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Genetic Landscape of Acute Myeloid Leukemia Interrogated by Next-generation Sequencing: A Large Cancer Center Experience

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Abstract. *Background/Aim: Acute myeloid leukemia (AML) represents a heterogeneous disease with varying morphologic, immunophenotypic, and genetic features, along with varying patient outcomes. The genomic tractability of AML makes it amenable for targeted next-generation sequencing (NGS) testing clinically. Materials and Methods: One hundred eights-seven unique patients with a diagnosis of acute myeloid leukemia between May 2011 and Oct 2014 and with mutational analysis by NGS were included in this study. The distribution of gene mutations was investigated in different subcategories of AML. Results: Most patients in this study (n=182) received Genoptix testing (either 5-gene panel or 21-gene panel). In 130/187 (70%) cases, there was an average of 2.3 mutations per case (range=0-7 mutations). We specifically mention mutations in 32 genes, their significance and co-occurrence as detected in different types of AML. Conclusion: The genetic heterogeneity of AML signifies the importance of taking a personalized-medicine approach to the management of patients with AML.*

Acute myeloid leukemia (AML) is a deadly cancer with a 5-year relative survival of approximately 25%. However, at both the biological and the clinical level, it represents a heterogeneous disease with varying morphological, immunophenotypic, and genetic features, along with varying patient outcomes (1, 2).

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Molecular testing plays a major role in AML and is routinely performed as a part of the diagnostic work-up. For example, cytogenetic testing is necessary for disease subclassification with certain aberrations being definitional in AML with recurrent cytogenetic abnormalities. Furthermore, the detection of select cytogenetic markers serves as the strongest predictive marker for determining prognostic subgroups in AML (3, 4). In the most recent iteration of the WHO classification of hematopoietic tumors in 2008, provisional categories for AML with certain gene mutations (*NPM1* and *CEPBA*) were also introduced. Furthermore, the 2016 revision of the WHO classification consisted of an additional section on myeloid neoplasms with germline predisposition, identifying germline mutations in *CEBPA, DDX41, RUNX1, ANKRD26, ETV6*, or *GATA2* (5). In clinical practice, *FLT3, NPM1* and *CEBPA* are often assayed given their role in stratification of cases of AML with normal cytogenetics (3). More recently, several other clinically-significant genes have been identified in AML (6). In fact, comprehensive sequencing of *de novo* AML has identified >2,300 mutations. Thankfully, in any given tumor, the number of mutations is on average less than 15 and overall only around 20 genes are significantly mutated in AML (7). In fact, the AML genome is one of the simplest cancer genomes.

Therefore, the genomic tractability of AML makes it amenable for targeted next-generation sequencing (NGS) testing clinically. Understanding of the frequencies of key AML-related gene mutations across different diagnostic subcategories is important because it can facilitate selection of targeted therapies, help us understand potential pathways or resistance mechanism, and serve as a scaffold to discover relevant clinical correlations as our mutation database is populated. At our high-volume cancer center which is home to many refractory AML patients, we instituted a policy of

routinely performing next generation sequencing testing on all new cases of AML in order to provide more refined predictive and prognostic information on our patients to guide therapy and clinical decision making. Depending on the clinical need, limited (5 genes) to extensive (>400 genes) NGS gene panels were utilized. Thus, we summarize the data collected from NGS reports on 187 AML patients.

Materials and Methods

All 187 unique patients with a diagnosis of acute myeloid leukemia between May 2011 and Oct 2014 and with mutational analysis by NGS were included in this study. The clinical history and pathologic diagnoses were retrieved from the PowerChart electronic medical records at the Moffitt Cancer Center (MCC). Pathologic diagnoses were reviewed and verified by slide review as necessary.

Next Generation Sequencing: Mutational analysis by NextGen Sequencing was performed by Genoptix, using a 5-panel gene panel initially and later, a 21-gene panel. The minimal depth of coverage was 500x. The genes tested by Genoptix panels include *ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1 IDH2, JAK2, KIT, MPL, NPM1, NRAS, PHF6, RUNX1, SF3B1, SRSF2, SETBP1, TET2, TP53, U2AF1, and ZRSR2*. *FLT-3, CALR*, and *CEBPA* mutations were ordered separately for some patients and identified by alternative molecular testing methods. Those data are also included. Some cases were sent for the more extensive FoundationOne panel. The 436 genes tested by this panel are listed in Table I.

