Complete Molecular Risk Stratification of De Novo Acute Myeloid Leukemia with Intermediate Cytogenetics Using a Nine-Gene Panel

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ABSTRACT

Background: Refining risk stratification of acute myeloid leukemia (AML) cases using molecular profiling, especially those with intermediate cytogenetic risk, is becoming standard of care. However, current WHO and ELN classifications are focused on few markers, mainly FLT3, NPM1, and CEBPA. While these abnormalities are relatively common, not all patients with AML and intermediate or normal cytogenetics will have abnormalities in these genes leaving large percentage of patients without refined risk stratification. We demonstrate that using 9 different AML-related genes are adequate to provide one or more molecular markers to further risk stratify patients with de novo AML.

Methods: Using direct sequencing we analyzed 211 samples referred from community practice with the diagnosis AML for molecular analysis. All samples were evaluated prospectively for mutations in FLT3, NPM1, IDH1, IDH2, CEBPA, DNMT3A, WT1, RUNX1, and TP53 using direct sequencing. Fragment length analysis was used in addition to sequencing for FLT3 and NPM1. Available morphology, cytogenetics, and clinical data along with history were reviewed.

Results: Of the 211 samples tested 103 (49%) had at least one or more molecular abnormality adequate for refining the risk classification. The mutations detected in these 103 patients were as follows: 27 (26%) FLT3-ITD, 10 (10%) FLT3-TKD, 30 (29%) NPM1, 7 (7%) CEBPA, 14 (14%) DNMT3A, 19 (18%) IDH1, 13 (13%) IDH2, 10 (10%) WT1, 38 (37%) RUNX1, and 2 (2%) TP53. There was significant overlap and most patients had more than one mutation as illustrated in the graph to the right. However, if the testing was restricted to FLT3, NPM1, CEBPA and DNMT3A, only 56 (54%) would have had refined risk classification and 46% of patients would have remained without subclassification. The most striking finding was that all the remaining patients, who had no molecular abnormality detected in any of these 9 genes, had either history of MDS evolved to AML, therapy-related AML, or cytogenetic abnormalities other than intermediate (multiplex cytogenetic abnormalities or core-binding factor abnormality).

Conclusions: Using FLT3, NPM1, CEBPA, and DNMT3A is inadequate for the molecular characterization of patients with AML. Patients with de novo AML and intermediate risk cytogenetics can be adequately prognostically subclassified and molecularly studied by testing only 9 genes. More importantly, this data confirms that the molecular biology driving de novo AML is significantly different from that driving MDS, AML with myelodysplasia-related changes, therapy-related AML, or AML with core binding factor or multiplex cytogenetics. Unlike de novo AML, these entities should be molecularly studied using MDS-specific driver genes. Furthermore, this data suggests that different therapeutic approaches should be developed for MDS and MDS-related AML.

OBJECTIVE

Explore the ability of molecular profiling in refining risk-stratification of patients with AML and intermediate-risk cytogenetics

Determine if only 9 genes (FLT3, NPM1, CEBPA, IDH1, IDH2, DNMT3A, WT1, RUNX1, and TP53) are adequate for refining risk stratification in patients with AML and intermediate-risk AML

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease. Based on cytogenetic findings, AML can be classified into three major risk groups: high (adverse), intermediate, and good. The intermediate-risk group constitutes approximately 60% of AML patients and includes patients with potential of long survival as well as patients with very short survival similar to the high-risk. Better and more refined risk classification has been the focus of numerous studies. The most successful risk stratification of the cytogenetic intermediate-risk AML patients appears to be based molecular findings. Next generation sequencing (NGS) made it possible to test mutations in multiple genes in reliable and cost-effective fashion. However, interaction between genes and the clinical relevance of a specific gene in the presence of other gene mutations is not well established. For routine clinical practice, there is a need to define a set of genes that are informative and clinically useful in refining the risk classification of patients with AML.

Current WHO and ELN classifications are focused on few markers, mainly FLT3, NPM1, and CEBPA. While these abnormalities are relatively common, not all patients with AML and intermediate or normal cytogenetics will have abnormalities in these genes leaving a large percentage of patients without refined risk stratification. Furthermore, there is a possibility that a patient with a mutation in one of these genes may have additional mutations that is currently well-established and may modify the risk stratification predicted by the one of the three genes.

SAMPLES AND METHOD

Bone marrow samples from 211 consecutive patients referred from community based practices for molecular evaluation with a diagnosis of AML were tested for mutations in the 9 different genes. Although not consistent, some data was provided on all samples, including clinical data, flow cytometry data, morphology evaluation, or cytogenetic and fluorescence in situ hybridization data.

Nucleic Acid extraction and mutation analysis

Total nucleic acid was extracted from all samples using either QIACube system or NucliSENS easyMAG Instrument.

Mutation analysis was performed using bidirectional standard Sanger sequencing. ABI 3730 sequencing instrument was used following standard procedures.

