Almac Diagnostics

Challenges of NGS Companion Diagnostic Development

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VP of Quality & Regulatory Affairs
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“Almac Diagnostics is a stratified medicine company specialising in biomarker-driven clinical trials”
Presentation Overview

- Quality Management Systems for NGS CDx Development
- Overview of the Companion Diagnostic Development
- Key Activities in the CDx Development Pipeline
  - Specific Challenges for NGS
- Regulatory Guidance/Paradigms
- Summary
Presentation Overview

• Quality Management Systems for NGS CDx Development

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• Regulatory Guidance/Paradigms

• Summary
Quality Management System

It's not just what is developed but how......

Quality Management Systems are essential

Laboratory Accreditations
- UKAS to ISO17025 & ISO15189
- CLIA (US Clinical Laboratory Improvement Amendments)
- College of American Pathologists (CAP)

Laboratory Licence & QMS
- Human Tissue Act UK (HTA License)
- US State Licences:
  - New York (CLEP Permit)
  - Florida
  - California
  - Pennsylvania
- Complies with:
  - GLP
  - GCP
  - GCLP

Manufacturing Certifications
- EN ISO 13485:2016
  For design, development & manufacture of in vitro diagnostic nucleic acid technique based assays for gene mutation and expression analysis
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Companion Diagnostic Development

- Biomarker Discovery
- Clinical Trial Assay Development
- CDx Development
- CDx Commercialisation
Companion Diagnostic Development Pipeline Overview

Adapted from FDA Draft guidance Principles for Co-Development of an In Vitro Companion Diagnostic Device with a Therapeutic Product
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Key Activities in the CDx Development Pipeline

**INPUTS**
- Design Controls
- Assay Development
- Assay Software
- Reagent & Control Manufacture
- Regulatory Interaction

**OUTPUTS**
- Design Input
- Review
- Design Output
- System Testing
- Component Selection
- Purchase Controls
- Manufacturing Pipeline
- Early Regulatory Engagement

**V&V**
- Design Review
- Design Validation & Verification
- Analytical Verification
- Clinical Validation
- User Acceptance Testing
- CTA CE/CLIA Certification
- CTA Review by RA

**TRANSFER & APPROVAL**
- Design Transfer
- Assay Delivery
- Deployment
- Commercial Scale Delivery
- Regulatory Submission
CDx Development Pipeline

**INPUTS**
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CDx Planning and Documentation

- Design planning
- Design input gathering
- Design output selection
- Verification of input versus output- 2 main types of Verification
  - Analytical verification
  - Clinical Validation
- Design review at key product stages and prior to product lock (i.e. platform selection and signature-panel lockdown)
- Risk Management to ISO14971 required throughout whole design process
Design Control

Design Input

- Reagent & Control Specification
  - RNA Extraction Kit Specification
  - Library Prep
  - Sequencing
  - Control Specification

- Analysis Software Specification
  - Custom Code
  - SOUP Items
  - Hardware Specification

Assay Requirements Document

Assay Specification

- Labelling Specification
  - Test Request & Patient Test Results form
  - Instructions for use (Clinical)
  - Environmental Specification
  - Clinical Specimen

- Instrument Specification
  - Sequencer

Performance Specification

Analytical Performance Specification

Clinical Performance Specification

Design Output
Design Control
NGS Considerations during Design Control

Intended Use of your NGS assay drives platform/technology selection

• Kit or Single Site NGS Offering
• Sample type will determine target enrichment and library prep method
  - E.g. FFPE- high multiplex PCR
• Software requirements-
  - Bioinformatics Pipeline
  - Reporting Software
• Analyte(s) to be detected
  - Targeted gene panel
  - Exome
  - Whole genome
  - Types of sequence variations to be detected
• Analytical Performance requirements
• TAT
# CDx Development Pipeline

<table>
<thead>
<tr>
<th>INPUTS</th>
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<th>TRANSFER &amp; APPROVAL</th>
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NGS Assay Development

Assay Design in Collaboration with Platform Vendor
- Tissue Sampling
- DNA / RNA Extraction
- Library Prep
- Sequencing
- Base Calling
- Alignment
- Variant Calling
- Annotation/Filtering/Classification
- Interpretation
- Report

Entire pipeline requires validation for a CDx assay approval

Wet Lab

Dry Lab
NGS Assay Validation Challenges

• How to validate all known mutation possibilities?
• Tumor cellularity requirements?
• Minimal mutant allele burden detection?
• Quantitative or Qualitative reporting?
• Quality control: read depth? Other parameters?
• Unknown variants?
• Many other questions and variables

