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Supplementary Tables

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Supplementary Table 1. Demographic characteristics of subjects without evaluable primary outcomes

Characteristic	Randomized (N=79)	Evaluable Primary Outcome (N=57)	Non-evaluable Primary Outcome (N=22)
Age – yr [IQR]	34 [26,47]	35 [26,51]	33 [26,38]
Female sex – no. (%)	54 (68.4)	36 (63.2)	18 (81.8)
Race – no. (%)*			
White	56 (70.9)	37 (64.9)	19 (86.4)
Black	2 (2.5)	2 (3.5)	0
Asian	10 (12.7)	8 (14.0)	2 (9.1)
Other	11 (13.9)	10 (17.5)	1 (4.5)
Hispanic – no. (%)*	9 (11.4)	9 (15.8)	0
Weight [IQR]*	150 [132,180]	160 [135,184]	140 [130,171]
Prior Donations in 1 yr	2 [2,3]	3 [2,4]	2 [1,3]

*Self-reported by subjects

Supplementary Table 2. Median laboratory values by intervention

	Iron	Placebo
No. of Subjects		
Screening	39	40
Donation-1*	39	40
PTR-1†	39	40
Donation-2‡	37	37
PTR-2§	33	35
Median hemoglobin (IQR) – g/dL		
Screening	12.7 (12.3-13.0)	12.7 (12.3-13.5)
Donation-1*	12.3 (11.8-13.1)	12.5 (11.8-13.2)
PTR-1†	11.3 (10.5-12.1)	11.4 (10.5-12.2)
Donation-2‡	13.9 (13.0-14.9)	12.4 (11.5-13.6)
PTR-2§	13.9 (12.9-14.4)	11.2 (10.2-12.7)
Median hematocrit (IQR) – %		
Screening	39.8 (38.6-41.2)	40.2 (38.6-41.3)
Donation-1*	38.7 (37.2-40.3)	38.6 (37.3-40.9)
PTR-1†	35.2 (33.8-37.6)	35.8 (32.6-38.4)
Donation-2‡	41.6 (39.5-44.8)	38.6 (36.8-41.7)
PTR-2§	41.7 (39.5-42.8)	35.8 (32.5-40.1)
Zinc protoporphyrin (IQR) – µMol/mol heme		
Screening	90 (73-100)	87 (75-106)
Donation-1*	84 (74-95)	90 (73-107)
PTR-1†	93 (90-112)	106 (88-123)

Donation-2‡	53 (49-58)	83 (74-108)
PTR-2§	54 (47-65)	102 (80-136)
Reticulocyte hemoglobin (IQR) – g/dL		
Screening	31.2 (28.5-33.4)	31.7 (28.7-33.5)
Donation-1*	31.9 (28.9-33.6)	32.1 (30.6-34.4)
PTR-1†	28.6 (24.9-31.2)	29.0 (25.2-31.3)
Donation-2‡	34.9 (33.8-35.9)	32.1 (29.8-33.8)
PTR-2§	33.2 (32.3-34.5)	29.0 (25.8-31.6)
Ferritin (IQR) – μg/L		
Screening	9.8 (8.3-12.2)	10.8 (9.0-11.9)
Donation-1*	11.4 (9.7-16.5)	12.7 (10.0-18.3)
PTR-1†	8.1 (6.6-10.8)	8.4 (6.4-11.6)
Donation-2‡	80.0 (47.8-96.5)	13.6 (9.9-17.7)
PTR-2§	25.1 (20.4-39.6)	8.4 (6.3-10.5)
Soluble transferrin receptor (IQR) – mg/L		
Screening	5.1 (3.6-6.5)	5.3 (4.0-6.5)
Donation-1*	4.8 (3.8-6.0)	4.6 (3.6-6.6)
PTR-1†	6.7 (4.7-8.0)	7.3 (5.4-8.5)
Donation-2‡	2.8 (2.4-3.6)	5.0 (3.8-7.1)
PTR-2§	3.6 (3.2-4.5)	7.0 (5.2-9.6)
Transferrin saturation (IQR) – %		
Screening	14.6 (9.3-18.4)	13.8 (9.8-18.3)
Donation-1*	12.8 (9.8-17.4)	16.4 (11.7-20.8)
PTR-1†	8.7 (6.2-13.2)	9.1 (6.8-12.4)

Donation-2‡	29.0 (25.2-35.6)	13.2 (7.6-20.2)
PTR-2§	18.8 (15.1-28.5)	9.1 (7-13.7)
Hepcidin (IQR) – ng/mL		
Screening	8.2 (6.1-14.1)	7.8 (6.1-13.6)
Donation-1*	14.7 (12.4-18.4)	16.0 (12.7-21.2)
PTR-1†	15.2 (13.3-17.3)	14.1 (10.9-17.6)
Donation-2‡	29.9 (22.2-63.2)	15.9 (12.5-18.9)
PTR-2§	20.3 (16.2-31.6)	15.5 (13.7-18.8)

*Donation-1 = First blood donation prior to randomization.
†PTR-1 = First post-transfusion recovery prior to randomization.
‡Donation-2 = Second blood donation occurring after randomization.
§PTR-2 = Second post-transfusion recovery occurring after randomization.

Supplementary Table 3. Median laboratory values by sex

	Iron		Placebo	
	Male	**Female**	**Male**	**Female**
No. of Subjects				
Screening	12	27	13	27
Donation-1*	12	27	13	27
PTR-1†	12	27	13	27
Donation-2‡	12	25	13	24
PTR-2§	11	22	13	22
Median hemoglobin (IQR) – g/dL				
Screening	12.9 (12.5-13.5)	12.6 (12.1-13.0)	13.2 (12.7-13.6)	12.6 (12.1-13.4)
Donation-1*	12.4 (11.9-13.2)	12.1 (11.7-13.0)	13.2 (12.7-14.0)	12.0 (11.7-12.8)
PTR-1†	12.0 (11.2-12.9)	11.0 (10.4-11.5)	12.2 (11.6-13.0)	11.0 (10.5-11.5)
Donation-2‡	15.1 (14.7-15.5)	13.2 (12.7-14.0)	13.5 (12.4-14.9)	12.1 (11.3-12.6)
PTR-2§	14.6 (14.1-15.3)	13.2 (12.7-14.0)	13.1 (11.6-14.2)	10.9 (10.2-11.5)
Median hematocrit (IQR) – %				
Screening	41.2 (40.0-43.0)	39.3 (38.4-40.4)	41.3 (40.5-41.8)	39.4 (38.5-40.6)
Donation-1*	40.0 (38.2-42.7)	38.6 (37.1-40.1)	41.8 (39.0-43.4)	38.0 (36.4-38.9)
PTR-1†	37.4 (35.8-42.4)	34.8 (33.2-36.5)	38.6 (37.3-41.3)	33.7 (32.1-36.6)
Donation-2‡	44.9 (43.1-47.1)	40.5 (38.6-42.0)	42.4 (39.2-45.5)	37.6 (36.5-39.4)
PTR-2§	43.9 (42.2-46.5)	40.5 (39.0-42.1)	40.1 (37.4-42.5)	33.5 (32.1-36.1)
Zinc protoporphyrin (IQR) – µMol/mol heme				
Screening	95 (88-107)	78 (72-96)	98 (81-106)	86 (72-105)
Donation-1*	92 (84-106)	80 (70-94)	96 (76-106)	80 (70-107)

PTR-1†	97 (89-121)	93 (90-109)	112 (86-114)	105 (89-124)
Donation-2‡	52 (50-54)	54 (48-63)	81 (74-106)	88 (75-126)
PTR-2§	49 (43-66)	54 (47-65)	80 (74-123)	103 (93-143)
Reticulocyte hemoglobin (IQR) – g/dL				
Screening	27.6 (25.5-30.7)	32.2 (30.3-34.0)	29.8 (27.9-31.6)	32.4 (30.2-34.6)
Donation-1*	29.0 (27.8-30.3)	32.4 (30.5-34.1)	28.1 (27.0-31.4)	33.0 (31.0-34.9)
PTR-1†	26.1 (24.8-29.0)	29.5 (25.2-31.5)	26.7 (24.9-30.1)	30.3 (25.7-33.1)
Donation-2‡	35.0 (33.6-35.6)	34.6 (33.9-35.9)	31.4 (26.8-34.3)	32.8 (30.8-33.7)
PTR-2§	33.2 (30.6-34.5)	33.6 (32.4-34.8)	28.8 (25.4-31.4)	29.2 (26.4-31.6)
Ferritin (IQR) – μg/L				
Screening	9.3 (8.0-10.2)	10.8 (8.8-13.0)	10.7 (9.4-11.2)	10.9 (8.7-12.1)
Donation-1*	10.1 (8.8-12.0)	11.6 (10.2-16.5)	11.3 (9.4-14.1)	13.8 (10.0-20.8)
PTR-1†	7.5 (6.7-10.0)	8.3 (6.6-11.0)	9.1 (7.7-11.8)	8.3 (6.2-11.3)
Donation-2‡	49 (36.6-81.0)	84.8 (66.3-100.8)	15.8 (13.2-19.3)	13.3 (9.7-17.0)
PTR-2§	22.7 (17.2-33.2)	25.5 (20.4-39.8)	10.5 (9.1-12.5)	7.9 (6.0-9.2)
Soluble transferrin receptor (IQR) – mg/L				
Screening	6.7 (6.2-8.6)	4.4 (3.4-5.5)	7.0 (5.6-8.0)	4.4 (3.7-5.8)
Donation-1*	6.1 (5.7-8.1)	4.2 (3.8-5.4)	6.6 (5.5-7.6)	3.8 (3.5-5.2)
PTR-1†	7.6 (7.0-9.8)	5.7 (4.5-7.4)	7.9 (7.0-9.2)	6.6 (4.3-8.1)
Donation-2‡	3.4 (2.9-4.1)	2.7 (2.4-3.2)	5.5 (4.2-7.9)	4.8 (3.4-6.1)
PTR-2§	4.5 (3.4-6.4)	3.5 (3.1-4.0)	8.2 (5.1-9.6)	6.7 (5.4-8.3)
Transferrin saturation (IQR) – %				

Screening	9.0 (7.7-13.1)	16.2 (11.6-20.1)	10.6 (9.5-13.8)	14.8 (10.8-21.7)
Donation-1*	10.2 (7.6-12.2)	14.1 (10.6-22.2)	16.1 (9.0-17.0)	18.1 (12.2-24.0)
PTR-1†	8.2 (6.8-10.9)	8.7 (6.1-15.1)	10.4 (7.3-12.7)	8.8 (6.5-11.3)
Donation-2‡	29.6 (21.9-43.8)	29.0 (26.0-35.0)	14.3 (9.1-19.9)	11.8 (7.4-22.3)
PTR-2§	16.7 (12.1-22.6)	22.2 (15.7-29.2)	11.1 (7.3-15.0)	8.7 (6.5-11.5)
Hepcidin (IQR) – ng/mL				
Screening	4.6 (3.7-9.7)	9.2 (6.5-14.7)	8.7 (5.0-14.0)	7.7 (6.4-12.8)
Donation-1*	14.2 (12.3-16.0)	16.9 (12.4-18.8)	14.9 (12.4-19.3)	16.9 (12.7-22.7)
PTR-1†	14.6 (12.0-17.8)	15.4 (13.3-17.1)	13.8 (11.2-17.5)	14.4 (10.5-19.2)
Donation-2‡	26.1 (14.1-49.2)	32.9 (23.6-79.2)	15.9 (12.5-18.9)	15.9 (12.4-18.8)
PTR-2§	16.2 (14.5-31.0)	22.9 (17.8-33.5)	15.5 (14.7-18.6)	15.2 (13.5-19.4)

*Donation-1 = First blood donation prior to randomization.
†PTR-1 = First post-transfusion recovery prior to randomization.
‡Donation-2 = Second blood donation occurring after randomization.
§PTR-2 = Second post-transfusion recovery occurring after randomization.

Supplementary Table 4. Median laboratory values by age

	Iron		Placebo	
	<50 years old	**≥50 years old**	**<50 years old**	**≥50 years old**
No. of Subjects				
Screening	30	9	30	10
Donation-1*	30	9	30	10
PTR-1†	30	9	30	10
Donation-2‡	28	9	27	10
PTR-2§	25	8	25	10
Median hemoglobin (IQR) – g/dL				
Screening	12.8 (12.3-13.0)	12.6 (12.4-12.9)	12.7 (12.3-13.3)	13.3 (12.2-13.7)
Donation-1*	12.3 (11.7-13.1)	12.2 (11.9-12.7)	12.3 (11.8-12.9)	13.5 (11.8-14.2)
PTR-1†	11.4 (10.5-12.1)	10.9 (10.4-11.4)	11.3 (10.6-11.8)	12.3 (10.0-13.4)
Donation-2‡	13.8 (13.0-15.1)	14.0 (13.0-14.2)	12.3 (11.5-13.6)	13.0 (11.3-15.2)
PTR-2§	14.0 (13.0-14.5)	13.4 (12.7-13.8)	10.9 (10.2-11.9)	11.8 (10.9-14.2)
Median hematocrit (IQR) – %				
Screening	39.9 (38.6-41.3)	39.5 (39.3-41.1)	40.0 (38.6-40.9)	41.1 (39.1-41.9)
Donation-1*	38.6 (37.2-40.4)	39.0 (38.7-40.1)	38.6 (37.5-39.5)	41.5 (36.8-44.4)
PTR-1†	35.4 (33.9-38.4)	34.8 (33.4-36.2)	35.5 (32.6-37.4)	37.8 (32.1-41.3)
Donation-2‡	41.8 (39.6-45.5)	41.5 (39.0-43.1)	38.6 (36.8-41.4)	39.9 (36.5-45.5)
PTR-2§	42.1 (39.5-43.4)	40.3 (39.7-41.7)	33.9 (32.5-38.0)	37.6 (35.4-42.5)
Zinc protoporphyrin (IQR) – µMol/mol heme				
Screening	88 (72-104)	96 (77-97)	86 (74-106)	93 (78-104)
Donation-1*	82 (74-95)	86 (82-95)	89 (70-107)	93 (76-99)

PTR-1†	97 (90-113)	92 (90-109)	106 (89-124)	106 (86-121)
Donation-2‡	52 (49-60)	54 (48-55)	83 (74-106)	85 (74-129)
PTR-2§	53 (47-60)	55 (46-74)	102 (86-123)	89 (72-136)
Reticulocyte hemoglobin (IQR) – g/dL				
Screening	31.4 (29.0-33.4)	28.8 (25.7-32.6)	31.9 (27.9-33.8)	31.6 (29.8-32.5)
Donation-1*	31.9 (29.0-33.6)	30.8 (28.6-33.6)	32.7 (30.3-34.6)	31.4 (30.8-32.6)
PTR-1†	29.4 (25.1-31.2)	26.6 (24.6-28.6)	30.2 (24.5-32.6)	27.4 (26.3-30.1)
Donation-2‡	35.0 (33.7-36.1)	34.4 (33.9-35.4)	32.9 (29.8-34.1)	31.3 (26.8-32.1)
PTR-2§	33.2 (32.4-34.9)	33.5 (31.2-34.4)	29.0 (26.4-31.6)	29.2 (25.4-32.2)
Ferritin (IQR) – μg/L				
Screening	9.8 (8.1-11.2)	11.0 (9.1-12.2)	10.7 (8.7-12.1)	10.8 (10.0-11.7)
Donation-1*	11.3 (9.7-16.5)	11.4 (10.4-14.0)	13.4 (10.0-20.0)	11.9 (10.0-14.1)
PTR-1†	8.0 (6.6-10.6)	8.6 (6.7-11.0)	8.6 (6.4-11.3)	8.1 (7.2-11.8)
Donation-2‡	72.1 (45.7-98.7)	81.0 (66.3-95.0)	13.9 (9.8-19.3)	13.4 (9.9-15.8)
PTR-2§	24.0 (21.2-30.3)	38.1 (15.7-47.8)	8.3 (6.3-9.7)	10.1 (8.2-12.0)
Soluble transferrin receptor (IQR) – mg/L				
Screening	5.2 (3.6-6.5)	5.1 (4.4-5.9)	4.5 (3.9-6.4)	5.7 (5.0-8.0)
Donation-1*	4.9 (3.8-6.0)	4.8 (4.4-6.4)	4.2 (3.5-5.8)	5.6 (4.5-7.4)
PTR-1†	6.7 (4.6-8.0)	6.9 (5.8-7.7)	6.9 (5.0-8.7)	7.9 (7.0-8.3)
Donation-2‡	2.9 (2.4-3.7)	2.8 (2.5-3.3)	4.6 (3.2-6.4)	5.8 (5.2-7.9)
PTR-2§	3.6 (3.1-4.5)	3.5 (3.4-4.4)	6.6 (5.2-9.7)	8.2 (6.8-9.1)
Transferrin saturation (IQR) – %				
Screening	13.4 (9.3-18.4)	14.6 (10.3-17.9)	14.7 (10.1-18.6)	11.1 (9.4-13.8)

Donation-1*	11.7 (9.6-14.4)	14.9 (14.0-18.0)	16.4 (12.2-23.8)	16.4 (10.6-19.6)
PTR-1†	9.3 (6.1-14.7)	8.5 (7.8-10.6)	8.9 (6.7-11.3)	10.2 (7.2-12.7)
Donation-2‡	28.8 (24.8-33.4)	35.0 (26.3-36.9)	14.1 (7.3-30.5)	12.5 (9.1-17.1)
PTR-2§	21.4 (15.1-29.5)	17.8 (14.8-25.5)	8.7 (6.6-13.7)	10.4 (9.1-11.6)
Hepcidin (IQR) – ng/mL				
Screening	9.1 (6.1-14.1)	7.6 (6.1-10.0)	8.4 (6.4-14.0)	7.4 (5.0-9.4)
Donation-1*	15.3 (12.7-18.6)	13 (11.2-18.1)	18.4 (13.9-23.3)	12.5 (12.2-12.7)
PTR-1†	15.8 (13.7-17.9)	12.7 (9.7-14.1)	14.9 (11.3-19.2)	13.6 (10.3-17.5)
Donation-2‡	27.3 (22.2-49.2)	49.5 (32.9-79.2)	16.4 (12.5-21.6)	14.8 (11.8-16.1)
PTR-2§	21.2 (16.2-31.0)	19.2 (16.3-31.9)	15 (13.5-20.2)	16.1 (14.7-17.8)

*Donation-1 = First blood donation prior to randomization.
†PTR-1 = First post-transfusion recovery prior to randomization.
‡Donation-2 = Second blood donation occurring after randomization.
§PTR-2 = Second post-transfusion recovery occurring after randomization.

