AML typical mutations (*CEBPA*, *FLT3*, *NPM1*) Identify a High-Risk Chronic Myelomonocytic Leukemia Independent Of CPSS Molecular

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Supplementary Methods.

We used an AML cohort to compare clinical characteristics with mutCFN CMML. In summary, the M4/M5 AML cases (n=65) were diagnosed between 2013 and 2021, with 35 (54.7%) of these patients, considered fit for chemotherapy, receiving intensive treatment under the CETLAM 2012 protocol (NCT #NCT04687098). The median age of this AML series was 71 years (range 25-97), with 40% belonging to the favorable risk category according to ELN 2017, 38.5% to the intermediate risk category, and 21.5% to the unfavorable risk category (see more details in (Supplementary Results, and Supplementary Tables S5-S8).

Supplementary Results

First, we compared mutCFN CMML with CMML-2, and considering that most mutCFN CMML belong to CMML-2 (17 out of 21), there were no observable clinical differences. At the molecular level, we observed that mutCFN CMML patients had *TET2* as a co-mutation less frequently (19% vs 61.5%, p=0.037), as well as *PHF6* (0% vs. 30.8%, p=0.023), but more frequently had *DNMT3A* mutation (42.9% vs 7.7%, p=0.05). Regarding prognosis, we did not observed statistical differences is median OS (23.5 months [95% CI, 9.6-28.5] vs 19.5 months [6.6-31], p=0.6) (Supplementary Table S5). When we included both CMML-2 and mutCFN individually in a multivariate analysis, it showed that both CMML-2 (HR 3.045, 95% CI 1.242-7.461, p=0.015) and mutCFN category (HR 2.511, 95% CI 1.042-6.051, p=0.04), remained as independent adverse prognostic factors (Supplementary Table S6). Secondly, we compared the mutCFN CMML group with M4/M5 AML. We observed no differences in clinical characteristics, except that AML patients were more frequently women (47.7% vs. 19%, p=0.02) (Supplementary Table S7). Median OS was not statistically different between both groups. In this series, we identified receiving intensive treatment as a prognostic factor (48.2 months [95% CI 27.8-NA] vs 7.1 months [95% CI, 4.2-11.8], *p*<0.001), acknowledging the implicit bias of age and better performance status in patients who received this treatment. In a multivariate analysis, age at diagnosis (HR 1.036, 95% 1.003-1.071, p=0.035) and receiving intensive treatment (HR 0.402, 95% CI 0.198-0.818, p=0.012) were identified as independent prognostic factors, while the diagnosis of AML or CMML did not (HR 0.632, 95% CI 0.345-1.158, p=0.138) (Supplementary Table S8).

Supplementary Table S1. Patient characteristics of the validation cohort.

Characteristics	**CMML (n=168) n (%)**
Age, years, median (range)	72 (26-89)
Sex (men/women)	47/121 (28/72)
Leucocytes, x10^{9}/L, median (range)	7.8 (2.4-59.4)
Platelets, x109/L, median (range)	115 (7-933)
Hemoglobin, g/dL, median (range)	11.7 (7-139)
Blasts BM, % median (range)	4 (0-19)
ICC/WHO 2022 classification	
MD-CMML	128 (76.2)
MP-CMML	40 (23.8)
ICC/WHO 2022 classifications	
CMML -1	138 (82.1)
CMML -2	30 (17.9)
CPSS-Mol	
Low	48 (28.7)
Intermediate-1	39 (23.4)
Intermediate-2	48 (28.7)
High	32 (19.2)
mutCFN, n (%)	11 (6.5)
mut*CEBPA*, n (%)	1 (0.6)
mut*FLT3,** n (%)	7 (4.2)
FLT3-ITD	4 (2.4)
FLT3-TKD	4 (2.4)
mut*NPM1*, n (%)	3 (1.8)

*One patient had both *FLT3*-ITD and *FLT3*-TKD.

Supplementary Table S2. Mutations in *CEBPA*, *FLT3* or *NPM1* detected at diagnosis in the validation cohort.

