Ibrutinib in combination with rituximab for indolent clinical forms of mantle cell lymphoma (IMCL-2015): a multicentre, open-label, single-arm, phase 2 trial

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SUPPLEMENTAL MATERIAL

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Supplemental Figure S1. PFS from the time of ibrutinib stop in patients in response and sustained undetectable MRD

PFS, progression-free survival; MRD, minimal residual disease

Supplemental Figure S2. PFS according to MRD status at PB (Cycle 12) (*P*=.063)

PFS, progression-free survival ;MRD, minimal residual disease; PB, peripheral blood

Supplemental Figure S3. **A)** OS by MIPI (*P*= .007) and **B)** OS by *TP53* mutational status (*P*= .0002)

OS, overall survival; MIPI, Mantle cell lymphoma International Prognostic Index; wt, wild-type; mut, mutated

1.- Description of genetic analyses performed in the IMCL-2015 study

Cytogenetic studies were obtained from 72-hour cultures with TPA, karyotypes were described according to the International System for Human Cytogenetic Nomenclature, and a complex karyotype (CK) was defined by the presence of at least three clonal abnormalities. FISH analyses were performed using XL t(11;14) CCND1/IGH dual fusion and XL CCND1 dual color break-apart probes (MetaSystems). Copy number alterations (CNA) were analyzed using Cytoscan and Oncoscan arrays (Thermofisher) for fresh and FFPE samples, respectively, and a high genomic complexity was defined as more than 5 CNA. *TP5*3 mutational analysis was retrieved from whole-genome and whole-exome sequencing of tumor/normal samples from fresh and formalin-fixed paraffin-embedded samples, respectively. All genetic analyses were done as previously described (Nadeu F, et al. Blood 136:1419-1432, 2020). cMCL and nnMCL gene expression signatures were studied using the L-MCL16 assay (NanoString Technologies) (Clot G, et al. Blood 132:413-422, 2018; Nadeu F, et al Blood 136:1419-1432, 2020)

2.- Treatment discontinuation events and relationship with IR combination

Focusing on the 10 patients (20%) having an event different from disease progression, in 6 cases a clearly related toxicity with ibrutinib treatment was observed (12%) leading to early discontinuation from the study in two of this cases (4%). This is in the range of what has been described with the use of ibrutinib with rituximab combination. In addition, the most serious complication was the severe aplastic anemia observed at 5.5 months of IR therapy. This patient had a normal bone marrow biopsy at screening with only a minimal involvement by MCL detectable by flow citometry and had not previously presented cytopenias related to the treatment neither required ibrutinib dose reduction. The detection of the aplastic anemia was done after the detection of an isolated thrombocytopenia that rapidly evolved to pancytopenia. The patient was in CR by CT scan and with no evidence of MCL by flow cytometry or molecular methods. We have not found previous reports of such a complication, at the best of our knowledge, and taking into account the baseline characteristics and the onset of the aplastic anemia in this patient it cannot be completely ruled out a sporadic coincidence instead of a treatment-related association (considered in the study as a SUSAR). For the other 3 events appearing under therapy, pancreatic adenocarcinoma (also considered SUSAR), ischemic stroke and vertebral fractures they were considered to be unrelated events to the treatment by the local PI.

3.- Table S1. Histological review according to the type of sample: bone marrow and lymph node or other samples

Patients and samples (N= 49)	
Cytological variant (%)	
Small cell	19 (39%)
Classical	30 (61%)
Cyclin D1 expression (%)	
Positive	48 (98%)
Negative*	1 (25)
Bone marrow samples (N=28)	
Pattern of infitration (%)	
Interstitial	21 (75%)
Interstitial, nodular	6 (21%)
Nodular	1 (4%)
Degree of infiltration (%)	
Extensive	13 (50%)
Moderate	9 (35%)
Focal	4 (15%)
SOX11 expression (N= 27)	
Negative	16 (59%)
Positive	11 (41%)
Ki67 expression (N=24)	
Low (< 30%)	24 (100%)
High (≥30%)	0 (0%)
TP53 expression (N=11)	
Negative	11 (100)
Positive(#)	0 (0%)
LN or other extranodal samples (N= 21)	
Pattern of infiltration (N= 21)	
Mantle zone	2 (10%)
Nodular	7 (33%)
Diffuse	5 (24%)
Combined&	6 (28%)
..NA (small biopsy)	1 (5%)
SOX11 expression (N=21)	
Negative	1(5%)
Positive	20 (95%)
Ki67 (N=21)	
Low (< 30%)	19 (90%)
High (≥30%)&	2 (10 %)