Supplementary Table 5. Median laboratory values by race

	Iron		Placebo	
	White	**Non-white**	**White**	**Non-white**
No. of Subjects				
Screening	25	14	31	9
Donation-1*	25	14	31	9
PTR-1†	25	14	31	9
Donation-2‡	23	14	28	9
PTR-2§	20	13	27	8
Median hemoglobin (IQR) – g/dL				
Screening	12.7 (12.4-13.2)	12.8 (11.9-13)	12.7 (12.3-13.5)	13 (12.3-13.3)
Donation-1*	12.2 (11.9-13.1)	12.4 (11.7-13.0)	12.5 (11.8-13.2)	11.9 (11.8-12.7)
PTR-1†	11.4 (10.9-12.1)	10.9 (10.0-11.5)	11.3 (10.5-12.4)	11.5 (11.0-11.8)
Donation-2‡	14.1 (13.2-15.1)	13.6 (12.6-14.9)	12.5 (11.6-14.2)	12.2 (11.3-12.7)
PTR-2§	13.9 (12.7-14.5)	13.9 (13.0-14.3)	11.5 (10.3-13.4)	10.6 (9.9-11.7)
Median hematocrit (IQR) – %				
Screening	40.1 (39.4-41.2)	39.3 (38.4-40.4)	39.9 (38.6-41.3)	40.5 (38.8-40.6)
Donation-1*	39.0 (37.2-40.2)	38.7 (37.5-40.3)	38.6 (37.5-41.8)	38.6 (36.4-39.5)
PTR-1†	36.0 (34.8-37.6)	34.5 (32.2-36.5)	35.7 (32.1-38.5)	36.6 (33.7-37.9)
Donation-2‡	42.0 (39.7-45.0)	41.1 (38.6-44.8)	38.4 (37.1-42.9)	38.6 (36.4-39.3)
PTR-2§	41.1 (39.1-42.7)	42.1 (40.3-43.4)	36.1 (33.4-40.6)	32.7 (32.3-36.7)
Zinc protoporphyrin (IQR) – µMol/mol heme				
Screening	88 (74-96)	94 (72-110)	86 (74-105)	99 (76-116)
Donation-1*	82 (70-89)	94 (76-112)	90 (72-99)	107 (75-118)

PTR-1†	92 (86-100)	111 (93-133)	104 (86-121)	116 (105-124)
Donation-2‡	52 (48-57)	55 (50-66)	81 (74-106)	92 (88-139)
PTR-2§	54 (44-59)	54 (49-66)	96 (77-130)	103 (100-145)
Reticulocyte hemoglobin (IQR) – g/dL				
Screening	31.5 (28.9-32.6)	30.8 (28.3-33.5)	32.1 (28.8-33.6)	30.7 (27.7-33.2)
Donation-1*	32.1 (29.1-33.9)	30.4 (28.4-33.4)	32.1 (30.8-34.6)	32.6 (28.1-34.2)
PTR-1†	29.4 (25.5-31.2)	26.6 (23.6-30.3)	29.0 (25.5-31.6)	28.9 (24.9-30.8)
Donation-2‡	35.1 (33.9-36.3)	34.5 (33.6-35.8)	32.3 (30.8-34.3)	31.2 (29.2-33.0)
PTR-2§	33.7 (32.6-34.7)	32.6 (31.4-34.5)	29.3 (25.8-32.4)	28.0 (24.8-31.5)
Ferritin (IQR) – μg/L				
Screening	9.7 (8.1-11.0)	10.9 (8.7-13.0)	10.4 (9.0-11.8)	11.5 (9.0-12.1)
Donation-1*	10.8 (9.9-16.5)	11.9 (9.7-15.8)	12.4 (9.4-16.0)	18.4 (14.3-23.4)
PTR-1†	7.1 (6.6-10.6)	8.5 (7.4-10.8)	8.3 (6.3-11.3)	8.9 (7.0-11.9)
Donation-2‡	73.8 (47.8-102.4)	84.6 (37.5-96.5)	14.5 (9.9-18.9)	13.6 (10.8-16.3)
PTR-2§	25.5 (20.2-40.0)	23.7 (20.4-30.3)	9.1 (7.0-10.7)	7.6 (5.4-8.8)
Soluble transferrin receptor (IQR) – mg/L				
Screening	5.1 (3.9-6.3)	5.3 (3.4-7.0)	5.5 (4.0-7.0)	4.4 (3.9-5.9)
Donation-1*	4.8 (3.8-6.0)	5.0 (3.9-6.1)	4.8 (3.7-7.2)	3.7 (3.5-4.9)
PTR-1†	6.7 (4.6-7.5)	7.0 (5.6-9.1)	7.3 (5.2-9.0)	7.2 (5.7-7.8)
Donation-2‡	2.8 (2.4-3.4)	3.0 (2.4-3.8)	4.9 (3.7-7.5)	5.5 (4.5-6.4)
PTR-2§	3.5 (3.0-4.5)	4.0 (3.4-4.6)	6.8 (5.2-9.1)	7.9 (5.3-9.8)
Transferrin saturation (IQR) – %				
Screening	12.8 (9.7-16.4)	15.9 (8.7-20.9)	13.8 (9.7-18.6)	12.4 (10.1-17.5)

Donation-1*	12.8 (9.8-18.0)	13.3 (10.1-15.0)	16.7 (11.8-19.8)	16.2 (10.7-25.9)
PTR-1†	9.9 (7.8-14.7)	7.0 (5.1-10.8)	10.0 (6.9-12.9)	7.3 (6.5-9.4)
Donation-2‡	30.1 (25.2-36.9)	28.9 (24.4-35.0)	13.6 (7.4-27.0)	12.4 (9.6-19.0)
PTR-2§	18.2 (15.3-27.3)	21.9 (15.1-29.2)	9.4 (7.6-13.7)	7.4 (5.0-14.0)
Hepcidin (IQR) – ng/mL				
Screening	7.6 (6.1-14.3)	9.1 (5.8-11.9)	7.7 (5.8-12.8)	10.4 (6.4-17.1)
Donation-1*	15.0 (12.6-18.4)	13.6 (11.9-18.4)	14.9 (12.4-19.0)	23.3 (20.1-29.0)
PTR-1†	14.7 (13.7-17.1)	15.8 (13.3-18.5)	13.3 (10.3-17.4)	15.9 (15.3-21.7)
Donation-2‡	32.8 (22.9-73.5)	27.1 (19.3-49.2)	15.4 (12.4-17.9)	16.8 (15.8-19.9)
PTR-2§	20.7 (16.6-32.2)	20.2 (15.6-26.4)	14.9 (13.4-17.8)	19.8 (15.6-21.5)

*Donation-1 = First blood donation prior to randomization.
†PTR-1 = First post-transfusion recovery prior to randomization.
‡Donation-2 = Second blood donation occurring after randomization.
§PTR-2 = Second post-transfusion recovery occurring after randomization.

Supplementary Table 6. Number of subjects who would have been deferred at second donation using FDA allogeneic donation criteria

	Iron		Placebo	
	Male (N=12)	**Female (N=25)**	**Male (N=13)**	**Female (N=24)**
No. of Subjects (%)				
Hematocrit criteria*	0	4 (16.0)	3 (23.1)	14 (58.3)
Hemoglobin criteria†	0	4 (16.0)	5 (38.5)	15 (62.5)

*Hematocrit criteria is ≥39% and ≥38% for males and females, respectively.
†Hemoglobin criteria is ≥13.0 g/dL and ≥12.5 g/dL for males and females, respectively.

Supplementary Table 7. Mean hemoglobin level in donated red blood cell units before and after randomization and by sex, age, and race

	Donation-1*			Donation-2†		
All	**Iron (N=39)**	**Placebo (N=37)**	**Mean difference (95% CI)**	**Iron (N=29)**	**Placebo (N=33)**	**Mean difference (95% CI)**
Hemoglobin, g/dL (95% CI)	17.6 (17.1-18.0)	17.5 (17.1-17.9)	0.05 (-0.5-0.6)	18.7 (18.4-19.1)	17.5 (17.0-18.0)	1.2 (0.6-1.8)
Male	**Iron (N=12)**	**Placebo (N=12)**		**Iron (N=10)**	**Placebo (N=13)**	
Hemoglobin, g/dL (95% CI)	17.9 (16.7-19.2)	18.1 (17.2-19.1)	-0.2 (-1.7-1.2)	19.4 (18.8-20.0)	18.3 (17.4-19.1)	1.1 (0.0-2.2)
Female	**Iron (N=27)**	**Placebo (N=25)**		**Iron (N=19)**	**Placebo (N=20)**	
Hemoglobin, g/dL (95% CI)	17.4 (17.1-17.7)	17.2 (16.8-17.6)	0.2 (-0.3-0.7)	18.4 (18.1-18.7)	17.0 (16.4-17.6)	1.4 (0.7-2.0)
<50 years old	**Iron (N=30)**	**Placebo (N=28)**		**Iron (N=21)**	**Placebo (N=23)**	
Hemoglobin, g/dL (95% CI)	17.7 (17.2-18.2)	17.2 (16.9-17.6)	0.4 (-0.2-1.0)	18.8 (18.4-19.2)	17.4 (16.8-18.0)	1.4 (0.6-2.1)
≥50 years old	**Iron (N=9)**	**Placebo (N=9)**		**Iron (N=8)**	**Placebo (N=10)**	
Hemoglobin, g/dL (95% CI)	17.1 (16.6-17.7)	18.3 (17.2-19.4)	-1.2 (-2.3-0.0)	18.7 (18.1-19.3)	17.7 (16.7-18.7)	1.0 (-0.2-2.1)
White	**Iron (N=25)**	**Placebo (N=28)**		**Iron (N=17)**	**Placebo (N=24)**	
Hemoglobin, g/dL (95% CI)	17.4 (17.1-17.8)	17.5 (17.0-18.1)	-0.1 (-0.7-0.5)	18.9 (18.5-19.3)	17.8 (17.2-18.4)	1.1 (0.3-1.8)
Non-white	**Iron (N=14)**	**Placebo (N=9)**		**Iron (N=12)**	**Placebo (N=9)**	
Hemoglobin, g/dL (95% CI)	17.8 (16.7-18.9)	17.4 (16.9-17.9)	0.4 (-1.0-1.8)	18.5 (17.9-19.1)	16.6 (15.6-17.7)	1.9 (0.8-3.0)

*Donation-1 = First whole blood donation, which occurred prior to randomization.
†Donation-2 = Second whole blood donation, which occurred after randomization.

Supplementary Table 8. Primary outcome with pre-specified subgroup analysis

	Iron	Placebo	Mean Difference (95% CI)
All	**N=29**	**N=28**	
PTR-1* (%)	82.7 (80.2 – 85.2)	85.1 (83.2 – 87.1)	-2.4 (-5.5 – 0.7)
PTR-2† (%)	84.3 (82·0 – 86.6)	84.7 (82·9 – 86.6)	-0.4 (-3.3 – 2.5)
ΔPTR‡ (%)	1.6 (-0.5 – 3.8)	-0.4 (-2.0 – 1.2)	2.0 (-0.6 – 4.6)
Female	**N=20**	**N=16**	
PTR-1* (%)	81.2 (77.9 - 84.6)	85.5 (82.6 - 88.5)	-4.3 (-8.7 - 0.1)
PTR-2† (%)	83.9 (80.8 - 87.1)	84.2 (81.1 - 87.3)	-0.3 (-4.6 - 4.1)
ΔPTR‡ (%)	2.7 (0.2 - 5.2)	-1.3 (-3.6 - 1.0)	4.0 (0.7 - 7.4)
Male	**N=9**	**N=12**	
PTR-1* (%)	86.0 (83.3 - 88.7)	84.6 (81.8 - 87.3)	1.4 (-2.3 - 5.1)
PTR-2† (%)	85.3 (82.0 - 88.5)	85.5 (83.6 - 87.3)	-0.2 (-3.4 - 3.1)
ΔPTR‡ (%)	-0.8 (-5.2 - 3.7)	0.9 (-1.4 - 3.1)	-1.6 (-5.9 - 2.7)
<50 years old	**N=21**	**N=19**	
PTR-1* (%)	83.5 (81.4 - 85.6)	84.5 (81.8 - 87.3)	-1.0 (-4.4 - 2.3)
PTR-2† (%)	85.8 (84.0 - 87.6)	83.9 (81.2 - 86.5)	1.9 (-1.1 - 5.0)
ΔPTR‡ (%)	2.3 (-0.1 - 4.7)	-0.7 (-2.9 - 1.6)	3.0 (-0.2 - 6.1)
≥50 years old	**N=8**	**N=9**	
PTR-1* (%)	80.6 (72.1 - 89.2)	86.4 (84.0 - 88.7)	-5.7 (-13.3 - 1.9)
PTR-2† (%)	80.6 (73.1 - 88.0)	86.6 (85.1 - 88.1)	-6.0 (-12.5 - 0.5)
ΔPTR‡ (%)	-0.1 (-5.6 - 5.4)	0.2 (-1.7 - 2.2)	-0.3 (-5.4 - 4.7)
White	**N=16**	**N=21**	
PTR-1* (%)	83.8 (79.5 - 88.1)	84.6 (82.7 - 86.6)	-0.8 (-5.0 - 3.3)
PTR-2† (%)	84.7 (80.9 - 88.5)	84.8 (82.9 - 86.8)	-0.1 (-4.0 - 3.7)

ΔPTR‡ (%)	0.9 (-2.5 - 4.3)	0.2 (-1.2 - 1.7)	0.7 (-2.6 - 3.9)
Non-White	**N=13**	**N=7**	
PTR-1* (%)	81.4 (78.8 - 84.0)	86.6 (80.3 - 93.0)	-5.3 (-10.5 - -0.1)
PTR-2† (%)	83.9 (81.2 - 86.6)	84.4 (78.5 - 90.4)	-0.5 (-5.7 - 4.6)
ΔPTR‡ (%)	2.5 (-0.2 - 5.3)	-2.2 (-7.7 - 3.3)	4.7 (-0.3 - 9.7)

*PTR-1 = First post-transfusion recovery outcome measure prior to randomization.
†PTR-2 = Second post-transfusion recovery outcome measure after randomization.
‡ΔPTR = Paired difference between each subject's first and second post-transfusion recovery outcome measure.

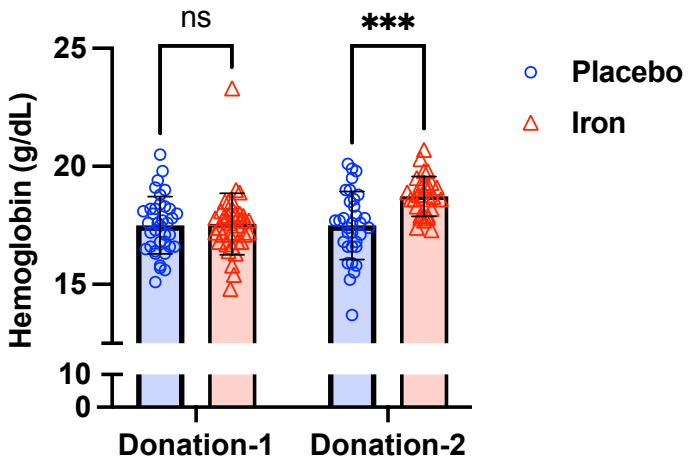
Supplementary Table 9. Adverse Events

Adverse event	Iron	Placebo
No. of patients randomized	39	40
No. of patients with at least 1 event (%)	1 (2.6)	4 (10.0)
No. of events	1	5
Diarrhea	0	1
Nausea	0	1*
Headache	0	1
Paresthesia	1	1
Cardiac stent procedure	0	1†

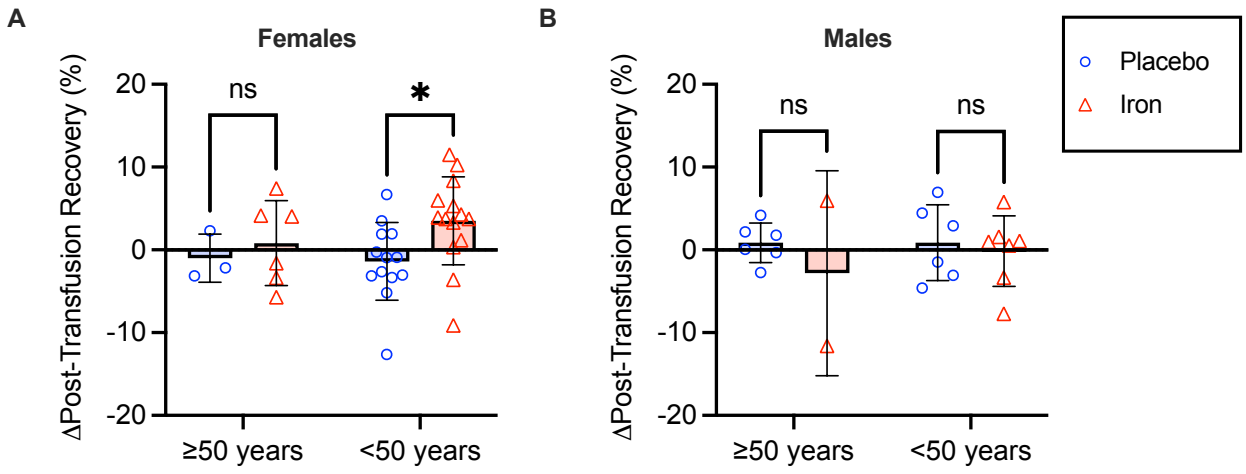
*Occurred at blood donation prior to randomization

†3 weeks following randomization, cardiologist cleared to complete study

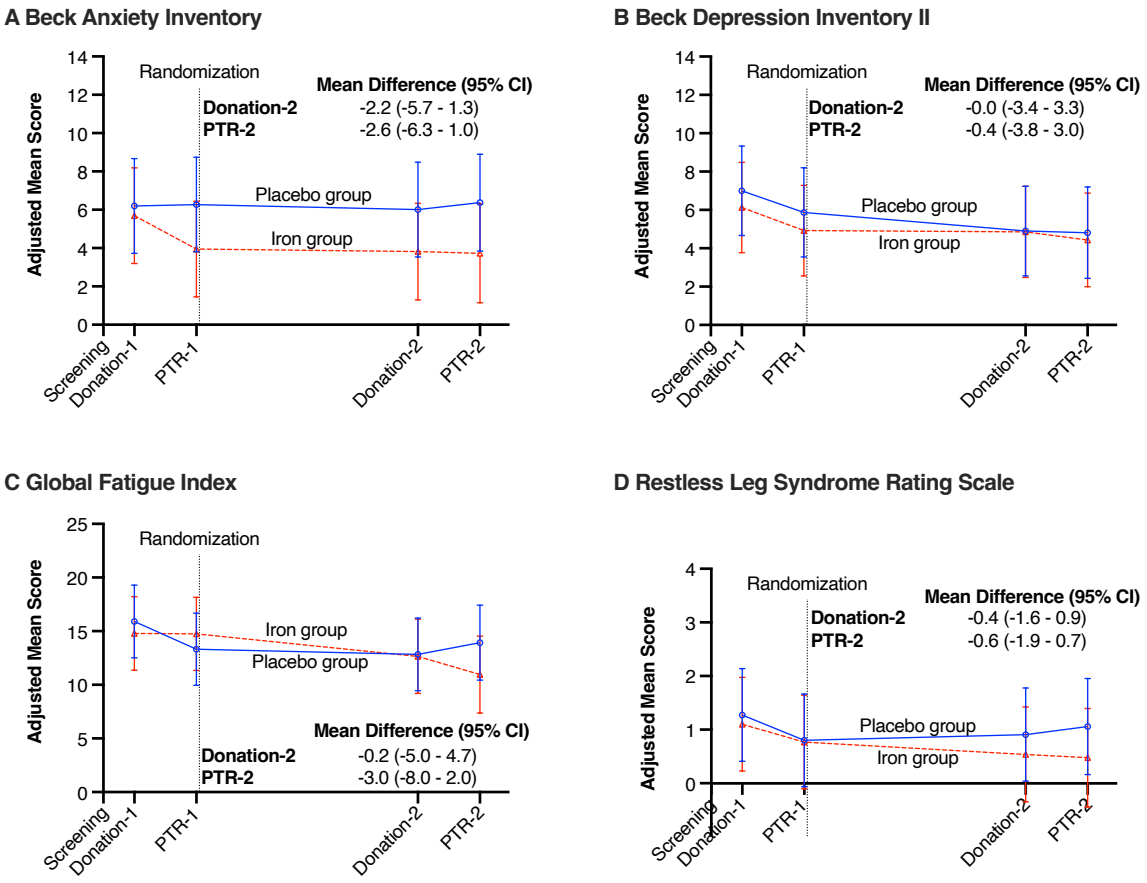
Supplementary Figure 1. Hemoglobin levels in donated red blood cell units at both donations. Bars represent mean hemoglobin levels in donated red blood cell units before (Donation-1) and after (Donation-2) randomization to placebo (open blue circles) or iron repletion (open red triangles). Error bars represent standard deviation. ****P*<0.001 in mixed-effects analysis.



Supplementary Figure 2. Post-transfusion recovery in males and females below and above 50 years of age. Bars represent mean change in red blood cell post-transfusion recovery between the second measure performed after randomization and the first measure before randomization to placebo (open blue circles) or iron repletion (open red triangles). (A) Overall change in post-transfusion recovery among female participants by age and (B) male participants by age, as indicated. Error bars represent standard deviation. *P<0.05 by unpaired t-test.

