_suppl.3504). In a study of diverse cancers (including patients with colon adenocarcinoma) treated with immunotherapy, higher TMB (≥20 mutations/mb) was independently associated with better response rate and better PFS (PMID- 28835386).

Anti-PD1 monoclonal antibody pembrolizumab is in phase II clinical trials for CRC patients with hypermutated MSI negative cancer (NCT01876511). Nivolumab alone or in combination with ipilimumab (anti-PD1 monoclonal antibodies) are in phase II clinical trials in solid tumor patients with high mutation load (NCT03668119; NCT02693535). Anti-PDCD1 monoclonal antibody IBI308 is in phase I/II trials for metastatic cancers (including colorectal cancer) with TMB level >20 mut/Mb (NCT03568539).

More Recent Interpretations

Example: Colon Cancer

MSI Qualitative: High; MSI Quantitative: High **MSI-H is classified to tier 1 based on level A evidence.**

Interpretation: In colon cancer, tumor specimens characterized as MSI-H are more common in stage II disease (22%) than in stage III disease (12%) whereas the percentage of stage IV colorectal tumors characterized as MSI-H ranges from 3.5% to 5.0% in clinical trials and was 6.5% in the Nurses' Health Study and Health Professionals Follow-up Study (NCCN, Colon Cancer v4.2018, NCCN, Rectal Cancer v3.2018). NCCN panel recommends MSI testing of all patients with a personal history of colon or rectal cancer to identify individuals with Lynch syndrome, to inform use of immunotherapy in patients with metastatic disease, and to inform decisions for patients with stage II disease (NCCN, Colon v4.2018, NCCN, Rectal Cancer, v3.2018). In colon cancer, MSI (MSI-H) and wild-type KRAS/BRAF have been associated with both improved prognosis and increased lymph node retrieval (NCCN, Colon v4.2018). In colon cancer, stage II MSI-H patients may have a good prognosis and do not benefit from 5-FU adjuvant therapy, and adjuvant therapy should not be given to patients with low-risk stage II MSI-H tumors (NCCN, Colon v4.2018). Also, in colon cancer, poorly differentiated histology is not considered a high-risk feature for patients with stage II disease whose tumors are MSI-H (NCCN, Colon v4.2018).

Substantial evidence shows that in patients with stage II disease, a deficiency in MMR protein expression or MSI-H tumor status is a prognostic marker of a more favorable outcome (NCCN, Colon Cancer, v4.2018). In rectal cancer, stage II MSI-H patients may have a good prognosis and do not benefit from 5-FU adjuvant therapy (NCCN, Rectal v3.2018). MMR/MSI status may be a prognostic marker for rectal cancer, with 5-year rectal cancer specific survival rates of 100% reported for patients with stage I/II mismatch repair-deficient tumors (NCCN, Rectal v3.2018). In a recent study, in a cohort of stage II/III resected rectal cancer patients, the MSI status was not reported to be associated with survival outcome (PMID- 30077598).

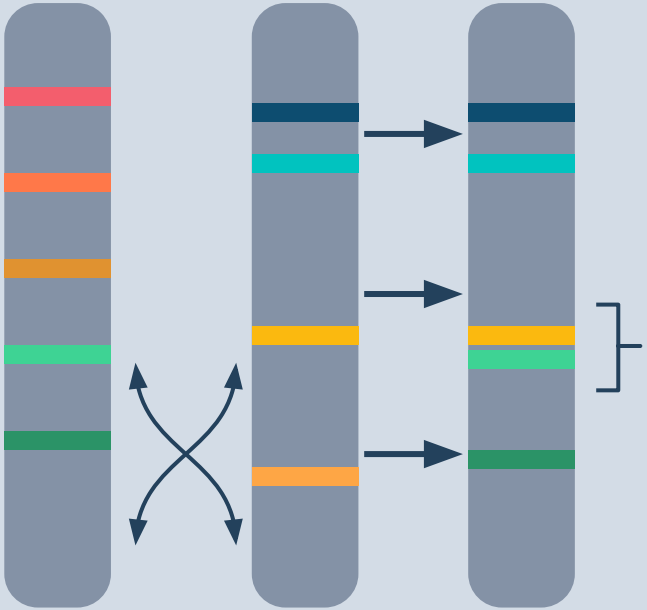
NCCN recommends pembrolizumab (FDA approved PD-1 blocking antibody for MSI-H or dMMR metastatic colorectal cancer), nivolumab (FDA approved PD-1 blocking antibody for MSI-H or dMMR metastatic colorectal cancer), or nivolumab plus ipilimumab as subsequent-line treatment options in patients with metastatic MMR deficient or microsatellite instability-high (dMMR/MSI-H) colorectal cancer who have not previously received a checkpoint inhibitor (NCCN, Colon v4.2018, NCCN, Rectal Cancer, v3.2018).

