SUPPLEMENTARY APPENDIX

Safety and efficacy of plerixafor dose escalation for the mobilization of CD34+ hematopoietic progenitor cells in patients with sickle cell disease: interim results

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Supplemental Methods

PB CD34 testing

Duplicate aliquots containing 0.5 x 10^6 mononuclear cells were incubated with anti-CD45 FITC (fluorescein isothiocyanate, BD#340664), anti-CD34 PE (phycoerythrin, Beckman Coulter #IM 1871) monoclonal antibodies at room temperature in the dark for 20 minutes. Red blood cells were lysed with fixative-free ammonium chloride (10x NH_4CL Lysing Solution, Beckman Coulter #IM 3514) diluted with reagent grade water to 1x. 7-amino-actinomycin D (7AAD) (Beckman Coulter 3IM3422) was then added. Samples were mixed, kept at room temperature in the dark for 15 minutes and then stored for <1 hr on wet ice in the dark until acquisition/ analysis. With analysis, compensations were set to avoid spectral overlap for CD34 PE and 7AAD; 7AAD versus FSC plots were used to define non-debris regions examined in subsequent dot plots. Similarly, the FL1 secondary threshold was adjusted on the CD45 FITC versus SSC dot plot to exclude red cells, platelets, and other debris but not any CD45 FITC cells. The sequential Boolean gating strategy defined by ISHAGE was then applied to identify CD34+ cells. Viability was then evaluated on the FSC versus 7AAD dot plot instead of the traditional SSC vs 7AAD plots as this technique avoids overestimation of percent viability which can sometimes be seen with traditional gating strategies. Absolute CD34+ cell numbers were calculated using the two platform method with total white blood cell counts measured on an Advia 2120 hematology analyzer (Siemens Healthcare, Germany)

Supplementary Table 1. Patient characteristics: other relevant data

Dose level	Subject ID	% Hb F	% Hb S	Hb (g/dl)	Hct (%)	Platelet count (10^3/ul)	Reticulocyte count (10^3/ul)	LDH (IU/L)	Spleen imaging
80 µg/kg	1	29.3	65.6	13.0	37.3	186	88.2	328	Not done
	2	17.2	79.6	8.2	24.2	337	248.5	365	Splenomegaly
	3 (1*)	**12.5**	**83.9**	**9.8**	**28.6**	**257**	**170.5**	**237**	**Atrophic**
	4	**9.3**	**87.2**	**7.5**	**20.8**	**195**	**292.5**	**822**	**Atrophic**
	5	10.9	85.7	8.4	24.2	406	201.5	385	Atrophic
	6	6.3	89.9	9.2	26.5	442	203.1	251	Not done
160 µg/kg	7	10	86	9.9	28.6	297	134.7	400	Not done
	8 (1*)	22.7	74.9	11.8	33.3	400	154.3	435	Not done
	9	**1.2**	**41.9**	**9.5**	**28.1**	**392**	**223.8**	**326**	**Atrophic**
240 µg/kg	**10**	**3.1**	**93.1**	**7.4**	**21.9**	**417**	**266.2**	**172**	**Atrophic**
	12	6.1	88	8.4	23.5	322	183.3	423	Atrophic
	13	17.9	79.6	8.2	23.4	402	257.4	432	Normal size
	14	19.2	77.8	10.7	30.7	352	151.4	273	Not done
	3 (2*)	**17.3**	**79.5**	**11.1**	**30.7**	**233**	**231.1**	**450**	**Atrophic**
	8 (2*)	26.6	71.1	12.0	34.1	217	152.9	486	Not done

*Indicates first and second enrollments for indicated subject.

Bolded patients were not on HU.