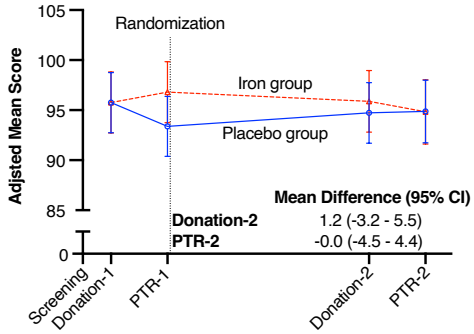


Supplementary Figure 3. Emotional wellbeing and health measurements during the trial. The datapoints represent the estimated means based on a mixed-model repeated-measures analysis after adjustment for the baseline value. The vertical bars denote 95% confidence intervals. The dependent variable was the quality-of-life survey score at each predetermined time point. Fixed effects included the interaction between treatment and time. Time was treated as a categorical variable. The subject was included in the model as a random effect. A first-order autoregressive covariance matrix was used to model the within-patient variance-covariance errors. Pre-specified secondary outcome measures were survey scores from the (A) Beck Anxiety Inventory, (B) Beck Depression Inventory II, (C) Global Fatigue Index, and (D) Restless Leg Syndrome Rating Scale.

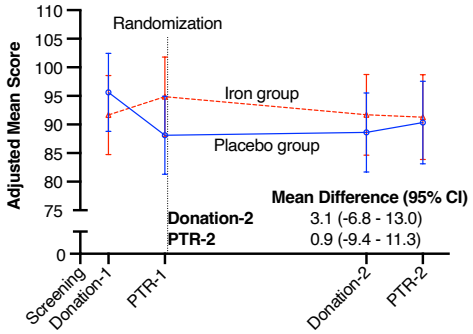


Supplementary Figure 4. RAND Short Form-36 (SF-36) quality of life measurements. The datapoints represent the estimated means based on a mixed-model repeated-measures analysis after adjustment for the baseline value. The vertical bars denote 95% confidence intervals. The dependent variable was the laboratory value at each predetermined time point. Fixed effects included the interaction between treatment and time. Time was treated as a categorical variable. The subject was included in the model as a random effect. A first-order autoregressive covariance matrix was used to model the within-patient variance-covariance errors. Pre-specified secondary outcome measures were the component SF-36 scores of (A) physical functioning, (B) role limitations due to physical health problems, (C) role limitations due to personal or emotional problems, (D) energy/fatigue, (E) emotional well-being, (F) social functioning, (G) bodily pain, (H) and general health.

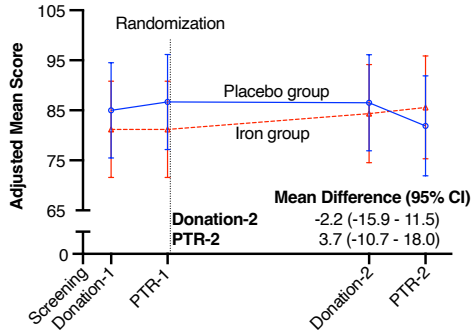
A Physical Functioning Score



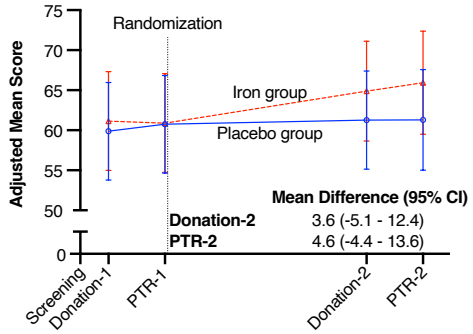
B Role Limitations/Physical Score



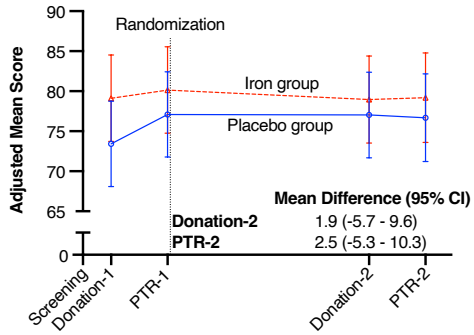
C Role Limitations/Emotional Score



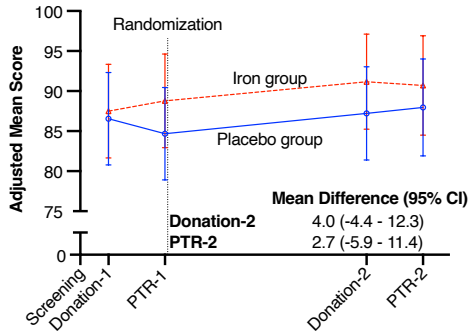
D Energy/Fatigue Score



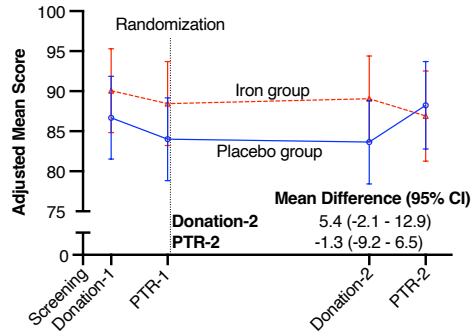
E Emotional Well-Being Score



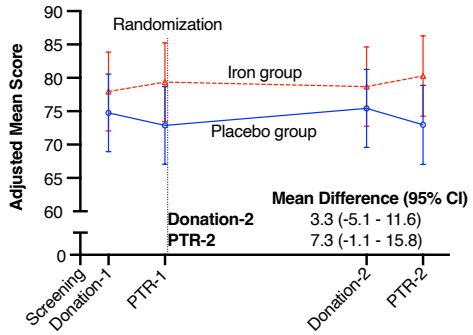
F Social Functioning Score



G Pain Score



H General Health Score



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on Page Number/Line Number	Reported on Section/Paragraph
Title and abstract				
	1a	Identification as a randomised trial in the title	page 1/line 1	
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see Table 2)	page 3	
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	page 4-5	
	2b	Specific objectives or hypotheses	page 5	
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	page 6	Randomization and
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	page 7	Procedures subsection
Participants	4a	Eligibility criteria for participants	page 6	Trial design and
	4b	Settings and locations where the data were collected	page 6	Trial design and
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	page 7	Procedures subsection
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	page 8	Outcomes subsection
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA	
Sample size	7a	How sample size was determined	page 9	Statistical analysis
	7b	When applicable, explanation of any interim analyses and stopping guidelines	page 9	Statistical analysis
Randomisation:				
Sequence generation	8a	Method used to generate the random allocation sequence	pages 6,7	Randomization and
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	pages 6,7	Randomization and
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	pages 6,7	Randomization and blinding section

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	page 6-7	Randomization and blinding subsection
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		Randomization and blinding subsection
	11b	If relevant, description of the similarity of interventions	NA	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	page 9	Statistical analysis
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	page 9	Statistical analysis
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	page 10	Patients subsection and Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons		Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	page 7	Procedures subsection
	14b	Why the trial ended or was stopped	NA	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group		Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups		supplemental Table 2-8
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	page 9	statistical analysis
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	page 12	laboratory outcomes subsection
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	page 13	supplemental Table 9
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	page 16	Discussion section
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	page 17	Discussion section
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	page 16-17	Discussion section
Other information				
Registration	23	Registration number and name of trial registry	page 3	abstract

Protocol	24	Where the full trial protocol can be accessed, if available	page 6	methods
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	page 10	role of funding source

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Table 2 Items to include when reporting a randomized trial in a journal or conference abstract

Item	Description	Reported on Page Number/Line Number	Reported on Section/Paragraph
Title	Identification of the study as randomized		
Authors *	Contact details for the corresponding author		
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)		
Methods			
Participants	Eligibility criteria for participants and the settings where the data were collected		
Interventions	Interventions intended for each group		
Objective	Specific objective or hypothesis		
Outcome	Clearly defined primary outcome for this report		
Randomization	How participants were allocated to interventions		
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment		
Results			
Numbers randomized	Number of participants randomized to each group		
Recruitment	Trial status		
Numbers analysed	Number of participants analysed in each group		
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision		
Harms	Important adverse events or side effects		

Conclusions	General interpretation of the results		
Trial registration	Registration number and name of trial register		
Funding	Source of funding		

** this item is specific to conference abstracts*

From: Hopewell S, Clarke M, Moher D, et al. CONSORT for reporting randomized controlled trials in journal and conference abstracts: explanation and elaboration. PLoS Med. 2008;5(1):e20

Please leave this space alone as it will be supplemented by the editorial office when needed.

TRIAL FULL TITLE	Donor Iron Deficiency Study
SAP VERSION	1.0
SAP VERSION DATE	2021/08/27
TRIAL STATISTICIAN	Donald McMahon
Protocol Version (SAP associated with)	2.5
TRIAL PRINCIPAL INVESTIGATOR	Eldad A. Hod, MD
SAP AUTHOR(s)	Eldad A. Hod, Donald McMahon, Gary Brittenham

SAP Signatures

I give my approval for the attached SAP entitled Donor Iron Deficiency Study dated 2021/08/27

Statistician (Author)

Name: Donald McMahon, PhD

Signature: [redacted]

Date: 08/27/2021

Principal Investigator

Name: Eldad A. Hod, MD

Signature: [redacted]

Date: 08/27/2021

1 Table of Contents

2 Abbreviations and Definitions

AE	Adverse Event
CPM	Counts Per Minute
CRF	Case Report Form
PTR	Post-Transfusion Recovery
RBC	Red Blood Cell
SAE	Serious Adverse Event

3 Introduction

This is a prospective, double-blind, placebo-controlled randomized trial to determine if red blood cells (RBCs) from donors with iron deficient erythropoiesis have decreased post-transfusion RBC recovery (PTR) and whether iron repletion improves PTR.

4 Study Objectives and Endpoints

The safety and efficacy of intravenous iron repletion for treating iron deficiency has already been demonstrated.[1,2] The main objective of this study is to assess whether blood donation-induced iron deficiency impairs the quality of RBCs. The primary endpoint will be the change in 24-hour post-transfusion recovery from the first to the second donation. The primary outcome will be the group mean difference in the primary endpoints between the group receiving intravenous iron repletion and the group receiving intravenous saline**.** Secondary outcomes will assess laboratory measures of iron status and surveys to assess quality of life, fatigue, and emotional health. In an ancillary study, cognition will also be evaluated.

The endpoints for the parent study are consistent with the Donor Iron Deficiency Study trial record ClinicalTrials.gov Identifier: NCT02889133

The endpoints for the ancillary study to assess cognition are consistent with the Neuroimaging of Donor Iron Deficient Study trial record ClinicalTrials.gov Identifier: NCT02990559

4.1 Primary Hypotheses

1) The 24-hour post-transfusion RBC recovery of units obtained from donors exhibiting iron-deficient erythropoiesis will not meet FDA standards for clinical use.
2) The 24-hour post-transfusion RBC recovery of units obtained after intravenous iron repletion will improve significantly and will meet FDA standards for clinical use.

4.2 Secondary Hypotheses

1) Iron repletion will decrease RBC zinc protoporphyrin and soluble transferrin receptor levels.
2) Iron repletion will increase hepcidin, ferritin, transferrin saturation, hemoglobin, hematocrit, and reticulocyte hemoglobin levels.
3) Iron repletion will improve the following quality-of-life RAND SF-36 scores:
    a) SF-36 Physical functioning score
    b) SF-36 Role functioning/physical score
    c) SF-36 Role functioning/emotional score
    d) SF-36 Energy/fatigue score

e) SF-36 Emotional well-being score
f) SF-36 Social functioning score
g) SF-36 Pain score
h) SF-36 General health score

4) Iron repletion will improve the following emotional well-being and other health scores:
a) Beck Depression Inventory (BDI)-II score
b) Beck Anxiety Inventory (BAI) score
c) Global Fatigue Index (GFI) score
d) Restless Legs Syndrome Rating Scale score

4.3 Primary Hypothesis for Ancillary Study

1) Iron repletion will improve the National Institutes of Health Toolbox® for Assessment of Neurological and Behavioral Function (NIH Toolbox)-derived uncorrected standard Cognition Fluid Composite Score.[3,4]

4.4 Secondary Hypotheses for Ancillary Study

1) Iron repletion will improve the following NIH Toolbox individual test component scores:
a) Dimensional Change Card Sort Test
b) Flanker Inhibitory Control and Attention Test
c) Picture Sequence Memory Test
d) List Sorting Working Memory Test
e) Pattern Comparison Processing Speed Test
f) Rey Auditory Verbal Learning Test

5 Study Methods

5.1 General Study Design and Plan

This is a randomized, controlled, double-blind clinical trial (Figure 1). Healthy regular donors who meet donation standards, while exhibiting iron-deficient erythropoiesis by laboratory test criteria, will donate a single standard RBC unit that will be leukoreduced and refrigerator stored under standard conditions in AS-3 for up to 42 days. After 40-42 days of storage, a 51Cr-radiolabeled 24-hour RBC recovery study will be performed. Briefly, a small aliquot of the donated RBCs will be radiolabeled and injected into the volunteers as per the standardized protocol.[5] RBC recovery will be calculated from samples obtained at 5min, 7.5min, 10min, 12.5min, 15min, 30-minutes, 1-hour and 24 hours after autologous infusion. In a prospective, randomized, double-blind manner, these donors will then receive either intravenous saline or intravenous iron (low-molecular weight iron dextran, INFeD or ferric carboxymaltose INJECTAFER; 1 gram of iron) from 1-day to 4-weeks (target 1-day) after the first post-transfusion recovery study. After five months, the participant will donate a second RBC unit, similarly stored for 40-42 days (target same as first recovery study), and a second autologous 51-chromium 24-hour post-transfusion RBC recovery will be determined.

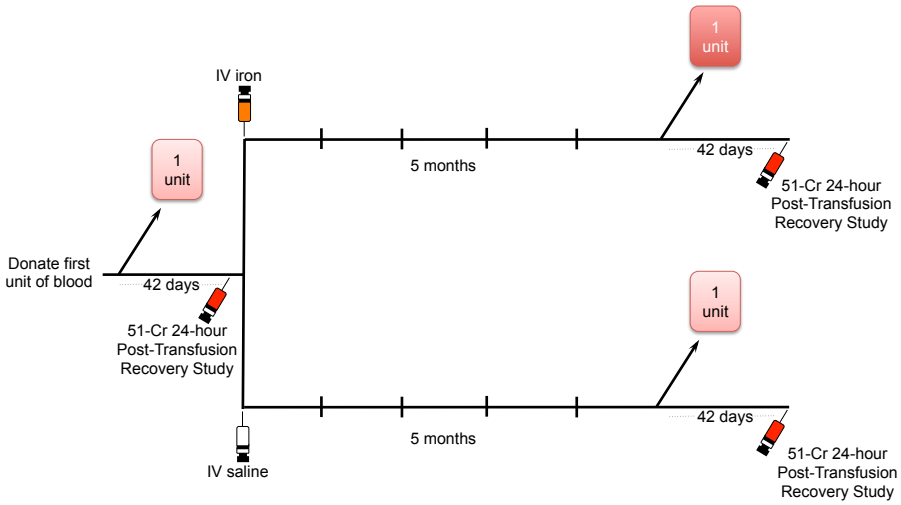


Figure 1. Study Schema. Frequent blood donors with iron-deficient erythropoiesis will donate one unit of RBCs and a ^{51}Cr-labeled post-transfusion RBC recovery study will be performed. Each volunteer will then be randomized to placebo (intravenous saline) or iron repletion (intravenous low molecular weight iron-dextran or ferric carboxymaltose) within 4-weeks of completing the post-transfusion RBC recovery study. 5 months ± 4 weeks later (target 5 months) they will donate another RBC unit. 40-42 days after the second blood donation, another ^{51}Cr-labeled RBC recovery study will be performed and the result compared to the first ^{51}Cr-labeled post-transfusion RBC recovery study. Quality-of-life and emotional well-being surveys will be administered in print format at both blood donation and post-transfusion recovery study visits (i.e., four evaluations for each assessment).

5.2 Inclusion-Exclusion Criteria and General Study Population

Inclusion criteria
18-75 years old
Healthy (by self report)
Body weight > 110 lbs
female hematocrit >38%, male hematocrit >39%
frequent blood donor (male ≥2 and female ≥1 RBC unit donations in past year)
ferritin <15 ng/mL
zinc protoporphyrin >60 µmol/mol heme
Exclusion criteria
ineligible for donation based on the New York Blood Center autologous donor questionnaire
C-reactive protein >10 mg/L
sickle cell trait (by self report)
systolic blood pressure >180 or <90 mm Hg, diastolic blood pressure >100 or <50 mm Hg
heart rate <50 or >100
temperature >99.5°F prior to donation (attempts will be made to reschedule donation, if possible)
temperature >100.4°F or subjective feeling of illness prior to ^{51}Cr-labeled 24-hour RBC recovery study
positive results on standard blood donor infectious disease testing
pregnancy
taking, or planning to take, iron supplements and not willing to stop for duration of study
history of severe asthma requiring hospitalization, allergic eczema (atopic dermatitis), or other atopic allergy associated with anaphylaxis

5.3 Randomization and Blinding

Randomization will be performed using a computerized system with equal allocation (1:1) to iron repletion or placebo. Randomization will be stratified by gender; randomly permuted block sizes of 4, 6, or 8 will be used. The moment of randomization will be recorded and will occur only after successful completion of the first post-transfusion RBC recovery study.

The study will be double-blind with the identity of the randomized groups known only to the Columbia University Irving Medical Center Research Pharmacy. A research pharmacist will provide the placebo (IV saline) or treatment (IV low molecular weight iron-dextran or ferric carboxymaltose), and the test dose of iron/placebo, in tinted infusion bags with tubing specifically designed to maintain blinding in clinical research studies (Medipak). A research nurse unaffiliated with the study team will be responsible for the test infusion and total dose iron infusion. With this design, volunteers and study investigators will be blinded to whether volunteers receive the active intervention or placebo. Subjects in both groups will receive similar discharge instructions as if they had received iron repletion. Scheduling and logistic communications with volunteers will be made by the study coordinator, who will also be blinded

to the treatment group.

Situations may arise in which breaking the blinding earlier would be in the best interest of the volunteer. In any situation in which a physician or the subject asks to be un-blinded to study treatment, the research pharmacy can be reached on an emergency basis to provide this information. Finally, any un-blinding that occurs will be communicated to the Data Safety Monitoring Board (DSMB) and ultimately reported in all related publications.

5.4 Study Assessments

The schedule of measurements is summarized in the Table below. Following successful screening and signed informed consent, subjects will donate a unit of autologous red blood cells (Donation1). This will be followed 40-42 days later by the baseline 51-Chromium post-transfusion recovery (PTR1) study. Following the 24-hour post-chromium infusion blood draw, the subjects will be randomized to iron infusion or placebo (Randomization). For logistical reasons this infusion may be delayed for up to 4 weeks (28 days) following the blood draw. Five months ± 4 weeks after randomization, subjects will donate the second unit of autologous blood (Donation2). The second 51-Chromium post-transfusion recovery study will be performed 40-42 days after this donation. Subjects will have to return 24-hours after chromium infusion to determine the 24-hour post-transfusion recovery. Secondary measures including laboratory measures, surveys, and ancillary cognitive assessments will be conducted at the Donation1, PTR1, Donation2, and PTR2 study visits.

Because an active type and screen must be obtained before the blood bank issues the stored RBC unit and the radioactive 51-Cr-labeling can occur, we will provide subjects with the option of coming in up to 30 days prior to the scheduled PTR study to obtain a type and screen sample (1 tube of blood by standard venipuncture). This will provide for a shorter day for the PTR study because there will no longer be the need to wait for the type and screen testing and the study can begin once the subject arrives. If a subject chooses not to provide this optional blood draw, a blood sample will be taken when the IV lines are placed for the PTR study and the PTR study will proceed after the blood bank issues the blood and it is radiolabeled per hospital guidelines.

