Optimizing FDA's Regulatory Oversight of Next Generation Sequencing Diagnostic Tests—Preliminary Discussion Paper

I. Overview

Next generation sequencing (NGS)[1] comprises a collection of new technologies that allow rapid sequencing of large segments of an individual's DNA and even an individual's entire genome. Reliable and accurate NGS technologies promise to accelerate "personalized" or "precision" medicine, the tailoring of medical treatment to the individual characteristics of each patient. Unlike other laboratory tests that typically detect a single or a defined number of substances to diagnose a limited set of conditions, a single NGS test can identify thousands -- even millions -- of genetic variants and the results of that test could be used to diagnose or predict an individual's risk of developing many different conditions or diseases. NGS technologies are used extensively in research and are rapidly entering clinical practice.

The capabilities of NGS tests and their rapid evolution pose challenges -- as well as opportunities – for FDA in carrying out its mission to both protect and promote public health. FDA is committed to drawing on the knowledge of the scientific community to help inform this oversight. Appropriately-tailored oversight should foster innovation in NGS technology, allow the public to have timely access to newly developed tests, and ensure that those tests are accurate, reliable and clinically relevant.

II. FDA's Oversight of In Vitro Diagnostics (IVDs)

Accurate and reliable diagnostic tests are a foundation of medicine. As with other IVDs, an inaccurate NGS test can lead to patients receiving the wrong diagnosis,[2] the wrong treatment or no treatment at all even when effective therapy is available, and can impose unnecessary costs on the healthcare system. Inaccurate tests could cause healthy individuals to seek further testing and treatment to address an erroneous belief that they have, or could develop a certain condition. Conversely, inaccurate tests could lead to individuals believing that they do not have, or are at low risk to develop a disease, and thus forgo screening or therapy that would benefit them. For reasons such as these, FDA has regulated in vitro diagnostic tests (IVDs) for decades as part of its mission to protect and promote public health.

IVDs are typically used in clinical laboratories, and utilize a wide range of technologies to detect or measure DNA, protein, or other substances found in the human body. When FDA reviews an IVD to determine whether it meets applicable safety and effectiveness requirements, and hence can be marketed for clinical use, it examines whether the test is accurate and reliable (analytical performance) and if the results from the test correctly identify the relevant disease or condition (clinical or diagnostic performance). When FDA determines that an IVD has acceptable

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[1] Next-Generation Sequencing, also referred to as "massively parallel sequencing" or "high throughput sequencing", refers to technologies that perform DNA sequencing in parallel, allowing for the production of thousands or millions of sequences concurrently.

[2] Here, "diagnosis" refers to the "diagnosis of disease and other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease" (21 Code of Federal Regulations 809.3(a)), and includes but is not limited to diagnosis, aid in diagnosis, prognosis, therapy selection/dosing, monitoring, and risk prediction.

analytical and clinical performance, the Agency clears or approves the test, and the manufacturer may then legally market the device for clinical use.

III. NGS Tests: Challenges and Opportunities

Most IVDs detect only a single or a defined number of substances to diagnose one or several specified conditions. In contrast, NGS tests are capable of detecting the over 3 billion bases in the human genome, and in doing so identify the approximately 3 million genetic variants an individual may have. A single use of an NGS test could enable the diagnosis of any one, or more, diseases or conditions a patient presents with. NGS tests can also help to predict a patient's risk for developing certain conditions. Because it is possible to sequence the whole genome, it is not necessary to know what variant one wishes to identify prior to running and successfully interpreting an NGS test—a concept which is very different from how traditional IVDs are used.

These critical differences raise certain challenges in the evaluation of NGS tests using FDA's traditional regulatory approach for determining analytical and clinical performance of IVDs, including:

- Unlike IVDs that detect only a single or a defined number of substances to diagnose one or several specified conditions, NGS tests can identify an essentially unlimited number of variants based on the over 3 billion base pairs that compose the human genome. Evaluating whether each data point is accurately measured would take years and thus delay the public's access to the benefits of this technology.

- FDA typically requires the test developer to establish that the variant identified and reported is clinically meaningful to any disease or condition the test is intended for use in diagnosing. The developer must submit data and/or information demonstrating the clinical significance of any variant for which it wishes to make clinical claims. In many instances, NGS tests are used precisely because they can routinely detect rare variants for which it may be impractical for test developers to provide conclusive evidence supporting clinical significance.

- The clinical relevance of many variants identified by NGS tests may have limitations because of the rarity of the mutations, and their co-existence with other possible causative variants in individuals with a given pathology. FDA's regulation of diagnostics is intended to ensure that test results are clinically meaningful. How to communicate information regarding the significance of the presence of genetic variants in a way that is understandable to physicians and consumers presents challenges.

