

Supplementary Appendix

This appendix has been provided by the author to give readers additional information about his work.

Supplement to: Olnes MJ, Scheinberg P, Calvo KR, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. *N Engl J Med* 2012;367:11-9. DOI: 10.1056/NEJMoa1200931.

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Supplement to Olnes et al, Improved Hematopoiesis after Eltrombopag in Refractory Aplastic Anemia

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Supplemental Figure Legends

Figure 1S. Clinical Trial Design

Figure 2S. Reticulin Staining of Bone Marrow Before and After Prolonged Eltrombopag Treatment. Core bone marrow biopsies stained with the Dako Reticulin Artisan Kit and counterstained with hematoxylin and eosin using the Dako Artisan instrument are shown at baseline and after eltrombopag treatment for all four individuals treated with eltrombopag for more than eight months: Patient 1 (baseline Panel A, 8 months Panel B), Patient 2 (baseline Panel C, 14 months Panel D), Patient 4 (baseline Panel E, 11 months Panel F), and Patient 5 (baseline Panel G, 9 months Panel H).

Figure 3S. Hematologic Response Summary Data. Mean +/- SEM shown for responding patients (n=9 for platelets, n=9 for neutrophils and n=6 for hemoglobin) over time in each lineage. Numbers below each time point indicate the number of patients available for analysis from each responding cohort. Individual data on patients is shown in Figure 2.

Figure 4S. CD34 Staining of Bone Marrow Before and After Prolonged Eltrombopag Treatment. Core bone marrow biopsies stained with the human CD34 mouse monoclonal antibody QBEnd10, using the Dako Autostainer system with Dako Envision+DAB for detection. Both CD34+ hematopoietic stem and progenitor cells as well as endothelial cells stain brown with this antibody. Marrow sections are shown at baseline and after eltrombopag treatment for all four individuals treated with eltrombopag for more than eight months, and compared to a normal control bone marrow (Panel A): Patient 1 (baseline Panel B, 8 months Panel C), Patient 2 (baseline Panel D, 14 months Panel E), Patient 4 (baseline Panel F, 11 months Panel G), and Patient 5 (baseline Panel H, 9 months Panel I). Note normalization of the frequency of CD34+ HSPCs in panels C, E and G compared to baseline lack of CD34 staining except blood vessels in panels B, D and F.

