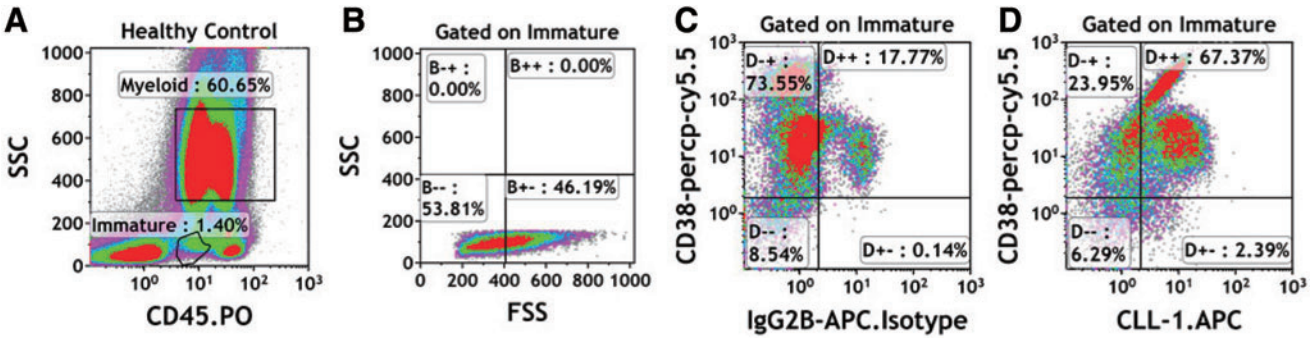
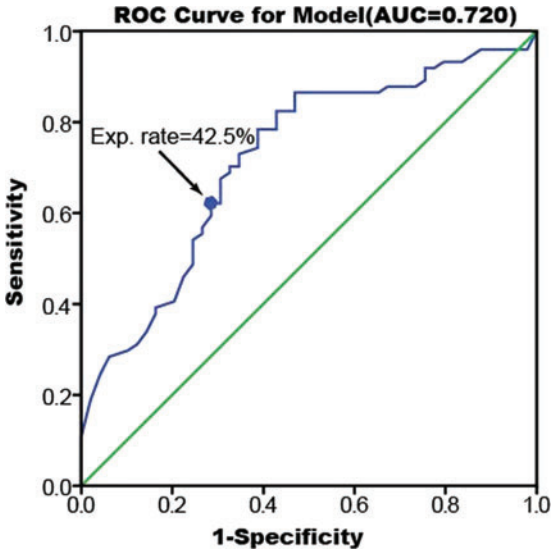
Supplementary Data