NOTE. Samples reviewed and listed in the table: BM (N= 28), lymph node (N=17), tonsil (N=2), colon (N=1), conjunctive (N=1). Insufficient material for MCL diagnosis confirmation (N=1, BM)
Abbreviations: LN, lymph node; NA, not available.
(*) One case had a *CCND2* translocation and overexpression
(#) Three *TP53* mutated cases had a negative TP53 expression (1 missense and 2 truncating mutations)
(&)Combined pattern: Nodular/marginal zone (3 cases); Nodular/diffuse (2 cases) and Marginal/diffuse (1 case); & High Ki67: 34% and 50% each

4.- Table S2. Clinical and biological features according to *TP53* mutational status

Features	***TP53* mutated (N=6)**	***TP53* wild-type(N= 35)**	***p***
Median age, years (range)	71 (65-75)	63 (41-84)	ns
Sex Male Female	 5 (83%) 1(17%)	 24 (69%) 11 (31%)	 ns
ECOG 0-1	6 (100%)	35 (100%)	ns
Spleen size (cm) (median, range)*	16.5 (10-29)	13.5 (9-25)	ns
Lymph node size (mm)* No enlarged and no FDG uptake Longest diameter, median (range)	 1 (17%) 28 (14-43)	 8 (23%) 21 (13-23)	 ns
Bone marrow involvement	5 (83%)	34 (97%)	ns
Ann Arbor I-II III-IV	 1 (17%) 5 (83%)	 0 (0%) 35 (100%)	 ns
WBC count (x 10^9/L) (median, range)	38.6 (7·4-126)	12.7 (4-111)	ns
PB involvement by flow cytometry	5 (83%)	33 (94%)	ns
Hemoglobin (< 110 g/L)	0 (0%)	2 (6%)	ns
Platelet count (< 100 x 10^9/L)	0 (0%)	4 (11%)	ns
Serum LDH (> ULN)	0 (0%)	4 (11%)	ns
Serum B2-microglobulin (> ULN)	4 (67%)	15 (43%)	ns
MIPI Low risk Intermediate risk High risk	 0 (0%) 1 (17%) 5 (83%)	 9 (26%) 15 (43%) 11 (31%)	 ns
Observation pre-treatment (months)	10 (4-25)	8 (3-126)	ns
Complex karyotype	5/5 (100%)	4 (25%)	***0.006***
High genomic complexity (>5)	5/6 (83%)	9 (28%)	***0.018***
Del17p/LOH	5/6 (83%)	1/32 (3%)	***< 0.001***
L-MCL-16 nnMCL cMCL	 3/5 (60%) 2/5 (40%)	 14 (58%) 10 (42%)	 ns

NOTE. Data are n(%) or median (range). Abbreviations: ECOG, Eastern Cooperative Oncology Group; FDG, ^{18}F-fluorodeoxyglucose; WBC, white-blood cell; PB, peripheral blood; LDH, lactate dehydrogenase; MIPI, Mantle Cell Lymphoma International Prognostic Index; *CCND1*, cyclin D1; *CCND2*, cyclin D2; LOH, loss of heterozygosity; nnMCL, non nodal MCL molecular subtype; cMCL, conventional MCL molecular subtype.

*Spleen and lymph node measurements according to centralized PET-CT review

5.- Table S3. Clinical and biological features according to nnMCl or cMCL molecular subtypes

Patients (N= 31)	nnCML (N= 17)	cMCL (N=14)	*p*
Median age, years (range)	62 (44-76)	68 (41-84)	***0.049***
Sex Male Female	 12 (71%) 5 (29%)	 10 (71%) 4 (29%)	 ns
ECOG 0-1	17 (100%)	14 (100%)	ns
Spleen size (cm) (median, range)	16 (9-29)	12.5 (9-22)	ns
Lymph node size (mm) No enlarged and no FDG uptake Longest diameter, median (range)	 6 (35%) 19 (13-28)	 3 (21%) 21 (17-28)	 ns
Bone marrow involvement	17 (100%)	12 (86%)	ns
Ann Arbor I-II III-IV	 0 (0%) 17 (100%)	 1 (7%) 13 (93%)	 ns
WBC count (x 10^9/L) (median, range)	25.5 (8-126)	12.8 (4-49)	ns
PB involvement by flow cytometry	17 (100%)	11 (79%)	0.08
Hemoglobin (< 110 g/L)	1 (6%)	1 (7%)	ns
Platelet count (< 100 x 10^9/L)	1 (6%)	1 (7%)	ns
Serum LDH (> ULN)	1 (6%)	1 (7%)	ns
Serum B2-microglobulin (> ULN)	7 (47%)	8 (66%)	ns
MIPI Low risk Intermediate risk High risk	 2 (12%) 7 (41%) 8 (47%)	 3 (21%) 4 (29%) 7 (50%)	 ns
Observation pre-treatment (months)	17 (4-126)	10 (3-7)	ns
Complex karyotype	5 (38%)	3 (75%)	ns
High genomic complexity (>5)	4 (24%)	6 (55%)	ns
Del17p/LOH	3 (18%)	2 (20%)	ns
TP53 mutation	3 (18%)	2 (17%)	ns