Day of study	**Urine pregnancy test**	**CBC with reticulocyte count**	**Iron parameters***	**C-reactive protein**	**Type and Screen**	**51-Cr PTR****	**Iron/Placebo infusion**	**Surveys/ Neuro-cognitive testing**
Screening		X	X	X	X			
Donation 1	X	X	X	X				X
Blood draw (optional) ***					X			
PTR1 (40-42 days	X	X	X	X	X	X		X

after Donation 1)								
Randomization (1-28 days after PTR1)	X						X	
Donation 2 (5months ± 4 weeks after iron infusion1)	X	X	X	X				X
Blood Draw (optional) ***					X			
PTR2 (40-42 days after Donation 2)	X	X	X	X	X	X		X
Final blood draw (1 day after PTR2)						X – collection of 24 hour (+/- 4hr) time point for final measurement		

*Iron parameters = Zinc Protoporphyrin (ZPP), iron, total iron binding capacity (TIBC), transferrin saturation, ferritin, soluble transferrin receptor, hepcidin.

**PTR = post-transfusion recovery study with associated blood draws for hemoglobin and Chromium-51 determination taken pre-infusion and 5, 7.5, 10, 12.5, 15, 30, 60 minutes and 24-hours post-infusion (+/- 4 hours).

***The option to come in up to 30 days prior to the PTR study will be provided to obtain a type and screen sample. This will provide an active type and screen specimen so that the PTR study can begin without delay. Alternatively, the type and screen blood sample will be drawn when the IV is paced for the PTR study and then the study will begin once the type and screen is tested and the blood is issued and labeled.

Assessments will occur at the following visits: Screening, Donation-1, PTR-1, Donation-2, and PTR-2. The following table describes the allowable time windows in between each of these study visits. The number of study visits occurring outside of these study visits will be described in the primary outcome manuscript and if below 10% of cases, will be included in the primary analysis.

Analysis Time Windows

Visit (target number of days from prior visit)	Lower bound (days from prior visit)	Upper bound (days from prior visit)
Screening (0)	N/A	N/A
Donation-1 (7)	1	60
PTR-1 (41)	40	42
Randomization (1)	1	28
Donation-2 (150)	120	180
PTR-2 (41)	40	42

6 Sample Size

Based on preliminary data from our prior ^{51}Cr RBC recovery studies,[6] the standard deviation of the measure in our single site is 5.0%. Furthermore, the expected mean difference in post-transfusion RBC recovery between iron replete mice and mice with iron-deficient erythropoiesis is 10.6%.[7] If the difference were this large in humans, we would require <6 subjects (alpha = 0.05, two-sided, power = 0.80). However, we expect the difference to be less conspicuous in humans than in inbred mice. Thus, we power the study to detect a clinically relevant difference in post-transfusion recovery of 4%. Under this assumption, the calculated sample size required for each arm is 26 evaluable subjects with both pre- and post-randomization PTR measures complete (alpha = 0.05, two-sided, power = 0.80). Furthermore, to allow for up to a 15% dropout rate (i.e., 4/26 subjects), we plan to randomize 30 subjects per arm for a total sample size of 60 subjects. Of note, randomization will occur after successful completion of the first PTR study; thus, we predict up to 70 individuals will need to be recruited.

7 General Analysis Considerations

7.1 Timing of Analyses

Final analysis will be performed once at least 26 subjects in each arm have completed both post-transfusion recovery measures required for primary outcome analysis and after all already enrolled subjects have completed all primary and secondary outcome measurements. Furthermore, all tables of measurements will be uploaded into a local encrypted database (https://www.sac-cu.org/CALM/Login.aspx?ReturnUrl=%2fCALM%2fEditProject.aspx) and error checked as compared to source documents prior to database locking.

7.2 Analysis Populations

7.2.1 Full Analysis Population (Modified Intention to Treat)

- For the primary outcome analysis, the full analysis population will be defined as all subjects who were successfully randomized and had an evaluable primary outcome measure (51-Cr post-transfusion recovery study) after randomization.

- For the secondary outcome analyses, the full analysis population will be defined as all subjects who were randomized.

7.2.2 Per Protocol Population

- *N/A*

7.2.3 Safety Population

- *All subjects who received any study treatment (including placebo control) or donated blood after enrollment.*

7.3 Covariates and Subgroups

Pre-specified demographic variables (gender [male/female], race [white/not white], age [<50/≥50 years]) will be explored in subgroup analyses for the primary and secondary outcomes. These subgroup analyses are exploratory and only summary statistics will be presented.

7.4 Missing Data

The primary outcome analysis will be limited to only those subjects with an evaluable primary outcome measure (i.e., evaluable pre- and post-randomization post-transfusion recovery measure).

For secondary analyses, it is assumed that missing data is Missing At Random. Linear mixed models for repeated measures will be utilized for these secondary analyses to handle the missing data.

7.5 Interim Analyses and Data Monitoring

Interim analyses will be performed twice (after every 20 subjects have completed study participation) in addition to the final analysis. The Data Safety Monitoring Board (DSMB) will conduct the analyses using a two-sided asymmetric Lan-DeMets alpha-spending approach with an O'Brien-Fleming two-sided symmetric stopping boundary and overall alpha = 0.05. The DSMB criteria for early stopping will include: (i) the Z-score at interim analysis lies outside of the group sequential boundaries as calculated (see Table below); (ii) major safety violations; and (3) convincing evidence of futility in the context of adverse events (AEs). Interim boundaries together with terminal criteria (z-scores and associated p-values) calculated using the WinLD

version 2 program are provided in the Table below.

Table. Lan-DeMets Group Sequential Boundaries Calculations

20	-3.7103	3.7103	0.00010	0.00021
40	-2.5114	2.5114	0.00601	0.01210
60	-1.9930	1.9930	0.02313	0.05000

7.5.1 Practical Measures to Minimize Bias

The interim analyses will be performed by the independent statistician (Donald McMahon). The analyses will be performed in blinded fashion (i.e., without knowing which group received iron repletion or placebo) and presented to the DSMB in closed session. The investigators and sponsor will not be provided results from the interim analyses. All final analyses will be performed and finalized before unblinding to study intervention.

7.6 Multiple Testing

No multiple comparison adjustments for the secondary end points are defined. Therefore, only point estimates and 95% confidence intervals will be provided. The confidence intervals will not be adjusted for multiple comparisons and will not be used to infer definitive treatment effects.

8 Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, and IQR. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by treatment and by visit number. All summary tables will be structured with a column for each treatment in the order (Iron, Placebo) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

8.1 Subject Disposition

Phone screening case report forms (CRF) will be tabulated as number of calls received. A CRF will be filled by study coordinators at each subsequent visit and used as source data to determine subject disposition. Those presenting for an in-person screening appointment, whether or not blood was successfully drawn, will be considered screened. Laboratory results will be examined to determine inclusion in the study based on level of iron deficiency. Only those with two evaluable PTR study measures (i.e., one before and one after randomization) will be tabulated for the primary outcome measure. For tabulating number of subjects for secondary outcomes, final totals will reflect any subject that completed at least one study survey after randomization.

8.2 Derived variables

The primary outcome measure, a 24-hour 51-chromium post-transfusion recovery will be

performed and derived as per the standardized protocol,[5] by a blinded assessor. RBC recovery will be calculated from samples obtained at 5min, 7.5min, 10min, 12.5min, 15min, 30-minutes, 1-hour and 24 hours after autologous infusion. Mean counts per minute (CPM) per gram hemoglobin per milliliter blood will be calculated from blood samples obtained at the above time points (8 replicate measurements taken). The background mean CPM per gram hemoglobin per milliliter blood present prior to infusion will be subtracted from that calculated at each timepoint. Using all the evaluable points up to 24 hours, best fit logarithmic regression through all the evaluable points will be performed (https://keisan.casio.com/exec/system/14059930226691) to extrapolate the T0 CPM per gram hemoglobin per milliliter blood (i.e., the time-zero amount of 51-chromium present immediately after infusion). The RBC recovery at any given timepoint X is given by the following formula:

%RBC recovery (Time = X) = CPM per gram hemoglobin per mL (Time =X) / CPM per gram hemoglobin per mL (Time = 0)

A blinded assessor will review plots of all data and determine whether particular points should be dropped as clear outliers.[8]

Transferrin saturation will be calculated using the following formula, and expressed as percent:

Transferrin saturation (%) = serum iron (mg/dL) / total iron binding capacity (mg/dL)

8.3 Protocol Deviations

The primary outcome analysis will be based on evaluable post-transfusion recovery studies performed 40-42 days after blood donation of a leukoreduced unit of RBCs refrigerator-stored in AS-3 storage solution. Major protocol deviations that will result in exclusion of data from analysis include: erroneous collection of non-leukoreduced RBC units, storage in a storage solution other than AS-3, and performance of the post-transfusion recovery measure outside of the 40-42 day window.

8.4 Demographic and Baseline Variables

The following demographic and baseline variables will be recorded at the initial screening visit: age, sex, race (white, black, Asian, other), ethnicity (Hispanic/non-Hispanic), weight, and number of donations in the prior year.

9 Efficacy Analyses

The primary null hypothesis will be tested in an intent-to-treat analysis using a t-test, or nonparametric equivalent, of the between-group difference in means of the within-subject change in the post-transfusion RBC recovery from the initial study under iron-deficient erythropoiesis conditions and the subsequent study performed after randomization to iron repletion or placebo. Confidence intervals will be calculated assuming equal variance in the two independent samples. We will also examine pre-specified demographic variables (gender [male/female], race [white/not white], age [<50/≥50 years]) in sub-group analyses. These will be presented as means with 95% confidence intervals in supplementary tables.

The secondary outcome null hypotheses will be tested in an intent-to-treat analysis using linear mixed models for repeated measures to compare the difference in the iron repletion and placebo group temporal course at the defined time points on the secondary outcome measures. We will also examine pre-specified demographic variables, as above, to see if we can identify

one or more that may be effect modifiers. Fixed effects will include the interaction between treatment (iron vs placebo) and time. Time will be treated as a categorical variable. The subject will be included in the model as a random effect. A first-order autoregressive covariance matrix will be used to model the within-patient variance-covariance errors. Ancillary cognitive outcomes will be handled similarly.

All secondary efficacy variables will be summarized by treatment group. N, Median, and IQR will summarize continuous efficacy variables, whereas number and percent will summarize categorical efficacy variables.

10 Safety Analyses

The safety and efficacy of intravenous iron repletion treatment for correcting iron deficiency has already been demonstrated. Nonetheless, safety analyses will be performed to assess any harm associated with iron repletion or placebo treatments. When calculating the incidence of adverse events, the number of subjects with at least one event along with the total number of events will be summarized. All randomized subjects will be included in this analysis and all reported adverse events occurring after enrollment will be presented. Results will be presented for the iron repletion group and the placebo group separately.

11 Quality Assurance of Statistical Programming

A second review statistician will independently reproduce the primary analyses and summary statistics. The reviewing statistician will have an overview of the entire analyses and will explicitly check the code producing all tables as well as any other pieces of code as desired.

12 Summary of Changes to the Protocol and/or SAP

Summary of Changes to Protocol

Version Number	Date Approved by IRB	Changes	Rationale
1.1	23 August 2016	Change of inclusion criteria from ferritin < 12 ng/mL to ferritin < 15 ng/mL	To be in line with WHO criteria for iron deficiency
		Addition of print versions of SF-36 quality-of-life, Beck Depression Index-II, Beck Anxiety Index, and Multidimensional Assessment of Fatigue (MAF) surveys as attachments to study protocol	To incorporate study instruments used into protocol
		Addition of NIH Toolbox ancillary study testing to main protocol and consent form	Adds minimal risk to main parent protocol and by not making it an optional

			ancillary study, will retain maximal power for detecting a difference in cognitive outcomes
2.0	21 December 2016	Page 2 - Explicit statement of secondary hypothesis: “Iron therapy will improve neurocognition and emotional well-being in donors with iron deficient erythropoiesis”	Additional ancillary hypothesis added to main study protocol
		Page 3 - Clarification of primary outcome: “The primary outcome will be the group mean difference in 24-hour post- transfusion recovery difference from after treatment to baseline, between the groups receiving intravenous saline and iron”	Elucidation of primary outcome
		Page 4 – Modification of study schema to remove “crossover” iron dextran infusion. This was suggested by the independent study statistician on the DSMB and was discussed and approved at the initial DSMB meeting. Overall, this will reduce risk to subjects and will increase comparability between groups	Prior to study start, this was a recommended modification by the DSMB to reduce risk of low molecular weight intravenous iron dextran to subjects
		Page 11 – More explicit statements of secondary outcomes	Cosmetic changes to specifications of secondary outcomes
		Page 12 – Change inclusion criteria from hemoglobin cutoff to hematocrit cutoff	New York Blood Center only measures hematocrit to determine eligibility to donate blood (FDA provides criteria for both)
		Page 12 – minor modifications to exclusion criteria to make more clear: “6) temperature >99.5°F prior to donation (attempts will be made to reschedule donation if possible); 7) temperature >100.4°F or subjective feeling of illness prior to 51Cr 24-hour RBC recovery study (to avoid the subject having a concurrent illness that may affect post-transfusion recovery); (attempts will be made to reschedule donation and 51Cr 24- hour RBC recovery study); 10) taking, or planning to take, iron supplements and not willing to stop for duration of study;”	Minor modifications to exclusion criteria to make more clear
		Page 14 – Clarification of how blinding will be managed and that independent study statistician on DSMB will be	Elucidation of procedures for blinding

		unblinded and will create randomization scheme.	
		Page 16 –Dosage of test dose of iron dextran provided as per research pharmacy.	To provide rationale for premedication.
		Page 17 – Rationale for 1 gram dose of iron dextran provided as per recommendation from DSMB.	To provide rationale for dose selected
		Page 20,21 – Addition of two optional study visits to simply provide a blood sample for a blood bank type and screen. Thus, subjects will be given the option of coming in for an extra blood draw within 30 days before the scheduled post-transfusion recovery study. Those not taking this option will have the type and screen drawn on the day of the post-transfusion recovery study and will simply have a later start time on that day.	This is for logistical purposes as there must be an active type and screen on record before the blood can be issued from the blood bank. Although this can be done on the same day as the post-transfusion recovery study is performed, this will lead to a delay on that day of participation.
		Page 21 – Zinc protoporphyrin levels, determining an important inclusion criteria, will be sent to a clinically-validated reference laboratory (ARUP) as opposed to being performed in our research laboratory.	Decision made for all laboratory measures performed for inclusion/exclusion purposes to be done in a CLIA-certified clinical laboratory.
		Page 22 – Section 8.0 detailing subject compensation was added to the protocol.	Elucidation of procedures
		Page 28 – Semi-annual DSMB report was modified to show results by group rather than by treatment to maintain blinding of the DSMB. Data entry completeness and data QA will also be reported.	Elucidation of procedures
		Page 44 - All of the Case Report Forms (CRFs) were moved to Appendix 7.	Elucidation of procedures
2.1	02 February 2017	Added a short 2-minute auditory hearing test as part of the cognitive assessments. This will be done on an iPad using headphones through the NIH toolbox tool already used for the cognitive testing. Protocol was updated to version 2.1 with the addition of section 5.4.3 to address this modification. A Hearing Severity survey was also attached.	Due to recent JAMA Article Schieffer KM, Chuang CH, Connor J, Pawelczyk JA, Sekhar DL. Association of Iron Deficiency Anemia With Hearing Loss in US Adults. JAMA Otolaryngol Head Neck Surg. Published online December 29, 2016. doi:10.1001/jamaoto.2016.3631
		Added restless leg syndrome severity survey to documents section and protocol (section 5.4.4)	Due to association of restless leg syndrome with iron deficiency

2.2	24 May 2017	Change of inclusion criteria from ZPP>80 to ZPP>60	With change of measurement to ARUP from research use only test performed in lab, pilot study performed prior to study start is more consistent with a cutoff of 60 for ZPP. Change in criteria reviewed and approved by DSMB.
		Change of inclusion criteria from requirement of at least 3 and 2 donations in past year for males and females, respectively, to at least 2 and 1 donations in past year for males and females, respectively	To increase generalizability to all repeat blood donors as opposed to just most active ones. Change in criteria reviewed and approved by DSMB prior to implementation.
		Allow re-screening of subjects who meet ZPP and ferritin inclusion criteria, but are too anemic to donate blood (i.e., hematocrit <38% for females, <39% for males). The re-screening will be scheduled from 2 weeks to 2 months following the initial screening. Subjects will be compensated $10 for re-screening.	To increase accrual and enrollment rates.
2.3	24 October 2018	Page 16,22 - Revised protocol better specifying window period for 24 hour post-transfusion recovery final blood draw.	Better specification of window periods to avoid protocol deviations.
		Page 15 - Specify that women older than 55 years do not require a urine pregnancy test prior to study participation.	To avoid unnecessary testing
2.4	14 November 2018	Addition of intravenous ferric carboxymaltose (INJECTAFER 1gram Fe) as option if low molecular iron dextran is unavailable due to supply chain issues	Address national shortage of INFeD
2.5	06 December 2019	Addition of exploratory metabolomics testing of biobanked transfusate samples	Specification of additional exploratory analyses planned to be performed

Rationale for Adjustments of Statistical Analysis Plan (adjustment to SAP published in Bitan et al.[9] 8/7/2019)

To better handle spurious missing data, the statistical analysis plan for all secondary outcomes was revised to utilizing linear mixed models as specified in section 9 of this SAP.

13 References

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Donor Iron Deficiency Study

Protocol Version 2.5 – December 6, 2019

Study Sponsor: National Heart, Lung, and Blood Institute

Grant: NIH R01-HL133049

A randomized trial to determine if RBCs from donors with iron deficient erythropoiesis have decreased post-transfusion RBC recovery and whether iron repletion improves recovery

Concept Synopsis and Study Schema
Each year, ~5 million regular donors meet almost half the transfusion needs of the United States by voluntarily donating their blood. Despite fulfilling all requirements for blood donation, almost two-thirds of the women and half of the men who are regular blood donors are iron deficient. In order to manage the inventory, red blood cell (RBC) units destined for transfusion can be stored for up to 42 days prior to transfusion based on FDA guidelines. This guideline is based on a post-transfusion recovery study in which one must prove to the FDA that, on average, using healthy volunteers, more than 75% of transfused red cells remain in circulation for 24 hours at outdate. **This research will determine if RBC units from iron-deficient volunteer blood donors fail to meet U.S. FDA standards for 24-hour post-transfusion recovery.** RBCs from iron-deficient donors may be specifically damaged by refrigerated storage. In studies with a mouse model of iron deficiency (with hemoglobin levels similar to those that are acceptable for human blood donation), we found a mean 10.6% further decrease in the 24-hour post-transfusion recovery of refrigerator-stored red cells from iron-deficient animals, as compared to the post-transfusion recovery from iron-replete littermates. A comparable decrement in post-transfusion recovery of refrigerator-stored red cells from iron-deficient human volunteer blood donors would result in many of these units failing to meet established quality standards for clinical use. This study will also test whether giving iron deficient donors their iron back will improve the quality of their red cells during refrigerator storage.

Research Question(s)/Hypothesis(es):

Primary
1. The 24-hour post-transfusion RBC recovery from units obtained from iron-deficient donors will not meet FDA standards for clinical use.

2. The 24-hour post-transfusion RBC recovery from units obtained after intravenous iron repletion will improve significantly and will meet FDA standards for clinical use.

Secondary
1. Iron therapy will improve neurocognition and emotional well-being in donors with iron deficient erythropoiesis.