Supplementary Table 2. Absolute PB CD34+, WBC, neutrophil, lymphocyte, and monocyte concentrations (baseline-12 hr -20 hr)

Dose Level	Patient ID	CD34 (per ul)			WBC (x10^3/ul)			ANC (x10^3/ul)			ALC (x10^3/ul)			AMC (x10^3/ul)		
80 µg/kg	1	20	48	6	4.0	9.1	6.0	1.9	3.7	3.1	1.3	3.8	2.0	0.6	1.5	1
	2	2	7	1	6.4	14.2	9.0	3.5	6.5	5.2	2.3	6.5	3.0	0.5	1	0.7
	3 (1*)	**1**	**8**	**3**	**6.0**	**13.0**	**8.0**	**2.9**	**5.7**	**3.6**	**2.0**	**4.6**	**2.5**	**0.6**	**1.5**	**1**
	4	**4**	**18**	**4**	**8.9**	**15.7**	**12.6**	**4.9**	**5.2**	**4.4**	**2.6**	**7.2**	**5.4**	**1.1**	**2.4**	**2**
	5	11	132	69	7.8	19.1	13.2	4.2	5.9	5.4	3.0	11.5	5.9	0.4	1.3	1.5
	6	4	37	11	6.9	12.4	8.2	2.8	3.3	3.0	2.9	7.3	3.5	1.1	1.6	1.2
160 µg/kg	7	6	43	18	9.9	18.6	13.6	5.0	7.9	6.8	3.1	7.9	4.3	1.2	2.1	1.7
	8 (1*)	1	27	30	9.4	18.5	16.9	6.3	11.1	9.9	1.8	4.9	4.9	0.9	1.5	1.5
	9	**19**	**251**	**178**	**11.3**	**28.2**	**23.2**	**6.8**	**19.7**	**15.4**	**2.9**	**5.1**	**4.5**	**1.4**	**2.3**	**2.6**
240 µg/kg	**10**	**5**	**95**	**81**	**6.9**	**17.8**	**18.2**	**3.5**	**7.6**	**9.2**	**2.7**	**2.1**	**5.3**	**0.9**	**2.9**	**2.4**
	12	1	19	17	12.8	26.7	20.7	8.8	13.3	10.5	2.8	11.2	7.7	1	1.1	1.7
	13	3	31	20	12.1	25.8	22.4	6.6	13.7	14.4	3.1	7.3	4.4	2	3.4	3
	14	5	63	59	7.2	19.9	13.1	4.0	7.3	6.4	2.4	10.2	5.0	0.8	1.9	1.5
	3 (2*)	**2**	**30**	**27**	**9.0**	**22.5**	**18.1**	**4.8**	**12.2**	**10.5**	**2.9**	**6.9**	**5.0**	**0.9**	**2.3**	**1.8**
	8 (2*)	0	10	9	4.9	11.3	10.2	1.9	4.0	4.5	1.8	4.4	3.1	1	2.2	2.1

PB= peripheral blood, WBC=white blood cell, ANC= absolute neutrophil count, ALC= absolute lymphocyte count, AMC= absolute monocyte count
Bolded patients were not on HU

Supplementary Table 3. Comparison of $CD34^+$ and $CD34^+CD38^-$ peripheral blood concentrations and fold increases.

Dose level	Subject ID	12 hr PB $CD34^+$/ul	12 hr PB $CD34^+CD38^-$/ul	Peak/pre $CD34^+$ ratio	12 hr/pre $CD34^+CD38^-$ ratio
80 µg/kg	1	48	0.9	2.4	2.73
	2	7	0.2	3.5	3.28
	5	132	0.95	12	2.4
160 ug/kg	7	43	0.7	7.2	2.3
240 ug/kg	10	95	0.2	19	3.5
	14	63	0.3	12.6	4.5

PB=peripheral blood

Supplementary Table 4. Adverse events (AE) at least possibly related to plerixafor

AE severity	Patient ID	Dose level (ug/kg)	WBC (ANC) closest in time to AE	Onset post-plerixafor injection	AE description	Additional considerations
SAE	2	80	10.1 (4.6)	47 hours	5 day hospitalization for uncomplicated typical pain crisis in chest and hips	Pt had withheld that 6 days prior to study admit, she had an ED admission for pain (which would have led to study admit postponement)
	13	240	18.5 (12.6)	80 hours	Abdominal pain requiring 10-hr ED visit where IV saline & hydromorphone (1 mg IV) given.	Pt admitted inconsistent HU use for 2 weeks prior to study admit. Also had tachycardia on admission to 102 bpm; pulse not rechecked before discharge.
AE	1	80	9.1 (3.7)	0 hours	Grade 1 injection site discomfort	No intervention required.
	9	160	23.2 (15.4)	24 hours	Grade 1 hip pain and myalgias	Resolved with hydromorphone (4 mg po) which is part of her standard outpatient pain regimen as needed