Number of patients	cDNA	Protein	VAF	Co-Mutations (VAF, %)
***CEBPA* (n=1)**				
1	c.68dup	p.(His24AlafsTer84)	51	*BCOR* (44), *DNMT3A* (42), *GATA2* (46), *RUNX1* (44), *SRSF2* (42), *TET2* (95)
***FLT3* (n=7)**				
1	c.1756_1788dup	p.(Asp586_Glu596dup)	42.22	*SF3B1* (33.9)
2	c.2503G>T	p.(Asp835Tyr)	• Patient 1: *FLT3* (29.6), *ASXL1* (28.1), *SRSF2* (51.9), *TET2* (51.6) • Patient 2: *FLT3* (12.4), *RUNX1* (NA)	
1	c.2503G>C	p.(Asp835His)	54	*ASXL1* (51), *SRSF2* (58)
1	c.1796_1797insCGTTGATTTCAGAGAATATGAATA	p.(Y599delinsYVDFREYEY)	6.65	*SRSF2* (46.85), *SETBP1* (97.74), *ASXL1* (29.36)
1	c.2508_2510del	p.(Ile836del)	3.42	*ASXL1* (36.2), *NRAS* (6.78, 1.38)
	c.1740_1837+7dup		3.38	
1	81bp ITD			*JAK2* (8), *SF3B1* (49)
***NPM1* (n=3)**				
1	c.860_863 dupTCTG	p.(Trp288Cysfs12)	41	*NRAS* (7), *PTPN11* (25), *GATA2* (41), *DNMT3A* (43), *TET2* (42)
2	c.859_860insTCTG	p.(Trp288CysfsTer12)	• Patient 1: *NPM1* (30.9), *TET2* (43.7, 44.81) • Patient 2: *NPM1* (30.23), *DNMT3A* (45.77)	

Supplementary Table S3. Features of allogeneic stem cell transplantation (alloHSCT).

Characteristics of alloHSCT	n (%)
Type of donor	11 (100)
Matched Sibling	1 (9)
Unrelated	
HLA matched	8 (73)
HLA mismatched	2 (18)
Conditioning type	11 (100)
Myeloablative	2 (18)
Reduced Intensity/Sequential	9 (82)
GVHD Prophylaxis	11 (100)
Cyclophosphamide + Tacrolimus	1 (9)
Tacrolimus + Mycophenolate	2 (18)
Tacrolimus + Rapamycin	3 (28)
Cyclosporin + Methotrexate	4 (36)
Methotrexate + Cyclosporin + ATG	1 (9)

HLA, human leukocyte antigens; GVHD, Graft-versus-host disease; ATG, Anti-Thymocyte Globulin.

Supplementary Table S4. Multivariate analysis of overall survival of the combined series (discovery series plus validation series)

Variable	**HR**	**95% CI**	***p***
Age at diagnosis (years)	1.042	1.023-1.061	<0.001
CPSS-Mol			<0.001
Low (reference)			
Intermediate-1	1.503	0.872-2.590	0.142
Intermediate-2	2.512	1.515-4.163	<0.001
High	3.746	2.204-6.365	<0.001
mutCFN	1.851	1.119-3.063	0.017

Supplementary Table S5. Characteristics of the mutCFN and wtCFN CMML-2 patients.

Characteristics	**mut*CFN* CMML (n=21) n (%)**	**wtCFN CMML-2 (n=13) n (%)**	***P***
Age, years, median (range)	63 (47-86)	69 (28-91)	NS
Sex (men/women)	17/4 (81/19)	10/3 (76.9/23.1)	NS
Leucocytes, x10^9/L, median (range)	19.6 (4-78)	22.7(2.4-54.3)	NS
Platelets, x109/L, median (range)	98 (6-207)	82 (19-371)	NS
Hemoglobin, g/dL, median (range)	8.6 (5-13)	11.4 (7.5-13.4)	NS
Blasts BM, % median (range)	12 (1-19)	13 (6-18)	NS
Cytogenetic risk			NS
Low	16 (76.2)	10 (76.9)	
Intermediate	3 (14.3)	0	
High	0	2 (15.4)	
NA	2 (9.5)	1 (7.7)	
Intensive treatment, n (%)	12 (57.1)	2 (15.4)	0.03
Allogeneic transplant, n (%)	12 (57.1)	2 (15.4)	0.03
Overall survival, median (95% CI)	23.5 (9.6-28.5)	19.5 (6.6-31)	NS
CIP to AML at 2 years, % (95% CI)	44.9 (21.9-65.6)	17.1 (2.3-43.9)	0.075

Supplementary Table S6. Multivariate analysis of overall in CMML patients.

