Benefit of intermediate-dose cytarabine-containing induction in molecular subgroups of acute myeloid leukemia

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Methods

Patients

Between September 1, 2010 to January 13, 2016, 591 patients aged 15-<55 years with de novo newly-diagnosed acute myeloid leukemia (AML) were enrolled in our study registered at www.chictr.org.cn (identifier: ChiCTR-TRC-10001202) as described in detail in our previous report(1). The median follow-up time of survivors in the current report was 70 months (range, 5-115 months). The primary endpoint of this trial was relapse-free survival (RFS). Co-secondary endpoints included rates of complete remission, event-free survival (EFS), and overall survival (OS).

Cytogenetic and mutational analyses

Cytogenetic analyses were done from bone marrow samples at diagnosis. Samples were analyzed by R-banding and classified according to the International System for Human Cytogenetic Nomenclature (ISCN2009). Cytogenetic risk was classified according to the Refined Medical Research Council criteria (2).

RUNX1-RUNX1T1 and *CBFbeta-MYH11* were also detected by RT-PCR and Fluorescence in situ hybridization (FISH). Mutations in *NPM1* and *CEBPA* were detected in DNA from bone marrow samples at diagnosis by PCR and direct sequencing. Mutations in *FLT3*-ITD was detected by PCR and electrophoresis on agarose gels.

Treatment

Eligible patients were randomly-assigned to conventional- (100 mg/mE+2/d days 1-7 as a 12-h IV infusion) or intermediate-dose cytarabine (100 mg/mE+2/d days 1-4 as a 12-h IV infusion and 1 g/mE+2 every 12 h as a 3-h IV infusion on days 5-7). Patients also received daunorubicin (40 mg/mE+2/d on days 1-3) and omacetaxine mepesuccinate (2 mg/mE+2/d on days 1-7). Patients achieving a complete remission were randomized to receive three courses of high-dose cytarabine (3 g/mE+2 days 1-3 every 12 h as a 3-h IV infusion) or two courses of intermediate-dose cytarabine (1.5 g/mE+2 at the same schedule) with daunorubicin (40 mg/mE+2/d on days 1-3) in the 1st and mitoxantrone (6 mg/mE+2/d on days 1-3) in the 2nd courses. The 2nd randomization was not stratified for induction regimen. The details of that trial were described in our previous report(1). The study was approved by the hospital ethics committee and conducted in accordance with the Declaration of Helsinki. Written informed consent for treatment and genetic testing was obtained from all patients.

Statistical analyses

RFS in patients achieving complete remission was defined as the interval from complete remission to relapse or death from any cause and censored at last follow-up visit or contact. EFS was defined as the interval from randomization to assessment of response after the induction cycle if patients failed to achieve a complete remission, the date of relapse in patients achieving a complete remission or the date of death, whichever occurred first. OS was defined as the interval from randomization to death from any cause.

All randomized patients were included in analyses of complete remission, EFS, and OS. All randomized patients achieving complete remission were included in RFS analyses by intent-to-treat. RFS, EFS, and OS were calculated by the Kaplan-Meier method. Log-rank test was used for univariable analyses and the proportional hazard model of Cox for multivariable analyses of RFS, EFS, and OS. Age and white blood cell count (WBC) were analyzed as continuous variables in multivariable analyses. Transplantation in the first complete remission (CR1) was handled as a time-dependent binary covariate for survival analyses. Statistical tests were two-sided with a significance level set at 0.05. Analyses were done with SPSS (version 20).

Reference

1. Wei H, Wang Y, Gale RP, et al. Randomized Trial of Intermediate-dose Cytarabine in Induction and Consolidation Therapy in Adults with Acute Myeloid Leukemia. Clin Cancer Res. 2020;26(13):3154-3161.
2. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 2010;116(3):354-365.

Table S1： The mutations and cytogenetic risk at study entry

Subgroup	CD (n=296) （%）	ID (n=295) （%）
Cytogenetic risk		
Favorable	81(27%)	83(28%)
Intermediate	179(60%)	182(62%)
Poor	18(6%)	15(5%)
Unknown	18(6%)	15(5%)
Gene mutation		
*CEBPA*dm	32(11%)	43(15%)
RUNX1-RUNX1T1	60(20%)	71(24%)
CBFbeta-MYH11	21(7%)	12(4%)
NPM1	51(17%)	38(13%)
FLT3-ITD	35(12%)	31(11%)

CD: conventional dose, ID: intermediate dose

Table S2： Outcomes by treatment (ID vs. CD) according to subgroup.