Supplementary Appendix A: Treatment Protocols

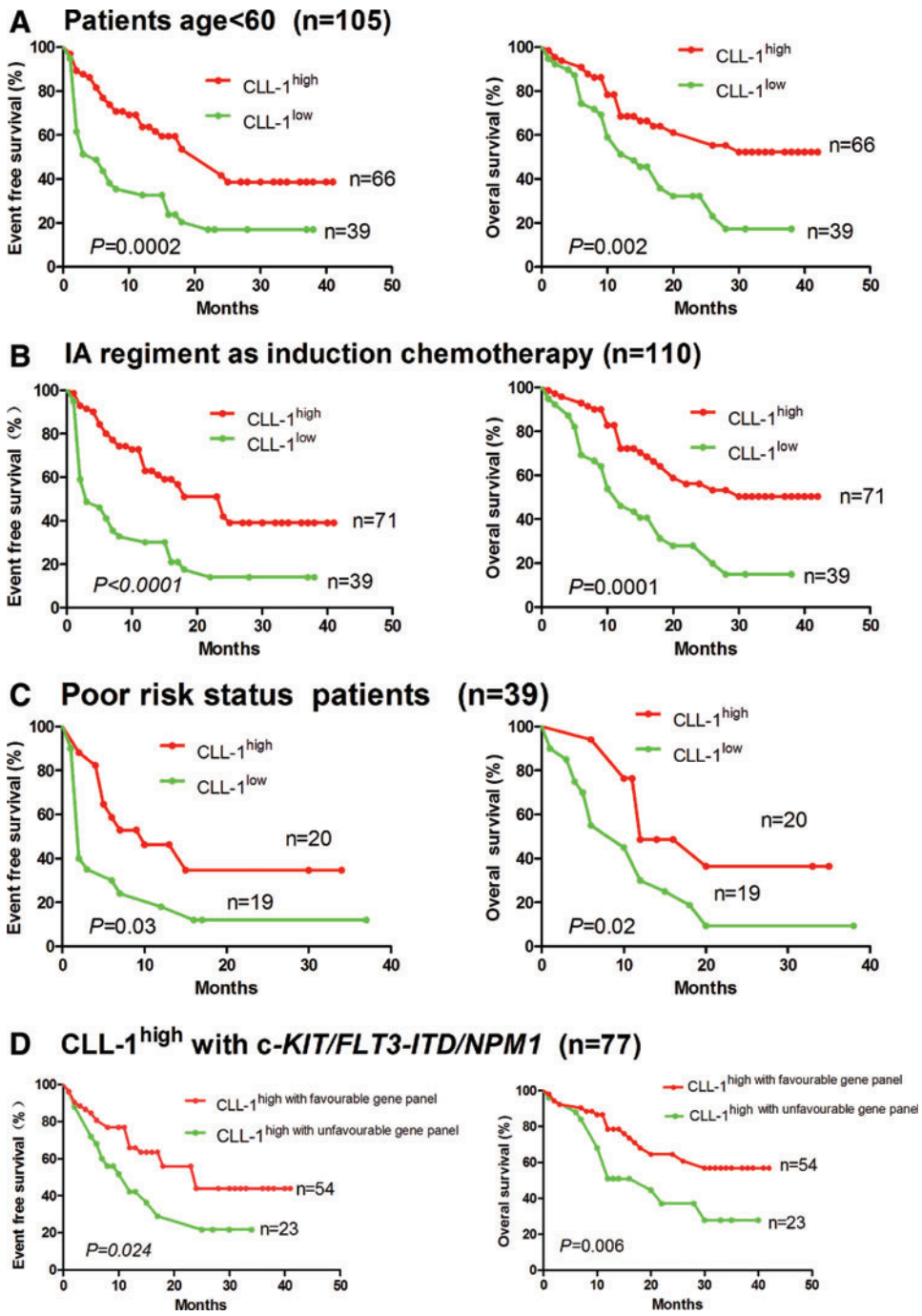
Treatment protocols of 123 cases patients were treated with the protocols as follows: 110 patients received standard DA (daunorubicin 45 mg/m^2/day for 3 days, Ara-C 100 mg/m^2/day for 7 days) regimen (''T1''), 13 received either HAA (homoharringtonine 2 mg/m^2/day for 7 days, Ara-C 100 mg/m^2/day for 7 days and aclarubicin 20 mg/m^2/day for 5 days) or HAD (the same as HAA excerpt for the replacement of aclarubicin with daunorubicin 45 mg/m^2/day for 3 days) protocols (''T2''). Subsequently, the patients received high-dose Ara-C-based consolidation therapy (Ara-C 2.0/m^2 q12 h × 6 times, whereas others experience the transplantation).



SUPPLEMENTARY FIG. S1. Flow cytometric analysis of CLL-1 expression on immature population in healthy control. **(A)** The immature population in healthy control was characterized by low expression of CD45 and low side scatter (CD45^{dim}/SSC^{low}). Granulocytes were excluded based on SSC properties. The percentage of immature population is 1.4%. **(B)** The immature population was back-gated into a forward scatter (FSC)/SSC plot in order to ensure homogeneous scatter property of the immature population. **(C)** Gate on immature population, IgG2B-APC isotope control was used as negative controls of CLL-1 expression in immature population. **(D)** According to the gate of isotope controls in immature population, percentage of CLL-1 positive cells are 69.70%. CLL-1, C-type lectin-like molecule-1.



SUPPLEMENTARY FIG. S2. The optimal cutoff values of CLL-1 expression in bulk blast were determined by means of receiver operating characteristic (ROC) curve analysis, the area under ROC curve is 0.72, the maximum Youden index corresponding to the optimal cutoff values with a sensitivity of 73.0% and a specificity of 65.3%.



