



Title	Low-dose antithymocyte globulin inhibits chronic graft-versus-host disease in peripheral blood stem cell transplantation from unrelated donors
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## Supplemental figure legends

### Supplemental figure 1. Selection process of the study cohort.

### Supplemental figure 2. Impacts of ATG on the incidence of engraftment and aGVHD.

The cumulative incidence of neutrophil engraftment (A), platelet engraftment (B), grade II - IV aGVHD (C), and grade III - IV aGVHD (D) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups (Neutrophil engraftment: 99.0% [95% CI, 80.6 - 100.0%] vs 96.8% [95% CI, 92.8 - 98.6%],  $P = 0.059$ , Platelet engraftment: 95.9% [95% CI, 88.5 - 98.6%] vs 89.4% [95% CI, 83.9 - 93.1%],  $P = 0.24$ ).

### Supplemental figure 3. Impacts of ATG on the incidence of relapse and NRM, and the probability of survivals.

The cumulative incidences of relapse (a), NRM (b), Kaplan-Meier plots of OS (c) and PFS (d) in the ATG (*solid lines*) and non-ATG (*dashed lines*) groups (2 years relapse rate: 32.5% [95% CI, 23.3 - 42.0%] vs 30.1% [95% CI, 23.7 - 36.7%],  $P = 0.46$ , 2 years NRM: 14.7% [95% CI, 8.4 - 22.5%] vs 17.4% [95% CI, 12.4 - 23.2%],  $P = 0.38$ ).

### Supplemental figure 4. Association of the interaction between the ALC and the dose of ATG with aGVHD, cGVHD and relapse.

The interaction between the ALC and the dose of ATG (ALC before ATG / a total dose of ATG, ALC/ATG) in patients with or without grade III

- IV aGVHD (a), M/S cGVHD (b), and relapse (c). The cumulative incidence of grade III - IV aGVHD (d), M/S cGVHD (e), and relapse (f) in patients with the low-ALC (*solid lines*, N = 32), intermediate-ALC (*dashed lines*, N = 30), and high-ALC (*dotted lines*, N = 28) groups.

ALC/ATG was significantly higher in patients with grade III - IV aGVHD (median: 0.173 vs. 0.027,  $P = 0.015$ ) and M/S cGVHD (median: 0.104 vs. 0.022,  $P = 0.002$ ) compared to those without it. ALC/ATG was significantly lower in patients with relapse compared to those without it (median: 0.075 vs. 0.105,  $P = 0.030$ ). Based on these results, ATG group was stratified into 3 groups according to ALC/ATG; low- ALC/ATG ( $< 0.012$ ), intermediate-ALC / ATG ( $0.012 \leq \text{ALC} < 0.077$ ), and high-ALC/ATG ( $0.077 \leq$ ) groups. The cumulative incidence of grade III - IV aGVHD at 100 days (0 vs. 0 vs. 14.3%,  $P = 0.011$ ) and M/S cGVHD at 2 years (9.5 vs. 20.7 vs. 39.3%,  $P = 0.013$ ) were significantly higher in the high-ALC/ATG group than those in the others, while relapse rate at 2 years was significantly higher in the low-ALC/ATG group than those in the others (44.7 vs. 24.1 vs. 25.0%,  $P = 0.044$ ).

**Supplemental figure 5. GRFS and M/S CRFS according to ALC before ATG.** The Kaplan-Meier plots of GRFS (a) and M/S CRFS (b) in the low-ALC (*solid lines*, N = 20), intermediate-ALC (*dashed lines*, N = 18), and high-ALC (*dotted lines*, N = 15) groups in patients transplanted in remission (2 years GRFS: 30.0 vs. 72.2 vs. 33.3%,  $P = 0.013$ , 2 years M/S CRFS: 30.0 vs. 72.2 vs. 26.7%,  $P = 0.008$ ).

**Supplemental table 1     ATG dose and schedule**

Total dose	Day -5	Day -4	Day -3	Day -2	Day -1	N
1 mg/kg (N = 13)				1		12
			0.5	0.5		1
1.25 mg/kg (N = 3)				1.25		2
					1.25	1
1.5 mg/kg (N = 11)				1.5		11
2 mg/kg (N = 25)				1	1	18
			1	1		3
				2		2
			2			1
					2	1
2.5 mg/kg (N = 43)		1.25	1.25			14
				1.25	1.25	14
			1.25	1.25		12
	1.25	1.25				1
				2.5		1
					2.5	1
3 mg/kg (N = 1)			1.5	1.5		1
Unknown						1

**Supplemental table 2 Causes of death**

<b>Causes of death</b>	<b>ATG group</b>	<b>Non-ATG group</b>	<b>P-value</b>
<b>Relapse / Disease progression</b>	22 (57.9%)	47 (51.6%)	0.57
<b>NRM</b>	16 (42.1%)	44 (48.4%)	
<b>Respiratory failure</b>	4	9	
<b>Infection</b>	3	4	
<b>aGVHD</b>	2	3	
<b>TMA/SOS</b>	1	6	
<b>Organ failure</b>	1	4	
<b>PTLD</b>	1	0	
<b>cGVHD</b>	0	4	
<b>Graft failure</b>	0	2	
<b>Neurologic complication</b>	0	2	
<b>Hemophagocytic syndrome</b>	0	1	
<b>Others</b>	0	4	
<b>Unknown</b>	4	5	