ED = Emergency Department, SAE= serious adverse events

Supplemental Figure Legends

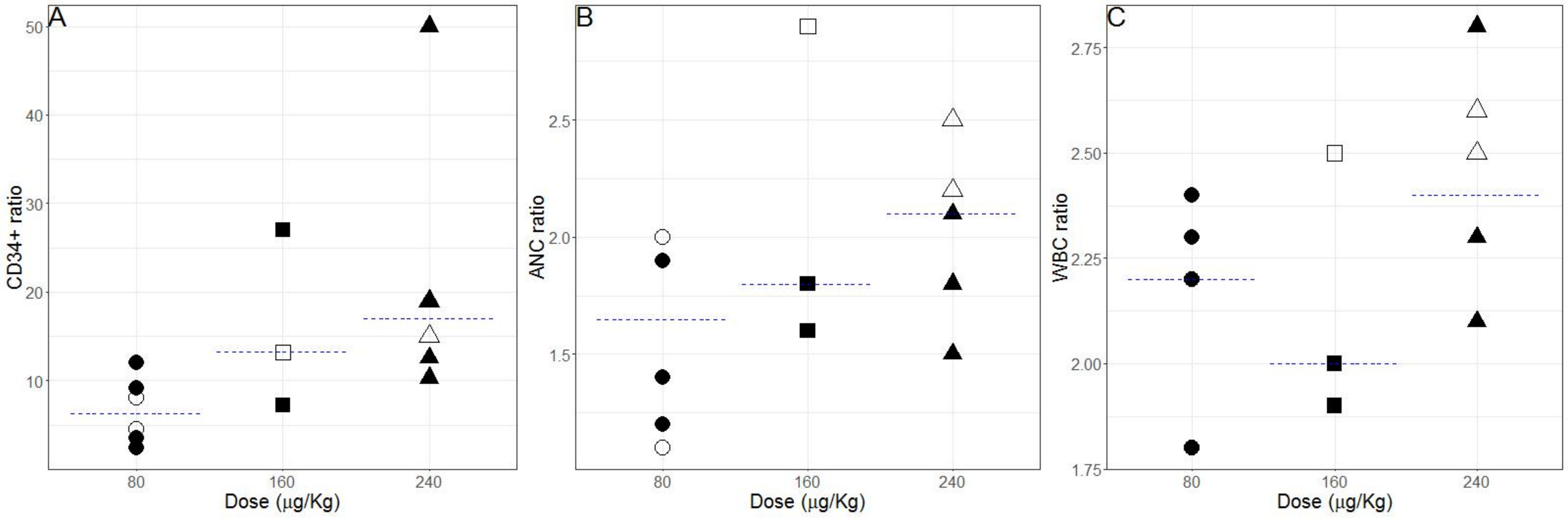
Supplemental Figure 1: There was a significant increase in the median CD34+ fold increase with dose (p=0.01). Effect of the plerixafor dose on the peak/baseline ratios at 12 hours post-plerixafor for peripheral blood CD34+ concentration (A), absolute neutrophil count (ANC) (B), and white blood cell count (WBC) (C) in 15 patients treated at 80 (circles), 160 (squares) and 240 (triangles) µg/Kg of plerixafor. Patients on hydroxyurea are in the filled circles, squares and triangles, patients off hydroxyurea in the open circles, squares and triangles.