	CR rate			RFS				EFS				OS			
Subgroup	CD	ID	*P*	HR of Univariate (95%CI)	*P*	HR of Multivariable (95%CI) *	*P*	HR of Univariate (95%CI)	*P*	HR of Multivariable (95%CI) *	*P*	HR of Univariate (95%CI)	*P*	HR of Multivariable (95%CI) *	*P*
All patients	77%	87%	0.003	0.680 (0.518-0.893)	0.006	0.670(0.509-0.881)	0.004	0.641 (0.512-0.802)	<0.001	0.630(0.503-0.789)	<0.001	0.732(0.570-0.938)	0.014	0.720(0.560-0.925)	0.010
Cytogenetic															
Favorable	95%	98%	0.655	0.586(0.345-0.996)	0.048	0.574(0.337-0.976)	0.040	0.575 (0.347-0.953)	0.032	0.563(0.339-0.934)	0.026	0.760(0.433-1.334)	0.339	0.752(0.427-1.326)	0.325
Intermediate	73%	84%	0.012	0.669(0.470-0.953)	0.026	0.656(0.459-0.936)	0.020	0.635 (0.480-0.841)	0.002	0.619(0.467-0.821)	0.001	0.673(0.491-0.921)	0.014	0.643(0.468-0.885)	0.007
Poor	33%	73%	0.037	0.975(0.317-2.998)	0.964	0.675(0.177-2.574)	0.565	0.542 (0.257-1.145)	0.108	0.313(0.124-0.791)	0.014	0.836(0.370-1.888)	0.667	0.604(0.249-1.463)	0.264
Unknown	83%	73%	0.674	0.873 (0.310-2.455)	0.797	1.135(0.375-3.439)	0.823	1.055 (0.455-2.445)	0.900	1.082(0.444-2.634)	0.863	1.424(0.591-3.431)	0.431	1.566(0.613-4.002)	0.348
Gene mutation															
*CEBPA*dm	100%	95%	0.504	0.287(0.110-0.747)	0.011	0.288(0.108-0.769)	0.013	0.387 (0.162-0.922)	0.032	0.328 (0.129-0.839)	0.020	0.348(0.119-1.017)	0.054	0.262(0.077-0.889)	0.032
RUNX1-RUNX1T1	93%	97%	0.528	0.523 (0.289-0.945)	0.032	0.428(0.228-0.804)	0.008	0.508 (0.291-0.887)	0.017	0.420 (0.232-0.760)	0.004	0.571(0.310-1.053)	0.073	0.490(0.258-0.930)	0.029
CBFbeta-MYH11	100%	100%	-	0.910 (0.274-3.024)	0.878	0.527(0.147-1.895)	0.327	0.915 (0.275-3.039)	0.884	0.535 (0.149-1.927)	0.339	2.565(0.574-11.470)	0.218	1.385(0.249-7.706)	0.710
NPM1	86%	92%	0.601	0.812(0.380-1.734)	0.590	0.865(0.390-1.917)	0.721	0.721 (0.373-1.395)	0.331	0.759 (0.382-1.508)	0.431	0.774(0.366-1.640)	0.504	0.787(0.357-1.737)	0.553
FLT3-ITD	69%	71%	0.833	0.605 (0.238-1.539)	0.292	0.679(0.259-1.781)	0.431	0.741 (0.391-1.403)	0.358	0.779 (0.405-1.500)	0.455	0.703(0.347-1.425)	0.329	0.864(0.410-1.820)	0.700

CD: conventional dose, ID: intermediate dose

*Adjusted for age, WBC, cytogenetics, and transplantation in CR1

Table S3： Outcomes by treatment (ID vs CD) in *NPM1* mutation and *FLT3*-ITD subgroup

	CR rate			RFS				EFS				OS			
Subgroup	CD	ID	*P*	HR of Univariate (95%CI)	Wald *P*	HR of Multivariable (95%CI) *	Wald *P*	HR of Univariate (95%CI)	Wald *P*	HR of Multivariable (95%CI) *	Wald *P*	HR of Univariate (95%CI)	Wald *P*	HR of Multivariable (95%CI) *	Wald *P*
NPM1+FLT3- ITD-	87%	92%	0.847	0.798（0.318-2.001）	0.630	0.656（0.241-1.784）	0.408	0.738（0.331-1.644）	0.457	0.602(0.252-1.437)	0.253	0.649(0.249-1.688)	0.375	0.409（0.136-1.233）	0.112
NPM1+FLT3- ITD+	83%	92%	0.593	0.812(0.203-3.254)	0.768	1.249（0.257-6.059）	0.783	0.660(0.201-2.168)	0.493	0.917（0.246-3.415）	0.897	0.976(0.262-3.642)	0.971	1.761（0.353-8.780）	0.490
NPM1-FLT3- ITD+	59%	56%	0.822	0.433(0.114-1.637)	0.217	0.657（0.151-2.852）	0.575	0.794(0.371-1.699)	0.553	0.940（0.411-2.149）	0.883	0.602(0.255-1.422)	0.247	0.734（0.279-1.928）	0.530

CD: conventional dose, ID: intermediate dose

*Adjusted for age, WBC, cytogenetics, and transplantation in CR1

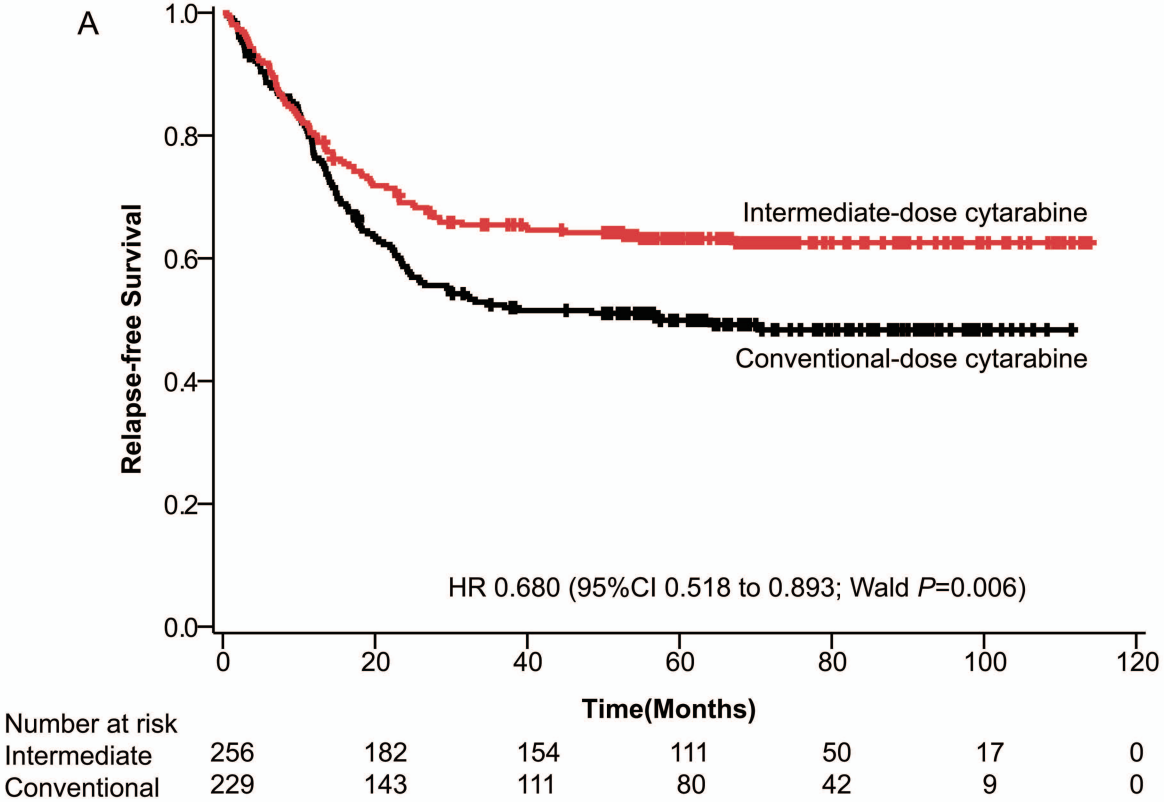
Table S4： Outcomes by treatment (ID vs. CD) with inclusion of the second randomization in multivariable analyses.

