RNA-seq for Identification of Targetable Alterations in AML

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Abstract

Background: Menin inhibition induces differentiation and antileukemic effects by downregulating aberrantly expressed *HOX* genes. While *KMT2A* rearrangements (*KMT2Ar*) and *NPM1* mutations are clinically identifiable, other alterations with similar dependency on menin are often missed. Clinical testing for *HOX* expression is lacking, and conventional cytogenetics (CG) has limited resolution, potentially missing cryptic or novel rearrangements. We hypothesized that some acute myeloid leukemia (AML) patients with normal karyotype and co-mutations in *WT1*, *FLT3*, or *RAS*, or chromosomal alterations, may harbor fusions leading to *HOX* upregulation.

Methods: We screened AML cases with normal karyotype and either *FLT3/WT1/RAS* co-mutations or chromosomal alterations identified by CG as mentioned above [example: 11p15 for *NUP98* rearrangements (*NUP98r*)]. RNA-seq was performed on banked bone marrow samples from 76 patients (pts). Data were aligned to GRCh38 using STAR and fusions were called with Arriba. HOX expression was analyzed via unsupervised hierarchical clustering using Ward's method.

Results: The median age of pts included was 58 years [range: 13-88], and 28 pts (36%) had therapy-related AML. Co-occurring mutations involved *WT1* (12/58 pts;20%), FLT3 (11/75 pts;15%), and *N/KRAS* (10/74;15%), 12 pts (16%) had normal karyotype by CG. Using unsupervised clustering, we identified four clusters based on *HOX* gene expression. We found high concordance of *HOXA9*, *HOXA10*, and *MEIS1* expression as previously described. We identified fusions in 38 pts (50%) and most were in the high *HOX* clusters (31/38 pts; 82%), among which 7 pts (9%) harbored novel fusions. Among pts with normal karyotype by CG, 4/12 (33%) had fusions identified by RNA-seq. The most common known fusions included *RUNX1::MECOM* (9 pts;12%), *KMT2Ar* (7 pts;9%), *NUP98r* (4 pts;5%), *MN1::ETV6* (3 pts;4%), *CBP::MOZ* (2 pts;3%), *NPM1::MLF1* (2 pts;3%), *ETV6::FLT3* (2 pts; 3%), *PICALM::AF10* (1 pt;1%). Notably, we detected a *UBTF-TD*. Novel fusions identified included *NPM1::PRDM16* in one pt; *PRDM16* is a transcription regulator essential for hematopoiesis, with previously described roles in leukemogenesis. Other novel fusions included *RUNX1* rearrangements (3 pts; 4%), *DDX3X::MLLT10* (1 pt;1%), and *HOXA3::DDX5* (1 pt;1%), all planned for validation with orthogonal methods. Altogether, in 31 pts (50%), a targetable alteration was identified, meeting eligibility criteria for menin inhibitor clinical trials for acute leukemia associated with *HOX* upregulation (NCT06229912; NCT05918913) or could additionally be targeted with a *FLT3* (i.e. *ETV6::FLT3*).

Based on clinical data including mutational and CG analysis, patients in this cohort formed ELN intermediate and adverse risk groups (5-year OS: 33% vs. 5%, p<0.001). However, RNA-seq provided additional prognostic information where *NPM1r* or *RUNX1r* (not including *MECOM*) had better a better prognosis with a 5-year OS of 60%, whereas all the other fusions detected not otherwise specified in ELN, or ELN adverse, had a poor prognosis with a 5-year OS of 9% (P=0.01).

Conclusion: In addition to improving prognostic predictions, these RNA-seq data highlight that many targetable genetic alterations are underdiagnosed in the clinic and that a gene expression-based method could comprehensively identify patients who may benefit from menin inhibitors.

Introduction

- Menin inhibition induces differentiation and antileukemic effects by downregulating aberrantly expressed *HOX* genes.
- *KMT2A* rearrangements (KMT2Ar) and *NPM1* mutations are clinically identifiable, but other alterations with similar dependency on menin are often missed.
- Clinical testing for *HOX* expression is lacking, and conventional cytogenetics (CG) has limited resolution, potentially missing cryptic or novel rearrangements.
- We hypothesized that some acute myeloid leukemia (AML) patients with normal karyotype and co-mutations in *WT1*, *FLT3*, or *RAS*, or chromosomal alterations, may harbor fusions leading to *HOX* upregulation.