Variable	**HR**	**95% CI**	***p***
Age at diagnosis (years)	1.067	1.032-1.103	<0.001
mutCFN	2.426	1.004-5.860	0.049
CMML-2	3.031	1.232-7.458	0.016
Transfusion dependence	1.050	0.507-2.176	0.895
MP-CMML subtype	0.870	0.410-1.845	0.716
Cytogenetic risk			
Low (reference)			
Intermediate+high	1.243	0.615-2.510	0.545
ASXL1	0.641	0.313-1.312	0.223
NRAS	1.769	0.750-4.175	0.193
RUNX1	2.329	1.083-5.006	0.03
SETBP1	5.268	1.941-14.299	0.001

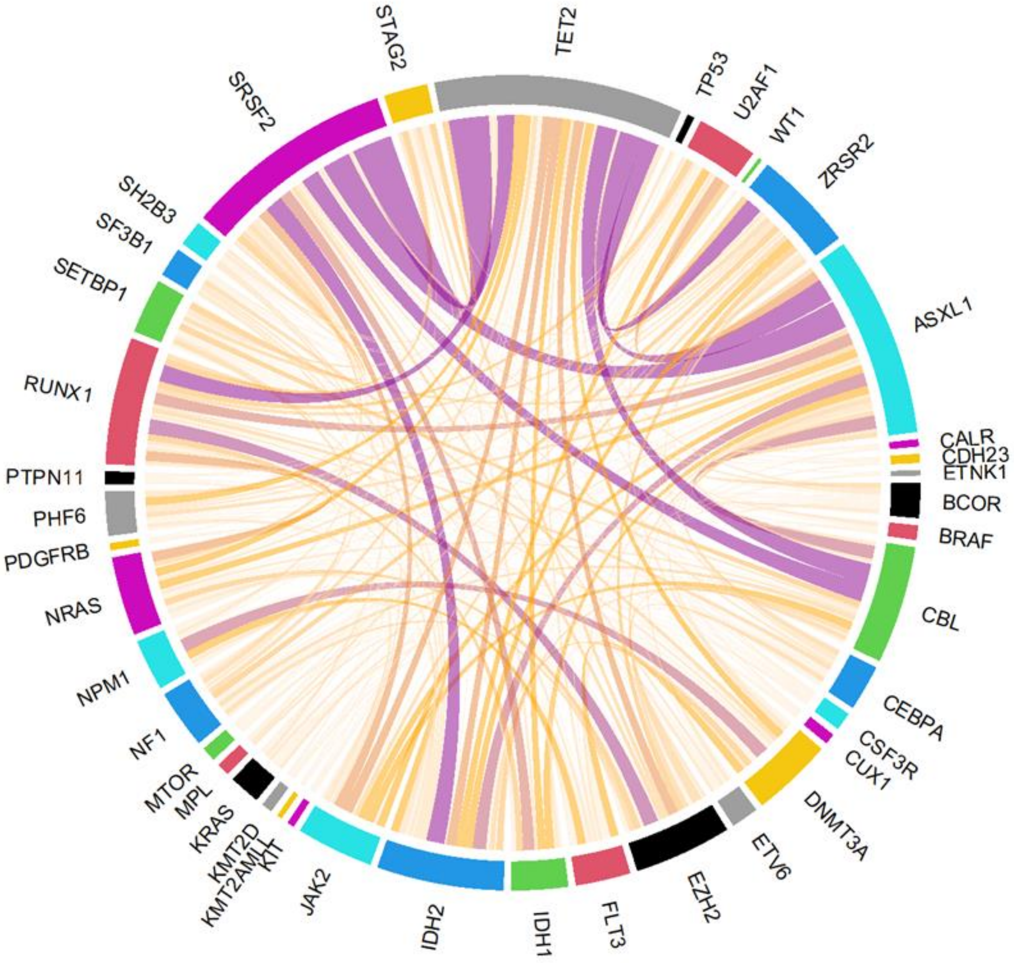
Supplementary Table S7. Characteristics of the mutCFN and M4/M5 AML patients.

