# Mutational landscape and clinical characterization of over 17000 patient samples with myeloid malignancies using real world data

Grant Hogg[1], Li Cai[2], Heidi M. Hoffmann[3], Kimberly A. Holden[1], Kerry D. Fitzgerald[1], Angela Kenyon[3], Michael Mooney[2], Sabrina Gardner[2], Wenjie Chen[3], Narasimhan Nagan[3], Deborah Boles[2], Scott Parker[2], Tamara J. Richman[3], Stanley Letovsky[3], Henry Dong[4], Steven M. Anderson[5], Marcia Eisenberg[6], Anjen Chenn[2], Taylor J. Jensen[1,2]

[1]Laboratory Corporation of America® Holdings, San Diego, CA; [2]Laboratory Corporation of America® Holdings, Durham, NC; [3]Laboratory Corporation of America® Holdings, Westborough, MA; [4]Laboratory Corporation of America® Holdings, New York, NY; [5]Labcorp Drug Development, Burlington, NC; [6]Laboratory Corporation of America® Holdings, Burlington, NC

## 1. Introduction

- Myeloid neoplasms represent a broad spectrum of hematological disorders.
- Somatic mutation status in key driver genes is important for diagnosis, prognosis and treatment.
- We summarize findings from 17181 clinical samples from 16133 patients analyzed by a next generation sequencing (NGS) laboratory developed test targeting 50 myeloid associated genes.
- Samples were analyzed comprehensively and as part of individual cohorts specific to acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN).

## 2. Methods

- Whole blood or bone marrow samples from patients with cause-for-testing for hematological symptoms were submitted for analysis by a referring clinician.
- DNA was extracted and assayed by a targeted, NGS panel to detect and report single nucleotide variants and small indels within 50 genes associated with myeloid malignancies.
- Sequenced on an Illumina MiSeq or NextSeq (Illumina, San Diego, CA).
- Multiple somatic variant classes were called including single nucleotide variants, insertions, and deletions. Copy number variants in the gene *KMT2A* were also reported.
- Results were reviewed, orthogonally confirmed unless previously validated, and reported by clinical laboratory directors.
- Disease status or symptoms used in this study were taken from test requisitions for each patient.

## 3. Summary

- 17181 patient samples assessed for somatic mutations in 50 genes.
- Disease status used in this study were taken from the test requisitions for each patient.
- 47.3% of patient samples had 2 or more somatic mutations.
- Pair-wise mutation analyses found 21 mutually exclusive pairs including between genes suggesting possible candidates for targeted therapy.
- The clinically favorable co-mutation of *NPM1* with *FLT3* internal tandem duplicate was significantly enriched in the AML population.
- The clinically favorable co-mutation of *NPM1* with *ASXL1* or *RUNX1* was significantly less common than expected in the AML population.
- Co-mutation of *ASXL1* with *RUNX1* significantly enriched in the AML population.
- Individual case studies of patients tested at multiple time-points show evidence of tumor evolution and/or therapeutic intervention.

## 4. Conclusion

Parallel testing of multiple genes in addition to the canonical driver mutations encompasses the mutations contributing to the etiology of myeloid neoplasms. Consistent patterns of mutations are routinely observed that can help the clinician tailor the treatment and chart the progression of myeloid disease for each patient.

## Tables + Figures

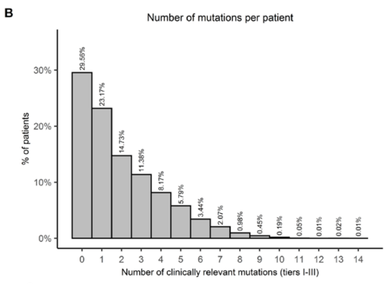
### Figure 1. Patient demographics and clinical mutations detected

