Regulatory Considerations for Next Generation Sequencing Based Tests

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Disclaimer

The views expressed during this presentation are those of the presenter and do not necessarily reflect the policy or position of the US FDA or the US government.
Presentation Topics

• What constitutes a good molecular submission?
• Examples of molecular submissions: lessons learned
• Regulatory considerations for NGS
FDA reviews tests for safety and effectiveness

Genetic tests should demonstrate:
- Analytical Reliability
- Clinical significance
- Benefits outweigh the risks

Patients and Physicians should know:
- How to interpret the information
- Clinical value of the information
- Limitations of the information
Molecular Diagnostics can be moderate risk (class II) or high risk (class III)

- Based on the indications for use
- Risks associated with wrong results

Class II Examples:
- CFTR mutations for cystic fibrosis
- Gene expression for risk of breast cancer recurrence
- Postnatal Chromosomal Microarray

Class III Examples:
- KRAS mutations in colorectal cancer for treatment
- Colon cancer screening test
Regulatory Process

**Class II** (moderate risk)

- **Traditional 510(k)**
  - FDA Clearance
    - Device shows substantial equivalence to a legally marketed predicate

**Class III** (high risk)

- **Pre-market Approval Application (PMA)**
  - **De novo 510(k)**
    - No Predicate
    - Special Controls
    - S&E
  - FDA Approval
    - Device demonstrates safety and effectiveness (S&E)
Presentation Topics

• What constitutes a good molecular submission?
• Examples of molecular submissions: lessons learned
• Regulatory considerations for NGS
What constitutes a Good Submission?

- Good science, innovative technologies
- Supportable Intended use/indications for use
- Excellent organization
  - Complete device description
  - Comprehensive analytical validation
  - Appropriate clinical validation
  - Complete software validation (as applicable)
  - Clear and truthful labeling (package insert)
- eCopy, forms and fees (as applicable)
- PMA extras (e.g., QSR, BIMO)
Basic Components of a Molecular Diagnostic Submission

• Intended use/indications for use
• Device description (platform, software)
• Specimen Handling and Pre-analytical
• Analytical Performance
• Clinical Performance
• Instrumentation, software validation (as applicable)
• Labeling (package insert)

For PMA - manufacturing, design controls, quality system requirements (QSR/GMP) (21 CFR 820; all products must follow), BIMO
Intended use/indications for use

• Clear understanding of what to expect from and deliver to end users (e.g., physicians, patients)
  – What does it measure?
  – What specimen type(s) does it need?
  – What clinical indications is it intended for?
  – What patient population is it intended for?
  – Is it a distributed kit/system, or a test performed in a lab?
  – What instrument platform does it use?
Device Description

• Detailed descriptions of the device
  - What components does it have?
  - How does it work to generate a result?
  - What are the limitations of the technology?
  - Does it include software, and if so, do you have sufficient software validation documentation?

• Follow the FDA guidance for premarket submission requirements for devices containing software and off-the-shelf software

Varieties of Molecular Devices

• Specimens
  – Whole blood/plasma
  – Buccal swab
  – Tumor Tissue
  – Urine/Fecal

• Methodologies
  – PCR/dPCR
  – FISH
  – Microarrays
  – Nextgen sequencing

• Analytes
  – DNA (including methylated DNA)
  – RNA
  – Proteins

• Outputs
  – Qualitative (e.g., SNP, mutation status)
  – Quantitative (e.g., copy number, transcript level)
  – Gene Signatures “score”
What FDA Reviews in Molecular Device

- **Sample Collection**
  - Whole blood/plasma
  - Buccal swab
  - Tumor Tissue
  - Urine/Fecal

- **Analyte Extraction**
  - DNA (including methylated DNA)
  - RNA
  - Proteins

- **Instrumentation**
  - PCR/dPCR
  - FISH
  - Microarrays
  - Nextgen sequencing

- **Software**
  - Qualitative (e.g., SNP, mutation status)
  - Quantitative (e.g., copy number, transcript level)
  - Gene Signatures “score”
Analytical Validation

• Comprehensive analytical validation studies (including pre-analytical)
  – Does it measure the analyte(s) accurately and reliably in the hands of the intended users with various sources of variability?
    • Intended type of clinical specimens
  – What specimen handling is required?
    • Sample type/matrix: Serum, EDTA/Heparin Plasma, urine
    • Collection /transport/storage: Preservative/stabilizer
    • Preparation: Fixation/sectioning, micro-/macro-dissection
    • Sample stability: Real-time, freeze thaw
      – Especially important for use of archived samples for clinical studies
Analytical Validation (cont.)