Characteristics	**mutCFN CMML (n=21) n (%)**	**M4/M5 AML (n=65) n (%)**	***p***
Age, years, median (range)	63 (47-86)	71 (25-97)	NS
Sex (men/women)	17/4 (81/19)	34/31 (52.3/47.7)	0.02
Leucocytes, x10^9/L, median (range)	19.6 (4-78)	26.4(0.5-285)	NS
Platelets, x109/L, median (range)	98 (6-207)	57 (11-481)	NS
Hemoglobin, g/dL, median (range)	8.6 (5-13)	8 (5.8-14.7)	NS
Blasts BM, % median (range)	12 (1-19)	68 (17-100)	<0.001
Abnormal cytogenetic, % median (range)	4 (20)	15 (25.4)	NS
Karyotype, n (%)	n=20	n=596	NS
Normal	16/20 (80)	50/59 (84.75)	NS
t(14;15)(q32;q22)	1/20 (5)	0	NS
Trisomy	2/20 (10)	4/59 (6.8)	NS
-7	1/20 (5)	0	NS
del(20q)	0	1/59 (1.7)	NA
Complex	0	3/59 (5.1)	NS
Others	0	1/59 (1.7)	NS
Intensive treatment, n (%)	12 (57.1)	35 (54.7)	NS
Allogeneic transplant, n (%)	12 (57.1)	15 (23.1)	<0.01
Overall survival, median (95% CI)	23.5 (9.6-28.5)	26.7 (13-48.2)	0.38

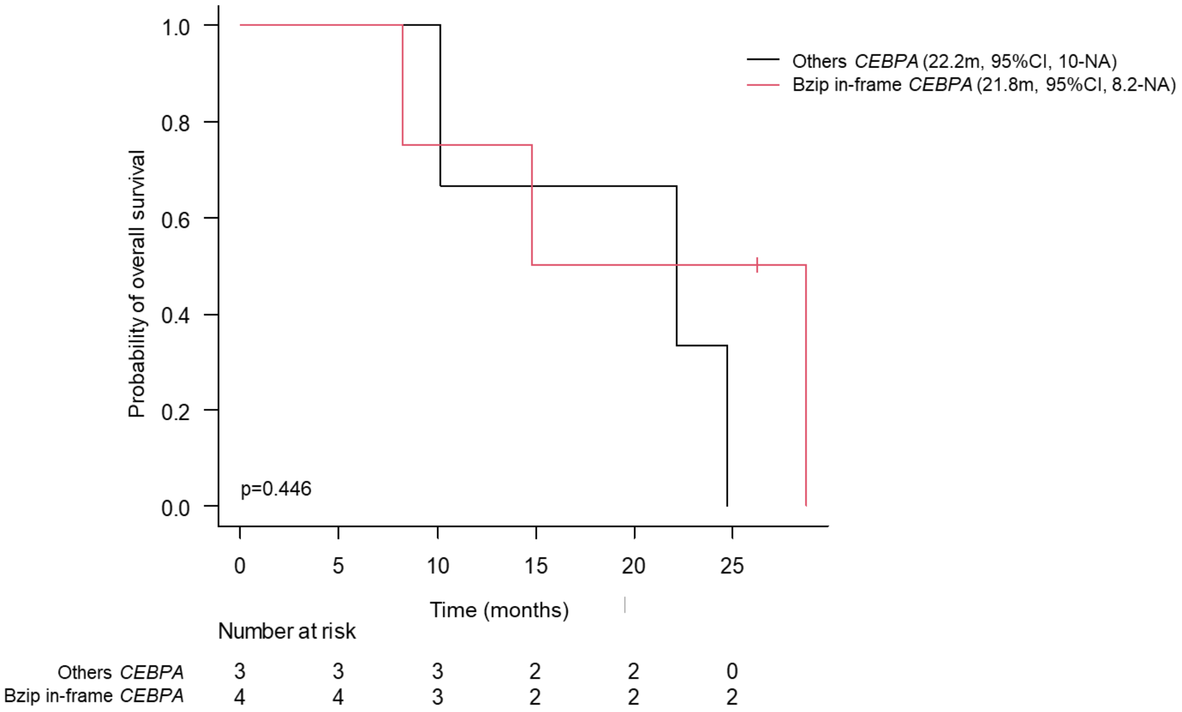
Supplementary Table S8. Multivariate analysis of overall survival of the mutCFN and M4/M5 AML patients.