NOTE. Data are n(%) or median (range). Abbreviations: ECOG, Eastern Cooperative Oncology Group; FDG, ^{18}F-fluorodeoxyglucose; WBC, white-blood cell; PB, peripheral blood; LDH, lactate dehydrogenase; MIPI, Mantle Cell Lymphoma International Prognostic Index; *CCND1*, cyclin D1; *CCND2*, cyclin D2; LOH, loss of heterozygosity; nnMCL, non nodal MCL molecular subtype; cMCL, conventional MCL molecular subtype.

*Spleen and lymph node measurements according to centralized PET-CT review

6. Table S4. Responses after 12 cycles of IR combination and according to genomic complexity

	All patients (N= 50)	CNA(N=40)	
Responses		**CNA (0-5) (N=25)**	**CNA (+5) (N=15)**
Overall response	42 (84%, 74-94)	31 (88%)	13 (87%)
Complete response	40 (80%, 69-91)	20(80%)	13 (87%)
Partial response	2 (4%, 0-9)	2 (8%)	0
Stable disease	3 (6%, 0-10)	3 (12%)	0
Progressive disease	1 (2%, 0-6)	-	1 (6.5%)
Non-evaluable	4 (8%, 0-15)		1 (6.5%)

NOTE. Data are n (%, 95% CI). Abbreviations: IR, ibrutinib- rituximab combination, CNA, copy number alterations.

* Four patients were non-evaluable at 12 months of treatment because treatment discontinuation: severe aplastic anemia, skin rash, withdrawal consent because of treatment intolerance and unrelated event with vertebral fractures

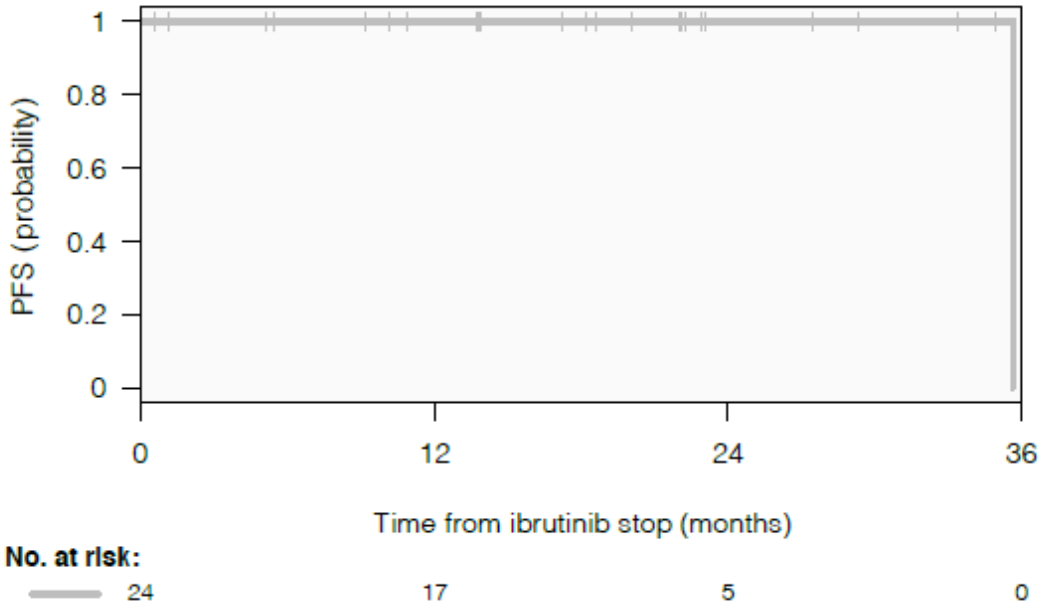
7.- Table S5: Molecular response in peripheral blood and bone marrow at 6,12, 18 and 24 months

