SUPPLEMENTARY APPENDIX

Predicting response of severe aplastic anemia to immunosuppression combined with eltrombopag

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Supplementary information

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Supplementary methods

Treatment

Horse ATG (ATGAM, Pfizer) was administered at a dose of 40 mg/kg of body weight per day for 4 days; cyclosporine was administered every 12 hours from day 1[1-3] or from day 14,[4] and was continued at least until 6 months with the dose adjusted to maintain trough blood levels of 200 to 400 ng/mL; mycophenolate mofetil was administered on day 1 of ATG at 600 mg/m^2 twice daily for patients aged <12 years and at 1 g twice daily for those ≥12 years, for 18 months; sirolimus at a dose of 2 mg/day in adults and 1 mg/m^2/day in children (<40 kg) was given on day 1 of ATG and continued for 6 months, with dose adjustment to maintain sirolimus levels between 5–15 ng/mL; EPAG was administered at a dose of 150 mg daily (75 mg daily for children aged 6–11 years, and 2.5 mg/kg of body weight per day for those who aged 2–5 years) and the duration varied in three subgroups, from day 14 until 6 months (cohort 1), from day 14 until 3 months (cohort 2) and from day 1 until 6 months (cohort 3 and extension cohort).

Table S1

Table S1. Logistic regression analysis for overall response

	Univariable logistic regression			Multivariable logistic regression		
	Odds ratio	95% CI	*P*	Odds ratio	95% CI	*P*
All patients (n = 416)						
Log (Age+1)	0.88	0.65–1.20	0.43			
Log (ARC+1)	2.24	1.76–2.90	<0.0001	2.06	1.60–2.68	<0.0001
Log (ANC+1)	7.27	2.53–22.2	0.0003	*	*	*
Log (ALC+1)	3.52	1.65–7.65	0.0013	2.95	1.33–6.65	0.0084
Log (Plt+1)	1.14	0.85–1.55	0.38			
Male sex	0.81	0.53–1.23	0.32			
PNH clone ≥1%†	1.32	0.84–1.09	0.24			
IST+EPAG group	2.75	1.74–4.42	<0.0001	2.39	1.48–3.94	0.0005
IST + EPAG group (n = 176)						
Log (Age+1)	1.15	0.64–2.01	0.63			
Log (ARC+1)	2.35	1.41–4.07	0.0015	2.12	1.15–4.01	0.018
Log (ANC+1)	26.9	3.31–307	0.0042	*	*	*
Log (ALC+1)	3.08	0.80–12.1	0.1	*	*	*
Log (Plt+1)	1.86	1.07–3.27	0.028	1.57	0.84–2.96	0.154
Log (TPO+1)‡	0.088	0.014–0.50	0.0071	0.14	0.02–0.87	0.039
Male sex	0.83	0.38–1.80	0.649			
PNH clone ≥1%†	1.19	0.48–3.14	0.71			
IST-only group (n = 240)						
Log (Age+1)	0.74	0.50–1.09	0.14	*	*	*
Log (ARC+1)	2.06	1.55–2.77	<0.0001	1.99	1.50–2.69	<0.0001
Log (ANC+1)	5.78	1.59–22.7	0.01	*	*	*
Log (ALC+1)	4.13	1.59–11.2	0.004	3.29	1.20–9.31	0.022
Log (Plt+1)	1.05	0.72–1.53	0.81			
Male sex	0.89	0.52–1.52	0.68			
PNH clone ≥1%†	1.4	0.81–2.43	0.23			

Abbreviations are explained in Table S1. *Variable included in initial multivariable logistic model but excluded by backward stepwise procedure in the final model. †PNH clone was not tested in 19 and 4 patients, treated with IST plus EPAG and IST alone, respectively. ‡TPO was studied in 140 patients treated with IST+EPAG.

