MOLECULAR ANALYSIS OF ACUTE PROMYELOCYTIC LEUKEMIA BY NEXT GENERATION SEQUENCING

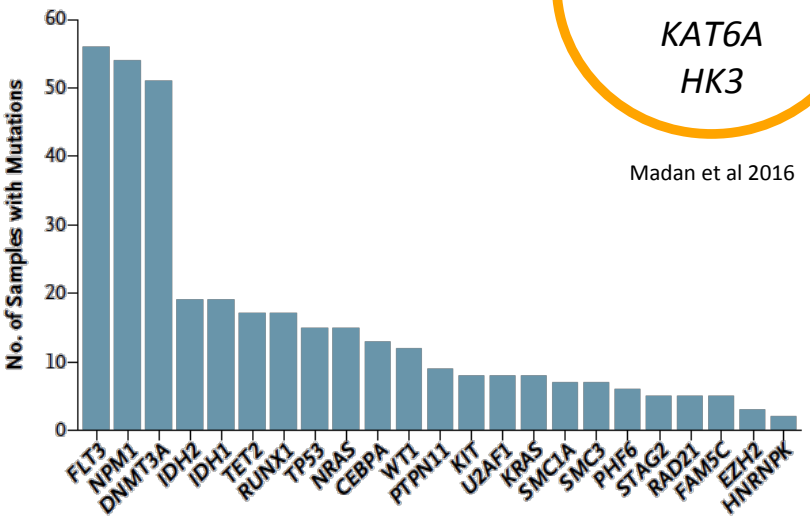
7th INTERNATIONAL SYMPOSIUM ON ACUTE PROMYELOCITIC LEUKEMIA

Llop M(1,9), Gil JV(2), Sargas C(2), Cervera J(3,9), Such E(3,9), Gil C(4), Sayas MJ(5), García R(6), Manso F(7), Fernández JM(8), Martínez-Cuadrón D(3), Rodríguez R(3), Boluda B(3), Montesinos P(3), Sanz MA(3,9) Barragán E(1,9).

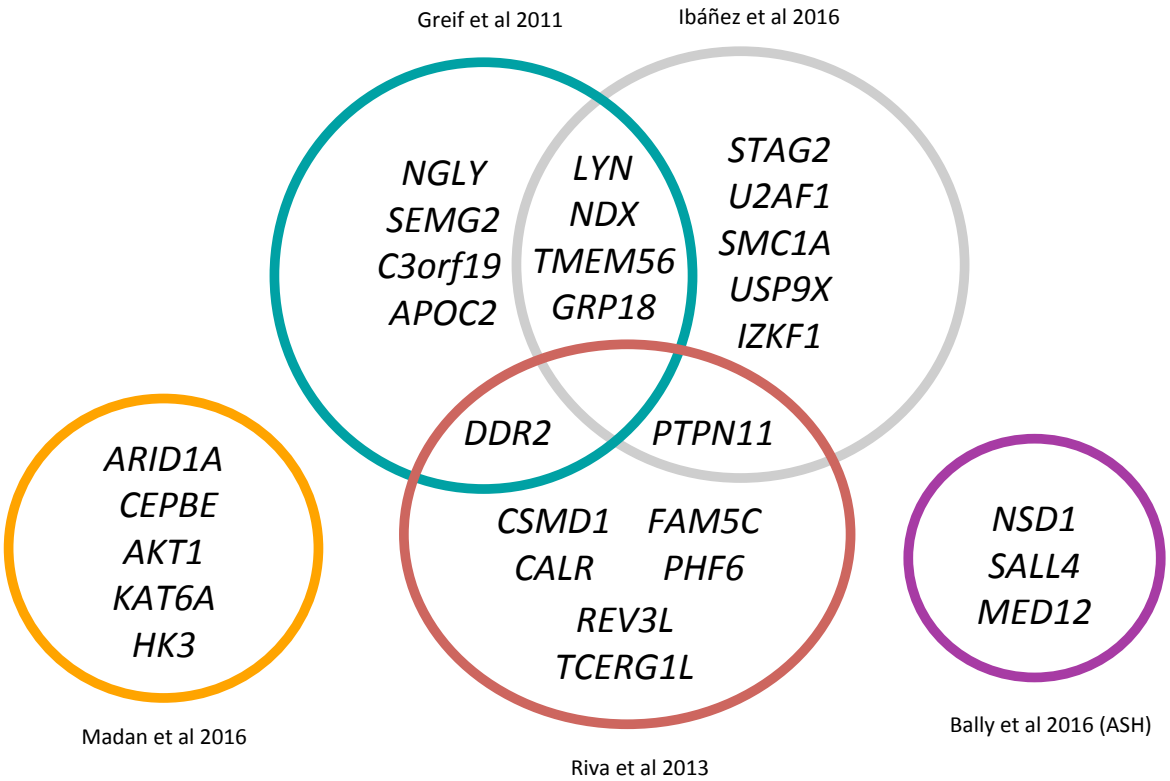
1 Molecular Biology Unit.. Hospital Universitari i Politècnic la Fe.
2 Research on Hematology Group. La Fe Health Research Institute.
3 Hematology Unit. Hospital Universitari i Politècnic la Fe.
4 Hematology Unit. Hospital General de Alicante.
5 Hematology Unit. Hospital Dr Peset.
6 Hematology Unit. Hospital General de Castellón.
7 Hematology Unit. Hospital General de Albacete.
8 Oncopediatric Unit. Hospital Universitar i Politècnic la Fe.
9 CIBERONC CB16/12/00284

Introduction

Revealing the genetic landscape of AML



TCGA, 2013.