Figure 1S

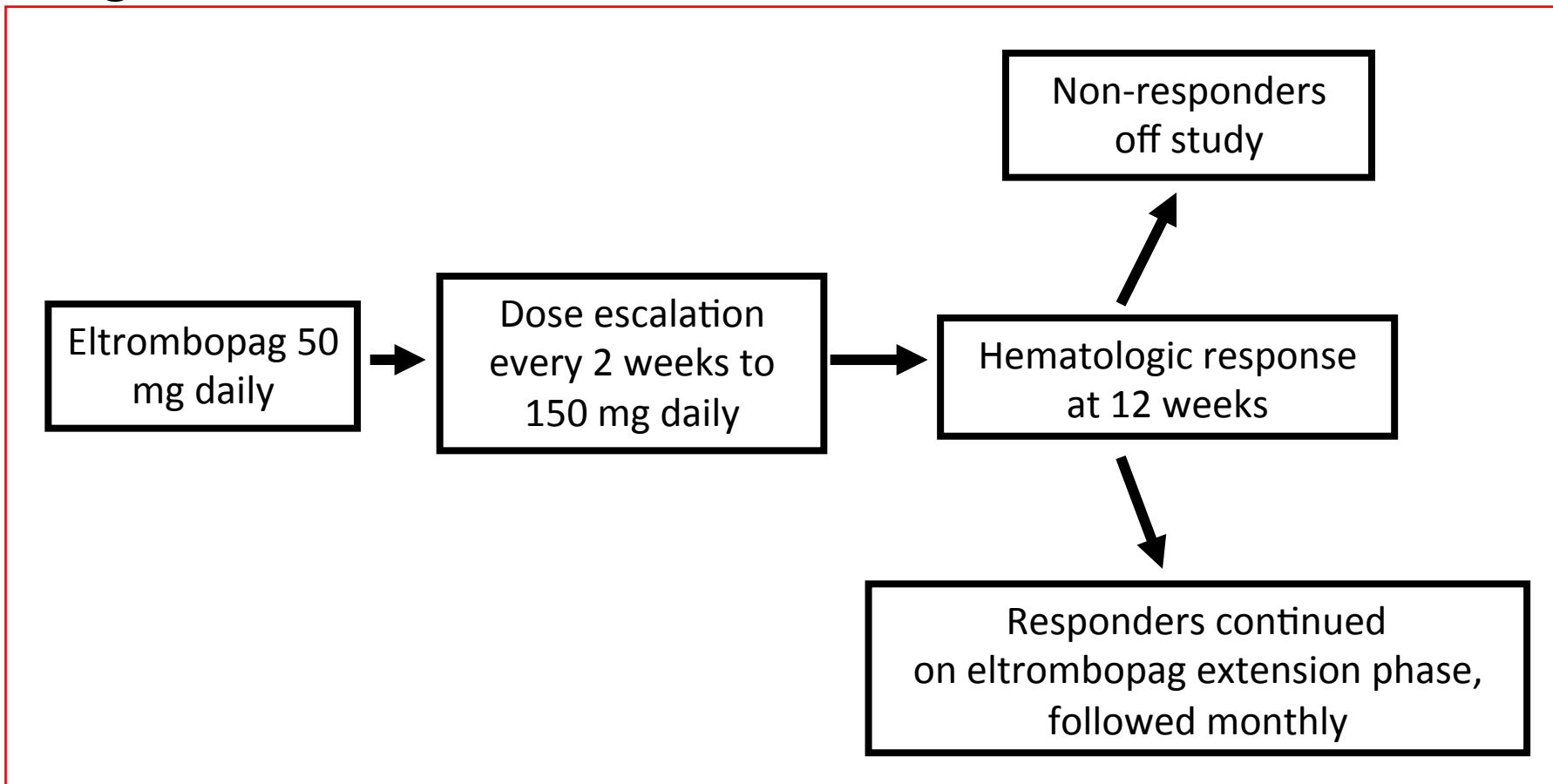


Figure 2S

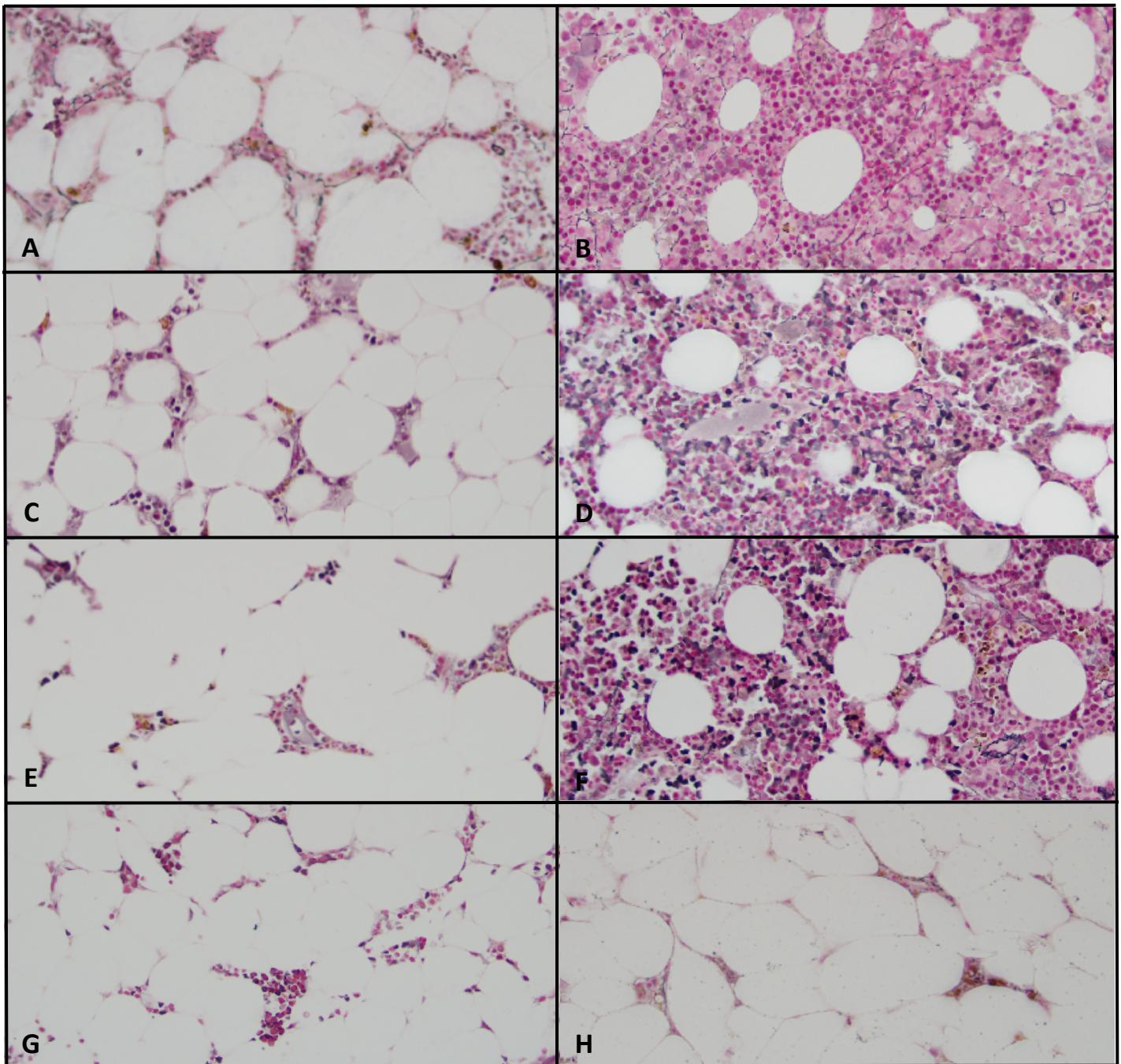