**Abbreviations:** TMA, thrombotic microangiopathy; SOS, sinusoidal obstruction syndrome.

**Supplemental table 3 Univariate analysis in all cohort**

Variables	Group	N	M/S cGVHD		Overall cGVHD		GRFS		M/S CRFS	
			Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>
Recipient age	< 53 years	134	0.39 (0.31 - 0.47)	0.013	0.49 (0.40 - 0.57)	0.051	0.32 (0.25 - 0.40)	0.36	0.29 (0.21 - 0.37)	0.38
	≥ 53 years	152	0.25 (0.18 - 0.32)		0.36 (0.29 - 0.44)		0.31 (0.23 - 0.38)		0.27 (0.20 - 0.34)	
Recipient sex	Female	99	0.25 (0.17 - 0.34)	0.057	0.38 (0.28 - 0.48)	0.40	0.30 (0.22 - 0.40)	0.80	0.29 (0.21 - 0.39)	0.56
	Male	187	0.35 (0.28 - 0.42)		0.44 (0.37 - 0.51)		0.32 (0.25 - 0.39)		0.27 (0.21 - 0.33)	
DRI	Low, Intermediate	198	0.31 (0.24 - 0.37)	0.66	0.42 (0.35 - 0.49)	0.73	0.32 (0.26 - 0.39)	0.15	0.31 (0.24 - 0.37)	0.017
	High, Very high	88	0.33 (0.24 - 0.43)		0.43 (0.32 - 0.53)		0.29 (0.20 - 0.39)		0.20 (0.12 - 0.29)	
HCT-CI	0, 1	205	0.33 (0.27 - 0.40)	0.53	0.43 (0.36 - 0.50)	0.98	0.35 (0.28 - 0.41)	0.011	0.29 (0.23 - 0.35)	0.25
	≥ 2	81	0.27 (0.18 - 0.38)		0.41 (0.30 - 0.51)		0.23 (0.14 - 0.32)		0.24 (0.15 - 0.34)	
Recipient CMV-IgG	Negative	42	0.36 (0.22 - 0.50)	0.60	0.46 (0.30 - 0.61)	0.49	0.48 (0.32 - 0.62)	0.072	0.38 (0.24 - 0.52)	0.16
	Positive	241	0.31 (0.25 - 0.37)		0.42 (0.36 - 0.48)		0.28 (0.23 - 0.34)		0.25 (0.20 - 0.31)	
Donor age	< 43 years	182	0.30 (0.24 - 0.37)	0.50	0.37 (0.30 - 0.45)	0.072	0.33 (0.27 - 0.40)	0.41	0.27 (0.21 - 0.33)	0.55
	≥ 43 years	104	0.34 (0.25 - 0.43)		0.51 (0.40 - 0.60)		0.28 (0.19 - 0.37)		0.29 (0.20 - 0.38)	
Donor recipient sex combination	No	241	0.32 (0.26 - 0.38)	0.71	0.42 (0.35 - 0.48)	0.91	0.33 (0.27 - 0.39)	0.41	0.28 (0.22 - 0.33)	0.76
	Yes	44	0.30 (0.17 - 0.44)		0.43 (0.28 - 0.57)		0.23 (0.12 - 0.37)		0.28 (0.16 - 0.42)	
HLA mismatch	Match	208	0.33 (0.26 - 0.39)	0.56	0.42 (0.36 - 0.49)	0.91	0.31 (0.24 - 0.37)	0.47	0.25 (0.20 - 0.31)	0.14
	Mismatch	78	0.29 (0.19 - 0.39)		0.42 (0.31 - 0.53)		0.34 (0.23 - 0.44)		0.34 (0.23 - 0.44)	
Conditioning	RIC	142	0.27 (0.20 - 0.34)	0.058	0.38 (0.30 - 0.46)	0.17	0.31 (0.24 - 0.39)	0.34	0.25 (0.18 - 0.32)	0.11
	MAC	144	0.36 (0.28 - 0.44)		0.46 (0.38 - 0.54)		0.32 (0.24 - 0.40)		0.31 (0.23 - 0.38)	
CD34 <sup>+</sup> cells	< 4.9×10 <sup>6</sup> /kg	125	0.27 (0.20 - 0.35)	0.099	0.39 (0.30 - 0.48)	0.38	0.33 (0.25 - 0.41)	0.89	0.30 (0.22 - 0.38)	0.98
	≥ 4.9×10 <sup>6</sup> /kg	72	0.38 (0.26 - 0.49)		0.44 (0.33 - 0.56)		0.31 (0.21 - 0.42)		0.26 (0.17 - 0.37)	
ATG administration	No	189	0.36 (0.29 - 0.43)	0.025	0.48 (0.40 - 0.55)	0.022	0.27 (0.21 - 0.33)	0.034	0.23 (0.18 - 0.30)	0.034
	Yes	97	0.22 (0.14 - 0.31)		0.32 (0.22 - 0.41)		0.40 (0.30 - 0.50)		0.36 (0.27 - 0.46)	

**Abbreviations:** RIC, reduced-intensity conditioning.

**Supplemental table 4      Multivariate analysis for OS, PFS, Relapse and NRM in all cohort**

<b>Variable</b>	<b>Reference</b>	<b>HR (95%CI)</b>	<b>P-value</b>
<b>Overall survival</b>			
DRI High, Very high	Low, Intermediate	1.91 (1.34 - 2.73)	< 0.001
HCT-CI $\geq$ 2	0, 1	1.66 (1.15 - 2.40)	0.007
Recipient age $\geq$ 53 years	< 53 years	1.56 (1.00 - 2.43)	0.048
<b>Progression-free survival</b>			
DRI High, Very high	Low, Intermediate	1.56 (1.11 - 2.19)	0.010
HCT-CI $\geq$ 2	0, 1	1.48 (1.04 - 2.08)	0.028
Recipient age $\geq$ 53 years	< 53 years	1.57 (1.04 - 2.37)	0.031
<b>Relapse</b>			
DRI High, Very high	Low, Intermediate	2.23 (1.48 - 3.35)	< 0.001
Recipient age $\geq$ 53 years	< 53 years	1.57 (1.04 - 2.38)	0.032
<b>Non-relapse mortality</b>			
HCT-CI $\geq$ 2	0, 1	1.96 (1.13 - 3.40)	0.016

