May 22, 2015

David Litwack, PhD
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health
Food and Drug Administration
Bldg. 66, Rm. 5544
10903 New Hampshire Ave.
Silver Spring, MD 20993


Dear Dr. Litwack,

The American Society of Hematology (ASH) appreciates the opportunity to comment on the Food and Drug Administration’s (FDA) Preliminary Discussion Paper on Optimizing FDA’s Regulatory Oversight of Next Generation Sequencing Diagnostic Tests issued on December 29, 2014.

ASH represents more than 15,000 hematologists who are committed to the treatment of blood and blood-related diseases. These diseases include anemias (such as sickle cell disease and thalassemia), thrombosis and bleeding disorders, as well as malignant hematologic disorders such as leukemia, lymphoma, and myeloma. ASH members include clinicians who order Next Generation Sequencing (NGS) diagnostic tests for their patients, as well as laboratory based hematologists and pathologists who develop, perform and interpret these tests. NGS testing is a broad, complex, and rapidly evolving area which has the potential to greatly impact diagnostics and therapies in clinical practice. Many malignant and non-malignant hematologic conditions are being studied by genomic analyses, and the field needs harmonized approaches for diagnostics and sequencing. Thus, the Society’s members have a vested interest in this draft guidance, its potential impact on patient care and future access to possibly life-saving diagnostic testing for patients with hematologic conditions.

ASH strongly supports the FDA’s mission of protecting public health by ensuring timely access to newly developed tests that are accurate, reliable, transparent, and clinically relevant. At the same time, ASH wants to make sure that oversight of NGS tests are not so burdensome and/or costly that patient access to necessary diagnostic tests are curtailed or the development of new and innovative tests are significantly stifled. To that end, ASH is pleased to submit comments in response to the questions outlined in the preliminary discussion paper.
How are labs currently developing NGS tests and assessing their analytical performance?

Many laboratories that develop and perform NGS tests generally focus on limited sets of genes or variants and use these tests to detect mutations. These laboratories take into account four key factors while performing NGS tests: (1) analytical sensitivity, where the proportion of biological samples that have a positive test result or known mutation are examined and found to be correctly classified; (2) analytical specificity, where the proportion of biological samples that have a negative test result or no identified mutation are examined and found to be correctly classified; (3) detection limits, which are used to determine both a) the minimal specimen requirements to generate enough DNA to complete the assay and any follow-up analysis, and b) mutations present at low level in a sample (low mutant allele frequency); and (4) assay precision and accuracy which requires robust measurement of the main assay components; i.e., library preparation and sequencing runs as well as proper adequate quality control measures.

Analytical performance of NGS tests is then assessed through Quality Assurance (QA) measures and programs developed by laboratories to support the routine analysis of samples, interpretation and reporting of NGS data. Predetermined QA checkpoints are established, and proper documentation of instruments used in each test along with documentation of all reagent lot numbers is also maintained. Laboratories also document any deviation from the standard procedures established by the laboratory during the validation process.

In order to develop QA programs for the analytical performance of NGS tests, laboratories define the intended use of the NGS test, define the assay system (define input/output, workflow, technologies), check the feasibility tests of the assay, define minimal analytical performance criteria, set up guidelines for quality control (e.g., library preparation, sequencing), identify and minimize sources of variance influencing the assay result, define the number of test cases, perform analytical validation, and evaluate assay performance.

Although the current processes involved in developing NGS tests and assessing their analytical performance enable these tests to have great potential for use in laboratories, there are still several shortcomings and challenges related to this technology. NGS tests fail to identify structural genetic alterations, like rearrangements, that are very important in many hematologic conditions since the tests generally focus on limited sets of genes or variants. More comprehensive NGS tests, including whole exome, genome and transcriptome sequencing may be useful in identifying multiple types of disease-associated genetic alterations, including sequence mutations, deletions and gains of DNA and chromosomal rearrangements. It may also help to detect inherited (germline) and somatic (tumor-acquired) mutations for both malignant and non-malignant diseases. This increasing complexity will have important implications for test regulation and oversight.

