Genetic Heterogeneity and Stratification of AML Samples with *NPM1* Mutation Detected by the MyAML® NGS Test

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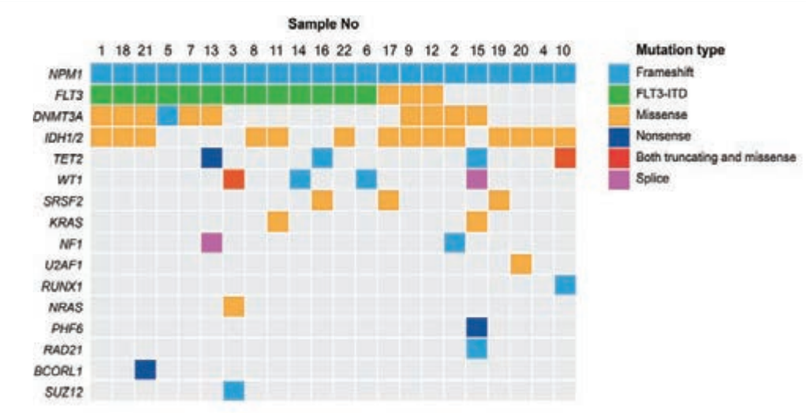
Abstract

Introduction: Molecular abnormalities in multiple genes cause acute myeloid leukemia (AML), a clinically and genetically heterogeneous disease. A recent study has shown that up to one-third of AML patients would have had their treatment altered using a knowledge bank of matched genomic – clinical data, as compared to the treatment they received using current practice recommendations[1]. With a frequency of ~30%, nucleophosmin (*NPM1)*-mutated AML is the largest genomic subgroup in this disease[2,3]. The key purpose of this study is characterization of the genetic heterogeneity and stratification of *NPM1*-mutated AML samples.

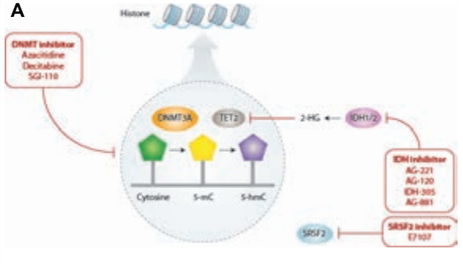
Methods: Using the MyAML® next generation sequencing (NGS) panel targeting 194 genes, we analyzed the genetic profile of 22 AML samples with driver mutations in the *NPM1* gene. The variants were annotated and interpreted following the Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, College of American Pathologists, and American College of Medical Genetics and Genomics .

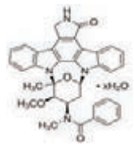
Results: In all cases the *NPM1* mutations co-occurred with mutations in the *FLT3* tyrosine kinase, *DNMT3A* methyltransferase, *IDH1* or *IDH2* isocitrate dehydrogenase genes. All *FLT3* mutations were located in the protein kinase catalytic domain and most mutations were internal tandem duplications (ITD). Four samples included more than one *FLT3*-ITD. Most of the *DNMT3A* mutations were detected at the mutational hotspot Arg882; all *IDH1* mutations were at the mutational hotspot Arg132; most *IDH2* mutations were at mutational hotspot Arg140, and all *SRSF2* mutations were found at mutational hotspot Pro95. Mutations in *IDH2* were more frequent than in *IDH1*, and mutations in these two genes did not co-occur in the same sample. *FLT3*-ITDs had the largest variability in allele frequency, when compared to mutations in *DNMT3A* and *IDH1/2*. The *NPM1*^{mut}/*FLT3*^{ITD}/*DNMT3A*^{mut} genotype, associated with poor prognosis in AML patients, was observed in 6 samples (~27%), close to a previously reported frequency[3]. All samples included mutations in the epigenetic modifiers *DNMT3A*, *IDH1/2*, *TET2* and *WT1*, which are involved in the DNA methylation/hydroxymethylation pathway[6].

Mutational Profiles of AML Samples



Combinational Therapies: Commercial and Developmental Drugs

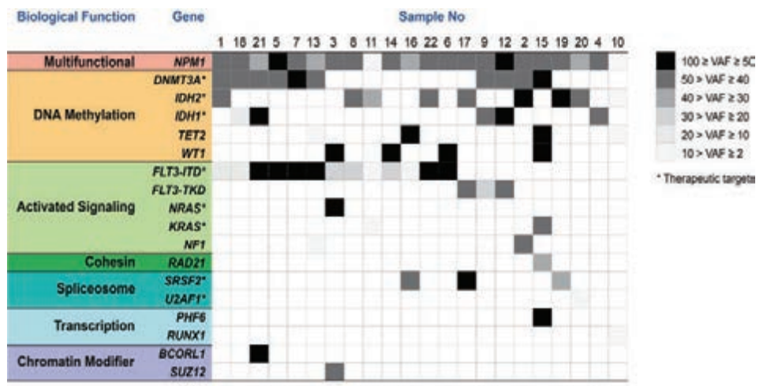




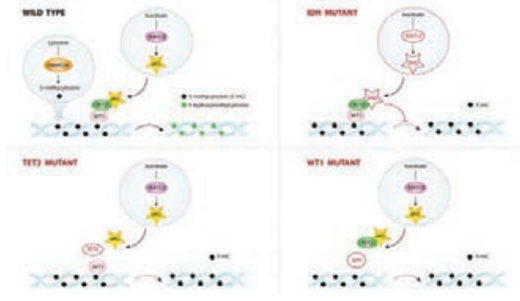
A. New agents targeting epigenetic processes.[13].