RESULTS

A. Mutations in one of the 9 genes observed in de novo AML with intermediate-risk cytogenetics

- Mutations in one or more of the 9 genes were detected in 103 of 211 (49%) patients.

- The remaining 108 patients without mutations in the 9 genes had either MDS or secondary leukemia that evolved from MDS (s-AML), or therapy-related leukemia (t-AML).

- Some of the patients without molecular abnormalities had de novo AML with cytogenetics characteristic of either adverse-risk or good-risk.

- This data suggests that de novo AML has specific molecular abnormalities that distinguish it from MDS, s-AML and t-AML.

B. Molecular Profile in de novo AML with intermediate-risk cytogenetics

- The mutations detected in the 103 patients were as follows: 27 (26%) FLT-ITD, 10 (10%) FLT3-TKD, 30 (29%) NPM1, 7 (7%) DNMT3A, 19 (18%) CEBPA, 14 (14%) IDH1, 13 (13%) IDH2, 10 (10%) WT1, 38 (37%) RUNX1, and 2 (2%) TP53. (Figure 1; pie chart)

- There was significant overlap and most patients had more than one mutation as illustrated in the graph below

- 15 of 27 (56%) patients with FLT3 ITD had a mutation in the NPM1 gene which significantly modifies the poor prognosis predicted by the FLT3 ITD mutation.

- 2 of the 7 (29%) patients with FLT3 TKD mutation had mutation in the NPM1 gene.

- Majority of patients with RUNX1 (24 of 38; 63%) had only mutation in the RUNX1 gene.

Pie chart legend:
- (37%) RUNX1
- (29%) NPM1
- (26%) FLT-ITD
- (19%) DNMT3A
- (14%) IDH1
- (13%) IDH2
- (10%) FLT3-TKD
- (10%) WT1
- (7%) CEBPA
- (2%) TP53
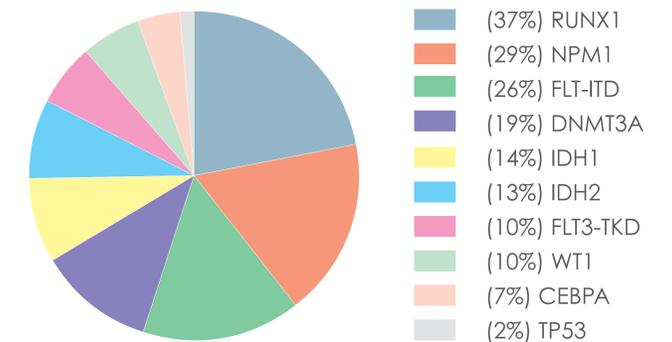
- 19 of 27 (70%) patients with IDH1/IDH2 mutation had no other mutations.

- Both patients with TP53 mutations had no other molecular abnormalities.
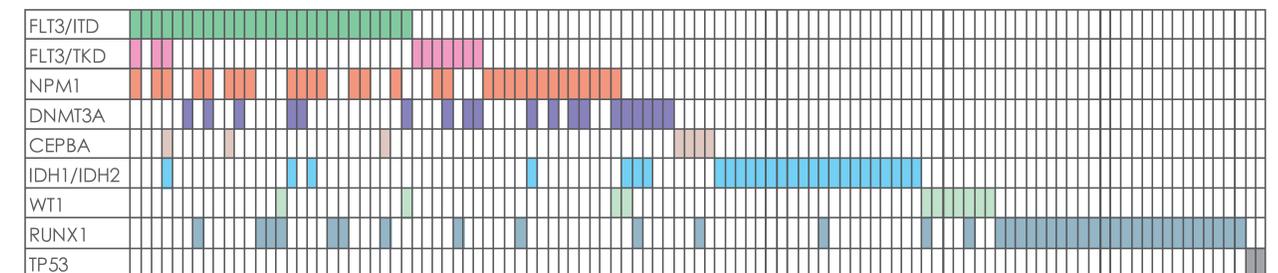
C. Inadequate profiling of de novo AML if only 4 genes are used

- If the testing was restricted to FLT3, NPM1, CEBPA and DNMT3A, only 54% (56 of 103) would have had refined risk classification and 46% of patients would have remained without subclassification.

- Clearly the use of the nine genes is inadequate for profiling t-AML or s-AML, and MDS specific molecular panel should be used for this group of patients.

- All patients with de novo AML without molecular abnormalities in any of the 9 genes had either good- or adverse-risk AML.

CONCLUSION

Using FLT3, NPM1, CEBPA, and DNMT3A is inadequate for the molecular characterization of patients with de novo AML and intermediate-risk AML. Using only the four genes will leave 46% of these patients without molecular risk subclassification.

A limited panel of 9 genes is adequate for molecular profiling of patients with de novo AML. Molecular abnormalities in patients with t-AML or s-AML cannot be captured using the standard de novo AML molecular profiling.

56% of patients with de novo AML and FLT3 ITD also have NPM1 mutation, which should be considered as it may modify the adverse prognostic significance of FLT3 mutation.