**Supplemental table 5 Patient and transplant characteristics in the ATG group**

	Low ALC group (N = 32)	Intermediate ALC group (N = 30)	High ALC group (N = 28)	P-value
<b>Sex (Male / Female)</b>	22 / 10	18 / 12	18 / 10	0.79
<b>Age (median, range)</b>	59.5 (17 - 67)	59 (39 - 68)	45 (20 - 70)	0.001
<b>Disease</b>				NA
<b>AML</b>	12	17	10	
<b>ALL</b>	6	0	7	
<b>MDS</b>	3	5	10	
<b>MPN</b>	1	0	0	
<b>CML</b>	2	0	1	
<b>ML</b>	8	8	0	
<b>Disease risk index</b>				0.28
<b>Low</b>	3	4	1	
<b>Intermediate</b>	22	22	17	
<b>High</b>	4	3	9	
<b>Very high</b>	3	1	1	
<b>HCT-CI</b>				0.97
<b>0</b>	16	14	17	
<b>1</b>	7	7	4	
<b>2</b>	3	4	3	
<b>3 ≤</b>	6	5	4	
<b>Recipient CMV serostatus</b>				0.53
<b>CMV IgG (+)</b>	26	25	20	
<b>CMV IgG (-)</b>	6	5	8	
<b>Donor age (median, range)</b>	40.5 (24 - 51)	35 (20 - 49)	45 (23 - 51)	0.002
<b>Donor–recipient sex combination</b>				0.85
<b>Female to Male</b>	3	4	4	
<b>Others</b>	28	26	24	
<b>Unknown</b>	1	0	0	
<b>HLA (GVH direction)</b>				0.65
<b>Match</b>	17	12	15	
<b>1-locus mismatch</b>	14	16	11	
<b>2-locus mismatch</b>	1	2	2	
<b>Conditioning</b>				0.011
<b>MAC</b>	10	11	20	0.022
<b>CY + TBI based</b>	6	1	5	
<b>BU + CY</b>	2	1	9	
<b>FLU + BU based</b>	2	8	6	
<b>FLU + MEL + TBI</b>	0	1	0	
<b>RIC</b>	22	19	9	0.62
<b>FLU + BU based</b>	12	15	6	
<b>FLU + MEL based</b>	7	4	3	
<b>FLU + BU + MEL</b>	2	0	0	
<b>Others</b>	1	0	0	
<b>GVHD prophylaxis</b>				NA
<b>TAC + MTX</b>	32	30	28	
<b>CD34<sup>+</sup> cells (×10<sup>6</sup>/kg) (median, range)</b>	4.60 (2.08 – 10.91)	4.55 (2.01 – 9.92)	4.61 (1.69 – 9.78)	0.99

**Abbreviations:** NA, not applicable; CY, cyclophosphamide; TBI, total body irradiation; BU, busulfan, FLU, fludarabine; MEL, melphalan; RIC, reduced-intensity conditioning; TAC, tacrolimus; MTX, methotrexate