Figure 3S

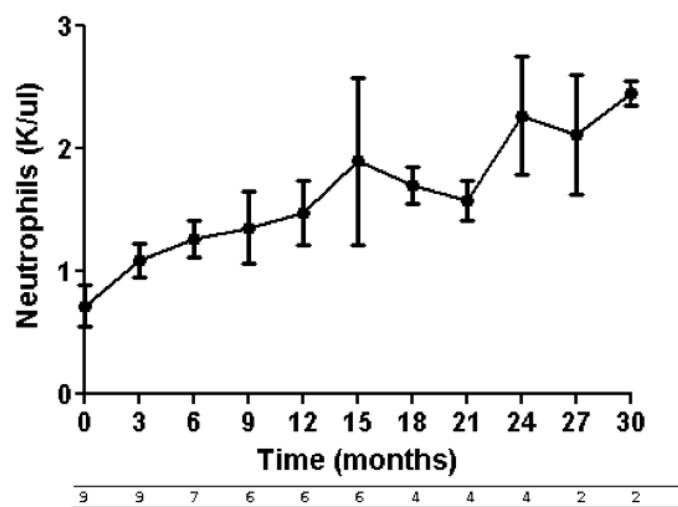
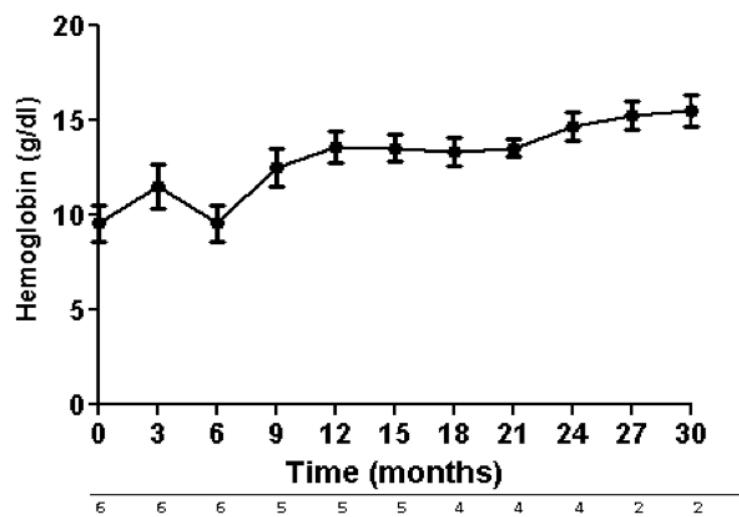
