SUPPLEMENTAL METHODS

DNA extraction

Clonal hematopoiesis mutational analysis was carried out on granulocytes (GN) recovered at baseline and post-ASCT timepoints using Ficoll Histopaque (Sigma-Aldrich/Merck, Darmstadt, Germany) stratification on PB samples. Alternatively, when granulocytes were not available, M-CH analysis was performed on mononuclear cells extracted by red blood cell lysis (in NH_4Cl solution, pH 7) in BM samples. (25). Conversely, tumor cells mutational analysis was performed on CD19+ cells samples sorted by immunomagnetic beads (CD19 MicroBeads, human-Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) and stocked as dry pellets at University of Torino. Genomic DNA was extracted using both DNAzol reagent (Life Technologies, Carlsbad, CA, USA) and a Maxwell semiautomated extraction instrument (Promega, San Francisco, CA, USA). (26) DNA quantity (ng) and purity (odds ratio A260/A280 and A260/A230) were evaluated by use of a NanoDrop2000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA), and housekeeping gene (TP53 exon 8) control amplification was performed to check DNA quality.(27)

Minimal residual disease (MRD), flow cytometry and tumor mutational analysis

MRD monitoring was assessed in the lab of Torino University in BM and PB samples by using an allele-specific oligonucleotide real-time quantitative polymerase chain reaction (ASO RQ-PCR) approach (either on immunoglobulin heavy chain (IGH) gene or B-cell lymphoma-1 (BCL-1)/IGH rearrangements) at the indicated time points and evaluated in accordance with the criteria of the EuroMRD standardization group.(18) MFC was performed by a 6-color panel for BM (K/L/CD19/CD23/CD5) and PB (also CD22/CD20/CD43/CD200) on FACSCanto II (BD Biosciences, San Jose, CA, USA).

TP53 disruptions were identified via next-generation targeted sequencing and copy-number alteration analysis on CD19 negative selected tumoral cells from BM baseline samples or as previously described.(28)

Statistics

Statistical analysis was carried out using R software version 4.2.2 (**https://www.r-project.org**) and Rstudio software version 2023.6.0.421 (**http://www.rstudio.com/**). For all statistical analyses, the level of significance was set at $P \leq 0.05$. For survival analysis, PFS and OS were used as clinical end points. PFS was calculated from the date of enrollment in the clinical study to the date of disease progression (event), death from any cause (event), or last follow-up (censoring). OS was measured from the date of enrollment in the clinical study to the date of death from any cause (event) or last follow-up (censoring). Survival was estimated with the Kaplan-Meier method, and the log-rank test was applied to compare the survival distributions of the patients. The Cox proportional hazards model was implemented for univariate and multivariate survival analyses. The outcome data of MCL0208 trial cohort for the present analysis has been updated with a median follow-up of 84 months from enrollment and 74 months for the randomized population (29)

SUPPLEMENTAL FIGURES

Figure S1 Survival plots of MCL0208 patient cohort analyzed vs not analyzed for M-CH mutations.

