# Diagnostic Quality Assurance Pilot

## Project Update

AMP Annual Meeting

Reference Materials Forum

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Barbara Zehnbauer, Emory University School of Medicine

# Quality pilot objectives

**Context:** The pilot emerged from the Sustainable Predictive Oncology Therapeutics and Diagnostics (SPOT/Dx) working group.

Launched in 2013, SPOT/Dx focused on precision medicine to improve patient outcomes by equipping healthcare leaders with tools to advance clinical decision-making for the diagnosis and treatment of cancer and the regulatory and reimbursement infrastructure.

In service to the SPOT/Dx mission, participants proposed a Diagnostic Quality Assurance Pilot ("the quality pilot") to recognize molecular diagnostic quality and improved standardization of laboratory processes.

# Background

- Current environment for precision medicine:
  - Advent of NIH Precision Medicine Initiative (PMI)
  - FDA January 2017 LDT / NGS oversight discussion paper
  - Existing standardization gap in personalized medical diagnostics
    - No process to compare performance of CDx and LDTs for targeted therapies in cancer treatment
    - Quality assurance of diagnostics is a key issue for reimbursement decisions
    - Impacts patient access to diagnostics

# Problem statement

- What are we solving for?
  - ***Vision:*** Help ensure that diagnostics will provide clinicians with consistent and correct answers, regardless of which lab conducts the test and which diagnostic platform the lab uses
  - ***Quality Pilot objective:*** Equip molecular pathology labs with traceable reference samples to assess whether participating labs' appropriately validated tests can achieve diagnostic performance comparable to a companion diagnostic (CDx) for targeted cancer therapy. Accuracy of genotyping will be determined regardless of whether labs use the FDA-approved CDx or an LDT

# Core principles and differentiators associated with the quality pilot

- **Sustainability:** quality control materials that are commercially maintainable
- **Transparency of results:** visibility of outcomes
- **Accelerated reference material creation/availability:** initiate at phase 3 of CDx/drug development, prior to market launch
  - *The Illumina Extended RAS Panel CDx was approved by FDA, June 2017
- **Collaborative dialogue:** diversity and balance of perspectives among stakeholders
- **Quick action:** test proof of concept as rapidly as possible, evolve process as needed
- **Efficiency:** work within existing mandates, use existing pathways and infrastructure as much as possible

# Pilot overview

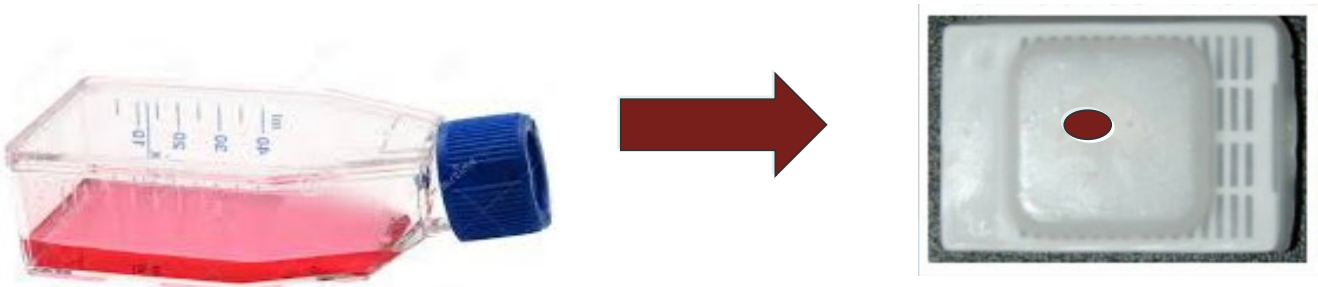
- Modeled on a CDx comprised of:
  - Two-gene, multiple variant NGS panel
  - Volunteered by Amgen and CDx partner, Illumina
- Performance standards specifications of:
  - Illumina CDx Extended RAS Panel CDx for a targeted colorectal cancer therapy - FDA approved June 2017
- Technical implementing partner is College of American Pathologists (CAP):
  - Selected vendors from RFP process for production of reference samples
  - Manages the distribution of samples to labs
  - Coordinates data collection and analysis
- Labs will demonstrate their ability to accurately:
  - Analyze reference samples for a variety of DNA variants
  - Report findings of clinical decision points for the targeted therapy

# Reference samples

- Includes “wet” challenge, a “dry” (*in silico*) challenge, and a neoplastic cellularity image-based challenge

- **Wet Challenge:** Blended cancer cell lines with pre-defined variant profiles
  - Total testing process
  - Limited number of genes, variants, and variant allele fractions
  - Expensive to design and develop

- Wet lab vendor: Horizon Discovery manufactured cell lines (CRISPR) and produced FFPE samples



