Clinical Chemistry

Development, Validation, and Regulatory Considerations for a Liquid Biopsy Test

Article:
Dana WY Tsui, et al.
Development, Validation, and Regulatory Considerations for a Liquid Biopsy Test.
Clin Chem 2020;66:408-14. https://doi.org/10.1093/clinchem/hvaa010
Guests: Dr. Dana Tsui is the Technical Director of the Molecular Diagnostics Service, Department of Pathology at Sloan-Kettering Cancer Center in New York City, and Dr. Reena Philip is Director of the Division of Molecular Genetics and Pathology at the U.S. Food and Drug Administration in Silver Spring, Maryland.

Bob Barrett: This is a podcast from *Clinical Chemistry,* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I am Bob Barrett.

Genome guided precision medicine has become an essential part of cancer management. However, tumor biopsies are not always accessible and often fail to capture complex genetic heterogeneity, especially in the relapse setting. Measuring circulating tumor cells and cell-free tumor nucleic acids, often called liquid biopsy tests, offer opportunities to explore the underlying evolving tumor in a safe non-invasive way. In a Q&A feature appearing in the March 2020 issue of *Clinical Chemistry*, experts from diverse areas including regulatory, pharmaceutical, and academic and commercial molecular diagnostic test providers, discussed the different aspects of development, validation, and regulatory considerations for liquid biopsy tests.

Two of the moderators of that article, Dr. Dana Tsui, the Technical Director of the Molecular Diagnostics Service, Department of Pathology at Memorial Sloan-Kettering Cancer Center in New York City, and Dr. Reena Philip, the Director of the Division of Molecular Genetics and Pathology at the U.S. Food and Drug Administration in Silver Spring, Maryland, join us in this podcast. We'll start with you, Dr. Tsui. What is the main clinical need for a liquid biopsy test?

Dr. Dana Tsui: The main clinical need is to supplement tumor testing when tissues are not available or failed. For example, at Memorial Sloan Kettering, we have profiled more than 40,000 tumor samples in our clinical molecular diagnostic lab and in around 10% of the time, the tumor tissue or the tumor specimen that was submitted -- the tumor testing has failed either because there was insufficient DNA or the DNA quality was not suitable for testing. So, in those scenarios, liquid biopsy test would be very helpful to help tumor profiling.

Bob Barrett: That sounds very useful. When was the first liquid biopsy test approved by the FDA to use in the clinic?

Dr. Reena Philip: Yes. In the last several years, FDA has worked closely with different stakeholders in the liquid biopsy area, including

academia, clinical community, and industry on the regulatory aspect of liquid biopsy tests to facilitate clinical implementation. FDA approved the first liquid biopsy test in June 2016, the eGFR mutation test for Exon 19 deletions and L858R, as a companion diagnostic test for Tarceva for non-small cell lung cancer patients. Later, the same test was approved for eGFR T790M as a companion diagnostic for Tagrisso. And last year, FDA also approved the liquid biopsy test for PIK3CA mutations as a companion diagnostic to Alpelisib for breast cancer patients.

Bob Barrett: Thanks, Dr. Philip. Now, what are the key challenges to consider when we develop a liquid biopsy test?

Dr. Reena Philip: The considerations to develop liquid biopsy test, it depends on the intended use/indications for use of the test. The whole process needs to be considered including the pre-analytics which define how the samples are being collected, stored, and processed, which we would discuss in the article. And clinical validation, it varies depending on the claims of the test. Claims could be to identify the variance to be used as a companion diagnostic test for a specific therapeutic product, or the clinical claim could be monitoring for recurrence or change to therapy, early detection or screening of cancer. So, each claim has a different intended population. And study design for each claim varies, and it should be conducted in the intended population.

Dr. Dana Tsui: The considerations would also depend on the purpose of the test. The analytics which define the type of molecular assay to be used, the genomic territory to be covered, and expected turnaround time. These factors would be governed by the exact clinical need the test is designed to address and the logistics of implementing the test in the clinic. For example, if one is to develop a test to screen for a set of well-defined hotspot mutations for a given cancer type in a local clinic and requires a few hours or a day turnaround time, then a PCR-based hotspot digital PCR assay might be the best. On the other hand, in an institution with accurate informatics infrastructure, an NGS-based approach to screen for cancer biomarker across multiple cancer types might be more feasible and appropriate.

Bob Barrett: How about the challenges when it comes to validation?

Dr. Reena Philip: The analytical and clinical validation of the test, it depends on the intended use and indications for use of a test. The assay should be accurate, reproducible, and clinically meaningful. So, as part of the analytical validation process, key studies include establishing the limit of blanks and limit of detection of the test and demonstrating reproducibility and accuracy of the test, which is described in the article. And clonal hematopoiesis of indeterminate potential (CHIP), different shedding rates based on tumor type/stage of the disease, and other technical challenges should also be addressed in optimizing a liquid biopsy assay. The key aspect is to establish cut-offs

appropriately, so it minimizes false-positives and false-negatives.

Dr. Dana Tsui: Yeah, I agree with Reena. If plasma samples have very low analyte concentrations, such as below the limit of detection, reproducibility would be particularly challenging because the target analytes are usually present at very low levels such as 0.1% and below. So, when the plasma sample is analyzed twice, the mutation may be missed even with the perfect test, simply due to sampling errors. Another consideration is specificity, because most of the liquid biopsy tests aim to detect mutation down to such a low level, which is the level very close to technical errors introduced during the molecular assay, such a sequencing or PCR errors. So, it's really important to understand the false-positive rate of a given test in negative control samples, so the choice of which material to be used as controls are very important. We discuss this in one of the sections of this article the choice of orthogonal assay and what controls may be used for validating the test.

Bob Barrett: What is the FDA's role in the regulation for liquid biopsy tests?

Dr. Reena Philip: The FDA recognizes the clinical benefits of liquid biopsies tests since the use of liquid biopsy assays and assessment of ctDNA is rapidly advancing in the clinical setting. These assays have the potential to allow doctors to identify patients whose tumors have specific mutations in the least invasive way possible. So, FDA has hosted public workshops with AACR. FDA has had numerous discussions and meetings with multiple sponsors focusing on development and validation considerations for tests utilizing ctDNA. And furthermore, FDA has been engaged in active discussions with consortia (examples are Friends of Cancer Research, fNIH, BloodPAC) on several projects including the development of reference materials and acceptable tools that can help accelerate the development of the liquid biopsy tests in clinical practice and also for drug development.

Bob Barrett: Well, finally who should be thinking about these considerations for liquid biopsy tests?

Dr. Reena Philip: Any organization or individual who plans to develop or use liquid biopsy tests should be thinking about these considerations. For example, test developers and clinical molecular diagnostic labs should fully validate the whole process from pre-analytics and all the analytical steps to ensure robust performance of the test. Physicians who are ordering the test should be careful how to interpret positive and negative results, such that they can better inform the patient to help them understand what the results mean and the considerations to think about when they transfer the results from one clinic or test provider to the other. We discussed these considerations in the article.

Bob Barrett: That was Dr. Reena Philip from the U.S. Food and Drug Administration and she was joined by Dr. Dana Tsui from the Memorial Sloan-Kettering Cancer Center in this podcast on the development, validation, and regulatory considerations for a liquid biopsy test. That Q&A feature appears in the March 2020 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.