Variable	**HR**	**95% CI**	***p***
Age at diagnosis	1.036	1.003-1.071	0.035
Belonging to the CMML or AML categories	0.632	0.345-1.158	0.138
Receiving intensive chemotherapy	0.402	0.198-0.818	0.012

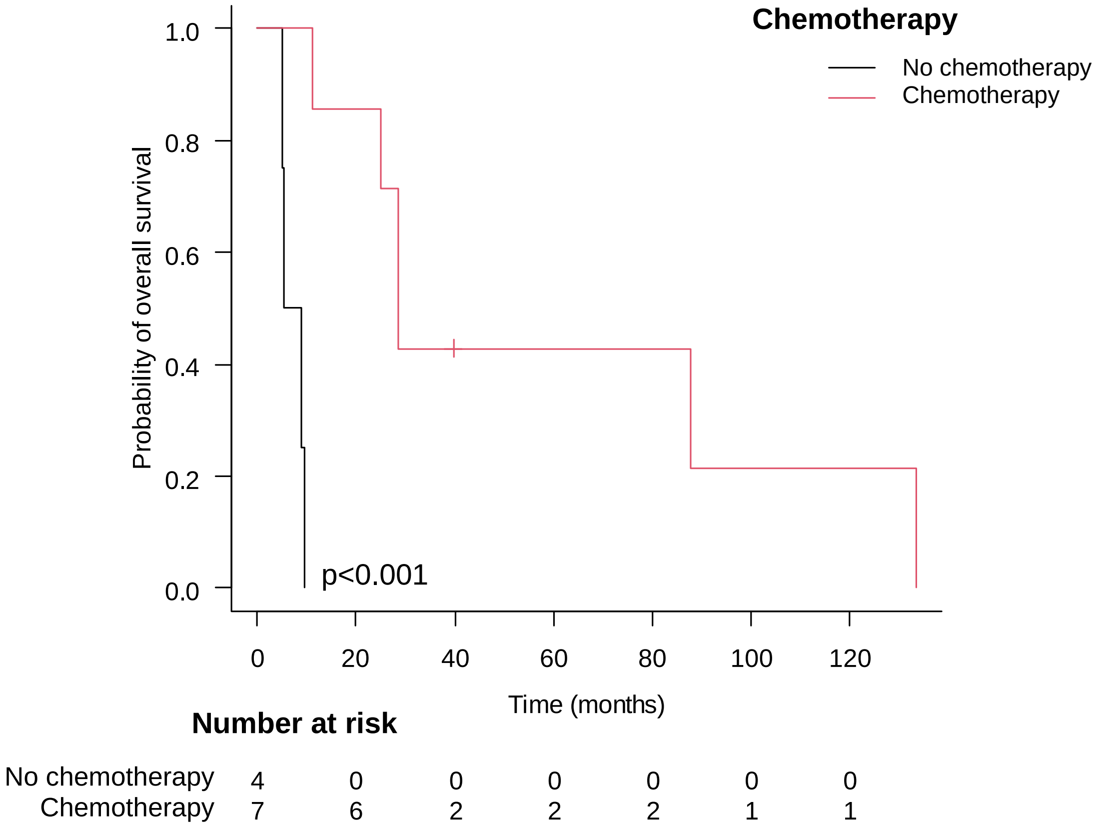
Supplementary Figure S1. Circos plot representation of genes at diagnosis of the entire cohort. The orange connections indicate that there are more than 1 but fewer than 10 patients with simultaneous mutations in the two genes. The purple connections indicate that there are more than 10 patients with simultaneous mutations in the two genes.