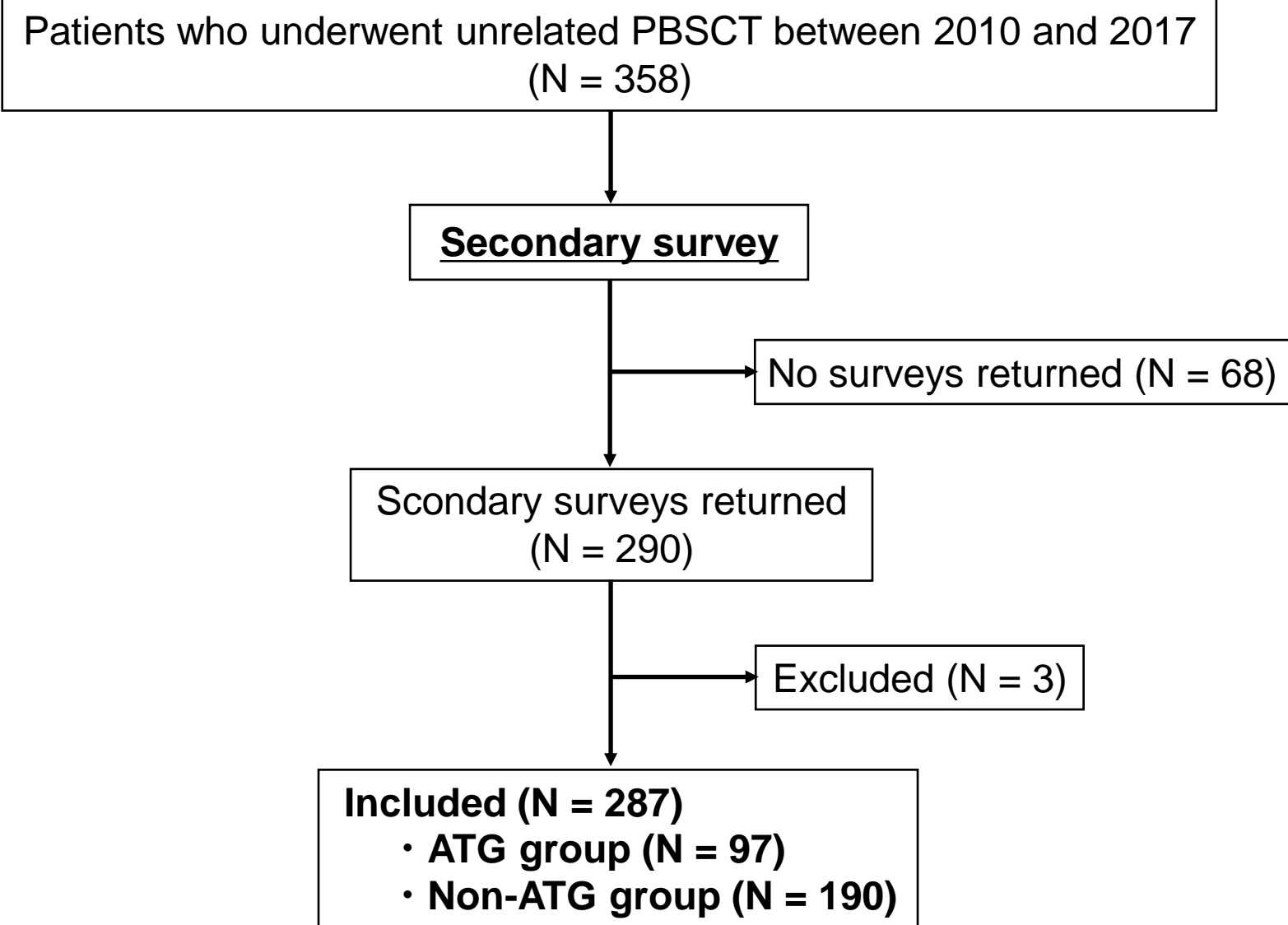


**Supplemental table 6 Univariate analysis in the ATG group**

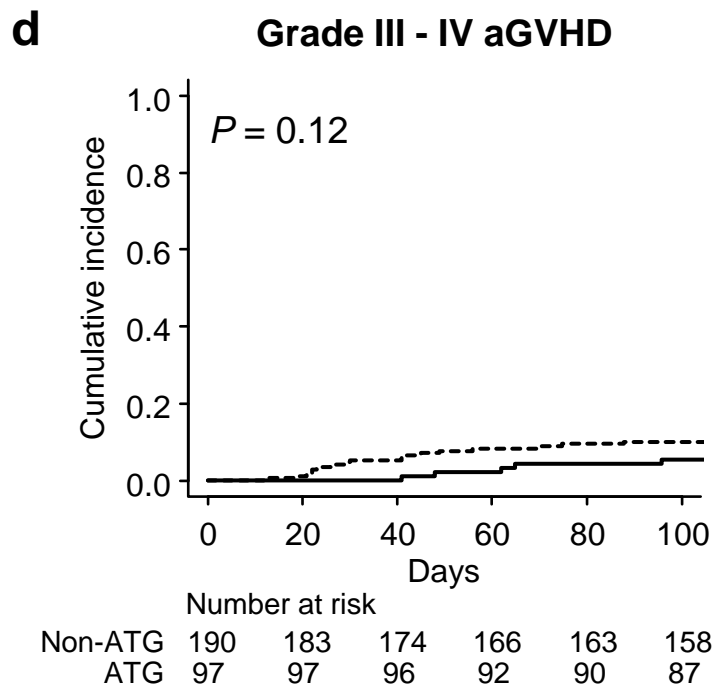
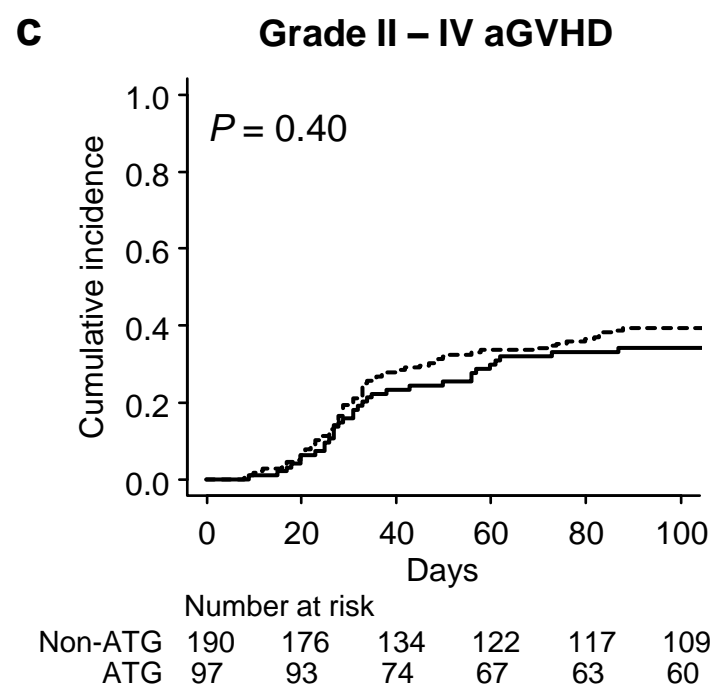
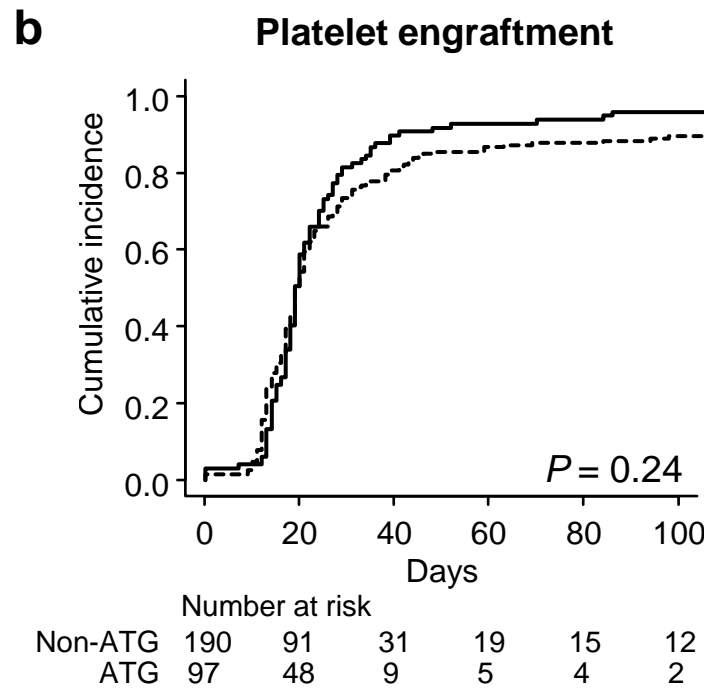
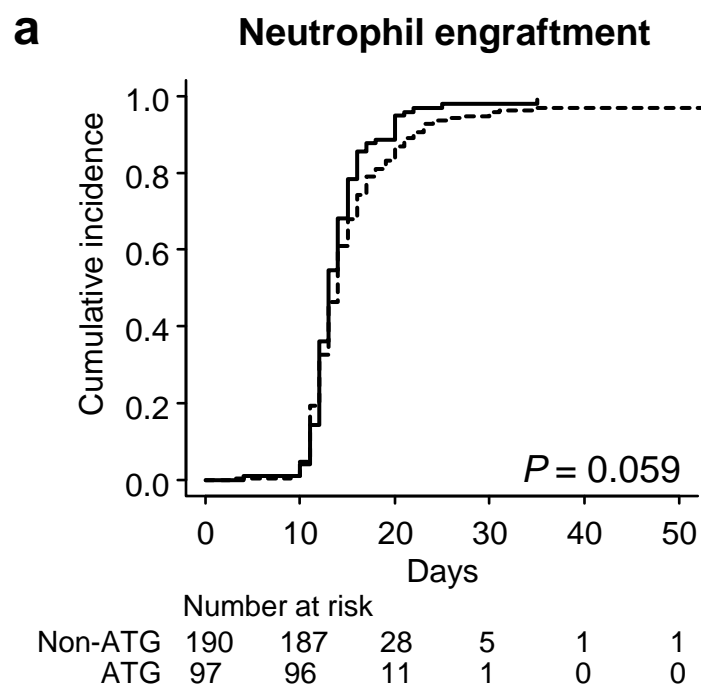
Variables	Group	N	M/S cGVHD		Relapse		GRFS		M/S CRFS	
			Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>	Incidence (95% CI)	<i>P</i>
Recipient age	< 53 years	37	0.25 (0.12 - 0.40)	0.77	0.31 (0.16 - 0.46)	0.33	0.39 (0.24 - 0.55)	0.63	0.39 (0.24 - 0.55)	0.26
	≥ 53 years	60	0.20 (0.11 - 0.32)		0.34 (0.22 - 0.46)		0.41 (0.28 - 0.53)		0.34 (0.22 - 0.46)	
Recipient sex	Female	36	0.17 (0.07 - 0.32)	0.33	0.34 (0.19 - 0.50)	0.61	0.40 (0.24 - 0.56)	0.81	0.34 (0.19 - 0.50)	0.64
	Male	61	0.25 (0.15 - 0.37)		0.31 (0.20 - 0.43)		0.40 (0.28 - 0.52)		0.37 (0.25 - 0.49)	
DRI	Low, Intermediate	75	0.23 (0.14 - 0.33)	0.62	0.30 (0.20 - 0.40)	0.25	0.39 (0.28 - 0.50)	0.92	0.35 (0.25 - 0.46)	0.79
	High, Very high	22	0.19 (0.06 - 0.38)		0.43 (0.21 - 0.63)		0.44 (0.23 - 0.63)		0.39 (0.19 - 0.59)	
HCT-CI	0, 1	70	0.25 (0.15 - 0.35)	0.52	0.32 (0.21 - 0.43)	0.73	0.45 (0.33 - 0.56)	0.030	0.39 (0.28 - 0.50)	0.16
	≥ 2	27	0.16 (0.05 - 0.33)		0.34 (0.17 - 0.53)		0.28 (0.12 - 0.45)		0.28 (0.12 - 0.45)	
Recipient CMV-IgG	Negative	20	0.20 (0.06 - 0.40)	0.72	0.30 (0.12 - 0.51)	0.85	0.50 (0.27 - 0.69)	0.58	0.45 (0.23 - 0.65)	0.49
	Positive	77	0.23 (0.14 - 0.33)		0.33 (0.23 - 0.44)		0.38 (0.27 - 0.48)		0.34 (0.23 - 0.44)	