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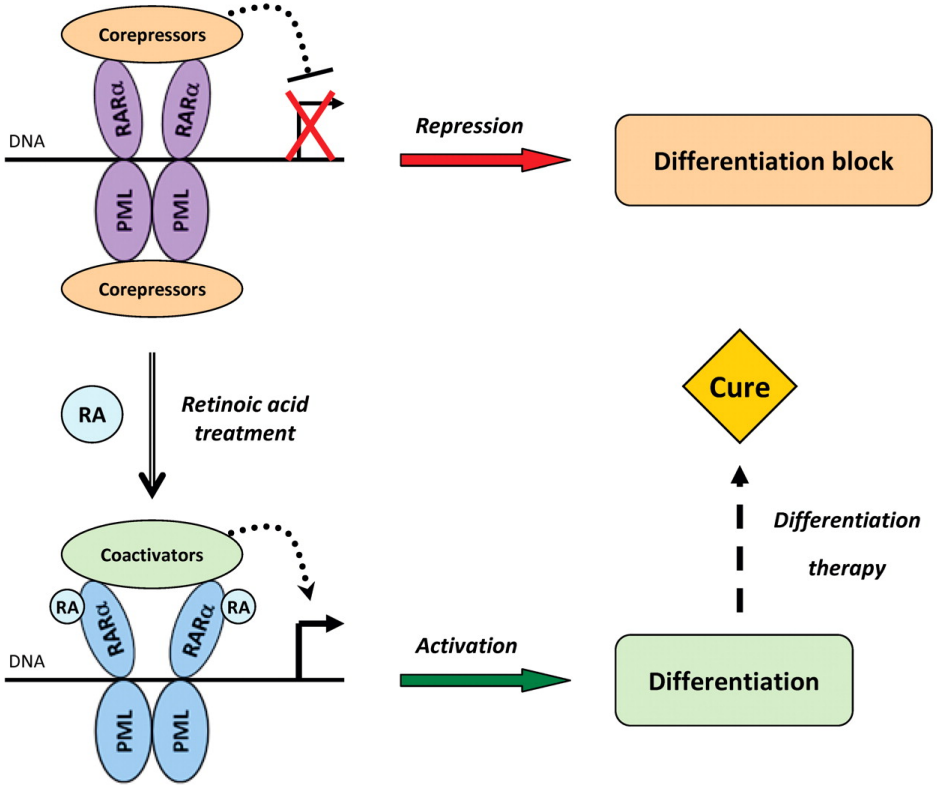
Revealing the genetic landscape of AML



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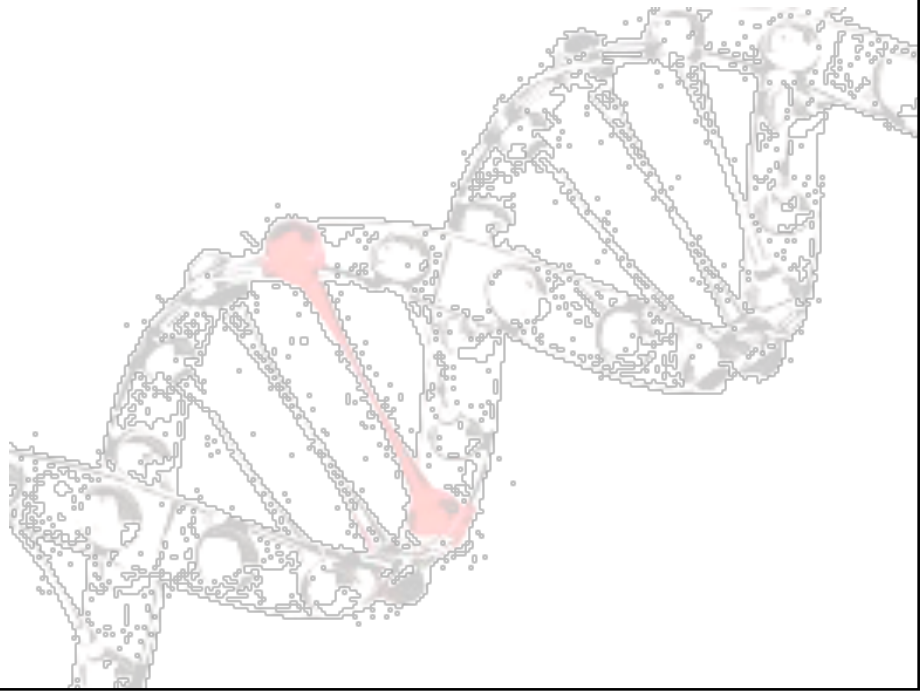
Aims

The aim of this study is to analyze the mutational landscape of favourable AML with NGS, focusing on APL