SUPPLEMENTARY FIG. S3. Kaplan–Meier survival analysis followed with log-rank test of de novo acute myeloid leukemia patients selected by age <60, IA regiment as induction chemotherapy, CLL-1^{high} with the status of *c-KIT*, *FLT3-ITD*, *NPM1*, and poor-risk status patients. **(A)** The EFS and the OS of CLL-1^{low} group is significantly lower than CLL-1^{high} group among age <60 patients ($n=105$, $P=0.002$ and $P=0.002$ respectively). **(B)** The EFS and the OS of CLL-1^{low} group is significantly lower than the CLL-1^{high} group among IA regiment ($n=110$, $P<0.0001$ and $P=0.0001$, respectively). **(C)** The EFS and the OS of CLL-1$^{high\ with\ unfavorable\ gene\ panel}$ group is significantly lower than the CLL-1$^{high\ with\ favorable\ gene\ panel}$ group among the patients of CLL-1^{high} group ($n=77$, $P=0.024$ and $P=0.006$, respectively). Gene panel included the status *of c-KIT*, *FLT3-ITD*, and *NPM1*. **(D)** The EFS and the OS of CLL-1^{low} group is significantly lower than the CLL-1^{high} group among poor-risk patients ($n=39$, $P<0.03$ and $P<0.02$, respectively). EFS, event-free survival; OS, overall survival.

Supplementary Table S1. Information and Panels of mAbs for Detection of De Novo Acute Myeloid Leukemia Bone Marrow

Source	*Catalogue no.*	*mAb and fluorochrome*	*Specification*	*mAb clone*
Invitrogen (Camarillo)	MHCD4530	CD45-PO	0.5 mL	HI30
Beckman Coulter (Indianapolis)	IM1135U	CD33 FITC	100T	D3HL60.251
Beckman Coulter (Indianapolis)	IM2732	CD117 PE	100T	104D2D1
R&D	FAB2946A	CLL-1 APC	100T	687317
Invitrogen (Camarillo)	MG130	Mouse IgG1-PO	0.5 mL	HI30
Beckman Coulter (Indianapolis)	A07795	Mouse IgG1-FITC	100T	D3HL60.251
Beckman Coulter (Indianapolis)	A07796	Mouse IgG1-PE	100T	104D2D1
R&D (Minneapolis)	IC0041A	Mouse IgG_{2B}-APC	200T	687317
Beckman Coulter (Indianapolis)	6603369	PBS		

Panels for immunophenotype of de novo AML bone marrow: Tube1: CD45-isotype (collecting cell 2×10^6). Tube2: CD45-Ab/CD117-isotype/CD33-isotype/CLL-1-isotype (collecting cell 2×10^6). Tube3: CD45-Ab/CD117-Ab/CD33-Ab/CLL-1-Ab (collecting cell 2×10^6).
AML, acute myeloid leukemia; CLL-1, C-type lectin-like molecule-1.

Supplementary Table S2. Immunophenotype Features of De Novo Acute Myeloid Leukemia Patients in C-Type Lectin-Like Molecule-1^{HIGH} and C-Type Lectin-Like Molecule-1^{LOW}

Immunophenotype	*CLL-1^{high}*	*CLL-1^{low}*	P
AML, no.	77	46	
CD11B, no. (%)	9 (11.68)	12 (26.08)	0.04

P value were calculated by means of Chi-square test.

Supplementary Table S3. Multivariate Analysis of Continuous C-Type Lectin-Like Molecule-1 Value for Complete Remission, Event-Free Survival, and Overall Survival