	RFS		EFS		OS	
Subgroup	HR of Multivariable (95%CI) *	*P*	HR of Multivariable (95%CI) *	*P*	HR of Multivariable (95%CI) *	*P*
All patients	0.645(0.482-0.862)	0.003	0.647(0.484-0.864)	0.003	0.682(0.493-0.945)	0.022
Cytogenetic						
Favorable	0.483(0.273-0.853)	0.012	0.481(0.272-0.850)	0.012	0.552(0.294-1.037)	0.065
Intermediate	0.647(0.444-0.942)	0.023	0.647(0.444-0.943)	0.024	0.682(0.445-1.046)	0.079
Poor	1.417(0.284-7.076)	0.671	1.495(0.288-7.765)	0.632	0.465(0.078-2.785)	0.402
Unknown	0.781(0.196-3.117)	0.727	0.785(0.195-3.156)	0.733	1.115(0.210-5.927)	0.899
Gene mutation						
*CEBPA*dm	0.301(0.101-0.896)	0.031	0.294 (0.098-0.881)	0.029	0.186(0.037-0.945)	0.043
RUNX1-RUNX1T1	0.287(0.142-0.582)	0.001	0.292 (0.145-0.591)	0.001	0.288(0.133-0.621)	0.001
CBFbeta-MYH11	0.753(0.179-3.171)	0.699	0766 (0.182-3.216)	0.715	1.962(0.322-11.947)	0.465
NPM1	0.701(0.300-1.640)	0.413	0.704 (0.301-1.647)	0.418	0.705(0.263-1.891)	0.487
FLT3-ITD	0.549(0.198-1.521)	0.249	0.552 (0.199-1.536)	0.255	0.584(0.179-1.906)	0.373

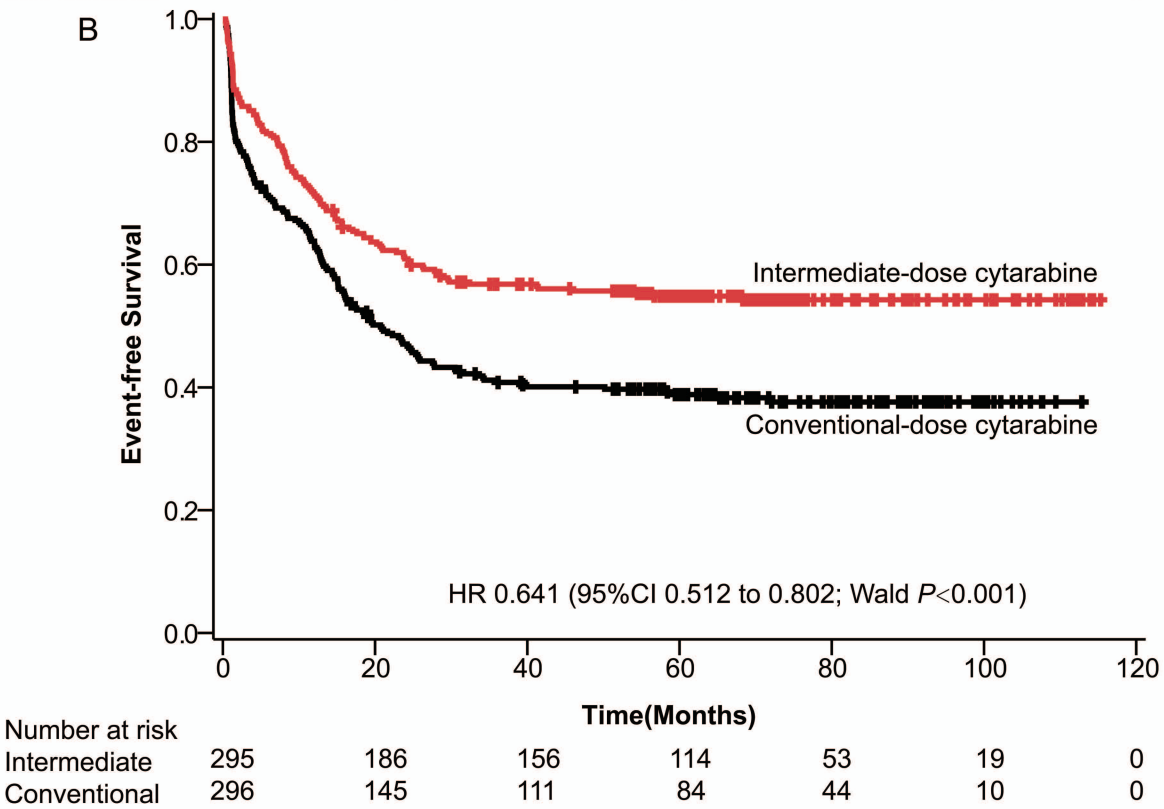
CD: conventional dose, ID: intermediate dose