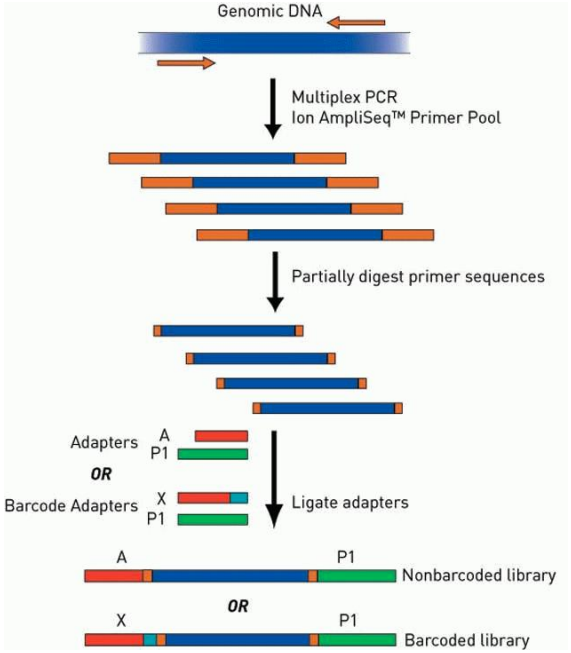


Patients and Methods

Next generation sequencing

AML AMPLISEQ COMMUNITY PANEL

Gen	Chr.	Amplicones	Bases	CDS exones
ASXL1	chr20	28	2947	12
BRAF	chr7	1	11	15 (V600E)
CBL	chr11	5	416	8, 9
CEBPA	chr19	9	1117	CDS
DNMT3A	chr2	42	3619	CDS
FLT3	chr13	3	66	16 (N676), 20-21 (830-850)
GATA2	chr3	20	1643	CDS
IDH1	chr2	3	332	4
IDH2	chr15	2	201	4
JAK2	chr9	1	128	14
KIT	chr4	8	632	8, 10, 11, 17
KRAS	chr12	4	370	2, 3, 4
NPM1	chr5	1	79	11
NRAS	chr1	3	370	2, 3, 4
PTPN11	chr12	5	427	3, 7 ,8, 13
RUNX1	chr21	15	1149	3-8
TET2	chr4	59	6369	CDS
TP53	chr17	24	1715	CDS
WT1	chr11	4	324	7,9



Ion Chef

PGM

Clonal amplification on ISPs by emPCR and template-positive ISP enrichment (+ chip load)

Sequencing by sinthesis. Semiconductor technology

Patients and Methods

Favorable-risk AML

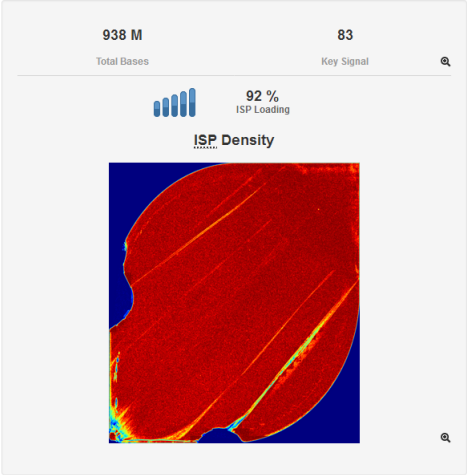
73 favorable risk AML
- 45 APL (61.64%)
- 28 no promyelocytic AML (38.36%)

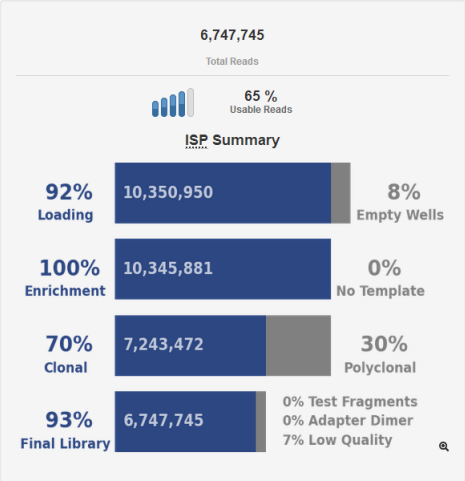
APL	N (%)	Median (range)
Age		47 (10-77)
Adult	39 (86.67)	
Pediatric	6 (13.33)	
Sex		
Male	18 (40)	
Female	27(60)	
APL type		
Primary	37 (82.22)	
Secondary	8 (17.78)	
***PML-RARA* type**		
BCR1	29 (64.44%)	
BCR3	16 (35.56)	

No APL AML	N (%)	Median (range)
Age		45.5 (19-67)
Adult	28 (100)	
Pediatric	0 (0)	
Sex		
Male	19(68)	
Female	9 (32)	
Rearrangement		
AML1-ETO	15 (53.57)	
CBFB-MYH11	13 (46.43)	