The percentage of patients with a mutation for each gene was calculated by dividing the number of patients who were positive for the mutation for each gene by the total number of patients who tested for that gene by NGS sequencing. This was done to account and compensate for the different genes tested in the different panels performed on patients. The mutation results and the diagnoses were analyzed to investigate the distribution of gene mutations in different subcategories of AML.

Results

A total of 187 unique AML patients were tested for gene mutations. Most patients in this study (n=182) received Genoptix testing (either 5-gene panel or 21-gene panel). A minority (n=8) patients were tested by FoundationOne panel, and 3 patients were tested on both platforms.

The most common type of AML was *de novo* AML with myelodysplasia related changes (MRC) (n=51), followed by secondary AML-MRC such as AML arising from a prior Myelodysplastic Syndrome (MDS) or MDS/Myeloproliferative Neoplasm (MDS/MPN) (n=29). Overall, mutations in 32 genes were detected: *AR, ARID2, ASXL1, CBL, CD36, CPS1, DNMT3A, ETV6, EZH2, FLT3, IDH1, IDH2, IKZF1, JAK2, KDR, KIT, KRAS, LRP1B, NF1, NPM1, NRAS, PDGFRB, PHF6, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1*, and *ZRSR2*. When compared to the Genoptix 5 or 21 gene panel, testing with FoundationOne panel resulted in the detection of 11 additional mutated genes, namely: *AR, ARID2, CD36, CPS1, IKZF1, KDR, KRAS, LRP1B, NPM1, PDGFRB*, and *WT1*. For cases which had both panels performed (n=3), FoundationOne testing yielded detection of 4 additional mutated genes, namely: *KDR, PDGFB, NRAS, IKZF1*. The overall data are summarized in Table I and graphically represented in Figure 1.

At least one non-synonymous gene mutation was detected. In 130/187 (70%) cases, there was an average of 2.3 mutations per case (range=0-7 mutations). The highest number of non-synonymous mutations in any case was from a case of chronic myelogenous leukemia (CML) that transformed to AML (7 mutations). In the AML subgroup, secondary AML-MRC showed the highest mutation rate (82.8%), followed by AML M6 (77.7%), M5 (75%), M4 (68.8%), *de novo* AML-MRC (64.7%), and M2 (64.3%). AML with inv(16) or t(16;16) showed the lowest mutation rate (25%). Most cases lacking any detectable mutation were from the AML not otherwise specified (NOS) category and *de novo* AML-MRC subgroups (49% of negative cases).

ASXL1 had the highest mutation rate (20.7%) in the entire cohort, followed by *TET2* (15.3%), *RUNX1* (15.2%), *DNMT3A* (14.8%), and *TP53* (14.4%), *IDH2* (12%), *NRAS* (11.9%), *FLT3* (11%), and *NPM1* (11%). The remaining genes were mutated at <10% frequency. No *MPL* mutation was detected in the 119 samples tested. In terms of which diseases were more likely to harbor certain gene mutations, *ASXL1* mutations occurred most frequently in AML arising from CML (75%) or chronic myelomonocytic leukemia (CMML) (60%). *DNMT3A* mutations appeared to be common in M5 (33.3%). *FLT-3* mutation appeared to be more frequent in M4 and M1 subtypes and was not seen in secondary AML-MRC. *IDH1* mutations are more common in M1 (19%) than other subcategories of AML, while *IDH2* was more common in M2 (23.1%). *NPM1* mutation was most common in M5 (25%) and M1 (23.8%). *NRAS* mutation was most commonly seen in secondary AML-MRC (20%). *RUNX1* mutation was most commonly seen in AML arising from prior CMML (75%). *SRSF2* was most common in M6 (33.3%). *TET2* mutations were most common in AML from CMML (60%). *TP53* was most common in M6 (42.9%).