Table S2

Table S2. Patient age and impact of ALC on IST outcomes

Author	Year, Publication (Enrollment)	ATG	Age, years, median (range)	n	ALC and OR	ALC and other outcomes
Gu C *et al.*[5]	2019 (2005–2018)	ATG-F or rATG	7 (3–14)	94	Lower ALC in OR vs NR (median, 1.8 vs 2.5 $\times 10^9$/L; *P*=0.24).	
Yoshida N *et al.*[6]	2011 (1997–2006)	hATG	8 (1–17)	312	Lower ALC in OR vs NR (median, 1.6 vs 2.0 $\times 10^9$/L; ***P*=0.0060**).	
Narita A *et al.*[7]	2015 (2001–2013)	hATG or rATG	9 (0–16)	113	Lower ALC in OR vs NR (median, 1.5 vs 2.0 $\times 10^9$/L; NS).	
Sakaguchi H *et al.*[8]	2014 (not shown)	hATG or rATG	10 (1–16)	64	Lower ALC in OR vs NR (median, 1.8 vs 2.0 $\times$ 10^9/L; NS).	ALC <2$\times 10^9$/L associated with higher FFS (***P*=0.04**) and TFS (***P*=0.011**).
Jain R *et al.*[9]	2019 (2012–2017)	hATG or rATG	10 (8–11*)	43	NS (data not shown)	NS in OS and FFS (data not shown).
Cabannes-Hamy A *et al.*[10]	2018 (2000–2013)	hATG or rATG	17 (3–65)	84	Odds ratio, 0.73 for continuous ALC (*P*=0.17)	Lower ALC associated with lower relapse rate (***P*=0.011**) and higher FFS (***P*=0.004**)
Kulagin A *et al.*[11]	2013 (2005–2012)	hATG or rATG	21 (1–65)	125	NS (data not shown)	
Scheinberg P *et al.*[12]	2009 (1989–2005)	hATG	31 (18–53*)	316	ALC >1$\times 10^9$/L associated with higher OR 70% (139 of 200) vs 47% (55 of 116; ***P*=0.0054**).	Higher ALC associated with higher OS (***P*=0.0013**).
Zaimoku Y *et al.*	Present study (2012–2019)	hATG + EPAG	32 (3–82)	176	Higher ALC in OR vs NR (median, 1.3 vs 1.0 $\times 10^9$/L; *P*=0.079 [***P*=0.016**‡]).	Higher ALC in CR vs no CR (*P*=0.065 [***P*=0.010**‡])
Shin SH *et al.*[13]	2013 (2001–2010)	hATG or rATG	36 (14–75)	99	Odds ratio, 0.81 (*P*=0.60) for ALC >0.9$\times 10^9$/L.	ALC >0.9$\times 10^9$/L tended to associate with higher OS (*P*=0.092).
Chang MH *et al.*[14]	2010 (1994–2007)	hATG or rATG	49 (15–78)	62	Odds ratio, 1.1 (*P*=0.86) for ALC >0.9$\times 10^9$/L,	ALC >0.9$\times 10^9$/L associated with higher OS (***P*=0.008**).
Boddu P *et al.*[15]	2017 (2000–2016)	hATG or rATG	49 (19–84)	62	Odds ratio, 12.3 (***P*=0.025**) for log ALC in the multivariate analysis.	Higher ALC tended to associate with higher OS (*P*=0.09)
Afable MG 2nd *et al.*[16]	2011 (1996–2010)	hATG or rATG	52† (3–80)	87	ALC >0.75$\times 10^9$/L associate with higher OR (*P*=0.070).	ALC >0.75$\times 10^9$/L associated with higher OS in multivariate analysis (***P*=0.03**).
Vaht K *et al.*[17]	2018 (2000–2011)	hATG or rATG	53 (2–85)	157	ALC >1.0$\times 10^9$/L showed higher OR 48% (58 of 121) vs 42% (15 of 36; *P*=0.51).	

The blue and red cells indicate association of better outcomes with lower and higher ALC, respectively. ATG-F indicates Jurkat cell-reactive ATG; FFS, failure free survival; hATG, horse ATG; HR, hazard ratio; OS, overall survival; rATG, rabbit ATG; TFS, transplant free survival. The other abbreviations are explained in Table S1. *IQR †Mean of median age in the two IST cohorts. ‡After exclusion of young children aged <10 years.