2. Metabolite levels in the transfusate will be associated with 24-hour post-transfusion RBC recovery and iron status.

Study Schema:
This is a randomized, controlled, double-blind clinical trial. 60 healthy regular donors who meet donation standards, while exhibiting iron-deficient erythropoiesis by laboratory test criteria, will donate a single standard RBC unit that will be leukoreduced and stored under standard conditions for 42 days. The FDA gold standard measure for the quality of RBCs destined for transfusion is the 51Cr-radiolabeled 24-hour RBC recovery study. Thus, a small aliquot of the transfused RBCs will be radiolabeled and injected into the volunteers on day 41 or 42 of storage. RBC recovery will be calculated from samples obtained at 5min, 7.5min, 10min, 12.5min, 15min, 30-minutes, 1-hour and 24 hours after autologous infusion as per the standardized protocol.[4] For secondary outcomes, study surveys and neurocognitive assessments to assess symptoms of anemia and iron deficiency will also be performed prior to donation and prior to infusion.

In a prospective, randomized, double-blind manner, these donors will then receive either intravenous saline or low-molecular weight iron dextran (or ferric carboxymaltose if iron dextran is unavailable due to supply) within 1-day to 4-weeks after the first post-transfusion recovery study. After five months, they will donate a second RBC unit, similarly stored for 42 days, and autologous 51-chromium 24-hour post-transfusion RBC recoveries will again be determined. The primary outcome will be the group mean difference in 24-hour post-transfusion recovery difference from after treatment to baseline, between the groups receiving intravenous saline and iron.

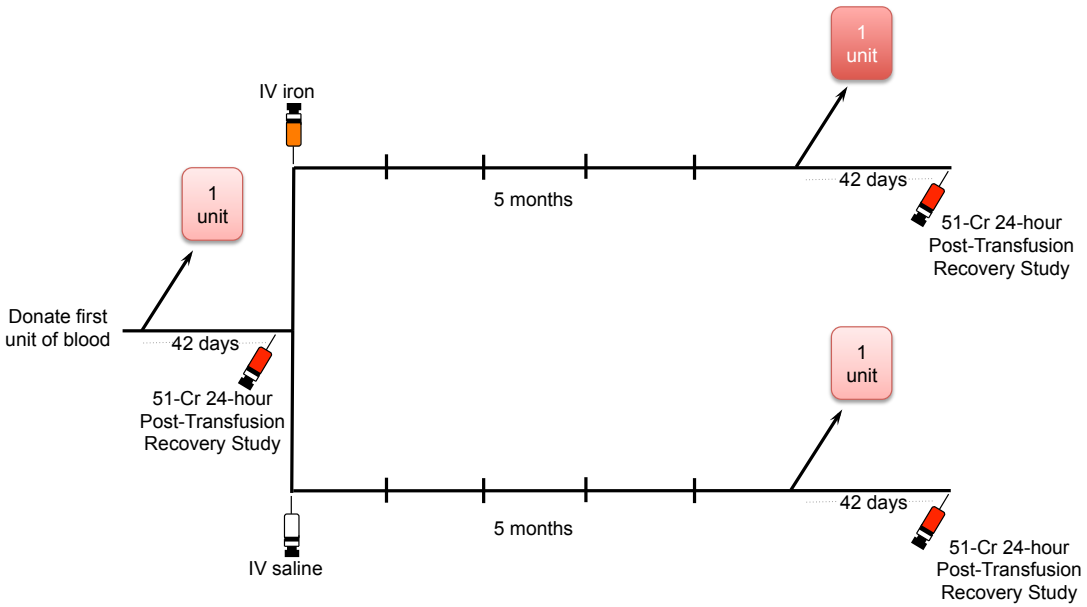

Figure 1. Study Schema. 60 frequent blood donors with iron-deficient erythropoiesis will donate one unit of RBCs and a ^{51}Cr-labeled post-transfusion RBC recovery study will be performed. Each volunteer will then be randomized to placebo (intravenous saline) or iron repletion (intravenous low molecular weight iron-dextran or ferric carboxymaltose) within 4-weeks of completing the post-transfusion RBC recovery study. 5 months ± 4 weeks later (target 5 months) they will donate another RBC unit. 42 days after the second blood donation, another ^{51}Cr-labeled post-transfusion RBC recovery study will be performed and the result compared to the first ^{51}Cr-labeled post-transfusion RBC recovery study.

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1. Background and Significance:

General Introduction. Iron deficiency is common among regular blood donors, but the recovery and quality of RBC units from iron-deficient donors has not been rigorously examined. Evidence from both animal and human studies indicate that when the iron supply for erythropoiesis is inadequate, the RBCs produced have multiple metabolic defects that impair their ability to tolerate refrigerated storage. Our studies in a mouse model demonstrated decreased post-transfusion recovery of refrigerator-stored RBCs obtained from iron-deficient donors. The planned studies will identify human donors at greatest risk of providing RBCs with poor post-transfusion recovery by using a combination of a decreased serum ferritin concentration and increased RBC zinc protoporphyrin, as described below. To evaluate unequivocally the role of iron deficiency in poor post-transfusion RBC recovery, intravenous iron will be used for iron repletion.

Serum ferritin and RBC zinc protoporphyrin detect iron-deficient erythropoiesis in volunteer blood donors. Iron deficiency, a decrease in the amount of body iron, is detected clinically by measuring indicators of iron storage (e.g., serum ferritin), and of iron supply (e.g., RBC zinc protoporphyrin). In the absence of complicating factors, as iron stores decrease, serum ferritin levels decline; a serum ferritin level less than 12 µg/L is virtually diagnostic of the absence of marrow iron stores. RBC zinc protoporphyrin monitors the supply of iron available for RBC production. In the developing RBC, the insertion of iron into protoporphyrin IX is the final step in producing heme for incorporation into hemoglobin. If iron is unavailable, divalent zinc is incorporated instead, producing zinc protoporphyrin, which binds to hemoglobin and persists for the life of the RBC as a biochemical indicator of an inadequate supply of iron for RBC production.[1,3]

Four successive stages of iron deficiency can be distinguished (Fig. 2). **Reduced iron stores (not shown)**: As iron stores decrease, serum ferritin levels decline proportionally. **Iron depletion**: Iron depletion develops when iron stores are absent, but iron delivery to the erythroid marrow for producing hemoglobin and other functional iron compounds is maintained by the combination of iron recycling from senescent RBCs and gastrointestinal iron absorption. With absent iron stores, the serum ferritin falls to <15 µg/L. Because the iron supply for RBC production is maintained, RBC zinc

protoporphyrin levels remain in the reference range. **Iron-deficient erythropoiesis**: With further reductions in body iron, the lack of iron limits production of hemoglobin and other iron-requiring compounds, resulting in iron-deficient erythropoiesis. Nonetheless, the effect on the circulating hemoglobin concentration is insufficient to be detected by the standards used to screen blood donors, which currently only includes a hemoglobin test. As newly formed RBCs replace senescent RBCs, RBC zinc protoporphyrin progressively increases, providing an index reflecting the severity and duration of the inadequate supply of iron for erythropoiesis. Further decreases in the nominal serum ferritin levels have no physiological meaning. Thus, **the combination of a serum ferritin <15 µg/L and an increased RBC zinc protoporphyrin (i.e., >80 µmol/mol heme), is highly specific for iron-deficient erythropoiesis**.[1,3]
Iron-deficiency anemia: Further diminution in body iron produces frank iron-deficiency anemia, which would result in donor deferral.

Iron deficiency is common in volunteer blood donors. In the United States in 2011, of the donors who provided the ~15.7 million units of RBCs that were collected, 69% were repeat donors.[5] In addition, in Canada, ~90% of RBC units collected for transfusion are provided by repeat donors.[6] Although iron deficiency is surprisingly prevalent in first-time donors,[7,8] its prevalence is even higher in the particularly altruistic frequent donors, especially among women of childbearing age.[9,10] In the REDS-II Donor Iron Status Evaluation (RISE) study,[11] up to 49% and 66% of male and female frequent donors, respectively, manifested either iron depletion (i.e., absent iron stores) or iron-deficient erythropoiesis. Similar frequencies of iron deficiency were also reported in Canadian,[6] Austrian,[12] Danish,[13] and Dutch[14] populations.

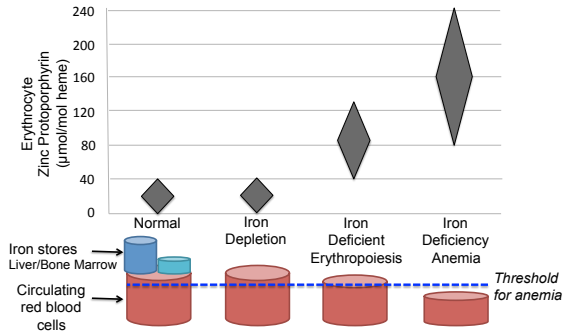

Figure 2. RBC zinc protoporphyrin progressively increases with increasing severity of iron deficiency. Iron deficiency progresses from reduced iron stores to iron depletion, in which there is normal erythropoiesis with normal zinc protoporphyrin levels, but decreased ferritin due to absent iron stores. The next stage is iron-deficient erythropoiesis, in which increased RBC levels of zinc protoporphyrin and very low serum ferritin levels reflect both absent iron stores and the lack of sufficient iron for normal hemoglobin production. As iron deficiency progresses further, frank iron deficiency anemia develops with insufficient iron for maintaining adequate hemoglobin levels. **Our target population is human donors with iron-deficient erythropoiesis**. Adapted from Hastka et al.[1] and Brittenham[3].

RBCs from donors with iron-deficient

erythropoiesis have impaired tolerance for refrigerated storage and decreased post-transfusion RBC recovery. RBCs from individuals with iron deficiency anemia have decreased levels of endogenous antioxidants,[15,16] have evidence of oxidative damage,[17,18] and are more sensitive to oxidative stress[15,18] and low pH;[16] the latter decreases progressively during RBC storage.[19] Furthermore, refrigerated storage induces oxidative stress in donor RBCs and inhibits their oxidative stress defense mechanisms.[17,20-24] Oxidative damage *per se* also impairs RBC deformability[25] and impaired deformability was seen in humans,[18] rats,[18] and rabbits with iron deficiency anemia[26] and in stored RBCs from healthy human donors.[27] Rigid RBCs are less able to pass through previously negotiable microcirculatory beds and are more prone to undergo extravascular hemolysis in the spleen.[28] Indeed, circulatory RBC lifespan is decreased in humans with iron deficiency anemia[16,29-31] and in relevant animal models.[26,32] In humans, decreased circulatory lifespan is most likely due to extravascular hemolysis in the spleen[29-31] and is corrected by iron repletion.[16,30]

Remarkably, in several older studies,[16,29,33] RBCs obtained from donors with iron deficiency anemia were transfused into healthy recipients, without prior refrigerated storage. In each study, the transfused iron-deficient RBCs had a decreased circulatory lifespan/recovery, most likely due to splenic clearance. In addition, when RBCs obtained from healthy donors were transfused into recipients with iron deficiency anemia, the transfused RBCs had a normal lifespan, suggesting that the iron deficiency-induced defect was intrinsic to the RBC and not due to enhanced clearance mechanisms.[33,34]

Iron deficiency decreased the 24-hour post-transfusion RBC recovery in a mouse model. Replicating the human studies described just above from the 1940-70's is no longer ethically permissible. Although the RBCs transfused in these studies were from donors with iron deficiency anemia, not from donors with iron-deficient erythropoiesis, deliberate allogeneic human transfusion studies using such donors and healthy volunteer recipients are no longer feasible. Instead, we used a mouse model to obtain proof-of-principle preliminary data. Three mouse donor cohorts were prepared: iron replete, iron-

deficient erythropoiesis, and iron deficiency anemia (Fig. 3). Similar to our prior studies,[35] refrigerator-stored, transfused RBCs from iron-replete donors had a normal 24-hour post-transfusion recovery (mean 77.1%). In addition, as expected from other publications,[16,29,33] the 24-hour post-transfusion recovery was poor using RBCs from donors with severe iron-deficiency anemia (mean 46.7%; $p<0.001$). In contrast, results using donors with iron-deficient erythropoiesis were subnormal (mean 66.5%; $p<0.05$) and would be less than the FDA-mandated minimum (in this mouse model). Taken together, **these data support our hypothesis that, after refrigerated storage, RBCs from donors with iron-deficient erythropoiesis without anemia are suboptimal**.

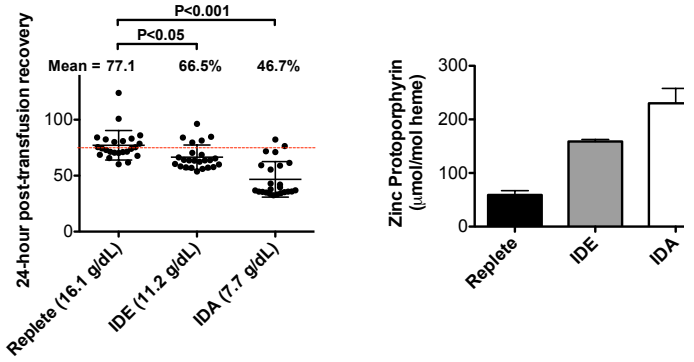

Figure 3. RBCs from iron-deficient donor mice have reduced 24-hour recovery and increased zinc protoporphyrin levels. Weanling male C57BL/6 mice were placed on a defined iron control diet of 45ppm iron (TD.110593, Harlan Laboratories) or an iron-deficient diet of 0-4ppm iron (TD.110592). A third group was fed the iron-deficient diet and was also phlebotomized weekly (50-100 µL per bleed) to generate a total of three groups of mice with varying iron status: (1) iron replete, (2) iron-deficient erythropoiesis (**IDE**) with mild iron deficiency, and (3) severe iron deficiency anemia (**IDA**). At ~10 weeks of age, blood was collected from each cohort, leukoreduced, packed, and refrigerator stored in CPDA-1. After 12 days of storage, 24-hour post-transfusion recovery was determined in recipient transgenic mice expressing enhanced Green Fluorescent Protein under the direction of the human ubiqutin C promoter using flow cytometric detection of transfused RBCs (left panel). The experiment was repeated three times in its entirety (N=24 per group total). Mean hemoglobin levels prior to blood donation are in parenthesis. The dotted red line represents 75% RBC recovery. Statistical significance determined by one-way ANOVA with Dunnett's

2. Objectives

2.1 Primary Hypothesis

1) The 24-hour post-transfusion RBC recovery of units obtained from donors exhibiting iron-deficient erythropoiesis will not meet FDA standards for clinical use.

2) The 24-hour post-transfusion RBC recovery of units obtained after intravenous iron repletion will improve significantly and will meet FDA standards for clinical use.

2.2 Secondary Hypothesis

1) The extent of iron-deficient erythropoiesis, as measured by RBC zinc protoporphyrin levels, will predict the 24-hour post-transfusion RBC recovery.

2) There will be a good correlation (R^2>0.5) between RBC zinc protoporphyrin levels and other markers of iron status: ferritin, hepcidin, soluble transferrin receptor, reticulocyte hemoglobin.

3) Iron repletion will improve fatigue score[36] and self-reported health and wellbeing score.[37]

4) Iron repletion will decrease RBC zinc protoporphyrin and soluble transferrin receptor levels.

5) Iron repletion will increase hepcidin, ferritin, and hemoglobin levels.

6) Iron repletion will improve the following neurocognitive scores:

a) Cognitive Function Composite Score
b) Executive Function
c) Attention
d) Episodic Memory
e) Language
f) Processing Speed
g) Working Memory

7) Iron repletion will improve the following emotional well-being scores:

a) quality of life
b) fatigue
c) depression
d) anxiety

8) Metabolite levels in the RBC unit will correlate with iron status and 24-hour post-transfusion RBC recovery.

3. Study Population

3.1 Enrollment Inclusion Criteria:

1) 18-75 years old;

2) healthy (by self report);

3) body weight >110 lbs;
4) female hematocrit >38%, male hematocrit >39%;
5) frequent blood donor (men ≥2 and female ≥1 RBC unit donations in past year);
6) ferritin <15 ng/mL;
7) zinc protoporphyrin >60 μmol/mol heme.

3.2 Enrollment Exclusion Criteria:

1) ineligible for donation based on the New York Blood Center donor autologous questionnaire;
2) C-reactive protein >10 mg/L;
3) sickle cell trait (by self report);
4) systolic blood pressure >180 or <90 mm Hg, diastolic blood pressure >100 or <50 mm Hg;
5) heart rate <50 or >100;
6) temperature >99.5°F prior to donation (attempts will be made to reschedule donation if possible);
7) temperature >100.4°F or subjective feeling of illness prior to ^{51}Cr 24-hour RBC recovery study (to avoid the subject having a concurrent illness that may affect post-transfusion recovery); (attempts will be made to reschedule donation and ^{51}Cr 24-hour RBC recovery study);
8) positive results on standard blood donor infectious disease testing;
9) pregnancy;
10) taking, or planning to take, iron supplements and not willing to stop for duration of study;
11) history of severe asthma requiring hospitalization, allergic eczema (atopic dermatitis), or other atopic allergy causing anaphylaxis.

4. Trial Enrollment

4.1 Screening/Recruitment

The New York Blood Center study staff will send a recruitment letter/email (see Appendix 1) to potential subjects who are 18-75 years old and frequent blood donors donating in the Manhattan catchment area. We will define frequent blood donors as (1): men who have donated the equivalent of at least 2, and women who have donated the equivalent of at least 1, RBC units in the past year.

Volunteers responding to the recruitment email will be screened for participation in the study by phone or email (see Appendix 7 for all screening forms) and then invited for a

screening meeting (P&S 14-434) to confirm eligibility and provide informed consent.

Screening day (P&S 14-434): All volunteers will be given the health questionnaire they would receive upon donation at the New York Blood Center (see Appendix 7). All volunteers who decide to proceed with screening and sign consent will have four tubes of blood drawn to determine baseline laboratory values and to perform a blood type and antibody screen. If the baseline hematocrit is >38% (female) or >39% (male), ferritin < 15ng/mL, zinc protoporphyrin level is > 60 umol/mol heme, and C-reactive protein is <10mg/L (see inclusion/exclusion criteria above) then the volunteer will be scheduled for an autologous whole blood donation at the New York Blood Center, and scheduled for an autologous 51-Chromium red cell recovery study 6 weeks after donation. At that time, neurocognitive assessments and emotional wellbeing will be performed as well (see Appendix 2-5 for surveys and section 5.4). Re-screening: Those subjects who meet the ferritin <15ng/mL and the zinc protoporphyrin > 60umol/mol heme criteria, but do not qualify due to a low hematocrit may be re-screened between 2 weeks to 3 months later.

4.2 Randomization

Randomization will be performed using a computerized system with equal allocation (1:1) to iron repletion or placebo. Randomization will be stratified by gender; randomly permuted block sizes of 4, 6, or 8 will be used. The moment of randomization will be recorded and will occur only after successful completion of the first post-transfusion RBC recovery study (i.e., between Day #43-71; target is the day after the post-transfusion recovery study; performed on Harkness Pavillion 10). Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA)). One peripheral intravenous line will be inserted and blood (2 tubes, 10mL) will be drawn for determining the 24-hour post-transfusion recovery. The subject will be randomized at this point. The randomization scheme will be created by a statistician unaffiliated with the study team and provided to the research pharmacy and DSMB prior to study initiation. The CUMC research pharmacy will be responsible for randomizing each subject to the appropriate treatment arm and will prepare either the iron treatment or saline placebo and provide it to the study team in a blinded

fashion. If a subject arrives on the day of randomization and has a fever (T>100.4F) or feels ill, then the 24-hour post-transfusion recovery sample will be drawn, but randomization may be held until the subject feels better and reschedules.