*Adjusted for age, WBC, cytogenetics, transplantation in CR1, and consolidation therapy

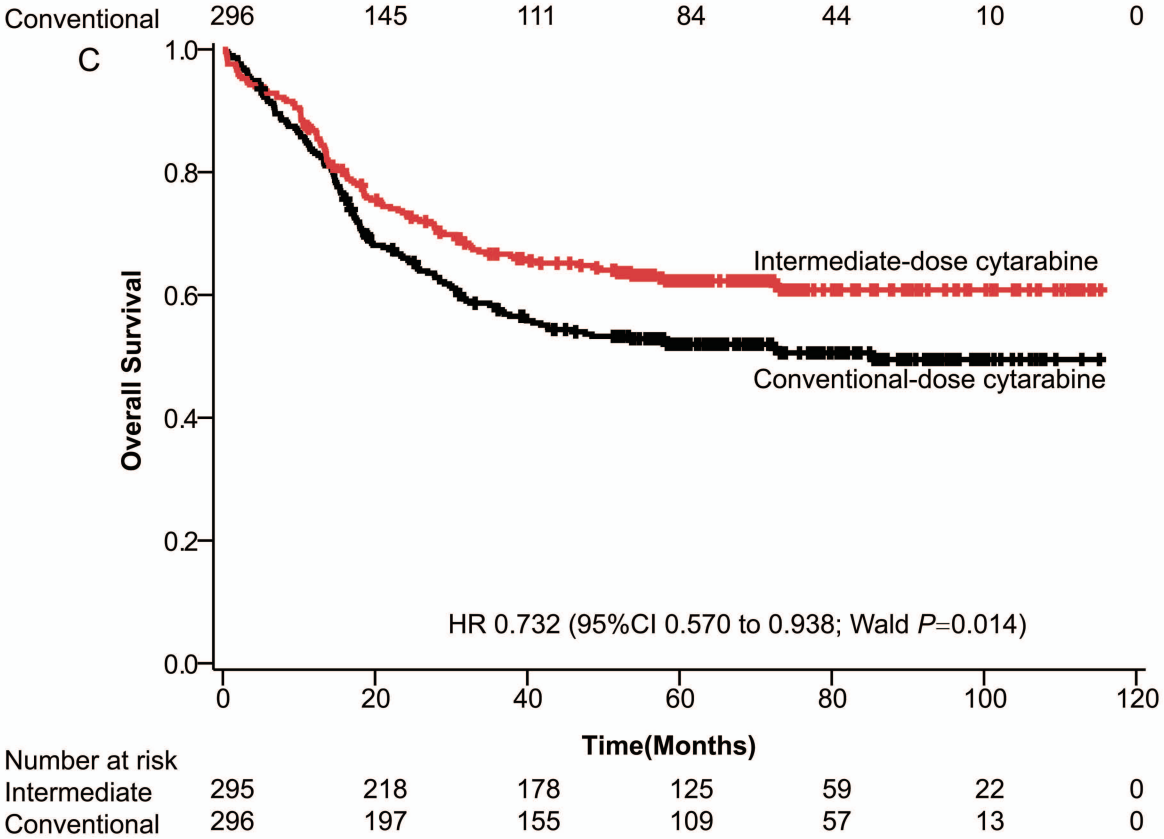
Figure S1. Outcomes of the entire cohort with longer follow-up by treatment assignment. (A) RFS, (B) EFS, and (C) OS.



Number at risk							
Intermediate	256	182	154	111	50	17	0
Conventional	229	143	111	80	42	9	0



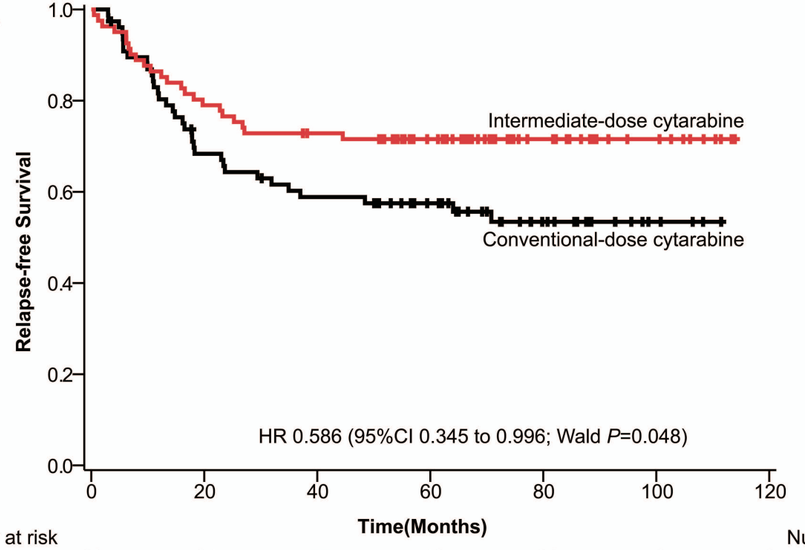
Number at risk							
Intermediate	295	186	156	114	53	19	0
Conventional	296	145	111	84	44	10	0