Donor age	< 43 years	59	0.12 (0.05 - 0.22)	0.003	0.42 (0.29 - 0.54)	0.019	0.43 (0.30 - 0.55)	0.77	0.39 (0.26 - 0.51)	0.50
	≥ 43 years	38	0.37 (0.22 - 0.52)		0.18 (0.08 - 0.32)		0.37 (0.22 - 0.52)		0.32 (0.18 - 0.46)	
Donor recipient sex combination	No	84	0.21 (0.13 - 0.30)	0.78	0.34 (0.24 - 0.44)	0.19	0.39 (0.29 - 0.50)	0.31	0.34 (0.24 - 0.45)	0.22
	Yes	12	0.25 (0.05 - 0.52)		0.17 (0.02 - 0.43)		0.50 (0.21 - 0.74)		0.50 (0.21 - 0.74)	
HLA mismatch	Match	49	0.21 (0.11 - 0.33)	0.94	0.37 (0.24 - 0.51)	0.27	0.40 (0.26 - 0.53)	0.71	0.36 (0.23 - 0.49)	0.68
	Mismatch	48	0.23 (0.12 - 0.36)		0.28 (0.16 - 0.41)		0.41 (0.27 - 0.54)		0.36 (0.23 - 0.50)	
Conditioning	RIC	51	0.23 (0.12 - 0.35)	0.88	0.34 (0.21 - 0.48)	0.44	0.37 (0.24 - 0.51)	0.32	0.29 (0.17 - 0.42)	0.064
	MAC	46	0.22 (0.11 - 0.35)		0.30 (0.18 - 0.44)		0.44 (0.29 - 0.57)		0.44 (0.29 - 0.57)	
CD34 <sup>+</sup> cells	< 4.9×10 <sup>6</sup> /kg	42	0.15 (0.06 - 0.28)	0.64	0.25 (0.13 - 0.39)	0.15	0.48 (0.32 - 0.62)	0.15	0.48 (0.32 - 0.62)	0.12
	≥ 4.9×10 <sup>6</sup> /kg	25	0.16 (0.05 - 0.33)		0.40 (0.21 - 0.59)		0.36 (0.18 - 0.54)		0.32 (0.15 - 0.50)	
ALC before ATG	Low-ALC group	32			0.45 (0.27 - 0.61)	0.013				
	Other groups	58			0.25 (0.14 - 0.36)					
ALC before ATG	Intermediate-ALC group	30					0.52 (0.33 - 0.68)	0.090	0.48 (0.30 - 0.65)	0.059
	Other groups	61					0.37 (0.25-0.49)		0.32 (0.21 - 0.44)	
ALC before ATG	High-ALC group	28	0.50 (0.30 - 0.67)	< 0.001						
	Other groups	62	0.10 (0.04 - 0.19)							