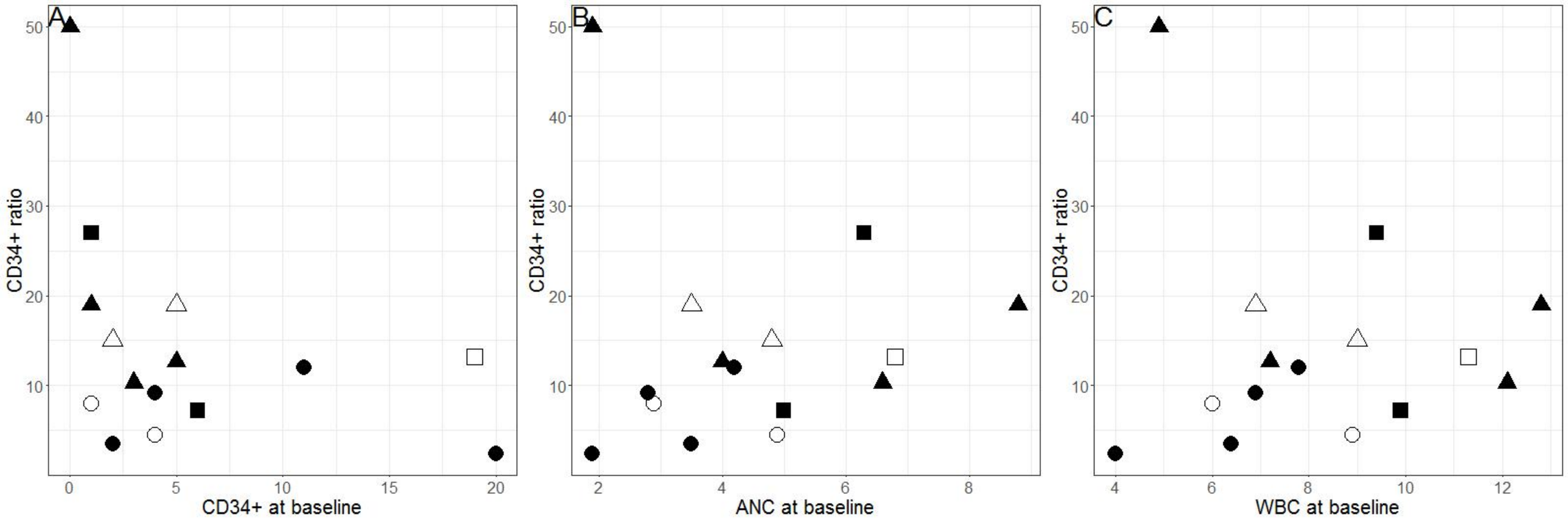
Supplemental Figure 2: There was no correlation between CD34 fold increase and baseline CD34+, absolute neutrophil (ANC), or white blood cell (WBC) concentrations. Association between peripheral blood CD34 peak/baseline ratios at 12h post-plerixafor and baseline values of CD34 (A), ANC (B) and WBC (C) ratios in 15 patients treated at 80 (circles), 160 (squares) and 240 (triangles) µg/Kg of plerixafor. Patients on hydroxyurea are in the filled circles, squares and triangles, patients off hydroxyurea in the open circles, squares and triangles.