Another challenge involves the translation of NGS into clinical practice which requires the establishment of robust, accurate and rapid pipelines for analysis of large volumes of data. Currently, there are no uniform standards for assessing analytical performance of NGS tests. Any techniques developed to detect important variants must be benchmarked by another
approach, such as performing whole genome or exome sequencing, or another non-NGS technique.

**What are the benefits and risks to public health of having FDA independently evaluate the analytical performance of NGS tests and/or platforms using data submitted by the developer on an agreed upon subset of data points for the test?**

The FDA’s independent evaluation of the analytical performance of NGS tests and/or platforms will be very important and beneficial to public health. The FDA’s evaluation of these tests could help to foster the standardization of testing practices as well as the reporting of results between laboratories, thus allowing for more accurate detection and identification of a range of genetic alterations that respond to treatment or drive disease onset and progression.

ASH believes that in order to facilitate the effective use of NGS tests, it will be imperative for the FDA’s evaluation process to include a variety of data points and parameters being submitted by developers. Furthermore, a rigorous yet expeditious evaluation process and the provision of clear regulations and procedures to NGS developers will help foster the development and analysis of NGS tests.

The FDA could also play a critical role in developing a common data platform containing reusable test cases, data sets, and benchmarks that will be a valuable resource to researchers developing and using NGS tests. This platform could be designed to provide researchers with the opportunity to submit information on identified variants and/or loci as well evidence-based data for verification. An external quality assurance program with similar quality standards as those used for various NGS tests could be used by the FDA to evaluate/validate the data submitted by researchers.

**Are the concepts for standards outlined above adequate to ensure that NGS tests have appropriate analytical performance? If not, what else should be included? Alternatively, are some of the concepts for standards listed above unnecessary, and if so, which ones and why?**

ASH believes that the FDA standards mentioned in the discussion paper require additional detail, especially with regard to the different types of sequencing, the types of variants salient to hematologic conditions, and issues related to clinical practice, such as turnaround time, verification and cost. Tests must also be evaluated in comparison to existing approaches.

Regarding different types of sequencing, Sanger sequencing is still the gold standard. However, its sensitivity level for detecting mutations is lower than that offered by NGS. The difference in sensitivity levels occurs because the depth of sequence coverage offered by NGS is at least one hundred times more than that offered by Sanger sequencing. With such high levels of sensitivity, NGS can be used to intensively evaluate leukemias, lymphomas and even minimal residual disease (MRD) so as to foster the development of targeted treatment strategies.

Examples of promising variants in hematologic conditions that could be detected by NGS sequencing include germline mutations responsible for bone marrow failure; sequence mutations
responsible for constitutional or acquired myelodysplastic syndromes, myeloid leukemias, and chromosomal rearrangements and gene fusions responsible for leukemia. Importantly, in a given disease individual genes may be subject to different types of genetic change in different patients (e.g., mutation, deletion or gene fusion in leukemia) and tests used must be able to detect the range of alterations.

ASH encourages the FDA to consider additional factors to ensure the appropriate analytical performance of NGS tests, including: (1) platform validation, which validates the lab procedure, quality of the sequencing, and the pipeline for calling variants; and (2) accounting for different performance expectations based on the region sequenced (i.e., GC-rich regions).

How should changes or advances in technology be managed utilizing a standards-based approach? What types of changes in technology pose the most concern and what are the best standards to address those concerns?

Technological advances foster the discovery of new variants and drive the field of diagnostics. Since new approaches to NGS testing may be developed that require evaluation and benchmarking, ASH recommends that all new advances in NGS techniques receive the same evaluation as other diagnostic tests regulated by the FDA.

It may be useful to have external experts re-evaluate tested data points to ensure that the existing parameters of all tests are up-to-date and compatible with the progression of NGS technology and assay development. Doing so would require a reliable and scheduled system of oversight and review.

Who should develop the standards: FDA, an ad hoc committee of experts, a Standards Development Organization, others, or a combination of these approaches?

