Clonal Hematopoiesis with Somatic Mutations in „AYA“ Generation of Patients With Chronic Myeloid Leukemia

Koblihova J.[1], Polivkova V.[1], Benesova A.[1], Zizkova H.[1], Curik N.[1,2], Pepek M.[3], Stoklosa T.[3], Stastna Markova M.[1], Klamova H.[1,4], Srbova D.[1], Machova Polakova K.[1,2]

[1]Institute of Hematology and Blood Transfusion, Prague, Czech Republic; [2]Institute of Pathophysiology, 1st Medicine Faculty, Charles University, Prague, Czech Republic; [3]Department of Immunology, Medical University of Warsaw, Warsaw, Poland; [4]Institute of Clinical and Experimental Hematology of the 1st Medicine Faculty, Charles University and Institute of Hematology and Blood Transfusion, Prague, Czech Republic

INTRODUCTION

During chronic myeloid leukemia (CML) hematopoiesis, the myeloid lineage development is primarily impaired and untreated CML progresses to a terminal myeloid or lymphoid blast phase. Before introduction of tyrosine kinase inhibitors (TKIs) into the clinical practice, the higher age was a negative prognostic factor (Cortes et al. 2003). In contrast, in the current era of TKIs, it seems that younger patients with chronic phase of CML at age 15-39, defined by National Comprehensive Cancer Network (NCCN) Guidelines as adolescents and young adults (AYA), have worse prognosis and response to TKIs. The clonal hematopoiesis with somatic mutations is an age-related phenomenon with a frequency around 10% for population older than 65 years in contrast to the population younger than 50 years with frequency of 1%. In recent years, mutations in genes involved in epigenetic modification and RNA splicing, which are recurrently mutated in myeloid neoplasms have been highly reported and seem to represent a premalignant condition (Branford et al. 2019; Kim et al. 2017). However, except for mutations in the kinase domain of BCR-ABL1, very little is known about the genomic landscape of CML AYAs and their potential effect on resistance to the TKI treatment and relapses.

AIM

To determine, whether the worse prognosis of CML AYAs, resulting in the therapy failure and disease progression, is associated with the clonal hematopoiesis with somatic mutations.

MATERIAL AND METHODS

Patients

- Samples from CML AYAs (N=23; aged 18-37 at the time of diagnosis; 10 women and 13 men; see Table)
- Two responders were included for comparison (patient #22 and #23)
- 21/23 patients failed on TKI or relapsed after allo-HSCT (allogeneic hematopoietic stem cells transplantation)
- In 6/21 AYAs, mutations in the kinase domain of BCR-ABL1 were detected at the time of TKI failure (M244V, T315I, E255K/V + Q252H, F317L + M351T, V379I, L284S)

Detection of mutations by myeloid panel sequencing (next generation sequencing)

- Retrospective analysis of samples by DNA-based custom panel (Roche) on MiSeq (Illumina)
- Data evaluation in NextGENe software (Softgenetics)
- Variant characterization in open-source databases (VarSome, Ensembl, COSMIC, NCBI – dbSNP)
- Variant confirmation by Sanger sequencing and/or ASO-ddPCR

RESULTS

Almost a half (9/21) of AYAs that in follow up failed on TKIs harbored somatic mutations at the time of diagnosis

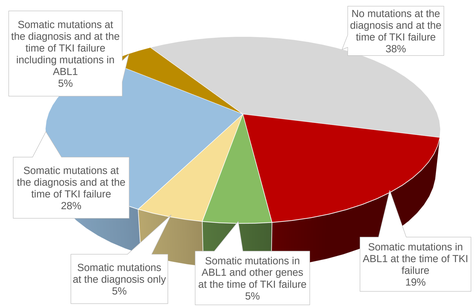
AYA patient	Age at diagnosis	Transcript	Diagnosis: BCR-ABL1 % IS	Diagnosis: Mutation	TKI failure: TKI	TKI failure: Months from dg	TKI failure: BCR-ABL1 % IS	TKI failure: Mutation	Relapse after allo-HSCT: Months from allo-HSCT	Relapse after allo-HSCT: BCR-ABL1 % IS	Relapse after allo-HSCT: Mutation
#1	30	b2a2	86	ASXL1 S795delinsCLfs (33%)	DASA	30	29	-			
#2	29	b2a2	191	-	IM	15	3	ABL1 M244V (56%)			
#3	22	b3a2	13	CSF3R A593V (53%)	IM	14	2	CSF3R A593V (55%)			
#4	26	b2a2	338	-	IM	98	40	-			
#5	20	b3a2	16	-	IM	7	8	ABL1 E255V (70%) ABL1 E255K (13%) ABL1 Q252H (3%)			
#6	28	b3a2	95	ASXL1 G645delinsGWfs (39%)	NILO (3rd line)	36	143	ASXL1 G645delinsGWfs (50%)	24	46	ASXL1 G645delinsGWfs (5%)
#7	33	b2a2	138	-	DASA (2nd line)	28	24	-			
#8	25	b2a2	37	- -	IM	9	50	ABL1 T315I positive by ddPCR RUNX1 D198N (25%)	51*	271	ABL1 T315I (28%) RUNX1 D198N (17%)
#9	19	b2a2	51	-	IM	55	23	-	4	30	-
#10	30	b3a2	325	ASXL1 E773X (47%)	IM	23	80	ASXL1 E773X (44%) ABL1 F317L (21%) ABL1 M351T (67%)	36	16	ASXL1 E773X (21%)
#11	30	b3a2	133	-	IM	36	37	-			
#12	37	b3a2	24	TET2 H1868D (9%)	NILO	33	82	TET2 H1868D (2%)			
#13	30	b2a2	44	SIRT1 E536K (49%)	IM	18	18	SIRT1 E536K (54%)			
#14	37	b2a2	101	-	IM	20	41	-			
#15	36	b3a2	63	ATRX H865Q (46%)	ongoing (failure)						
#16	33	b3a2	17	-	IM	92	0.3	-			
#17	35	b3a2	11	-	IM	64	0.3	ABL1 V379I (100%)			
#18	36	b2a2	96	-	IM	39	5.4	- (in follow up 43M ABL1 L284S 21%)			
#19	18	b2a2	22	ASXL1 T1372delinsTCfs (37%)	IM	17	1.2	ASXL1 T1372delinsTCfs (0.3%)			
#20	36	b2a2, b3a2	53	-	NILO	10	1.1	-			
#21	31	b3a2	52	DNMT3A R635Q (45%)	IM	10	1	DNMT3A R635Q (0.6%)			
#22	31	b2a2	14	-	IM (lower dose)	13	0.9	-			
#23	22	b3a2	81	-	NILO	12	0.1	-			

