Genomic characterization of chronic lymphocytic leukemia patients with *TP53* alterations from a Spanish real-world cohort treated with targeted agents

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1 INTRODUCTION

- *TP53* alterations [17p13 deletions (*TP53*^{del}) and *TP53* mutations (*TP53*^{mut})] are adverse prognostic factors in chronic lymphocytic leukemia (CLL). Double-hit cases (*TP53*$^{del+mut}$ or multiple *TP53*^{mut}) have a particularly poor prognosis (Brieghel et al., 2021)
- Complex karyotype (CK, ≥3 chromosomal alterations) predicts worse outcome in chemoimmunotherapy (CIT)-treated patients. Low/intermediate CK (3-4 alt.) worsens evolution only in *TP53*-altered cases, while high CK (≥5 alt.) confers an aggressive outcome independently of other risk factors (Baliakas et al., 2019).
- The impact of CK in patients treated with targeted therapies is under analysis (Kang et al., 2024). Although CK associates with *TP53* alterations, the link between *TP53*-alteration type and genomic complexity remains unclear.

2 OBJECTIVES

1. To characterize the genomic profile of *TP53*-altered CLL patients in a Spanish real-world cohort treated with targeted agents (COMPLEX project).
2. To describe numerical/structural alterations in the *TP53* genomic region and genomic complexity in a subgroup by optical genome mapping (OGM).

3 PATIENTS AND METHODS

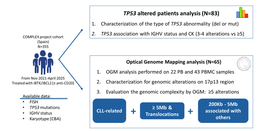

Figure 1. Workflow of the study: Overview of the methodological steps including patient selection, data collection, TP53 status assessment and OGM analysis.

4 RESULTS

1. *TP53*-altered patient distribution (Figure 2):

2. Genomic characteristics of *TP53*-altered patients (N=83) (Figure 3):

- CK was found in 40 (48%) patients, including 31 (37%) high CK **(Table1).**
- Double-hit *TP53* vs single-hit *TP53:*
  - High CK was more frequent in double-hit *TP53* patients (50% vs. 27%, p=0.018).
  - No differences on IGHV status (71% vs. 60% unmutated, p=0.326).

3. OGM analysis (N=65)

- **Cytogenomic characterization of the 17p13 deletion (Figure 4):**
  - OGM detected the deletion in 33 (80%) patients.
  - In eight cases, deletion was undetected due to infiltration below the method's sensitivity threshold (<20%)
  - The median size of the deletion was 17Mb [range: 480Kb-25.1Mb].
  - Associated with structural alterations in 8/33 (24%):
    - As simple translocation (n=1)
    - As a complex three-way translocation (n=1)
    - As a 17q gain compatible with isochromosome 17q (n=6)
- **Genomic complexity by OGM (GC):**
  - Identified in 43/65 (66%) → 34/43 (79%) also CK by conventional cytogenetics.
  - GC was more frequent in double-hit than in single-hit *TP53* groups (81% vs. 51%, p=0.011).
  - Chromoanagenesis in 22/65 (34%) cases, mostly in double-hit *TP53* (77%, 17/22).
    - Chromothripsis was observed in 20 cases; one also had chromoanasynthesis, and three showed chromoplexy.
    - Chromoplexy represented the only chromoanagenesis event in two cases

Table 1. Clinical and biological characteristics of the 83 patients with TP53 abnormalities.