	CR		*EFS*		*OS*	
Variables	P	*OR (95% CI)*[a]	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.44	0.98 (0.95–1.02)	0.83	0.97 (0.97–1.02)	0.91	1.00 (0.97–1.02)
WBC[b]	0.21	0.99 (0.98–1.00)	0.44	1.00 (0.99–1.01)	0.69	0.99 (0.99–1.00)
Risk status 1 vs. 2[c]	0.06	10.69 (0.88–12.56)	0.20	0.35 (0.07–1.77)	0.15	0.20 (0.02–1.76)
Risk status 3 vs. 2[c]	0.05	3.74 (0.94–14.85)	0.52	1.26 (0.61–2.59)	0.43	1.34 (0.64–2.81)
Transplantation[d]			0.01	0.20 (0.08–0.45)	0.01	0.14 (0.05–0.36)
Biallelic *CEBPA*[e]	0.67	1.49 (0.22–9.86)	0.01	0.14 (0.03–1.06)	0.02	0.09 (0.01–0.71)
FLT3-ITD[e]	0.05	8.97 (0.99–8.94)	0.86	1.09 (0.60–2.93)	0.83	1.11 (0.39–3.14)
c-KIT[e]	0.14	0.26 (0.04–1.59)	0.05	0.37 (0.13–1.03)	0.07	0.39 (0.14–1.10)
T1 vs. T2[f]	0.11	1.02 (1.01–1.04)	0.12	1.95 (0.82–4.62)	0.42	1.41 (0.60–3.31)
CLL-1 level[b]	0.01	4.98 (3.95–6.02)	0.02	0.68 (0.48–0.99)	<0.01	0.98 (0.98–0.99)
RLC[b]			<0.01	1.03 (1.01–1.05)	<0.01	1.05 (1.03–1.06)

[a]Odds ratio (OR) >1 corresponds to an increased tendency of complete remission compared with the lower values of continuous variables or the reference group of categorical. Hazard ratios (HR) >1 correspond to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.
[b]Age, WBC, CLL-1 level, RLC were analyzed as continuous variables.
[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor risk, respectively, RLC after induction chemotherapy.
[d]Stands for transplantation as consolidation treatment.
[e]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants or WT. Mutant versus WT.
[f]T1, T2 as previously reported.
CI, confidence interval; CR, complete remission; EFS, event-free survival; OS, overall survival; RLC, residual leukemia cell; WBC, white blood cell; WT, wild type.

Supplementary Table S4. Univariate Analysis of Clinical Parameters and C-Type Lectin-Like Molecule-1 Levels for Complete Remission, Event-Free Survival, and Overall Survival

	CR		*EFS*		*OS*	
Variables	P	*OR (95% CI)*[a]	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.03	0.97 (0.94–0.99)	0.01	1.02 (1.00–1.03)	0.01	1.02 (1.00–1.03)
WBC[b]	0.11	0.99 (0.98–1.00)	0.01	1.00 (1.001–1.009)	0.01	1.00 (1.00–1.01)
Risk status 1 vs. 2[c]	0.01	13.00 (1.70–19.37)	0.01	0.12 (0.03–0.55)	0.01	0.08 (0.01–0.61)
Risk status 3 vs. 2[c]	0.01	0.09 (0.01–0.67)	0.65	0.88 (0.52–1.50)	0.84	0.94 (0.53–1.66)
Transplantation[d]			0.01	0.43 (0.23–0.82)	0.01	0.39 (0.20–0.78)
Biallelic *CEBPA*[e]	0.01	0.09 (0.01–0.67)	0.00	0.14 (0.03–0.60)	0.01	0.09 (0.01–0.67)
FLT3-ITD[f]	0.49	0.72 (0.29–1.80)	0.32	1.36 (0.74–2.50)	0.25	1.44 (0.76–2.73)
c-KIT[f]	0.24	1.92 (1.23–2.45)	0.56	1.77 (1.57–1.92)	0.29	2.91 (1.39–5.36)
T1 vs. T2[g]	0.21	1.22 (0.89–1.66)	0.01	2.86 (1.53–5.32)	0.03	2.09 (1.07–4.10)
CLL-1^{high} vs. CLL-1^{low}	0.01	3.10 (1.85–5.20)	<0.01	0.37 (0.24–0.59)	<0.01	0.39 (0.24–0.63)
RLC[b]			0.01	1.04 (1.03–1.03)	0.01	1.05 (1.03–1.06)

[a]OR >1 corresponds to an increased tendency of complete remission compared with the lower values of continuous variables or the reference group of categorical. HR >1 corresponds to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.
[b]Age, WBC, RLC were analyzed as continuous variables.
[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor risk, respectively, RLC after induction chemotherapy.
[d]Stands for transplantation as consolidation treatment.
[e]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants/WT.
[f]Mutant versus WT.
[g]T1, T2 as previous reported.