A

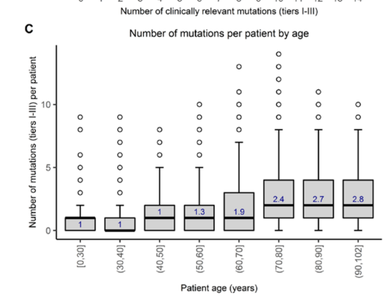
| Characteristics | Results |
|---|---|
| Total patient samples, n | 17181 |
| Total patients | 16133 |
| Age, mean (SD) | 67.17 (15.67) |
| Gender, n (%) | |
| Male | 9525 (55.4) |
| Female | 7656 (44.6) |
| Number of clinical indications, mean (SD) | 1.26 (0.51) |
| Select reported clinical indications[a], n (%) | |
| AML | 2225 (13.0) |
| MDS | 4176 (24.3) |
| MPN | 2018 (11.6) |
| MDS/MPN | 590 (3.43) |
| Symptoms only (including anemia, thrombocytopenia and leukopenia) | 8159 (47.5) |
| Patients with positive NGS Result, n | 12102 (70.4) |
| Patients with negative NGS Result, n | 5079 (29.6) |
| Tier I-III mutations detected, n | 34581 |

a. Patients with multiple clinical indications are counted for all indications on test requisition form.

