Targeted NGS as a Frontline Strategy to Accelerate the Precision Treatment of Solid and Heme Tumors

A GenomeWeb / Pillar Biosciences Webinar

Speakers

Mark Ewalt, MD

Associate Medical Director, Laboratory Operations for Diagnostic Molecular Pathology, Molecular Diagnostics Service, Department of Pathology and Laboratory Medicine
Memorial Sloan Kettering Cancer Center

Pamela Ward, PhD

Scientific Director
USC Keck Hospital and Norris Comprehensive Cancer Center

Recent data and professional guidelines are driving a need to accelerate testing and reporting to inform the delivery of precision medicine. Clinical laboratories are looking for ways to efficiently streamline their tumor profiling platforms and more effectively deliver local NGS testing.

This white paper is based on "Targeted NGS as a Frontline Strategy to Accelerate the Delivery of Precision Medicine for Solid and Heme Tumors," a GenomeWebinar, sponsored by Pillar Biosciences. The program featured insights from speakers Mark Ewalt, associate pathologist and associate medical director for laboratory operations in the Diagnostic Molecular Pathology Laboratory at Memorial Sloan Kettering (MSK) Cancer Center, and Pamela Ward, scientific director at University of Southern California Keck Hospital and the Norris Comprehensive Cancer Center.

To set the stage, Ewalt described MSK's Diagnostic Molecular Pathology Laboratory. MSK's lab administered more than 37,000 molecular oncology tests in 2023. Many of these tests were performed with their flagship solid tumor assay, MSK-IMPACT.

MSK-IMPACT is a comprehensive genomic profiling (CGP) assay that supports tumor and normal sequencing of 400 genes. The assay generates somatic information about mutations, copy number alterations, structural variance, as well as some meta-mutational information, such as tumor mutation burden (TMB) and microsatellite instability (MSI) status. The assay has enabled MSK to develop and participate in many clinical studies and validate biomarkers.

However, MSK identified the need for a faster test for frontline biomarkers more immediately useful for clinical management. The organization had a few rapid assays, but there were limitations associated with these. For example, rapid PCR assays are specific for only a few variants and can't detect the whole spectrum of actionable mutations within a gene.

So, Ewalt's team started considering the development of a new assay. The team's goals were to consolidate the rapid test offerings onto a single platform, address unmet needs, and fit the assay into the organizational workflow. Of course, rapid turnaround time (TAT) was a top concern.

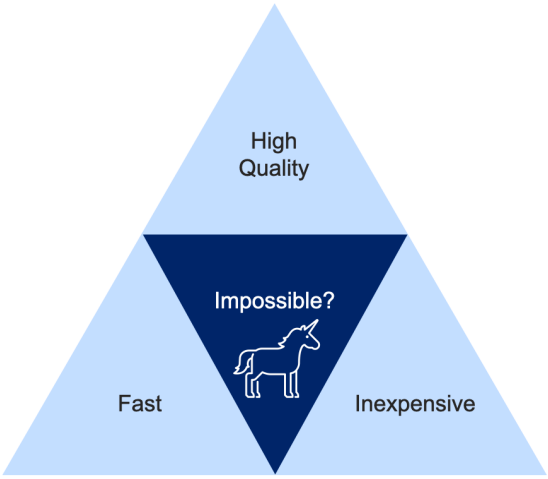
"Our initial goal was a TAT of three to five days, and to have flexible batch sizes," Ewalt said. "And then we wanted to meet the goals of the infamous iron triangle of fast, high-quality, and inexpensive. ... The question [was]: Is this even possible? And so, we were looking for something that would be this unicorn for us."

What were our goals in a new assay?

Consolidate existing rapid offerings
- *EGFR/KRAS* Idylla
- *ERBB2/EGFR* exon 20 sizing assay
- *IDH1/2* Idylla

Address unmet needs
- Full gene *TP53*
- *POLE* hotspots

Fit our workflow
- Rapid TAT (3 – 5 day goal)
- Flexible batch sizes



The "unicorn" assay would require only a small footprint of genomic space; complement the current assay; support CGP, both for research and for patients who are negative for frontline markers; provide a similar limit of detection (LOD) to MSK-IMPACT to avoid discordant results; support fingerprint SNPs for identity and quality purposes; and provide flexible batch sizes that are easy to implement.