Nivolumab and ipilimumab (FDA approved anti-CTLA4 antibody) with radiation therapy is under phase II trial for MSI High colorectal cancer (NCT03104439). Pexa-Vec in combination with durvalumab, and tremelimumab (anti-CTLA4) is in phase I/II clinical trial for MSI-H colorectal cancer (NCT03206073). M7824, anti PD-L1/TGF-β antibody, is under phase I/II trial for MSI-H metastatic colorectal cancer or solid tumors (NCT03436563). TSR-042 (anti-PD-1 monoclonal antibody) is currently in phase I clinical trial recruiting MSI-H solid tumors (NCT02715284). LY3300054 (anti-PD-L1 checkpoint antibody) alone or in combination with LY3321367 (anti-TIM2 monoclonal antibody) is currently in phase I clinical trial in patients with MSI-H/ MSI-H and PD-1/PD-L1 Naïve solid tumors (NCT02791334).

Clinical Trials

SELECT CLINICAL TRIALS

Variant	Trial ID	Title	Phase	Disease	URL
MSI-High	NCT03104439	Nivolumab and Ipilimumab and Radiation Therapy in Microsatellite Stable (MSS) and Microsatellite Instability (MSI) High Colorectal and Pancreatic Cancer	II	Malignant tumor of colon; Malignant tumor of rectum	https://clinicaltrials.gov/show/NCT03104439
MSI-High	NCT03206073	A Phase I/II Study of PexaVec Oncolytic Virus in Combination With Immune Checkpoint Inhibition in Refractory Colorectal Cancer	I/II	Malignant tumor of colon; Malignant tumor of rectum	https://clinicaltrials.gov/show/NCT03206073
MSI-High	NCT03436563	A Phase Ib/II Trial of M7824 in Solid Tumors With Microsatellite Instability or With Consensus Molecular Subtype 4 Metastatic Colorectal Cancer	I/II	Malignant tumor of colon; Malignant tumor of rectum	https://clinicaltrials.gov/show/NCT03436563
MSI-High	NCT02715284	A Phase 1 Dose Escalation and Cohort Expansion Study of TSR-042, an Anti-PD-1 Monoclonal Antibody, in Patients With Advanced Solid Tumors	I	Malignant neoplastic disease; Neoplastic disease	https://clinicaltrials.gov/show/NCT02715284
MSI-High	NCT02791334	A Phase 1a/1b Study of a Novel Anti-PD-L1 Checkpoint Antibody (LY3300054) Administered Alone or in Combination With Other Agents in Advanced Refractory Solid Tumors (Phase 1a/1b Anti-PD-L1 Combinations in TumorsPACT)	I	Malignant neoplastic disease; Neoplastic disease	https://clinicaltrials.gov/show/NCT02791334
TMB-High	NCT01876511	Phase 2 Study of MK-3475 in Patients With Microsatellite Unstable (MSI) Tumors	II	Malignant tumor of colon; Malignant tumor of rectum	https://clinicaltrials.gov/show/NCT01876511
TMB-High	NCT03668119	A Randomized, Open-Label, Phase 2 Study of Nivolumab in Combination With Ipilimumab or Nivolumab Monotherapy in Participants With Advanced or Metastatic Solid Tumors of High Tumor Mutational Burden (TMB-H)	II	Malignant neoplastic disease; Neoplastic disease	https://clinicaltrials.gov/show/NCT03668119
TMB-High	NCT02693535	Targeted Agent and Profiling Utilization Registry (TAPUR) Study	II	Malignant neoplastic disease; Neoplastic disease	https://clinicaltrials.gov/show/NCT02693535
TMB-High	NCT03568539	An Open-label, Phase 1b Multicenter Study of IBI308 in Subjects With Advanced/ Metastatic Solid Malignancies	I/II	Malignant neoplastic disease; Neoplastic disease	https://clinicaltrials.gov/show/NCT03568539



1. Co-occurring Variants
2. Splice Site, Exon Skipping
3. Gene Fusions
4. Copy Number Variants
5. Tumor Mutational Burden & Microsatellite Instability

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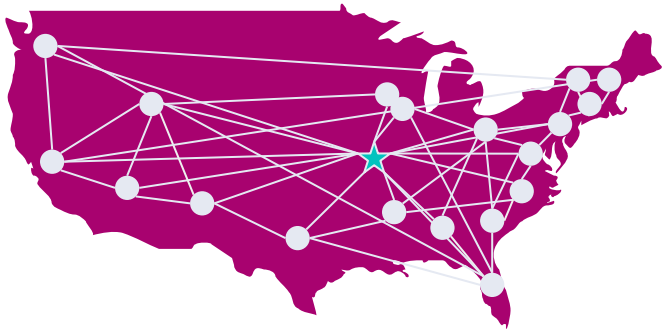

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