Clinical, Genomic Profile and Recurrent Somatic Mutations in 205 Chronic Lymphocytic Leukemia Treatment-Naive Patients

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Introduction

NGS approaches have transformed our understanding of the genetic heterogeneity of CLL. However, the detection of somatic mutations and their relative frequencies is variable, which possibly reflects differences in the composition of the cohorts studied worldwide and the time point during the course of the disease when they were tested.

Considering the landscape of genomic alterations recently identified in CLL as well as the availability of new targeted therapies, it is warranted to accurately predict outcome at the individual patient level.

An international prognostic index (CLL-IPI) and the molecular index (M-IPI) are promising scoring systems to improve the precision of prognostic counseling.

The aims of the study were:

1) to characterize CLL patients not previously treated according to their biological profile and their impact on the prognosis
2) to test the hierarchical of both stratification models in general practice.

Methods

- We included all patients diagnosed of CLL according to the NCIWG guidelines in our institution between 1986 and 2016 with available DNA for MiSeq sequencing.
- Amplification and sequence analysis of IGH rearrangements were performed on either gDNA or cDNA according to the updated ERIC recommendations.
- A custom panel designed by Sequencing Multiplex was used to investigate somatic mutations with MiSeq platform. It included the following genes; TP53 (all), BIRC3 (ex 7–9), SF3B1 (ex 14–16), MYD88 (ex 5), and NOTCH1 (ex 34), ATM (all), XPO1 (ex 15-16), EGR2 (ex 2), POT1 (ex 4-9) and NFKBIE (ex 1).
- Clinical and biological data were extracted from medicaL.
- Times to first-treatment (TFT) curves were plotted using the Kaplan-Meier method and compared by the log-rank test.

Results

- 205 CLL patients were included. The median age was 64 years (range, 32–93) with a 1.2:1 ratio of males to female.
- The main clinical characteristics are detailed in **Table 1.** The median follow-up was 76 (1-295) months.

Table 1: Associations between gene mutations, clinical and biological variables

Variable			*ATM (n=61, 29,8%)*		*TP53 (n=30, 14,6%)*		*NOTCH1 (n=26, 12,7%)*		*NFKBIE (n=24, 11,7%)*		*SF3B1 (n=22, 10,7%)*		*XPO1 (n=18, 8,8%)*		*EGR2 (n=13, 6,3%)*		*BIRC3 (n=7, 3,4%)*		*POT1 (n=6, 2,9%)*		*MYD88 (n=2, 1%)*	
			%	P-value	%	P-value	%	P-value	%	P-value	%	P-value	%	P-value	%	P-value	%	P-value	%	P-value	%	P-value
Binet	A	n=166, 83%	23	0,38	11	0,13	11	0,81	10,5	0,89	7,5	0,14	7	0,54	5	0,55	2,5	0,41	3	0,26	1	0,52
	B	n=34, 17%	6		4		2		2		3		2		1,5		1		0		0	
	C																					
ECOG	0	n=168, 84,4%	24,1	0,77	11,6	0,20	12,1	0,23	11,1	0,60	8	0,57	8,5	0,22	6	0,42	2,5	0,33	3	0,29	1	0,54
	>0	n=31, 15,6%	5		3,5		1		1,5		2		0,5		0,5		1		0		0	
Age	≤65 years	n=110, 55%	16	0,98	8	0,84	**4,5**	**0,025**	7,5	0,59	6,5	0,50	6	0,28	5	1	1,5	0,51	1	0,28	1	0,20
	>65 years	n=90, 45%	**13**		7		**8,5**		5		4		3		1,5		2		2		0	
IGVH status	M	n=85, 41,9%	**9,4**	**0,042**	**3**	**0,009**	3,9	0,22	4,4	0,52	**2,5**	**0,054**	**1**	**0,006**	**1**	**0,045**	1	0,47	**0**	**0,035**	1	0,09
	UM	n=118, 58,1%	**20,7**		**11,8**		8,9		7,9		**8,4**		**7,9**		**5,4**		2,5		**3**		0	
LDH value	normal	n=171, 88,3%	**9,8**	**0,035**	13,8	0,75	10,7	0,20	10,2	0,84	10,7	0,27	8,7	0,39	4,6	0,14	2,6	0,16	3,1	0,36	0,5	0,72
	increased	n=23, 11,7%	**26.1**		1,5		2,6		1,5		0,5		0,5		1,5		1		0		0	
b-2-m	Normal	n=150, 76,5%	21,9	0,73	**9,7**	**0,06**	**7,7**	**0,015**	7,7	0,17	9,7	0,25	7,1	0,9	5,1	0,57	2,6	0,75	2	0,56	0	0,07
	increased	n=46, 23,5%	6,1		**5,6**		**5,6**		4,1		1,5		2		1		1		1		0,5	
ZAP70	≤20%	n=54, 38,3%	12,1	0,84	5,7	0,57	2,8	0,069	5	0,80	4,3	0,64	2,8	0,43	1,4	0,43	0	0,07	0,7	0,39	NV	
	>20%	n=87, 61,7%	18,4		7,1		11,3		7,1		8,5		7,1		4,3		3,5		2,8			
CD38	≤30%	n=109, 63,7%	15,2	0,78	7	**0,03**	**5,8**	**0,004**	7	0,71	**5,8**	**0,056**	5,3	0,75	4,1	0,67	2,3	0,71	2,3	0,88	0,6	0,45
	>30%	n=62, 36,3%	9,4		**10,5**		**9,4**		4,7		**7**		3,5		1,8		1,8		1,2		0	
del(17p13)	No	n=179, 94,2%	26,3	0,96	**12,1**	**<0,0001**	11,6	0,57	12,1	0,61	10	0,87	8,4	0,31	**5,3**	**0,006**	3,2	0,54	3,2	0,54	1,1	0,73
	Yes	n=11, 5,8%	1,6		3,2		1,1		1,1		0,5		1,1		**1,6**		0		0		0	