To meet these requirements, the team collaborated with Pillar Biosciences to customize one of their existing assays oncoReveal Nexus into a 21-gene panel with 16 fingerprint SNPs. The team named the implementation of this assay MSK-REACT, or Rapid Evaluation of Actionable Cancer Targets.

Enter Pillar...

Collaborative design
- Customized existing content
  - 21 gene panel
  - 15 fingerprint SNPs
  - ~20kb footprint

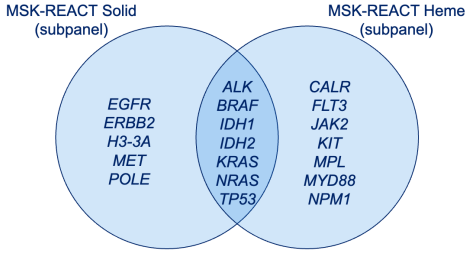
Easy implementation
- Pivat® bioinformatics included
- <8 hour and simple hands-on process

oncoReveal™ Nexus 21 Gene Panel

ALK	EGFR	H3-3A (H3F3A)	JAK2	MET	NPM1	TP53
BRAF	ERBB2	IDH1	KIT	MPL	NRAS	PDGFRA
CALR	FLT3	IDH2	KRAS	MYD88	POLE	TERT

Genes marked in green indicate full CDS coverage

oncoReveal™ Nexus 21 becomes MSK-REACT Rapid Evaluation of Actionable Cancer Targets



oncoReveal™ Nexus 21 Gene Panel

ALK	EGFR	H3-3A (H3F3A)	JAK2	MET	NPM1	TP53
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Pursuing Validation

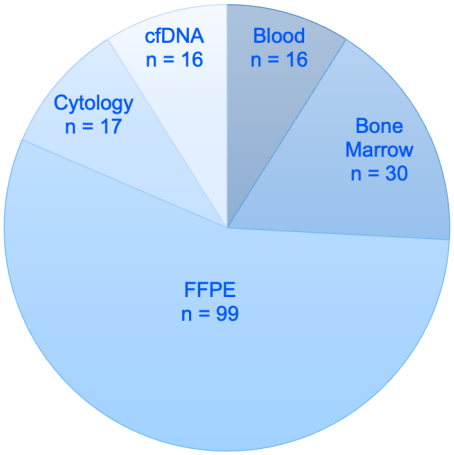
The MSK team then worked to validate this assay, using 196 clinical samples and five commercial controls. One of the first validation studies involved input testing. The assay delivered reproducible detection of various mutation types with an input from 0.6 to 100 ng at low and high variant allele frequencies.

Validation Cohort

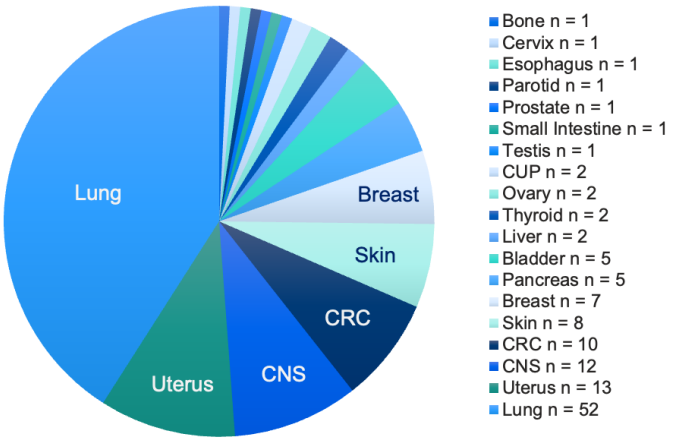
196 Clinical Samples
- 178 patient positives
- 18 patient negatives (healthy donor blood)

5 Commercial Controls
- 3 multiplex positive controls
  - Horizon HD753 Structural Multiplex
  - Horizon HD829 Myeloid
  - Seracare SeraSeq® Tri-Level
- 2 negative controls
  - NA12878
  - NA24385