A) Progression free survival (PFS); **B)** Overall survival (OS)**.**

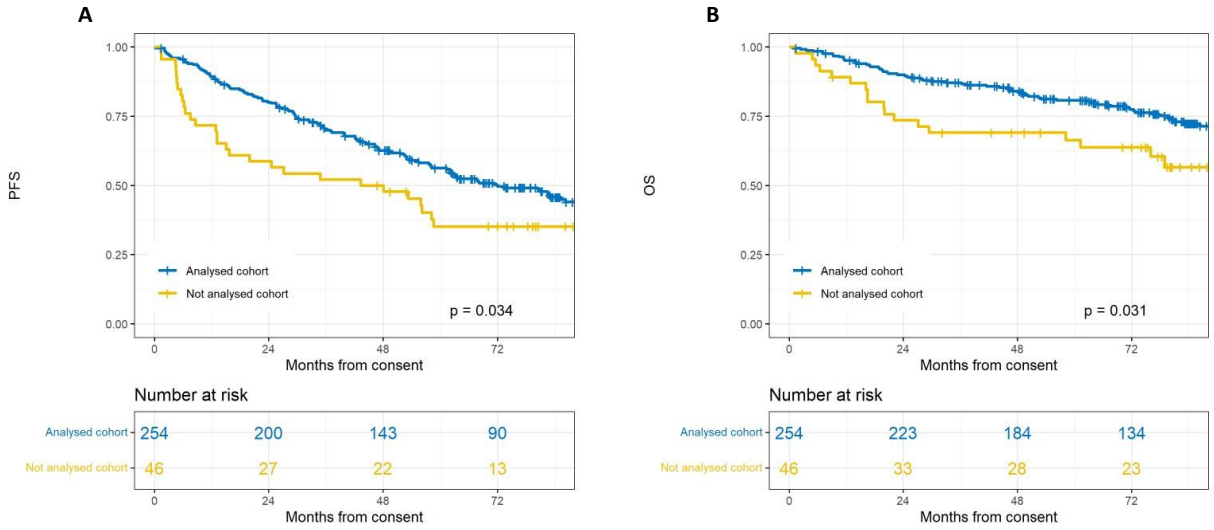


Figure S2 Trajectories of M-CH clones from diagnosis throughout autologous stem cell transplantation. Lines represent variant allele fraction (VAF) trajectories of myeloid clonal hematopoiesis (M-CH) mutation from diagnosis to time-points with 12 months from autologous stem cell transplantation (ASCT). The colors of lines in each box represent different cases.

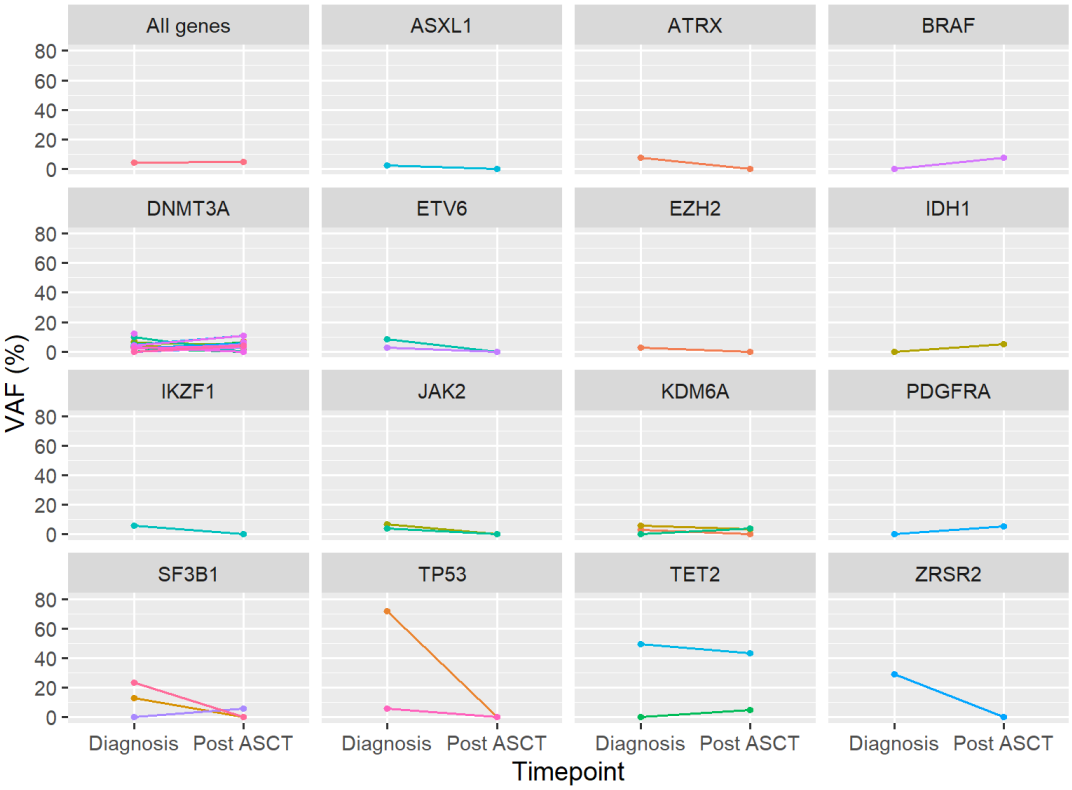


Figure S3 PFS and OS did not differ between M-CH+ and M-CH- patients at baseline. A) Progression free survival (PFS); **B)** Overall survival (OS)**;** CH- patients: patients without any myeloid clonal hematopoiesis (M-CH) mutation with minimum variant allele fraction of 2% at baseline. CH+ patients: patients with at least one myeloid clonal hematopoiesis mutation with minimum variant allele fraction of 2% at baseline.

