Florina__.__.2019

Dear Colleague,

We report the results for patient **Ms/Mr** _____, suffering from _____cancer, whose sample receipt on _____, 2019. The sample we received for analysis was a XXX of whole blood that contained EDTA-Ca as anti-coagulant and packed with an ice pack *(Or alternative for FFPE or Fresh tissue)*. Upon arrival there were performed:

- Malignant cell isolation and then positive and negative selection using multiple cell markers.
- DNA and RNA isolation from the above cells/ *tissue* and evaluation of the above with molecular-based assays as well with spectrophotometry.
- NGS experiments for DNA and RNA samples.

Mr/Ms_____
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Report Summary

GENOMIC FINDINGS BY TIER + LEVEL				TMB		MSI		CLINICAL TRIALS
2 IA	0 IB	1 IIC	0 IID	24 muts/Mb	high status	5% Unstable Sites	stable status	13

GENOMIC FINDINGS

Tier I - Strong Clinical Significance

VARIANT	LEVEL	VAF %	CLINICAL IMPACT
NCOA4-RET fusion	A	-	**Responsive To** - Cabozantinib, Vandatinib *in non-small cell lung cancer*
KRAS p.G12D c.35G>A	A	10.0	**Non-Responsive To** - Erlotinib, Gefitinib, Afatinib, Osimertinib, Dacomitinib **Unfavorable Prognosis In** - non-small cell lung cancer

Tier II - Potential Clinical Significance

VARIANT	LEVEL	VAF %	CLINICAL IMPACT
PDGFRA p.D842V c.2525A>T	C	15.0	**Responsive To** - Dasatinib *in gastrointestinal stromal tumor* **Non-Responsive To** - Sunitinib, Imatinib *in gastrointestinal stromal tumor*

Other Biomarkers

BIOMARKER	STATUS	VALUE	CLINICAL IMPACT
TMB	High	24 muts/Mb	**Responsive To** - Nivolumab, Nivolumab + Ipilimumab *in non-small cell lung cancer*
MSI	Stable	5% Unstable Sites	

Appendix:

Tier I - Strong Clinical Significance

VARIANT	INTERPRETATION
NCOA4-RET fusion A	RET encodes a receptor tyrosine kinase involved in cell growth and differentiation which is known to undergo oncogenic activation in vivo and in vitro by cytogenetic rearrangement (provided by RefSeq, Jul 2008). NCOA4 encodes an androgen receptor coactivator which interacts with the androgen receptor in a ligand-dependent manner to enhance its transcriptional activity. Chromosomal translocations between NCOA4 and RET, both located on chromosome 10, have been associated with papillary thyroid carcinoma (provided by RefSeq, Feb 2009). RET rearrangements resulting in fusion with partner genes including KIF5B, CCDC6 and NCOA4 have been reported in non-small cell lung cancer (NSCLC) patients(PMID- 29128428). A NCOA4-RET fusion is identified in this case. The N terminus of the NCOA4 gene fuses with the C terminus of the RET gene in this fusion (PMID- 28011461). In PCCL3 cells, expression of NCOA4-RET fusion was reported to simultaneously activate DNA synthesis and apoptosis apart from interfering with thyroid differentiation at steps distal to the TSH-R (PMID- 12690093, 2003). The NCOA4-RET fusion has been reported in patients with NSCLC specifically in lung adenocarcinoma patients (COSMIC, February 2019, PMID- 23150706). RET rearrangements are one of the emerging biomarkers to identify novel therapies for patients with metastatic NSCLC (NCCN, NSCLC v.3.2019). NCCN recommends cabozantinib and vandatinib (category 2A) as targeted agents for NSCLC patients harbouring RET rearrangements (NCCN, NSCLC v.3.2019).
KRAS p.G12D c.35G>A A NM_004985.3 VAF % 10.0 DEPTH 5663	The KRAS protein has intrinsic GTPase activity and is an important mediator of growth factor receptor signaling resulting in the activation of several downstream pathways such as PI3K-mTOR and RAS-RAF-MEK pathway (RefSeq, Jul 2008). A missense alteration in KRAS, G12D, is identified in this case. Codon 12 lies within a GTP binding region of the KRAS protein (UniProt.org). Mutations in KRAS at codon 12 (within the GTP binding region), including KRAS G12D, result in reduced GTPase activity, which in turn leads to constitutive activation of KRAS and its downstream PI3K-AKT and MAPK signaling pathways (PMID- 26902995; 25705018). In ClinVar, KRAS G12D has been classified as 'Pathogenic' in several malignancies ('Pathogenic' for somatic in malignancies including non-small cell lung cancer) (Variation ID: 12582). KRAS G12D is reported in malignancies including non-small cell lung cancer (COSMIC, February 2019). Approximately 25% of patients with lung adenocarcinomas in a North American population have KRAS mutations (NCCN, NSCLC v3.2019). KRAS mutation prevalence has been associated with cigarette smoking (NCCN, NSCLC v3.2019). In NSCLC, the presence of a KRAS mutation is prognostic of poor survival when compared to patients with tumors without KRAS mutation, independent of therapy (NCCN, NSCLC v3.2019). KRAS mutations have a predictive role in brain metastases incidence, recurrence and outcome in Caucasian NSCLC patients (PMID- 27999344; 26616848). Mutations in KRAS have been associated with reduced responsiveness to EGFR TKI therapy and do not appear to affect chemotherapeutic efficacy (NCCN, NSCLC v3.2019). Targeted therapy is currently not available for patients with KRAS mutations, although immune checkpoint inhibitors appear to be effective; MEK inhibitors are in clinical trials (NCCN, NSCLC v3.2019).