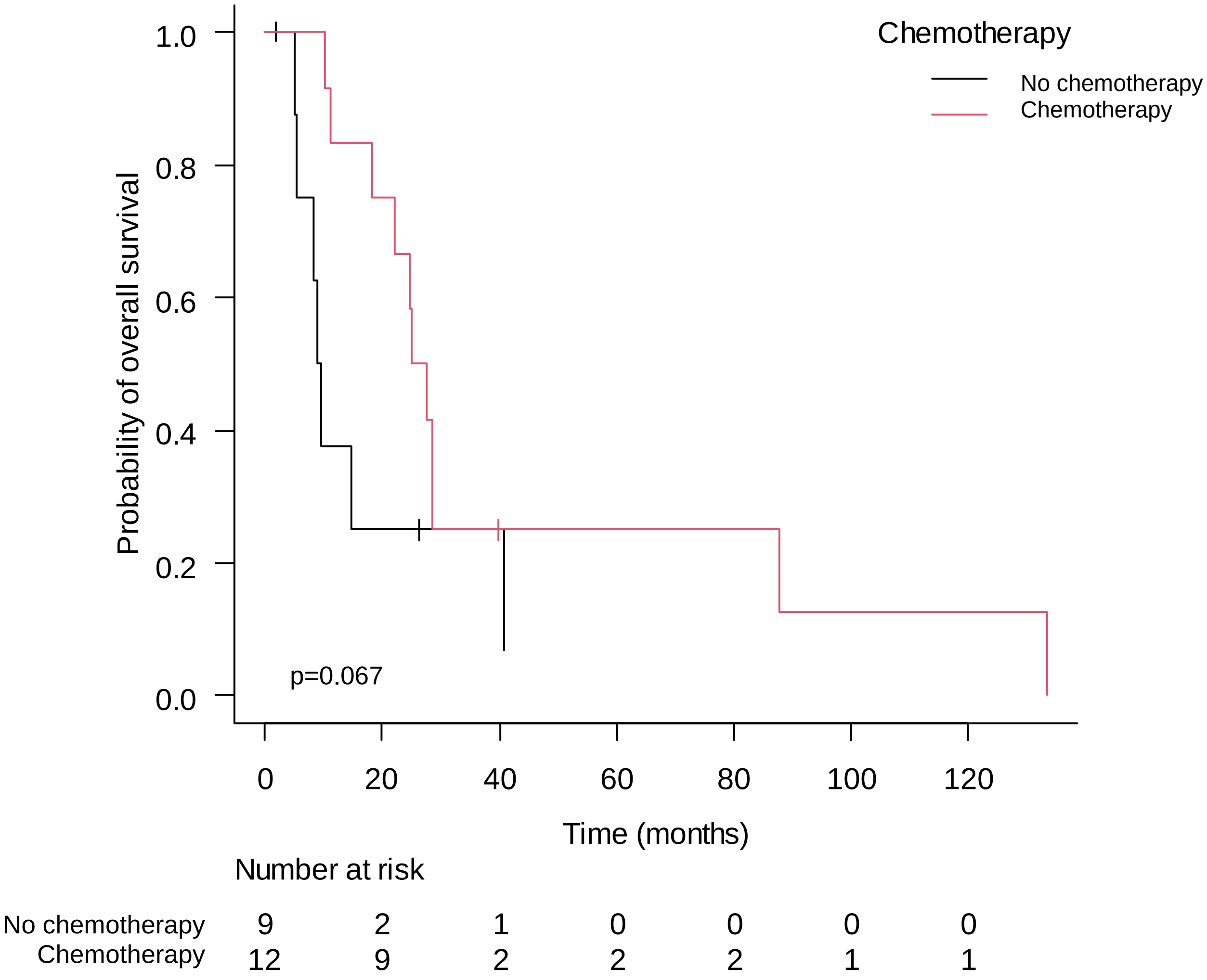
Supplementary Figure S2. Survival of CMML patients with bZIP in-frame *CEBPA* versus others *CEBPA.*



