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RNA Fusions and Their Association with DNA Alterations in Myeloid Neoplasia Patients Identified By a Single Tube Multimodal Comprehensive Genomic Profiling Test

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Background: Recent updates into NCCN professional guidelines included several genomic biomarkers for myeloid disorders. Detecting SNVs, indels, select CNVs and genomic rearrangements in a single comprehensive genomic profiling test is invaluable for clinical care. Moreover, WHO recognizes 23 genomic rearrangements or fusions which define subclasses of AML, MDS/MPN and related neoplasms, and their detection is essential for patient management. Here we present joint prevalence data of gene fusions and other genomic alterations in myeloid disorders in a cohort of 312 patients analyzed by a CLIA grade single-tube NGS assay capable of concurrent analysis of DNA and RNA alterations.

Methods: Total nucleic acid (TNA) from bone marrow or peripheral blood was analyzed by a CLIA grade custom amplicon-based multimodal NGS test reporting DNA mutations in 126 genes and gains/losses in 17 genes by DNA-seq, and RNA fusions from 40 genes by RNA-seq. Libraries were sequenced on a NovaSeq6000 instrument, and fusions were called with in-house developed BI pipeline using the distribution of AI-assisted fusion confidence scores to improve the signal to noise discrimination for fusion calls before validation. Deidentified patient data was used according to an IRB approved protocol.

Results: Analytical validation of RNA fusion calling against FISH and Sanger-seq in 74 hematologic disorder cases demonstrated 96.7% sensitivity and 98.2% specificity with 100% reproducibility. This improved fusion detection module was added to our CLIA validated NGS assay, which at tumor purity of ≥20% detects SNVs, Indels (<81 bp) and CNVs with sensitivities and specificities of 95-100% (Fig. 3). Data from 789 patients serially tested with this assay was used to study the distribution of myeloid fusion events in community cases, which included 312 adult patients with confirmed/suspected myeloid disorders, such as AML, CML, MDS, etc, and 477 lymphoid leukemia cases. 55% of the myeloid disorder patients were male and 45% were female, with a median age of 67.5 (22-87) and 71 (22-89) years, respectively. 27% (84/312) presented a gene fusion, 85% of which (71/84) involved a gene from WHO/NCCN fusion gene recommendations. Prevalence for common fusions were 6.7% for BCR::ABL1; 5.1% for PML::RARA; 2.6% for KMT2A; 1.9% for RUNX1::RUNX1T1; 1.6% CBFB::MYH11; 1.3% for PICALM::MLLT10 and 1 % for NUP98. Other fusions were detected in <1% patients. Fusions of PDGFRA, ETV6, ZNF384, FGFR1 and other genes were also observed. BCR::ABL1 were seen not only in CML patients but also in a patient with AML. 25% (2/8) of KMT2A fusions detected by NGS were confirmed by Sanger-seq but missed by FISH. Interestingly, the prevalence of fusion positive cases in lymphoid leukemia patients tested concurrently was 17% (80/477). Novel fusions were called in ~8% of all patients with high confidence. An interesting case of an AML patient with a potentially oncogenic CCND2::MGP fusion removing CCND2's degradation signal was observed and was validated by Sanger-seq. Finally, we analyzed the relationship between fusions and recurrent mutations. DNA alterations in CALR, EZH2, FLT3, KIT, and ZRSR2 were enriched in fusion positive cases, while alterations in CEBPA, IDH1/2, KMT2A, MPL, NPM1, BCOR, IKZF1, FBXW7 STAG2, CSF3R, PDGFRA, PHF6, PTPN11 were specifically found in fusion negative cases.

Conclusions: A robust low-noise RNA fusion detection coupled with DNA alterations testing for myeloid disorders in a single assay enables to fully molecularly characterize acute myeloid leukemias and other myeloid disorders. Frequencies of well-known fusions in a small community-based cohort were similar to studies performed in academic settings with subsets of gene alterations being mutually exclusive from fusions. Larger studies are needed to confirm those associations.

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- A single-tube comprehensive NGS LDT was used to study the prevalence of myeloid disease related RNA fusions, as well as SNV/indels in a large cohort (789) of hematological malignancy patients
- All well-known recurring myeloid fusions were detected, with frequencies similar to those seen in prior studies in academic settings. Mutual exclusivity/enrichment was determined between the presence of fusions and specific SNV/indels
- The assay showed robust performance in clinical validation against FISH and qPCR as independent orthogonal assays for SNV/indels, structural variants (CNV and splice site) and RNA fusions. A number of new RNA fusions was also detected and validated, some being potentially relevant for clinical care.