Although the unique features of NGS tests create regulatory challenges, these same features that give rise to these challenges also provide opportunities for novel solutions:

- The accumulation of data from NGS testing is enabling scientists, clinical labs, and regulators to better understand NGS outputs and error modes. NGS used in research and in diagnostic testing is generating a large amount of data that can be leveraged in further research, clinical trials, databases, and learning health systems to further evaluate the analytical and clinical performance of NGS tests.

- The large amount of cross-genome data generated by NGS tests could allow unique approaches, such as novel metrics and computational approaches, for assessing test performance.

- More generally, the cumulative generation of data through the increased use of NGS testing could help spur additional research in genomics and precision medicine.

FDA recognizes both the regulatory challenges of evaluating NGS tests described above and the unique importance of these tests to the research and clinical communities. Given its mission to protect and promote public health, the Agency has worked for several years to implement efficient and appropriate oversight of these tests, with the goal of assuring safety and effectiveness while enabling innovation in the field and supporting the advancement of precision medicine. FDA has hosted several public workshops examining various aspects of NGS, beginning in 2011, and has interacted extensively with scientists and other subject matter experts at conferences and in other professional venues. FDA personnel have also participated in developing standards and tools for the scientific community such as the Next Generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) Workgroup and the Genome in a Bottle Consortium. In 2013, FDA cleared the first NGS instrument as well as two NGS tests for cystic fibrosis. In doing so, the Agency focused on how to use representative variants to assess the analytical performance of the instrument, and the use of aggregated publically available data to support the clinical relevance of variants in the CFTR gene.

The challenges and opportunities described above are now presenting themselves as realities because of the critical mass of genomic data that has been accumulated by researchers and clinicians. For this reason, FDA is now exploring new regulatory approaches that will enable the Agency to provide appropriate oversight, in a way that is more suitable to the complexity and data-richness of this new technology, to assure that NGS tests have adequate analytical and clinical performance. Appropriate oversight of NGS testing may help improve the validity and comparability of genomic data, and in doing so, could improve the quality of the evidence base used by scientists when designing studies and computational strategies and in interpreting research results. Instituting appropriate oversight is therefore necessary both to assure safe and effective tests and to advance FDA's mission of facilitating innovation to benefit public health, particularly in the area of precision medicine.

IV. Exploring New Regulatory Approaches for NGS Tests

FDA recognizes that any new approach to regulating NGS tests should be designed to assure that the public has timely access to tests that have adequate analytical and clinical performance. As mentioned above, the very features of NGS that pose challenges to evaluating test performance also present the possibility of novel solutions.

In this request for public input, FDA is considering new regulatory approaches only for NGS tests because this technology allows broad and indication-blind testing and is capable of generating vast amounts of data, both of which present issues that traditional regulatory approaches are not well-suited to address. Other technologies capable of detecting genetic variation, including but not limited to PCR and SNP arrays, are generally designed to capture predefined data points that are known in advance of testing, and therefore are more suited to regulation under traditional approaches. However, even these technologies may benefit from a

different approach for capturing data related to clinical performance. Furthermore, these new approaches would not apply to the manufacturing of components of NGS tests (e.g., stand-alone software, instrumentation, reagents).

For the purposes of addressing the questions presented below, an NGS test is defined as a human DNA sequencing assay performed on a particular NGS instrument (e.g., MiSeqDx) with a workflow defined by standard operating procedures that specify all materials and procedures. This includes all steps from defining the patient sample type and method of DNA extraction to computational processing of sequencing data, and, if offered, any portion of interpretation of the clinical meaning of individual variants identified in that patient that is performed within the test system (including software) rather than by a healthcare professional. The intended use of the NGS test may be specific for certain types of specimens, patient populations, etc., but does not necessarily include any claims about the clinical relevance of specific variants.

A. Analytical Performance of NGS Tests

FDA has authorized the marketing of one NGS instrument (Illumina MiSeqDx™) and its universal sequencing reagents[3], and two accompanying assays for the diagnosis of cystic fibrosis (Illumina MiSeqDx™ Cystic Fibrosis 139 Variant and Clinical Sequencing Assays). In doing so, FDA and the developer worked together to successfully address some of the challenges noted above. For instance, because it was impractical to provide data on the ability of the instrument to accurately and reliably detect every possible variant that might exist in a genomic sequence, analytical test performance for the MiSeqDx system was demonstrated for a representative subset of types of variants in various sequence contexts. Demonstrating adequate analytical performance for this subset provided reasonable assurance that the test would be able to successfully identify relevant variants in the genome without requiring the company to submit data for every possible variant the test could identify. FDA plans to continue to use this subset-based approach when evaluating the analytical performance of NGS platforms, but is considering novel and efficient approaches for establishing analytical performance for specific NGS tests developed using FDA cleared or approved components in clinical diagnostic laboratories.