B. Rydapt (midostaurin) is used for treatment of AML patients who are *FLT3* mutation-positive.

Variant Allele Frequencies



Effect of Mutations in Mediators of the DNA Methylation Pathway



IDH mutations lead to production of 2-hydroxyglutarate (2HG), which inhibits TET2. TET2 mutations disrupt interaction with WT1. WT1 mutations cause failure to properly direct TET2 to target sites, either by disruption of the interaction itself or of DNA binding. aKG, alpha ketoglutarate[6].

Risk Stratification of AML Samples Based on Genetics

No	Genes				Guidelines		Scientific Publications (examples)		
	NPM1	FLT3	DNMT3A	TET2	NCCN[4]	ELN[5]	NPM1/FLT3/DNMT3A[3]	DNMT3A/FLT3[14]	DNMT3A/TET2[14b]
1	A					favorable	worse OS	worse DFS	
18	A					favorable	worse OS	worse DFS	
21	D					intermediate	worse OS	worse DFS	
5	A					intermediate	worse OS	worse DFS	
7	A					intermediate	worse OS	worse DFS	
13	A					intermediate	worse OS	worse DFS	worse DFS
3	A					favorable	better OS		better DFS
8	A					favorable	better OS		better DFS
11	A					favorable	better OS		better DFS
14	A					favorable	better OS		better DFS
16	A					favorable	better OS		
22	1					intermediate	better OS		better DFS
6	A					intermediate	better OS		better DFS
17	A				favorable				better DFS
9	A				favorable			worse DFS	
12	A				favorable			worse DFS	
2	A					favorable			
15	A				favorable	favorable			worse DFS
19	A				favorable	favorable		better DFS	better DFS
20	A				favorable	favorable		better DFS	better DFS
4	A				favorable	favorable		better DFS	better DFS
10	A				favorable			better DFS	

ELN Recommendations for AML Treatment Based on Genetics

European LeukemiaNet - Conventional Care Regimens[5]	
Consolidation therapy‡,§	
Younger patients (18-60/65 y)	
Favorable-risk genetics	2-4 cycles of IDAC (1000-1500 mg/m^2 IV over 3 h q12h, d1-3; or 1000-1500 mg/m^2 IV over 3 h d1-5 or 6)
Intermediate-risk genetics	Allogeneic HCT from matched-related or unrelated donor
	2-4 cycles of IDAC (1000-1500 mg/m^2 IV over 3 h q12h, d1-3; or 1000-1500 mg/m^2 IV over 3 h d1-5 or 6), or
	High-dose therapy and autologous HCT
Adverse-risk genetics	Allogeneic HCT from matched-related or unrelated donor
Older patients (> 60/65 y)	
Favorable-risk genetics	2-3 cycles of IDAC (500-1000 mg/m^2 IV over 3 h q12h, d1-3; or 500-1000 mg/m^2 IV over 3 h d1-5 or 6)
Intermediate/adverse-risk genetics	
	No established value of intensive consolidation therapy; consider allogeneic HCT in patients with low HCT-Comorbidity Index, or investigational therapy
Patients considered not candidates for intensive chemotherapy	
Low-dose cytarabine**	Low-dose cytarabine (20 mg q12h, SC, d1-10, q4 wk; until progression); not recommended in patients with adverse-risk genetics

‡ Patients, at least those aged 18 to 60 y, with newly diagnosed AML and activating *FLT3* mutations may be considered to receive additional therapy with midostaurin (administered after the chemotherapy). § Results from assessment of MRD should be taken into account for selecting consolidation therapy. ** In some countries used in a dosage of 20 mg/m^2 SC once daily. HCT, hematopoietic cell transplantation

Conclusions

• The MyAML® NGS test allows a comprehensive, rapid, and cost-effective molecular profiling of AML samples.

• We confirmed the previously reported occurrences and statistically significant associations of driver mutations in AML samples[2,3,8,11]. *NPM1* mutations associate with mutations in the mediators of the DNA methylation/hydroxymethylation pathway and *FLT3*. Aberrant DNA methylation is a crucial process during tumorigenesis.

• The complexity of molecular interactions and their role in AML is a major challenge for clinical applications of genetic data. The classification "AML with *NPM1* mutation" only partially contributes to the risk stratification, based on the 2017 Recommendations by NCCN[4] and ELN[5], as well as on literature analysis. This classification does not inform about currently available treatments and clinical trials, which depend on the mutational profile of the AML sample obtained from sequencing of multiple genes.

• Therapies that combine conventional treatment and drugs targeting driver mutations are a promising strategy for improving the disease outcome. A combination of DNA methyltransferase and *FLT3* inhibitors have already been shown to be effective in AML patients[9].

• *NPM1*-positive minimal residual disease (MRD) has recently emerged as a sole prognostic factor for relapse, regardless of the presence of other gene alterations[8]. MRD monitoring allows precise and sensitive tracking of leukemia clones in order to apply personalized medicine[7]. Patients whose leukemia evolves from a pre-leukemic clone (e.g. *DNMT3A*, *TET2* mutations)[10] may require therapy that exceeds standard chemotherapy. Monitoring of the pre-leukemic clone for acquisition of driver mutations (e.g. *NPM1*, *FLT3*^{ITD}) during remission might prompt initiation of aggressive therapy, such as allogenic hematopoietic stem cell transplantation[12].

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