Robust fusion detection with the Neo Comprehensive : Myeloid Disorders assay

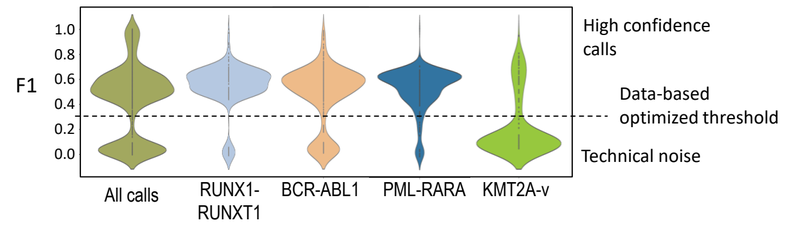
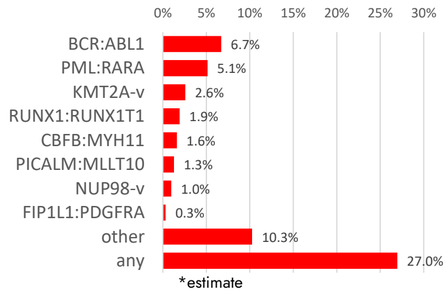


Figure 1. Data from a total of 2628 fusion calls was used to improve discrimination between real calls and technical noise. Violin plots summarizing machine learning F1 scores across all calls, or specific fusions are plotted. Large numbers of calls with F≥0.3 were confirmed positive, while those with F1<0.3 were negative, thus representing assay technical noise.

Myeloid disorder cases - fusion prevalence*



All cases - fusion prevalence

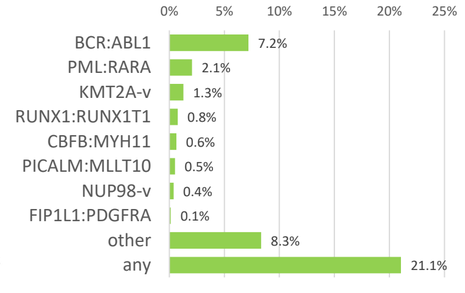
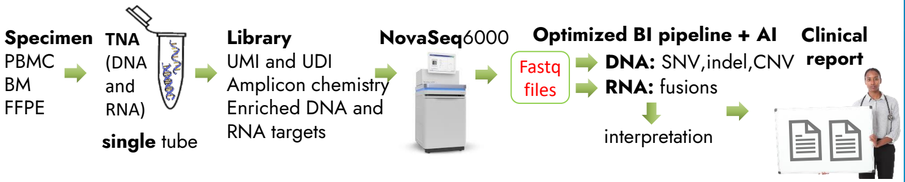


Figure 2. Observed frequencies of myeloid disease RNA fusions in myeloid cases (left) and overall in all hematologic malignancy cases (right). Fusions were observed in 27% of myeloid cases, with BCR::ABL1 and PML::RARA, characteristic of CML and APL, respectively, being the most common, followed by several well-known fusions typical for AML/MDS.

Improved assay: Neo Comprehensive : Myeloid Disorders



Variant Type	Reportable Range (%) (20% abnormal cells)	Sensitivity (%)	Specificity (%)	Reproducibility at the LOD (%)
SNVs	≥3%*	98.31%	99.96%	100%
Indels ≤81bp	≥3%*	98.85%	99.99%	100%
Splice site variants	≥5%*	94.87%	99.96%	100%
CNV (gain/loss)	Gain: ≥trisomy, loss: ≤ monosomy)	95.98%	98.36%	95.83%
RNA fusions	calls: ≥5/5 reads + ML : F1≥0.3	96.70%	98.20%	100%

Figure 3. Top, workflow of the Neo Comprehensive: Myeloid Disorders assay used in this study. Bottom, performance across different assay modalities

Novel putative oncogenic fusion between cyclinD2 and matrix Gla-domain protein

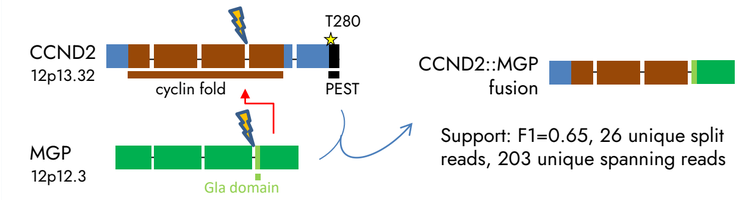


Figure 4. An example of a discovery of a new fusion in a 75 year old male AML patient. The detection was confirmed by qPCR. CCND2-MGP fusion is predicted to be highly expressed and is likely oncogenic.