IM imatinib; NILO nilotinib; DASA dasatinib; - no mutations detected
*from 2nd allo-HSCT

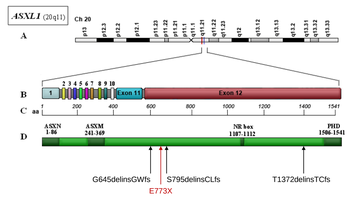
At the time of diagnosis, somatic mutations were identified in *ASXL1* (n=4), *CSF3R* (n=1), *TET2* (n=1), *DNMT3A* (n=1), *ATRX* (n=1), and *SIRT1* (n=1) in 9/21 AYAs, who subsequently failed on TKI treatment. Overall, 5 missense, 3 frameshift mutations and one nonsense mutation were detected. According to VarSome database (Kopanos et al. 2018), detected mutations in *ASXL1*, *RUNX1*, *DNMT3A* were reported as pathogenic/likely pathogenic. Mutation R635Q in DNMT3A gene lies within the SAM-dependent MTase C5-type domain of the Dnmt3a protein and probably result in a loss of function.

The detected mutations were categorized into mutation patterns according their presence at the diagnosis and/or at the time of TKI failure.

Distribution of mutation patterns of 21 AYAs



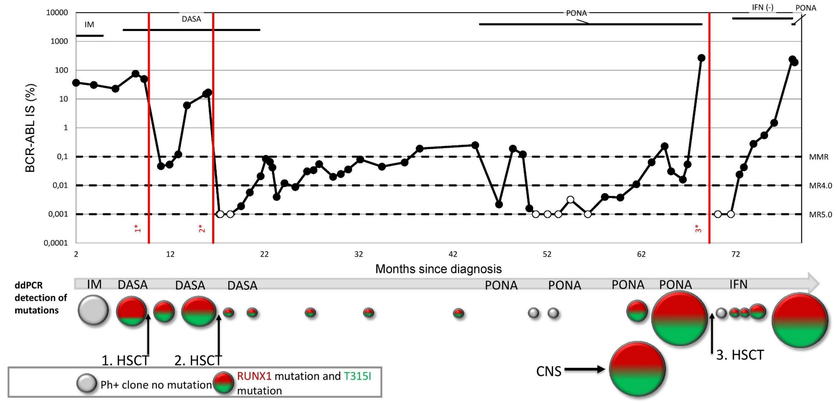
ASXL1 was the most frequently mutated gene at the time of diagnosis



Adopted and edited from http://atlasgeneticsoncology.org/Genes/ASXL1ID44553ch20q11.html

Four patients harbored a mutation in *ASXL1* gene at the time of diagnosis. All the mutations in *ASXL1*, gene involved in epigenetic modification, were found in exon 12. In patient #6, G645delinsGWfs was found at the diagnosis and on the 3rd line nilotinib treatment. In patient #10, nonsense mutation E773X was confirmed at the time of TKI failure and also at the allo-HSCT relapse. Another *ASXL*1 mutation, S795delinsCLfs, was found in a patient #1 only at diagnosis. In patient #19, *ASXL1* mutation T1372delinsTCfs was found at both time points.

Mutation D198N in RUNX1 gene in T315I-BCR-ABL1-mutated clone was responsible for the resistance to TKIs and recurrent relapses after allo-HSCTs



The patient 25 years old at the time of diagnosis relapsed after 3rd allo-HSCTs due to Ph+ clone with a quick development of D198N mutation in RUNX1 and T315I in BCR-ABL1 kinase domain on imatinib therapy first line. The clone persisted after 2nd allo-HSCT even though the patient was in major molecular response (MMR). After ponatinib start, the patient achieved undetectable levels of BCR-ABL1, but subsequently relapsed to blast phase in CNS followed by the blast phase relapse in bone marrow. After the third allo-HSCT, patient quickly relapsed to blast phase and died.

CONCLUSION

The preliminary data of this work outlined that somatic mutations in the myeloid genes are frequently found in CML AYAs, who failed on the TKI or relapsed after allo-HSCT, alone or together with mutated *BCR-ABL1*. The most frequently mutated gene was *ASXL1*, which is in line with the work by Ernst et al. (2018) even though on younger patients including children. Despite the clonal hematopoiesis with somatic mutations is considered as age-related phenomenon, in AYA CML patients, it may represent a critical problem in achieving sustained molecular response on solo TKI therapy, or even worse, it may result in higher risk of therapy failure and disease progression.

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CONTACT

jitka.koblihova@uhkt.cz