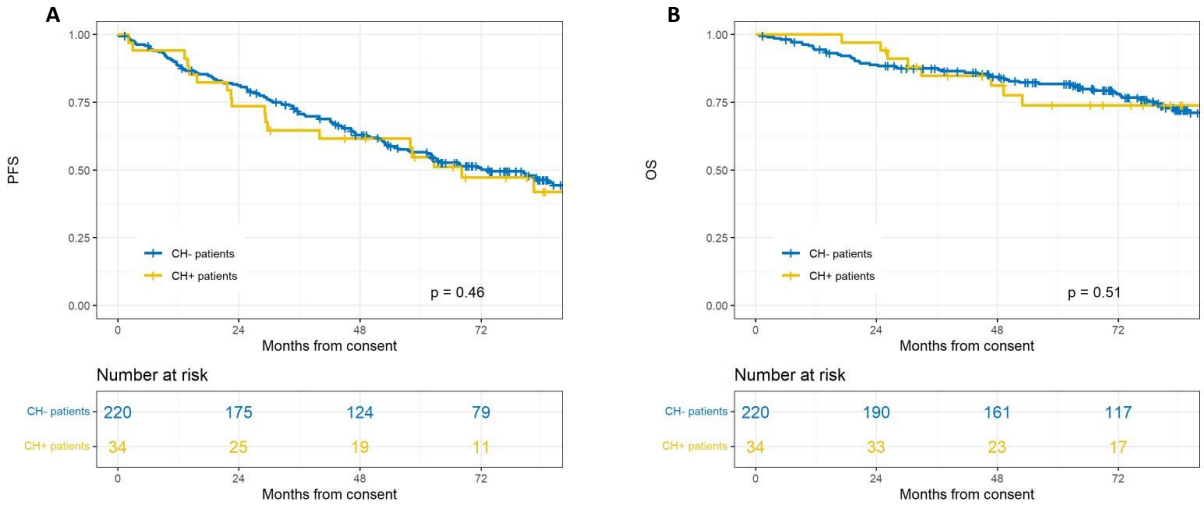


Figure S4 M-CH+ patients within 12 months from autologous stem cell transplantation do not show any difference in PFS and OS compared M-CH- patients. A) Progression free survival from time of ASCT (PFS); **B)** Overall survival from time of ASCT (OS)**;** CH- patients: patients without any myeloid clonal hematopoiesis (M-CH) mutation with minimum variant allele fraction of 2% within 12 months from autologous stem cell transplantation (ASCT). CH+ patients: patients with at least one myeloid clonal hematopoiesis mutation with minimum variant allele fraction of 2% within 12 months from ASCT.

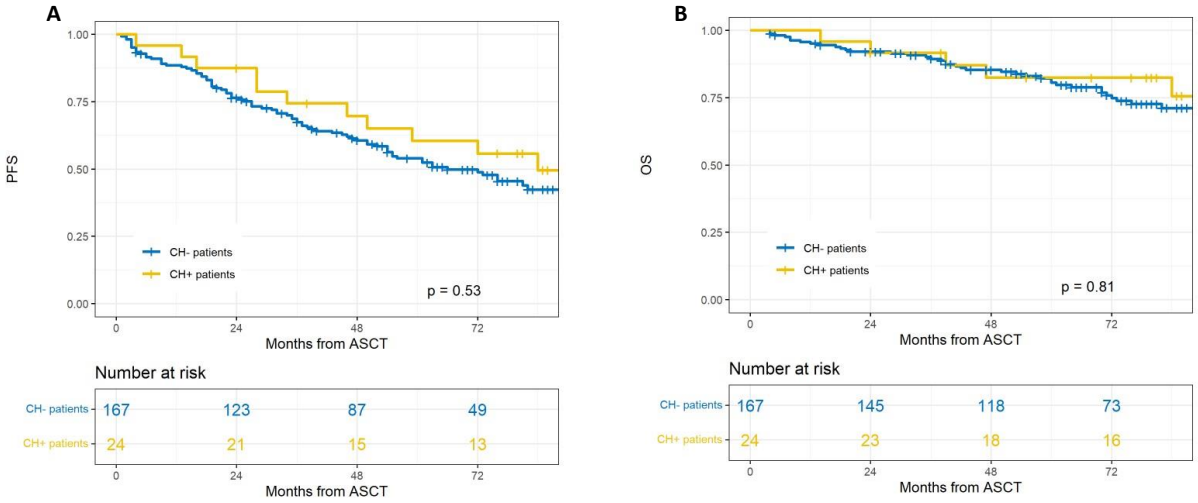
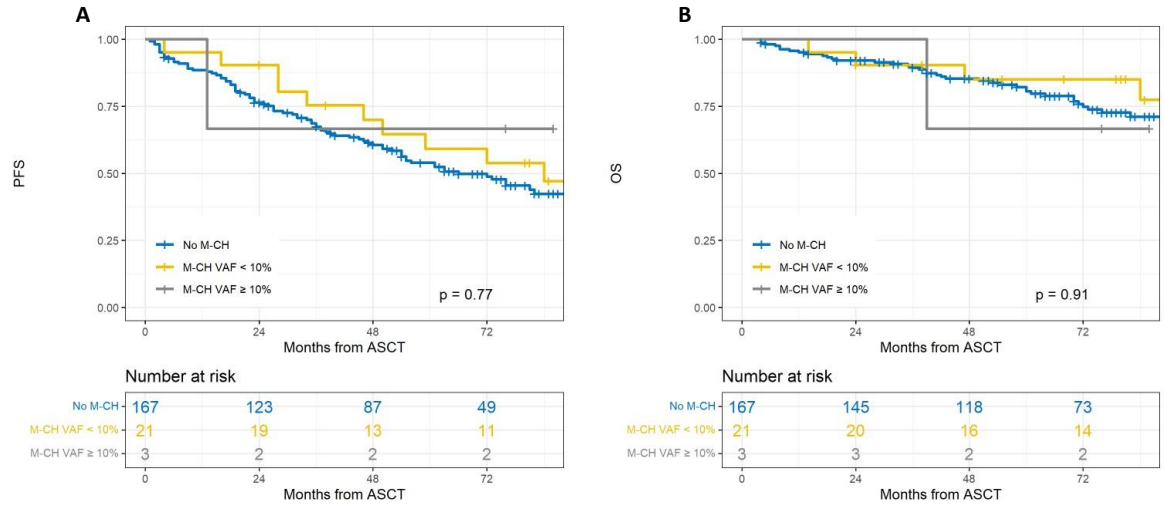