	Double hit *TP53*	Single hit	
	(N=37)	*TP53*^{mut} **(N=25)**	*TP53*^{del} **(N=21)**
Men	22/37 (59%)	19/25 (76%)	14/21 (67%)
Median age (years)	72 [59-89]	71 [46-88]	65 [44-85]
Clinical stages			
Binet A	12/34 (35%)	5/25 (20%)	2/21 (10%)
Binet B/C	22/34 (65%)	20/25 (80%)	19/21 (90%)
Genomic alterations			
***TP53* abnormalities**			
Median of % nuclei with del(17)[p13]*	61 [11-98]*	-	13 [7-94]
Median of % *TP53* mutation VAF **	29 [2-91]**	9 [1.1-69]	-
FISH			
del(13)(q14)	24/35 (69%)	14/25 (56%)	14/21 (67%)
Trisomy 12	5/37 (13%)	6/25 (24%)	7/21 (33%)
del(11)(q22q23)	4/37 (11%)	6/25 (24%)	4/21 (19%)
Unmutated IGHV	23/32 (72%)	18/24 (75%)	11/21 (52%)
CK(≥ 3 abn.)	23/36 (64%)	9/24 (38%)	8/21 (38%)
CK-low/intermediate (3-4 abn.)	4/36 (11%)	3/24 (13%)	2/21 (10%)
CK-high (≥ 5 abn.)	19/36 (53%)	6/24 (25%)	6/21 (28%)
Treatment			
Type of treatment			
Ibrutinib	8/37 (21%)	5/25 (20%)	9/21 (43%)
Acalabrutinib	14/37 (38%)	4/25 (16%)	6/21 (29%)
Zanubrutinib	4/37 (11%)	6/25 (24%)	2/21 (9%)
Venetoclax	1/37 (3%)	1/25 (4%)	-
Venetoclax + obinutuzumab	1/37 (3%)	3/25 (8%)	3/21 (14%)
Venetoclax + rituximab	8/37 (21%)	6/25 (24%)	-
Ibrutinib + venetoclax	1/37 (3%)	1/25 (4%)	1/21 (5%)
Line of treatment			
First-line	22/37 (59%)	14/25 (56%)	13/18 (71%)
Second-line	6/37 (16%)	6/25 (24%)	6/21 (29%)
Third-line	4/37 (11%)	3/25 (20%)	-
≥3 lines	5/37 (14%)	-	-
Prior chemoimmunotherapy treatment	10/37 (27%)	9/25 (36%)	4/21 (19%)
Follow-up			
Median follow-up (months)	17 [0-60]	9.5 [0-72]	15 [1-40]
End of treatment	10/35 (29%)	7/25 (32%)	9/21 (42%)
Fixed-duration therapy	9/37 (24%)	5/25 (20%)	3/21 (14%)
Discontinuation by CLL progression	3/37 (8%)	-	2/21 (9%)
Discontinuation by toxicity, intolerance or other	3/37 (8%)	2/25 (12%)	7/21 (33%)
Exitus	6/37 (16%)	3/25 (12%)	2/21 (10%)
Cause			
CLL progression	2/6 (33%)	1/3 (33%)	-
Infection	-	1/3 (33%)	2/2 (100%)
Second neoplasm	-	1/3 (34%)	-
Others	4/6 (66%)	-	-

**del(17)[p13] identified in 33 patients*
*** A VAF of the most frequently identified mutation is indicated*

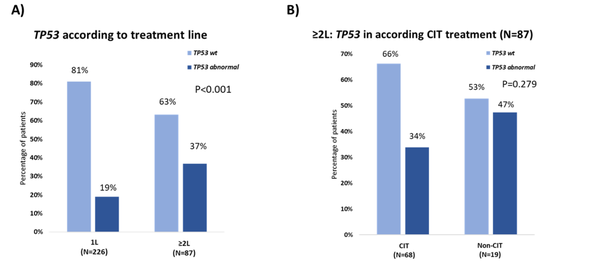

Figure 2. Distribution of patients by *TP53* status and line of treatment (A), and by chemoimmunotherapy exposure (B).

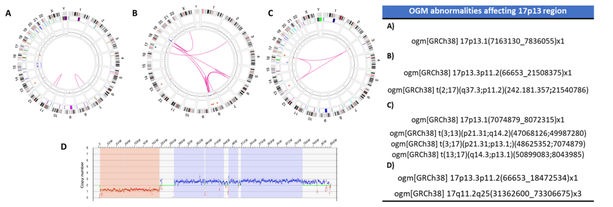

Figure 4. Examples of the OGM results. (A) Patient with a simple 17p13 deletion; (B) Patient with a 17p13 deletion associated with translocation affecting 2q37.3; (C) Patient with a 17p13 deletion associated with a complex translocation [t(3;13;17)(p21;q14.2;p13.1)]; (D) Patient with a 17p13 deletion associated with a gain of 17q region (probably corresponding to i(17q)).

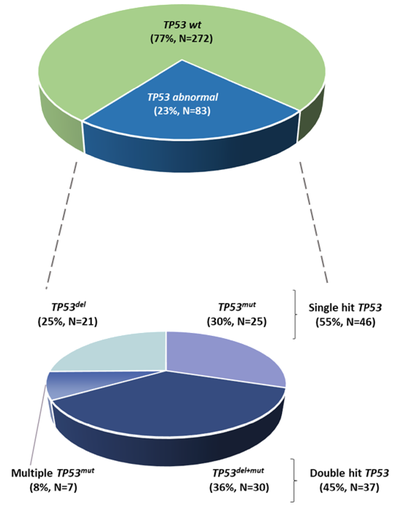

Figure 3. Representation of *TP53*-alterred patients in the global cohort and classification of 83 *TP53* altered patients according to the type of *TP53* alteration.

5 CONCLUSIONS

1. The COMPLEX cohort is enriched in *TP53*-altered patients, mainly *TP53*$^{del+mut}$, who showed higher frequencies of high CK and chromoanagenesis.
2. Deletions in 17p13 are often large and generally not associated with other 17p structural alterations, though mostly found in a complex genomic context.
3. Although OGM is a robust technique that allows the identification and characterization of 17p13 deletions, FISH should be maintained to detect minor deleted clones.

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