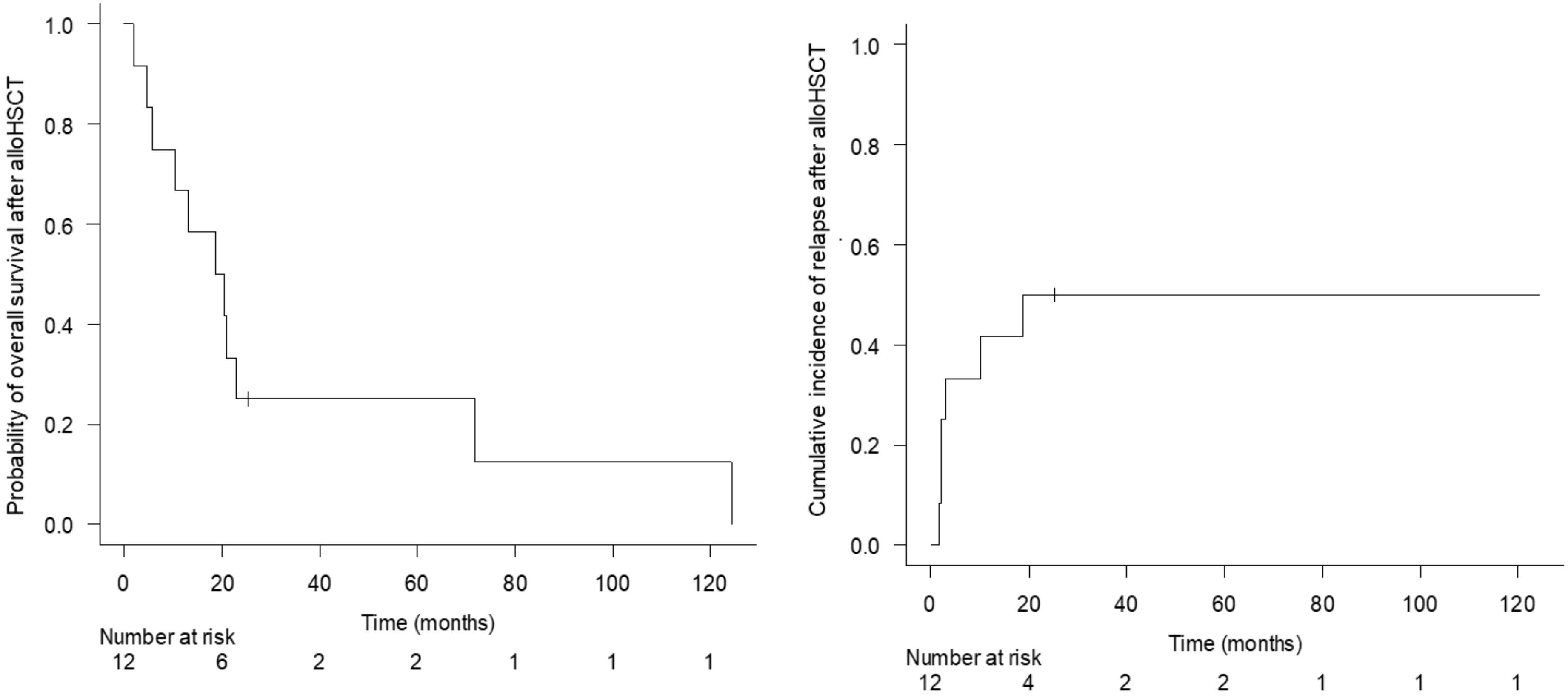
Supplementary Figure S3. Survival of CMML patients with *NPM1* mutations who received (red) or did not receive (black) chemotherapy.



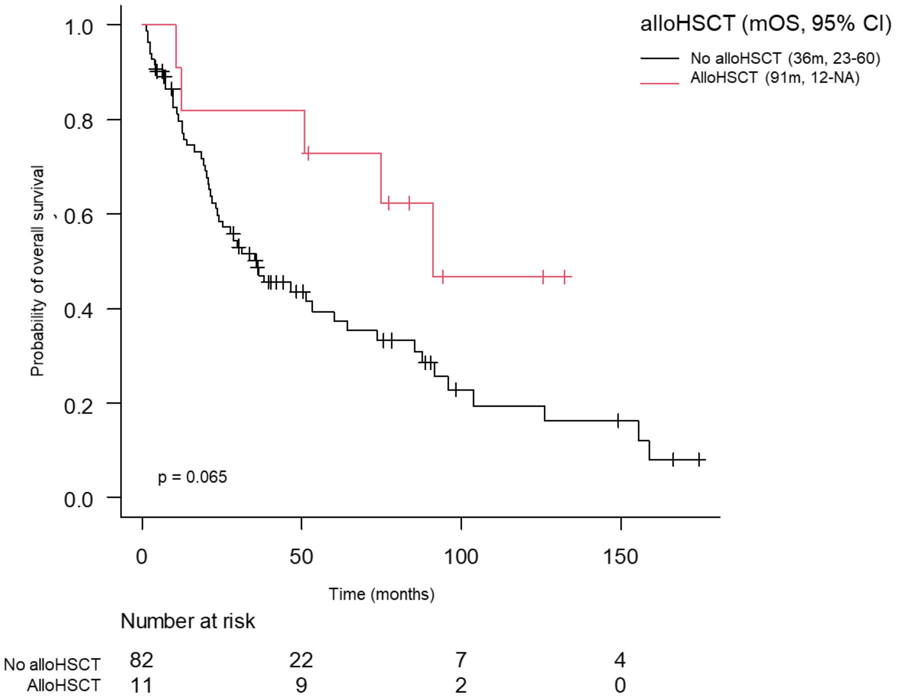
Supplementary Figure S4. Survival of CMML patients with *CEBPA*, *FLT3* and/or *NPM1* mutations who received (red) or did not receive (black) chemotherapy.