Supplementary Table S5. Refitted Multivariate Cox Models for Event-Free Survival and Overall Survival Analysis

	EFS		*OS*	
Variables	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.04	1.01 (0.99–1.03)	0.03	1.02 (1.00–1.04)
WBC[b]	0.00	1.00 (1.00–1.01)	0.00	1.00 (1.00–1.01)
Risk status 1 vs. 2[c]	0.12	0.46 (0.17–1.22)	0.97	1.01 (0.36–2.80)
Risk status 3 vs. 2[c]	0.03	0.42 (0.19–0.95)	0.16	2.18 (0.72–6.64)
Biallelic *CEBPA*[d]	0.00	0.13 (0.02–0.60)	0.02	0.09 (0.01–0.77)
FLT3-ITD[e]	0.03	0.30 (0.10–0.90)	0.05	0.32 (0.10–1.04)
c-KIT[e]	0.39	2.41 (0.31–18.30)	0.15	4.34 (0.56–33.65)
T1 vs. T2[f]	0.13	1.87 (0.83–4.22)	0.37	1.45 (0.63–3.35)
CLL-1^{high} vs. CLL-1^{low}	0.03	0.57 (0.32–1.00)	0.01	0.48 (0.26–0.88)

[a]HR >1 corresponds to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.
[b]Age, WBC were analyzed as continuous variables.
[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor risk, respectively.
[d]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants/WT.
[e]Mutant versus WT.
[f]T1, T2 as previous reported.

Supplementary Table S6. Multivariate Analysis of Clinical Parameters and C-Type Lectin-Like Molecule-1 Levels for Complete Remission, Overall Survival, and Event-Free Survival in Patients with Age <60

	CR		*EFS*		*OS*	
Variables	P	*OR (95% CI)*[a]	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.47	0.98 (0.94–1.02)	0.57	0.99 (0.96–1.01)	0.92	0.99 (0.97–1.02)
WBC[b]	0.18	0.99 (0.97–1.00)	0.63	1.00 (0.99–1.01)	0.96	1.00 (0.99–1.00)
Risk status 1 vs. 2[c]	0.12	8.12 (0.56–17.15)	0.14	0.29 (0.05–1.49)	0.10	0.17 (0.02–1.44)
Risk status 3 vs. 2[c]	0.02	5.92 (1.28–7.27)	0.62	1.22 (0.54–2.79)	0.64	1.21 (0.52–2.79)
Transplantation[d]			0.00	0.21 (0.09–0.49)	0.00	0.16 (0.06–0.43)
CEBPA.Bi[e]	0.04	16.59 (1.08–25.47)	0.02	0.17 (0.03–0.76)	0.06	0.14 (0.02–1.13)
FLT3-ITD[f]	0.74	1.41 (0.18–10.89)	0.69	1.24 (0.41–3.76)	0.79	1.16 (0.37–3.58)
c-KIT[f]	0.10	6.71 (1.67–10.58)	0.07	0.34 (0.10–1.11)	0.13	0.40 (0.12–1.33)
CLL-1^{high} vs. CLL-1^{low}	<0.01	6.89 (2.11–10.47)	0.01	0.43 (0.22–0.86)	0.03	0.46 (0.22–0.92)
T1 vs. T2[g]	0.05	0.04 (0.00–1.08)	0.03	4.14 (1.12–15.27)	0.43	1.69 (0.45–5.31)
RLC[b]			<0.01	1.03 (1.01–1.05)	<0.01	1.04 (1.02–1.06)

[a]OR >1 corresponds to an increased tendency of complete remission compared with the lower values of continuous variables or the reference group of categorical. HR >1 corresponds to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.
[b]Age, WBC, RLC were analyzed as continuous variables.
[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor risk, respectively, RLC after induction chemotherapy.
[d]Stands for transplantation as consolidation treatment.
[e]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants/WT.
[f]Mutant versus WT.
[g]T1, T2 as previous reported.