Figure S5 M-CH+ patients carrying clones with VAF ≥ 10% detected post ASCT present similar PFS and OS comparing with M-CH+ patients with VAF 2%-10% and M-CH- patients.



SUPPLEMENTAL TABLES

Table S1 M-CH mutations identified in MCL0208 patients at baseline. Mutations with VAF ≤ 2% were excluded. Furthermore, M-CH variants were classified as "Potential MCL mutation" or not according to M-CH VAF and MCL infiltration in sample as assessed by flow cytometry (see Methods section). MCL: Mantle cell lymphoma; VAF: Variant allele frequency; PB: Peripheral blood; BM: bone marrow.

Case	Potential MCL mutation	Sample type	VAF (%)	Gene	Codon	Protein
1	No	PB	2.1	DNMT3A	c.G1906A	p.V636M
2	Yes	BM	7.71	TP53	c.C742T	p.R248W

4	Yes	PB	28.48	BCOR	c.A4274G	p.N1425S
5	No	PB	2.59	EZH2	c.G647A	p.R216Q
5	No	PB	2.85	KDM6A	c.G3868T	p.E1290X
5	No	PB	7.66	ATRX	c.3737-1G>A	
5	Yes	PB	3.76	BCOR	c.G4330A	p.V1444M
5	Yes	PB	3.95	BCOR	c.A3659G	p.E1220G
5	Yes	PB	36.8	BCOR	c.A4274G	p.N1425S
7	Yes	PB	30.33	TP53	c.A715G	p.N239D
14	No	PB	71.96	TP53	c.C844T	p.R282W
96	Yes	PB	52.7	TP53	c.G670T	p.E224X
98	No	PB	2.65	DNMT3A	c.2323-2A>G	
98	No	PB	3.11	DNMT3A	c.C1204T	p.Q402X
98	No	PB	3.55	DNMT3A	c.C2396G	p.P799R
98	No	PB	6.99	DNMT3A	c.C2099G	p.P700R
104	No	PB	13.11	SF3B1	c.T1972C	p.W658R
197	No	PB	5.92	KDM6A	c.G3332C	p.R1111P
199	Yes	PB	4.66	TP53	c.T584C	p.I195T
207	No	PB	3.51	DNMT3A	c.G2182A	p.G728S
207	No	PB	7.49	JAK2	c.G1849T	p.V617F
299	No	PB	15.68	ASXL1	c.C1773G	p.Y591X
304	No	PB	2.78	DNMT3A	c.G2645A	p.R882H
497	No	PB	4.72	DNMT3A	c.C2309T	p.S770L
497	No	PB	6.64	JAK2	c.G1849T	p.V617F
599	No	BM	6.12	DNMT3A	c.G2645A	p.R882H
799	No	PB	2.17	ASXL1	c.C2077T	p.R693X
999	No	PB	6.07	ETV6	c.C1105T	p.R369W
1002	No	PB	3.83	JAK2	c.G1849T	p.V617F
1006	No	PB	2.19	DNMT3A	c.G1906T	p.V636L
1096	No	PB	2.24	WT1	c.C1321T	p.R441X
1097	No	PB	6.86	DNMT3A	c.T1978C	p.Y660H
1106	Yes	PB	40.47	TP53	c.363delT	p.S121fs
1108	Yes	PB	28.91	BCOR	c.1562delA	p.N521fs

