SPOT/Dx Diagnostic Quality Assurance Pilot

Project Update

AACR Annual Meeting
Cancer Genomic Reference Samples
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Diagnostic Quality Assurance Pilot\(^1\) overview

- **Context:** The pilot emerged from the Sustainable Predictive Oncology Therapeutics and Diagnostics (SPOT/Dx)\(^2\) working group launched in 2013.

- **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.

- **Objective:** Equip molecular pathology labs with traceable reference samples as a baseline to assess how participating labs’ appropriately validated tests’ diagnostic performance compares 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

Background

- Current environment for precision medicine:
  - Advent of NIH Precision Medicine Initiative (PMI)
  - FDA January 2017 LDT 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
Pilot overview

- Model: compare lab developed test performance to CDx comprised of:
  - Two-gene, multiple variant NGS panel volunteered by Amgen & Illumina – KRAS and NRAS
  - Performance specifications of Illumina CDx Extended RAS Panel CDx for a targeted colorectal cancer therapy - FDA approved June 2017

- Steering Committee
  - Multiple stakeholders – oncologists, patient advocates, payors, laboratory professionals, and liaisons from regulatory agencies

- Scientific and Technical Working Group partners with College of American Pathologists
  - Selected vendors from RFP process for production of reference samples
  - Manages the distribution of samples to labs
  - Coordinates data collection and analysis
Core principles of the 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
- **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
Common reference samples

- “Wet” samples are commonly residual patient specimens
  - Are not inexhaustible
  - Do not represent spectrum of clinical disease

- Human cell lines are relatively inexhaustible
  - Blend parent cell lines with defined genetic variant cell lines
  - Specific design for genes, variants, and VAFs
  - Represent the pre-analytic stage of testing (DNA isolation)
  - Expensive and time-consuming to develop

By Bernstein0275 - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=25453056
https://www.geneticistinc.com/ffpe-blocks
SPOT/Dx Quality Assurance pilot includes
- “wet” challenge = total NGS testing process
- “dry” (in silico) challenge = data interpretation
- a neoplastic cellularity, image-based challenge = specimen requirements
**Wet Challenge**: Blended cancer cell lines with pre-defined variant profiles
- Total testing process
- Cell lines harvested and formalin-fixed
- Paraffin embedded

**Wet lab vendor**: Horizon Discovery manufactured cell lines (CRISPR) and produced FFPE 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
- To examine bioinformatics interpretation

Dry lab vendor: P&V Licensing designed custom in silico files
Labs will demonstrate their ability to accurately:
- Analyze reference samples for a variety of KRAS and NRAS sequence variants
- Different VAFs are included in these challenges

Provide some details of their assay, such as:
- 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?

Report findings of clinical decision points for the targeted therapy:
- Does your laboratory report "mutation detected" results in the case of variants detected below the assay lower limit of the VAF?
Four (4) Proof of Concept (PoC) labs, representing experts of the Scientific Technical Working Group, pre-tested the manufactured 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
Twenty laboratories were enrolled for this pilot
  – Sequencing platform diversity
  – Selection approach (amplicon based vs. hybrid capture)
  – Selection method

Both academic and commercial labs were selected, wide range of annual test volumes

Lab accounts established (with CAP) for in silico files
Lesson learned – technical - 1

- **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 were successfully produced and analyzed
  - Customized files
    - Variants are introduced into the individual laboratory’s sequence files of the parent cell line DNA
    - Retains the intrinsic characteristics* of the submitted lab’s sequence files
  - Data files were exchanged via the CAP Movelt platform
    - *(in terms of assay design, platform, target region, bioinformatics pipeline, etc.)*
Dry lab challenge (contd)

- CAP Movelt 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
Dry lab challenge *(contd)*

- Due to the complexity of introducing external data files into certain NGS platform informatics pipelines, extra steps and guidance were necessary for labs
- Lab challenges uploading FASTQ files to produce a VCF file
- FASTQ files returned were not compatible with the secondary analysis pipeline, most likely due to some characters in the headers that were not supported
- BAMs with non-standard tags/features may have been an issue
Utilizing Proof of Concept 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.
Lesson learned – process

- Multistakeholder approach connects 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 – Qtr 2 2019.

- Results and findings will be published – Qtr 3/4 2019.

- Manuscript(s) will summarize data and multi-stakeholder perspectives:
  - Lab data - analytical findings
  - Pilot procedures and process
  - Implications of pilot for broader practices/model for further studies
The way forward

- 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 Cancer Genomic 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/](http://mdic.org/clinicaldx/somatic-reference-samples/))
  - JD Alvarez will summarize in this symposium
Appendix

Additional details
Steering Committee

- Jeff Allen, PhD, CEO, Friends of Cancer Research
- Naomi Aronson, PhD, Executive Director, Clinical Evaluation, Innovation and Policy, Blue Cross and Blue Shield Association (BCBSA)
- 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
- Erick Lin, MD, PhD, MBA, Medical Director, Clinical Content, Office of Clinical Affairs, BCBSA
- 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, Chief Medical Officer, Freenome
- Richard L. Schilsky, MD, FACP, FASCO, SVP 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*)

Liaisons:

- 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
- 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

Advisors to the Steering Committee and Chair:

- Lindee Goh, PhD, Partner, Tapestry Networks
- Elizabeth Shaughnessy, Senior Associate, Tapestry Networks
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)
- Barbara Zehnbauer, PhD, Adjunct Professor of Pathology, Emory University School of Medicine and Journal of Molecular Diagnostics, Editor in Chief (Liaison to the Steering Committee)