# Reference samples

- **Dry Challenge (*in silico* files):** Pre-defined variant profiles introduced by a computerized process into the participating lab's own BAM and/or FASTQ files (from either amplification-based or capture-based assay designs, run on either Torrent-based or Illumina-based platforms)
  - Limited to bioinformatics component of the test
  - Virtually unlimited flexibility
  - Less expensive to create

- Dry lab vendor: P&V Licensing designed custom *in silico* files

# Quality pilot progress (I)

- Diversity of *KRAS* and *NRAS* sequence variants and VAFs are included in the challenges
- Wet challenge samples designed to examine total testing process
- Dry challenge included to examine bioinformatics interpretation
- Details about lab assay characteristics are also collected and sorted for insight into accuracy of performance

# Sample Assay Characteristics Questions

- Is a sensitivity control included in each run for the lower limit of the VAF for which your laboratory's assay is validated?
- If your laboratory performs targeted sequencing of cancer genes or mutation hotspots, which selection method is used for this assay?
- What is the read length in base pairs for this assay used for somatic variant detection?
- Does your laboratory report "mutation detected" results in the case of variants detected below the assay lower limit of the VAF?

# Quality pilot progress (II)

- Four(4) Proof of Concept (PoC) labs, representing experts of the Scientific Technical Working Group, have pre-tested the reference samples to verify:
  - Performance specifications of the FFPE cell lines
  - Design criteria of variants and VAFs were met
  - Processes for data file submissions for *in silico* mutagenesis and return to testing lab for data analysis

# Quality pilot progress (III)

- Twenty laboratories have been enrolled for this pilot
- Laboratory Selection Criteria:
  - Sequencing platform diversity
  - Selection approach (amplicon based vs. hybrid capture)
  - Selection method
  - Laboratory setting
  - Test volume for the NGS assay being used to examine KRAS and NRAS
- Both academic and commercial labs were selected, wide range of annual test volumes
- Lab accounts established (with CAP) for *in silico* files

# Lessons learned – technical (I)

- **Wet lab challenge**
  - All PoC labs correctly identified the sequence variants from the wet lab samples
  - Reported VAFs were within acceptable ranges
  - Neoplastic cellularity was also assessed by POC labs
- **Dry lab challenge**
  - Customized *in silico* reference samples (in terms of assay design, platform, target region, bioinformatics pipeline, etc.) can successfully be performed
  - Customized files are preferred because the variants are introduced into the individual laboratory's sequence files of the parent cell line
  - Retains the intrinsic characteristics of the submitted lab's sequence files
  - Data files were exchanged via the CAP Movelt platform

# Lessons learned – technical (II)

- **Dry lab challenge *(contd)***
  - **First**, the CAP MoveIt platform was significantly expanded to accept and manage the large data files (BAM or FASTQ)
    - Labs perform NGS on parent cell line DNA
    - Submit files to CAP
    - Transfer to P&V for in silico mutagenesis
    - P&V uploads to CAP
    - Labs access files from CAP
    - Labs analyze mutagenized data files
  - Excellent agreement among POC labs for *in silico* findings although a few variants were not included in some lab assays

# Lessons learned – technical (III)

- **Dry lab challenge *(contd)***
  - **Second**, due to the complexity of introducing external data files into certain NGS platform informatics pipelines, extra steps and guidance were necessary for labs
    - Lab was having trouble uploading FASTQ files to produce a VCF file
    - FASTQ files returned are not compatible with the secondary analysis pipeline, most likely due to some characters in the headers that are not supported
    - Problem may have been BAMs with non-standard tags/features

# Lessons learned – technical (IV)

- **Utilizing PoC labs**
  - Verifying reference sample performance and validating the data analysis processes was a very worthwhile internal quality check
  - *In silico* files are easy to generate; more detail is needed to instruct labs about effectively introducing them into their NGS informatics pipelines for analyzing variants

# Lessons learned – process

- **Multi-stakeholder approach comprises many perspectives**
  - Each sector's concerns constructively informed the work of other groups, providing greater transparency about priorities and processes

# Expected outcomes

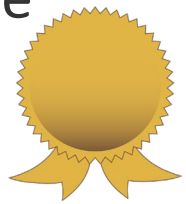
- The pilot will report on the percentage of labs able to achieve high levels of concordance and data on types of platforms and laboratories linked with results
- Data will be gathered about identity of variant calls, threshold of detection, aspects of NGS assay details and interpretation of clinical decision significance
- Results and findings will be reported in a peer-reviewed manuscript but will omit specific lab names

# Projected timeline

- Qtr 3 2018
  - Vendors completed reference samples manufacture
    - Parent cell line DNA
    - Blended cancer cell lines with defined variants FFPE samples
    - Custom *in silico* variant data files
- Qtr 4 2018
  - All reference samples tested by proof of concept labs
  - Reference samples distributed to 20 Quality Pilot labs
- Qtr 1/2 2019
  - Data submitted to CAP from pilot labs
  - Pilot data analyses
  - Manuscript preparation
  - Dissemination of results; perhaps through a workshop