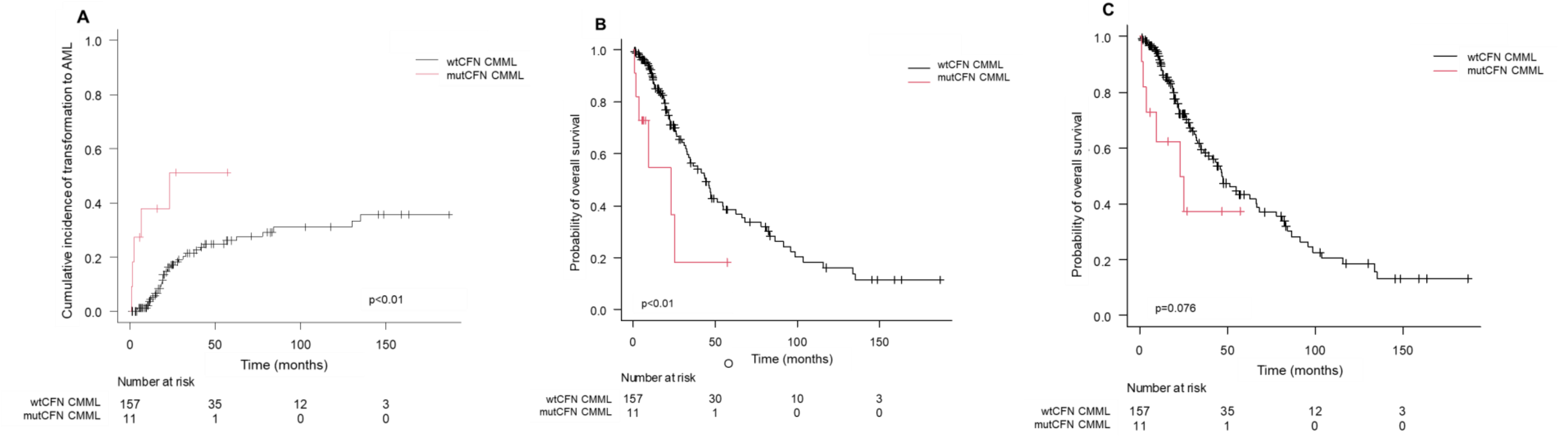
Supplementary Figure S5. Overall survival (A) and cumulative incidence of relapse (B) of mutCFN patients after alloHSCT.



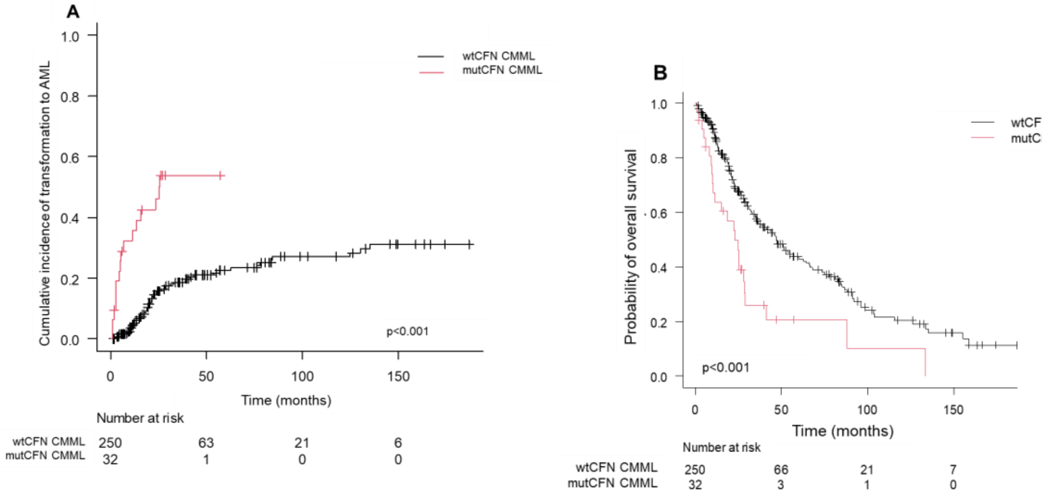
Supplementary Figure S6. Overall Survival of wtCFN patients with and without alloHSCT.



Supplementary Figure S7. Cumulative incidence of transformation to AML (A), overall survival censored at the time of alloHSCT (B), and overall survival (C) in the validation cohort.



Supplementary Figure S8. Cumulative incidence of transformation to AML (A), and overall survival (B) of the combined series (discovery series plus validation series).



Supplementary Figure S9. VAF (variant allele frequency) representation of the mutated genes detected at CMML diagnosis and at progression to AML in five patients with available paired samples. The symbols (* and ^) represent different mutation variants.