Number at risk							
Intermediate	295	218	178	125	59	22	0
Conventional	296	197	155	109	57	13	0

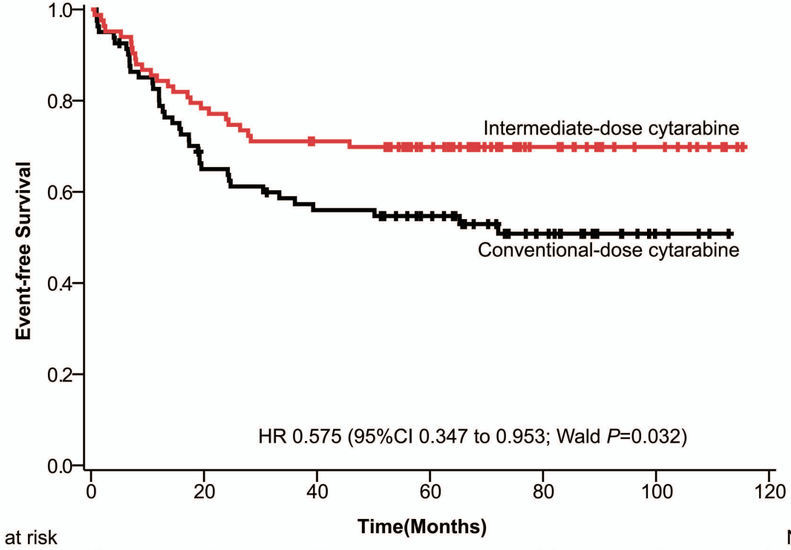
Figure S2. Outcomes of cytogenetic risk subgroup by treatment assignment. (A) RFS, (B) EFS, and (C) OS of patients with favorable risk. (D) RFS, (E) EFS, and (F) OS of patients with intermediate risk. (G) RFS, (H) EFS, and (I) OS of patients with poor risk. (J) RFS, (K) EFS, and (L) OS of patients with unknown risk.

A



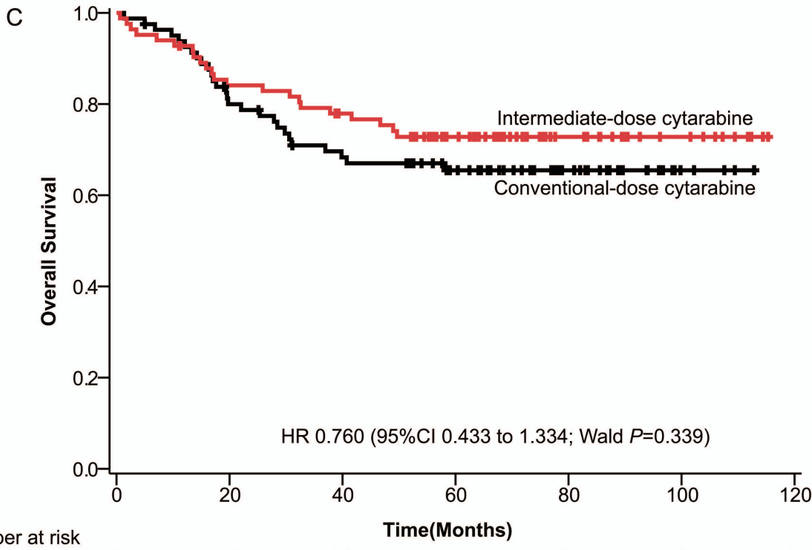
Number at risk							
Intermediate	81	64	57	43	21	9	0
Conventional	77	51	43	34	17	4	0

B



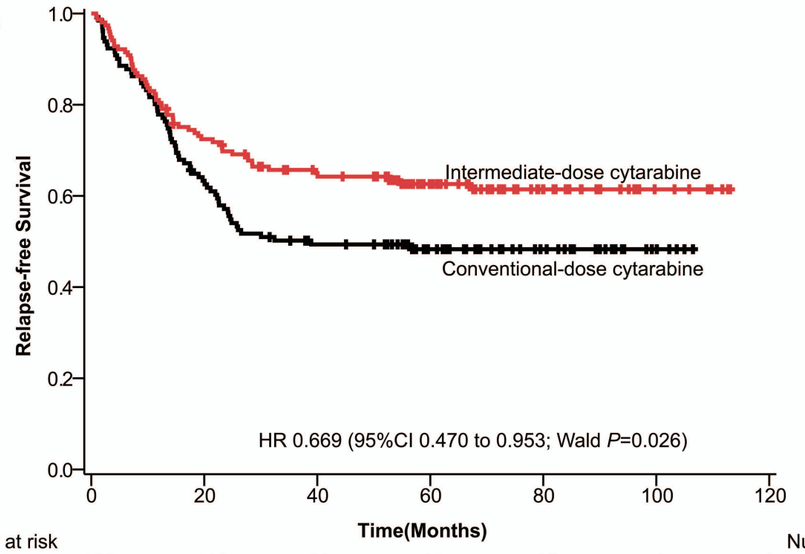
Number at risk							
Intermediate	83	65	57	44	21	9	0
Conventional	81	51	43	35	18	4	0

C



Number at risk							
Intermediate	83	68	61	44	21	9	0
Conventional	81	63	52	41	23	4	0

D



Number at risk							
Intermediate	153	109	89	61	27	8	0
Conventional	131	82	60	39	20	5	0

