Liver Cancer

Informing the best treatment decisions faster with plasma molecular profiling

CIRCULOGENE

According to the American Cancer Society, in 2020 an estimated 42,810 Americans will be diagnosed with cancer of the liver. Liver cancer incidence rates have more than tripled since 1980, while the death rates have more than doubled during this time. Getting these patients on the right treatment faster can make all the difference.

The adoption of precision medicine can have a substantial effect on survival in patients with liver cancer. However, the ability of patients with liver cancer to undergo tumor molecular profiling or receive targeted therapies is a challenge in the U.S. healthcare system.

The advent of next-generation sequencing (NGS) has dramatically revolutionized the molecular knowledge of cancer by increasing the feasibility and possibility of DNA sequencing.

CIRCULOGENE'S *comprehensive liver panel is a non-invasive technique that can be combined with traditional tissue biopsy to track cell-free DNA and detect disease biomarkers in blood faster and more accurately.*

Clinical Cancer Research, September 2019 – "Comprehensive Liquid Profiling of Circulating Tumor DNA and Protein Biomarkers in Long-Term Follow-Up Patients with Hepatocellular Carcinoma"

"Our strategy of comprehensive mutation profile integration could accurately and better evaluate patients' prognostic risk…"

"Real-time monitoring of tumor burden for patients with HCC, which could greatly benefit prognostic evaluation and treatment selection, remains a critical challenge."

"One major advantage of cfDNA is its unique ability for containing comprehensive somatic information with regard to primary HCC and/or metastatic lesions, thus allowing it to overcome the inference of tumor heterogeneity."

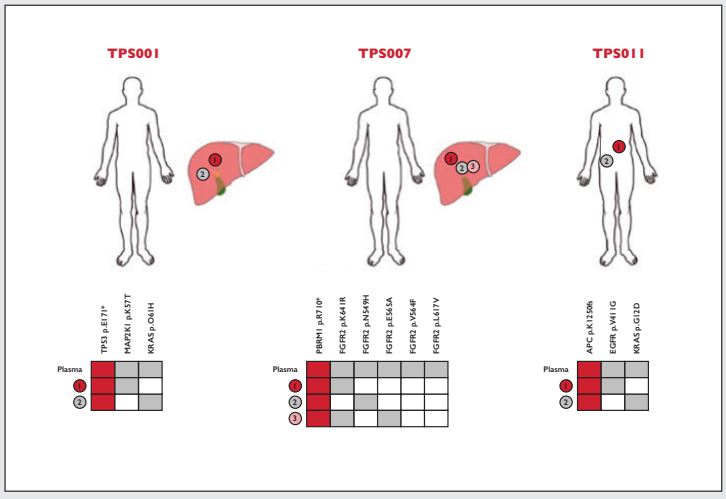
Oncogene 2020 – "Immune-based Therapies for Hepatocellular Carcinoma"

"The systemic management of cancer has been recently revolutionized by the advent of immune checkpoint inhibitors (ICPI)."

"…PD1/PDL1 as forerunner molecular targets of cancer related immune exhaustion has rapidly extended to HCC based on promising results of ICPI therapy in multiple indications."

Nature Medicine, September 2019 - "Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers"

"In patients with multiple post-progression tumor biopsies…in all patients, individual resistance mechanisms emerging in distinct metastatic lesions were detectable in plasma cfDNA."



80-Year-Old Patient

LARGE LIVER MASS WITH TISSUE BIOPSY 'LOW-GRADE' NEUROENDOCRINE TUMOR WITH LACK OF MITOTIC FIGURES

This case presents a divergence of the histologic tissue pathology and plasma ctDNA findings, forcing a treatment dilemma that is tough enough given her age. Typically, low-grade/grade 1 neuroendocrine tumors carry an indolent tumor biology and prolonged survival potentials even with radiographically bulky disease. That is what the tissue histology is indicating. However, the circulating tumor DNA is portending a far different tumor biology.