Tier II - Potential Clinical Significance

VARIANT	INTERPRETATION
PDGFRA p.D842V c.2525A>T C NM_006206.4 VAF % 15.0 DEPTH 7986	PDGFR-alpha (PDGFRA) is a receptor protein kinase that activates the PI3K/AKT/mTOR and MAPK/ERK pathways and promotes activation of STAT family members STAT1, STAT3 and STAT5A and/or STAT5B (UniProt.org). A missense alteration in PDGFRA, D842V, is identified in this case. Codon 842 lies in exon 18, within the protein kinase domain of PDGFRA (UniProt.org). PDGFRA D842V is reported to be an activating, in vitro (PMID- 27349873; 12949711, 2003). In ClinVar, somatic PDGFRA D842V is reported as 'Pathogenic' in gastrointestinal stromal tumor (GIST) (Variation ID: 13543). PDGFRA D842V has been reported in Non-small cell lung cancer (COSMIC, February 2019). About 5% to 10% of GISTs have a mutation in the gene encoding PDGFRA receptor tyrosine kinase and PDGFRA exon 18 mutations are common in gastric GISTs (NCCN, Soft Tissue Sarcoma, v1.2019). Identification of activating kinase mutations in PDGFRA is an ancillary technique useful in the diagnosis of sporadic and familial GIST (NCCN, Soft Tissue Sarcoma, v1.2019). PDGFRA exon 18 mutations (including D842V) are associated with a better prognosis in GIST patients (NCCN, Soft Tissue Sarcoma, v1.2019). Primary imatinib resistance is commonly seen in GIST patients with mutations including PDGFRA D842V (NCCN, Soft Tissue Sarcoma,v1.2019; PMID- 30506540). A small number of GIST patients with a primary or secondary D842V mutation did not respond to sunitinib treatment (NCCN Soft Tissue Sarcoma v1.2019; PMID- 30224936). Dasatinib has demonstrated activity against PDGFRA D842V mutation, and it could be an effective treatment option for imatinib-resistant GIST patients (NCCN, Soft Tissue Sarcoma v1.2019).

Other Biomarkers

BIOMARKER	INTERPRETATION
TMB High 24 muts/Mb	Tumor mutational burden is an emerging quantitative genomic biomarker used to predict sensitivity to checkpoint inhibitors. NCCN recommends nivolumab with or without ipilimumab for patients with high TMB based on a recent study and the results of a Phase III clinical trial, NCT02477826 (NSCLC v3.2019, PMID: 29658845, 28636851)
MSI Stable 5% Unstable Sites	Microsatellite Instability is caused by a failure of the DNA mismatch repair system (MMR) and a predictor of favorable response to immunotherapies (PMID: 26028255). This patient does not exhibit evidence of High Microsatellite Instability (MSI).