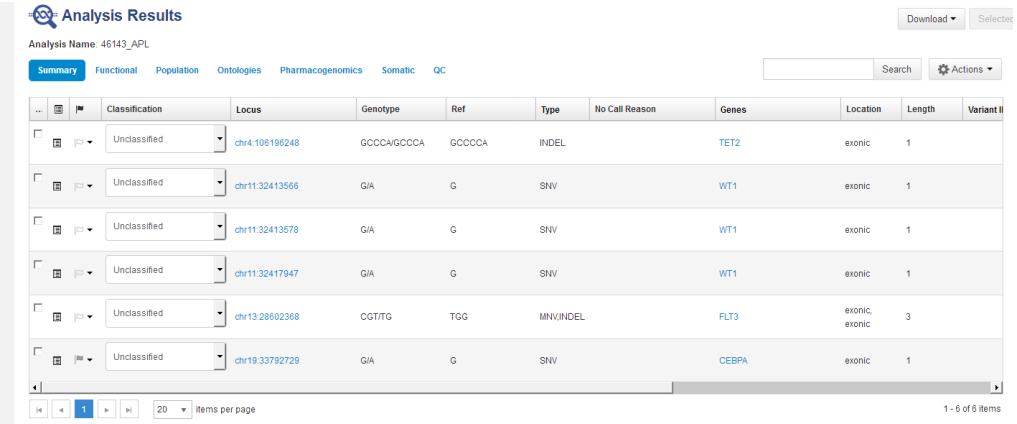
Results

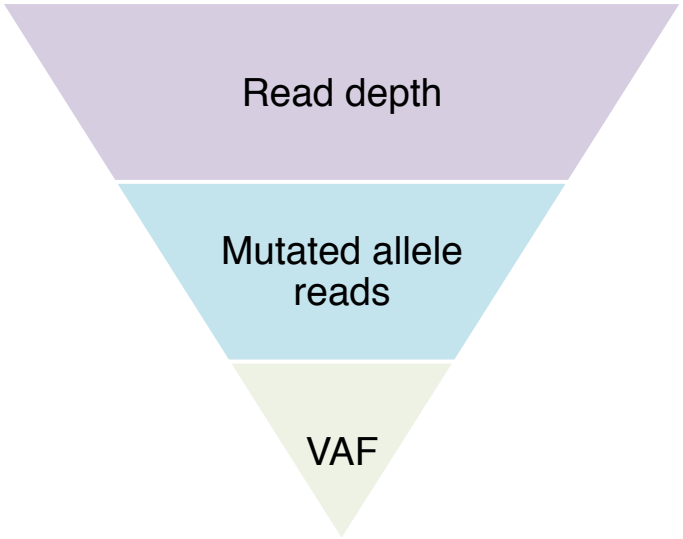
Sequencing metrics and variant analysis

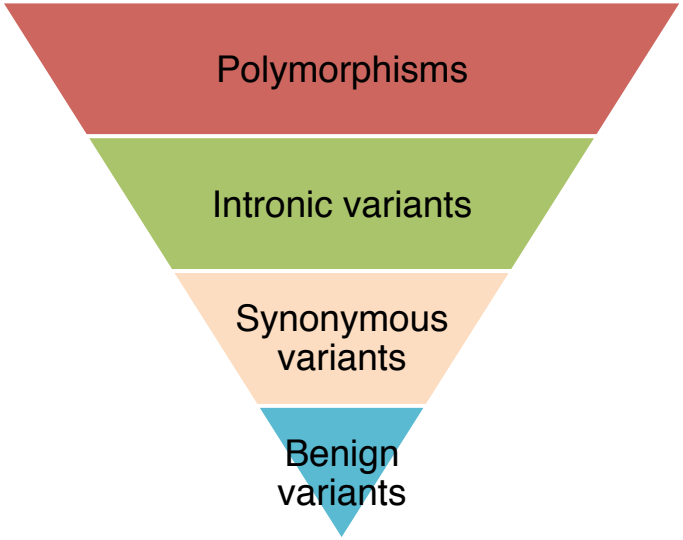




	MAPPED READS (bp)	ON TARGET (%)	MEAN DEPTH (reads)	UNIFORMITY (%)
Mean	820350	90.28	3231	93.19
Min	669428	56.56	1028	89.44
Max	986560	98.66	4760	96.50