1199	Yes	PB	15.33	TET2	c.C3508T	p.Q1170X
1201	Yes	PB	5.29	TET2	c.C286T	p.R96C
1202	No	PB	2.19	DNMT3A	c.T2308G	p.S770A
1299	No	BM	3.1	DNMT3A	c.C2309T	p.S770L
1299	No	BM	3.82	DNMT3A	c.A1900T	p.I634F
1299	No	BM	4.04	JAK2	c.G1849T	p.V617F
1299	No	BM	9.6	DNMT3A	c.G2259A	p.W753X
1404	Yes	PB	2.54	TP53	c.G743A	p.R248Q
1696	No	PB	25.28	DNMT3A	c.G886A	p.V296M
1697	No	PB	8.17	ETV6	c.A1168G	p.T390A
1799	No	PB	6.08	IKZF1	c.C331T	p.R111X
1799	Yes	PB	5.7	NOTCH1	c.C7375T	p.Q2459X
1900	No	PB	2.39	ASXL1	c.G3195A	p.W1065X
1900	Yes	PB	3.05	BCOR	c.G3973A	p.G1325R
1900	Yes	PB	4.46	BCOR	c.G4421A	p.G1474D
1900	Yes	PB	6.14	BCOR	c.C4385A	p.A1462E
1902	No	PB	49.51	TET2	c.C4165T	p.Q1389X
1997	No	PB	4.58	DNMT3A	c.C1903T	p.R635W
2000	No	PB	2.54	CUX1	c.C2634G	p.Y878X
2096	Yes	PB	41.28	BCOR	c.4834dupC	p.L1612fs
2197	No	PB	2.2	DNMT3A	c.T2444A	p.L815Q
2197	No	PB	2.2	DNMT3A	c.C2446T	p.Q816X
2197	No	PB	28.91	ZRSR2	c.T614C	p.M205T
2197	Yes	PB	17.12	TP53	c.376-1G>C	
2497	Yes	PB	6.93	TP53	c.G422A	p.C141Y
2696	No	PB	2.47	ETV6	c.T1138G	p.W380G
2897	No	PB	2.61	GNAS	c.G602A	p.R201H
2897	No	PB	2.92	DNMT3A	c.T2308C	p.S770P
2897	No	PB	6.89	DNMT3A	c.C2222G	p.A741G
2996	No	PB	11.98	DNMT3A	c.C1551A	p.C517X
2996	No	PB	4.2	DNMT3A	c.G2207A	p.R736H
3099	Yes	PB	3.25	TP53	c.A394G	p.K132E

3298	No	PB	2.97	DNMT3A	c.T2128A	p.C710S
3896	Yes	PB	19.95	TP53	c.T590A	p.V197E
3998	No	PB	2.1	DNMT3A	c.G2053A	p.G685R
4000	No	PB	5.74	TP53	c.T379A	p.S127T
4801	No	BM	23.58	SF3B1	c.G2244C	p.K748N
4801	Yes	BM	17.43	TP53	c.C832A	p.P278T

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114

115 **Table S2 M-CH mutations identified in MCL0208 patients within 12 months of ASCT.** Mutations
116 with VAF $\leq$ 2% were excluded. MCL: Mantle cell lymphoma; VAF: Variant allele frequency; PB:
117 Peripheral blood; BM: bone marrow.