Supplementary Table S7. Multivariate Analysis of Clinical Parameters and C-Type Lectin-Like Molecule-1 Levels for Complete Remission, Event-Free Survival, and Overall Survival in IA Regiment

	CR		*EFS*		*OS*	
Variables	P	*OR (95% CI)*[a]	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.27	0.97 (0.93–1.01)	0.98	1.00 (0.98–1.02)	0.67	1.00 (0.98–1.02)
WBC[b]	0.20	0.99 (0.97–1.00)	0.95	1.00 (0.98–1.01)	0.81	1.00 (0.98–1.01)
Risk status 1 vs. 2[c]	0.14	7.68 (0.50–16.41)	0.10	0.26 (0.05–1.31)	0.09	0.16 (0.01–1.37)
Risk status 3 vs. 2[c]	0.03	5.61 (1.16–7.15)	0.89	0.95 (0.46–1.92)	0.79	0.90 (0.41–1.96)
Transplantation[d]			<0.01	0.20 (0.09–0.46)	<0.01	0.13 (0.05–0.33)
CEBPA.Bi[e]	0.04	0.08 (0.01–0.60)	0.01	0.08 (0.01–0.60)	0.96	0.06 (0.02–0.10)
FLT3-ITD	0.07	8.10 (7.08–8.91)	0.44	0.54 (0.11–2.55)	0.37	0.49 (0.10–2.37)
c-KIT[f]	0.05	10.84 (3.28–35.76)	0.05	4.57 (0.96–7.01)	0.00	3.87 (1.07–5.89)
CLL-1^{high} vs. CLL-1^{low}	0.02	7.68 (0.50–16.41)	0.04	0.55 (0.29–1.05)	0.03	0.45 (0.22–0.92)
RLC[b]			0.01	1.03 (1.01–1.05)	0.01	1.04 (1.02–1.06)

IA regimen stands for idarubicin (10 mg/m^2/day, days 1–3) or daunorubicin (45 mg/m^2/day, days 1–3) and cytarabine (Ara-C 100 mg/m^2/day, days 1–7).

[a]OR >1 corresponds to an increased tendency of complete remission compared with the lower values of continuous variables or the reference group of categorical. HR >1 corresponds to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.

[b]Age, WBC, RLC were analyzed as continuous variables.

[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor, respectively, RLC after induction chemotherapy.

[d]Stands for transplantation as consolidation treatment.

[e]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants/WT.

[f]Mutant versus WT.

Supplementary Table S8. Multivariate Cox Analysis for Event-Free Survival and Overall Survival When Gene Panel Was Enrolled

	EFS		*OS*	
Variables	P	*HR (95% CI)*[a]	P	*HR (95% CI)*[a]
Age[b]	0.96	1.00 (0.95–1.03)	0.59	1.00 (0.99–1.01)
WBC[b]	0.18	1.00 (0.99–1.02)	0.47	1.00 (0.98–1.02)
Risk status 1 vs. 2[c]	0.48	1.38 (0.57–3.31)	0.36	1.57 (0.58–4.53)
Risk status 3 vs. 2[c]	0.48	1.51 (0.51–4.46)	0.57	1.43 (0.43–4.76)
Transplantation[d]	<0.01	0.20 (0.07–0.43)	<0.01	0.28 (0.12–0.60)
Biallelic *CEBPA*[e]	<0.01	0.14 (0.04–0.60)	0.02	0.12 (0.04–0.82)
T1 vs. T2[f]	0.14	1.97 (0.86–4.50)	0.24	1.58 (0.71–3.58)
CLL-1^{high} vs. CLL-1^{low}	0.02	0.47 (0.25–0.86)	0.01	0.48 (0.21–0.83)
RLC[b]	<0.01	1.04 (1.01–1.06)	<0.01	1.05 (1.03–1.07)
Favorable vs. unfavorable[g]	0.59	0.78 (0.31–1.93)	0.57	0.70 (0.29–1.95)

[a]OR >1 corresponds to an increased tendency of complete remission compared with the lower values of continuous variables or the reference group of categorical. HR >1 corresponds to an increased risk of death/relapse compared with the lower values of continuous variables or the reference group of categorical.

[b]Age, WBC, RLC were analyzed as continuous variables.

[c]Risk status 1, 2, 3 stand for risk stratification of favorable, intermediate, and poor risk, respectively.

[d]Stands for transplantation as consolidation treatment.

[e]Biallelic *CEBPA* mutant versus monoallelic *CEBPA* mutants/WT.

[f]T1, T2 as previous reported.

[g]Favorable versus unfavorable, favorable gene panel includes *mutate NPM1 with WT c-KIT and FLT3-ITD,* unfavorable gene panel includes *WT NPM1 with mutate c-KIT and FLT3-ITD.*