**Abbreviations:** RIC, reduced-intensity conditioning.

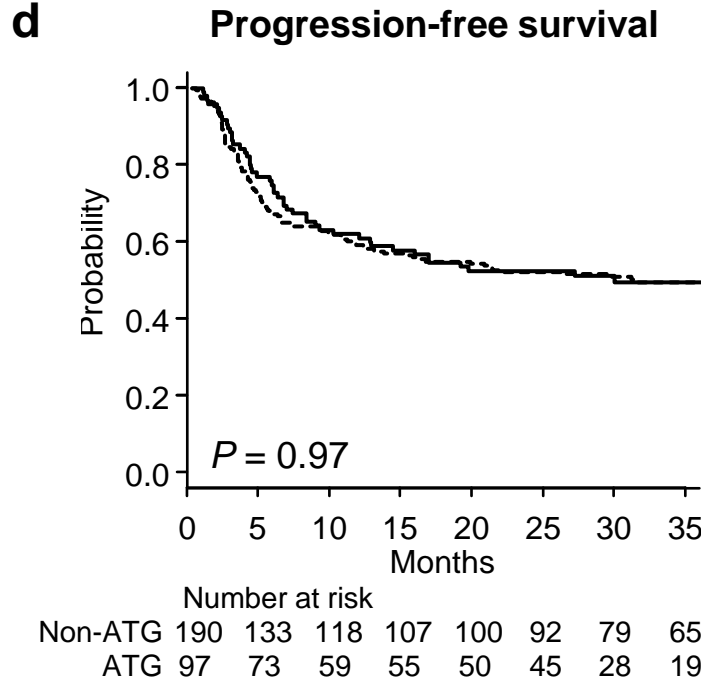
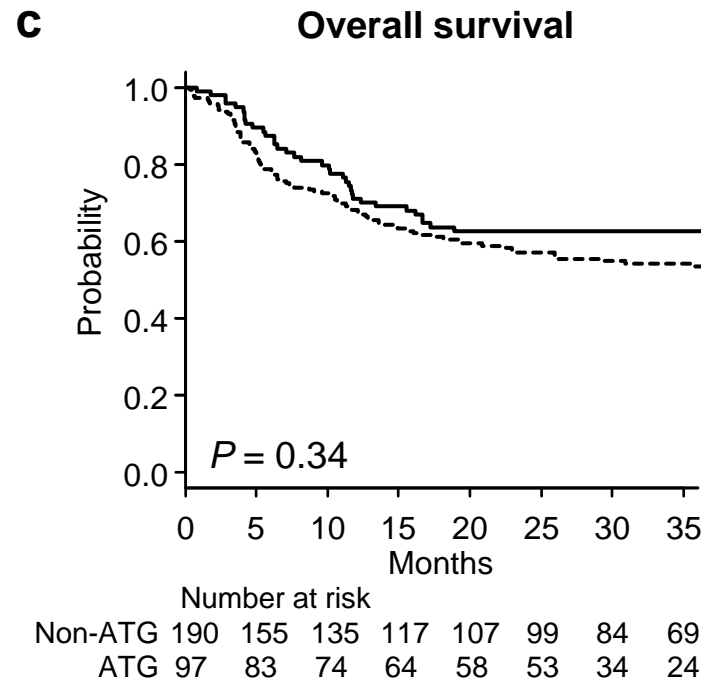
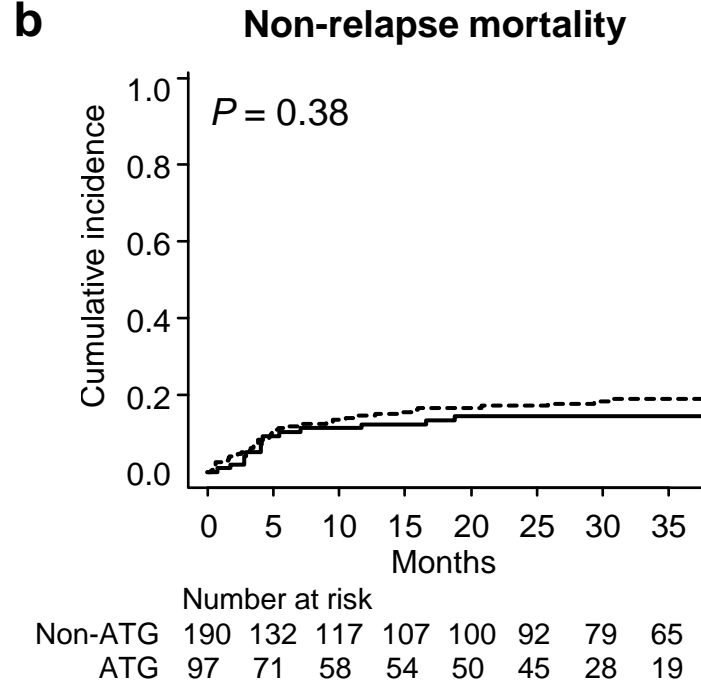
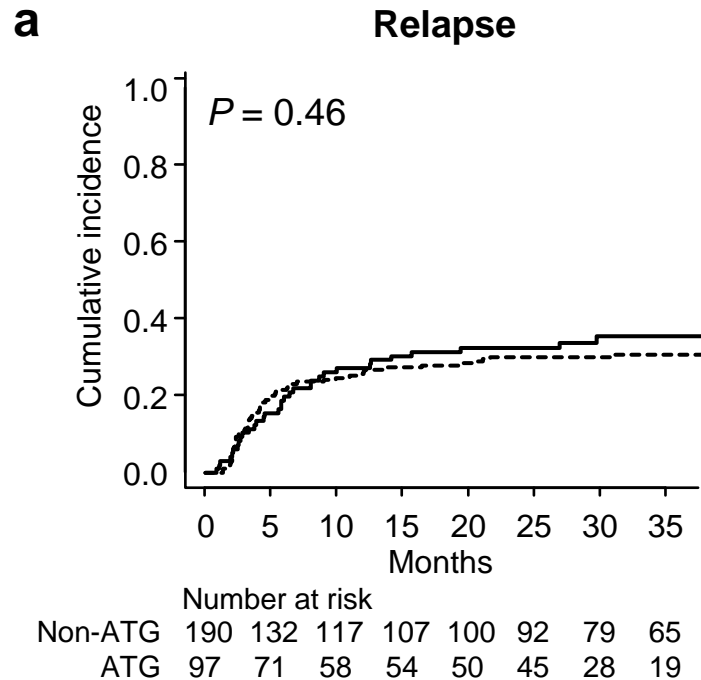
# Supplemental fig 1.