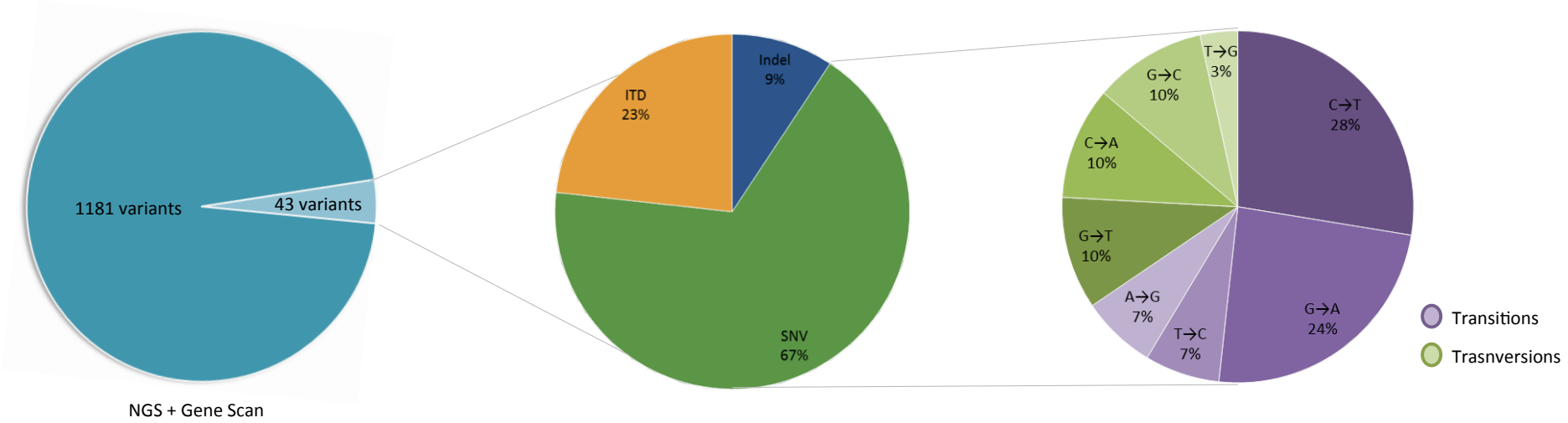


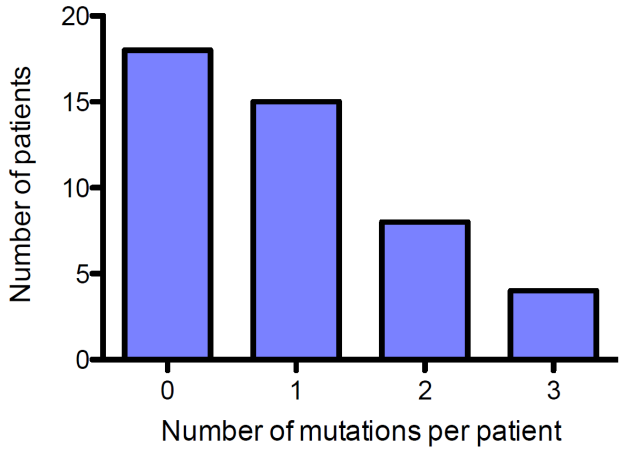




Results

Mutational distribution in APL

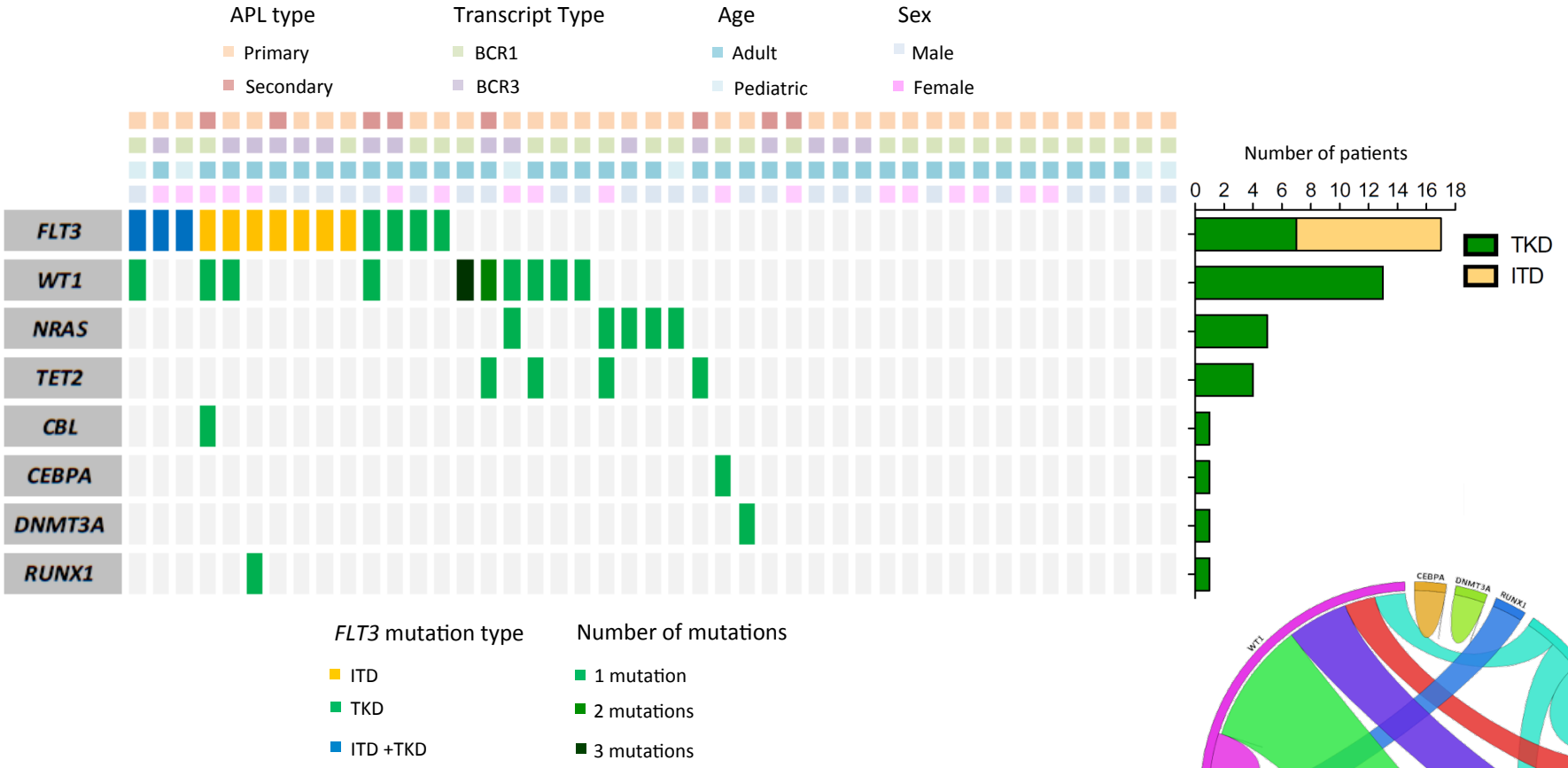


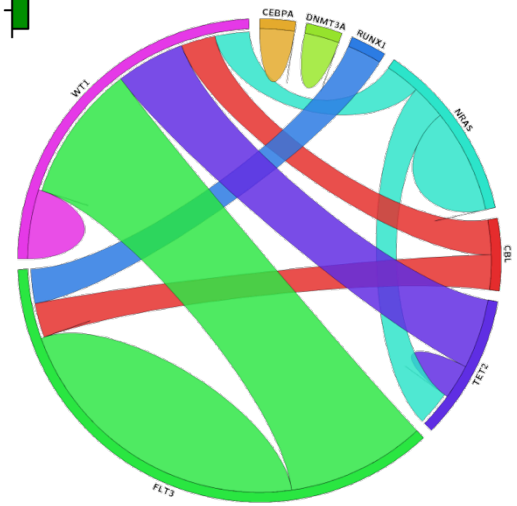


- 27/45 (60.0%) of patients showed at least one mutation
- The mean number of mutations per patient vas 0.96