Figure S1

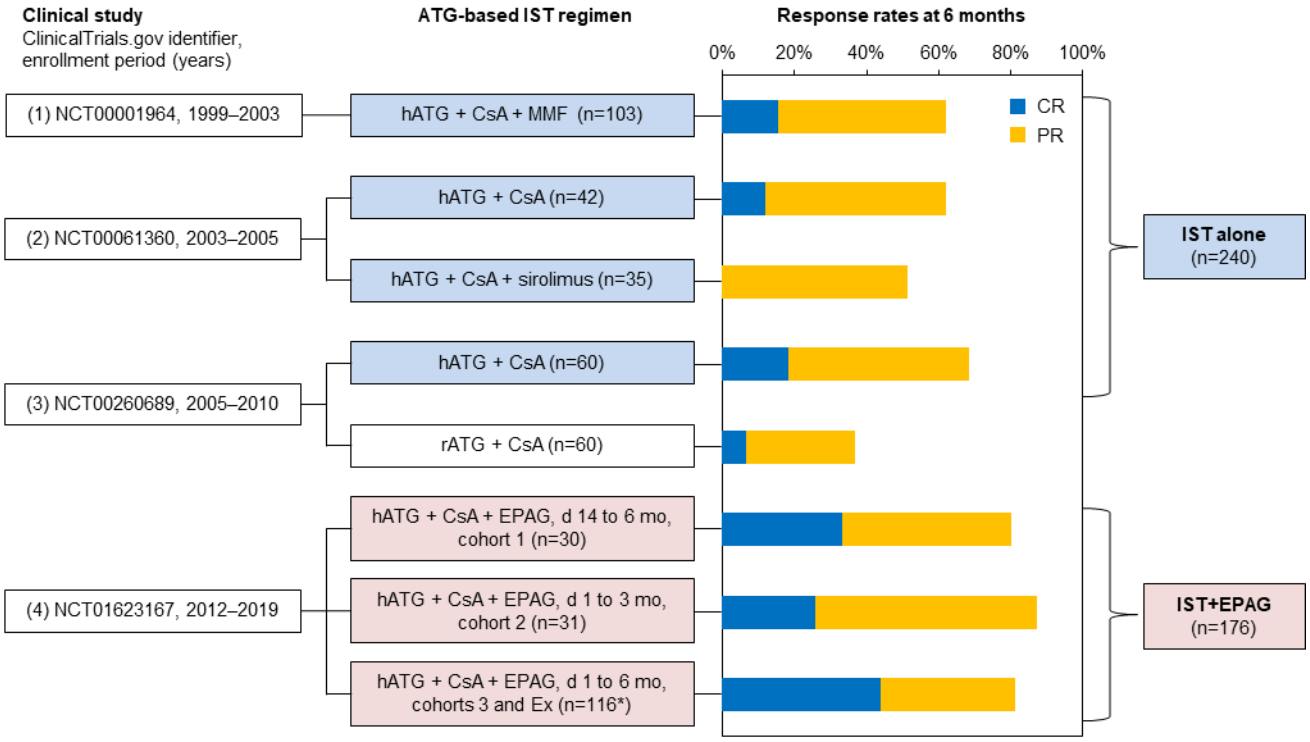

Figure S1. IST protocols and responses. Immunosuppressive therapy (IST) alone group included four horse antithymocyte globulin ATG (hATG)-based regimens from three clinical studies. IST plus eltrombopag (EPAG) group was consisted of four cohorts with three different administration duration of EPAG. CR indicates complete response; Ex, extension cohort; MMF, mycophenolate mofetil; PR, partial response; and rATG, rabbit ATG. *One patient in Ex cohort who did not reach 3-month evaluation after a hematological response was excluded from the statistical analysis.