ASH believes a multitude of stakeholders should be involved in the development of standards for NGS tests. Potential groups to be represented are the relevant federal agencies, including the FDA; researchers focused on molecular biology, bioinformatics, hematology, pathology, and genetics; industry; professional organizations like ASH, the College of American Pathologists (CAP) and the American Society for Clinical Pathology; and, relevant non-scientific professionals such as ethicists; and patients. ASH would be pleased to provide the FDA with additional input regarding the makeup of a future standards development committee.

What are current practices for clinical interpretation of variant information from NGS tests?

ASH fully supports the FDA’s efforts in reviewing this important issue. Currently, there are no uniform practices for clinical interpretation of variant information from NGS tests; however, there are a few standard areas of current practice that are worth the Agency’s consideration.

First, alignment software tools and reference genomic data from curated databases like COSMIC, dbSNP, ClinVar, IARC, OMIM and HGMD, and disease/gene specific databases (e.g., TP53, RB1 mutation databases) are used to facilitate accurate identification of variants from test sequences.
Although these databases are important, they can be imperfect reference sources and may contain inaccurate tumor or germline variants.

Second, several variant calling tools are used to detect mutations, DNA copy number alterations and chromosomal rearrangements. The accuracy of each approach is dependent on the type and complexity of the alteration – in general, single nucleotide alterations are more reproducibly identified than insertion deletion mutations and chromosomal rearrangements. Tools differ in their ability to detect somatic (tumor acquired) and germline mutations. Finally, the ability to distinguish somatic from germline mutations in cancer genomes depends on the availability of matched non-tumor sequence data, and the use of public data for mutation annotation, filtering and interpretation. Standard practice uses the absolute chromosomal coordinates of a Genome Reference Consortium (GRC) genome assembly to facilitate the curation of genome assemblies based on the sequence overlaps of long, high quality sequences thus allowing for a query to be run against a curated database and the position where a variation has occurred.

Third, with the large amount of data produced by NGS, the possibility of predicting the functional impact of variants in an automated fashion is increasingly important. Computer-aided annotation enables the filtering and prioritization of potential disease-causing mutations for further analysis. These tools (e.g., PolyPhen, SIFT, MutationTaster, FATHMM) try to predict possible impact of an amino acid substitution on the structure and function of human proteins.

Fourth, in order to interpret the identified variant data with high confidence and to reduce the margin for a sequencing artifact, all called variants should reside in a region with a baseline coverage (100x recommended) and should be read from the sequencer independently from both directions (5’ to 3’ as well as 3’ to 5’). For a minimum read depth, a low-end mutated allele frequency (mutation load) should be stipulated (e.g., 1% - 3%). Finally, variant calls are validated against gold standard approaches with known samples and outcomes from Sanger sequencing, qPCR or an orthogonal NGS approach. In cases of extreme low-level mutation, the variant may be validated in an independent second sequencing run.

Would the use of ClinVar/ClinGen or other curated databases by all test developers incentivize data sharing and provide a more efficient way to establish clinical significance for different variants? Are there other steps that should be taken to facilitate sharing of this data? Is ClinVar/ClinGen the appropriate resource for FDA to utilize? Are there other resources that FDA should consider?

The current challenges in NGS data analysis are partly based on the fact that many variants are unknown and unclassified or scattered across various databases. In this context, conducting variant analysis coercively requires review of data from different data providers. This becomes problematic when each data provider applies different rules and conventions while collecting submissions, making it difficult to compare datasets for specific variants. More importantly, naming conventions (e.g., Human Genome Variation Society (HGVS) nomenclature for naming variants on DNA and amino-acid level) are not adhered to across data providers, making it challenging to directly compare various datasets even when the data are available.
Although no single site will be comprehensive, creating a centralized and curated hub for data collection with a fixed set of conventions should alleviate the problem of having different data providers using different rules to collect submissions, and using different naming conventions that complicate cross-data system comparisons. This could also decrease the number of discordant data sets for the same variant, and will allow users to use recognized, clinically significant datasets supported by evidence-based assessments. The Society recommends the hub be freely and publicly accessible, and data curation transparent.