118

Case	Sample type	VAF (%)	Gene	Codon	Protein
1	PB	2.63	DNMT3A	c.G1906A	p.V636M
98	PB	2.3	DNMT3A	c.C1204T	p.Q402X
98	PB	3.8	DNMT3A	c.2323-2A>G	
98	PB	6.02	DNMT3A	c.C2099G	p.P700R
98	PB	6.82	DNMT3A	c.C2396G	p.P799R
109	PB	4.3	DNMT3A	c.G1930A	p.A644T
197	PB	3.36	KDM6A	c.G3332C	p.R1111P
401	PB	5.03	IDH1	c.G395T	p.R132L
401	PB	6.53	DNMT3A	c.C2141G	p.S714C
502	PB	4.9	DNMT3A	c.C1135T	p.R379C
599	PB	4.62	DNMT3A	c.G2645A	p.R882H
796	PB	3.23	DNMT3A	c.G2146A	p.V716I
1002	PB	6.37	DNMT3A	c.G989A	p.W330X
1199	PB	4.78	TET2	c.C3508T	p.Q1170X
1298	PB	3.98	KDM6A	c.A3623G	p.Y1208C
1896	PB	3.96	DNMT3A	c.T1031C	p.L344P

1902	PB	43.35	TET2	c.C4165T	p.Q1389X
1997	BM	10.53	DNMT3A	c.C1903T	p.R635W
2001	PB	5.66	PDGFRA	c.G2470A	p.V824I
2197	BM	5.53	DNMT3A	c.T2444A	p.L815Q
2197	BM	5.95	DNMT3A	c.C2446T	p.Q816X
2198	PB	2.83	DNMT3A	c.G2645A	p.R882H
2497	BM	3.99	DNMT3A	c.C2396A	p.P799H
2598	PB	5.16	DNMT3A	c.G1685A	p.C562Y
2598	PB	5.91	SF3B1	c.A2294G	p.Y765C
2899	PB	7.49	BRAF	c.A1742G	p.N581S
2996	PB	10.78	DNMT3A	c.C1551A	p.C517X
2996	PB	6.65	DNMT3A	c.G2207A	p.R736H
3301	PB	3.19	DNMT3A	c.A2204G	p.Y735C
3998	PB	4.34	DNMT3A	c.G2053A	p.G685R
4797	PB	4.07	DNMT3A	c.G2116A	p.G706R

Table S3 Baseline and follow-up clinical and laboratory features of patients according to presence of M-CH clones at MCL diagnosis (VAF threshold 2%) M-CH+: patients carrying myeloid clonal hematopoiesis mutations with VAF ≥ 2%)**;** M-CH-: patients without myeloid clonal hematopoiesis mutations with VAF ≥ 2%)**;** BMI: body mass index; WBC: white blood cells; LDH: lactate dehydrogenase; ULN: upper limit of normal; MIPI: Mantle Cell Lymphoma International Prognostic; MIPIc: Mantle Cell Lymphoma International Prognostic Index combined; ASCT: Autologous stem cell transplantation; MRD: minimal residual disease, PCR: Polymerase chain reaction; G1-G2: Grade 1 or grade 2 toxicity; G3-G4: Grade 3 or grade 4 toxicity; R-CHOP: Rituximab in combination with cyclophosphamide, doxorubicin, vincristine and prednisone; R-CTX: Rituximab in combination with cyclophosphamide; NEUT: neutrophils; PLTS: platelets.

131

Characteristic	M-CH- patients at diagnosis, N = 220[1]	M-CH+ patients at diagnosis, N = 34[1]	p-value[2]
Gender			**0.051**
Female	52 (24%)	3 (8.8%)	
Male	168 (76%)	31 (91%)	
Age (years)			**0.011**
Median (Range)	56 (32, 66)	60 (46, 66)	
BMI (kg/m^2)			0.2
Median (Range)	24.9 (16.3, 42.2)	26.2 (19.4, 35.5)	
WBC (cells/µL)			>0.9
Median (Range)	7 (1, 196)	7 (3, 106)	
Neutrophils (cell/µL)			0.8
Median (Range)	3.85 (0.18, 22.00)	3.91 (0.12, 15.95)	
NA	1	0	
Lymphocytes (cells/µL)			0.7
Median (Range)	2 (0, 186)	2 (0, 73)	
NA	2	0	
Hemoglobin (g/dL)			0.3
Median (Range)	13.00 (5.80, 16.90)	12.90 (7.00, 16.50)	
Platelets (10^9/L)			0.6
Median (Range)	190 (18, 624)	186 (26, 490)	
NA	0	1	
Histology			
Blastoid	19 (8.6%)	1 (2.9%)	0.5
Classic	201 (91%)	33 (97%)	
Ki67 index			0.5
<30	142 (72%)	21 (66%)	
>=30	56 (28%)	11 (34%)	

