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Clinical sequencing of more than 2,500 patients with myeloid disorders using a targeted next generation sequencing platform

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I. Introduction

Conditions associated with abnormal myeloid proliferation and/or differentiation can be broadly categorized as myeloid malignancies. This group of disorders is heterogeneous and includes acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and others. These disorders are often driven by somatic mutations in a subset of genes and the detection of these mutations can have diagnostic, prognostic, and therapeutic relevance to the patient.

II. Methods

We designed, validated, and implemented a laboratory developed test (IntelliGEN® Myeloid) based on a next generation sequencing (NGS) panel that targets mutations within 50 genes previously linked to myeloid malignancies. Within these genes, multiple somatic variant classes were called including single nucleotide variants, insertions, and deletions. Whole blood, bone marrow, or cell pellets from 4,277 consecutive patients were submitted for testing during the evaluation period. DNA was extracted from each sample and used as the template for creating targeted NGS libraries (ArcherDx, Boulder, CO) that were subsequently sequenced on Illumina Miseq DNA sequencers (Illumina, San Diego, CA). Results were reviewed, orthogonally confirmed unless previously validated, and reported by clinical laboratory directors. Clinical indication(s) for each sample were identified based upon the information listed on the test requisition form. All results were de-identified prior to inclusion for the analysis presented herein.

III. Figures and Table Legends

Figure 1. Overview of IntelliGEN Myeloid

List of genes included as part of the assay and the association of each gene with three clinical indications: myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), and myeloproliferative neoplasms (MPN).

IntelliGEN® MYELOID PANEL GENES

Gene*	Association		
	MDS	AML	MPN
ABL1[1]		●	●
ASXL1	●	●	●
BCOR	●	●	●
BCORL1		●	
BRAF[2]			●
CALR			●
CBL			●
CDKN2A	●	●	
CEBPA		●	
CSF3R		●	●
CUX1	●	●	
DNMT3A	●	●	●
ETV6	●	●	●
EZH2	●	●	●
FBXW7			
FLT3[3]		●	
GATA1		●	
GATA2	●	●	
IDH1	●	●	●
IDH2[4]	●	●	●

Gene*	Association		
	MDS	AML	MPN
IKZF1		●	●
JAK2	●		●
JAK3		●	
KDM6A	●	●	●
KIT[1]		●	
KMT2A		●	
KRAS[5]		●	
MPL			●
NF1	●	●	
NOTCH1			
NPM1		●	
NRAS	●	●	
PDGFRA[1]		●	
PHF6		●	
PML		●	
PTPN11		●	
RAD21		●	
RUNX1	●	●	●
SETBP1	●	●	●

Gene*	Association		
	MDS	AML	MPN
SF3B1	●	●	●
SMC1A	●	●	
SMC3	●	●	
SRSF2	●		●
STAG2	●	●	
TET2	●	●	●
TP53	●	●	
U2AF1	●	●	
WT1		●	
ZRSR2	●		●

Genes associated with FDA approved therapies

- ● Frequently mutated in MDS
- ● Diagnostic and/or prognostic significance in AML
- ● Diagnostic and/or prognostic significance in MPN

*identification of mutations should always be used within the context of clinical findings and bone marrow evaluation

REFERENCES

1. GLEEVEC® (imatinib mesylate). Novartis Pharmaceuticals Corporation East Hanover, NJ; 2017.
2. ZELBORAF® (vemurafenib). Genentech USA, Inc., A Member of the Roche Group, S. San Francisco, CA: 2017.
3. RYDAPT® (midostaurin). Novartis Pharmaceuticals Corporation, East Hanover, NJ: 2017.
4. IDHIFA® (enasidenib). Celgene Corporation, Summit, NJ: 2017.
5. Vectibix® (panitumumab). Amgen Inc., Thousand Oaks, CA; 2009.

Figure 2. Patient demographic information and analysis

A: Patient demographics for all samples included in this analysis

A total of 4,277 consecutive clinical samples were analyzed and reported. The number (n) and proportion (%) of patients for each factor are listed. All clinical indications were included as noted within the test requisition form.

