Validation of a Rapid Targeted Next Generation Sequencing Oncology Panel

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Introduction

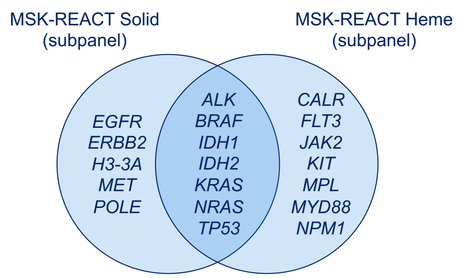
Problematic: Prompt identification of oncogenic drivers is crucial for clinical management, particularly targeted therapies and clinical trials, yet traditional next-generation sequencing (NGS) assays have long turnaround times, and current rapid methods are limited by their low throughput.

Objective: Validate a rapid targeted DNA-based NGS oncology panel to detect single nucleotide variants (SNVs) and indels of 19 genes associated with solid and hematologic malignancies.

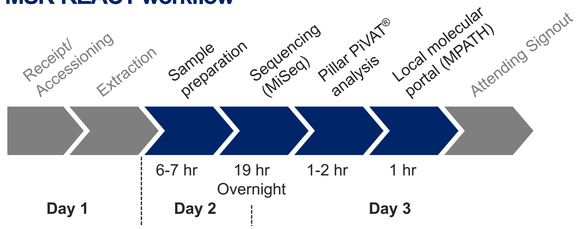
Material and Methods

MSK-REACT (Rapid Evaluation of Actionable Cancer Targets) is a targeted amplicon based NGS assay using Pillar oncoReveal™ Nexus 21 Gene kit for detection of SNVs and indels in hotspot regions of 19 cancer-related genes.

MSK-REACT panel



MSK-REACT workflow

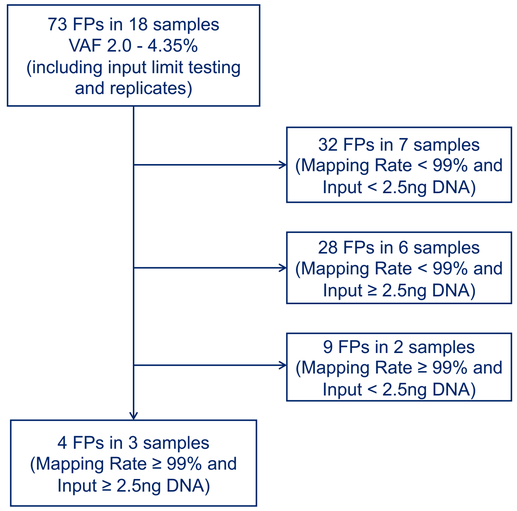


Criteria for Positive Calls

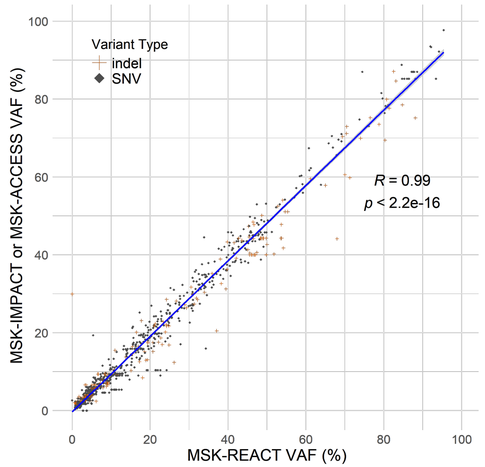
Mapping Rate %	First-Tier "Hotspot" Events	Second-Tier Events
≥ 99%	Depth ≥ 20 Variant Reads ≥ 10 VAF ≥ 2%	Depth ≥ 50 Variant Reads ≥ 10 VAF ≥ 5%
< 99%	Depth ≥ 50, Variant Reads ≥ 10, VAF ≥ 5%	