Results

Mutational distribution in APL



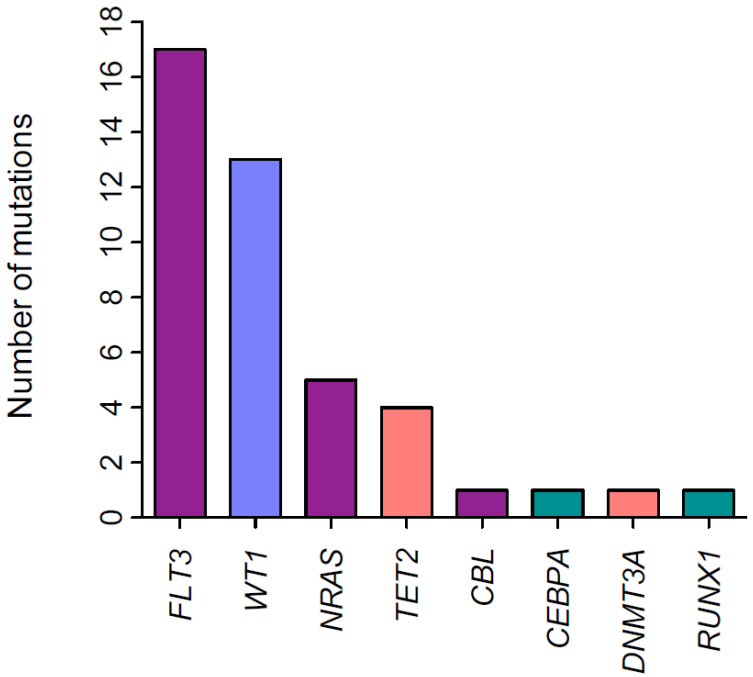


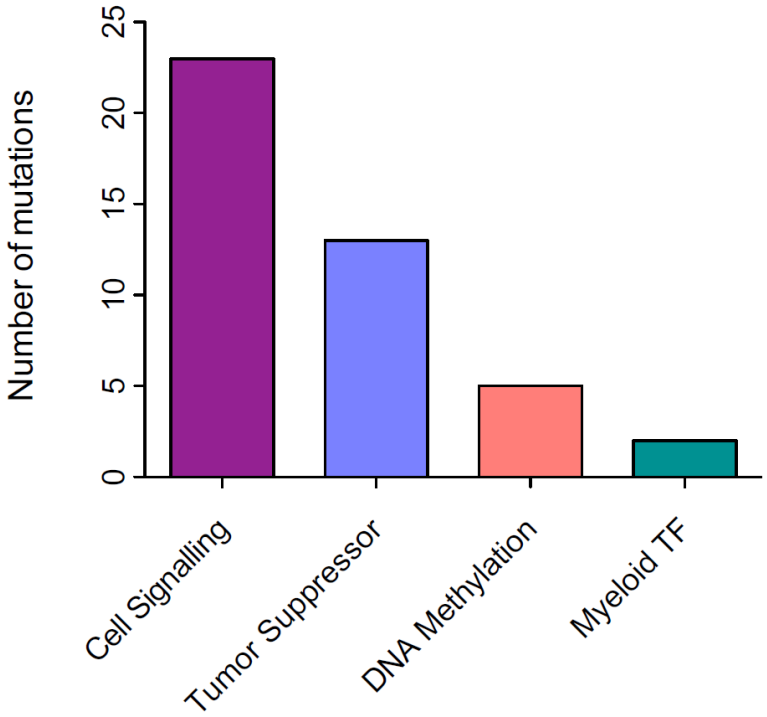
In 10/45 (22%) patients, *FLT3* was the only genetic mutation

Mutations in *RUNX1, DNMT3A, CEBPA* and *CBL* were found in less than 10% of cases

Results

Functional categories (TCGA)