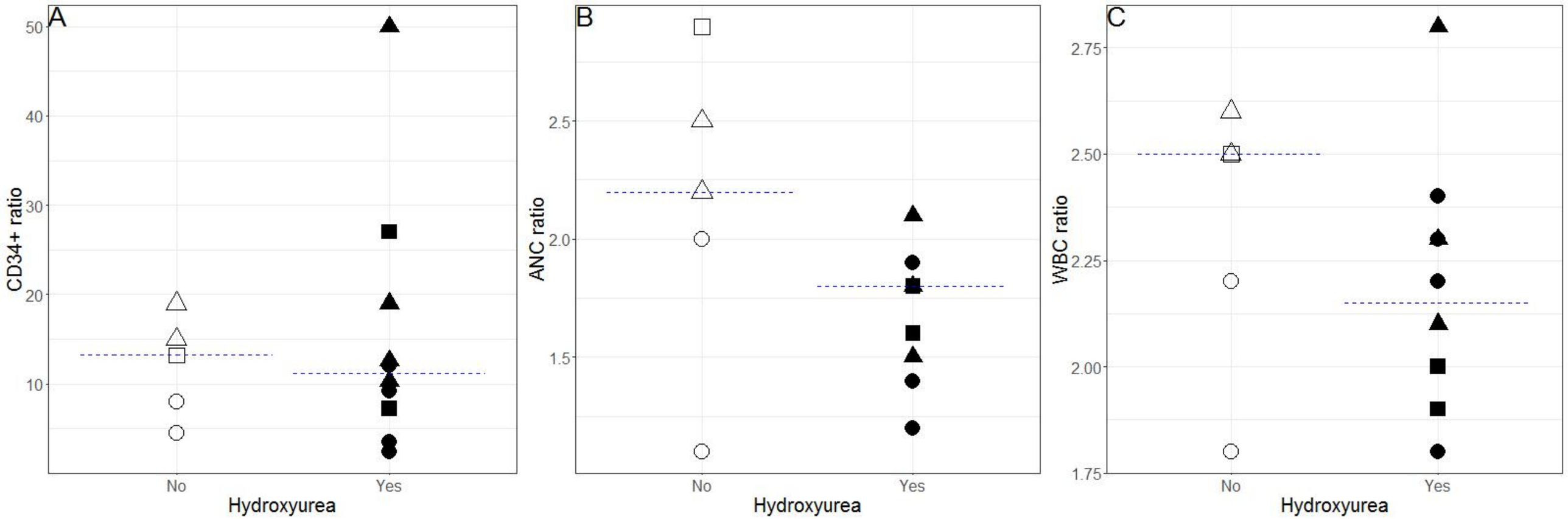
Supplemental Figure 3: Hydroxyurea was not associated with differences in the fold increases of CD34+ (p=0.64), absolute neutrophil (ANC, p=0.12), or white blood cell (WBC, p=0.36) concentrations. Correlation between no hydroxyurea or hydroxyurea and peak/baseline ratios at 12h post-plerixafor of peripheral blood CD34 (A), ANC (B), and WBC (C) ratios in 15 patients treated at 80 (circles), 160 (squares) and 240 (triangles) µg/Kg of plerixafor. Patients on hydroxyurea are in the filled circles, squares and triangles, patients off hydroxyurea in the open circles, squares and triangles.

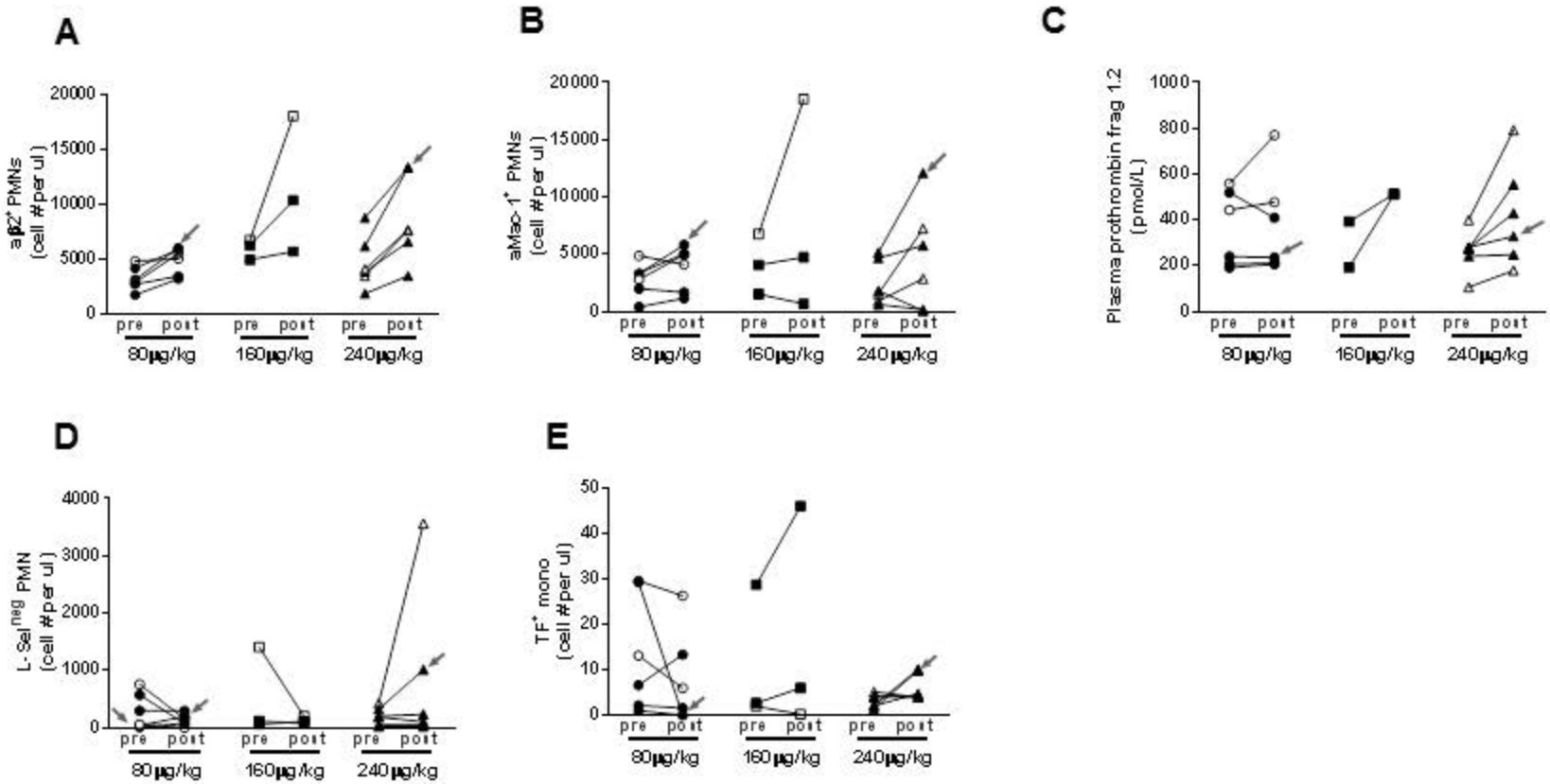
Supplementary Figure 4. 12 hr post-plerixafor, there were significant increases in absolute numbers of aβ2+ neutrophils (A) and aMac-1+ neutrophils (B) and plasma prothrombin fragment 1.2 levels (C). Absolute numbers of L-Sel^{neg} neutrophils and TF+ monocytes were not particularly high in patients with serious adverse events (D-E). Significant increases in activation markers at 12 hr post-plerixafor (A-C). Absolute values for activation markers with high fold increases in SAE patients (D-E). Filled shapes are HU-treated, open shapes are not HU-treated. Grey arrows point to the values for the two patients with serious adverse events. aβ2^{+} PMN= activated β2 integrin-positive neutrophils, aMac-1^{+} PMN = activated Mac-1-positive neutrophils, L-Sel^{neg} PMN=L-selectin-negative neutrophils, TF^{+} mono=tissue-factor-positive monocytes