Figure S2

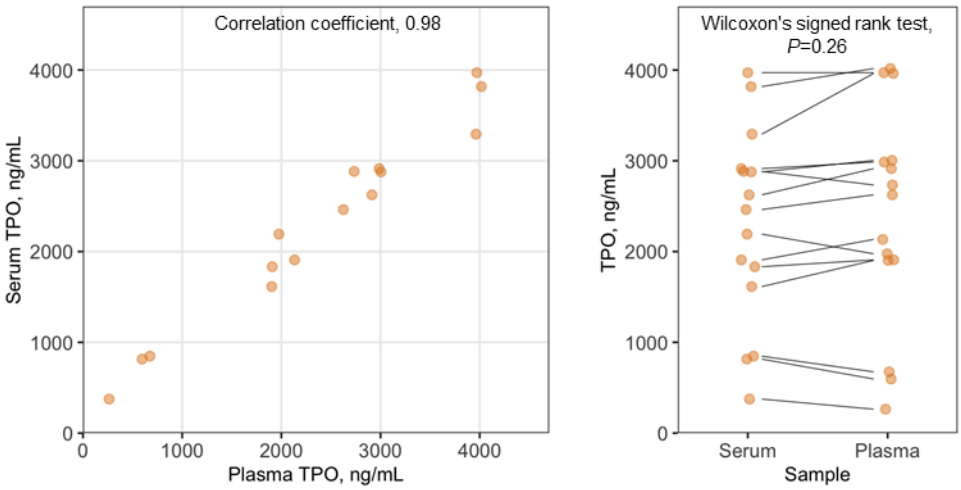

Figure S2. TPO levels in plasma and serum. Plasma and serum sampled at the same time contained almost equal levels of thrombopoietin (TPO) in 15 patients with immune aplastic anemia.