• Analytical performance
  – Sensitivity/Linearity (LOB/LOD/LOQ)
  – Specificity (Exclusivity / Cross Reactivity / Interference)
  – Accuracy ← Valid comparator
  – Precision (Repeatability / Reproducibility)
    • Performance around cut-off
  – Guard band studies/robustness studies
  – Matrix/Method/Instrument Equivalency (Serum vs plasma, method comparison, etc)
  – Stability (test, calibrators, controls)
Challenges with Analytical Validation

- Specimen handling variability
- Difficulty obtaining clinical samples for rare alleles
- Multiplex assays often require complex validation
- Lack of reproducibility/high analytical variability
- Analytes are not stable
- Lack of comparators, calibrators and standards
- Whole genome technologies present unique challenges to validation strategies
Clinical Validation

• Strong clinical validation studies
  – Is your study design appropriate to support your claim?
    • Diagnosis, residual disease, etc. (current state)
    • Monitoring, recurrence (change in state)
    • Risk of disease, prognosis, prediction (future state)
  – Does your test result correlate with the result from a predicate device or expected clinical endpoint for your Intended Use in the hands of intended users?
  – Does your clinical study incorporate additional elements that will support/strengthen your claim?
    • Comparator method testing
Clinical Validation (cont.)

• Clinical Performance
  - Clinical Cutoff (if applicable)
    • Training set
    • Test set
  - Clinical Sensitivity and Clinical Specificity
  - Positive and Negative Predictive Values
  - Positive and Negative Percent Agreements
  - Correlation coefficient
    • Quantitative assay
    • Infrequent for molecular devices (except IVDMIA, transcript etc)
  - Statistical analysis plan
Clinical Validation (cont.)

• When peer-reviewed literature is used to support each claimed allele
  – Should be summarized and organized
  – Describe genotypes and associated phenotypes
  – Information about prevalence in diseased and carrier population summarized by ethnicity
  – Biological in vitro data about effect may be useful
    • e.g., CFTR2 database used in mutation tests for Cystic Fibrosis
Challenges for Clinical Validation

- Specimen availability
- Clinical study design and data don’t support intended use
- Clinical studies to validate molecular diagnostic test often have unique statistical challenges
- Pre-specified clinical/statistical analysis plan is crucial
- Sensitivity of assay vs. comparator method
- Appropriate determination of clinical cut-offs

The pivotal validation study should investigate test use in the claimed clinical population in intended setting using the final test configuration!
Companion Diagnostics

- Tests required to determine whether a specific drug should or should not be administered to a patient
- Test used in therapeutic trial
- Validation of test comes from a successful drug trial
- Problems can arise when the final test intended for marketing not the one used to screen patients for trial
  - Prescreening
  - Test changes
  - Missing outcome data in CTA negative population
- Plan to retest all patient specimens screened with new test and conduct a “bridging study” – re-analysis of drug efficacy based on new test results.
Package Insert (21CFR 809.10)

- FDA cleared/approved intended use(s)
  - Truth in labeling
- How the test works/test principle, controls
- How to perform the test
- Test validation data
- How to interpret the information
- Clinical value of the information
- Limitations of the information
- Contraindications and warnings
Good Submission = Favorable Outcome

• Sponsor and FDA work together through pre-submission process and interactive reviews to realize the good science and/or innovative technologies for better patient care
  - 510(k) clearances
  - De novo petitions granted
  - PMA approvals
Presentation Topics

• What constitutes a good molecular submission?
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First FDA clearances of NGS

- **Platform** - De novo for the Illumina MiSeqDx instrument and the Illumina Universal Kit reagents
  - The Universal Kit reagents isolate and create copies of genes of interest from patient blood samples; the MiSeqDx platform analyzes the genes. The software compares the patient’s genomic sequence to a reference sequence and reports back any differences.