4.3 Blinding

The randomized group will only be known to the Columbia University Medical Center Research Pharmacy and the statistician who generated the randomization scheme. The study will be double-blind. As described in section 5.3, both a test dose of the iron treatment/placebo and the treatment/placebo will be blinded. The test dose will be administered prior to giving the full dose to ensure safety of treatment. A research pharmacist will provide the placebo (IV saline) or treatment (IV low molecular weight iron-dextran or ferric carboxymaltose) and test dose of iron/placebo in a tinted infusion bag and tubing specifically designed to maintain blinding in clinical research studies (Medipak). A research nurse unaffiliated with the study team, from the Outpatient or Inpatient Center at the Columbia General Clinical Research Center (Harkness Pavillion 10th Floor), will be responsible for the test infusion and total dose iron infusion. With this design, volunteers and study investigators will be blinded to whether volunteers receive the active intervention or placebo. Subjects in both groups will receive similar discharge instructions as if they had received low molecular weight iron-dextran (see Appendix 6 for discharge instructions). Only the research pharmacist and the statistician on the DSMB will not be blinded. Scheduling and logistic communications with volunteers will be made by the study coordinator, who will also be blinded to the treatment group. The research pharmacy will provide a standard 1-gram dose of low molecular weight iron-dextran, ferric carboxymaltose, or saline in special bags designed to conceal group assignment.

Situations may arise in which breaking the blind earlier would be in the best interest of the volunteer. In any situation in which a physician or the subject asks to be un-blinded to study treatment, a sealed copy of the randomization scheme for each subject will be kept in the investigator's office and will be broken upon request. Furthermore, the research pharmacy can be reached on an emergency basis to provide this information. Finally, any un-blinding that occurs will be reported to the DSMB and ultimately reported in the resulting publication.

5. Interventions

5.1 Blood Donation:

The blood donation will be performed at the New York Blood Center at 310 East 67th Street, New York, NY per standard protocol for autologous RBC donations. A urine pregnancy test will be performed on all female participants < age 55 on the day of donation. A positive pregnancy test will result in exclusion from the study (see Appendix 7 for donation day eligibility form). At the New York Blood Center, subjects will be screened per the standard protocol for autologous RBC donations. This will include health questionnaire, finger stick hemoglobin determination, temperature, heart rate and blood pressure measurement. Following donation, the blood will be leukoreduced, packed, and stored in AS-3 solution. The unit will be transferred to the CUMC-NYPH blood bank for storage following standard hospital procedures.

5.2 ^{51}Chromium 24-hour post-transfusion RBC recovery study

Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA) on Harkness Pavillion 10). A urine pregnancy test will be performed on all female participants < age 55 years on the day of infusion. A positive pregnancy test will result in exclusion from the study (see Appendix 7 for infusion eligibility form). Two intravenous lines will be placed in contralateral arms. Four tubes of blood will be drawn to determine laboratory values and to perform a repeat blood type and antibody screen. If the subject chooses to come in for an optional blood draw within 30 days of this study visit, only three tubes will be drawn as the type and screen will not be necessary.

A 30 mL aliquot of the autologous blood unit donated 6 weeks prior will be removed using sterile technique by a licensed radiopharmacist into a syringe. This syringe will be labeled with the volunteer's identifying information per hospital policy and radiolabeled in the Kreitchman PET Center. The radiolabeling will be performed using 20 microcurie of sodium chromate (^{51}Cr) based on the methods of Moroff et al[4] and the recommendations from the International Committee for Standardization in Hematology.[38] The ^{51}Cr labeled red cells will be washed with saline and then infused intravenously

(over 1 minute through one IV line) into the volunteer. For calculating counts per minute per ml of RBC at time zero (T0), samples (two tubes, 10 mL) from the contralateral arm will also be obtained after 5, 7.5, 10, 12.5, 15 minutes, 30 minutes, and 1-hour after infusion. Thus, in total, the volunteers will be infused approximately 30mL of packed radiolabeled RBCs and will have 16 tubes or 80 mL of blood drawn overall (8 time points x 2 tubes x 5mL per tube = 80 mL). All blood draws will be performed from the peripheral intravenous line to avoid multiple needle sticks. Finally, because 30mL of blood will be infused and only 80mL of blood removed, this will leave the volunteer with a net loss of only 50mL of blood on the day of infusion. Following the 1-hour timed blood draw, the subject will be discharged.

5.3 Low Molecular Weight Iron Dextran (LMWID) or Ferric Carboxymaltose (INJECTAFER) Infusion

Volunteers will be admitted to the Columbia General Clinical Research Center (GCRC) (supported by the Columbia Clinical and Translational Science Award (CTSA) on Harkness Pavillion 10). One peripheral intravenous line will be inserted and blood (2 tubes, 10mL) will be drawn for determining the 24-hour post-transfusion recovery. The allowable time frame for this blood draw will be from 20-28 hours of infusion the prior day. The subject will be randomized from this point to 4-weeks later by the research pharmacy. A research pharmacist will provide the placebo (IV saline) or treatment (IV low molecular weight iron-dextran or ferric carboxymaltose) and test dose (25 mg LMWID, 12.5 mL of a 2 mg/mL solution of iron dextran or 0.5 mL of 50 mg/mL solution of ferric carboxymaltose, diluted in normal saline) in tinted infusion bags and tubing specifically designed to maintain blinding in clinical research studies (Medipak). If low molecular weight iron dextran is unavailable due to shortages, the research pharmacist will provide ferric carboxymaltose instead. A research nurse unaffiliated with the study team, from the Outpatient or Inpatient Center at the Columbia General Clinical Research Center (Harkness Pavillion 10th Floor), will be responsible for the test infusion and total dose iron infusion. The research pharmacy will provide a standard 1-gram dose of low molecular weight iron-dextran, ferric carboxymaltose, or saline in special bags designed to conceal group assignment. In addition, the clinical pharmacy will provide Solumedrol 125

mg IV both before and after infusion and Tylenol 650mg PO and Benadryl 25mg PO before the infusion. The Solumedrol has been shown to reduce the risk of myalgias and arthralgias after LMWID infusions.[39] Premedication with Tylenol and Benadryl have also been shown to be effective in reducing adverse events,[40] and this is the current standard of care at Columbia University Irving Medical Center for adults receiving LMWID. After the intravenous test dose (25 mg of LMWID or ferric carboxymaltose, 12.5 mL of the complete dose, infused over 20 minutes), patients will be observed for any side effects for 40 minutes (one hour from start of infusion); if no adverse effects are seen, then the entire dose diluted in 500 mL normal saline (i.e., 2 mg/mL of LMWID) will be infused over a period of 2 to 6 hours as tolerated (target 2 hours). If ferric carboxymaltose is used, the same test dose and the full 1 gram dose will be administered in 250 mL of normal saline over the same time frame. The same premedication will be provided to minimize differences in treatment effects. Adverse events will be identified by observation, direct inquiry, and physical examination of each volunteer. Vital signs will be measured before, during (after 15 minutes and then hourly), and after each infusion. Resuscitation equipment and personnel trained in the detection and treatment of anaphylactic-type reactions will be readily available during drug administration.

Recent studies support the convenience, safety, and efficacy of a single infusion of 1g of LMWID as therapy for iron deficiency in adults.[41] The calculation for iron repletion using LMWID (from INFeD package insert) in adults is as follows:
Dose (mL) = 0.0442 (Desired Hb - Observed Hb) x LBW + (0.26 x LBW)

Based on:
Desired Hb = the target Hb in g/dl.
Observed Hb = the patient's current hemoglobin in g/dl.

LBW = Lean body weight in kg. A patient's lean body weight (or actual body weight if less than lean body weight) should be utilized when determining dosage.

For males: LBW = 50 kg + 2.3 kg for each inch of patient's height over 5 feet

For females: LBW = 45.5 kg + 2.3 kg for each inch of patient's height over 5 feet
To calculate a patient's weight in kg when lbs are known: patient's weight in pounds = weight in kilograms/2.2

Using this formula, the minimum dose that would be required to raise the hemoglobin by 2 g/dL would be ~800mg (minimum donation requirement is 110lbs = 50 kg) in a female and almost 900mg in a male. Thus, given that all subjects will be healthy volunteers with ferritin < 15 ng/mL with evidence of iron-deficient erythropoiesis, and will be dosed following a whole blood donation for study purposes, 1 gram of LMWID was chosen for simplicity, safety, consistency, and design considerations. Based on similar considerations, 1 gram of ferric carboxymaltose would provide equivalent total dose iron repletion with a similar safety profile.[42]

5.4 Cognitive and Emotional Status Assessment

As outlined in the *Schedule of Measurements* section below, cognitive and emotional functioning will be assessed with a thorough battery of neuropsychological tests and questionnaires assessing overall wellbeing. Tests will be administered in person by a psychometrician who has received training on test administration.

Neuropsychological tests will be administered to subjects on an iPad or Tablet. Subjects will be supervised at all times during this portion of the evaluation and will be encouraged to complete the tests within one session (i.e. without interruption). Total time for test administration is approximately 30 minutes and the time of start and completion of the test battery will be recorded. When the neuropsychological battery has been completed, the subject will be provided with a clipboard containing printed copies of four questionnaires (see appendices 4-7). The subject will be asked to complete the surveys on his/her own without interruption. The time of start and completion of the surveys will be recorded.

Neuropsychological tests included were selected based on the following criteria: All (1) meet high psychometric standards, (i.e. are reliable, valid, and well standardized); (2) have appropriate normative data; (3) maintain the lowest potential

for patient burden, e.g. duration; (4) can be administered serially without significant practice effects.

5.4.1 Cognition

Subjects will be administered subtests from the NIH Toolbox Cognition Battery (NIHTB-CB), a computerized battery of neuropsychological tests. The NIH Toolbox provides a standard set of royalty-free, comprehensive assessment tools that can be used by researchers and clinicians in a variety of settings, with a particular emphasis on measuring outcomes in longitudinal epidemiologic studies and prevention or intervention trials. The battery has been normed and validated across the lifespan in subjects age 3-85 and its use ensures that assessment methods and results can be used for comparisons across existing and future studies. By providing a "common currency" for the study of neurological research, the NIH Toolbox enables economies of scale and enhances efficiency. The NIH Toolbox is capable of monitoring neurological and behavioral function over time, and measuring key constructs across developmental stages.

Scores from the NIH Toolbox Cognition Battery will produce a Cognitive Function Composite Score. Individual measure scores reflecting Executive Function, Attention, Episodic Memory, Language, Vocabulary, Processing Speed and Working Memory will also be obtained.

NIH Toolbox Cognition Battery (NIHTB-CB)

Vocabulary

NIH Toolbox Picture Vocabulary Test

Memory

NIH Toolbox Auditory Verbal Learning Test (Rey)
NIH Toolbox Picture Sequence Memory Test

Attention/Executive Functioning

NIH Toolbox Flanker Inhibitory Control and Attention Test

NIH Toolbox Dimensional Change Card Sort Test (DCCS)

Processing Speed
NIH Toolbox Pattern Comparison Processing Speed Test

Working Memory
NIH Toolbox List Sorting Working Memory Test

5.4.2 Emotion

Subjects will complete four paper and pencil self-administered questionnaires to evaluate quality of life, fatigue, depression, and anxiety.

Short Form 36 Health Survey (SF-36)
36-item, patient-reported survey assessing overall health status and quality of life (Appendix 4).[43]

Multidimensional Assessment of Fatigue (MAS)
16 item scale used to measure fatigue according to four dimensions: degree/severity, distress that it causes, timing of fatigue, and its impact on ADLs (Appendix 5).[44]

Beck Depression Inventory-II (BDI-II)
21 item self-report multiple choice inventory used to assess for depression (Appendix 6).[45]

Beck Anxiety Inventory (BAI)
21-question multiple-choice self-report inventory that is used for measuring how the subject has been feeling in the last week, focusing primarily on somatic symptoms (Appendix 7).[46]

5.4.3 Hearing

Words-In-Noise (WIN) Test
Measures a person's ability to recognize single words amid varying levels of background noise, measuring difficulty a person might have hearing in a noisy environment. A recorded voice tells the participant to listen and repeat words. Background noise gets louder, reducing the signal-to-noise ratio.
Hearing Handicap Inventory Screening
10 questions to assess hearing impairment.

5.4.4 Restless Leg Syndrome (RLS)

Restless Leg Syndrome Rating Scale
10 questions to assess restless leg symptoms.

6. Schedule of Measurements

6.1 Summary Timeline

The schedule of measurements is summarized in **Table 1**. Following successful screening and signed informed consent, subjects will donate a unit of autologous red blood cells (Donation1). This will be followed 40-42 days later by the baseline 51-Chromium post-transfusion recovery study. Following the 24-hour post-chromium infusion blood draw, the subjects will be randomized to iron infusion or placebo (Iron infusion1). Five months ± 4 weeks later, subjects will donate the second unit of autologous blood (Donation2). The second 51-Chromium post-transfusion recovery study will be performed 40-42 days after this donation. Subjects will have to return 24-hours after chromium infusion to determine the 24-hour post-transfusion recovery. Surveys and neurocognitive assessments will be conducted prior to Donation1, PTR1, Donation2, and PTR2.

Because an active type and screen must be obtained before the blood bank issues the stored RBC unit and the radioactive 51-Cr-labeling can occur, we will provide subjects with the option of coming in up to 30 days prior to the scheduled PTR study to obtain a type and screen sample (1 tube of blood by standard venipuncture). This will provide for a shorter day for the PTR study because there will no longer be the need to wait for the type and screen testing and the study can begin once the subject arrives. If a subject chooses not to provide this optional blood draw, a blood sample will be taken when the IV lines are placed for the PTR study and the PTR study will proceed after the blood bank issues the blood and it is radiolabeled per hospital guidelines.

Table 1: Schedule of Measurements

Day of study	Urine pregnancy test	CBC with reticulocyte count	Iron parameters*	C-reactive protein	Type and Screen	51-Cr PTR**	Iron/Placebo infusion	Surveys/ Neuro-cognitive	Metabolomics****
Screening		X	X	X	X				
Donation1	X	X	X	X				X	X
Blood draw (optional)***					X				
PTR1 (40-42 days after Donation1)	X	X	X	X	X	X		X	
Iron infusion1 (1 day after PTR1)	X						X		
Donation2 (5months ± 4 weeks after iron infusion1)	X	X	X	X				X	X
Blood Draw (optional)***					X				
PTR2 (40-42 days after Donation2)	X	X	X	X	X	X		X	
Final blood draw (1 day after PTR2)						X – collection of 24 hour (+/- 4hr) time point for final measurement			

*Iron parameters = Zinc Protoporphyrin (ZPP), iron, total iron binding capacity (TIBC), transferrin saturation, ferritin, soluble transferrin receptor, hepcidin.

**PTR = post-transfusion recovery study with associated blood draws for hemoglobin and Chromium-51 determination taken pre-infusion and 5, 7.5, 10, 12.5, 15, 30, 60 minutes and 24-hours post-infusion (+/- 4 hours).

***The option to come in up to 30 days prior to the PTR study will be provided to obtain a type and screen sample. This will provide an active type and screen specimen so that the PTR study can begin without delay. Alternatively, the type and screen blood sample will be drawn when the IV is paced for the PTR study and then the study will begin once the type and screen is tested and the blood is issued and labeled.

****Metabolomics will be performed by mass spectrometry on blood obtained from the unit at the time of radiolabeling by metabolomics facilities at BloodWorks NW and University of Colorado.

6.2 Assessment Procedures

6.2.1 Zinc Protoporphyrin, Serum Iron, Total Iron Binding Capacity, Transferrin Saturation, Ferritin, C-Reactive Protein, Soluble Transferrin Receptor

Samples for zinc protoporphyrin and soluble transferrin receptor will be sent to ARUP Laboratories for testing. The other parameters will be measured in the Columbia University Medical Center Automated Core Laboratory using clinically-validated instruments.

6.2.2 Hepcidin

This will be measured using an ELISA kit (Bachem) following the manufacturer's instructions.

6.2.3 Complete Blood Counts

Blood counts will be performed in the Columbia University Medical Center Automated Core Laboratory using clinically-validated instruments.

6.2.4 Urine Pregnancy Test

Point of care urine pregnancy testing will be performed using QuickVue One-Step hCG Urine tests (Quidel Corp) following the manufacturer's instructions.

6.2.5 Type and Screen Testing

All type and screen testing will be performed by licensed medical technologists in the Columbia University Medical Center – New York Presbyterian Hospital blood bank following standard procedures.

6.2.6 Metabolomics Testing

Comprehensive metabolomics testing will be performed for research use only at the metabolomics core facilities at the University of Denver and Bloodworks NW using coded specimens. CUIMC investigators will not share identifying information with the collaborating investigators.

7. Specimen Collection Procedures

All blood samples will be obtained by peripheral venipuncture or from an indwelling peripheral intravenous line following institutional protocols.

8. Compensation

Subjects will be provided monetary compensation in cash for participation per visit. $20 will be provided for the screening. $10 will be provided for re-screening for those who meet criteria to be invited to be screened again. $80 will be provided for each donation and PTR study ($320 total). $80 will be provided for the iron/placebo infusion and for the final blood draw at the end of the study ($160 total). Finally, $40 will be provided for each of the optional blood draws. Thus, the maximum total compensation for subjects is $590 and the minimum compensation is $500 for completing the study. Subjects who dropout of the study prior to completion will keep any compensation received up to the dropout.

9. Adverse Event Criteria and Reporting

9.1 Definitions

Adverse Event (AE) – Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Grades were developed using the following guidelines:
Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

Serious Adverse Event (SAE) – Any adverse event temporally associated with the subject's participation in

research that meets any of the following criteria: results in death; is life-threatening (places the subject at immediate risk of death from the event as it occurs); requires inpatient hospitalization or prolongation of existing hospitalization; results in a persistent or significant disability/incapacity; results in a congenital anomaly/birth defect; or any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Note that seriousness and severity are separate concepts. The term "severe" refers to the intensity of a specific event; a severe event may be of minor medical significance (e.g., a severe leg cramp). The term "serious" is based on outcome or action criteria that are usually associated with events that pose a threat to the patient's life or functioning. An event that is mild in severity is serious if it leads to one of the outcomes defined above.

Grade 4 and 5 events will always be considered Serious Adverse Events. Many Grade 3 events and some Grade 1 and 2 events may meet the definition of a Serious Adverse Event.

Unexpected Adverse Event – Any adverse event occurring in one or more subjects in a research protocol, the nature, severity, or frequency of which is not consistent with either: the known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in (a) the protocol–related documents, such as the IRB-approved research protocol or the current IRB-approved informed consent document, and (b) other relevant sources of information, such as product labeling and package inserts; or the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others (UP): Any incident, experience, or outcome that meets all of the following criteria: unexpected (in terms of nature,

severity, or frequency) given (a) the research procedures that are described in the protocol-related

Attribution – the determination of whether an adverse event is related to a medical treatment procedure.
Attribution categories:

1. Not Related - Event clearly related to other factors (e.g., clinical state, other therapies; concomitant drugs)
2. Possibly Related - Sequence of event is compatible with study drug, device, or procedure, but could have been produced by other factors
3. Probably Related - Sequence of event is compatible with study drug, device, or procedure and cannot be explained by other factors without much doubt
4. Definitely Related - Sequence of event is compatible with study drug, device, or procedure and beyond doubt cannot be explained by other factors

9.2 Types of Adverse Events Reported

Strict definitions and monitoring protocols of adverse effects (AEs) and serious AEs (SAEs) will be developed with the Data Safety Monitoring Board (DSMB). An SAE defined as being one or more of the following: (i) life threatening, (ii) results in hospitalization, or (iii) causes irreversible, persistent, or significant disability/incapacity; these will be reported to regulatory agencies within 7 days of their occurrence. Any AE or SAE temporally related with study procedures will be reported by the site investigators or coordinators. An alert will be sent to the DSMB, and the Institutional Review Board and Sponsor (NIH-NHLBI). Safety outcomes will be assessed at each study visit and with a follow-up phone call the day after each visit using a checklist of known adverse events and an open-ended question for volunteers to describe other adverse events. Participants will be encouraged to call the coordinator throughout the study if they experience 1) a new symptom, 2) change in severity of an existing symptom, 3) see a doctor/ER, 4) start a new medication. The most common serious acute adverse reaction of blood donation and insertions of intravenous lines is a vasovagal reaction, which may lead to loss of consciousness. Witnessed or un-witnessed vasovagal reactions will be considered as AEs, unless resulting in hospitalization (e.g., due to loss of consciousness causing

head trauma), in which case the event will be considered an SAE. Permanent or persistent peripheral arm nerve damage (in a phlebotomized arm) causing disability will be considered an SAE. Bruising and pain at the site of needle insertion that results in volunteer complaint at the follow-up phone call a day after phlebotomy will be considered an AE. Anaphylactoid reactions to iron infusions are usually evident within a few minutes, and close observation is necessary to ensure recognition. If at any time during the intravenous administration, any signs of a hypersensitivity reaction or intolerance are detected, administration will be stopped immediately. The reaction will be considered an SAE if it meets one of the criteria listed above. Furthermore, subjects will be called one day and one week after infusion to inquire about delayed side effects, such as myalgias, arthralgias, and gastrointestinal problems. Finally, iron deficiency is associated with fatigue, restless leg syndrome, decreased physical endurance and work capacity, and impaired concentration, attention, and other cognitive functions. Thus, subjects randomized to the placebo group may have AEs due to continued iron deficiency. These will be assessed using the validated Multidimensional Assessment of Fatigue, Beck Depression Inventory-II, Beck Anxiety Inventory, and the overall well-being SF-36 Health Survey incorporating both physical and mental summary measures. Each subject will take these short surveys as outlined in Table 1.