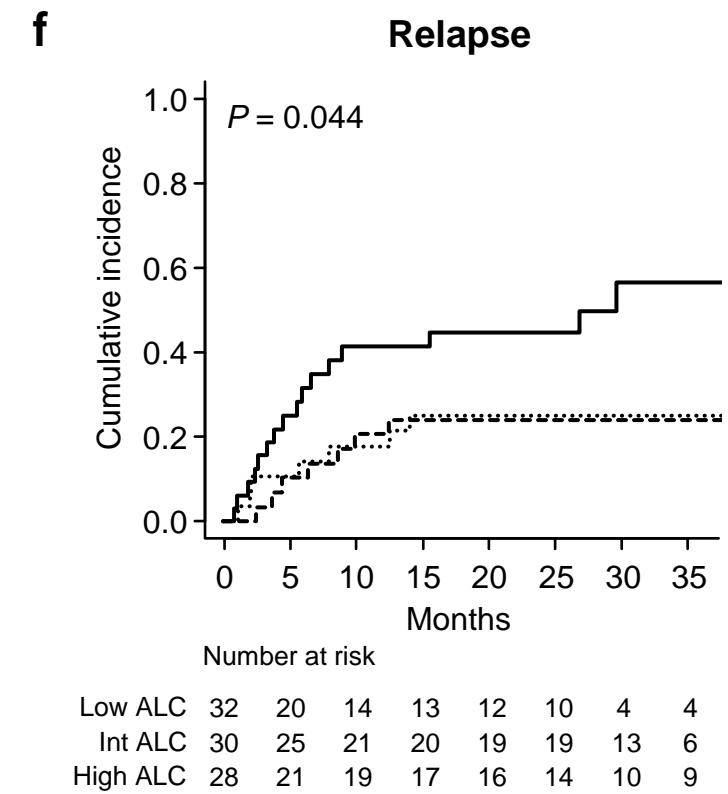
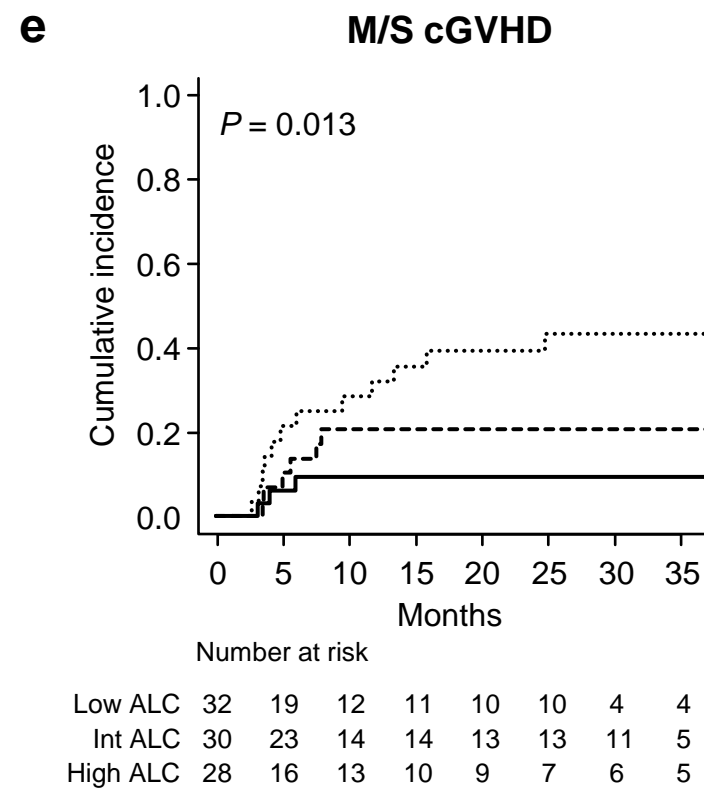
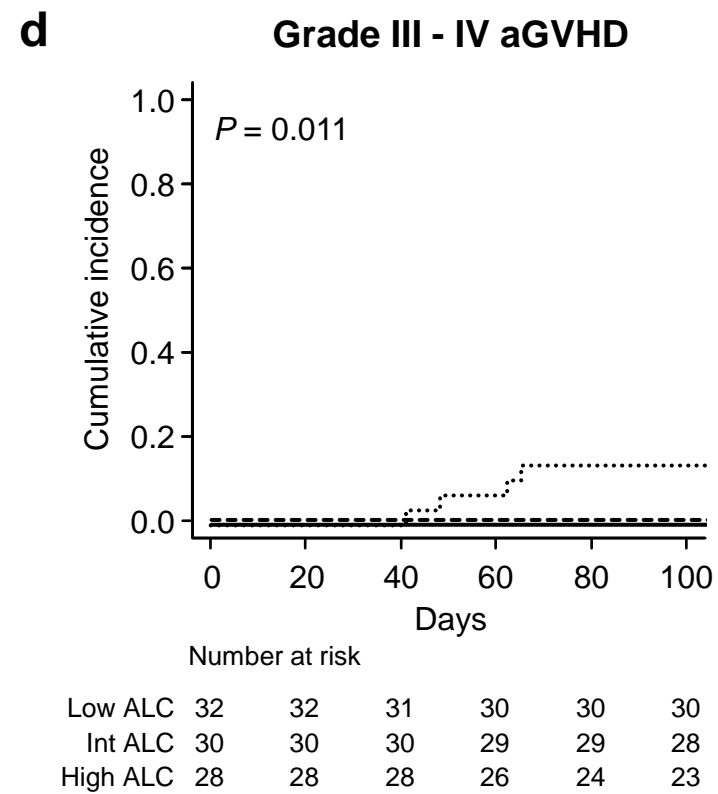
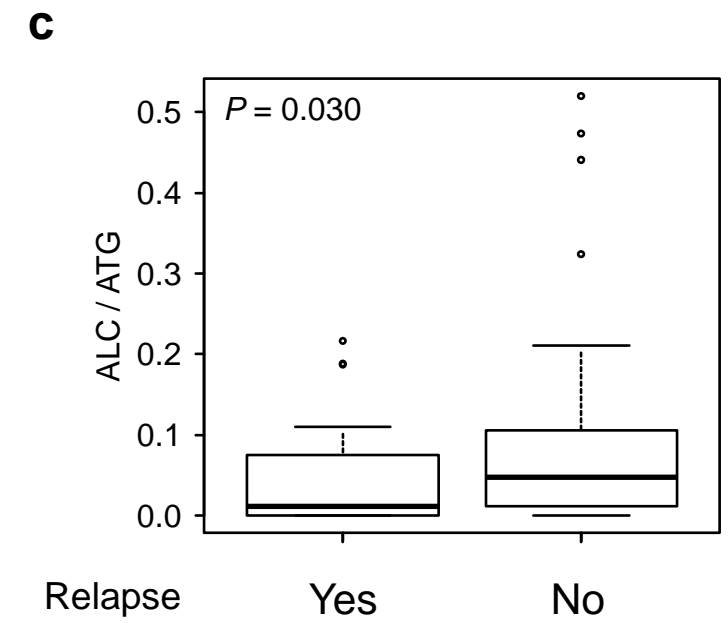
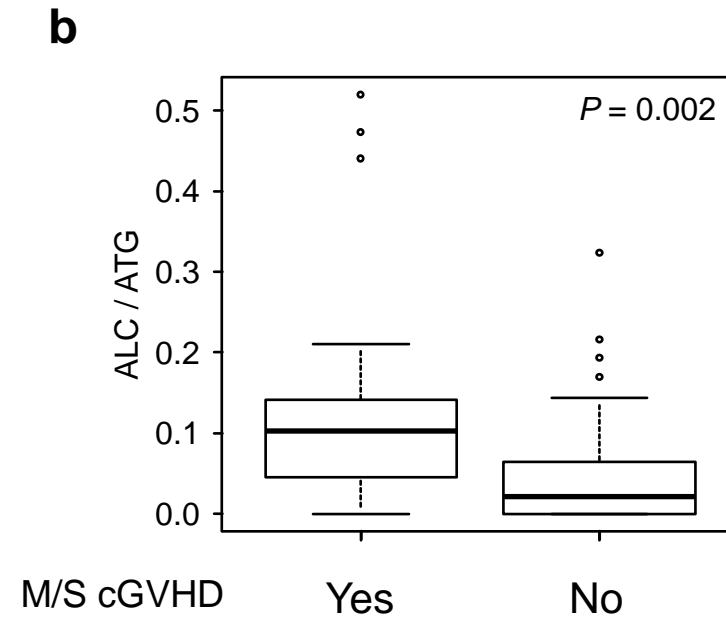
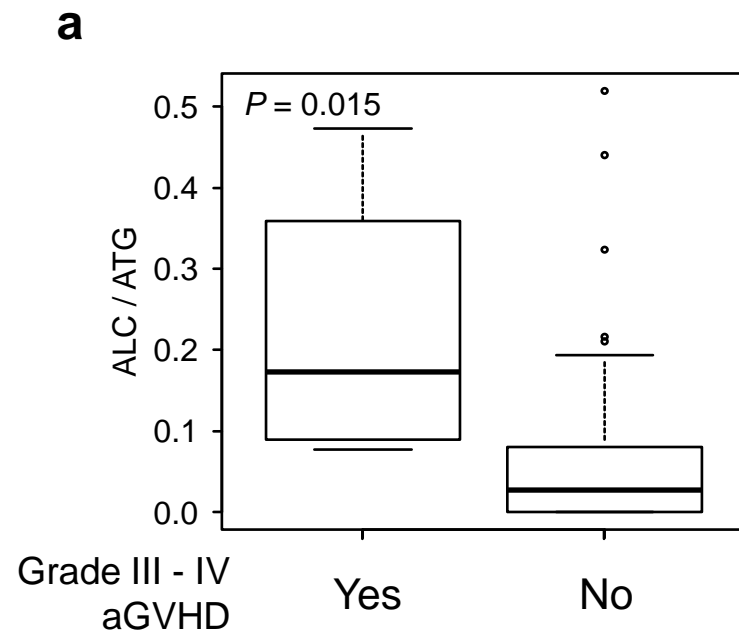
# Supplemental fig 2.



