RAPID CLINICAL MUTATION SCREENING FOR THE NCI MYELOMATCH TRIAL USING THE GENEXUS PLATFORM

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INTRODUCTION

- The promise of "precision medicine" in acute myeloid leukemia (AML) demands fast and accurate assessment of the mutation and cytogenetic landscape of each AML case.
- The NCI Myeloid Malignancies Molecular Analysis for Therapy Choice program (myeloMATCH) is a precision medicine clinical trial initiative across the NCI National Clinical Trials Network for newly diagnosed patients.
- In myeloMATCH, the goal is to have cytogenetic and mutation assays performed **within 72 hours** of central lab receipt, allowing for a rapid assignment of the appropriate clinical trial from the myeloMATCH trial portfolio.

AIM

- The NCI-Myeloid Assay version 2 (NMAv2) is performed on the Ion Torrent Genexus System, a fully automated next generation sequencing (NGS) platform which provides a turnaround time of **<48 hours** from specimen receipt to clinical reporting.
- The NMAv2, which includes 93.3% of genes with mutations at ≥3% frequency and 72% of genes with mutations at >1% frequency in AML, was analytically validated at the Molecular Characterization Lab (MoCha) and Fred Hutchinson Molecular Oncology Lab (MO).

GENE COVERAGE

Hotspot Genes (N=28)	ANKRD26, ABL1, BRAF, CBL, CSF3R, DDX41, DNMT3A, FLT3 (ITD+TKD), GATA2, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, MYD88, NPM1, NRAS, PPM1D, PTPN11, SMC1A, SMC3, SETBP1, SF3B1, SRSF2, U2AF1, WT1
Full Genes (N=17)	ASXL1, BCOR, CALR, CEBPA, ETV6, EZH2, IKZF1, NF1, PHF6, PRPF8, RB1, RUNX1, SH2B3, STAG2, TET2, TP53, ZRSR2
Fusion Driver Genes (N=35)	ABL1, ABL2, BCL2, BRAF, CCND1, CREBBP, EGFR, ETV6, FGFR1, FGFR2, FUS, HMGA2, JAK2, KAT6A, KAT6B, KMT2A, KMT2A-PTD, MECOM, MET, MLLT10, MRTFA, MYBL1, MYH11, NTRK2, NTRK3, NUP214, NUP98, PAX5, PDGFRA, PDGFRB, RARA, RUNX1, TCF3, TFE3, ZNF384

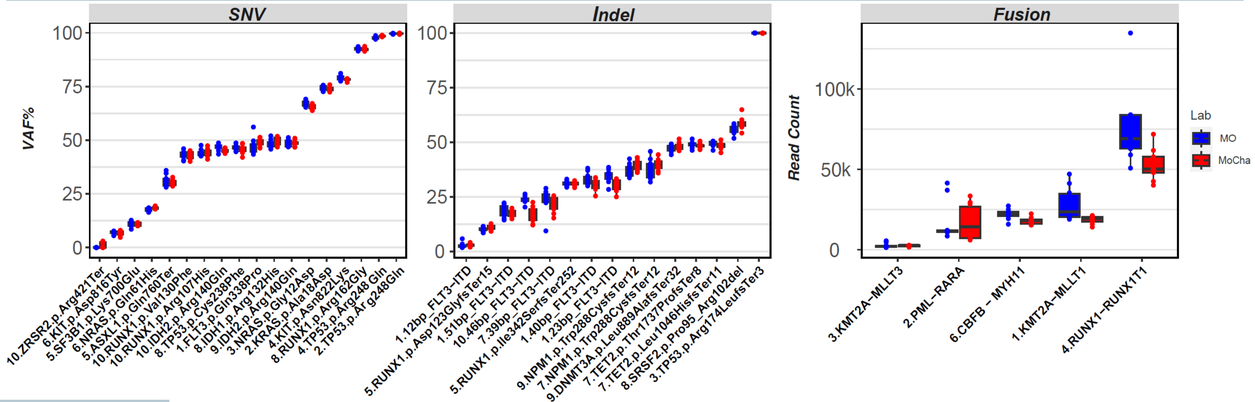
The NMAv2 provides targeted coverage of relevant gene mutations and fusions for accurate classification of myeloid disorders per WHO guidelines, covering 45 DNA genes and 35 fusion genes.