- **Whole system with specific indications**
  - The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay - detects known CFTR variants (based on the CFTR2 database information).
    - The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay - sequences a large portion of the CFTR gene.
Next Generation Sequencing

- Rapid development, anticipated to change how clinical practice/diagnosis will be performed
- Multi-variant/multi-gene in a single assay
- Sensitivity, speed, reduced cost, and overcome limited sample availability
- Whole genome, exome, targeted-sequencing, RNA-Seq, methylation sequencing, germline vs. somatic
- Regulatory challenges for NGS
  - High throughput, complex, evolving, big data, software standardization, etc.
Next Generation Sequencing test steps

- Specimen
- DNA
- Library
- Sequencing
- Base calling
- Alignment
- Variant calling

Data analysis
- Annotation / filtering / classification
- Interpretation
- Report

extraction
amplification/capture

Fill-in of missing data
orthogonal confirmation
Special issues with NGS

- Unprecedented ability to detect rare and novel variants
- Rapidly evolving technology
- Challenges for NGS-based test analytical validation
  - Unit of validation – specimen source, analyte type, specific gene variants, specific exons, variant categories, genomic landscape
  - Comparator methods
  - Rare specimens
  - Lack of reference materials
  - One platform may have different uses (SNVs/indels/CNVs; Germline vs. somatic)
Cystic Fibrosis Variant Panel Assay
21 CFR§866.5900

Variant Panel Assay

• The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis

• http://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf

Clinical Sequencing Assay

• The test is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations

• http://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf
Review Considerations

• More than 2X number of variants in panel than previously cleared
• Sequencing for identification of known and unknown variants
• Inclusion of extremely rare variants
  – Clinical Validity
  – Lack of specimens for validation
• Previous submission requirements and model for CF assays are different
Analytical Validation

- Accuracy established for common variants using clinical samples, cell lines, and extremely rare variants with plasmid blends.
- Assay reproducibility established with only a subset of variants in both assays. Clinical sequencing assay also assessed performance in the problematic regions.
- 139-variant panel utilized bi-directional sequencing of all variant positions in all specimens; while the sequencing assay utilized bi-directional sequencing of 5,206 callable positions for all specimens.
Clinical Validity

Variant Panel Assay

• Clinical validity established for already cleared variants
• Clinical validity for new variants established using a well documented/curated database [CFTR2 Project Database (http://cftr2.org)]

Clinical Sequencing Assay

• No clear-cut clinical validity for novel variants
• Assay reports variants from reference with no annotations on significance
• Large burden on assay interpretation
CFTR2 Database

- **Key features leading to acceptance of database**
  - All patients enrolled diagnosed with CF
    - Specific clinical data collected on all patients
    - Variants classified as:
      - CF-causing mutation, Non CF-causing mutation, Mutation of varying clinical consequence (MVCC), Mutation of unknown significance (MOUS)
    - Variant classification based on
      - Clinical data using prespecified clinical & functional characteristics (i.e., sweat chloride, lung function, pancreatic status, and pseudomonas infection rates)
      - *In vitro* functional studies (i.e., CFTR protein synthesis, maturation, expression, function, and chloride conductance)
      - Evaluation of penetrance
CF Assays: Lessons Learned

• Utilization of a well documented/curated database for demonstrating clinical validity

• Variant classification based on literature-based evidence

• Representative subsets of variants that cover the range of variant types, sizes and genomic regions were assessed in analytical validation studies
• **Next-Generation Sequencing Platform** – 21 CFR 862.2265 High throughput genomic sequence analyzer for clinical use
  - Class II exempt from the premarket notification requirement subject to the limitations in 21 CFR 862.9
  - Product Code: PFF – High throughput DNA sequence analyzer
  - [http://www.accessdata.fda.gov/cdrh_docs/reviews/K123989.pdf](http://www.accessdata.fda.gov/cdrh_docs/reviews/K123989.pdf)

• **MiSeqDx Universal Kit** – 21 CFR 862.3800 Reagents for molecular diagnostic instrument test systems
  - Class I exempt: general controls, including current good manufacturing practices
  - Product Code: PFT – Reagents for molecular diagnostic test systems
  - [http://www.accessdata.fda.gov/cdrh_docs/reviews/K133136.pdf](http://www.accessdata.fda.gov/cdrh_docs/reviews/K133136.pdf)
**Regulatory Information/Resources**

- **MiSeqDx CF Assays** – 21 CFR 866.5900 CFTR (cystic fibrosis transmembrane conductance regulatory) gene mutation detection system
  - Class II: general controls + special controls*
  - Product Codes:
    - PFR – System, cystic fibrosis transmembrane conductance regulator gene, mutations & variants panel sequencing detection
      - [http://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf](http://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf)
    - PFS – System, Cystic Fibrosis Transmembrane Conductance Regulator Gene, Variant Gene Sequence Detection
      - [http://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf](http://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf)
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New Developments and Efforts

• **Public Workshop on NGS**
  
  – February and November 2015, public meetings on NGS
    
    http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm427296.htm
    
    http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm459449.htm
    
  – February 2016, NGS Oncology Panel
    
    http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm480046.htm
New Developments and Efforts (cont.)