Medical follow-up of any Serious Adverse Event will be provided by the medical staff of Columbia University Medical Center in the New York Presbyterian Hospital or, if needed, in the out-patient clinic at appropriate intervals until resolution of the condition related to the Serious Adverse Event. An Adverse Event that does not meet the criteria of a Serious Adverse Event will be reported promptly to Drs. Spitalnik and Hod, appropriate management will be provided by the medical staff at Columbia University Medical Center, and the occurrence of any adverse events reviewed periodically by Dr. Spitalnik, Dr. Hod, and their Co-Investigators. A Data Safety Monitoring Board will be established and will review all reported events at least annually and all serious adverse events within one month of occurrence. A report summarizing the Data Safety Monitoring Board meeting and

recommendations will be submitted to the Institutional Review Board.

9.3 Reporting timelines

Table 2 below details the SAE and UP safety reporting requirements and timelines. Reporting timelines for all non-serious AEs will follow the Data and Safety Monitoring Plan for the study.

Table 2: SAE and UP event reporting timelines

What Event is Reported	When is Event Reported	By Whom is Event Reported	To Whom is Event Reported
Fatal or life-threatening unexpected, suspected serious adverse reactions	Within **7 calendar days** of initial receipt of information	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	NHLBI
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Within **15 calendar days** of initial receipt of information	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	NHLBI
Unanticipated adverse effects	Within **10** working days of investigator first learning of effect	Investigator	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
		Sponsor	Local/internal IRBs and Data Safety Monitoring Board (DSMB)
Unanticipated Problem that is not an SAE	Within **14 days** of the investigator becoming aware of the problem	Investigator	Local/internal IRBs/Institutional Officials, NHLBI and/or DCC
All Unanticipated Problems[1]	Within **30 days** of the IRB's receipt of the report of the UP from the investigator.	IRB	OHRP
		Investigator	External IRBs

[1.] Per OHRP guidance: only when a particular AE or series of AEs is determined to meet the criteria for an UP should a report of the AE(s) be submitted to the IRB at each institution under the HHS regulations at 45 CFR part 46. Typically, such reports to the IRBs are submitted by investigators.

10. Interim Reporting

This section describes scheduled reports that will be sent to the DSMB. Reporting requirements for events that will be monitored continuously (i.e. all fatal events and all serious adverse events possibly, probably, or definitely related to red cell transfusion) are described above.

10.1 Semi-Annual DSMB Reports

The DSMB will meet twice a year, either in-person or via teleconference. Reports will include:

- Baseline characteristics overall and by group
- Primary study endpoint overall and by group, (p-values to compare to early stopping boundary will only be provided after 20, 40, and 60 volunteers have been completed)
- Number, type, and severity of serious adverse events, overall and by treatment arm, with p-value for comparison of number of serious adverse events per group. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Number, type, and severity of adverse events attributed as possibly, probably, definitely related to donation, iron or red cell infusion, overall and by treatment arm, with p-value for comparison of number of such adverse events per group. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Proportion of subjects in each treatment arm with at least one serious adverse event possibly, probably, or definitely related to a donation, red cell or iron infusion, with p-value. This p-value will be compared to the p-value boundary from an alpha-spending approach approximating O'Brien-Fleming boundaries. No formal stopping rule is set for this comparison.
- Unexpected adverse events, and unanticipated problems overall and by treatment arm
- Site status

- Accrual
- Site/study compliance issues
- Data entry completeness and data QA

11. Statistical Considerations

11.1 Analysis Plan

The primary null hypothesis will be tested in an intent-to-treat analysis using a t-test, or nonparametric equivalent, of the between-group difference in means of the within-subject change in the post-transfusion RBC recovery from the initial study (under iron-deficient erythropoiesis conditions) and the subsequent study performed after randomization to iron repletion or placebo. A similar approach will be used to test the secondary objectives (i.e., the between-group difference in means of the within-subject change in fatigue score,[36] self-reported health and wellbeing score,[37] zinc protoporphyrin, hepcidin, soluble transferrin receptor, serum ferritin and hemoglobin concentration).

Furthermore, because we expect variable responses to iron repletion in the experimental group, and some crossover in the placebo group, we will also perform a secondary analysis to explore the effect of iron status on post-transfusion RBC recovery. We will use multiple regression to assess whether RBC zinc protoporphyrin level increases the R^2 of a model of post-transfusion recovery predicted by treatment group membership. We will examine pre-specified demographic variables (gender [male/female], race [white/not white], age [<50/≥50 years]) to see if we can identify one or more that may be effect modifiers. These factors will be considered for inclusion in an adjusted model. The specific criteria for inclusion are: (i) difference by treatment group significant at $\alpha = 0.10$ two-sided, and (ii) related to outcome at level $\alpha = 0.10$ two-sided. If any of the pre-specified covariates meet the criteria for inclusion, they will be incorporated in an adjusted model, and that model will become the primary analysis. Otherwise, the simple model will be primary. With 60 subjects, we will have 80% power to detect a partial correlation coefficient increase of 0.33 for the unique contribution of RBC zinc protoporphyrin level.[47]

11.2 Sample Size Estimate

Based on preliminary data from our prior ^{51}Cr RBC recovery studies (IRB protocol #AAAI-0835), the standard deviation of the measure in our single site is 5.0%. Furthermore, the expected mean difference in post-transfusion RBC recovery

between iron replete mice and mice with iron-deficient erythropoiesis is 10.6%. If the difference were this large in humans, we would require <6 subjects (alpha = 0.05, two-sided, power = 0.80). However, we expect the difference to be less conspicuous in humans than in inbred mice. Thus, we power the study to detect a clinically relevant difference in post-transfusion recovery of 4%. Under this assumption, the calculated sample size required for each arm is 26 (alpha = 0.05, two-sided, power = 0.80). Furthermore, to allow for up to a 15% dropout rate (i.e., 4/26 subjects), we plan to randomize 30 subjects per arm for a total sample size of 60 subjects. Of note, randomization will occur after successful completion of the first post-transfusion recovery study; thus, we predict up to 70 individuals will need to be recruited.

11.3 Interim Analyses

Interim analyses will be performed twice (after every 20 subjects have completed study participation) in addition to the final analysis. The DSMB will conduct the analyses using a two-sided asymmetric Lan-DeMets alpha-spending approach with an O'Brien-Fleming two-sided symmetric stopping boundary and overall alpha = 0.05. The DSMB criteria for early stopping will include: (i) the Z-score at interim analysis lies outside of the group sequential boundaries as calculated (Table 3); (ii) major safety violations; and (3) convincing evidence of futility in the context of AEs. Interim boundaries together with terminal criteria (z-scores and associated p-values) calculated using the WinLD version 2 program are provided in Table 3.

Table 3. Lan-DeMets Group Sequential Boundaries Calculations

Volunteers completed	Lower boundary	Upper boundary	Nominal upper alpha	Cumulative alpha
20	-3.7103	3.7103	0.00010	0.00021
40	-2.5114	2.5114	0.00601	0.01210
60	-1.9930	1.9930	0.02313	0.05000

12. Data Collection and Validation

Data will be collected and entered into an encrypted web-based data management system (DMS). Reports of outstanding edits, generated upon completion of data entry, will enable continuous cleaning of the data.

Confidentiality – each subject is assigned a unique number to assure confidentiality. Any publication or presentation will refer to subjects by this number and not by name. The medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files will be kept in a locked office in a cabinet.

Data Management – The principal investigators will monitor timely entry of data into the study database. Access to all source documentation maintained by the Investigator, including correspondence and source data, will be available for monitoring and audit purposes.

Data archives – at all times, appropriate backup copies of the database and related software files will be maintained and the information will be appropriately protected from illegitimate access.

13. Protection of human subjects

All aspects of this proposed research will be conducted according to the defined protocol, relevant FDA regulations, ICH-GCP Guidelines, and HIPAA for protection of human subjects under local IRB oversight.

14. Investigator Responsibility

14.1 Institutional Review Board (IRB) Approval

No patient will be enrolled in the study until the Columbia University Medical Center and New York Blood Center IRB has approved the protocol and the Informed Consent Form. Copies of all submissions to and correspondence (approvals and disapprovals) from the IRB must be maintained on file at the study site.

14.2 Informed Consent

If a subject is potentially eligible for the study and responds to the letter sent by the New York Blood Center, the subject will be contacted to be screened and obtain written informed consent. The background of the study and the potential benefits and risks will be explained. The subject or legally authorized representative must sign the consent form that has been approved by the IRB prior to enrollment. Failure to obtain signed informed consent renders the subject ineligible for the study. Copies of the signed informed consent shall be kept in the study files.

14.3 Subject Data Protection

Subjects will be identified in the electronic case report form (eCRF) by a subject identification number. All information and data sent to NHLBI, concerning subjects or their participation in this study, will be considered confidential. All data used in analysis and reports will be used without identifiable reference to the subject. At all times throughout the study, confidentiality shall be observed by all parties involved. All data shall be secured against unauthorized access. All subjects enrolled in this study will be informed and must agree to the use and disclosure of their study information by the institution and investigators to NHLBI, their agents and representatives, or other review boards.

15. Records and reports

15.1 Case Report Forms

Case Report Forms (CRFs) will be used to collect all subject data during the course of the study. The Principal Investigator or predetermined designated individual shall be responsible for completion of the CRFs. All protocol deviations shall be documented and a justification for any missed assessments shall be provided on the protocol deviation log. The Investigator will allow regulatory bodies to review the study files, subject CRFs, medical records and other study-related documents.

15.2 Source documents

Good Clinical Practice Guidelines require that investigators maintain information in the subject's medical records, laboratory reports, clinic charts, etc. that corroborate data recorded on the CRFs. In order to comply with these requirements, the following information should be maintained:

- Laboratory data before enrollment sufficient to verify protocol entry criteria
- Dated and signed notes for specific results of procedures and exams

15.3 Record Retention

NHLBI and the investigators must establish and maintain records and reports. The Investigator must maintain the signed Informed Consent Forms, CRFs, study documentation (listed above) and source documents for at least 3 years and 3 months after study completion or termination. In addition, the

Investigator must not discard or destroy any study-specific materials unless otherwise instructed by NHLBI.

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Appendices

17. Appendix 1: Recruitment letter

Dear [Mr. / Ms. LAST NAME],

I am writing to tell you about a Blood Donor Study being conducted by the New York Blood Center and Columbia University Medical Center – New York Presbyterian Hospital. The purpose of this research study is to determine whether iron deficiency from frequent blood donation affects the quality of red blood cells. You may be eligible for this study since you are a frequent blood donor.

Your participation in this study is voluntary. The decision whether or not to participate in the study is up to you. Whether or not you participate in this study will have no effect on your relationship with the New York Blood Center as a blood donor.

For this study, you will be asked to donate blood twice over approximately 6 months and may receive iron therapy. If you take part in this study, you will receive a monetary incentive based on how many visits you make as part of your participation.

If you are interested in learning more, please email the investigators at Columbia University Medical Center at CALM@columbia.edu . You may also call them at (212) 342-5648.

Thank you for your time and consideration. We hope to hear from you. If we don't hear from you we may try to contact you by email or letter again. You may disregard these contacts if you are not interested in participating.

Sincerely,

Debra Kessler RN, MS
Director, Special Donor Services

18. Appendix 2: SF-36 Health and well-being survey

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

---

2. **Compared to one year ago**, how would you rate your health in general **now**?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	1	2
14. **Accomplished less** than you would like	1	2
15. Were limited in the **kind** of work or other activities	1	2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	1	2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	1	2
18. **Accomplished less** than you would like	1	2
19. Didn't do work or other activities as **carefully** as usual	1	2

---

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- 1 - Not at all
- 2 - Slightly
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

---

21. How much **bodily** pain have you had during the **past 4 weeks**?

- 1 - None
- 2 - Very mild
- 3 - Mild
- 4 - Moderate
- 5 - Severe
- 6 - Very severe

---

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- 1 - Not at all
- 2 - A little bit
- 3 - Moderately
- 4 - Quite a bit
- 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

---

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- 1 - All of the time
- 2 - Most of the time
- 3 - Some of the time
- 4 - A little of the time
- 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5

19. Appendix 3: Multidimensional Assessment of Fatigue (MAS)

MULTITIDIMENSIONAL ASSESSMENT OF FATIGUE (MAF) SCALE

Instructions: These questions are about fatigue and the effect of fatigue on your activities.

For each of the following questions, circle the number that most closely indicates how you have been feeling during the past week.

For example, suppose you really like to sleep late in the mornings. You would probably circle the number closer to the "a great deal" end of the line. This is where I put it:

Example: To what degree do you usually like to sleep late in the mornings?

1	2	3	4	5	6	7	(8)	9	10

Not at all — A great deal

Now please complete the following items based on the past week.

---

1. To what degree have you experienced fatigue?

1	2	3	4	5	6	7	8	9	10

Not at all — A great deal

If no fatigue, stop here.

2. How severe is the fatigue which you have been experiencing?

1	2	3	4	5	6	7	8	9	10

Mild — Severe

3. To what degree has fatigue caused you distress?

1	2	3	4	5	6	7	8	9	10

No distress — A great deal of distress

MULTIDIMENSIONAL ASSESSMENT OF FATIGUE (MAF) SCALE (Continued)

Circle the number that most closely indicates to what degree fatigue has interfered with your ability to do the following activities in the past week. For activities you don't do, for reasons other than fatigue (e.g. you don't work because you are retired), check the box.

In the past week, to what degree has fatigue interfered with your ability to:

(NOTE: Check box to the left of each number if you don't do activity)

☐ **4. Do household chores**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

☐ **5. Cook**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

☐ **6. Bathe or wash**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

☐ **7. Dress**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

☐ **8. Work**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

☐ **9. Visit or socialize with friends or family**

1	2	3	4	5	6	7	8	9	10
Not at all								A great deal	

MULTIDIMENSIONAL ASSESSMENT OF FATIGUE (MAF) SCALE (Continued)

(NOTE: Check box to the left of each number if you don't do activity)

☐ **10. Engage in sexual activity**

1 2 3 4 5 6 7 8 9 10

Not at all A great deal

☐ **11. Engage in leisure and recreational activities**

1 2 3 4 5 6 7 8 9 10

Not at all A great deal

☐ **12. Shop and do errands**

1 2 3 4 5 6 7 8 9 10

Not at all A great deal

☐ **13. Walk**

1 2 3 4 5 6 7 8 9 10

Not at all A great deal

☐ **14. Exercise, other than walking**

1 2 3 4 5 6 7 8 9 10

Not at all A great deal

15. Over the past week, how often have you been fatigued?

4 Every day

3 Most, but not all days

2 Occasionally, but not most days

1 Hardly any days

16. To what degree has your fatigue changed during the past week?

4 Increased

3 Fatigue has gone up and down

2 Stayed the same

20. Appendix 4: Beck Depression Inventory-II (BDI-II)

Beck Depression Inventory II (BDI II)

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, andthen pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today.** Circle the number beside the statement you have picked. If several statements in the groupseem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness

0) I do not feel sad.
1) I feel sad much of the time.
2) I am sad all the time.
3) I am so sad or unhappy that I can't stand it.

2. Pessimism

0) I am not discouraged about my future.
1) I feel more discouraged about my future than I used to be.
2) I do not expect things to work out for me.
3) I feel my future is hopeless and will only get worse.

3. Past Failure

0) I do not feel like a failure.
1) I have failed more than I should have.
2) As I look back, I see a lot of failures.
3) I feel I am a total failure as a person.

4. Loss of Pleasure

0) I get as much pleasure as I ever did from the things I enjoy.
1) I don't enjoy things as much as I used to.
2) I get very little pleasure from the things I used to enjoy.
3) I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings

0) I don't feel particularly guilty.
1) I feel guilty over many things I have done or should have done.
2) I feel quite guilty most of the time.
3) I feel guilty all of the time.

6. Punishment Feelings

0) I don't feel I am being punished.
1) I feel I may be punished.
2) I expect to be punished.
3) I feel I am being punished.

7. Self-Dislike
0) I feel the same about myself as ever.
1) I have lost confidence in myself.
2) I am disappointed in myself.
3) I dislike myself.

8. Self-Criticalness
0) I don't criticize or blame myself more than usual.
1) I am more critical of myself than I used to be.
2) I criticize myself for all of my faults.
3) I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
0) I don't have any thoughts of killing myself.
1) I have thoughts of killing myself, but I would not carry them out.
2) I would like to kill myself.
3) I would kill myself if I had the chance.

10. Crying
0) I don't cry any more than I used to.
1) I cry more than I used to.
2) I cry over every little thing.
3) I feel like crying, but I can't.

11. Agitation
0) I am no more restless or wound up than usual.
1) I feel more restless or wound up than usual.
2) I am so restless or agitated that it's hard to stay still.
3) I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest
0) I have not lost interest in other people or activities.
1) I am less interested in other people or things than before.
2) I have lost most of my interest in other people or things.
3) It's hard to get interested in anything.

13. Indecisiveness
0) I make decisions about as well as ever.
1) I find it more difficult to make decisions than usual.
2) I have much greater difficulty in making decisions than I used to.
3) I have trouble making any decisions.

14. Worthlessness
0) I do not feel I am worthless.
1) I don't consider myself as worthwhile and useful as I used to.
2) I feel more worthless as compared to other people.
3) I feel utterly worthless.

15. Loss of Energy
0) I have as much energy as ever.
1) I have less energy than I used to have.
2) I don't have enough energy to do very much.
3) I don't have enough energy to do anything.

16. Changes in Sleeping Pattern
0) I have not experienced any change in my sleeping pattern.
la) I sleep somewhat more than usual.
lb) I sleep somewhat less than usual.
2a) I sleep a lot more than usual.
2b) I sleep a lot less than usual.
3a) I sleep most of the day.
3b) I wake up 1-2 hours early and can't get back

17. Irritability
0) I am no more irritable than usual.
1) I am more irritable than usual.
2) I am much more irritable than usual.
3) I am irritable all the time.

18. Changes in Appetite
0) I have not experienced any change in my appetite.
la) My appetite is somewhat less than usual.
lb) My appetite is somewhat greater than usual.
2a) My appetite is much less than before.
2b) My appetite is much greater than usual.
3a) I have no appetite at all.
3b) I crave food all the time.

19. Concentration Difficulty
0) I can concentrate as well as ever.
1) I can't concentrate as well as usual.
2) It's hard to keep my mind on anything for very long.
3) I find I can't concentrate on anything.

20. Tiredness or Fatigue

0) I am no more tired or fatigued than usual.
1) I get more tired or fatigued more easily than usual.
2) I am too tired or fatigued to do a lot of the things I used to do.
3) I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0) I have not noticed any recent change in my interest in sex.
1) I am less interested in sex than I used to be.
2) I am much less interested in sex now.
3) I have lost interest in sex completely.