Cell signalling

Tumor Suppressor

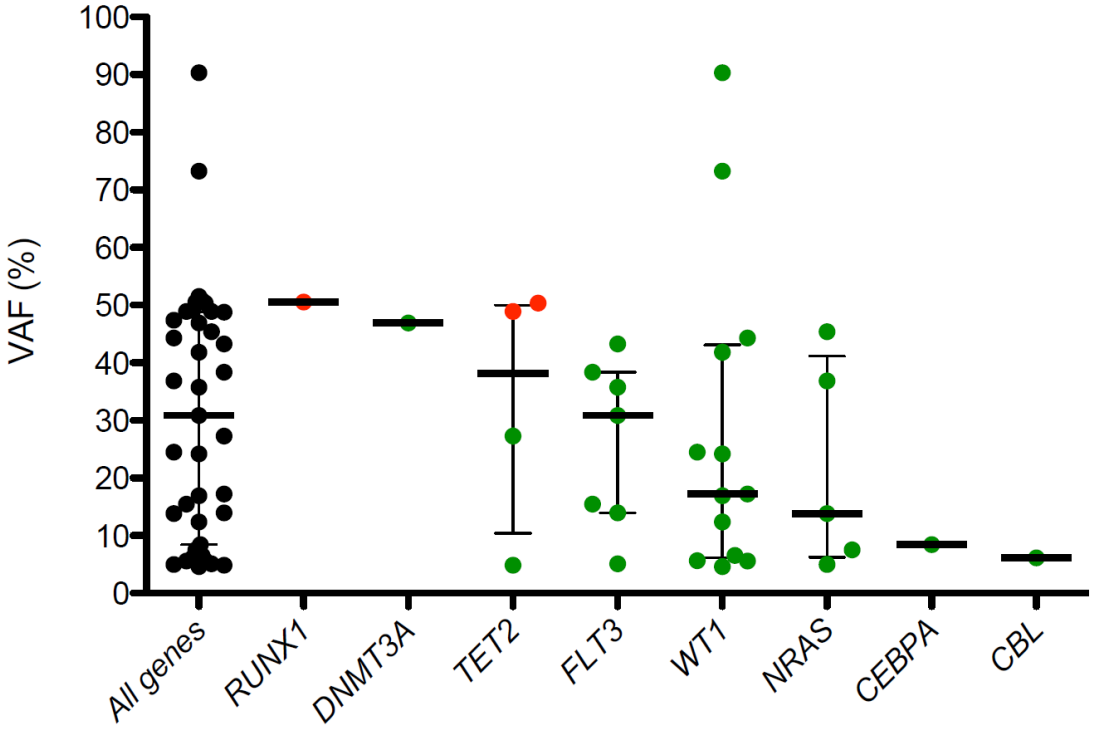
DNA methylation

Myeloid Transcription Factor

Transcription factor fussions

Results

Variant allele frequency (VAF)



	VAF (%)	APL type	PML-RARA type
RUNX1 p.Arg306Cys (rs202037007)	57.57	P	BCR3
TET2 p.Val1718Leu (rs142312318)	48.91	S	BCR1
TET2 p.Gln810Arg (rs28555446)	50.38	P	BCR3

Results

Early mutations *vs.* germ line mutations

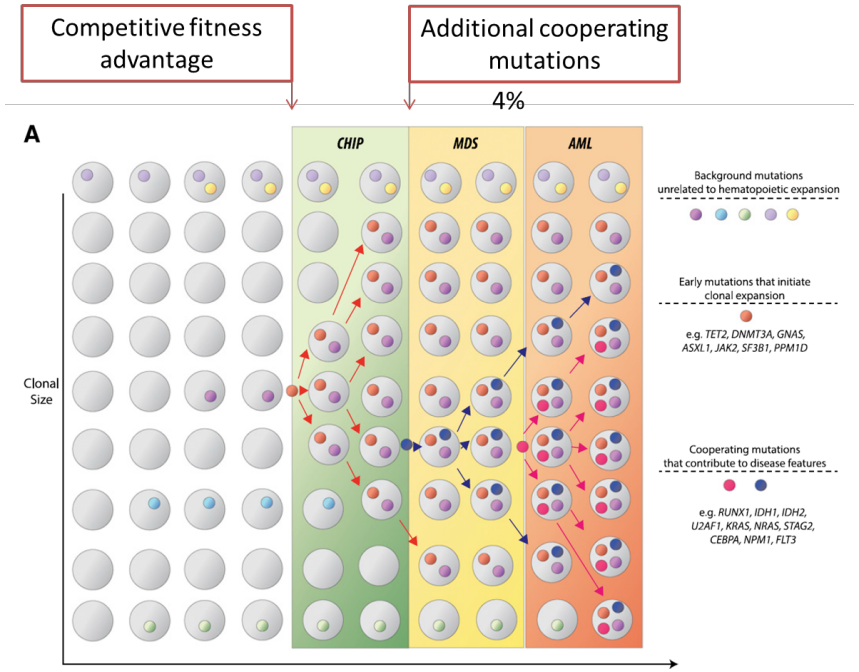
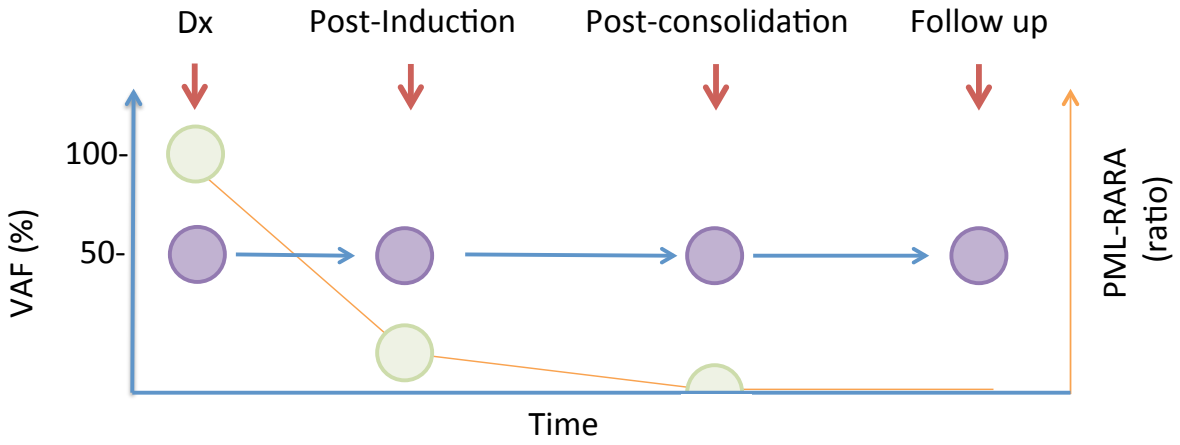