Relationship between presence of fusions and SNV/indels

	recurrent mutated genes	Fusion containing cases		Fusion negative cases	
Exclusive/more frequent in fusion+ cases	EZH2	3 (5.4%)	p.K568E, p.R298H, p.K740Gfs*30	-	-
	FLT3	7 (12.5%)	ITD (4), p.D835H, p.E611_F612ins19, p.N609_L610ins19	7 (6.8%)	ITD, p.T582_E608dup, p.R961H, p.D839G, p.V852I, p.I867S, p.L601_K602ins16
	ZRSR2	4 (7.1%)	p.E54*, p.R437G, p.Y274Vfs*15, splice c.203+1G>A	1 (1.0%)	p.R169*
	KIT	3 (5.4%)	p.D816Y, p.T417_D419delinsI, p.T417_D419delinsL	1 (1.0%)	p.L18F
	CALR	2 (3.6%)	p.P233L, p.Q365Rfs*50	1 (1.0%)	p.K368del
Exclusive/more frequent in fusion negative cases	IDH1	-	-	8 (7.8%)	p.R132C (2), p.R132H (2), p.W92R, p.R20*, p.K413E, p.R132L
	KMT2A	-	-	6 (5.8%)	p.M1926I (2), p.S215P, p.P562S, p.L126_R127delinsPS, p.F148L
	MPL	-	-	6 (5.8%)	p.W515L (2), p.S228R, p.S505N, p.V501M, p.W515K
	NPM1	-	-	6 (5.8%)	p.W288Cfs*12 (5), p.I269Kfs*7
	BCOR	-	-	5 (4.9%)	p.Q1110H, p.T936N, p.F876Lfs*3, p.E829D, p.G1568D
	IKZF1	-	-	4 (3.9%)	p.Y180C, p.S361A, p.G128R, p.R468G
	FBXW7	-	-	3 (2.9%)	p.I605M, p.S18C, p.P153S
	STAG2	-	-	3 (2.9%)	p.R216*, splice c.462+2_462+6delins13, p.V343*
	CSF3R	-	-	2 (1.9%)	p.W818*, p.T618I
	ETV6	-	-	2 (1.9%)	p.W360R, p.I176Hfs*3
	PDGFRA	-	-	2 (1.9%)	p.V224M, p.P278S
	PHF6	-	-	2 (1.9%)	p.R274*, p.H329R
	PTPN11	-	-	2 (1.9%)	p.D61A, p.A72T
	SH2B3	1 (1.8%)	p.R371K	5 (4.9%)	p.S18Y, p.L347Afs*38, p.S559A, p.R371K, p.R562Q
	DDX41	2 (3.6%)	p.Y340N, p.R525H	10 (9.7%)	p.R525H (3), p.D140Gfs*2 (2), p.S543*, p.M1?, p.Y259C, p.P78Qfs*3, p.R369*
	CEBPA	1 (1.8%)	p.Q83Sfs*77	5 (4.9%)	p.Q207Lfs*113, p.E10K, p.Y67Lfs*41, p.E144G, p.K313dup
	IDH2	2 (3.6%)	p.R140Q, p.A416V	9 (8.7%)	p.R140Q (6), p.I290M, p.V8L, p.R172K
	SRSF2	3 (5.4%)	p.P95H, p.P95R, p.P95L	12 (11.7%)	p.P95H (6), p.P95L (4), p.P95R (2)
	SETBP1	1 (1.8%)	p.D868G	4 (3.9%)	p.T195P, p.R942W, p.D868N, p.Q378R

Patient information

myeloid leukemia/MDS	
total cases*	312
female	45%
age (median)	22-89 (71)
male	55%
age (median)	22-87 (67.5)
Fusions	84 (27%)
BCR::ABL1	21 (6.7%)
PML::RARA	16 (5.1%)
KMT2A	8 (2.6%)
KMT2A::AFF1	5
KMT2A::MLLT4	1
KMT2A::IGH@	1
KMT2A::MLLT1	1
RUNX1::RUNX1T1	6 (1.9%)
CBFB::MYH11	5 (1.6%)
PICALM::MLLT10	4 (1.3%)
NUP98	3 (1%)
NUP98::NSD1	2
NUP98::HOXA9	1
FIP1L1::PDGFRA	1 (0.3%)
TFG::GPR128	4 (1.3%)
CCND2::MGP	1 (0.3%)
CXCR4::RARA	1 (0.3%)
ETV6::APOLD1	1 (0.3%)
other	13 (4.2%)
lymphoid leukemia	
total cases*	477
female	44%
age (median)	4-86 (58)
male	56%
age (median)	3-85 (51)
Fusions	80 (17%)
BCR::ABL1	34 (7.2%)
TFG::GPR128	6 (1.3%)
P2RY8::CRLF2	3 (0.6%)
TCF3::PBX1	3 (0.6%)
other	29 (5.5%)

Figure 5. **Left**, Co-existence or exclusivity of fusions and SNV/indels in myeloid disorder cases. Number of cases (% of all) and SNVs/indels are listed for each gene in fusion positive and negative samples. **Right**, Characteristics of patients used in this study. *Total numbers are extrapolated as the full diagnosis was not available for ~half of the patients

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