Figure S3

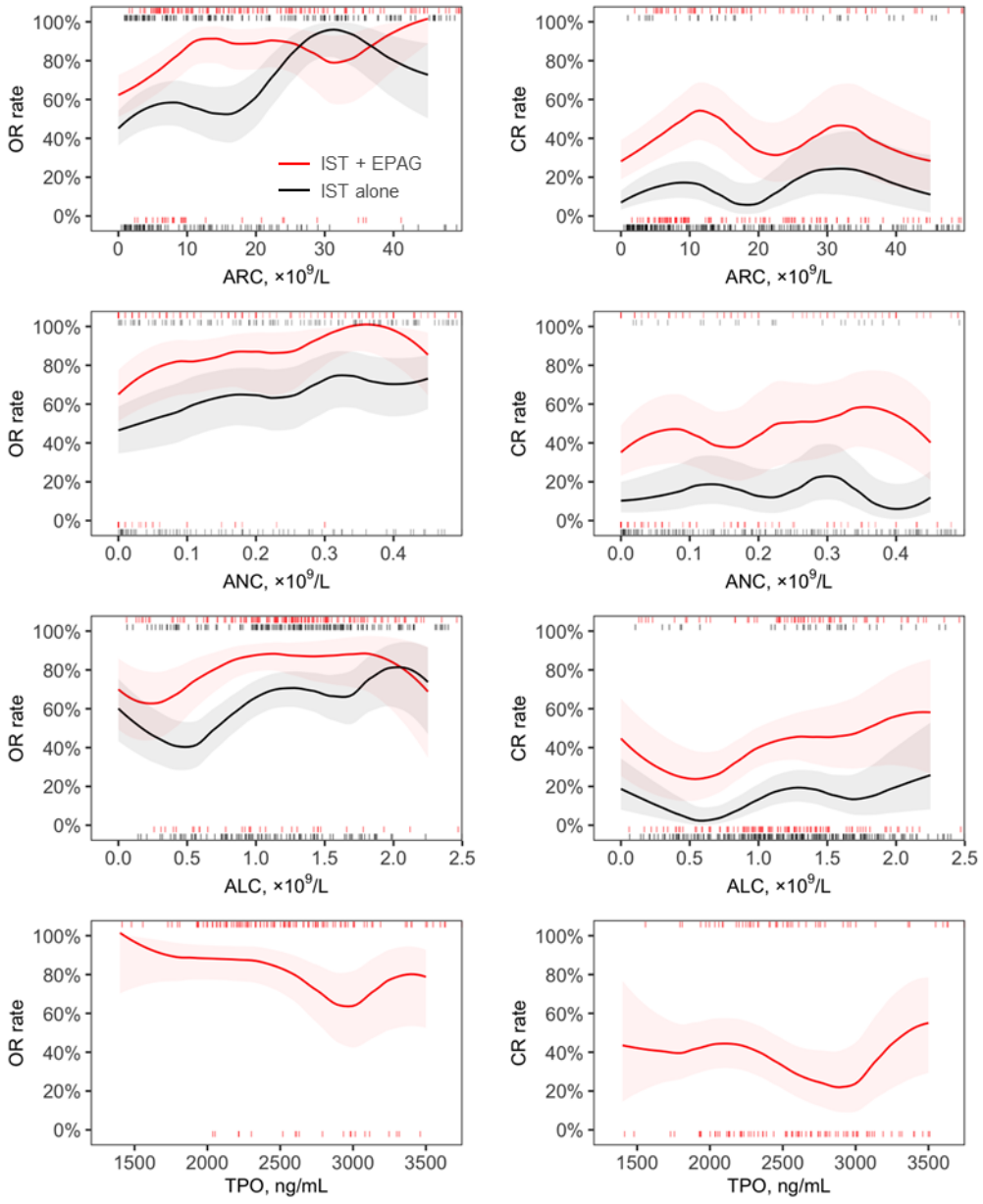

Figure S3. Response curves according to initial blood counts. Overall response (OR) rate and CR rate and their 90% confidence interval of individuals with initial blood counts between X and X + α in groups of IST plus EPAG (red line) and IST alone (black line) were shown. Absolute reticulocyte count (ARC), $\alpha = 10 \times 10^9$/L; absolute neutrophil count (ANC), $\alpha = 0.1 \times 10^9$/L; absolute lymphocyte count (ALC), $\alpha = 0.5 \times 10^9$/L; plasma thrombopoietin level (TPO), α = 500 ng/mL.