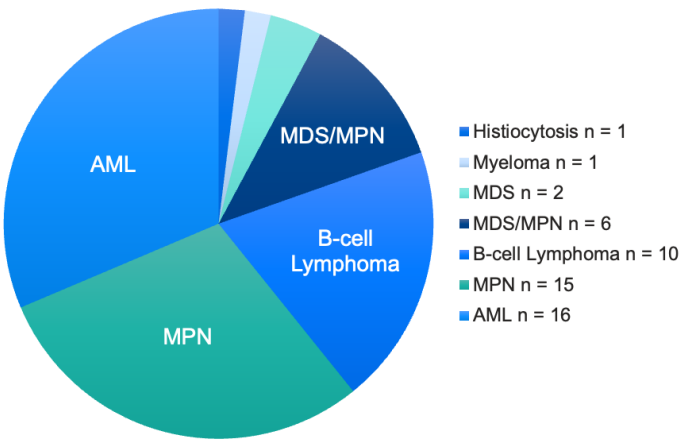
Clinical Positive Sample Distribution



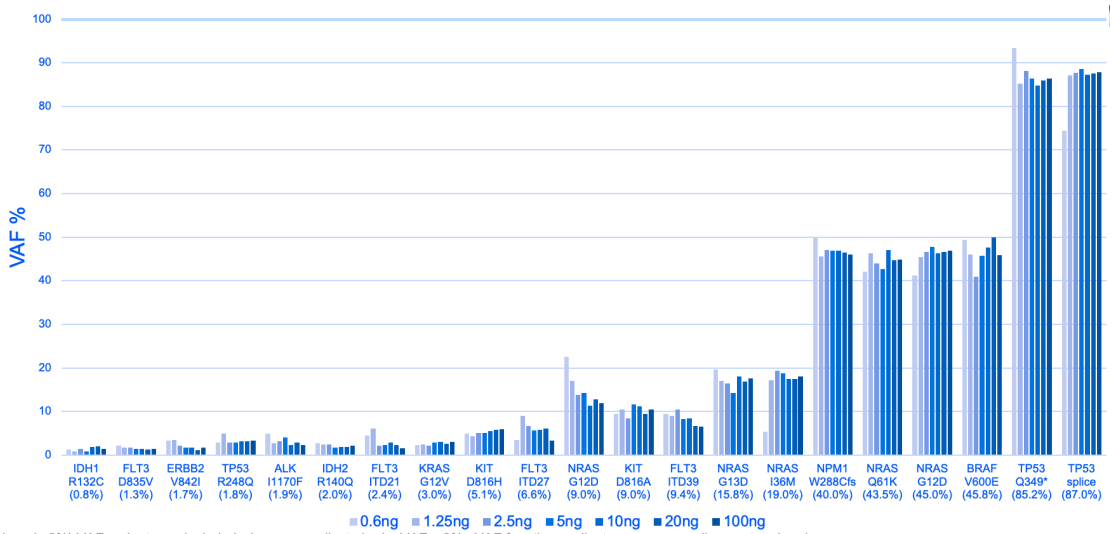
Solid Sample Distribution



Heme Sample Distribution



Validation – Limit of Input



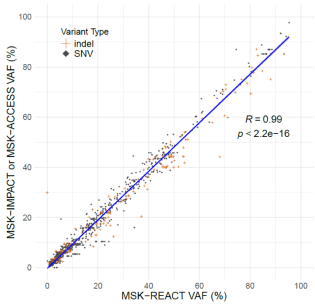
Low (< 2%) VAF variants are included when one replicate had a VAF ≥ 2%. VAF for other replicates were manually annotated and included from manual review of MSK-IMPACT or MSK-ACCESS BAM files.

The lab also examined quality metrics while doing the validation studies and found that lower mapping rates were correlated with the presence of false positives and noise in the samples. Mapping rates varied with the DNA input amounts, however, and the lab found they could overcome a low mapping rate by adding more input DNA. Low mapping rates were most pronounced, Ewalt said, with cell-free DNA (cfDNA) samples, but performance improved when using 10 ng or more of cfDNA.

The test performed well on blood, bone marrow, and FFPE samples at inputs as low as 1 ng. "Overall, taking this information, we set a goal input of 10 nanograms for our samples, and a minimum input of 2.5 ng, and this got us to a 99 percent mapping rate in the vast majority of cases," Ewalt said.

Intra-run precision was excellent, he added, with three samples repeated in triplicate, as well as an inter-run reproducibility with five samples repeated in quadruplicate. After screening out false positives with a mapping rate under 99 percent, tests that had low DNA input, or both, only four false positives in three samples.