MRD	**6 months**		**12 months**		**18 months**		**24 months**	
	PB (n=44)	BM (n=33)	PB (n=46)	BM (n=43)	PB (n=38)	BM (n=2)	PB (N=37)	BM (n=6)
Undetectable	30	16	40	28	34	1	29	3
Detectable	14	17	6	15	4	1	8	3
Molecular remission (%)	68%	48%	87%	65 %	89%	50%	78%	50%

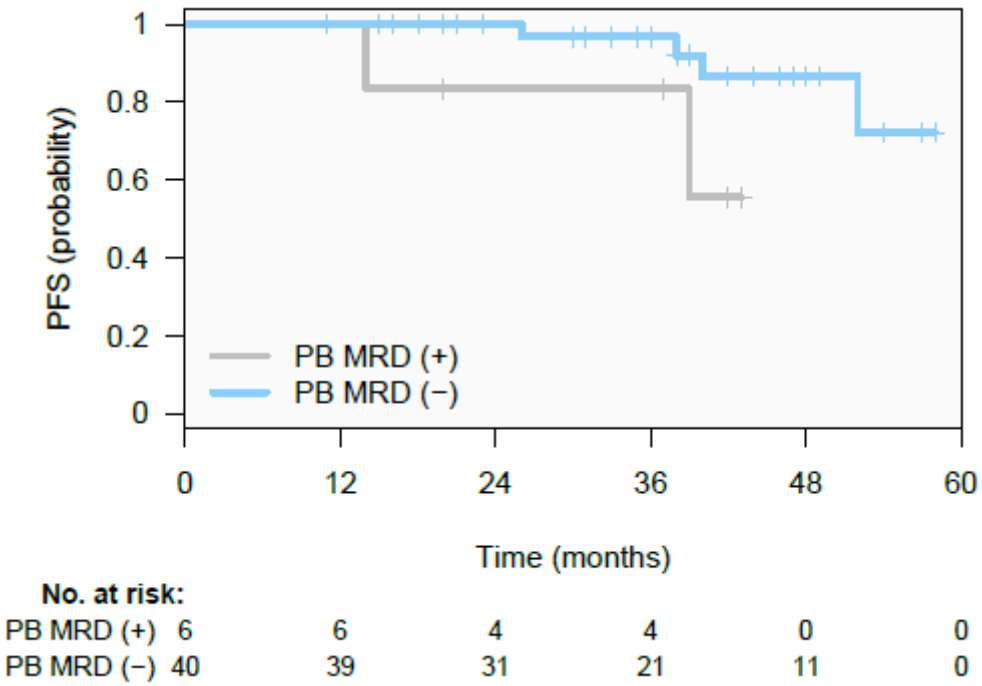
Regarding MRD studies, all 50 patients had a clonal marker identified in 75 basal samples and were evaluable for MRD. Two hundred and forty-nine follow-up samples were finally analyzed in 46 evaluable patients. ASO-RT-PCR and NGS were applied in 30 and 16 patients, respectively. Minimal sensitivity achieved was ≥ 10^{-5}, $5 \cdot 10^{-5}$ and 10^{-4} for the MRD evaluations of 37 (80%), 6 (13%) and 3 (7%) patients, respectively. All samples analyzed by NGS were assessed with a minimum sensitivity of 10^{-5}. Evaluation of the MRD status was made following the EuroMRD guidelines (van der Velden VH et *al*. Leukemia. 2007 Apr;21(4):604-11. doi:10.1038/sj.leu.2404586).

Abbreviations: MRD, minimal residual disease; PB, peripheral blood; BM, bone marrow

8.- Figure S1. Progression-free survival from the time of ibrutinib stop in patients in response and sustained undetectable MRD