21. Appendix 5: Beck Anxiety Inventory (BAI)

Beck Anxiety Inventory

Instructions: Below is a list of common symptoms of anxiety.

For each of the following questions, circle the number that most closely indicates how you have been bothered by that symptom during the past month, including today.

1. Numbness or tingling
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

2. Feeling hot
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

3. Wobbliness in legs
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

4. Unable to relax
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

5. Fear of worst happening
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

6. Dizzy or lightheaded
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

7. Heart pounding/racing
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

8. Unsteady
0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

Beck Anxiety Inventory (cont)

9. Terrified or afraid

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

10. Nervous

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

11. Feeling of choking

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

12. Hands trembling

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

13. Shaky or unsteady

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

14. Fear of losing control

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

15. Difficulty breathing

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

16. Fear of dying

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

17. Scared

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

Beck Anxiety Inventory (cont)

18. Indigestion

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

19. Feeling faint

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

20. Face flushed

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

21. Hot/Cold sweats

0 Not At All
1 Mildly but it didn't bother me much
2 Moderately – it wasn't pleasant at times
3 Severely – it bothered me a lot

22. Appendix 6: Discharge instructions

Discharge instructions

There are no restrictions regarding foods, beverages, or activities.

Flushing, tingling of the hands/feet, shivering, or dizziness may occur. The area around the injection site may be tender, irritated, or discolored (brown). If any of these effects persist or worsen, contact the study staff promptly.

Some people may experience a delayed reaction 1-2 days after their treatment. These side effects usually lessen within 3 to 4 days. Tell your doctor if any of these side effects persist or worsen more than 4 to 7 days after your treatment: back/joint/muscle aches, chills, moderate to high fever, headache, nausea/vomiting. You can take Tylenol (1000mg; 2 extra strength pills) every 6 hours while symptoms last.

Tell the study staff right away if you have any serious side effects, including: abdominal pain, fast/slow/irregular heartbeat, severe headache, or blurred vision.

Get medical help right away if any of these rare but very serious side effects occur: chest pain, seizures.

A very serious allergic reaction to this drug may occur. Get medical help right away if you notice any of the following symptoms of a serious allergic reaction: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.
This is not a complete list of possible side effects. If you notice other effects not listed above, contact the study staff.

You can reach the lead investigators by cell phone at:
Dr. Eldad Hod – [redacted]
Dr. Steven Spitanlik – [redacted]

23. Appendix 7: Case Report Forms

Donor Iron Deficiency Study – AAAQ8875

Phone Pre Screening Form

Name _____ Date of phone call____/____/____ (MM / DD / YY)

☐ FEMALE
☐ MALE

Age _____
Height _____

Date of Birth ____/____/____ (MM / DD / YY)

Contact Info:

Email _____ Phone_____

Address _____

Race (Check one or more):

☐ White
☐ African American
☐ Native Hawaiian/Other Pacific Islander
☐ Asian
☐ American Indian or Alaska Native
☐ Unknown
☐ Other

Ethnicity:

☐ Hispanic or Latino
☐ Not Hispanic or Latino
☐ Unknown

ELIGIBILITY CHECKLIST

INCLUSION CRITERIA (Eligible only if all are checked Yes or N/A)

1. Age 18 – 75 _____ ☐ Yes ☐ No
2. Weight >110 lbs _____ ☐ Yes ☐ No
3. Number of donation in past year? _____ (≥2♂; ≥1♀) ☐ Yes ☐ No
4. Healthy (by self report) ☐ Yes ☐ No
5. Not pregnant (or planning in next year) ☐ N/A ☐ Yes ☐ No
6. No history of asthma, allergic eczema, or atopic allergy ☐ Yes ☐ No

EXCLUSION CRITERIA (Eligible only if all are checked No)

1. Taking Iron Supplementation** ☐ Yes ☐ No
2. Sickle Cell Trait (by self report) ☐ Yes ☐ No
3. Allergies associated with anaphylaxis? ☐ Yes ☐ No

**And NOT willing to stop for the study

Eligibility Status: ☐ **Eligibile** ☐ **Not Eligible**

Investigator Signature:_____ Date:_____

Verbal consent for anwering the questions on this form and for screening was obtained:

Signature:_____ Date:_____

Donor Iron Deficiency Study – AAAQ8875
Screening Form

Subject ID: _____ Date of Visit: ____/____/____
(MM / DD / YY)

Subject initials | | | |

Eligibility Checklist

ELIGIBLE ONLY IF ALL ARE CHECKED YES (Y)

1. Hematocrit > 38% ♀; Hematocrit > 39% ♂	☐ Yes	☐ No
2. Ferritin <15.0 ng/mL	☐ Yes	☐ No
3. Zinc Protoporphyrin > 60 uMol/mol heme	☐ Yes	☐ No
4. C-reactive protein < 10 mg/L	☐ Yes	☐ No

Eligibility Status: ☐ **Eligibile** ☐ **Not Eligible**

Investigator Signature:_____ Date:_____

The following aspects of the study were specifically discussed and the subject was given the opportunity to ask questions and provided voluntary informed consent:

1) Timeline of the visits
2) Risks of needle sticks, blood donation, and IV placement
3) Iron Dextran/Placebo intervention with risk of anaphylaxis and joint pain
4) Radioactivity exposure
5) Post-transfusion recovery study and risks of infusing blood
6) Loss of confidentiality including pregnancy tests and infectious disease testing at donation

Signature:_____ Date:_____

Donor Iron Deficiency Study – AAAQ8875
Donation Eligibility Form

Subject ID: _____ Date of Donation____/____/____
(MM / DD / YY)

Donation ☐ #1 ☐ #2

Eligibility Checklist

ELIGIBLE ONLY IF ALL ARE CHECKED Yes (Y)

1. Eligible for donation based on NYBC donation criteria ☐ Yes ☐ No
2. Negative urine pregnancy test ☐ Yes ☐ No ☐ N/A

Eligibility Status: ☐ **Eligible** ☐ **Not Eligible**

Investigator Signature:_____ Date:_____

Donor Iron Deficiency Study – AAAQ8875

51-Cr RBC Post Transfusion Recovery Eligibility Form

Subject ID: _____ Date of Study: ____/____/____
(MM / DD / YY)

Post Transfusion Recovery Study ☐ #1 ☐ #2

Eligibility Checklist

ELIGIBLE ONLY IF ALL ARE CHECKED NO (N)

1. SBP >180 or <90mm Hg, DBP >100 or <50mm Hg	☐ Yes	☐ No	
2. Heart rate <50 or >100	☐ Yes	☐ No	
3. Temperature >100.4 F or feeling ill before transfusion	☐ Yes	☐ No	
4. Positive results on blood donor infectious disease testing	☐ Yes	☐ No	
5. Positive urine pregnancy test	☐ Yes	☐ No	☐ N/A

Eligibility Status: ☐ **Eligible** ☐ **Not Eligible**

Investigator Signature:_____ **Date:**_____

Donor Iron Deficiency Study – AAAQ8875

Iron Dextran/Saline Infusion Eligibility Form

Subject ID: _____

Date of Infusion: ____/____/____
(MM / DD / YY)

Eligibility Checklist

ELIGIBLE ONLY IF ALL ARE CHECKED NO (N)

1. SBP >180 or <90mm Hg, DBP >100 or <50mm Hg	☐ Yes	☐ No	
2. Heart rate <50 or >100	☐ Yes	☐ No	
3. Temperature >100.4 F or feeling ill before infusion	☐ Yes	☐ No	
4. Positive urine pregnancy test	☐ Yes	☐ No	☐ N/A

Eligibility Status: ☐ **Eligible** ☐ **Not Eligible**

Investigator Signature:_____ **Date:**_____

Donor Iron Deficiency Study – AAAQ8875

Randomization

Subject ID: _____

Name:_____

MRN:_____

DOB:_____

Date: _____

Time: _____

MALE FEMALE

Randomization # _____

Signature: _____

Donor Iron Deficiency Study – AAAQ8875

Lab results post Randomization

Subject ID: _____ Date of Blood Draw: ____/____/_____
(MM / DD / YY)

Visit: ☐ Donation2 ☐ PTR2

Post randomization lab results recorded? ☐ Yes ☐ No

The following have been recorded and verified:

- ☐ CBC w/reticulocytes
- ☐ Zinc Protoporphyrin
- ☐ Fe Serum
- ☐ UIBC / TIBC
- ☐ Transferrin Saturation
- ☐ Ferritin
- ☐ Soluble Transferrin Receptor
- ☐ CRP
- ☐ Samples stored for Hepcidin testing

Aliquots stored:_____
Labeled:_____

Notes:_____
_____
_____
_____
_____
_____
_____

Signature _____ Date _____

Confirmatory Co-Signature _____ Date _____

Donor Iron Deficiency Study – AAAQ8875

Timed Blood Draws

Subject ID: _____ **Date:** _____

Timed Blood Draws ☐ **PTR Study #1** ☐ **PTR Study #2**

Timed Draw	Time
Pre-infusion of Cr-51	
5-min post	
7.5-min post	
10-min post	
12.5-min post	
15-min post	
30 min post	
1 hour	
24 hour post	

Completed by _____

Donor Iron Deficiency Study – AAAQ8875

PTR Vital Signs

Subject ID: _____ **Date: _____**

☐ **PTR Study #1** ☐ **PTR Study #2**

	Time	Temp	HR	RR	BP
Pre-infusion					
End of infusion					

Weight:_____ **Height:_____**

RN Signature:_____

Donor Iron Deficiency Study – AAAQ8875

Fe-Dextran Infusion Vital Signs

Subject ID: _____ **Date: _____**

Test Dose over 20 minutes

	Time	Temp	HR	RR	BP
Pre-infusion					
10-min into infusion					
20-min (at end of infusion)					
40-min post infusion					

Is volunteer stable to proceed? Yes No _____

Investigator Signature

Therapeutic Dose

	Time	Temp	HR	RR	BP
15 m into infusion					
1 hr into infusion					
2 hr into infusion					
3 hr into infusion					
4 hr into infusion					
5 hr into infusion					
6 hr into infusion					

RN Signature:_____

Time therapeutic dose infusion start:_____

Time therapeutic dose infusion end:_____

Donor Iron Deficiency Study – AAAQ88875

Note To File

Subject ID: _____

RE:

Written by: _____

Date: __ __ / __ __ / __ __ __ __

Explanation of Event/Issue:

Description of the issue/process/problem being documented, including (if applicable) how, when, and by whom the issue was identified, cause of issue (if known), corrective and preventive actions taken (when and by whom).

Description of related forms/documents *(if applicable)*:

_____ _____

Author's Signature Date

_____ _____

PI's Signature Date

Note to File Guidance:
Notes to the Study File are written to acknowledge a discrepancy or problem with the study's conduct, or for other administrative purposes (such as to document where study materials are stored). Notes to the Study File should be written by the individual responsible for its content, and the author should sign and date the note. If the Note to Study File pertains to an item for which the PI is responsible (subject protection, data integrity, etc.), the PI should co-sign and date the note to acknowledge his/her awareness of the issue. Notes to the Study File should be kept on file in the study records and made available to study monitors or auditors reviewing the site's documents and procedures.

Donor Iron Deficiency Study – AAAQ8875

Subject Off Study Form

Subject Initials ☐☐☐ **Subject ID** ☐☐☐☐☐ **Date:** ☐☐ / ☐☐ / ☐☐
Month Day Year

Date subject went Off Study: ☐☐ / ☐☐ / ☐☐
Month Day Year

Last Visit Completed:

☐ Visit #
☐ Visit #
☐ Visit #

☐ Visit #
☐ Visit #
☐ Visit #

☐ Visit #
☐ Visit #
☐ Visit #

INDICATE OFF STUDY REASON: (select only one)

☐ Study Activities Completed

If the subject was withdrawn prior to completing the study (i.e. early withdrawal), select one of the following:

☐ Subject withdrawn – by Subject PRIOR to randomization**
☐ Subject withdrawn – by Subject AFTER randomization**
☐ Subject withdrawn – by PI PRIOR to randomization**
☐ Subject withdrawn – by PI AFTER randomization**
☐ Death
☐ Other**

If the subject was withdrawn, indicate specific reason(s):

☐ Subject lost to follow-up
☐ Subject refused follow-up
☐ Due to adverse events or complications
☐ Other**

**Additional explanation required:

_____
_____
_____

FORM COMPLETED BY: _____

Donor Iron Deficiency Study – AAAQ8875

Telephone Contact Form

Subject Initials ☐☐☐ **Subject ID** _____

	Date of Contact Attempt			Time	Contact Occurred	Outcome
	Month (MO)	Day (DD)	Year (YYYY)			
Contact Attempt #1				☐ AM ☐ PM	☐ Yes ☐ No	☐ No answer ☐ Left Voice message ☐ Left Message w/_____ ☐ Line Busy ☐ Other: _____
Contact Attempt #2				☐ AM ☐ PM	☐ Yes ☐ No	☐ No answer ☐ Left Voice message ☐ Left Message w/_____ ☐ Line Busy ☐ Other: _____
Contact Attempt #3				☐ AM ☐ PM	☐ Yes ☐ No	☐ No answer ☐ Left Voice message ☐ Left Message w/_____ ☐ Line Busy ☐ Other: _____
Contact Attempt #4				☐ AM ☐ PM	☐ Yes ☐ No	☐ No answer ☐ Left Voice message ☐ Left Message w/_____ ☐ Line Busy ☐ Other: _____
Contact Attempt #5				☐ AM ☐ PM	☐ Yes ☐ No	☐ No answer ☐ Left Voice message ☐ Left Message w/_____ ☐ Line Busy ☐ Other: _____

Date telephone contact completed: ☐☐ / ☐☐ / ☐☐

Month Day Year

Telephone Contact *(continued)*

QUESTION(S) TO BE ASKED	
Since your last study contact, have you had any changes in health status, medical conditions, or adverse events?	☐ Yes ☐ No
Concomitant Medications Log completed?	☐ Yes ☐ No
Adverse Event Symptoms reviewed with Subject?	☐ Yes ☐ No
Adverse Event Tracking Log Completed (same log form for all visits)?	☐ Yes ☐ No
If any AE has 'Yes' in Serious column, complete SAE form	☐ Yes ☐ No
Does the medical history form need to be updated?	☐ Yes ☐ No

COMMENTS:

_____

_____

_____

_____

_____

_____

_____

_____

TELEPHONE CONTACT CONDUCTED BY: _____

FORM COMPLETED BY: _____ **DATE:** _____

Donor Iron Deficiency Study – AAAQ8875

Unblinding Form

Date of Unblinding ____/___/___ (MM/DD/YY) Time of Unblinding _____AM/PM

Subject ID # _____ Date of Birth ____/___/___ (MM/DD/YY)

Name of person who has performed unblinding;

_____ _____

Print Name Signature

Has the IRB been notified? ☐ Yes ☐ No

Has the PI been notified? ☐ Yes ☐ No

Reason for unblinding:

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Adverse Event Tracking Log

Subject Initials | | | | **Subject ID#** | | | | | |

#	Date Reported	Adverse Event Description	Adverse Event Category** (**reference corresponding AE Category in the DSMP)	Start Date	End Date	Ongoing (Y or N)	Outcome[1]	Severity/ Grade[2]	Serious (Y or N)	Expected (Y or N)	AE Treatment[3]	Action Taken[4]	Attribution[5]	PI Initials	Date of PI Initials

- AE number. "1" indicates the first adverse event documented on the form, 2 = the second, etc. If the adverse event changes in severity, enter it as a separate adverse event row on the paper form using the same AE number as the one that ended.

Outcome[1]
0 – Fatal
1 – Not recovered/not resolved
2 – Recovered w/sequelae
3 – Recovered w/o sequelae
4 – Recovering/Resolving

Severity/Grade[2]
1 – Mild
2 – Moderate
3 – Severe
4 – Life Threatening
5 – Death (Fatal)

AE Treatment[3]
0 – None
1 – Medication(s)
2 – Non-medication TX

Action Taken[4] with Study Intervention
0 – None
1 – Interrupted
2 – Discontinued
3 – Dose reduced
4 – Dose increased
5 – Not Applicable

Attribution/ Relatedness[5]
0 – Definite
1 – Probable
2 – Possible
3 – Unlikely
4 – Unrelated

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Concomitant Medication Log

Subject Initials | | | | **Subject ID** | | | | | |

Are you currently taking any medications (prescription, over the counter, vitamins, minerals, supplements), or non-drug therapy?

Date	Medication/ Non-drug Therapy	Indication	Dose (per admin)	Dose Units[1]	Schedule/ Frequency[2]	Dose Form[3]	Route of Administration[4]	Start Date	End Date	Continuing at end of study (Y/N)

Dose Units[1]

1 - g (gram)
2 - mg (milligram)
3 - µg (microgram)
4 - L (liter)
5 - mL (milliliter)
6 - IU (International Unit)
7 - Other

Schedule (frequency)[2]

1 - QD (once a day)
2 - BID (twice a day)
3 - TID (three times a day)
4 - QID (four times a day)
5 - QOD (every other day)
6 - QM (every month)
7 - QOM (every other mo)
8 - QH (every hour)
9 - AC (before meals)
10 - PC (after meals)
11 - PRN (as needed)
12 - Other

Dose Form[3]

1 - Tablet
2 - Capsule
3 - Ointment
4 - Suppository
5 - Aerosol
6 - Spray
7 - Suspension
8 - Patch
9 - Gas
10 - Gel
11 - Cream
12 - Powder
13 - Implant
14 - Chewable
15 - Liquid
99 - Other

Route of Administration[4]

1 - Oral
2 - Topical
3 - Subcutaneous
4 - Intradermal
5 - Transdermal
6 - Intraocular
7 - Intramuscular
8 - Inhalation
9 - Intravenous
10 - Intraperitoneal
11 - Nasal
12 - Vaginal
13 - Rectal
14 - Other

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Serious Adverse Events Log

Subject ID# | | | | | |

Principal Investigator_____

SAE Number	SAE Description	AE Category *reference corresponding AE Category in the DSMP	SAE Classification	Event Start Date	Event End Date	Date Site Became Aware of Event (Reported Date)	Grade	Unexpected (Y or N)	Attribution	Outcome

SAE Classification	Grade	Attribution	Outcome
1 - Fatal (resulted in death)	1 - Mild	0 – Definite	0 – Fatal
2 - A life-threatening occurrence	2 - Moderate	1 – Probable	1 – Not recovered/not resolved
3 - Requires inpatient hospitalization or prolongation of existing hospitalization	3 - Severe	2 – Possible	2 – Recovered w/sequelae
4 - Results in persistent or significant disability/incapacity	4 - Life Threatening	3 – Unrelated	3 – Recovered w/o sequelae
5 - Results in congenital anomaly/birth defect	5 – Death (Fatal)		4 – Recovering/Resolving
6 - A significant medical incident that, based upon appropriate medical judgment, may jeopardize the subject and require medical or surgical intervention to prevent one of the outcomes listed above.			
7 - Loss of confidentiality that results in criminal or civil liability for participation or damage to financial standing, employability, insurability or reputation of the participant			

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Subject Deviation Tracking Log

Subject Initials ☐☐☐ **Subject ID#** ☐☐☐☐☐

#	Deviation Start Date	Deviation End Date	Deviation Description	Deviation Category (see below)	Does the Deviation: Impact Subject Safety*	Does the Deviation: Affect Data Integrity*	Does the Deviation: Affect Subject's Willingness to participate?*	Reported to IRB	PI Initials	Date of PI Initials
					☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	__ / __ / ____ ☐ Not Applicable		
					☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	__ / __ / ____ ☐ Not Applicable		
					☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	__ / __ / ____ ☐ Not Applicable		
					☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	__ / __ / ____ ☐ Not Applicable		
					☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	☐ Yes ☐ No ☐ Not Applicable	__ / __ / ____ ☐ Not Applicable		

If one or more is answered yes for any deviation, it must be reported to the IRB promptly (14 business days from notification of or becoming aware of the event).

Deviation Category:

1. Consent Deviation
2. Enrollment Deviation
3. Other (describe)