B

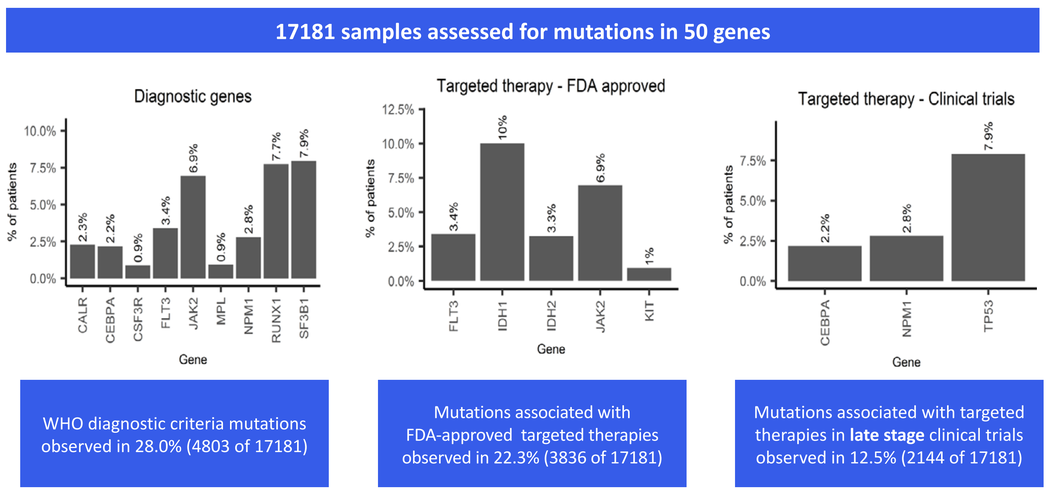



C



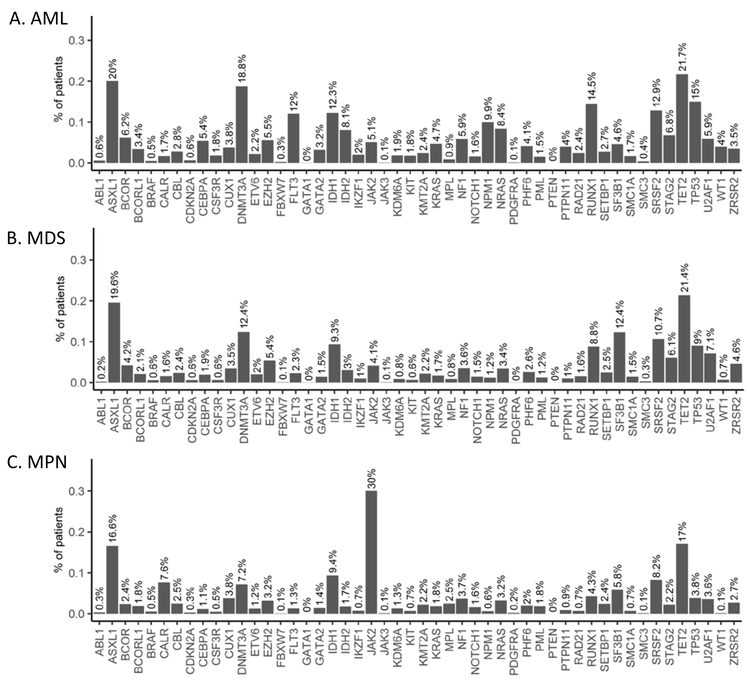

**A) Patient demographics for all samples included in this analysis.** The number (n) and proportion (%) of patients for each factor are listed. All clinical indications were included as noted within the test requisition form. **B) Distribution of the number of clinically relevant mutations detected per clinical sample.** The mean number of mutations per test was 1.98 (95% CI: 1.95 to 2.01) with a range of 0-14. **C) Boxplot showing number of mutations by detected by age of the patient at testing**. The number of clinically relevant (Tiers I-III) mutations identified for each of the 17181 patient samples was compared to the age of the patient at testing and were found to be positively correlated (Spearman's rank correlation coefficient, ρ=0.30, p<0.0001). Text in blue is the mean number of mutations per patient for that particular age group.

### Figure 2. Summary of diagnostic and therapeutic information




Histograms show percentage of 17181 patient samples with a mutation in genes associated with WHO diagnostic criteria, FDA-approved targeted therapies, and for late stage clinical trials of targeted therapies.

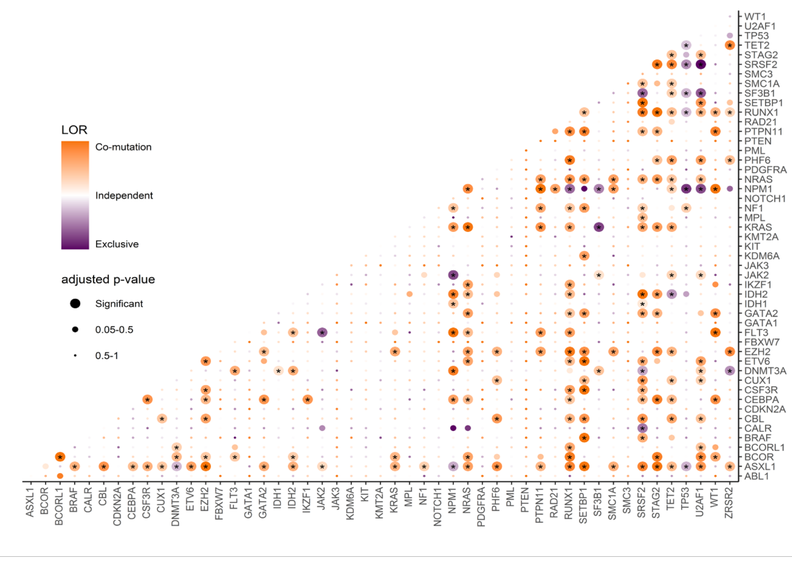
### Figure 3. Mutation frequencies by cause-for-testing




Samples were categorized into cohorts for Acute Myeloid Leukemia (AML), Myelodysplastic Syndrome (MDS) and myeloproliferative neoplasms (MPN) based upon the clinical indication(s) listed on the test requisition form. **A) % of patients with a mutation in each gene for AML cohort.** 2225 (13.0%) samples had an indication for AML. Mutations that are diagnostic for AML were observed as follows: *FLT3* in 12.0% (267 of 2225), *NPM1* in 10.0% (221 of 2225), and *RUNX1* in 14.5% (322 of 2225) of AML samples. FDA approved targeted therapies associated with mutated genes in the panel include midostaurin (*FLT3*), ivosidenib (*IDH1*), and enasidenib (*IDH2*). *IDH1* was observed in 12.3% (273 of 2225) and *IDH2* in 8.0% (180 of 2225) of AML patients. **B) % of patients with a mutation in each gene for MDS cohort.** 4176 (24.3%) samples had an indication for MDS. *SF3B1* mutations which are diagnostic for MDS with ring sideroblasts were found in 12.4% (516 of 4176) of MDS patients. **C) % of patients with a mutation in each gene for MPN cohort.** 2018 (11.6%) of samples had an indication for MPN. The canonical MPN mutations were observed as follows: *JAK2* (606 of 2018, 30.0%), *CALR* (154 of 2018, 7.6%), and *MPL* 2.5% (50 of 2018, 2.5%).