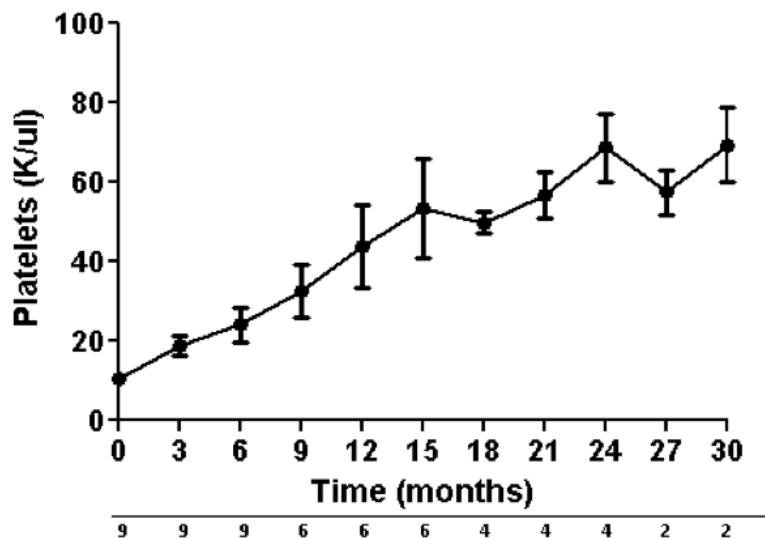
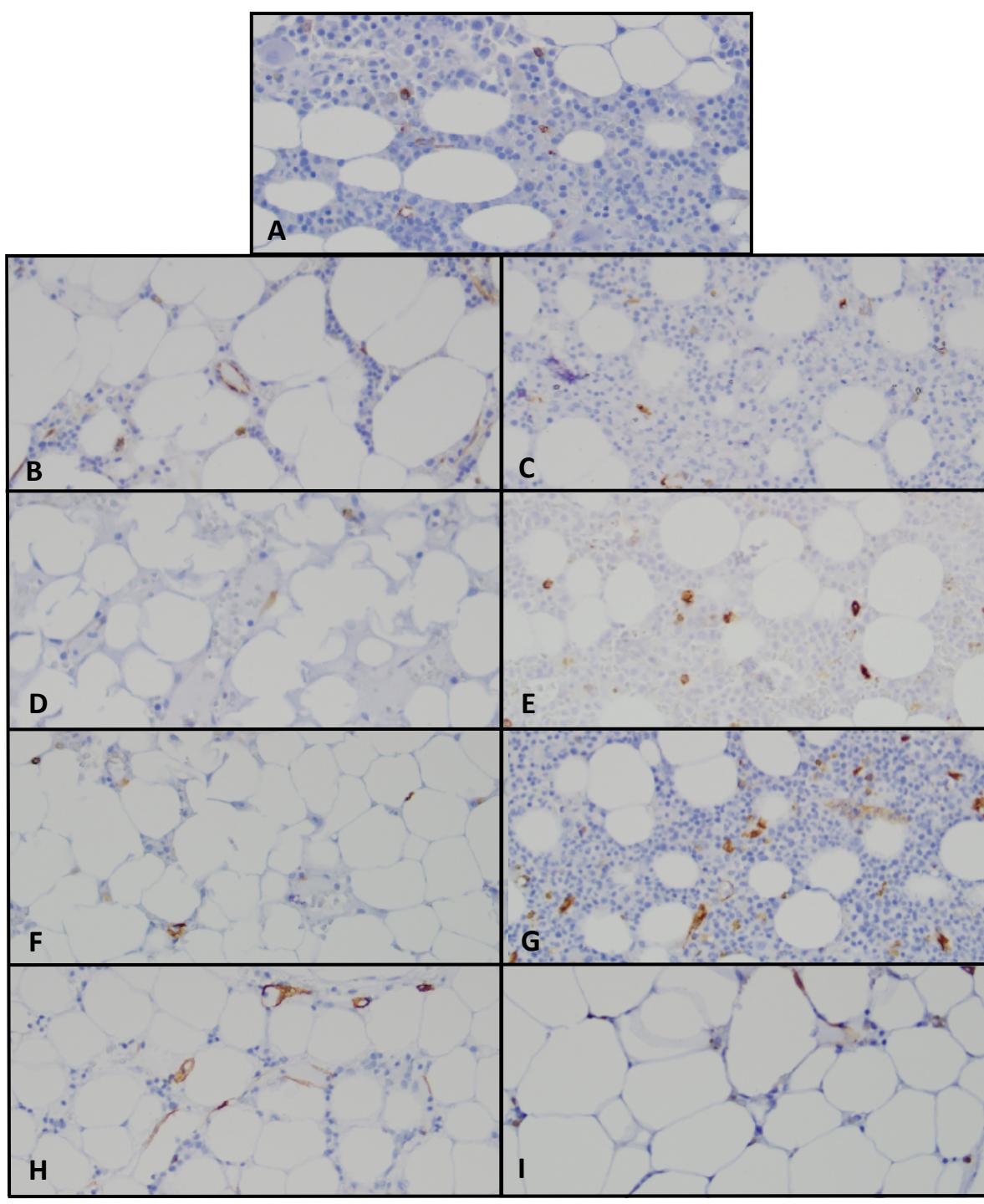