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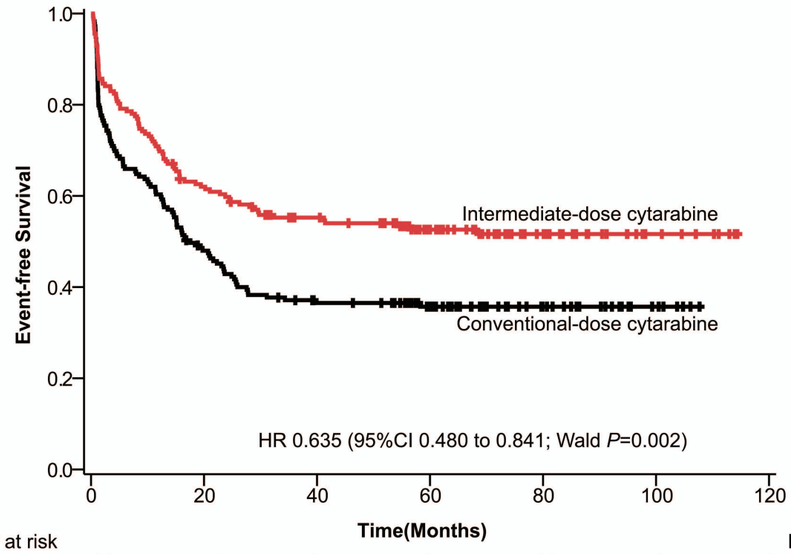
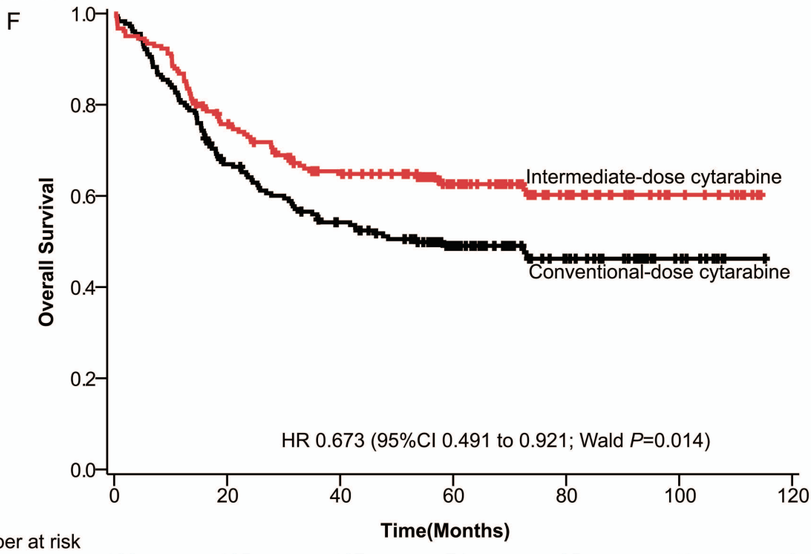


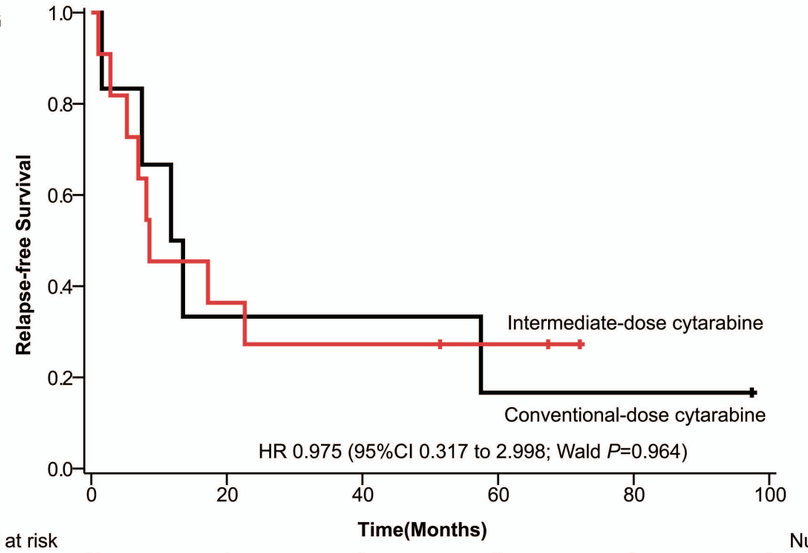
Number at risk							
Intermediate	182	111	91	61	30	9	0
Conventional	179	84	60	39	21	6	0

F



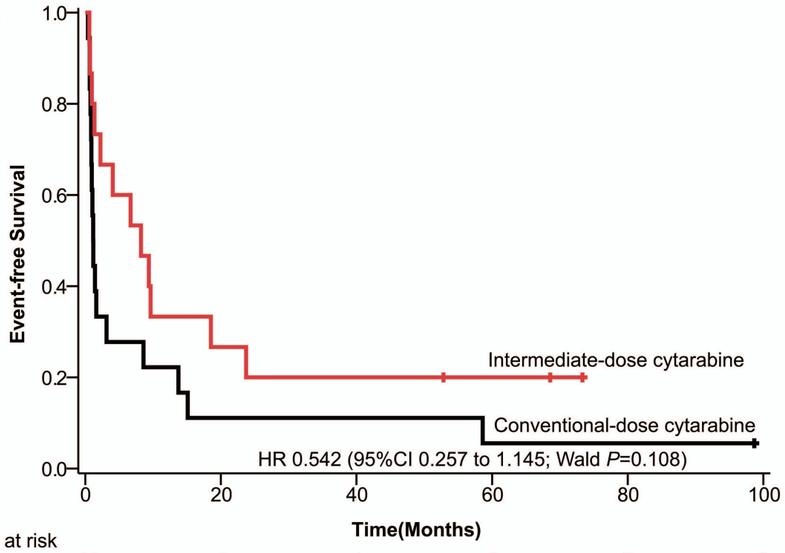
Number at risk							
Intermediate	182	135	107	74	35	11	0
Conventional	179	117	90	55	27	9	0