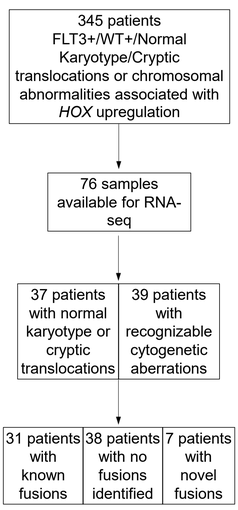
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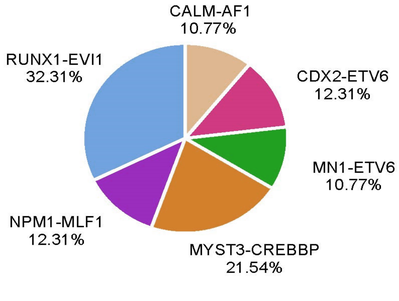
- Identify cryptic and novel fusions in AML using RNA-seq and evaluate the association with HOX expression and outcomes.

Methods

- We screened AML cases with normal karyotype and either *FLT3*/*WT1*/*RAS* co-mutations or chromosomal alterations identified by CG as mentioned above [example: 11p15 for *NUP98* rearrangements (*NUP98r*)].
- RNA-seq was performed on banked bone marrow samples from 76 patients.
- Data were aligned to GRCh38 using STAR and fusions were called with Arriba.
- *HOX* expression was analyzed via unsupervised hierarchical clustering using Ward's method.

Patient selection





Results

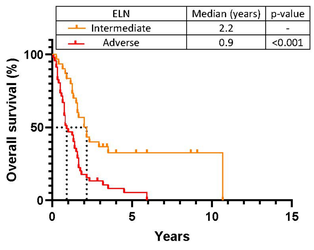
Baseline characteristics

Characteristics	N=76
Median age, years [range]	58.5 [13-88]
Sex, n%	
Male	59
Female	41
Leukemia subtype, n%	
AML	79
MPAL	2.6
ALL	2.6
CML	1.3
MDS	13
BPDCN	1.3
Therapy-related leukemia, n%	37
Co-occurring mutations, n% Fusions distribution	
FLT3	14.5
WT1	15.8
RAS	13
Cytogenetics, n%	
Diploid	23.7
Miscellaneous	22.4
-7/7q-	26.3
Previous therapies, n%	
Hypomethylating agents	21
Venetoclax	21

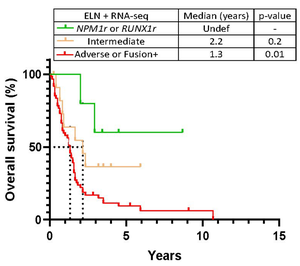
Known Fusion	Frequency	Novel Fusion	Frequency
RUNX1::EVI1	9/31	*DDX3X::MLLT10*	1/7
KMT2Ar	7/31	*HOXA3::DDX5*	1/7
NUP98r	4/31	*NPM1P27::MLF1*	1/7
MN1::ETV6	3/31	*NPM1::PRDM16*	1/7
ETV6::FLT3	2/31	*RUNX1::ENSG00000291214*	1/7
KAT6A::CREBBP	2/31	*RUNX1::RUNX1*	1/7
NPM1::MLF1	2/31	*RUNX1::USP42*	1/7
PICALM::AF10	1/31		
UBTF-ITD	1/31		

Survival

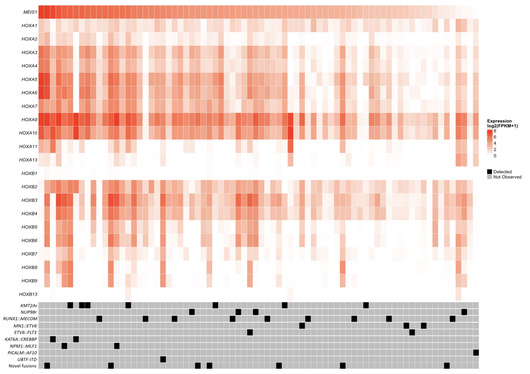
OS-ELN preRNAseq



OS-ELN postRNAseq



Heat map



Conclusion

- In addition to improving prognostic predictions, these RNA-seq data highlight that many targetable genetic alterations are underdiagnosed in the clinic and that a gene expression-based method could comprehensively identify patients who may benefit from menin inhibitors.