Figure 4S



Supplemental Table 1: Inclusion and Exclusion Criteria, Eltrombopag for Refractory Severe Aplastic Anemia

Inclusion

Diagnosis of refractory aplastic anemia, confirmed by marrow and cytogenetic studies within one month of study entry*

Prior treatment with at least one course of horse or rabbit ATG with cyclosporine, \geq 6 months prior to study entry

Platelets $< 30,000/\mu\text{L}$

Age ≥ 18 years

Exclusion

Fanconi anemia

PNH clone size in neutrophils of $\geq 50\%$

HIV positivity

Creatinine > 2.5 , Bilirubin > 2.0

SGPT or SGPT greater than 5 times the upper limit of normal

ECOG performance status of 3 or greater

Arterial or venous thrombosis within one year

Myocardial infarction within three months

Congestive heart failure or arrhythmia requiring chronic treatment

Active infection not responding to appropriate therapy

Inability to give informed consent

Nursing or pregnant females

Females of childbearing potential unwilling to take oral contraceptives

* Trisomy 8 or other abnormalities excluding monosomy 7 present on repeated marrows in a minority of metaphases in patients with profound hypocellularity and no dysplastic morphologic features are considered consistent with aplastic anemia.

Supplemental Table 2: Metaphase Cytogenetics

Patient #	Response	Baseline	3 Months	Most Recent Analysis
1	Y	46XYt(1;3) (3) 46XY(17) #	46XYt(1;3) (1) 46XY(9)	46XYt(1;3) (3) 46XY(17): 27 months
2	Y	46XY (20)	46XY (20)	46XY (20): 27 months
3	N	46XY (20)	46XY (20)	
4	Y	46XY (20)	46XY (20)	46XY (14): 21 months
5	Y	46XY (20)	46XY (20)	46XY (20): 27 months
6	N	46XY (20)	ND (refused)	
7	N	46XY (20)	45XY,-7 (20)	
8	N	46XX (6)	46XX, +8 (2), 46XX (18)^	transplanted
9	N	46XX (20)	46XX (20)	
10	N	46XX (20)	46XX (20)	
11	N	46XX (20)	46XX (20)	
12	Y	46XY (20)	46XY (20)	46XY (20): 15 months
13	Y	46XY (20)	46XY (20)	46XY (20): 15 months
14	N	46XX (20)	46XX (20)	
15	N	47XX,+8(2)/46XX(19)^	47XX,+8(3)/46XX(17)^	
16	N	NM*	ND (refused)	
17	N	46XX (20)	46XX (20)	
18	N	46XX (20)	46XX (20)	
19	N	46XY (20)	46XY(12), 45XY, -7 (5), 46XY, t(1;16) (3)	transplanted
20	Y	46XX (20)	46XX (20)	
21	N	46XY (20)	46XY (20)	
22	Y	46XX (20)	46XX (20)	
24	Y	46XX (20)	46XX (5)	
25	Y	46XY (10), 46XYY(10)	46XX (20)	
26	Y	46XY (20)	46XY (20)	

Grey shading indicates patients meeting criteria for clinical response at 3 months

#This patient has had a minority of metaphases with this translocation detected on every marrow examination since at least 2005, without any morphologic evidence for myelodysplasia.