A

Chacteristics	Results
Total patients, N	4,277
Age, mean (SD)	67.6 (15.3)
Gender, n (%)	
Male	2,436 (57)
Female	1,841 (43)
Specimen Type, n (%)	
Bone Marrow	3,093 (72.3)
Whole Blood	1,172 (27.4)
Other	12 (0.3)
Number of Clinical Indications, mean (SD)	1.4 (0.7)
Select reported clinical indications, n (%)	
Anemia	1,279 (29.9)
Myelodysplastic Syndrome	898 (20.1)
Pancytopenia	680 (15.9)
Thrombocytopenia	597(14.0)
Acute myeloid leukemia	571 (13.4)
Myeloproliferative neoplasms	314 (7.3)
Leukopenia	278 (6.5)
Polycythemia Vera	101 (2.4)

B: Clinically relevant mutations per patient

Histogram describing the number of clinically relevant (Tiers I-III) mutations identified for each of the 4,277 patients. Patients with more than 20 clinically relevant mutations were censored for visualization purposes only.

C: Clinically relevant mutations relative to patient age

The number of clinically relevant (Tiers I-III) mutations identified for each of the 4,277 patients was compared to the age of the patient. There is a statistically significant ($p<2.2e^{-16}$; Pearson's product-moment correlation) relationship between patient age and the number of clinically relevant mutations per patient. The blue number represents the mean number of mutations per patient for that particular age group. Patients with more than 20 clinically relevant mutations were censored for visualization purposes only.

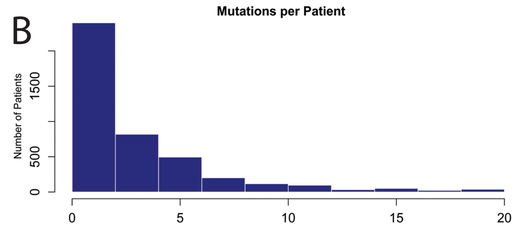


Figure 3. Overview of mutation results for each gene (column) and each patient (row)

Dark blue color represents that a clinically relevant (Tiers I-III) was detected in each gene for that patient. Dark red barplot across the top represents the cumulative number of patients where each particular gene has been mutated. Mutations have been detected in all 50 tested genes within this patient cohort, ranging from 1-935 patients for each gene.

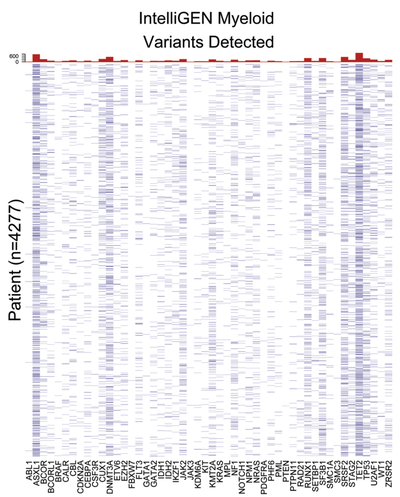
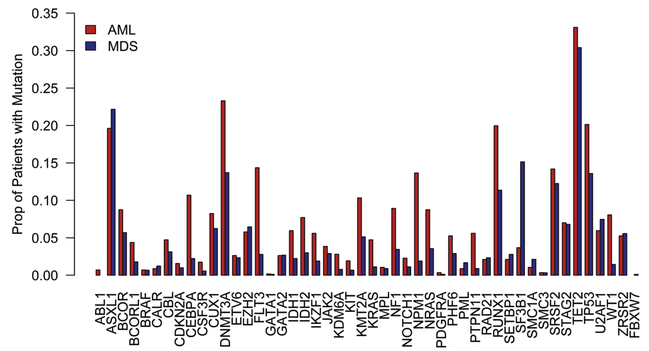


Figure 4. Mutation frequencies by reported indication

Each sample was categorized based upon the clinical indication(s) listed on the test requisition form. The proportion of patients with a mutation in each listed gene is shown for patients listing ML (red) or MDS (blue).



IV. Conclusion

Taken together, these data demonstrate that multi-gene NGS panels can be used to provide physicians that are treating patients with myeloid malignancies with diagnostic, prognostic, and therapeutic information that may help facilitate patient care

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