One such approach would be to promote the development of methodologic quality-based standards[4] that laboratories could meet as a means to demonstrate analytical performance of any NGS test that is developed by the lab. Such standards could:

- Define the technical metrics of NGS data quality and test performance (e.g., error rates, coverage, depth) that must be satisfied no matter how NGS is performed;[5]
- Include computational approaches to establish analytical performance;
- Provide guidelines for quality systems that laboratories must have in place to develop NGS tests;
- Provide current best practices for quality assurance and control in NGS sequencing, and specify requirements for proficiency testing;

[3] FDA also cleared the MiSeqDx™ Universal Kit 1.0 for use with the platform.

[4] Here, the term "standards" encompasses: metrics and tools that can assess the metrics, best practices, and more specific technical or other standards that would be developed by a recognized body.

[5] Alternatively there could be a different standard for each test, technology, and/or intended use.

- Provide for change control processes that would accommodate changes to technology while retaining accurate and reliable performance.

Standards could be created by an *ad hoc* committee of experts, FDA, or through a Standards Development Organization (SDO), such as the Clinical Laboratory and Standards Institute (CLSI), or a combination of the above. If the standards were created by an SDO, FDA would review and recognize those standards using its already established Standards Programs. FDA is seeking public comment on various aspects of this approach.

FDA would like specific feedback with regard to: (1) value of a standards-based approach to regulatory review of NGS tests (2) content of standards to be developed that will assure that conformity to the standard will assure test accuracy and reliability, (3) who should develop such standards, and (4) appropriate mechanisms to ensure compliance.

1. Questions for Public Comment on Analytical Performance

1. How are labs currently developing NGS tests and assessing their analytical performance?
2. 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?
3. What are the benefits and risks to public health of a standards-based approach to regulating NGS analytical performance?
4. Would a standards-based approach limited to NGS tests that use FDA cleared or approved components encourage continued development and consumer access to NGS tests with appropriate analytically performance?
5. To what extent could computational approaches be used to assess analytical performance? If possible, who should develop such approaches, how could FDA facilitate their development, and how could they be validated?
6. 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?
7. 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?
8. Who should develop the standards: FDA, an ad hoc committee of experts, a Standards Development Organization, others, or a combination of these approaches?
9. What measures should be put in place to monitor progress and impact, both positive and negative, if a standards-based approach were adopted?
10. How should conformity with standards be assessed?

B. Assessing the Clinical Performance of NGS Tests

Clinical or diagnostic claims for particular tests are typically evaluated by FDA based on a sponsor's submission of adequate data or information in a premarket application for their device or device platform. Data and information may come from clinical trials, appropriately curated databases, published literature, and/or other sources of valid scientific evidence. When clearing Illumina's NGS-based cystic fibrosis assays, FDA and the developer addressed the challenge of providing evidence for the clinical relevance of rare variants by allowing the developer to make use of a well-curated third party database consisting of evidence from multiple sources to establish clinical significance[6], as an alternative to conducting a new study or using existing literature. The two cystic fibrosis assays that were cleared differ in their indications and approaches to clinical performance:

- The variant panel assay reports only a discrete number of variants with established clinical significance.[7]
- The clinical sequencing assay reports any variant in the cystic fibrosis gene, regardless of whether clinical significance of the specific variant is known, and therefore has more limited indications for use.[8]

FDA is interested in public input on how this approach could be applied more broadly to enable access to timely, accurate, and reliable genomic information in a manner that is meaningful for clinical decision-making.

1. Leveraging Genetic Databases to Evaluate Clinical Performance

FDA believes that it could use high quality curated genetic databases that provide information on genetic variants and their association with disease to better establish the clinical performance of NGS tests by providing evidence about such associations and the strength of that evidence. FDA is particularly interested in those databases that actively engage the scientific community to provide ongoing evidence assessment using the best available data at any given time (e.g., continuous learning). FDA has initiated a dialogue with the National Institutes of Health (NIH) aimed at determining whether the data curated by the ClinGen program[9] and deposited in ClinVar[10] could be leveraged to support clinical significance for variants detected by NGS tests. ClinVar is a freely accessible, public archive of reports of the relationships among human variations and phenotypes annotated with supporting evidence. ClinVar facilitates access to and communication about the relationships asserted between human variation and observed health status, and the history of that interpretation. ClinGen is an NIH-funded effort dedicated to expert evaluation of research data and the data from the hundreds of thousands of clinical genetics tests

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[6] Clinical and Functional Translation of CFTR; www.cftr2.org
[7] Illumina MiSeqDx™ CF139-Variant Assay (CF139) 510(k) Substantial Equivalence Determination – Decision Summary (K124006); http://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf
[8] Illumina MiSeqDx™ Cystic Fibrosis Clinical Sequencing Assay link - 510(k) Substantial Equivalence Determination – Decision Summary (K132750); http://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf
[9] ClinGen is an NIH-funded resource for the development and implementation of a framework for evaluating the clinical validity of human genomic variants; http://iccg.org/about-the-iccg/clingen/.
[10] ClinVar is an NIH-supported database containing human variants and evidence supporting their relationship to phenotypes; http://www.clinvar.com/.