### Figure 4. Pairwise associations of mutual exclusivity and co-mutation among genes in the NGS panel

Associations are shown for the entire sample cohort regardless of indication. Associations are colored by odds ratio showing a gradient from orange (co-mutation) to white (no association) to purple (mutual exclusivity) as shown on the legend on the left. Circular labels are sized by the p-value calculated from the Fisher Exact test with the largest size corresponding to the lowest probability. Associations that are significant after correction for multiple hypotheses are indicated by *. Pair-wise mutation analyses found 21 mutually exclusive pairs including between genes associated with RNA splicing (*SF3B1*, *SRSF2* and *U2AF1*; OR<0.29, p <0.001) suggesting possible candidates for targeted therapy. For gene pairs co-mutated, the largest significant effect size was between the AML driver mutations *FLT3* and *NPM1* (OR=23.4, p <0.001).




### Table 1. *NPM1* and *FLT3* internal tandem duplicate mutation status amongst patients with an indication of AML

| Mutation 1 | Mutation 2 | ELN Prognosis | Samples with both | Mutation 1 Only | Mutation 2 Only | Samples with neither | Odds Ratio | Adjusted p value |
|---|---|---|---|---|---|---|---|---|
| *FLT3*-ITD$^{low}$ | | Intermediate | | | | | | |
| *FLT3*-ITD$^{high}$ | | Adverse | | | | | | |
| *FLT3*-ITD$^{low}$ | *NPM1* | Favorable | 50 | 77 | 171 | 1927 | 7.3 | $1.17 \times 10^{-19}$** |
| *FLT3*-ITD$^{high}$ | *NPM1* | Intermediate | 6 | 11 | 215 | 1993 | 5.1 | 0.013** |
| *FLT3*-ITD | *NPM1* | | 56 | 88 | 165 | 1916 | 7.4 | 5.24 x 10-22** |

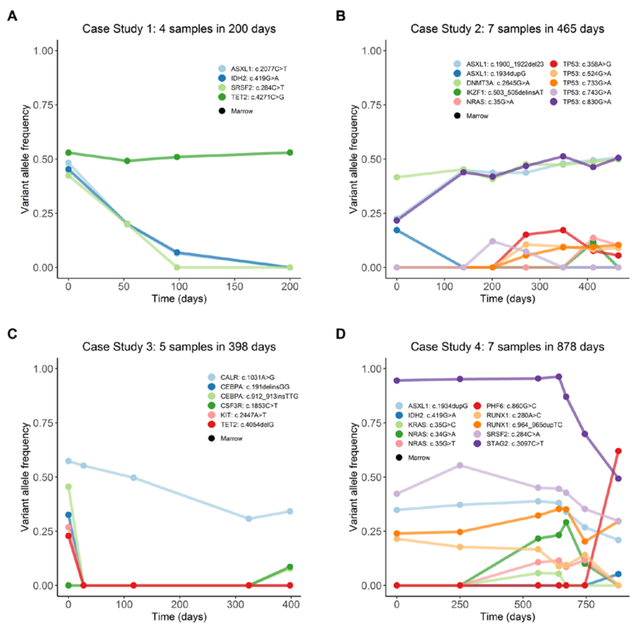
The European Leukemia Net (ELN) assigns a favorable, intermediate, or adverse risk based on a patient's *FLT3* internal tandem duplicate (ITD) and *NPM1* mutation status. The clinically favorable co-mutation of *NPM1* with *FLT3* internal tandem duplicate was significantly enriched in the AML population. *FLT3*-ITD$^{high}$ denotes allele ratio >0.5 while *FLT3*-ITD$^{low}$ denotes allele ratio <= 0.5.
** denotes statistical significance.