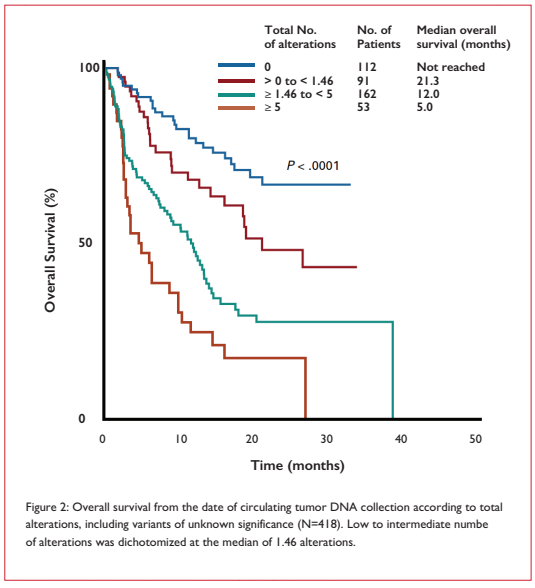
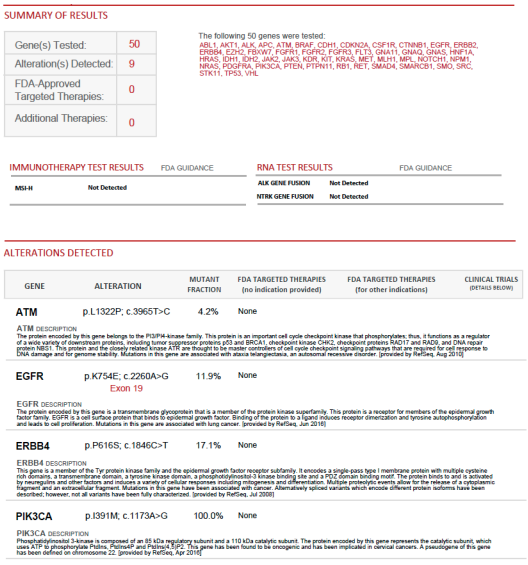


Figure 2: Overall survival from the date of circulating tumor DNA collection according to total alterations, including variants of unknown significance (N=418). Low to intermediate numbe of alterations was dichotomized at the median of 1.46 alterations.

The findings of 9 ctDNA mutations reflect an aggressive tumor biology and shortened survival. In a study of over 400 patients with a variety of advanced cancers, the 20-30% who were non-shedders of any ctDNA had a markedly longer median overall survival, whereas those shedding more than 5 ctDNA mutations had a survival of just 5 months. A cancer shedding ctDNA has a far worse prognosis than those not shedding. The genomic make-up of grade 1 neuroendocrine tumors is different than grade 3 tumors. SMAD4 mutations are associated with poorer survival in a variety of GI cancers, including colorectal and pancreatic adenocarcinoma. Although a full elucidation of the genomics of neuroendocrine tumors is still a work in progress, rarely are SMAD4 and/or p53 mutations part of the low-grade tumor genomics; rather, they are associated with a larger tumor burden and the high-grade/grade 3 neuroendocrine tumor spectrum.

This is what we often have seen clinically…a tumor biology that ends up having a much more aggressive tumor biology than the histologic biopsy would suggest. Intra-tumoral heterogeneity is always limiting for tissue biopsies. Only one small area at one point in time is sampled. The metastatic clone is different than the stationary clone. Plasma ctDNA can identify the more aggressive clone that reflects the tumor biology and has the most important treatment need.



SUMMARY OF RESULTS

Gene(s) Tested:	50
Alteration(s) Detected:	9
FDA-Approved Targeted Therapies:	0
Additional Therapies:	0

The following 50 genes were tested:
ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL

IMMUNOTHERAPY TEST RESULTS — FDA GUIDANCE

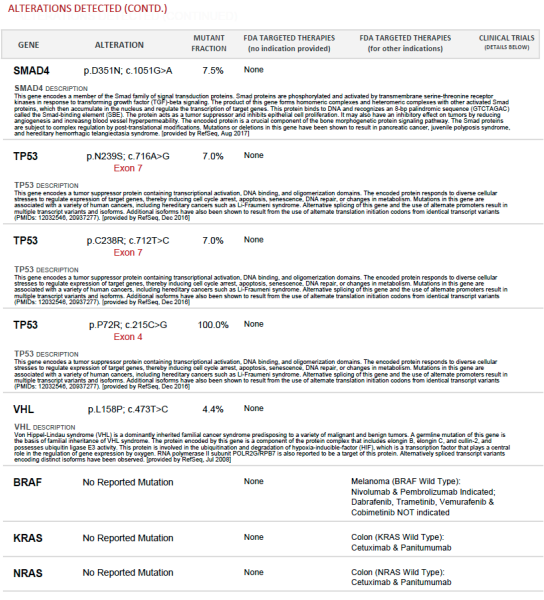
MSI-H	Not Detected

RNA TEST RESULTS — FDA GUIDANCE

ALK GENE FUSION	Not Detected
NTRK GENE FUSION	Not Detected

ALTERATIONS DETECTED

GENE	ALTERATION	MUTANT FRACTION	FDA TARGETED THERAPIES (no indication provided)	FDA TARGETED THERAPIES (for other indications)	CLINICAL TRIALS (DETAILS BELOW)
ATM	p.L1322P; c.3965T>C	4.2%	None		
ATM DESCRIPTION: The protein encoded by this gene belongs to the PI3/PI4-kinase family. This protein is an important cell cycle checkpoint kinase that phosphorylates; thus, it functions as a regulator of a wide variety of downstream proteins, including tumor suppressor proteins p53 and BRCA1, checkpoint kinase CHK2, checkpoint proteins RAD17 and RAD9, and DNA repair protein NBS1. This protein and the closely related kinase ATR are thought to be master controllers of cell cycle checkpoint signaling pathways that are required for cell response to DNA damage and for genome stability. Mutations in this gene are associated with ataxia telangiectasia, an autosomal recessive disorder. [provided by RefSeq, Aug 2015]				
EGFR	p.K754E; c.2260A>G (Exon 19)	11.9%	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]					
ERBB4	p.P616S; c.1846C>T	17.1%	None		
ERBB4 DESCRIPTION: This gene is a member of the Tyr protein kinase family and the epidermal growth factor receptor subfamily. It encodes a single-pass type I membrane protein with multiple cysteine-rich domains in the extracellular region, a transmembrane domain, a conserved tyrosine kinase domain, a C-terminal domain containing motif. The protein binds to and is activated by neuregulins and other factors and induces a variety of cellular responses including mitogenesis and differentiation. Multiple proteases can evidently allow for the release of a soluble fragment and an extracellular fragment. Mutations in this gene have been associated with cancer. Alternatively spliced variants which encode different protein isoforms have been described; however, not all variants have been fully characterized. [provided by RefSeq, Jul 2008]					
PIK3CA	p.I391M; c.1173A>G	100.0%	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]					



ALTERATIONS DETECTED (CONTD.)

GENE	ALTERATION	MUTANT FRACTION	FDA TARGETED THERAPIES (no indication provided)	FDA TARGETED THERAPIES (for other indications)	CLINICAL TRIALS (DETAILS BELOW)
SMAD4	p.D351N; c.1051G>A	7.5%	None		
SMAD4 DESCRIPTION: This gene encodes a member of the Smad family of signal transduction proteins. Smad proteins are phosphorylated and activated by transmembrane serine-threonine receptor kinases in response to transforming growth factor (TGF)-beta signaling. The product of this gene forms homomeric complexes and heteromeric complexes with other activated Smad proteins, which then accumulate in the nucleus and regulate the transcription of target genes. This protein binds to DNA and recognizes an 8-bp palindromic sequence (GTCTAGAC) called the Smad-binding element (SBE). The protein acts as a tumor suppressor and inhibits epithelial cell proliferation. It may also have an inhibitory effect on tumors by reducing angiogenesis and increasing blood vessel hyperpermeability. The encoded protein is a crucial component of the bone morphogenetic protein signaling pathway. The Smad proteins are subject to complex regulation by post-translational modifications. Mutations or deletions in this gene have been shown to result in pancreatic cancer, juvenile polyposis syndrome, and hereditary hemorrhagic telangiectasia syndrome. [provided by RefSeq, Aug 2017]					
TP53	p.N239S; c.716A>G (Exon 7)	7.0%	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]					
TP53	p.C238R; c.712T>C (Exon 7)	7.0%	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]					
TP53	p.P72R; c.215C>G (Exon 4)	100.0%	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]					
VHL	p.L158P; c.473T>C	4.4%	None		
VHL DESCRIPTION: Von Hippel-Lindau syndrome (VHL) is a dominantly inherited familial cancer syndrome predisposing to a variety of malignant and benign tumors. A germline mutation of this gene is the basis of familial inheritance of VHL syndrome. The protein encoded by this gene is a component of the protein complex that includes elongin B, elongin C, and cullin-2, and possesses ubiquitin ligase E3 activity. This protein is involved in the ubiquitination and degradation of hypoxia-inducible-factor (HIF), which is a transcription factor that plays a central role in the regulation of gene expression by oxygen. RNA polymerase II subunit POLR2G/RPB7 is also reported to be a target of this protein. Alternatively spliced transcript variants encoding distinct isoforms have been observed. [provided by RefSeq, Jul 2008]					
BRAF	No Reported Mutation		None	Melanoma (BRAF Wild Type): Nivolumab & Pembrolizumab Indicated; Dabrafenib, Trametinib, Vemurafenib & Cobimetinib NOT indicated	
KRAS	No Reported Mutation		None	Colon (KRAS Wild Type): Cetuximab & Panitumumab	
NRAS	No Reported Mutation		None	Colon (NRAS Wild Type): Cetuximab & Panitumumab	

Very sadly, given the ctDNA findings, her prognosis is extremely poor with a much shorter survival outcome than the typical grade 1 neuroendocrine tumors, also limiting any benefit of the standard-of-care, low-grade neuroendocrine tumor treatment approaches. However, this can also spark a goals-of-care and end-of-life-with-quality discussion. Sometimes, even with our advances in cancer treatment, that is still the kindest personalized cancer care there is.

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

Sources:
- JCO Precis Oncol 4:192-201
- Cell-Free DNA From Metastatic Pancreatic Neuroendocrine Tumor Patients Contains Tumor-Specific Mutations and Copy Number Variations. Front. Oncol. 8:467. doi: 10.3389/fonc.2018.00467
- Arch Pathol Lab Med. 2020;144:816–828; doi: 10.5858/arpa.2019-0654-RA