TIER III - VARIANTS OF UNKNOWN SIGNIFICANCE

AKT3 p.P449S NM_001206729.1 c.1345C>T	**AKT3** p.E450K NM_001206729.1 c.1348G>A	**APC** p.V2194I NM_000038.5 c.6580G>A	**APC** p.D1794V NM_000038.5 c.5381A>T	**APC** p.A1793E NM_000038.5 c.5378C>A	**APC** p.N1792K NM_000038.5 c.5376T>A	**APC** p.L148H NM_000038.5 c.443T>A	**APC** p.L148I NM_000038.5 c.442C>A
ATM p.N1240Kfs*4 NM_000051.3 c.3720_3736del17	**ATM** p.G301Vfs*19 NM_000051.3 c.900delA	**BRCA2** p.S2984* NM_000059.3 c.8951C>A	**BRCA2** p.S2984T NM_000059.3 c.8950T>A	**BRCA2** p.E2301K NM_000059.3 c.6901G>A	**BRCA2** p.I2296M NM_000059.3 c.6888A>G	**BRCA2** p.D2294E NM_000059.3 c.6882C>G	**BRCA2** p.N2291D NM_000059.3 c.6871A>G
BRCA2 p.P2283H NM_000059.3 c.6848C>A	**BRCA2** p.P2283T NM_000059.3 c.6847C>A	**BRCA2** p.G1761E NM_000059.3 c.5282G>A	**BRCA2** p.D1737V NM_000059.3 c.5210A>T	**BRCA2** p.D1737Y NM_000059.3 c.5209G>T	**BRCA2** p.E1734* NM_000059.3 c.5200G>T	**BRCA2** p.E1734K NM_000059.3 c.5200G>A	**BRCA2** p.L1732P NM_000059.3 c.5195T>C
BRCA2 p.H1731N NM_000059.3 c.5191C>A	**BRCA2** p.Y1313* NM_000059.3 c.3939C>A	**BRCA2** p.Y1313C NM_000059.3 c.3938A>G	**BRCA2** p.T1310I NM_000059.3 c.3929C>T	**BRCA2** p.T1310Mfs*25 NM_000059.3 c.3929delC	**BRCA2** p.N1297K NM_000059.3 c.3891T>A	**BRCA2** p.N1287Ifs*6 NM_000059.3 c.3860delA	**BRCA2** p.S1284R NM_000059.3 c.3852T>G
BRCA2 p.S1284R NM_000059.3 c.3852T>A	**BRCA2** p.V1283I NM_000059.3 c.3847G>A	**BRCA2** p.V1283* NM_000059.3 c.3847delG	**BRCA2** p.E866K NM_000059.3 c.2596G>A	**BRCA2** p.P606Q NM_000059.3 c.1817C>A	**BRCA2** p.Q347K NM_000059.3 c.1039C>A	**CCND3** p.S178A NM_001136017.2 c.532T>G	**CTNNB1** p.N287S NM_001098209.1 c.860A>G
KRAS p.R164Q NM_004985.3 c.491G>A	**KRAS** p.G174S NM_004985.3 c.520G>A	**KRAS** p.M188L NM_004985.3 c.562A>C	**MSH2** p.N566K NM_000251.2 c.1698T>A	**MSH2** p.T564N NM_000251.2 c.1691C>A	**MSH2** p.Y563S NM_000251.2 c.1688A>C	**MSH2** p.Y563N NM_000251.2 c.1687T>A	**MSH2** p.E562D NM_000251.2 c.1686G>C
MSH2 p.E562V NM_000251.2 c.1685A>T	**MSH2** p.E562* NM_000251.2 c.1684G>T	**MSH2** p.E562Q NM_000251.2 c.1684G>C	**MSH2** p.E561* NM_000251.2 c.1681G>T	**MSH2** p.E561K NM_000251.2 c.1681G>A	**MSH2** p.N560I NM_000251.2 c.1679A>T	**MSH2** p.S558F NM_000251.2 c.1673C>T	**MSH2** p.S558Y NM_000251.2 c.1673C>A
PIK3R1 p.Q92K NM_001242466.1 c.274C>A	**RB1** p.S302Y NM_000321.2 c.905C>A	**RB1** p.W195C NM_00321.2 c.58G>T					

CLASSIFICATION AND LEVELS OF EVIDENCE

The variant classification system used in this report is based on joint consensus recommendations of the Association for Molecular Pathology, American Society of Clinical Oncology, and the College of American Pathologists (J Mol Diagn 2017, 19:4-23). Tiers IA, IB, IIC, IID, III and IV describe variant categories of descending clinical significance in the patient. Variants in Tier IV are not reported in accordance with the consensus recommendations.

IA	IB	IIC	IID
Variant of strong clinical significance, Level A evidence (FDA approved therapy or practice guideline in patient's tumor type)	Variant of strong clinical significance, Level B Evidence (consensus in the field based on well-powered studies in patient's tumor type)	Variant of potential clinical significance, Level C evidence (FDA approved therapy or practice guideline in other tumor type(s), evidence from multiple small published studies, or based on availability of investigational therapies)	Variant of potential clinical significance, Level D evidence (case reports or preclinical studies)

III	Variant of unknown clinical significance
IV	Benign or likely benign variant