CIRCULOGENE
www.circulogene.com
info@circulogene.com
855-614-7083

# Plasma NGS Can Clarify Tissue and Treatment Uncertainty

"Medicine is the science of uncertainty and an art of probability." Sir William Osler

All of cancer medicine is fraught with treatment-decision-making uncertainties. Will my patient respond? Will treatment be tolerated? How long will a response be maintained? Are staging studies missing disease? Even sometimes, is this the right diagnosis? This case emphasizes how plasma NGS molecular profiling can clarify tissue and treatment uncertainty.

A 62-year-old female presented with a gastric mass and extensive peritoneal carcinomatosis. EGD forceps biopsy confirming a malignancy. However, limited biopsy cellularity precluded full IHC staining. The multi-disciplinary GI tumor board recommendation was palliative systemic chemotherapy.

## IMMUNOTHERAPY TEST RESULTS

**PD-L1 EXPRESSION** **Positive (≥50%)**

| GENE | ALTERATION | MUTANT FRACTION |
|---|---|---|
| **KIT** | p.V654A; c.1961T>C<br>V654A | 10.4% |
| **KIT** | p.T670I; c.2009C>T<br>Exon 14 | 2.3% |

Plasma NGS returned with a total of 15 somatic ctDNA mutations including the unexpected findings of KIT exon 13 V654A and exon 14 T670I mutations with cell-free RNA PD-L1 expression greater than 50%. These KIT mutations are pathogenic and known gatekeeper and ATP pocket binding GIST/gastrointestinal stromal tumor imatinib resistant mutations. With the plasma NGS molecular testing findings, the assumed certainty of the pathologic diagnosis of a gastric carcinoma radically changed, now supporting a GIST, and confirmed with a repeat tissue biopsy confirming CD117 positivity.

Two other plasma NGS results are impactful in this case. First, the number of ctDNA mutations is clearly indicating an aggressive tumor biology warranting aggressive treatment consideration. Second, the liquid biopsy PD-L1 expression reflects shedding of cell-free DNA PD-L1 indicative of a high tumor burden and aggressive tumor biology and also opens up thinking to integrate anti-PD-1/L1 therapy with a GIST TKI.

Given the known imatinib resistance, following group guidelines treatment falls to sunitinib. However, following the individual tumor biology with precision oncology guiding treatment seems far more enticing. Axitinib displayed better activity than sunitinib and regorafenib in overcoming these two identified imatinib resistant KIT mutations in both in vitro and in vivo models. PD-1/PD-L1 blockade has shown enhanced T-cell activity and anti-tumor efficacy in combination with imatinib in GIST, and salvage immune checkpoint blockade has achieved durable salvage benefit in GIST. At a minimum upon PDs with sunitinib, but why not up front, given the tumor biology guides a more favorable benefit of axitinib in combination with an anti-PD1 immune checkpoint inhibitor?

To reap the best outcome treatment benefits for individuals with cancer, stepping forward with treatment guided by the precision of tumor biology seems a far better probability than the science uncertainty of group guidelines.

Case Study Prepared by Doctor Paul Walker
Chief Medical Officer, Former Director of Thoracic Oncology at East Carolina University

Sources:
- Clin Cancer Res 2017;23:454-465. Published Online First July 28, 2016
- Ther Adv Med Oncol 2019, Vol. 11: 1–15 DOI: 10.1177/1758835919849757 © The Author(s), 2019`
- British Journal of Cancer (2019) 121:819–826; https://doi.org/10.1038/s41416-019-0596-1

Gastric Adenocarcinoma —Case Study

**Real Patients, Real Results**

# Plasma NGS Guiding a Different and Better Treatment

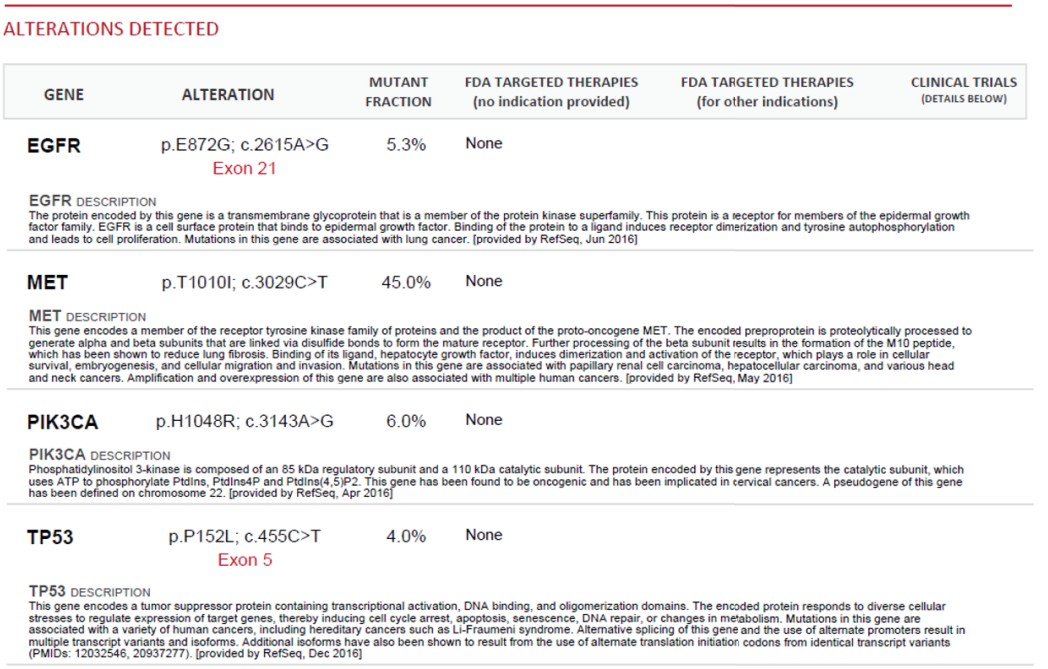
A 60-year-old female presented with a gastric adenocarcinoma and laparoscopic peritoneal studding.