### Table 2. *NPM1* and *ASXL1/RUNX1* mutation status amongst patients with an indication of AML

| Mutation 1 | Mutation 2 | ELN Prognosis | Samples with both | Mutation 1 Only | Mutation 2 Only | Samples with neither | Odds Ratio | Adjusted p value |
|---|---|---|---|---|---|---|---|---|
| *ASXL1* | | Adverse | | | | | | |
| *RUNX1* | | Adverse | | | | | | |
| *ASXL1* | *NPM1* | Favorable | 18 | 428 | 203 | 1576 | 0.33 | Mutual exclusive: $3.8 \times 10^{-4}$** |
| *RUNX1* | *NPM1* | Favorable | 8 | 314 | 213 | 1690 | 0.20 | Mutual exclusive: $3.9 \times 10^{-5}$** |
| *ASXL1* | *RUNX1* | | 138 | 308 | 184 | 1595 | 3.88 | Co-mutation: $1.5 \times 10^{-19}$** |

The European Leukemia Net (ELN) assigns an adverse risk based on *ASXL1* or *RUNX1* mutation. Co-mutation of *ASXL1* or *RUNX1* with *NPM1* mutation status gives a favorable outcome however this co-mutation was significantly less common than expected. In contrast, co-mutation of *ASXL1* and *RUNX1* was enriched in AML (OR=3.9, p <0.001). Further investigation is required to determine whether this co-mutation is clinically significant.
** denotes statistical significance.

### Figure 5. Mutation status in patients tested at multiple time-points




During the evaluation period, 1021 patients were tested at multiple time-points. These patients provided an average of 2.24 samples (range 2-7) which were taken an average of 233 days (range 0–1141 days) after the initial sample. 498 (48.8%) patients showed loss or gain of a mutation between sample dates, potentially the result of tumor evolution and/or therapeutic intervention. The following four individual case studies show specific examples of possible changes in tumor evolution and clonal architecture. For each case study, the variant allele frequency (VAF) of the detected mutations is shown on the y-axis and is plotted against time (days) on the x-axis. Each unique variant measured is colored according to the key and is described according to the gene and nucleotide change observed. Dots represent the test dates. Dots with time=0 days represent the first test date. VAF=0 means that a variant is not detected on the particular test date. **(A)** Patient with 4 unique variants detected over 4 test dates spanning 200 days. Changes in tumor fraction may be represented by changes in VAF for mutations in *ASXL1* (c.2077C>T), *IDH2* (c.419G>A), and *SRSF2* (c.284C>A). These 3 mutations are absent by test date four (200 days). **(B)** Patient with 10 unique variants detected over 7 test dates spanning 465 days. Mutations in *ASXL1* (c.1900_1922del23), *DNMT3A* (c.2645G>A), and *TP53* (c.830G>A) are present in all 7 test dates. Over the course of testing, the patient shows gains and losses of mutations in *ASXL1* (c.1934dupG), *IKZF1* (c.503_505delinsAT), *NRAS* (c.35G>A) and *TP53* (c.358A>G, c.524G>A, c.733G>A, and c.743G>A) which may correspond to the evolution of individual sub-clones. **(C)** Patient with 6 unique variants detected over 5 test dates spanning 398 days. Patient shows the loss of a mutation in *CEBPA* (c.912_913insTTG) after 27 days along with three other mutations in *CEBPA* (c.191delinsGG), *KIT* (c.2447A>T), and *TET2* (c.4054delG). The same *CEBPA* (c.912_913insTTG) mutation reemerges at 398 days alongside a mutation in *CSF3R* (c.1853C>T) potentially representing an expansion of a new sub-clone. **(D)** Patient with 10 unique variants detected over 7 test dates spanning 878 days. Four mutations are ever-present over the course of testing: *ASXL1* (c.1934dupG), *RUNX1* (c.964_965dupTC), *SRSF2* (c.284C>A), and *STAG2* (c.3097C>T). Six other mutations are either gained or lost on subsequent dates possibly reflecting gains or losses of individual sub-clones. The differing VAFs observed for mutations detected on the same test date suggest a complex clonal architecture for this patient.

Correspondence: Grant Hogg hoggg@labcorp.com

*CGC 13th Annual Meeting July 31 – August 3, 2022*