[^]Subclonal trisomy 8 without morphologic evidence for myelodysplasia, and with profound hypocellularity and is considered severe aplastic anemia in our clinical studies, as per WHO criteria

*NM-no mitoses

Cell Description	Designation in Table
T cells	T cells
T cytotoxic	CD8+
Double positive T cells	CD4+CD8+
T helpers	CD4+
T immature	CD4-CD8-
Activated T helper cells CD25+	CD4 CD25
Activated T helper cells CD38+	CD4 CD38
Activated T helper cells CD39+	CD4 CD39
Activated T helper cells CD103+	CD4 CD103
Activated T helper cells CD127+	CD4 CD127
Activated T helper cells CD146+	CD4 CD146
Activated T helper cells HLA-DR+	CD4 DR
T helper effector	CD4 EC
T helper Naïve	CD4 naïve
Total Memory T helper	CD4 mem
Th17 cells	Th17
T regulatory cells	Treg
Activated T cytotoxic CD25+	CD8 CD25
Activated T cytotoxic CD38+	CD8 CD38
Activated T cytotoxic CD39+	CD8 CD39
Activated T cytotoxic CD103+	CD8 CD103
Activated T cytotoxic CD127+	CD8 CD127
Activated T cytotoxic HLA-DR+	CD8 DR
T cytotoxic Total memory	CD8 mem
T cytotoxic Naïve	CD8 naïve
T cytotoxic Effector Memory expressing RA	CD8 EMRA
T cytotoxic Effector	CD8 EC

Table 3 Supplemental

Percentage of Lymphoid Gate

		T cells	CD8+	CD4+CD8+	CD4+	CD8-CD4-	CD4 CD25	CD4 CD38	CD4 CD39	CD4 CD103	CD4 CD127	CD4 CD146	CD4 DR	CD4 EC	CD4 naïve	CD4 mem	Th17	Treg	CD8 CD25	CD8 CD38	CD8 CD39	CD8 CD103	CD8 CD127	CD8 DR	CD8 mem	CD8 naïve	CD8 EMRA	CD8 EC
ALL patients (n=21)																												
Pre	Mean	52.3	49.3	2.6	42.0	6.1	6.1	12.0	10.2	1.1	12.0	8.5	3.0	23.4	21.1	51.7	6.3	6.9	6.9	4.0	4.1	2.3	1.8	1.2	27.9	25.6	17.4	29.0
	SD	18.2	17.0	1.8	18.5	4.3	2.9	6.5	6.7	0.8	9.2	3.7	2.5	19.8	18.8	19.5	3.3	3.2	3.2	2.9	2.3	1.3	1.8	1.0	16.0	22.1	17.7	23.1
12 weeks	Mean	52.5	50.6	3.0	39.1	7.3	6.0	13.1	9.1	1.1	14.1	8.1	2.3	27.6	19.7	48.9	6.0	6.7	8.1	4.7	3.6	2.3	1.6	0.9	27.7	21.6	20.4	30.4
	SD	20.8	16.0	2.4	17.5	4.8	2.5	10.1	6.7	1.0	13.5	4.1	1.8	21.2	16.2	16.7	3.6	2.6	4.3	3.3	2.2	1.0	1.6	0.7	14.3	21.6	18.9	19.3
Pre vs. 12 weeks (paired t test)		0.93	0.54	0.12	0.30	0.26	0.85	0.43	0.39	0.98	0.46	0.64	0.13	0.09	0.54	0.29	0.71	0.70	0.11	0.43	0.21	0.54	0.49	0.25	0.91	0.09	0.18	0.47

Responders [R] vs Nonresponders[NR]