Figure S4

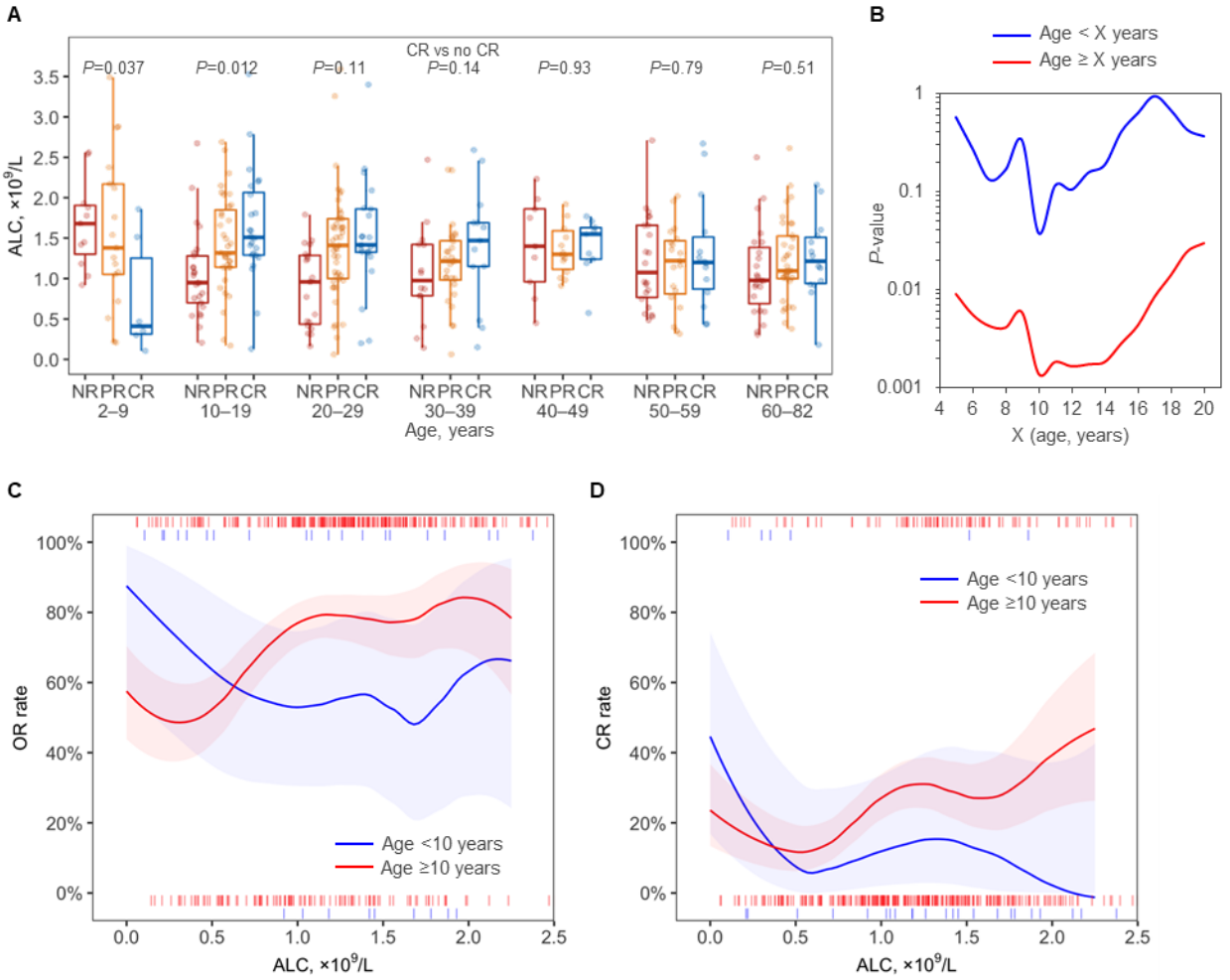

Figure S4. Inverse correlation of ALC with complete response in young children. (A) Pretreatment ALC and IST responses according to age-subgroups in the 416 patients treated with and without EPAG. Outlier for ALC > 4.0 × 10^9/L, 1 in PR, aged 40 to 49 years. (B) *P*-values for the comparisons of ALC between CR and non-CR among patients younger than X years (blue line; lower ALC correlates with CR) and among those at or older than X years (red line; higher ALC correlates with CR). The age of 10 years was the best cut-off for both positive and negative correlations of ALC with CR. (C and D) OR and CR rates and their 90% confidence interval in individuals with initial ALC between X and (X + 1.0) × 10^9/L, aged younger than 10 years (blue line) and those with initial ALC between X and (X + 0.5) × 10^9/L, aged 10 years or older (red line).