- In our cohort, 166 (83%) patients were in Binet A, and 34 (17%) Binet B-C. Concerning Rai stage 123 (61,5%) were Rai 0 and 77 (38,5%) Rai I-IV. In 118 out of 203 (58,1%) IGVH was germline.
- The most frequent cytogenetic abnormalities was del(13q) in 61 (32,1%), following +12 in 39 (10,5%), del (17p) in 11 (5,9%) and del(11q) in 8 (4,2%) and 17 missed data.
- Regarding the molecular data, 61 (29,8%) carried *ATM* mutation, 30 (14,6%) harbored a *TP53* mutation, 26 (12,7%) *NOTCH1*, 24 (11,7%) *NFKBIE*, 22 (107 (3,4%) *BIRC3*, 6 (2,9%) *POT1 and* 2 (1%) carried a *MYD88* hotspot p.L265P mutation.
- Among the 40 patients with *TP53* disruption, 29 had *TP53* mutations, 11 had only del(17p) and 6 had both of them.
- At least 1 mutation was identified in 109 of 171 (63,7%) patients; 60 (35,1%) patients had 1, 34 (19,9%) had 2, 13 (7,6%) had 3, and two cases had 4 and 5 mutations respectively.
- Frequencies and distributions of mutations, genomic aberrations, IGHV status and need of treatment are illustrated in **Figure 1.**
- Cooccurrence of genomic aberrations with mutations for all patients are shown in **Figure 2**.

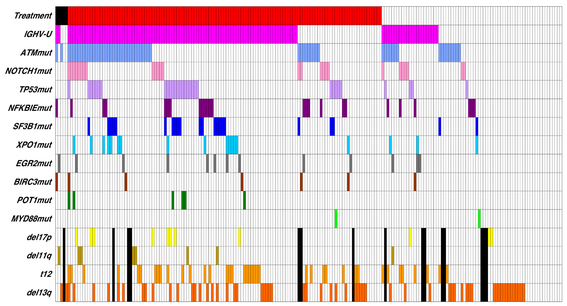
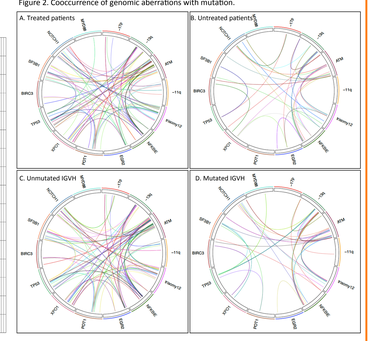
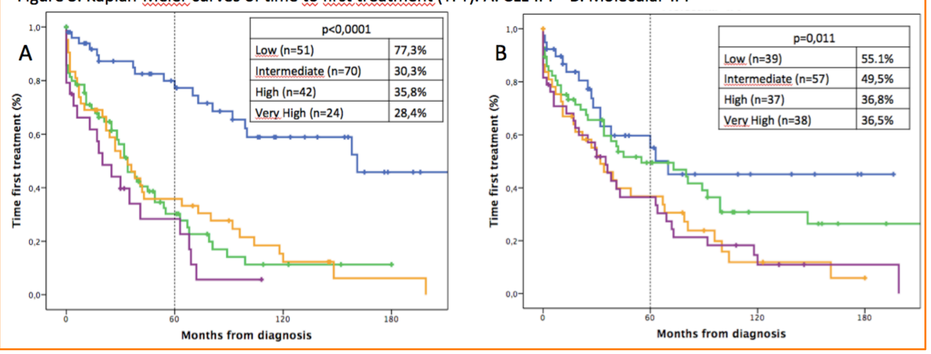


Figure 2. Cooccurrence of genomic aberrations with mutation.



- Most frequent chemotherapy schedules were: Fludarabine-cyclophosphamide-rituximab (FCR) 39 (36,1%), Chlorambucil 36 (33,3%), bendamustine-rituximab 26 (24,1%), Obinutuzumab-Rituximab 4 (2%) and Ibrutinib 3 (1,5%). Five patients transformed into Richter syndrome.
- Either, IPI-CLL and molecular-IPI identified 4 groups with significantly different outcome in terms of TFT although this difference was marginal in the high and very high categories. Detailed in **Figure 3**.
- In our series, IPI-CLL discriminated better than molecular-IPI, (p<0,0001 and 0.011, respectively).

Figure 3. Kaplan-Meier curves of time to first treatment (TFT). A. CLL-IPI B. Molecular-IPI



Conclusions

- In summary, in our cohort , gene mutations frequencies were very similar to those previously described in the literature.
- Our study highlights that both CLL-IPI and M-IPI are useful tools for real-life practice as both identified four group of patients with significantly different TFT times.
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