MSK-REACT results correlate well to MSK-IMPACT® and MSK-ACCESS®



914 Clinical variants
- 715 SNVs
- 199 Indels (2 – 52bp)

912 Concordant variants
- 2 false negatives (1 SNV, 1 indel)
- 4 false positives (4 SNVs, <5% non-hotspot)
- Sensitivity 99.8%
- PPV 99.6%

The lab also created input interpretation guidelines for this assay to ensure accurate results. A tiered reporting approach was adopted as well.

"Not only is the assay rapid, but the development and validation of this assay was done at light speed," Ewalt noted, with study design, collaboration with Pillar Biosciences, validation, New York State approval, and rollout all happening between September 2023 and August 2024.

MSK- REACT Clinical Rollout and Laboratory Testing Consolidation

MSK-REACT was rolled out across tumor types in phases. Phase I included lung adenocarcinoma and hematologic neoplasms, and Phase II covered endometrial cancer and pancreatic cancer trials. The test will next be rolled out to all tumor types, with the team trying to limit uptake while they further automate the test.

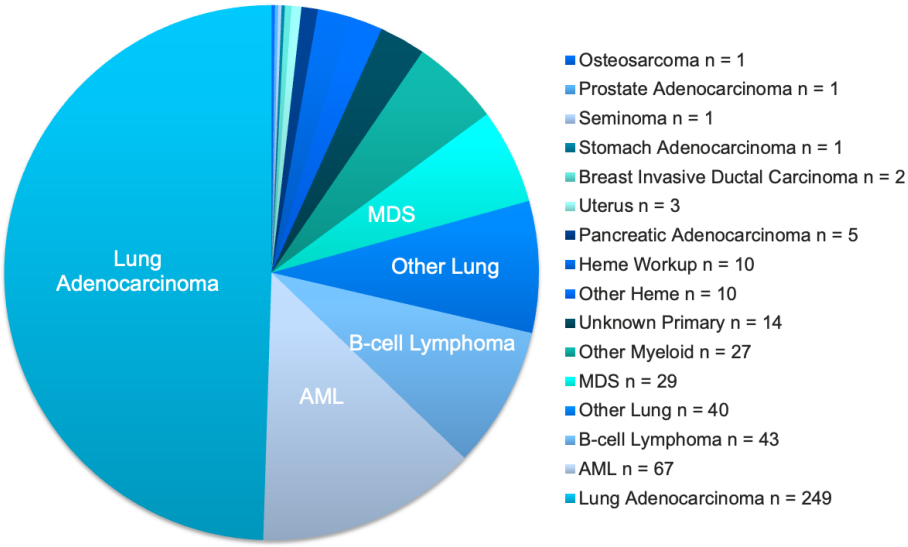
MSK-REACT has allowed the team to consolidate biomarker testing, ending routine Biocartis Idylla testing for KRAS and ERBB2/EGFR exon 20 sizing assays in lung cancer as well as heme hotspot TP53, IDH1/2, and NPM1 qPCR testing in hematologic malignancies.

The clinical uptake of MSK-REACT has been significant. "We have had gangbusters uptake," Ewalt said. From August 26 to November 11, 2024, the lab supported 503 total tests, which included 319 MSK-REACT Solid and 184 MSK-REACT Heme.

Clinical uptake has been significant

August 26 – November 11, 2024

- 503 total tests:
  - 319 MSK-REACT Solid
  - 184 MSK-REACT Heme
  - Rescued 25 IMPACT insufficient cases
  - Called variants in 9 cases where Idylla did not cover variants
- 3 cases failed all methods including MSK-REACT



"Over 80 percent of our patients were able to identify a driver alteration in their non-small cell lung cancer in under a week, which is really phenomenal," Ewalt said. In addition, MSK-REACT identified drivers in the majority of cases during this time period. Additionally, the test identified drivers in 25 samples with insufficient material for MSK-IMPACT testing, and called variants in nine cases in which PCR testing did not cover the variants.

Perhaps most importantly, turnaround times have been excellent, averaging a total of 3.8 days from test order to clinical report, with solid-tumor testing averaging about one day longer than liquid testing due to limitations in extracting from FFPE tissue.

