

How Liquid Biopsies Are Advancing Our Understanding of Hepatocellular Carcinoma



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Hepatocellular carcinoma (HCC) is a common cancer, estimated to be the fourth most common cause of cancer-related deaths globally¹. Unfortunately, our understanding of the molecular architecture of advanced HCC is limited due to imaging being accepted as a common diagnostic method, rendering advanced HCC tissue samples scarce. In a recent peer-reviewed article, Dr. Augusto Villanueva addressed these limitations using circulating tumor DNA (ctDNA)-based liquid biopsies powered by our custom Agilent SureSelect next-generation sequencing (NGS) panels.

In this technical overview, we provide a synopsis of this article² and describe how Dr. Villanueva used SureSelect targeted NGS panels to characterize the mutational landscape of patients with advanced-stage HCC. Additionally, we cover how his group leveraged NGS data to identify mutations that were predictive of therapeutic responses, and how the mutational profile of a subset of patients changed over the course of treatment.

Addressing Bottlenecks in Understanding HCC

While traditional molecular analyses of cancers are performed using tissue biopsies, these methods can be both invasive and (depending on the type of cancer) limited when tumor tissue is not available. In recent years, however, liquid biopsies. These assays are minimally invasive, relatively simple to collect, and can use ctDNA (a subtype of cell-free DNA) released from cancerous cells. Additionally, the low concentration of ctDNA (and subsequent low yield from liquid biopsy samples) has traditionally meant low-complexity libraries.

To address these challenges, Dr. Villanueva developed a custom SureSelect NGS panel for 25 genes, all of which were either:

- Part of the PI3K/MTOR signaling pathway
- Part of the WNT signaling pathway
- Frequently mutated in HCC patients
- Previously associated with immune exclusion in HCC patients

During their library preparation with the SureSelect XT HS kit, Dr. Villanueva's group used both unique molecular identifiers and dual indices to minimize PCR amplification bias and index hopping events, respectively.

Targeted Next-Generation Sequencing of ctDNA from HCC Patients

Following ctDNA sequencing and analysis using the Agilent SureCall software, researchers noted that mutational profile and mutation frequencies in ctDNA from advanced HCC patients are consistent with those seen in tissue-based screens of early-stage HCC patients.

Encouraged by these results, Dr. Villanueva's group proceeded to analyze whether specific mutations in the PI3K/MTOR or WNT pathways were able to predict treatment responses to tyrosine kinase inhibitors (TKI) or immune checkpoint inhibitors (CPI), respectively. While patients with WNT mutations showed no significant difference in survival times when treated with CPIs, patients with mutations in genes of the PI3K/MTOR pathway showed significantly shorter progression-free survival than those without mutations in this pathway.

Dr. Villanueva's group followed up on these results by performing sequential mutation profiling of eight patients with advanced HCC before and during treatment. They observed that:

- Three treatment-resistant patients exhibited a 300% increase in mutation variant frequency with no other changes – Part of the WNT signaling pathway
- Three patients that partially responded to treatment showed mutations at baseline, but these were undetectable following treatment response
- Two patients exhibited lengthy treatment response prior to disease progression; both patients retained at least one 'baseline' mutation, but either developed a new mutation or regained previously undetectable 'baseline' mutations following disease progression

Finally, researchers sought to validate their ctDNA-based profiling approach by comparing ctDNA samples to paired advanced HCC tumor tissues. Out of the eight mutations tested, six were validated. The authors noted that this success rate was in line with prior liquid biopsy approaches in HCC patients and that these discrepancies could be due to molecular heterogeneity.

Taking the Next Steps

The work performed by Dr. Villanueva's lab demonstrates the wealth of information that ctDNA can provide in better understanding cancers such as HCC. While liquid biopsies are a relatively new method, this approach compares favorably to traditional tissue biopsies and simplifies the sequential profiling of cancers. By enabling the non-invasive profiling of the mutational landscape of HCC and monitoring its response to treatments, Dr. Villanueva's methods offer a way to better predict treatment outcomes and the possibility of improved, guided treatments for HCC.

References

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