- Internal Working groups
  - DMGP: working primarily on oncology panels
  - Precision Medicine Initiative

- Guidance Documents (April, 2015)
  - Balancing Pre-market and Post-market Data Collection for Devices Subject to Premarket Approval
  - Expedited Access for Premarket Approval and De Novo Medical Devices Intended for Unmet Medical Need for Life Threatening or Irreversibly Debilitating Diseases or Conditions

- Developing Guidance for NGS
NGS-based Oncology Panels

Single Test, Multiple Biomarkers, Multiple Indications

- Increasingly employed in the clinical setting

- One panel can be used for multiple indications
  - Potential to detect rare and novel variants

- Challenges the regulatory paradigm for CoDx
  - Clinical validity, analytical validity, robust across tissue types

- ‘Follow-on’ CoDx
Current Thinking for NGS-based Oncology Panels

- Claims sought
  - *Follow-on CoDx* claim for specific variants
  - *Analytical claim* for remaining variants (FDA does not have a path for approval of analytical only claim for a panel)

- QC at each step of the NGS process required

- *Representative subsets of variants* covering the range of variant types, sizes and genomic regions should be assessed

- Analytical accuracy should utilize *well-validated orthogonal methods*

- Assessment of difficult and challenging tumor types (e.g., bone, brain, pancreas, thyroid, etc.), and representative tumors, required for ‘*pan-cancer*’ claim

- Clinical validation plan for a follow-on CoDx claim should include agreement analysis (i.e., *method comparison study*) with the original FDA-approved test

- Appropriate validation for modifications/changes to approved panel
Precision Medicine Initiative (PMI) –
Modernizing FDA Regulation of NGS Tests

• New regulatory strategies for next generation sequencing
  – Develop and implement *standards* to assure quality
  – Develop *open-source tools* to help test developers meet standards
  – Support the development of a *data commons* for evidence on the clinical relevance of genetic variation

• Goals
  – Develop and implement an adaptive standards-based regulatory approach
  – Enable patient access to their own health information and the software needed for its safe and accurate analysis
Germline NGS tests –
A spectrum of approaches from performance standards to design concept standards as a regulatory tool for NGS

Performance Standards
• Finalized test evaluation
• Establishing specific metrics and acceptance criteria that the test would have to satisfy

Design Concept Standards
• Test design and development
• Ensure that the test developer can achieve the proper design and validation of an NGS test

• Performance standard elements
• Overall workflow of clinical NGS test
• Existing guidelines and standards for NGS tests
• Cloud based, open source bioinformatics tools repository to facilitate standard development
• Stakeholder communities building some of the standards
**PrecisionFDA**

- **What is precisionFDA?**
  - A community R&D platform for NGS assay evaluation

- **Why precisionFDA?**
  - help advance the regulatory science needed to assess the accuracy of genomic tests and capabilities of software

* _Regulatory Science is the science of developing tools, standards, and approaches to assess safety, efficacy, quality, and performance*_
Advancing the accuracy and reproducibility of NGS

- Crowd-sourced, cloud-based platform
- Will provide tools and open access resources
- Will allow the community to test, pilot, and validate approaches to NGS

precisionFDA

Community
NGS-Based Test Developers (large and small), NIST, FDA Scientists, Standards Bodies, Academic Centers, Physicians, Consortiums

Security and Privacy
HIPAA/HITECH, CAP, ISO27001
Uniquely identified and immutable data
Version-controlled applications

Courtesy of Taha Kass-Hout, FDA Chief Health Informatics Officer
Current Activities on PrecisionFDA

- Currently available tools on PrecisionFDA
  - Data simulators
  - VCF comparators
  - NGS analysis tools
  - Datasets from reference material sequenced by different organizations
  - Forum for user communications

- Initial focus
  - Assess reproducibility of a test
  - Assess accuracy using reference samples
  - Assess agreement with other methods
  - Assess test performance on synthetic data

- PrecisionFDA challenges
  - Consistency challenge
  - Truth challenge
Thank you!

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