tapestry NETWORKS

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

1 https://www.tapestrynetworks.com/our-work/healthcare/diagnostic-quality-assurance-pilot
2 https://www.tapestrynetworks.com/our-work/healthcare/spotdx-working-group

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

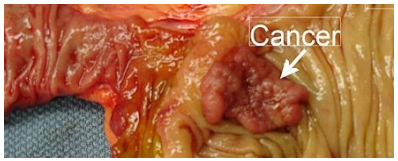
- Model: compare lab developed test performance to CDx comprised of:
  - Two-gene, multiple variant NGS panel volunteered by Amgen & Illumina – KRAS and NRAS
  - Performance specifications of Illumina CDx Extended RAS Panel CDx for a targeted colorectal cancer therapy - FDA approved June 2017
- Steering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

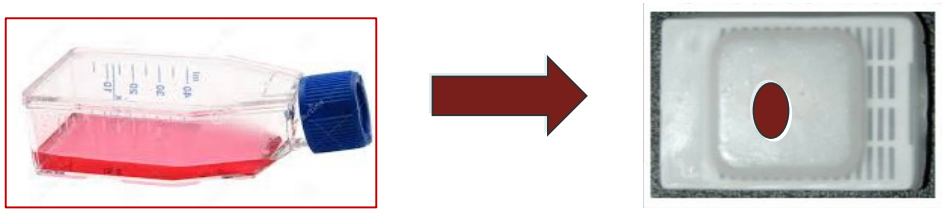
https://www.geneticistinc.com/ffpe-blocks

Pilot reference samples - 1

- 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

Pilot reference samples - 2

- **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



Pilot reference samples - 3

- 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

Pilot measurements

- 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?

Pilot progress - 1

- 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

Pilot progress - 2

- 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 MoveIt platform
    - *(in terms of assay design, platform, target region, bioinformatics pipeline, etc.)

Lesson learned – technical - 2

- Dry lab challenge *(contd)*
  - 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

Lesson learned – technical - 3

- 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

Lesson learned – technical - 4

- 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/)
  - 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)