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VIEWPOINT

TECHNOLOGY SPOTLIGHT

Genomic Analysis of Plasma Cell-Free DNA in Patients With Cancer

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Technology

The increased importance of cancer genotyping in guiding cancer treatment has created a need for efficient methods for genomic analysis of patients' cancers. This increased dependence on DNA-based tumor genotyping assays (eg, sequencing, polymerase chain reaction [PCR], fluorescence in situ hybridization [FISH]) has triggered a growing interest in the analysis of free-floating DNA present in the blood of patients with cancer—plasma cell-free DNA (cfDNA). Sensitive PCR techniques together with high-throughput next-generation sequencing (NGS) technologies have evolved to a point where genetic analysis of cfDNA is possible.

Strengths

Genotyping of plasma cfDNA is compelling for a number of reasons.[1] Most importantly, it can noninvasively provide clinically-relevant genomic information that is usually only available after an invasive tumor biopsy procedure. It can be very fast[2]—the blood specimen is sent directly to a molecular laboratory for DNA analysis, potentially faster than the complex journey of a tumor specimen from scheduled biopsy procedure to pathology review to molecular testing. Plasma genotyping can be highly quantitative, such that measurement of key cancer genes can potentially offer cancer-specific information on the response to therapy.[3] The method is readily scalable given the relative ease of specimen processing and handling. Finally, plasma specimens do not undergo formalin fixation, resulting in a reduced level of background "noise" compared with analysis of DNA from formalin-fixed tumor specimens.

Limitations

There are fundamental differences between the genomic analysis of tumor DNA and plasma cfDNA. While a tumor biopsy specimen is enriched for malignant cancer-derived material, a plasma specimen may contain limited representation of the tumor. Even in patients with cancer, plasma cfDNA is comprised predominantly of patient-derived germline DNA, and careful handling of the blood is important to minimize cell lysis and further contamination by germline DNA. In addition, adequacy assessment is a standard part of tumor genotyping, such that a specimen is only studied if tumor content is adequate for the intended assay. In contrast, such adequacy assessments are challenging for plasma cfDNA, and it is entirely possible that a given cfDNA specimen being studied has no cancer-derived DNA present.

Data Generated

Most cfDNA genotyping assays are designed to be highly sensitive to overcome the challenge of low levels of cancer-derived DNA within plasma. Some cancers may shed very little DNA into circulation because of small size, limited metastatic spread, or other biological factors. As a result, the clinical sensitivity of plasma genotyping (compared with tumor genotyping) has been reported in the range of 60% to 80% in patients with advanced cancer.[2,4,5] Specificity is also very high for most plasma genotyping assays, which is critical because even low false-positive rates can be problematic when testing for relatively rare molecular alterations. If a mutation is present in 5% of patients tested and a test has a 5% false-positive rate (95% specificity), then half of all positive results will be erroneous (50% positive predictive value). It is therefore essential that the expected level of background "noise" is clearly established during assay validation to minimize the risk of false-positive results and maximize specificity. When the false-positive rate approximates 0%, a positive result from plasma cfDNA testing can potentially be used on its own to guide therapy. Negative results may require either repeated plasma testing or a standard tumor biopsy procedure for genotyping.

Alternate Approaches

There are several types of plasma genotyping assays that are available for the care of patients with cancer, each addressing the challenges of sensitivity and specificity somewhat differently (**Table**). Allele-specific PCR, widely used for tumor genotyping, uses unique assay design to preferentially amplify a mutant DNA molecule, thus making it detectable over the background noise from wildtype DNA.[4] Emulsion PCR assays, such as droplet digital PCR or bead-based digital PCR in emulsion, use surfactant technologies to emulsify DNA into thousands of droplets at limiting dilution, resulting in thousands of individual PCR reactions and allowing absolute quantification of the number of mutant and wildtype variants present.[2,6] While PCR assays have clinically appealing features, including speed and low cost, these evaluate only known genomic alterations, cannot detect complex alterations like gene fusions, and can be difficult to multiplex. Targeted NGS of cfDNA has the potential to overcome these limitations, with multiplexed detection of a range of genomic alterations, but can be limited by false-positive results generated from sequencing artifacts. Through use of molecular barcoding and stringent bioinformatic approaches, plasma NGS is now moving into the clinical

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Table. Clinically Available Assays for Genotyping of Plasma Cell-Free DNA (cfDNA)

Characteristic	PCR Assays		NGS Assays	
	Allele-Specific PCR	Emulsion PCR	Amplicon-Based Targeted NGS	Capture-Based Targeted NGS
Variants potentially detected	Known recurring mutations	Known recurring mutations	Any exonic mutations, copy number gains	Exonic mutations, intronic gene fusions, copy number gains
Quantitation	Semiquantitative (against standard curve)	Absolute or relative quantitation, wide dynamic range	Quantitation of relative AF, but vulnerable to PCR amplification bias	Quantitation of relative AF
Speed and complexity	Rapid, relatively easy to interpret	Rapid, relatively easy to interpret	Potentially rapid, less complex bioinformatics	Potentially slower, more complex bioinformatics
Examples	Cobas (Roche); therascreen (Qiagen)	Droplet digital PCR (Biorad); BEAMing (Sysmex Inostics)	Tam-seq (Invivata)	Guardant360 (Guardant); cancerselect (Personal Genome Diagnostics)

Abbreviations: AF, allelic fraction; BEAMing, bead-based digital PCR in emulsions; cfDNA, cell-free DNA; NGS, next-generation sequencing; PCR, polymerase chain reaction.

space and can make accurate and precise calls for variants present in less than 1% of sequencing reads.[5]

Examples of Use

One intuitive application for plasma genotyping will be for understanding drug resistance. Already, genotyping of plasma cfDNA has become an important supplement to tumor genotyping for the discovery of resistance mechanisms, report of C797S mutations in the epidermal growth factor receptor (*EGFR*) gene, acquired after resistance to osimertinib in patients with lung cancer.[3] With repeated biopsy procedures after drug resistance becoming increasingly standard to test for targetable resistance mechanisms, there is potential for plasma genotyping to become a routine part of managing resistant cancers. For example, a retrospective analysis[7] of the phase I trial of osimertinib in patients with lung cancer suggested excellent clinical outcomes in those who were positive for *EGFR*T790M mutation in plasma; but in those with negative plasma genotyping, tumor genotyping for *EGFR*T790M was then needed to identify additional patients who were likely to benefit.

There is also potential for serial plasma genotyping to noninvasively monitor response to therapy and anticipate clinical progression. In cancers with an oncogenic driver mutation (eg, *KRAS, EGFR, BRAF*), the levels of this mutation in plasma may be representative of tumor burden and metastatic potential.[2] Future studies will be needed to determine if monitoring of plasma mutation burden contributes to or supersedes standard approaches involving clinical assessment, tumor imaging, and serum tumor markers. The cost of plasma genotyping currently exceeds that of standard serum tumor markers, so it will be crucial to find settings where this approach adds value. The overall promise of plasma genotyping is clear—there is now need for prospective clinical studies to demonstrate the benefit to facilitate broad and appropriate clinical application.

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