G



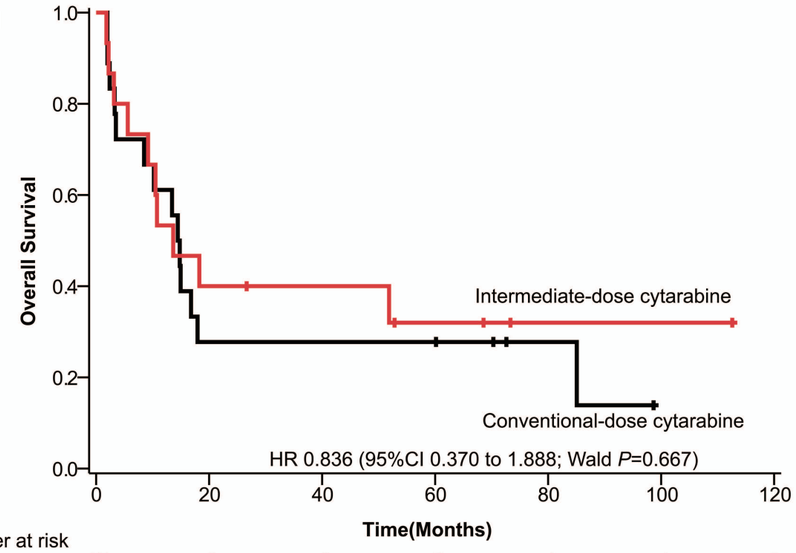
Number at risk							
Intermediate	11	4	3	2	0	0	
Conventional	6	2	2	1	1	0	

H



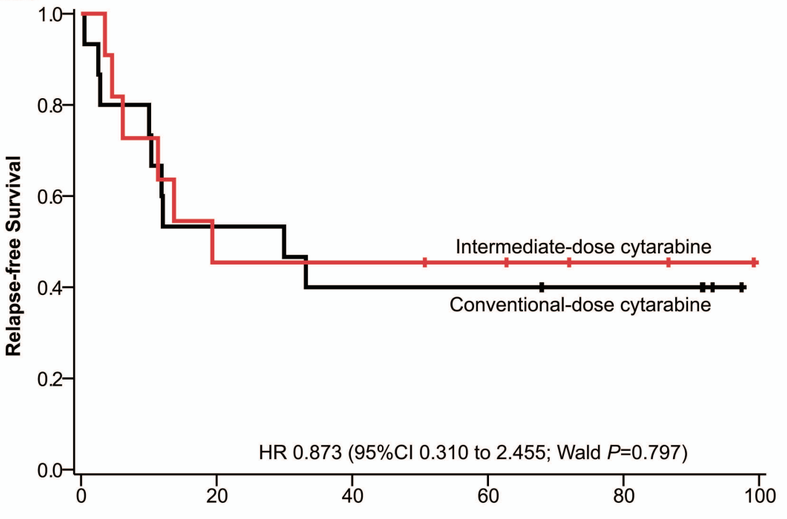
Number at risk							
Intermediate	15	4	4	2	0	0	
Conventional	18	2	2	1	1	0	

I



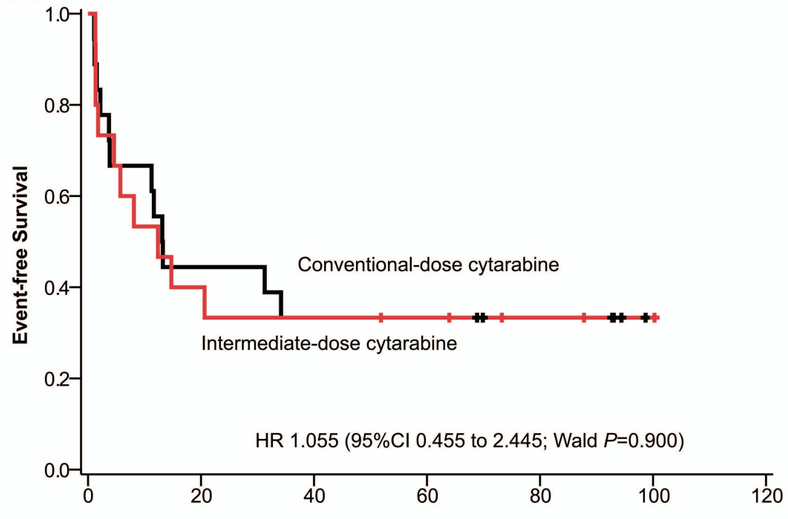
Number at risk							
Intermediate	15	6	6	3	1	1	0
Conventional	18	5	5	5	2	0	0

J



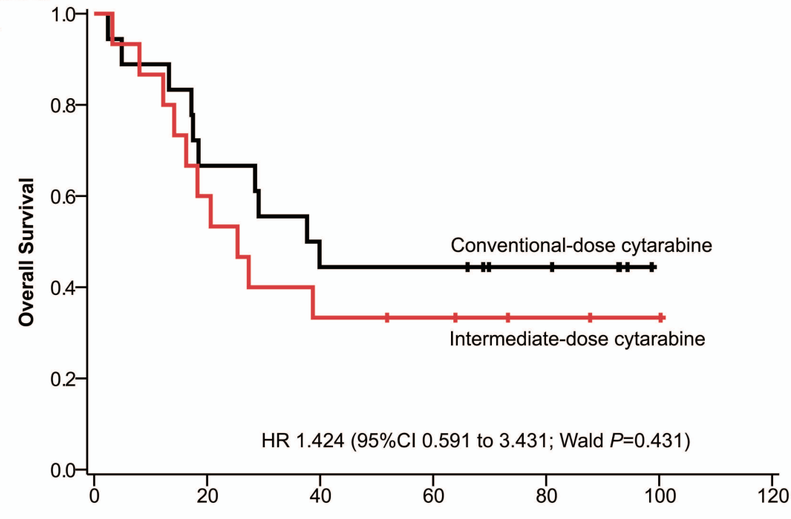
Number at risk						
Intermediate	11	5	5	4	1	0
Conventional	15	8	6	6	3	0