Table 17. Classification of myeloid neoplasms with germ line predisposition

Myeloid neoplasm classification
Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction
AML with germ line *CEBPA* mutation
Myeloid neoplasms with germ line *DDX41* mutation*
Myeloid neoplasms with germ line predisposition and preexisting platelet disorders
Myeloid neoplasms with germ line *RUNX1* mutation*
Myeloid neoplasms with germ line *ANKRD26* mutation*
Myeloid neoplasms with germ line *ETV6* mutation*
Myeloid neoplasms with germ line predisposition and other organ dysfunction
Myeloid neoplasms with germ line *GATA2* mutation
Myeloid neoplasms associated with BM failure syndromes
Myeloid neoplasms associated with telomere biology disorders
JMML associated with neurofibromatosis, Noonan syndrome or
Noonan syndrome-like disorders
Myeloid neoplasms associated with Down syndrome*

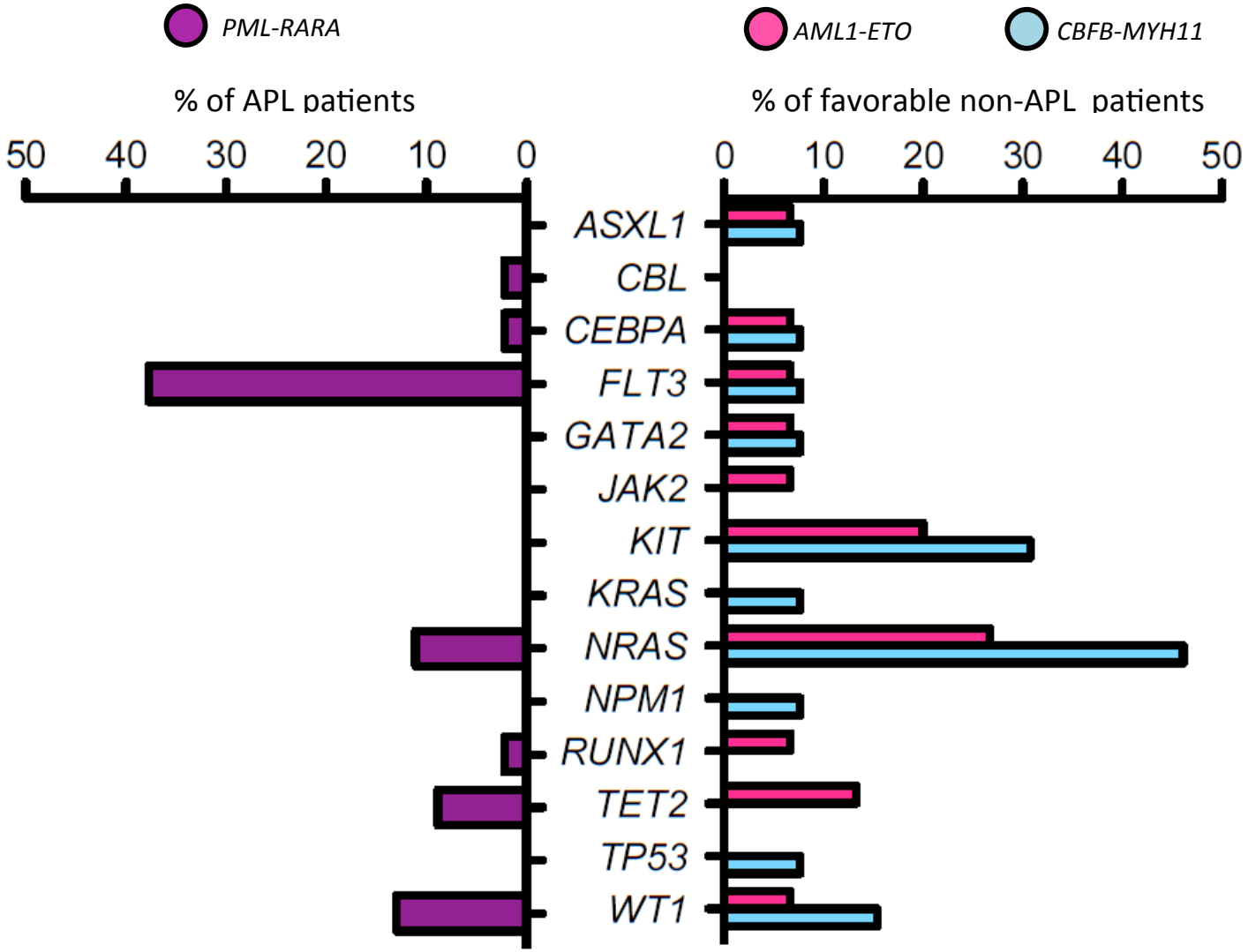
*Lymphoid neoplasms also reported.

Arber et al 2016



Results

Mutational patterns in APL *vs.* other favourable risk AML



Conclusions

- Our data shows that NGS is a valid method to detect recurrenty mutated genes in AML.
- APL patients harbor somatic mutations in *FLT3*, *WT1*, and *NRAS*.
- Mutations in genes involved in signaling processes are usually found at a low VAF, and patients harboring them could benefit of combined targeted therapy.
- APL patients harbor germ-line mutation in *TET2* and *RUNX1*.
- The mutational spectrum of APL is different from that of other favourable AML
- Further research is needed in order to understand the involvement of these mutations in the clinical management of APL.

Thank you for your attention!

The Research Group in Haematology and Haemotherapy

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