Analytical and Clinical Validation of Pharmacogenetic tests

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Outline

- Analytical validation
- Clinical validation
- Considerations
  - Clinical considerations
  - Analytical considerations for both genotyping tests and next generation sequencing

Premarket FDA review of IVDs

- Driven by the intended use of the device
  - The types of validation studies that are needed depend on the claims that are made in the intended use

- The risk of an IVD is based on the consequences of a false result
  - **Class I** = Low risk: Usually exempt from Premarket FDA review
  - **Class II** = Moderate risk: Requires a predicate device and 510(k) clearance
  - **Class III** = High risk and novel intended uses: Requires premarket approval (PMA)

Intended use

- Pharmacogenetic test example:
  - "is a qualitative genotyping assay which can be used as an aid to clinicians in determining therapeutic strategy for the therapeutics that are metabolized by the CYP2C19 gene product, specifically *2, *3, and *17."
  - Class II example; 510(k)

Pharmacogenetics

- Different from “classic” genetic tests
  - Many potential patients to be tested
  - “phenotype” usually not obvious prior to treatment
  - Wide population differences in alleles and frequencies
  - Rare allele combinations often hard to validate
  - *Test results could drive drug safety/effectiveness*

Test Performance

- Analytical validity
  - Does my test measure the analyte(s) I think it does?
  - Correctly?
  - Reliably?

- Clinical validity
  - Does my test result correlate with the expected clinical presentation?
  - How reliably?

Analytical Performance

- Repeatability/Reproducibility
  - Will I get the same result in repeated tests over time?
  - Will I get the same result as someone else testing the same sample?

  - Repeated testing of a set of samples. Tested from sample extraction through test result to capture entire testing process. Should include multiple operators, instruments, lots and days.
  - For a distributed kit, testing of the same samples at multiple sites.

Analytical Performance

- Accuracy
  - Will I get results that are the same as “Truth”?
  - “Truth” is typically bi-directional sequencing results
  - Study should include samples with all possible genotypes unless genotype is rare
  - Study should have sufficient samples to determine accuracy with set confidence

Analytical Performance

- DNA input study
  - What is the minimum and maximum amount of DNA that can be the input for the test and still provide an accurate result?
  - Test what you recommend on the package insert

Analytical Performance

- Potential Interferences
  - Endogenous and exogenous interferences could depend on:
    - Sample type
      - Impact of eating, drinking, etc. on DNA from saliva
    - Extraction method
    - Intended use population
      - Candidate for Plavix could have high cholesterol, triglycerides

Examples of Clinical Validity Support

- Information from peer-reviewed, published studies demonstrating a relationship between the genetic test result and the selected clinical presentation, e.g., Cystic fibrosis and ΔF508
- Prospective analysis of a retrospective study (e.g., using banked samples)
- Prospectively performed study

Clinical Considerations

- Often genetic studies are performed in homogenous populations. Other variants/genetic factors could be important in other races/ethnicities (see COAG study). Use of a limited genetic panel could cause harm.
- Different interpretations of the clinical validity of genetic variants
  - Which genotypes are PM? Should IMs be included?
- Results of studies evaluating CYP450 status and clinical outcomes have discrepant results (e.g., 2D6 and tamoxifen, 2C9 + VKORC1 and warfarin)
- Lack of improvement in clinical presentation/outcome over standard of care that does not incorporate genetic information

Analytical Considerations for Pharmacogenomics Testing

- Technical issues
  - Pseudodeficiencies
  - Rare variants not detected by a test
    - Assumption that *1 call means wild type (rare variants could occur)
    - Rare variants could prevent primer binding
    - One SNP may occur in >1 CYP450 genotype; are you calling a *2 or *10 for CYP2C19?

Analytical Considerations for Pharmacogenomics Testing

- Some tests take two days from sample processing through test result
  - Added time for shipping to laboratory
- The shortest test has a one hour turnaround (performed in a clinical laboratory)

Analytical Considerations for Pharmacogenomics Testing

- Next generation sequencing
  - Different technology may lead to different results, especially outside consensus sequences
  - Different interpretations of pathogenic, likely pathogenic, benign, etc.
  - Gene panels from different laboratories include different variants

Summary

- Analytical validation of pharmacogenetic tests should be robust
  - Including assessment of accuracy, reproducibility/repeatability, appropriate DNA input, potential interferences
- Clinical validity information can come from several sources
- There are analytical and clinical considerations to keep in mind for pharmacogenetic tests

Thank you!

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