METHODS & RESULTS

Methods

- Samples included 7 myeloid cell lines, 5 normal HapMap cell lines, 57 unique healthy donors, 91 AML/MDS patients and 3 contrived samples
- Nucleic acid extracted using Qiagen AllPrep DNA/RNA Mini kit and diluted to 1.11ng/µl for 27.75ng input onto the Genexus System
- Ion Torrent Genexus Software 6.6.0.3 used with the hg19 genome reference sequence to generate data for single nucleotide variants (SNVs), insertions/deletions (indels), and fusions
- The assay calling thresholds set to VAFs of 2.5% for SNVs, 3% for indels, 1% for FLT3-ITDs, and 100 read counts for fusions (KMT2A-PTD >= 2000 read counts)

Reproducibility Assessment Results



Results of Validation Experiments for the NMAv2

Experiment / Lab	Sensitivity	Specificity	Accuracy	Reproducibility	
				Mean PPA	Mean NPA
MO (FHCC)	98.62%	100%	>99.99%	100%	100%
MoCha (FNLCR)	98.97%	100%	>99%	99%	100%
Acceptance criteria	≥90%	≥99%	≥99%	≥90%	≥90%

Sensitivity

- A total of 76 clinical specimens, 7 cancer cell lines, and 2 contrived sample analyzed
- 291 total variants spanned 54 genes and represented 225 unique variants
- The assay met all sensitivity acceptance criteria for each variant type and overall, with 3 false negatives at MoCha and 4 false negatives at MO, with 1 FN common between both labs
- False negative variants detected below the LoR, between 2.1% and 2.4% VAF

Specificity

- 5 normal HapMap cell lines, 51 healthy donor blood samples, and 6 healthy BMA donor samples analyzed at both labs
- No false positives detected after blacklisting the following 3 variants in earlier feasibility studies: *ASXL* p.G646Wfs*12, *PRPF8* p.R56Efs*28, *RUNX1* p.S445Afs*149

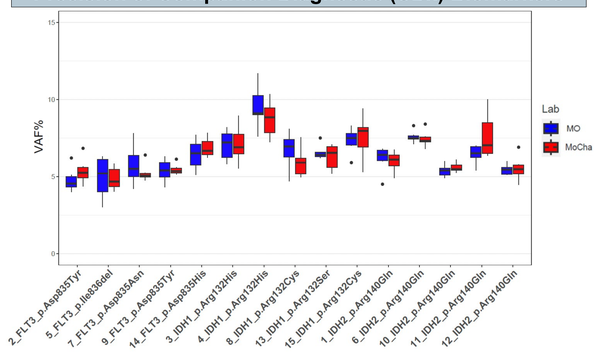
Reproducibility

- Analyzed 9-10 replicates of 10 samples from sensitivity; total of 38 variants expected (18 SNVs, 15 indels, 5 fusions)
- Replicates tested by 2 operators on 2 sequencers over 8 days for 6 reproducibility assessment types at each lab
- From a total of 172 SNVs expected from all replicates, 6 out of 9 replicates of *ZRSR2* p.Arg421Ter detected but not reported above the LoR at MoCha
- Total of 143 indels and 49 fusions expected and successfully reported at each lab
- No false positives detected

Reproducibility Assessment Types

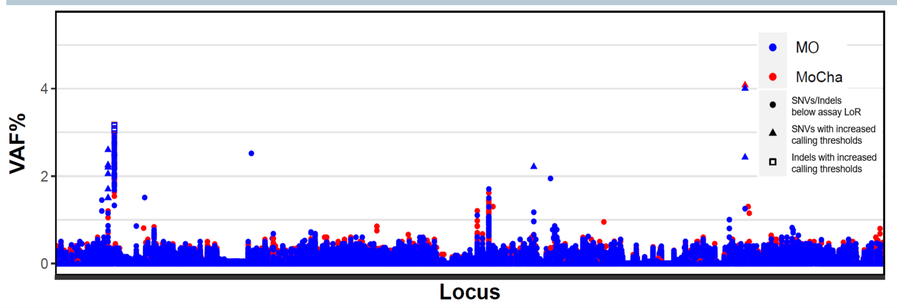
1. Intra-operator: day, instrument, and operator fixed
2. Between run: operator and instrument are fixed
3. Between instrument: day and operator fixed
4. Inter-operator: same day, each operator uses a different instrument
5. Inter-operator: different days, same instrument used by both operators
6. Reproducibility when day, instrument, and operator all differ

Precision of Companion Diagnostic (CDx) Biomarkers



- Evaluated 15 CDx biomarkers near the clinical reporting cutoff (5% VAF)
- Each sample run in 2 replicates per run over 3 days, yielding 6 replicate results

Limit of Blank Assessment Results



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- 57 healthy donor samples and 5 HapMap samples from the specificity set used to assess the noise of the assay
- The background noise for the assay, including CDx biomarkers, was at least **3 standard deviations below the LoR**
- Highest fusion read count observed at 17 reads (data not shown)
- The false positive rate for the qualitative claim was 0% for all variant classes at both the sample and variant levels, as there were no false positives reported

CONCLUSIONS

The NMAv2 has high specificity, sensitivity, accuracy and reproducibility (inter-lab and intra-lab) with sequencing results generated within 48 hours of assay initiation.

The assay is well suited for use to rapidly categorize the genomic alteration of AML and MDS to support evaluation and assignment of those with active myeloid malignancies to treatment sub studies in the NCI myeloMATCH precision medicine initiative.