NA	22	2	
LDH above ULN			0.8
<ULN	154 (70%)	23 (68%)	
>ULN	66 (30%)	11 (32%)	
MIPI			0.2
high risk	31 (14%)	7 (21%)	
intermediate risk	48 (22%)	11 (32%)	
low risk	141 (64%)	16 (47%)	
MIPIc			0.2
high risk	12 (6.1%)	5 (16%)	
intermediate-high risk	25 (13%)	4 (12%)	
intermediate-low risk	57 (29%)	10 (31%)	
low risk	104 (53%)	13 (41%)	
NA	22	2	
ECOG			0.3
>=1	48 (22%)	10 (29%)	
0	172 (78%)	24 (71%)	
Stage			>0.9
Stage II-III	12 (5.5%)	2 (5.9%)	
Stage IV	208 (95%)	32 (94%)	
Bulky	68 (31%)	13 (38%)	0.4
TP53 status in tumor cells			0,2
Mutation or deletion	22 (15%)	6 (26%)	
WT	121 (85%)	17 (74%)	
NA	77	11	
ASCT			0,4
ASCT not received	26 (12%)	6 (18%)	
ASCT received	194 (88%)	28 (82%)	
Clinical response after ASCT			0,5
CR	173 (90%)	28 (97%)	
PR	12 (6.2%)	0 (0%)	
SD or PD	7 (3.6%)	1 (3.4%)	

NA	28	5	
MRD post ASCT (Nested PCR)			0,5
Negative	81 (53%)	11 (46%)	
Positive	71 (47%)	13 (54%)	
NA	68	10	

[1] n (%)

[2] Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test

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133 **Table S4 Baseline and follow-up clinical and laboratory features of patients according to presence**
134 **of large M-CH clones at MCL diagnosis (VAF threshold 10%)** BMI: body mass index; WBC: white
135 blood cells; LDH: lactate dehydrogenase; ULN: upper limit of normal; MIPI: Mantle Cell Lymphoma
136 International Prognostic; MIPIc: Mantle Cell Lymphoma International Prognostic Index combined;
137 ASCT: Autologous stem cell transplantation; MRD: minimal residual disease, PCR: Polymerase chain
138 reaction; G1-G2: Grade 1 or grade 2 toxicity; G3-G4: Grade 3 or grade 4 toxicity; R-CHOP: Rituximab
139 in combination with cyclophosphamide, doxorubicin, vincristine and prednisone; R-CTX: Rituximab
140 in combination with cyclophosphamide; NEUT: neutrophils; PLTS: platelets.

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Characteristic	**M-CH- or M-CH VAF < 10% patients at diagnosis, N = 246**	**Patients with M-CH large clones (VAF ≥ 10%), N = 8**	**p-value**
Gender			0,7
Female	53 (22%)	2 (25%)	
Male	193 (78%)	6 (75%)	
Age (years)			0,8
Median (Range)	57 (32, 66)	58 (46, 64)	
BMI (kg/m^2)			0,2
Median (Range)	25.0 (16.3, 42.2)	26.8 (21.8, 35.5)	

WBC (cells/µL)			0,079
Median (Range)	7 (1, 196)	23 (3, 106)	
Neutrophils (cells/µL)			0,5
Median (Range)	3.85 (0.12, 22.00)	4.56 (1.40, 15.95)	
NA	1	0	
Lymphocytes (cells/µL)			**0,01**
Median (Range)	2 (0, 186)	13 (2, 73)	
NA	2	0	
Hemoglobin (g/dL)			0,3
Median (Range)	13.00 (5.80, 16.90)	12.40 (7.00, 15.50)	
Platelets (10^9/L)			**0,035**
Median (Range)	191 (18, 624)	129 (26, 249)	
NA	1	0	
Histology			0,5
Blastoid	19 (7.7%)	1 (12%)	
Classic	227 (92%)	7 (88%)	
Ki67 index			0,2
<30	160 (72%)	3 (43%)	
>=30	63 (28%)	4 (57%)	
NA	23	1	
LDH above ULN			**0,011**
<ULN	175 (71%)	2 (25%)	
>ULN	71 (29%)	6 (75%)	
MIPI			**0,003**
high risk	33 (13%)	5 (62%)	
intermediate risk	58 (24%)	1 (12%)	
low risk	155 (63%)	2 (25%)	
MIPIc			**0,002**
high risk	13 (5.8%)	4 (57%)	
intermediate-high risk	29 (13%)	0 (0%)	
intermediate-low risk	66 (30%)	1 (14%)	
low risk	115 (52%)	2 (29%)	