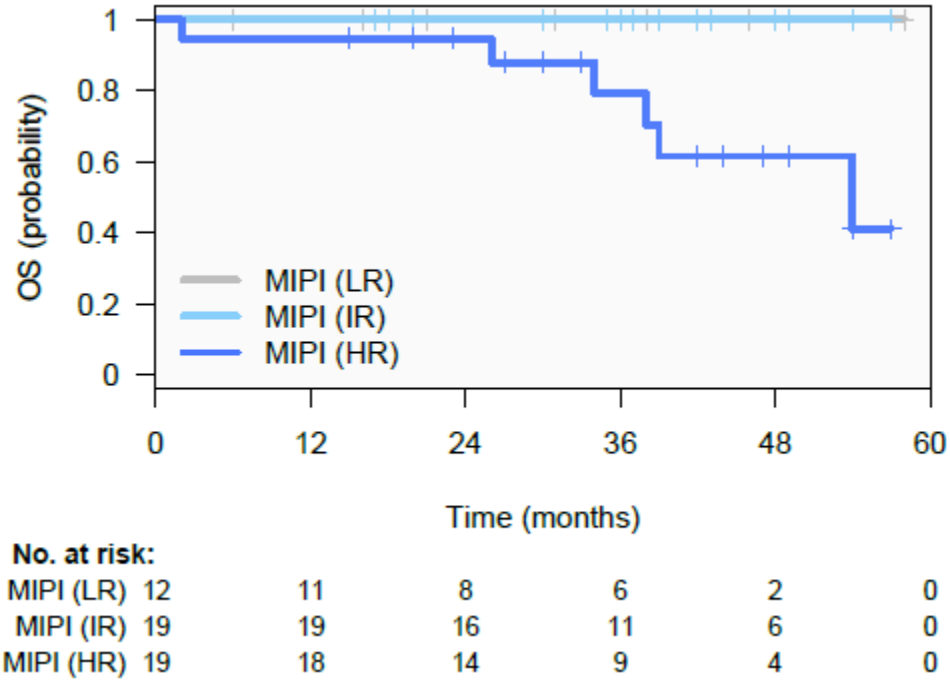
9.- Figure S2. Progression-free survival according to MRD status at PB (Cycle 12)



No. at risk:						
PB MRD (+)	6	6	4	4	0	0
PB MRD (−)	40	39	31	21	11	0

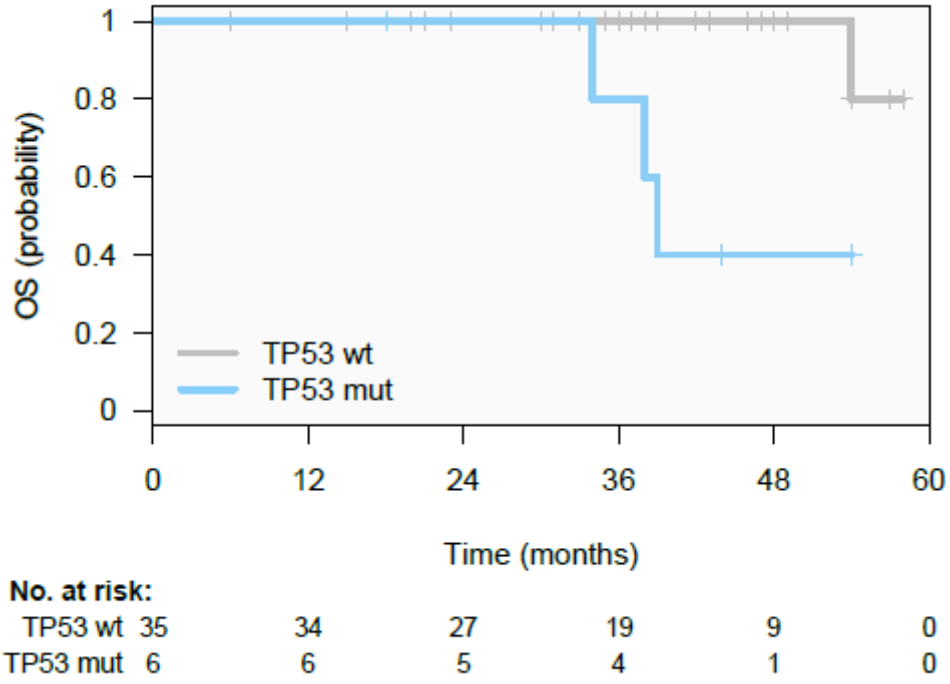
10.- Figure S3. Overall survival by MIPI (A) and *TP53* mutational status (B)

A)



No. at risk:						
MIPI (LR)	12	11	8	6	2	0
MIPI (IR)	19	19	16	11	6	0
MIPI (HR)	19	18	14	9	4	0

B)



No. at risk:						
TP53 wt	35	34	27	19	9	0
TP53 mut	6	6	5	4	1	0