Secondary AML-MRC and *de novo* AML-MRC showed different mutational profiles. The most frequently mutated genes in secondary AML-MRC were: *ASXL1* (34.5%), *TET2* (32%), *NRAS/SETBP1/TP53* (20% each), *DNMT3A* (16%), *IDH2* (12%), *EZH2* (10%). On the other hand, the most frequently mutated genes in *de novo* AML-MRC with MDS were: *TP53* (27%), *IDH2* (14.6%), *NPM1* (12.8%), *DNMT3A, TET2* (12.5%), FLT-3 (11.8%), RUNX1 (10.8%). Of these genes, the mutation rate of *ASXL1* was significantly higher in secondary AML-MRC than *de novo* AML-MRC (p=0.0051). The mutation rate of *SETBP1* was also significantly higher in secondary AML-MRC (20%) than in *de novo* AML-MRC (0%) (p=0.0247). The other mutation rates for other genes were not statistically significantly

Table I. *Summary of genes mutated in AML by AML subcategory.*

	AR	*ARID2*	*ASXL1*	*CBL*	*CD36*	*CPS1*	*DNMT3A*	*ETV6*	*EZH2*	*FLT3*	*IDH1*	*IDH2*	*IKZF1*	*JAK2*	*KDR*	*KIT*	*KRAS*
ACUTE UND LEUK							1										
AML, not classified			1								1	1					
AML FROM CML			3	1				1		1							
AML FROM CMML			3				1						1				
AML FROM ET											1			1			
AML FROM MDS			10		1		4	1	2		1	3					1
AML FROM MPN							1							1			
AML WITH INV(16)																1	
AML WITH INV(3)			1														
AML WITH MDS			4				6	1		2	2	7		1		1	
AML WITH T(9;22)																	
M0																	
M1			3				4	1	1	2	4	3					
M2			4	1			2	1			1	3				1	
M3			1														
M4			5			1	3			2	1	3			1	1	
M5	1	1	1				4	1		1	1						
M6			2					1	1			1					
Total	1	1	38	2	1	1	26	7	4	8	12	21	1	3	1	4	1

	LRP1B	*NF1*	*NPM1*	*NRAS*	*PDGFRB*	*PHF6*	*RUNX1*	*SETBP1*	*SF3B1*	*SRSF2*	*TET2*	*TP53*	*U2AF1*	*WT1*	*ZRSR2*	*Total*
ACUTE UND LEUK											1					2
AML, not classified												1				4
AML FROM CML						2	1	1		1	1	1	1			14
AML FROM CMML				1	1	1	3	1	1	1	3					17
AML FROM ET																2
AML FROM MDS		1	2	3		1	5	3		1	8	4	1		1	53
AML FROM MPN						1										3
AML WITH INV(16)				1												2
AML WITH INV(3)				1									1			3
AML WITH MDS			6	3		2	4		1	2	6	10	1			59
AML WITH T(9;22)														1		1
M0													1			1
M1			5	1			2			2	3		1			32
M2						1	1		1		2					18
M3																1
M4	1		3	2		1	2			1			1			28
M5			3	2			1			1	3		1		1	22
M6							1			2		3				11
Total	1	1	19	14	1	9	20	5	3	11	27	19	8	1	2	

Acute UND Leuk: Acute undifferentiated leukemia; AML, not classified: AML not otherwise classified; CML: chronic myeloid leukemia; CMML: Chronic myelomonocytic leukemia; ET: essential thrombocythemia; MDS: myelodysplastic syndrome; MPN: AML from myeloproliferative neoplasms; INV(16): inversion in chromosome 16; INV(3): inversion in chromosome 3; T(9;22): translocation between chromosomes 9 and 22; M0: AML with minimal differentiation; M1: AML without maturation; M2: AML with maturation; M3: acute promyelocytic leukemia; M4: acute myelomonocytic leukemia; M5: acute monocytic leukemia; M6: acute erythroid leukemia.

different between secondary AML-MRC and *de novo* AML-MRC in this limited study.

The most commonly co-mutated genes in AML were ASXL1 with *RUNX1* (5.3% co-occurrence), *TET2* (5.1% co-occurrence), or *NRAS* (5.1% co-occurrence). *DNMT3A* was most commonly co-mutated with *NPM1* (4.6% co-occurrence), *IDH2* (4% co-occurrence), or *TET2* (3.4% co-occurrence). Co-occurrence was not found to be specific for or significantly enriched in any AML subcategory.