NA	23	1	
ECOG			>0.9
>=1	56 (23%)	2 (25%)	
0	190 (77%)	6 (75%)	
Stage			>0.9
Stage II-III	14 (5.7%)	0 (0%)	
Stage IV	232 (94%)	8 (100%)	
Bulky			0,7
Bulky	78 (32%)	3 (38%)	
Non bulky	168 (68%)	5 (62%)	
TP53 disruption			0,061
Mut or del	25 (16%)	3 (50%)	
WT	135 (84%)	3 (50%)	
NA	86	2	
ASCT			0,3
ASCT not received	30 (12%)	2 (25%)	
ASCT received	216 (88%)	6 (75%)	
Clinical response after ASCT			>0.9
CR	195 (91%)	6 (100%)	
PR	12 (5.6%)	0 (0%)	
SD or PD	8 (3.7%)	0 (0%)	
NA	31	2	
MRD post ASCT (Nested PCR)			0,4
Negative	90 (53%)	2 (33%)	
Positive	80 (47%)	4 (67%)	
NA	76	2	
Randomization	176 (72%)	6 (75%)	>0.9
Hematological toxicity after I R-CHOP			0,1
G1-G2	35 (14%)	3 (38%)	
G3-G4	210 (86%)	5 (62%)	
NA	1	0	
Hematological toxicity after II R-CHOP			0,2

G1-G2	28 (11%)	2 (25%)	
G3-G4	217 (89%)	6 (75%)	
NA	1	0	
Hematological toxicity after III R-CHOP			0,077
G1-G2	31 (13%)	3 (38%)	
G3-G4	214 (87%)	5 (62%)	
NA	1	0	
Hematological toxicity after R-CTX			0,14
G1-G2	132 (56%)	6 (86%)	
G3-G4	104 (44%)	1 (14%)	
NA	10	1	
Hematological recovery after ASCT	157 (89%)	6 (100%)	>0.9
NA	69	2	
Days from ASCT to NEUT ≥ 500 (cells/µL)			0,3
Median (Range)	10 (3, 70)	11 (10, 13)	
NA	53	2	
Days from ASCT to NEUT ≥ 1000 /Ul			0,8
(cells/µL)			
Median (Range)	11 (4, 132)	11 (10, 14)	
NA	65	2	
Days from ASCT to PLTS ≥ 20 (10^9/L)			**0,026**
Median (Range)	13 (3, 145)	16 (13, 54)	
NA	52	2	
Days from ASCT to PLTS ≥ 50 (10^9/L)			0,3
Median (Range)	19 (6, 726)	21 (19, 36)	
NA	79	3	
Randomized to Lenalidomide	92 (52%)	2 (33%)	0,4
NA	70	2	
Lenalidomide dose reduction (%)			0,6
Median (Range)	35 (0, 100)	62 (31, 92)	
NA	154	6	
Lenalidomide completed cycles n.			0,2

Median (Range)	20 (0, 24)	8 (3, 12)	
NA	154	6	
Skin secondary cancer	2 (0.8%)	0 (0%)	>0.9
Secondary cancer (no skin)	19 (7.7%)	1 (12%)	0,5
Therapy-related hematologic cancer	7 (2.8%)	0 (0%)	>0.9

n (%)

Fisher's exact test; Wilcoxon rank sum test

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144 **Table S5 M-CH clones in patients with secondary hematological cancer**

145 VAF: variant allele frequency; MDS: myelodysplastic syndrome; ALL: Acute lymphoblastic leukemia;

146 AML: Acute myeloid leukemia; PB: Peripheral blood; BM: Bone marrow.

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Second cancer n.	Hematological cancer type	M-CH at follow-up	M-CH at follow-up VAF (%)	Sample at follow-up	M-CH at diagnosis	M-CH at diagnosis VAF (%)	Sample at diagnosis	Lenalidomide	Time to second cancer from ASCT (months)
#1	MDS	DNMT3A p.S714C IDH1 p.R132L	6.53 5.03	PB	(DNMT3A p.S714C)[#] NA	0.61 NA	PB	Not randomized	39
#2	MDS	No	NA	PB	No	NA	PB	No	34
#3	ALL	No	NA	PB	No	NA	BM	Yes	NA
#4	MDS	No	NA	PB	No	NA	PB	Yes	21
#5	AML	No	NA	PB	No	NA	PB	No	63
#6	MDS	No	NA	PB	No	NA	BM	Yes	29
#7	MDS	No	NA	PB	No	NA	PB	No	52

[#] Findings from retrospective analysis of NGS detected mutations below the 2% VAF threshold

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