R: n=10, NR: n=11

		Pre-R	Mean	57.1	49.6	3.0	41.7	5.8	5.9	11.9	7.6	1.0	10.7	8.3	3.2	25.6	23.9	43.0	6.1	5.9	6.7	3.8	3.4	2.4	2.0	1.4	21.9	22.1	22.4	33.6
		Pre-NR	Mean	48.0	49.1	2.3	42.3	6.4	6.4	12.1	12.5	1.3	13.3	8.6	2.8	21.3	18.6	59.6	6.4	7.9	7.1	4.1	4.7	2.3	1.6	1.0	33.4	28.8	13.0	24.9
		12 weeks-R	Mean	56.0	50.2	3.8	39.9	6.1	5.8	12.2	8.2	0.9	14.9	7.7	2.5	26.8	20.9	45.2	5.8	6.7	7.2	3.8	3.2	2.3	1.5	0.8	22.1	20.6	24.0	33.4
		12 weeks-NR	Mean	49.3	50.9	2.4	38.4	8.3	6.2	13.9	10.0	1.3	13.4	8.4	2.1	28.4	18.6	52.2	6.2	6.7	8.8	5.4	3.9	2.2	1.6	1.1	32.7	22.5	17.1	27.7
			SD	21.3	15.5	1.2	18.1	5.2	2.5	13.5	7.1	1.3	8.7	4.6	1.5	23.2	16.5	14.0	4.2	2.7	5.2	4.3	2.0	0.9	1.1	0.8	16.5	23.5	12.5	17.1
Pre vs 12 weeks R (paired t test)				0.74	0.74	0.13	0.44	0.67	0.95	0.74	0.46	0.64	0.38	0.37	0.07	0.72	0.21	0.39	0.45	0.35	0.63	0.97	0.86	0.49	0.38	0.16	0.96	0.13	0.39	0.94
Pre vs 12 weeks NR (paired t test)				0.67	0.62	0.70	0.44	0.31	0.83	0.49	0.26	0.92	0.98	0.89	0.40	0.06	1.00	0.10	0.88	0.24	0.08	0.43	0.04	0.84	0.98	0.78	0.88	0.16	0.30	0.35

Absolute counts per mm³

		T cells	CD8+	CD4+CD8+	CD4+	CD8-CD4-	CD4 CD25	CD4 CD38	CD4 CD39	CD4 CD103	CD4 CD127	CD4 CD146	CD4 DR	CD4 EC	CD4 naïve	CD4 mem	Th17	Treg	CD8 CD25	CD8 CD38	CD8 CD39	CD8 CD103	CD8 CD127	CD8 DR	CD8 mem	CD8 naïve	CD8 EMRA	CD8 EC
ALL patients (n=21)																												
Pre	Mean	466	205	16	218	27	9	28	15	2	26	15	4	32	78	103	10	10	13	8	8	5	3	2	55	57	39	54
	SD	354	123	21	245	24	6	40	11	2	30	13	4	29	151	96	7	7	8	7	6	4	3	1	55	69	62	52
12 weeks	Mean	537	231	19	255	31	11	30	14	4	34	20	5	52	68	131	14	12	16	10	8	5	3	2	68	58	42	63
	SD	455	137	25	338	29	12	40	11	10	51	33	9	93	142	176	26	9	10	8	8	4	3	1	72	80	52	62
Pre vs. 12 weeks (paired t test)																												
		0.24	0.20	0.14	0.42	0.21	0.40	0.73	0.56	0.39	0.38	0.51	0.83	0.32	0.18	0.28	0.45	0.36	0.04	0.31	0.80	0.38	0.88	0.27	0.06	0.90	0.42	0.24

Responders [R] vs Nonresponders [NR]

R: n=10, NR: n=11

		Pre-R	Mean	545	221	21	275	28	9	42	15	2	30	18	6	38	119	108	11	9	13	7	7	5	4	2	39	53	58	72
		Pre-NR	Mean	393	189	12	167	25	9	16	15	2	22	12	3	26	41	98	8	12	12	9	8	5	2	2	69	60	22	38
		12 weeks-R	Mean	600	250	27	289	33	11	38	16	2	45	15	4	41	106	135	10	12	17	10	9	5	3	2	52	60	58	81
		12 weeks-NR	Mean	480	213	12	225	30	12	23	12	6	25	24	5	63	33	128	18	12	15	10	8	5	3	2	83	55	28	47
			SD	355	132	12	281	26	14	32	7	14	26	44	12	128	38	162	36	10	8	9	5	5	2	1	92	66	20	30
Pre vs 12 weeks R (paired t test)																														
		0.50	0.47	0.10	0.72	0.49	0.44	0.25	0.84	0.51	0.45	0.44	0.16	0.77	0.10	0.42	0.69	0.13	0.19	0.36	0.66	0.88	0.05	0.17	0.27	0.61	0.94	0.55		
Pre vs 12 weeks NR (paired t test)																														
		0.36	0.19	0.86	0.49	0.21	0.57	0.50	0.21	0.35	0.72	0.41	0.53	0.36	0.55	0.48	0.40	0.89	0.13	0.64	0.52	0.31	0.08	0.83	0.15	0.64	0.16	0.18		