• Nex-stoCT: Workgroup set up to address gaps in metrics and processes for test validation, quality control

Source: Assuring the quality of next-generation sequencing in clinical laboratory practice (Nat Biotechnol.2012, Nov30(11))
Nex-stoCT:

Workgroup set up to address gaps in:

1. Metrics
2. Test Validation
3. Quality Control
4. Quality Assurance
## Performance Characteristics

<table>
<thead>
<tr>
<th>Performance Characteristics</th>
<th>Workgroup established definitions for NGS applications</th>
<th>Workgroup established metrics and processes for evaluation of NGS analytic performance</th>
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<tr>
<td>Accuracy</td>
<td>The closeness of agreement between a measured value and the true value, which for NGS is the accepted reference sequence.</td>
<td>• Coverage - The number of independent overlapping base calls made at a given position</td>
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<tr>
<td></td>
<td></td>
<td>• Depth of coverage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Average coverage</td>
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<tr>
<td></td>
<td></td>
<td>• Uniformity or distribution of coverage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Quality scores - The confidence in a base or variant call</td>
</tr>
<tr>
<td>Precision</td>
<td>The degree to which repeated measurements give the same result (repeatability and reproducibility).</td>
<td>• Monitor performance for:</td>
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<tr>
<td></td>
<td></td>
<td>• Library variability: independent library preparations</td>
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<tr>
<td></td>
<td></td>
<td>• Intra-run variability: same sample, same library, same run</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Inter-run variability: same sample, same library, different runs</td>
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<td></td>
<td></td>
<td>• Inter-operator variability</td>
</tr>
<tr>
<td>Analytic Sensitivity</td>
<td>The likelihood that the assay will detect a sequence variation, if present.</td>
<td>Depth of coverage must be sufficient to minimize a loss of sensitivity and specificity. The depth of coverage achieved with NGS will vary across the genome and therefore should be established across all regions of the sequence targeted for the clinical application. Analysis of RMs possessing comparable types of sequence variations across the targeted region by an orthogonal technique can provide a useful comparator.</td>
</tr>
<tr>
<td>Analytic Specificity</td>
<td>The probability that the assay will not detect a sequence variation, if not present.</td>
<td>Define areas of difficulty (e.g. repeat regions, insertions and deletions, allele dropouts) near the regions of interest. Biases introduced by capture-based or enrichment methods should be identified.</td>
</tr>
<tr>
<td>Reportable Range</td>
<td>The regions of the genome for which the NGS technology can accurately produce sequence information (e.g. multiple genes, exome, large genomic regions)</td>
<td>Materials containing the type of sequence variation(s) appropriately distributed within the target sequence may establish the capacity of the test to detect similar disease-associated mutations.</td>
</tr>
<tr>
<td>Reference Range</td>
<td>Establishment of reportable sequence variations expected to occur in the target population that the assay can detect.</td>
<td></td>
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Quality Control / Quality Assurance Considerations

- Monitoring established performance specifications
  - Coverage
  - Quality Score
  - Allelic Read Percentage
  - Mapping Quality
  - Strand Bias
  - GC Skew
  - Decline in Signal Intensity

- Use reference materials to monitor assay performance and for PT/AA

- Reference materials are needed that contain the range and distribution of sequence variations comparable to those which the assay is designed to detect

- Method-based PT may be component of an inter-laboratory comparison because of the size of the genome interrogated and the number of potential sequence variants targeted

- PT/AA Materials useful for NGS:
  - DNA from a well characterized cell line or patient sample (PT)
  - Electronic data (PT)
  - Inter-laboratory sample or electronic data exchange (AA)
Assay Delivery Within a Clinical Trial Setting

- Quality assurance must be maintained and monitored through the pre-analytical, analytical and post analytical phases of testing

- Require the correct assay result for the correct patient at the correct time
NGS Analytical Validation for CTA use

• Aim to complete CLIA-compliant analytical validation, generate quality control material and establish quality control limits

- NGS Analytical Validation (Nex-StoCT)
  - Next-generation sequencing: Standardisation of Clinical Testing (Nex-StoCT)  
    (www.cdc.gov/ophss/csels/dlpss/genetic_testing_quality_practices/ngsqp.html)

- CAP
  - CAP All Common Checklist and Molecular Pathology Checklist  
    www.cap.org

- CLEP
  - The Wadsworth Center’s CLEP NGS guidelines  
    (www.wadsworth.org/labcert/TestApproval)

- Accuracy
- Precision
- Sensitivity & specificity
- Limit of detection
- Reportable range
College of American Pathologist (CAP) Molecular Pathology Checklist

New standards for:

• Documentation
• Validation
• Quality assurance
• Confirmatory testing
• Exception logs
• Monitoring of upgrades
• Variant interpretation and reporting
• Incidental findings
• Data storage
• Version traceability,
• Data transfer confidentiality
CLEP Guidelines

Similar to CDC but also:

Controls

• NTC
• Negative control - HapMap cell line: Accuracy, Specificity (Validation only)
• Positive Control - Low positive near sensitivity level (Validation and periodically thereafter)

Reporting

• Statements detailing assay limitations
• Recommend reporting all variants to absolve labs of having to do this at later time or
• “This test is designed to detect x, y and z... Variants identified other than the ones listed above are not described in this clinical report. If interested, the other variants can be released upon request”.


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NGS Assay Development - Bioinformatics

- Run metrics e.g. Q-score distribution and error rate assessed
- Read trimming/removal as required
- Mapping
- Coverage is checked prior to variant calling to ensure sufficient depth
- Only calls observed in both strands pass variant call filter
- Annotation using VEP and filtration depending on trial/assay requirements
- Sample only reported if controls display correct calls
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For CDx these processes must be locked, validated and controlled
Manufacturing

Product Development & Lock

- Design and development of Product IFU, Assay Software and if required Test Request Form, Patient Test Report
- Development of batch manufacturing controls and specifications
- Design and development of packaging in conjunction with design control & marketing requirements
- Packaging/storage validation studies (shipping validation)
- Legal agreements with suppliers
Manufacturing - Ongoing

Ongoing Manufacturing - Process to GMP and ISO13485 standards to ensure patient and user safety is not compromised throughout
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CDx Development Path - Regulatory Approaches

Assay Development

CLIA Analytical Validation

PMA Validation

Regulatory Approval

PROVISIONAL PRODUCT LOCK

FINAL PRODUCT LOCK

GO / NO GO

PRE-SUBMISSION
Information for FDA: proposed development plan

IDE SUBMISSION
If Required
If assay deemed significant risk

PRE-SUBMISSION
Review analytical & clinical plans

FINAL SUBMISSION

REGULATORY PATHWAY
Regulatory Aspects for CDx (including NGS)

Quote from latest EMA concept paper:

“The potential to align technical assay validation and clinical evidence requirements for drug approval with technical and clinical performance requirements for CE marking will be discussed.”
Regulatory Aspects for CDx (including NGS)

**Technical** Performance requirements of assays used to measure predictive Biomarkers depend on:

- Stage of development (early vs pivotal study)
- Whether Biomarker status affects study entry
- Subject eligibility and treatment allocation
- Timing of the assay development in relation to drug development
- Use of central laboratory testing (complex tests)

- It is anticipated that the draft guideline will be available 9-12 months after the end of the public consultation of the concept paper (August-November 2018) and will be released for 6 months external consultation.
Other Pertinent Regulatory Guidance

• E18 Genomic Sampling and Management of Genomic Data Guidance for Industry

• Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases FDA Draft Guidance, July 8, 2016

• Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics, FDA draft guidance
FDA Fact Sheet on Tumour Profiling NGS

• Recent FDA approvals of 2 tumour profiling NGS assays:
  - Oncomine Dx Target Test Kit
  - MSK-IMPACT single site assay approval

• Defined 3 levels of biomarkers:
  1. Companion Diagnostics
  2. Cancer Mutations with Evidence of Clinical Significance
  3. Cancer Mutations with Potential Clinical Significance
FDA Fact Sheet on Tumour Profiling NGS

- Fluid approach to Reporting within levels 2 and 3

- Following FDA review and authorization of a tumor profiling NGS test, the test developers will be able to report additional variants of the same type post-market within the existing analytically validated genes in the panel, for claims consistent with the clinical criteria established in the original submission, without an additional FDA submission.

- As evidence of clinical significance becomes recognized by the clinical community, and provided that the analytical validity of the test was reviewed and established in the initial or a subsequent submission, mutations can be moved from Level 3 to Level 2 without an additional FDA submission.

- Allows use of NGS as a screening tool for multiple biomarkers across multiple trials on one panel without undue regulatory burden.
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Summary - Challenges for NGS for CDx

• The High Complexity of NGS pipeline and the results generated causes challenges for:
  - Assay validation, requirements of assay performance needs to meet regulatory requirements as a CTA and then subsequently as a CDx
  - Selection of suitable target capture approach and sequencing platform
  - Bioinformatics challenges for analysis and reporting
  - Revalidation of pipeline after upgrades/changes
  - Long term storage and retrieval of data
Thank you for your attention.
Any questions?
Thank You

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Almac Diagnostics

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