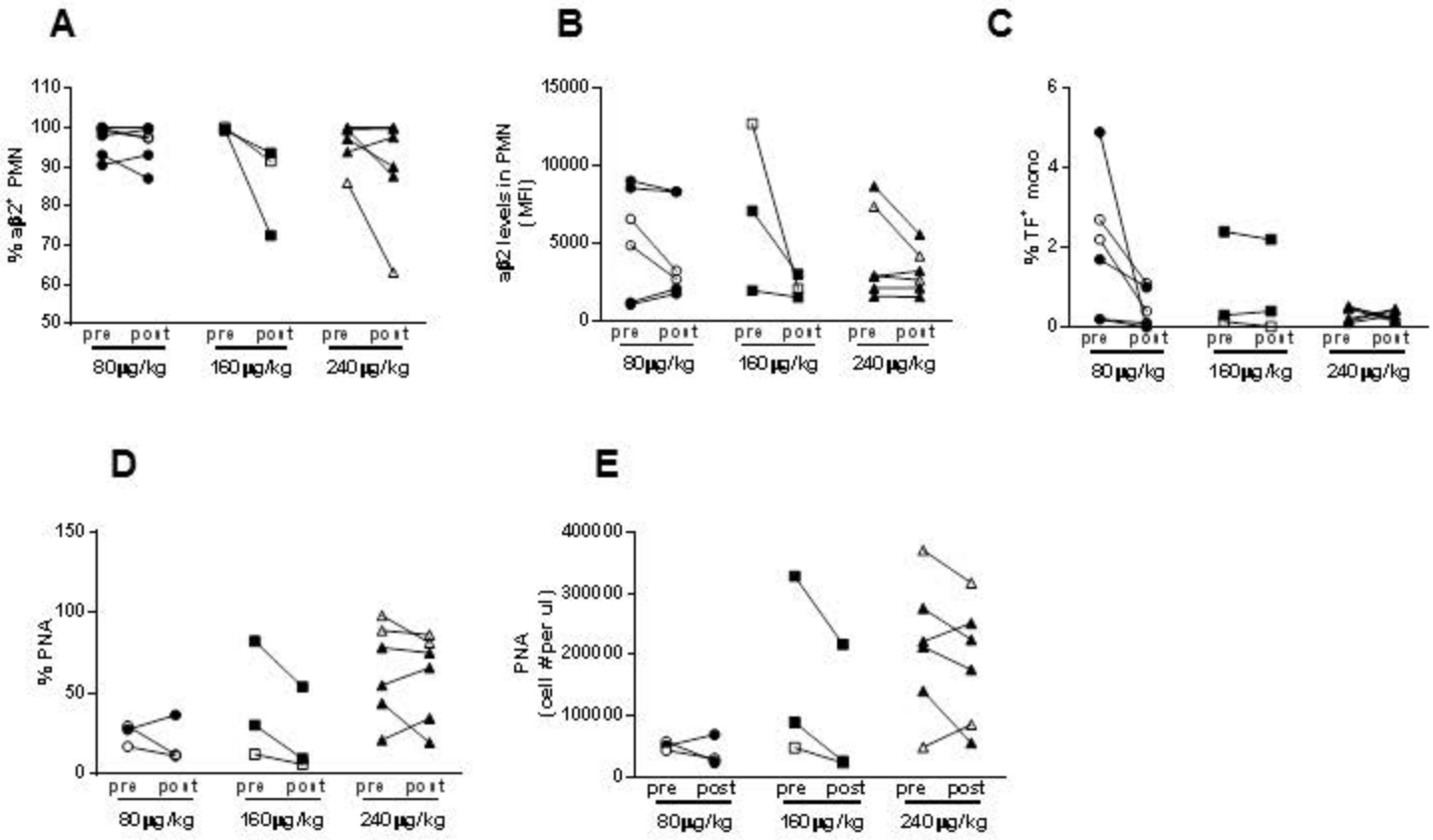
Supplementary Figure 5. 12 hr post-plerixafor, there were significant decreases in % aβ2^{+} neutrophils (A) aβ2^{+} MFI (B), % TF^{+} monocytes (C), % PNA (D) and absolute numbers of PNA (E). Filled shapes are HU-treated, open shapes are not HU-treated. aβ2+ PMN= activated β2 integrin positive neutrophils, MFI= median fluorescent intensity, TF+ mono=tissue-factor positive monocytes, PNA=platelet-neutrophil aggregates.