# The way forward

- Manuscript will summarize data and multi-stakeholder perspectives on findings, process, implications, and next steps
- Approach, if proven successful, could be scaled-up to include
  - Adding more labs
  - Comparing pilot lab use of both the CDx and their own LDTs
  - Potential for other labs to access these reference samples
  - Patient samples (to demonstrate commutability)
  - Focus on a different CDx for a different disease
- Process could be institutionalized via a “gold star / good housekeeping seal of approval” for labs that demonstrate equivalent performance of their LDTs to the CDx



# Sustainability

- Standards generated could be used globally;
  - Cancer Drug Development Forum (CDDF) members are very interested in the SPOT/Dx and Quality Pilot models
- Pilot has helped inform the Somatic Reference Samples project of the Medical Device Innovation Consortium (MDIC SRS) launched by the US FDA and medical device manufacturers (http://mdic.org/clinicaldx/somatic-reference-samples/)
  - JD Alvarez will speak about this in this forum

# Appendix

Additional details

# Steering Committee

- Jeff Allen, PhD, Executive Director, Friends of Cancer Research
- Naomi Aronson, PhD, Executive Director, Clinical Evaluation, Innovation and Policy, Blue Cross and Blue Shield Association
- Karen Gutekunst, PhD, Vice President of Diagnostic Development, Illumina
- Daniel F. Hayes, MD, FASCO, Stuart B. Padnos Professor of Breast Cancer Research, University of Michigan Comprehensive Cancer Center and President, American Society of Clinical Oncology (ASCO) 2016- 2017
- Robert Loberg, PhD, Executive Director, Head of Clinical Biomarkers & Diagnostics, Medical Sciences, IVD, Amgen
- John Pfeifer, MD, PhD, Vice Chair for Clinical Affairs, Pathology and Immunology, Washington University School of Medicine *(Liaison to the STWG)*
- Girish Putcha, MD, PhD, Director of Laboratory Science, MolDX, Palmetto GBA
- Richard L. Schilsky, MD, FACP, FASCO, Senior Vice President and Chief Medical Officer, ASCO
- Patricia Vasalos, Technical Manager, Proficiency Testing, College of American Pathologists *(Liaison to the STWG)*
- Barbara Zehnbauer, PhD, Adjunct Professor of Pathology, Emory University School of Medicine and Journal of Molecular Diagnostics, Editor in Chief *(Chair)*

**Advisor to the Steering Committee and Chair:**

- Lindee Goh, PhD, Partner, Tapestry Networks
- Elizabeth Shaughnessy, Senior Associate, Tapestry Networks

**Liaisons**:

- Julia A. Beaver, Associate Director, Division of Oncology Products 1, Office of Hematology Oncology Products, Center for Drug Evaluation and Research (CDER), U.S. Food and Drug Administration (FDA)
- Gideon Blumenthal, Associate Director, Precision Therapeutics, Office of Hematology Oncology Products, CDER, FDA
- Yun-Fu Hu, Deputy Director of the Division of Molecular Genetics and Pathology, Office of In Vitro Diagnostics and Radiological Health Center for Devices and Radiological Health, CDRH
- Eunice Lee, PhD, Office of In Vitro Diagnostics and Radiological Health, Center for Devices and Radiological Health (CDRH), FDAU.S. Food and Drug Administration (FDA)
- Lisa Meier McShane, PhD, Chief, Biostatistics Branch, Biometric Research Program, Division of Cancer Treatment and Diagnosis, U.S. National Cancer Institute
- Michael Pacanowski, PharmD, MPH, Associate Director, Genomics and Targeted Therapy, Office of Clinical Pharmacology, Office of Translational Sciences, CDER, FDA
- Julie A. Schneider, Regulatory Scientist, Office of Hematology and Oncology Products, CDER, FDA
- Katherine Szarama, Presidential Management Fellow, Coverage and Analysis Group, Centers for Medicare and Medicaid Services
- Zivana Tezak, PhD, Associate Director for Science and Technology, Office of In Vitro Diagnostics and Radiological Health, CDRH, FDA

# Scientific and Technical Working Group

- Julia A. Bridge, MD, Professor, College of Medicine, Department of Pathology and Microbiology, University of Nebraska Medical Center
- Suzanne Kamel-Reid, PhD, University of Toronto, Laboratory Medicine and Pathology and Toronto Genera Hospital and Research Institute
- Robert Loberg, PhD, Executive Director, Head of Clinical Biomarkers & Diagnostics, Medical Sciences, IVD, Amgen
- Jason Merker, MD, PhD, Assistant Professor of Pathology, Stanford University Medical Center
- John D. Pfeifer, MD, PhD, Vice Chair for Clinical Affairs, Department of Pathology, Washington University School of Medicine *(Chair)*
- Patricia Vasalos, Technical Manager, Proficiency Testing, College of American Pathologists *(STWG Project Manager)*
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