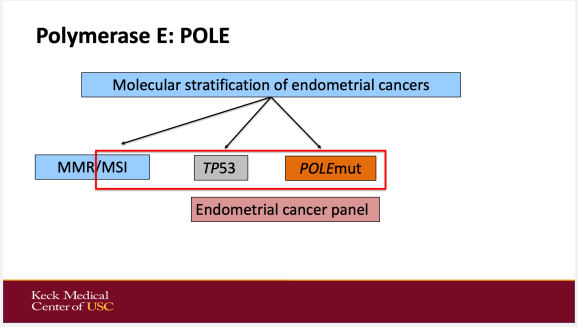
In summary, Ewalt said, MSK-REACT provides a robust and rapidly targeted NGS panel to complement CGP, and low input requirements enable the rescue of scant samples. The lab also discovered that pre-analytical variables are critical considerations in all rapid assay designs. Perhaps most importantly, the team learned that clinicians would use innovative assays if organizations take the time to build them. In the future, the lab is planning to pursue continued innovation; integrate SNP QC all NGS assays; expedite extraction; support laboratory automation; and roll out the assay to all tumor types.

Further Exploring the Clinical Utilization of oncoReveal Nexus

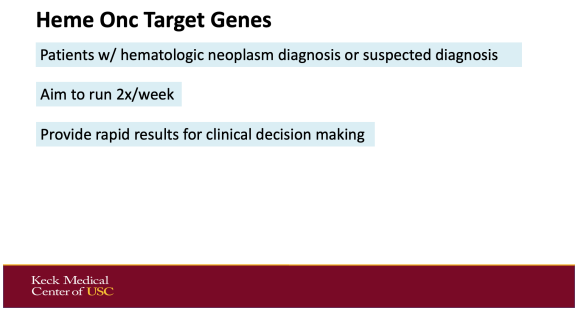
Ward discussed the clinical utilization of the Pillar oncoReveal Nexus 21 targeted gene panel at the USC Keck Hospital and the Norris Comprehensive Cancer Center. Ward explained that her lab was seeking an NGS panel that included POLE for endometrial cancer patients.

"We started to work on a Sanger sequencing assay, which was too tedious and lacked the required sensitivity, so we wanted to move to NGS. But to bring economy and efficiency into the lab, we also needed to have an NGS panel that included a number of [hematology oncology] targets. And so, this Pillar oncoReveal Nexus panel was a perfect fit for our lab," Ward said.

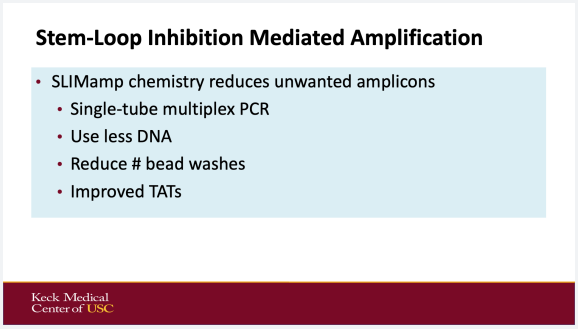
The prognosis and management of endometrial patients is dependent upon molecular classification, Ward explained, with labs needing to interrogate three biomarkers in each case: microsatellite instability (MSI), TP53 mutations, and POLE mutations. Typically, Ward explained, MSI and TP53 status are investigated with IHC. However, the lab worked with Pillar to create an NGS panel incorporating full gene coverage of TP53 and POLE, which it will offer alongside MSI testing by PCR fragment analysis.



For patients with diagnosed or suspected hematologic neoplasm, the lab's goals were to have a test they can run twice a week and that can provide rapid results for clinical decision making. "The trend is now moving to have these small rapid panels to get patients on clinical treatment earlier," Ward noted.



The lab chose to use Pillar's panel not only because of the content, but also because of the chemistry. The stem-loop inhibition mediated amplification (SLIMamp), Ward explained, reduces the unwanted amplicons that are typicallyfound in a tile-based, amplicon-based approach.



"The downstream effect of this is that we can have just a single multiplex tube going into this assay rather than two. So obviously, this means we need less DNA, but importantly it reduces the number of bead-based washes," Ward said. "With the reduced washes, we have improved [turnaround times] for anything that's going to be ordered on this panel".

The lab validated the panel on 59 samples, which included bone marrow, peripheral blood, and FFPE solid tumors. For interpretation and reporting, the lab will use Pillar's PiVAT bioinformatics pipeline to generate VCF files and the Sophia Genetics DDM platform for tertiary analysis.

Ward's lab is offering testing using the panel under 18 different Z-codes, including an AML, MPN, and endometrial panel, plus 11 single-gene tests, four of which have separate approvals for solid and heme cancers.