Figure S5

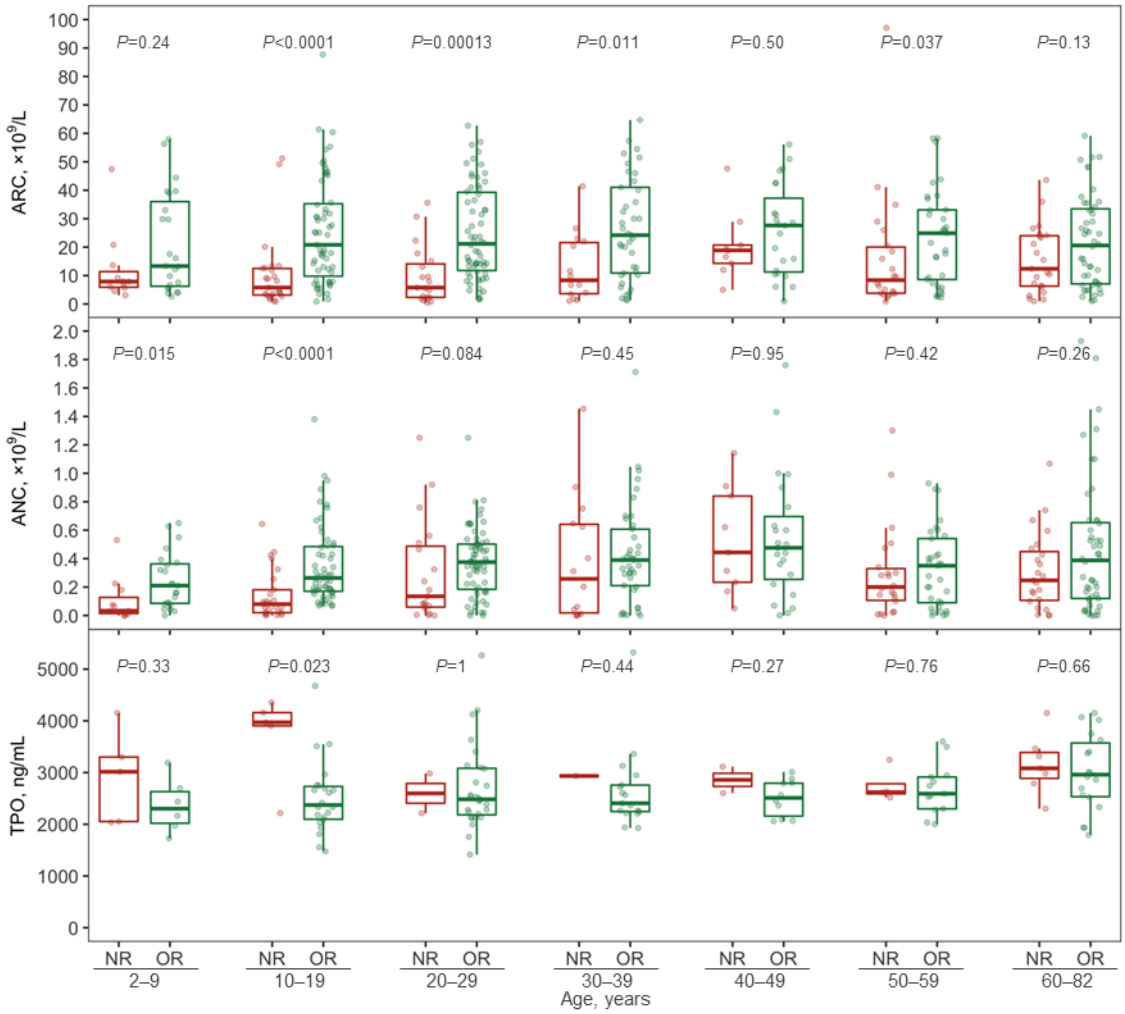

Figure S5. Age-subgroup and overall response. Pretreatment ARC (n=416; total patients), ANC (n=416; total patients) and TPO (n=140; IST+EPAG group) were compared between OR and NR according to age groups. Outlier for ARC > 100 × 10^9/L, 2 in OR.

Figure S6

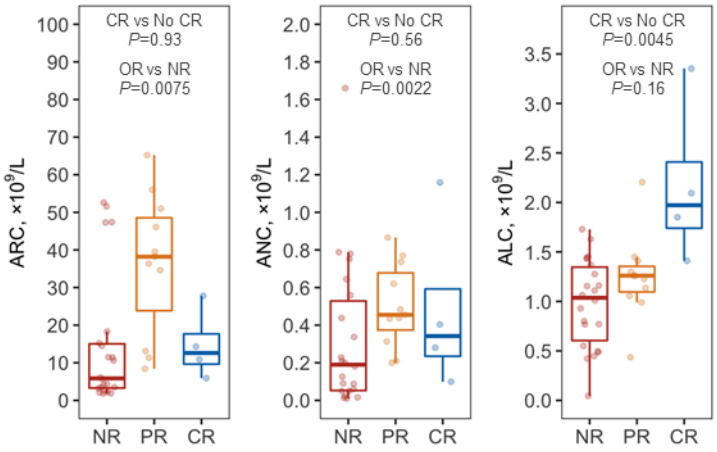

Figure S6. Rabbit ATG-based IST. Pretreatment ALC, ARC and ANC of 54 patients, aged 10 years or older (median, 27 years), initially treated with rabbit ATG and cyclosporine, were compared across hematological responses at 6 months.

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