Landscape of known and novel myeloid neoplasia fusions identified by a multimodal comprehensive genomic profiling test in 789 patients

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Background: 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. Discerning true fusions from artificial calls in RNAseq-based tests is challenging due to biological and technical noise. We previously developed a method to identify fusion transcripts by a single-tube NGS assay capable of concurrent analysis of DNA and RNA alterations in ALL patients. We expanded the assay with an improved fusion calling algorithm and used it to study the landscape of myeloid RNA fusions in the clinical setting.

Methods: Total nucleic acid (TNA) from bone marrow or peripheral blood was analyzed in our clinical laboratory by a CLIA grade custom amplicon-based multimodal NGS assay, targeting 302 genes by DNA-seq and 185 genes by RNA-seq. Libraries were sequenced on a NovaSeq6000 instrument, and fusions were called from RNA: de-duplicated and error-corrected UMI reads were processed by an in-house developed BI pipeline leveraging machine learning, to assign a final confidence score (F1). Deidentified patient data was used according to an approved IRB.

Results: Distribution of F1 scores was used to improve the discrimination between technical noise and real fusion calls. Analytical validation of RNA fusion calling against FISH and Sanger-seq in 74 hematologic disorder samples demonstrated 98.2% specificity and 96.7% sensitivity. Data from 789 patients was used to study the distribution of myeloid fusion events in community cases. 17% of patients had fusions involving genes from WHO/NCCN recommendations. Frequencies for most common fusions were 7.2% for BCR::ABL1 (56/789), 2.1% for PML::RARA, 1.3% for KMT2A-v, 0.8% for RUNX1::RUNX1T1, 0.6% for CBFB::MYH11 and 0.4% for NUP98. Fusions of PDGFRA, ETV6, ZNF384, FGFR1 and other genes were also observed and BCR::ABL1 fusions were seen not only in CML patients but also in a patient with AML. For KMT2A, 1 of 8 fusions detected by NGS were confirmed by Sanger-seq but missed by FISH, which correlates with higher sensitivity of the NGS assay. Novel fusions were called in ~8% of patients. This included an AML patient with a CCND2::MGP fusion, resulting in cyclin D2 (CCND2), frequently activated by DNA mutations in AML, fused to matrix Gla protein, a highly expressed gene in hematopoietic progenitor cells. The fusion was confirmed by Sanger-seq, and shown to lack exon 5 of CCND2, which contains Thr280, a residue required for ccnd2 degradation. This fusion is thus predicted to generate high cellular levels of oncogenic ccnd2-mgp.

Conclusions: Frequencies of well-known fusions in real world data obtained by a robust low-noise RNA fusion assay were similar to other studies done in academic setting. Reliable detection of bona-fide RNA fusions with this clinical test is invaluable for patient care and novel fusion identification.

- A single-tube comprehensive NGS LDT assay 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 (Figure 2)
- Mutual exclusivity/enrichment was determined between the presence of fusions and specific SNV/indels (Figure 5)
- The assay showed robust performance in clinical validation against FISH and qPCR as independent orthogonal assays for SNV/indels, CNVs and RNA fusions (Figure 3)
- A number of new RNA fusions was also detected and validated, some being potentially relevant for clinical care (Figure 4)

Robust fusion detection with the Neo Comprehensive : Myeloid Disorders assay

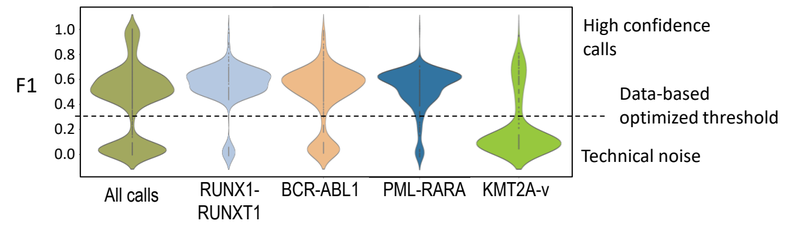
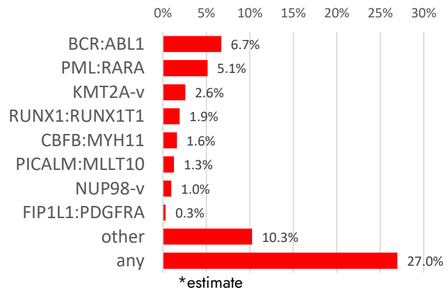


Figure 1. Data from a total of 2628 fusion calls was used to improve discrimination between real calls and technical noise. Violin plots summarizing machine learning F1 scores across all calls, or specific fusions are plotted. Large numbers of calls with F≥0.3 were confirmed positive, while those with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

Myeloid disorder cases - fusion prevalence*



All cases - fusion prevalence

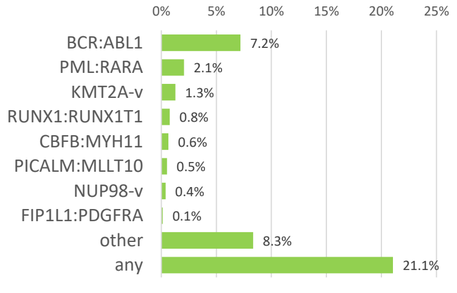
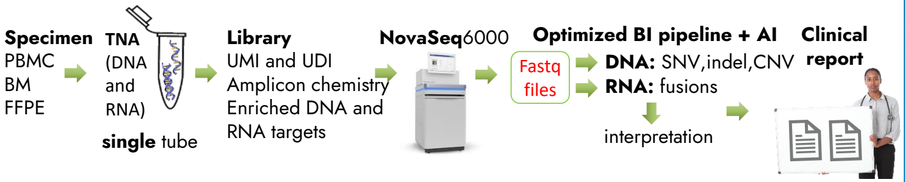


Figure 2. Observed frequencies of myeloid disease RNA fusions in myeloid cases (left) and overall in all hematologic malignancy cases (right). Fusions were observed in 27% of myeloid cases, with BCR::ABL1 and PML::RARA, characteristic of CML and APL, respectively, being the most common, followed by several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

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

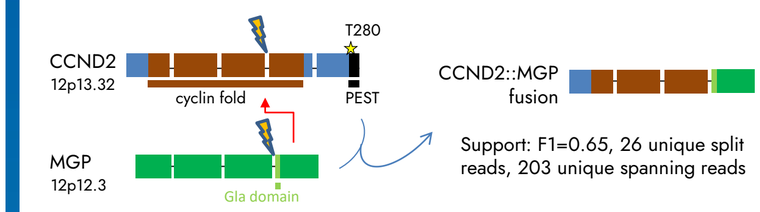

Figure 4. An example of a discovery of a new fusion in a 75 year old male AML patient. The detection was confirmed by qPCR. CCND2-MGP fusion is predicted to be highly expressed and is likely oncogenic.

Relationship between the 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