Results

Establishing input criteria ≥ 2.5ng DNA and mapping rate ≥ 99% correctly removes 95% of FPs



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



914 Clinical variants:
- 715 SNVs
- 199 Indels (1 – 52bp)

912 Concordant variants (99.8%)

	SNV	Indel
Precision	100% (4/4)	100% (4/4)
Reproducibility	100% (14/14)	100% (4/4)

2 false negatives

Tumor type – Sample type	Gene	cDNA change	Observed VAF	Reference VAF	Comment
Uterine Carcino-sarcoma – FFPE	*EGFR*	c.2248G>A	0	2.64	Non-hotspot alteration, eight reads (just above reporting threshold) in reference method; *POLE* mutated case with 275 mutations on MSK-IMPACT
AML – Blood	*FLT3*	c.1749_1784 dup	0	29.90	36 bp ITD mutation

4 "false positives" (not clinically reported with calling criteria)

Tumor type – Sample type	Gene	cDNA change	Observed VAF	Reference VAF	Comment
Uterine Endometrioid Carcinoma - FFPE	*H3-3A*	c.58C>T	2.72	0	Non-hotspot, VAF <5%
AML – Bone Marrow	*H3-3A*	c.58C>T	3.81	0	Non-hotspot, VAF <5%, input 100ng
Glioblastoma – FFPE	*H3-3A*	c.58C>T	2.84	0	Non-hotspot, VAF <5%
	H3-3A	c.86G>C	3.66	0	Non-hotspot, VAF <5%

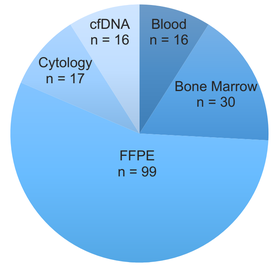
Material and Methods

Validation cohort

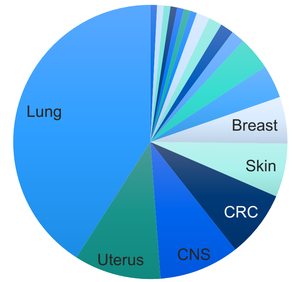
196 Clinical Samples:
- 178 patient positives
- 18 patient negatives (healthy donor blood)

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

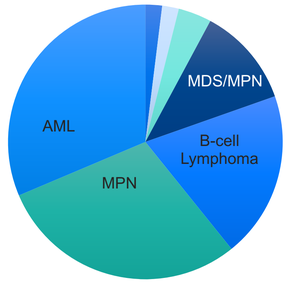


Solid Sample Distribution



- Bone n = 1
- Esophagus n = 1
- Prostate n = 1
- Testis n = 1
- Ovary n = 2
- Liver n = 2
- Pancreas n = 5
- Skin n = 8
- CNS n = 12
- Lung n = 52
- Cervix n = 1
- Parotid n = 1
- Small Intestine n = 1
- CUP n = 2
- Thyroid n = 2
- Bladder n = 5
- Breast n = 7
- CRC n = 10
- Uterus n = 13

Heme Sample Distribution



- Histiocytosis n = 1
- Myeloma n = 1
- MDS n = 2
- MDS/MPN n = 6
- B-cell Lymphoma n = 10
- MPN n = 15
- AML n = 16

Conclusions

- Validated rapid, DNA-based NGS oncology panel for detecting SNVs and indels in 19 genes
- High sensitivity, predictive value, precision, and reproducibility
- Compatible with diverse sample types (FFPE, bone marrow, blood, cfDNA, cytology)
- Supports clinical decisions with actionable results in 3-7 business days

List of abbreviations:
AML, acute myeloid leukemia; cfDNA, cell-free DNA; CNS, central nervous system; CRC, colorectal cancer; CUP, cancer of unknown primary; FFPE, formalin-fixed paraffin-embedded; FP, false positive; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; NGS, next-generation sequencing; PPV, positive predictive value; SNV, single nucleotide variant; VAF, variant allele frequency.