We also noted that some genes never co-occurred (Table II). For example, 176 patients were tested for both *IDH2* and *TET2* mutations, in which 21 patients were found to be positive for *IDH2* mutations and 27 patients were found to be positive for *TET2* mutations, but none of them were found to

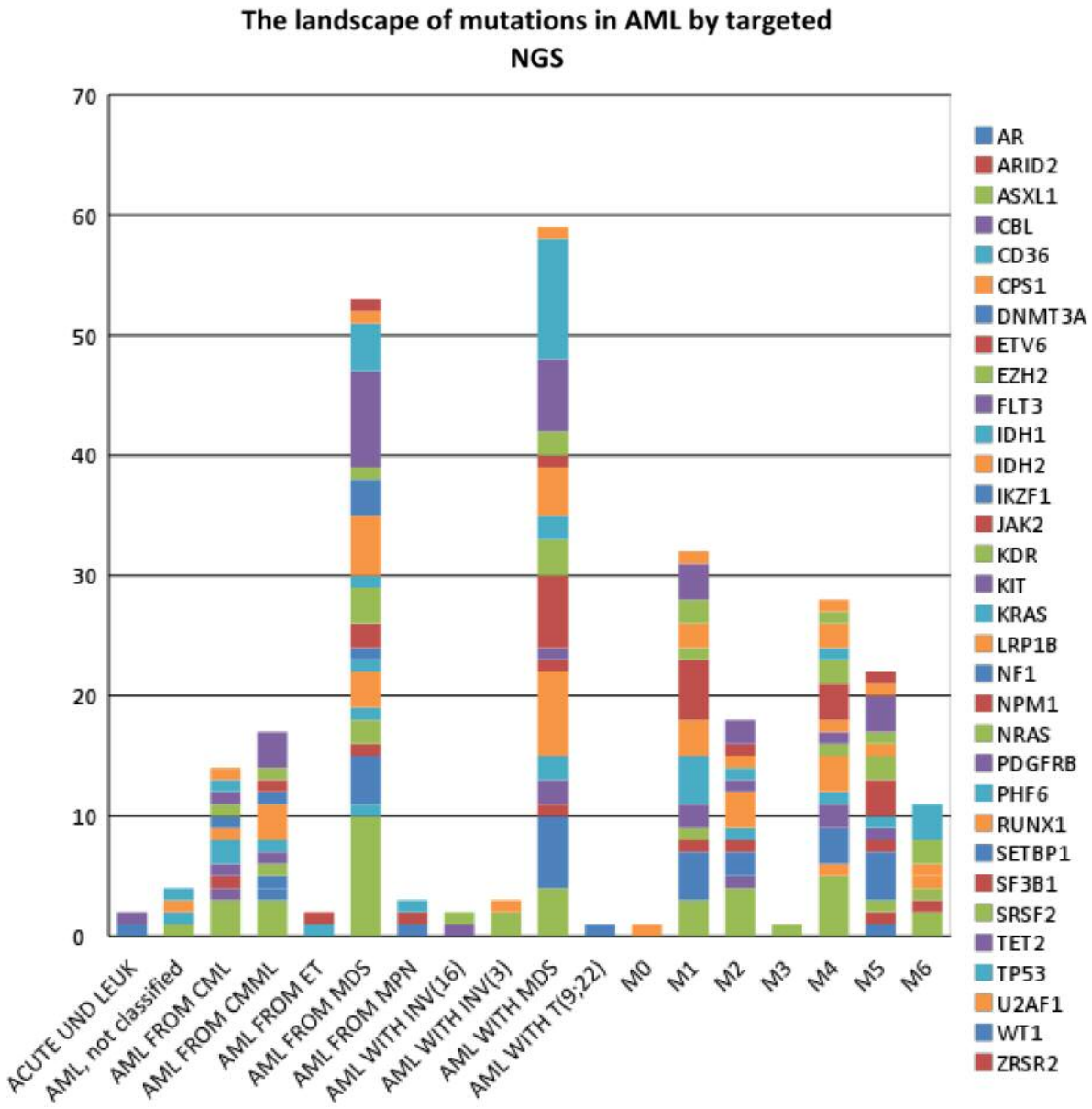

Figure 1. *The landscape of non-synonymous mutations in AML by AML subcategory.*

be positive for both. Therefore, *IDH2* may be potentially mutually exclusive of *TET2* and *TP53*. *TP53* may be potentially mutually exclusive of *IDH2, NRAS, NPM1*, and *SRSF2*. Mutations in *NPM1* and *RUNX1*, as well as *DNMT3A* and *SRSF2* were also potentially mutually exclusive.