being performed each year to determine which variants are most relevant to patient care. NIH has been engaged with the clinical and scientific communities to establish criteria for the strength of association between genetic variants and a disease that exceeds a minimum standard to assure there is credible evidence of some association. Once the ClinGen process has been applied to the evidence on variant-disease association, it would issue a conclusion regarding the levels and adequacy of the evidence to support a valid association between a genetic variant and a disease/condition. The ClinGen process allows the strength of the variant-disease link to be updated as new evidence is generated and entered into ClinVar.

FDA has focused on the use of ClinVar and ClinGen because they are publically accessible, transparent, scalable, and evidence is continually curated by the scientific community using the most current data available. However, other databases could meet the same needs, provided that they met certain criteria for curation, versioning, updating, and other processes. For example, FDA utilized a database to support clearance of the Illumina cystic fibrosis assays that was developed and supported in part by a disease advocacy foundation. The Agency is seeking public comment on the use of other curated databases to support clinical claims for genetic and genomic diagnostic tests, as well as on creating a public process based on important curation criteria so that other databases could be recognized as having sufficient quality to support clinical significance for variants detected by NGS tests.

In considering the concept of using externally-generated evidence assessments to support clinical performance, FDA believes that it should participate with NIH and the scientific community in the evidence assessment process, with a goal of streamlining steps the Agency would need to utilize this factual information in its risk-benefit assessments for potential clinical claims. Ultimately the Agency's goal is to allow test developers to leverage FDA-recognized evidence-based assessments of the clinical significance of genetic variants. This would minimize the need for sponsors to generate their own data in order to support a premarket submission to the FDA.

This approach would introduce a number of efficiencies, which, ideally, would provide providers and patients with better clinical understanding of the relevance of a larger number of variants much more rapidly than would occur without a shared database and centralized evidence assessment. It would facilitate data sharing and common use of information that has previously existed in silos, and it would allow multiple developers to rely on the same evidence supporting clinical relevance, rather than have each developer generate or aggregate the evidence individually.

2. Whether and How to Communicate Information About Less Well-Understood Variants

The evidence for association of many genetic variants and particular diseases is limited because of the rarity of the variant, or its coexistence with other variants in the presence of a given pathology. Some stakeholders have asserted that information regarding genetic variants for which there is not sufficient evidence to demonstrate clear clinical significance, but for which there is evidence to establish a likely association, may have some value to physicians and their patients in clinical decision-making in certain circumstances. FDA would like to further explore this concept, and is interested in public comment about: 1) the value of conveying information about genetic variants for which there is limited evidence of clinical significance, 2) whether some caveat as to the limitations of the available data may be needed, and, 3) if this can be of value, how and under what circumstances the information should be conveyed, to assure the

information is effectively communicated, the benefit to medical decision-making is maximized, and risks to patients are minimized.

3. Questions for Public Comment on Clinical Performance

1. What are current practices for clinical interpretation of variant information from NGS tests?
2. What are the benefits and risks to public health of the use of information from curated databases such as ClinVar/ClinGen in supporting clinical claims made by NGS tests?
3. 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?
4. Can curation and evidence evaluation standards be constructed in a manner that would allow interested developers to create databases that could support clinical significance?
5. 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?
6. 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?
7. How should FDA ensure that laboratories are accurately reporting the strength of variant-disease associations?
8. How can FDA incentivize test developers and clinical laboratories to deposit their information in ClinVar to improve the ability of the community to understand the genetic basis of disease?
9. 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?
10. What measures should be put in place to monitor progress and impact, both positive and negative, of the proposed use of well curated databases?
11. What other options should FDA consider to assure that the clinical significance of variants reported by NGS tests is accurate?

V. Conclusion

NGS tests are unique among existing IVDs in the amount of data that can be generated, the lack of an *a priori* definition of what will be detected, and the number of clinical interpretations that can be made from a single patient sample. In order to continue to support the development of

useful medical information, FDA believes the most efficient possible approaches to regulating NGS tests should be considered. Among the possibilities, a standards-based approach to analytical performance of NGS tests and the use of centralized curated databases containing up-to-date evidence to support clinical performance are under discussion. Because stakeholder feedback is important as FDA considers new approaches, the Agency will hold a public meeting on February 20, 2015, and open a public docket to accept comments on these approaches and the questions raised above. After review of input gathered by these efforts, FDA will determine the types of changes, if any, that it should initiate with respect to its oversight to NGS tests, and communicate its findings and conclusions to the public.