Tissue IHC HER2 negative; QNS additional molecular testing. Plasma NGS molecular profiling identified MSI-H and MET T1010I, exon 5 TP53, PIK3CA H1048R, EGFR E872G mutations.

Initial multi-disciplinary GI tumor board treatment recommendation before the results of the plasma NGS were reviewed was for an aggressive multi-modality treatment approach with induction chemotherapy with a full re-staging assessment and surgical consideration given her young age. The liquid biopsy plasma NGS results, however, guide a completely different and better treatment approach.

The two most striking NGS results are first and foremost the MSI-H finding but also the MET T1010I mutation with a MAF of 45%. MSI-H can be present in up to 15% of gastric cancers, particularly in distal cancers, with direct treatment, surveillance, and potential hereditary impact.

| IMMUNOTHERAPY TEST RESULTS | | FDA GUIDANCE |
|---|---|---|
| MSI-H | Detected | Pembrolizumab |



ALTERATIONS DETECTED

| GENE | ALTERATION | MUTANT FRACTION | FDA TARGETED THERAPIES (no indication provided) | FDA TARGETED THERAPIES (for other indications) | CLINICAL TRIALS (DETAILS BELOW) |
|---|---|---|---|---|---|
| EGFR | p.E872G; c.2615A>G Exon 21 | 5.3% | None | | |

EGFR DESCRIPTION
The protein encoded by this gene is a transmembrane glycoprotein that is a member of the protein kinase superfamily. This protein is a receptor for members of the epidermal growth factor family. EGFR is a cell surface protein that binds to epidermal growth factor. Binding of the protein to a ligand induces receptor dimerization and tyrosine autophosphorylation and leads to cell proliferation. Mutations in this gene are associated with lung cancer. [provided by RefSeq, Jun 2016]

| | | | | | |
|---|---|---|---|---|---|
| MET | p.T1010I; c.3029C>T | 45.0% | None | | |

MET DESCRIPTION
This gene encodes a member of the receptor tyrosine kinase family of proteins and the product of the proto-oncogene MET. The encoded preproprotein is proteolytically processed to generate alpha and beta subunits that are linked via disulfide bonds to form the mature receptor. Further processing of the beta subunit results in the formation of the M10 peptide, which has been shown to reduce lung fibrosis. 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. Mutations in this gene are associated with papillary renal cell carcinoma, hepatocellular carcinoma, and various head and neck cancers. Amplification and overexpression of this gene are also associated with multiple human cancers. [provided by RefSeq, May 2016]

| | | | | | |
|---|---|---|---|---|---|
| PIK3CA | p.H1048R; c.3143A>G | 6.0% | None | | |

PIK3CA DESCRIPTION
Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of this gene has been defined on chromosome 22. [provided by RefSeq, Apr 2016]

| | | | | | |
|---|---|---|---|---|---|
| TP53 | p.P152L; c.455C>T Exon 5 | 4.0% | None | | |

TP53 DESCRIPTION
This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons from identical transcript variants (PMIDs: 12032546, 20937277). [provided by RefSeq, Dec 2016]

Neoadjuvant immune therapy is also showing dramatic pathologic complete responses in a variety of cancers including a very notable 95% major pathologic responses and 60% pathologic complete responses in colorectal cancers and 85% in other GI cancers. The use of neoadjuvant immune therapy in MSI-H gastric cancers will open up new and potential organ preserving horizons.

The MSI-H tumor biology is unique with notable chemotherapy ineffectiveness but dramatic immune therapy benefit. In the MAGIC trial, peri-operative chemotherapy was detrimental in the MSI-H gastric cancer patients. The 2-year survival was 70% in MSI-H patients with surgery alone, decreasing to only 20% in those receiving chemotherapy. In the advanced gastric cancer KEYNOTE-061/062 trials, a 60% 3-year survival was achieved with immune checkpoint inhibitor therapy compared to 20% with chemotherapy. KEYNOTE-062 even highlighted the caveat that adding chemotherapy to first-line immune checkpoint therapy had an early 30% drop in survival compared to the anti-PD-1 therapy alone.

Any MSI-H finding needs to be assessed for Lynch Syndrome and the MET T1010I mutation at this MAF of 45% also sparks a germline concern, further emphasizing the need for formal germline testing.

Initial immune checkpoint inhibitor therapy alone is clearly the best starting point of treatment in this case. Any cytotoxic chemotherapy would be ineffective and potentially detrimental to her outcome. Immune therapy has a real potential of giving her durability. Any hope of durability would be minimal if the MSI-H tumor biology were not known, and chemotherapy given.

Case Study Prepared by Doctor Paul Walker
Chief Medical Officer, Former Director of Thoracic Oncology at East Carolina University

Sources:
- European Journal of Surgical Oncology 46 (2020) e33ee39
- JAMA Oncol. doi:10.1001/jamaoncol.2021.0275 Published online April 1, 2021
-JAMA Oncol. 2017;3(9):1197-1203. doi:10.1001/jamaoncol.2016.6762
Published online February 23, 2017;J Clin Oncol. 35:3065-3074. 
-Chalabi, M., Fanchi, L.F., Dijkstra, K.K. et al. Nat Med 26, 566–576 (2020)