As an additional note, ClinVar/ClinGen meets several of these criteria and is considered the most reliable choice as of now. As of February 2015, however, only 12% of overall variants (77,000) had been submitted by more than a single submitter. And since ClinVar/ClinGen does not curate the submitted data itself, approximately 20% of the existing data is currently discordant. Also, some nascent databases (e.g., the NCI Genomic Data Commons, the International Cancer Genome Consortium, Sanger, and St. Jude Children's Research Hospital - Washington University Pediatric Cancer Genome Project) are developing rich resources of clinically annotated data. ASH would be pleased to work with the FDA to convene a group of interested parties to discuss how database information can be best harmonized for the use of NGS test developers.

**Can information about the clinical meaning of variants be of value to physicians and patients when there is uncertainty about the strength of the association between the variant and disease? If so, why and under what circumstances?**

Regarding the strength of association between variants and disease, much of the current uncertainty stems from the rapid evolution of genome sequencing and the still limited set of discovery data. ASH is aware that variants with unknown significance may become important in the future and believes it is important for laboratories to provide relevant information about variations to physicians. Further, ASH supports the development of procedures to accurately record new variations.

**Can information regarding variants of unknown significance or variants with conflicting evidence regarding significance be of value to providers and patients? If so, why and under what circumstances?**

ASH supports that variants of unknown significance be reported to patients and providers. Not reporting these unknown variants could be misunderstood as a negative result of NGS testing, which could impede a subsequent re-analysis if the specific variant came to be associated with a disease. Furthermore, variants thought to have unknown significance could have important functional consequences, some of which may include the variant’s role as a specific mutation in causing the disease in question, or it could play a predictive role — predicting a deleterious mutation not previously described in a gene in the right disease, or help identify a new mutation in a cancer gene that may not necessarily be related to the cancer being studied.

In order to determine the functionality of variants currently regarded to have unknown significance, functional annotation experiments as well as drug target experiments will need to be performed on these variants in order to determine their functional effects.
What controls should be in place, if any, for laboratories who wish to implement their own interpretive process, rather than relying on FDA-recognized evidence assessments?

ASH recognizes that this is a rapidly evolving area and wants to ensure that laboratories have the flexibility to interpret NGS tests while promoting the integrity of the results and the standard of care given to patients. The implementation of baseline rules by the FDA addressing the selection, reporting, interpretation and communication of identified genomic variants will provide appropriate guidance to laboratories wishing to devise their own interpretive process. Further, the FDA should be involved in ensuring that the interpretative process in each laboratory is sound, precise, and results in easily understandable reporting for physicians and patients.

What other options should FDA consider to assure that the clinical significance of variants reported by NGS tests is accurate?

Since the bulk of the data obtained from NGS is still unclear, ASH encourages the FDA to implement systems that will allow for proper interpretation of the current available data. One of these systems could include asking researchers to provide comments along with their results when an NGS test has identified a specific variant.

Following this, ASH encourages the FDA to identify and convene expert panels that could systematically cross-examine the data from the NGS tests, as well as the information found in the published literature. If there is sufficient evidence in the literature to support the findings of the identified variant, (e.g., a diagnosis or prognosis), an endorsement from the FDA stating that fact could be added to the result. If the literature is inconclusive, that information should be included as well. The availability of such a system will enable physicians to be better equipped with the tools to interpret the NGS data and to better communicate the results to their patients.

ASH appreciates FDA’s ongoing efforts to gather input from stakeholders on this important issue and welcomes the opportunity to meet with relevant staff to further discuss ASH’s perspective. If you have any questions or would like additional information, please contact ASH Government Relations and Practice Manager, Stephanie Kaplan (skaplan@hematology.org or 202-776-0544) or ASH Scientific Affairs Coordinator, Alice Kuaban, MS (akuaban@hematology.org or 202-776-0544).

Sincerely yours,

David A. Williams, MD
ASH President