K



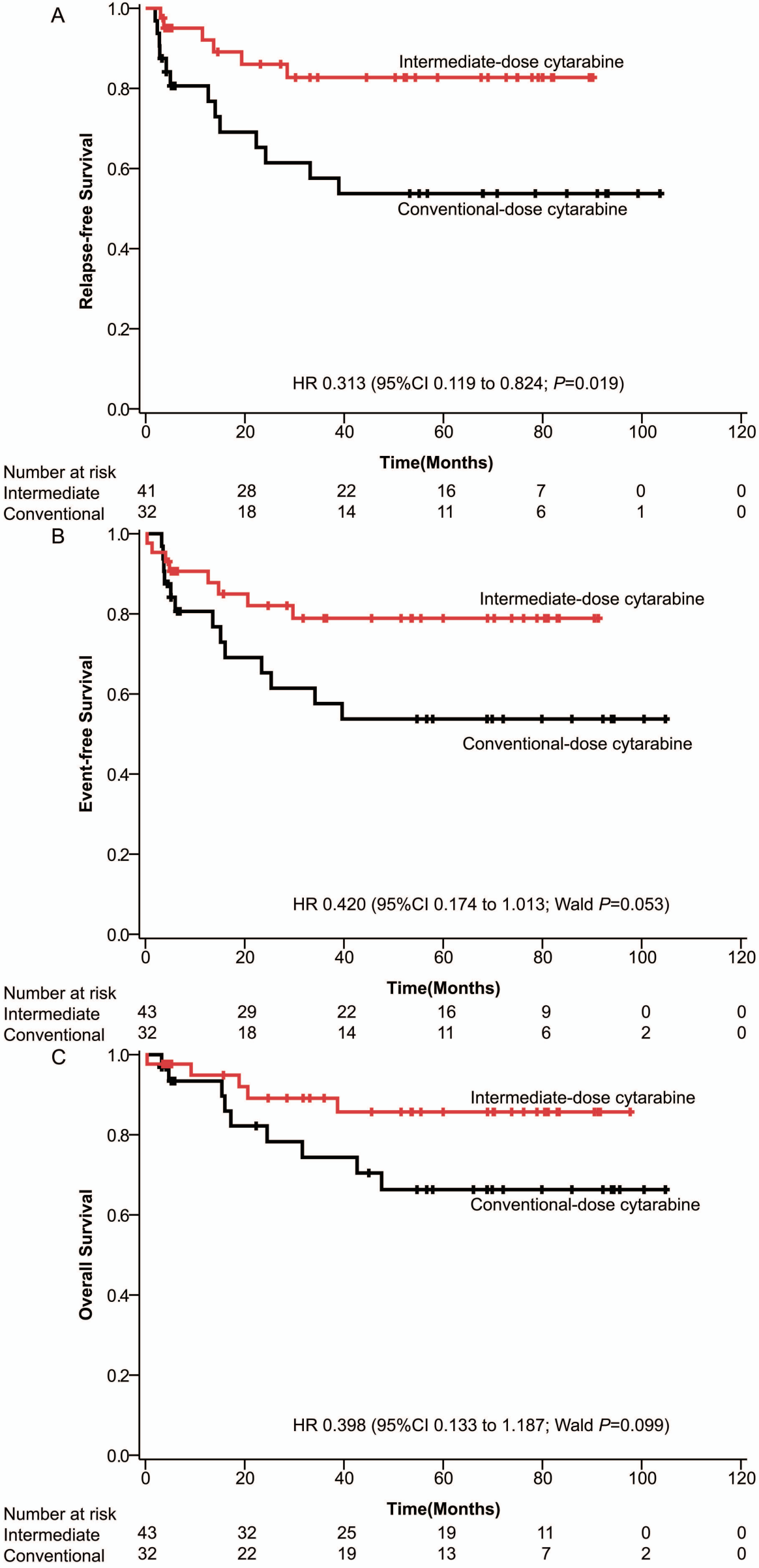
Number at risk							
Intermediate	15	6	5	4	2	1	0
Conventional	18	8	6	6	4	0	0

L



Number at risk							
Intermediate	15	9	5	4	2	1	0
Conventional	18	12	8	8	5	0	0

Figure S3. RFS(A), EFS(B), and OS(C) censored at the date of transplantation of *CEBPA*dm AML by treatment assignment.


A
Relapse-free Survival
1.0
0.8
0.6
0.4
0.2
0.0
Intermediate-dose cytarabine
Conventional-dose cytarabine
HR 0.313 (95%CI 0.119 to 0.824; P=0.019)
0
20
40
60
80
100
120
Time(Months)
Number at risk
Intermediate 41 28 22 16 7 0 0
Conventional 32 18 14 11 6 1 0
B
Event-free Survival
Intermediate-dose cytarabine
Conventional-dose cytarabine
HR 0.420 (95%CI 0.174 to 1.013; Wald P=0.053)
Time(Months)
Number at risk
Intermediate 43 29 22 16 9 0 0
Conventional 32 18 14 11 6 2 0
C
Overall Survival
Intermediate-dose cytarabine
Conventional-dose cytarabine
HR 0.398 (95%CI 0.133 to 1.187; Wald P=0.099)
Time(Months)
Number at risk
Intermediate 43 32 25 19 11 0 0
Conventional 32 22 19 13 7 2 0