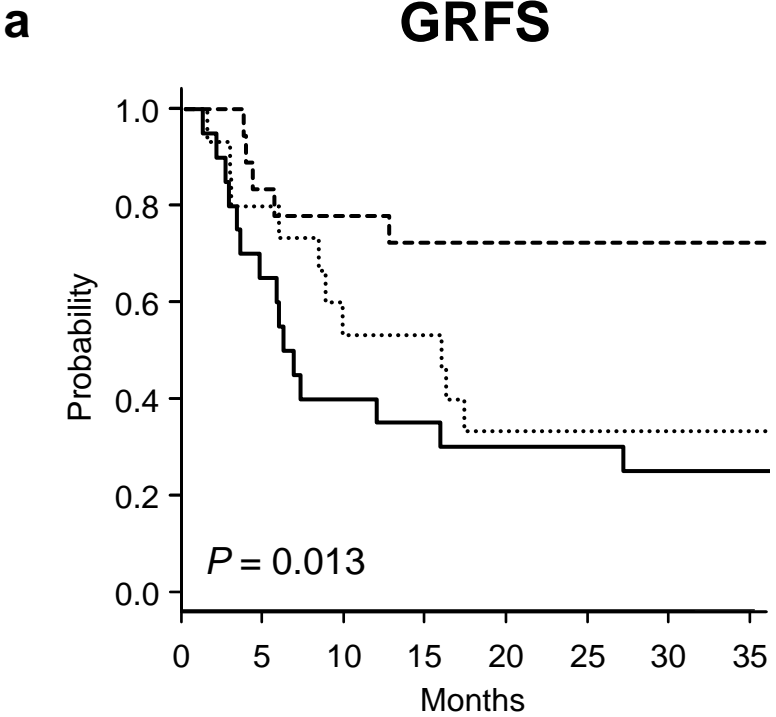
# Supplemental fig 3.



# Supplemental fig 4.

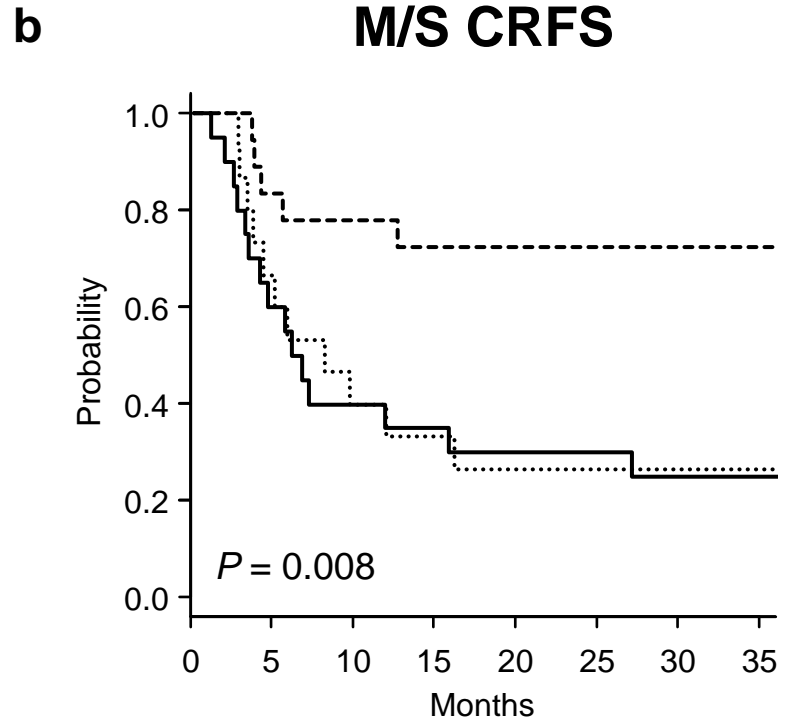


# Supplemental fig 5.



Number at risk

Low ALC	20	13	8	7	6	6	3	3
Int ALC	18	15	14	13	13	12	10	5
High ALC	15	12	8	8	5	5	4	2



Number at risk

Low ALC	20	12	8	7	6	6	3	3
Int ALC	18	15	14	13	13	12	10	5
High ALC	15	9	6	5	4	4	3	2