A
CD34+ ratio
50
40
30
20
10
80
160
240
Dose (μg/Kg)
B
ANC ratio
2.5
2.0
1.5
80
160
240
Dose (μg/Kg)
C
WBC ratio
2.75
2.50
2.25
2.00
1.75
80
160
240
Dose (μg/Kg)


A
B
C
CD34+ ratio
CD34+ at baseline
ANC at baseline
WBC at baseline
0
5
10
15
20
2
4
6
8
12
10
20
30
40
50


A
CD34+ ratio
50
40
30
20
10
No
Yes
Hydroxyurea
B
ANC ratio
2.5
2.0
1.5
No
Yes
Hydroxyurea
C
WBC ratio
2.75
2.50
2.25
2.00
1.75
No
Yes
Hydroxyurea


A
aβ2+ PMNs (cell #per ul)
20000
15000
10000
5000
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
B
aMac-1+ PMNs (cell #per ul)
20000
15000
10000
5000
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
C
Plasma prothrombin frag 1.2 (pmol/L)
1000
800
600
400
200
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
D
L-Sel neg PMN (cell #per ul)
4000
3000
2000
1000
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
E
TF+ mono (cell #per ul)
50
40
30
20
10
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg


A
% aβ2+ PMN
110
100
90
80
70
60
50
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
B
aβ2 levels in PMN (MFI)
15000
10000
5000
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
C
% TF+ mono
6
4
2
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
D
% PNA
150
100
50
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg
E
PNA (cell #per ul)
400000
300000
200000
100000
0
pre post pre post pre post
80µg/kg 160µg/kg 240µg/kg