Discussion

In our study, 70% of AML patients were found to have at least one mutation detected by targeted next-generation sequencing for genes previously described in hematopoietic malignancies or cancer in general. This figure is similar to what is previously reported in the literature for targeted NGS sequencing in AML (8). This high incidence of mutation detection further justifies the performance of NGS testing on AML cases as it can yield, at high frequency, genetic information that may be clinically actionable. For example, *ASXL1* mutations were detected in approximately 1 out of every 5 cases. In MDS and AML, *ASXL1* variants have been associated with worse prognosis (9, 10). In CMML, *ASXL1*

Table II. *Co-occurrence and mutual exclusivity amongst gene mutations in AML.*

	RUNX1	TET2	NRAS	SRSF2	NPM1	TP53	IDH2	FLT3	SETBP1	U2AF1	PHF6	DMNT3A	ETV6	EZH2	CBL	IDH1
ASXL1	7	9	6	6		4	4	2	3	3	4	4	3	3		2
DNMT3A	3	6		0	8		7	2			2					
FLT3					3		0									2
SRSF2	3	4							3							
RUNX1	3	4			0				2	2						
IDH1				2	5											
ETV6		2	2	3											2	
NRAS	3	2		2	2	0			2	2		2				
TET2					3	2			3					2	2	
IDH2			2		4	0					3					
ZRSR2	2	2														
TP53					0											

variants are associated with poorer survival and transformation to AML (11). ASXL1 variants have also been reported in CML associated with accelerated or blast phase (12). The maximum number of genes mutated in one patient was seven, which occurred in one patient with blast phase CML. This is congruent with our current understanding of leukemogenesis which posits the sequential acquisition of driver mutations.

ASXL1 was found to have the highest mutation rate in AML patients, followed by *TET2, RUNX1, DNMT3A,* and *TP53*. Mutations of *ZRSR2, CBL, KIT, JAK2, SF3B1, EZH2, SETBP1* were much less common. *MPL* mutation was not found in any of the AML patients tested. We detected *DNMT3A* mutations at a frequency of 15% which is similar to that reported by Thol *et al*. in 489 AMLs patients <60 years old (13). However other groups have reported frequencies ranging from 19% -36% (14). We detected *IDH2* and *IDH1* mutations at 12% and 7% frequency, respectively. *IDH* mutations have been reported at an incidence of 6%-16% for *IDH1* and 8-19% for *IDH2* (15), 25924101. *TET2* mutations were detected at 15% and in the literature the mutation rate ranges from 13.2%-34% (16, 17). ASXL1 mutations have been identified at 5-17% in the literature in sequencing studies of large cohorts (9, 18, 19). Our detection rate was slightly higher at 21%. This may be due to the disproportionate number of AML cases associated with MDS in our cohort or because we have more refractory AML patients in our cancer institute. It has been previously reported that *ASXL1* mutations are more common in intermediate-risk karyotype AML as compared to copy number-neutral AML (19). Our data confirms the inverse relationship of *ASXL1* mutations (less common in M5 and M1) with *FLT3* (most common in M4 and M1) and/or *NPM1* (most common in M5 and M1) mutations.

Interestingly, patients with AML-MRC that evolved from MDS showed a different mutational profile compared to *de novo* AML-MRC, with mutations most often involving *ASXL1* and *TET2* as opposed to *TP53* and *IDH2*. This may indicate that either mutation in *ASXL1* or mutation in *IDH2* are sufficient to dysregulate DNA methylation patterns in AML pathogenesis (20). Furthermore, secondary AML-MRC had a somewhat higher mutation rate (82.8%) than *de novo* AML-MRC (64.7%). Overall, the data suggest possibly different leukemogenic pathways for these two diseases. The higher incidence of genetic mutations in MDS-related AML *versus* those with AML with recurrent genetic abnormalities is in line with previously reported findings (8).

We also found that some genes were more likely to be co-mutated; however, given these combinations were not specific to any subcategory of AML, this may simply be due to the higher incidence of these genes being mutated in AML. On the other hand, some genes appear to be potentially mutually exclusive (*e.g*., *NPM1* and *RUNX1; DNMT3A* and *SRSF2*). Mutual exclusivity and co-occurrence for gene mutation in AML has previously been analyzed using Dendrix++. The previously reported exclusivity for *NMP1* with *TP53, NPM1* with *RUNX1* was corroborated in our study (7).

In summary, clinical NGS sequencing frequently detects mutations in genes associated with myeloid disease (~70%). Importantly, different gene mutations occur at different frequencies across different subcategories of AML underscoring the genetic heterogeneity of AML and the importance of taking a personalized-medicine approach to the management of patients with AML.

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