SUPPLEMENTAL MATERIALS

Thadhani et al. Removal of Soluble Fms-Like Tyrosine Kinase-1 by Dextran Sulfate Apheresis in Preeclampsia

Supplemental Table 1. Individual baseline characteristics of women undergoing apheresis treatment(s) Abbreviations: BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure

	Maternal Age (years)	Race	Smoking Status	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)
One Treatment						
A	27	Caucasian	No	34	152	104
B	24	Caucasian	No	34	144	95
C	33	Caucasian	No	34	171	112
D	28	Caucasian	No	37	170	100
E	38	Caucasian	No	27	170	100
F	36	Caucasian	No	26	150	90
Two Treatments						
G	31	Caucasian	No	29	169	119
H	26	Caucasian	No	23	159	96
I	23	Caucasian	No	28	179	105
J	26	Caucasian	No	34	155	95
Three Treatments						
K	20	Caucasian	No	36	150	95

Apheresis Protocol

The apheresis protocol (**Supplemental Table 2** below) included several measures to minimize adverse events, including withholding antihypertensive medications the night before and morning of apheresis treatments, administering saline boluses prior to start of the apheresis treatments, and reducing blood flow rates through the extracorporeal device during the treatments. We balanced the potential for adverse effects (namely transient hypotension) with the goal of optimizing sFlt-1 removal. Additionally, dialysis nurses at each site underwent extensive supervised training with the plasma-specific dextran sulfate device before apheresis treatments were carried out.

Supplemental Table 2. Protocol for extracorporeal apheresis in preterm preeclampsia using the LA-15 system

PRIOR TO START	**Preparation of Device**	1. LA-15 cartridge is washed with 3-4 L normal saline (NS) prior to starting. 2. LA-15 cartridge is primed with ≤ 2000 units of heparin. The syringe pump above the LA-15 device is set at 500 units per hour maximum. The syringe infusion is stopped at 90 minutes, so that no heparin is given during the last 30 minutes of apheresis. TMP (transmembrane pressure) is followed during entire treatment. Observe for clots in the plasma separator. 3. Fill tubing with NS to avoid patient bleeding and hypotension before start.
	Preparation for Mother	1. All anticoagulation is held (e.g. low molecular weight heparin) the night before and morning of treatment. 2. Hold blood pressure (BP) medications the morning of treatment. 3. All treatments occur in the AM in the obstetrics unit near the surgical suite (OR-team in stand-by). 4. Void of urine and/or insert bladder catheter prior to start 5. Place cardiotocography (CTG)-probes and evaluate CTG before proceeding. 6. IV needle placement – 1 steel needle 16G or 18G for “artery” side in the antecubital fossa, one steel 16G or 18G “venous” side return on the opposite arm (placing both on the same arm leads to re-circulation). In case of access problems venous return may be achieved by inserting 18G venous (plastic) catheters. 7. Wrap venous side in a warm blanket. 8. Prepare NS bags (1-2L) on the venous return side for volume resuscitation, <u>administer 250 ml– 1L of NS prior to start depending on BP.</u> 9. Check BP using wrist or leg, calibrate with arm BP prior to start.
	Laboratories (in addition to routine lab parameters)	1. Check hematocrit, platelet count, PT and PTT 2. Check urine P/C ratio and plasma sFlt-1 levels

DURING TREATMENT	**Monitoring and Management**	1. Monitor maternal blood pressure every 15 minutes 2. Monitor fetus with cardiotocography (CTG's) before, during, and after treatment, and continuously with fetal heart rate monitor. 3. In the first 20-25 minutes, arterial flow can be increased (accord. to patient's tolerance) to 50-60 ml/min. At ~25 minutes blood contacts the LA-15 cartridge and begins to return to patient (after 150 ml of plasma fills the cartridge), which is when to expect the first slight drop in BP. Therefore, decrease flow after <u>20-25 minutes to 30-40 ml/min for 7-10 minutes</u>. 4. The plasma extraction ratio should be ~ 20-23% during the first 20-25 minutes, then reduced to 15-17% when the flows are reduced at minute 25. The more plasma that is extracted at low flows, the higher the chance of clot in the plasma separator. 5. A few minutes after the first drop in BP (minute 25-30), flows can be increased back up to 50-60 ml/min with plasma ratio at 20-23% so long as BP remains stable. 6. Exactly when the LA-15 cartridges are switched (after 500 ml of plasma is cleared, about 60 min into treatment), the BP can again be expected to drop for a few minutes. Reduce flow to 30-40 ml and plasma ratio of ~15-17% at the switch period for 7-10 min. The next BP drop occurs when plasma from the second cartridge returns to patient (at minutes 80-90). 7. Within 30 minutes into the procedure and at the time of the plasma cartridge switch (500 ml plasma cleared), consideration should be made to administer additional NS (250 ml-1L) to avoid relative hypotension. 8. Target 1-1.5 L of plasma over 2 hours. 9. If the system is interrupted for any reason (e.g. IV access problems), flushed lines with NS as the system must be re-circulated to keep continuous flows through the membrane.
END OF TREAT-MENT	**Device**	1. Turn heparin syringe off 30 minutes before the end of the treatment. 2. Return blood to patient depending on the clinical situation. <u>Do not let NS flush or flow through return back to the patient.</u>
	Maternal/Fetal Evaluation	1. Check CTG's at the end of treatment, ultrasound evaluation can be used additionally. 2. Check sFlt-1 levels at ~4 hours post treatment (this should represent steady state levels after the procedure) and repeat daily. 3. sFlt-1 cannot be measured during the procedure due to interference with heparin. 4. Hold subcutaneous heparin the day after the treatment. Also, if sq heparin is to be started in the course, sFlt-1 should be drawn before administration of heparin. *Treatment regimens are typically 1-2 treatments per week, guided by circulating sFlt-1 levels.

Supplemental Table 3. Summary of baseline and demographic characteristics of women in apheresis group and matched control groups

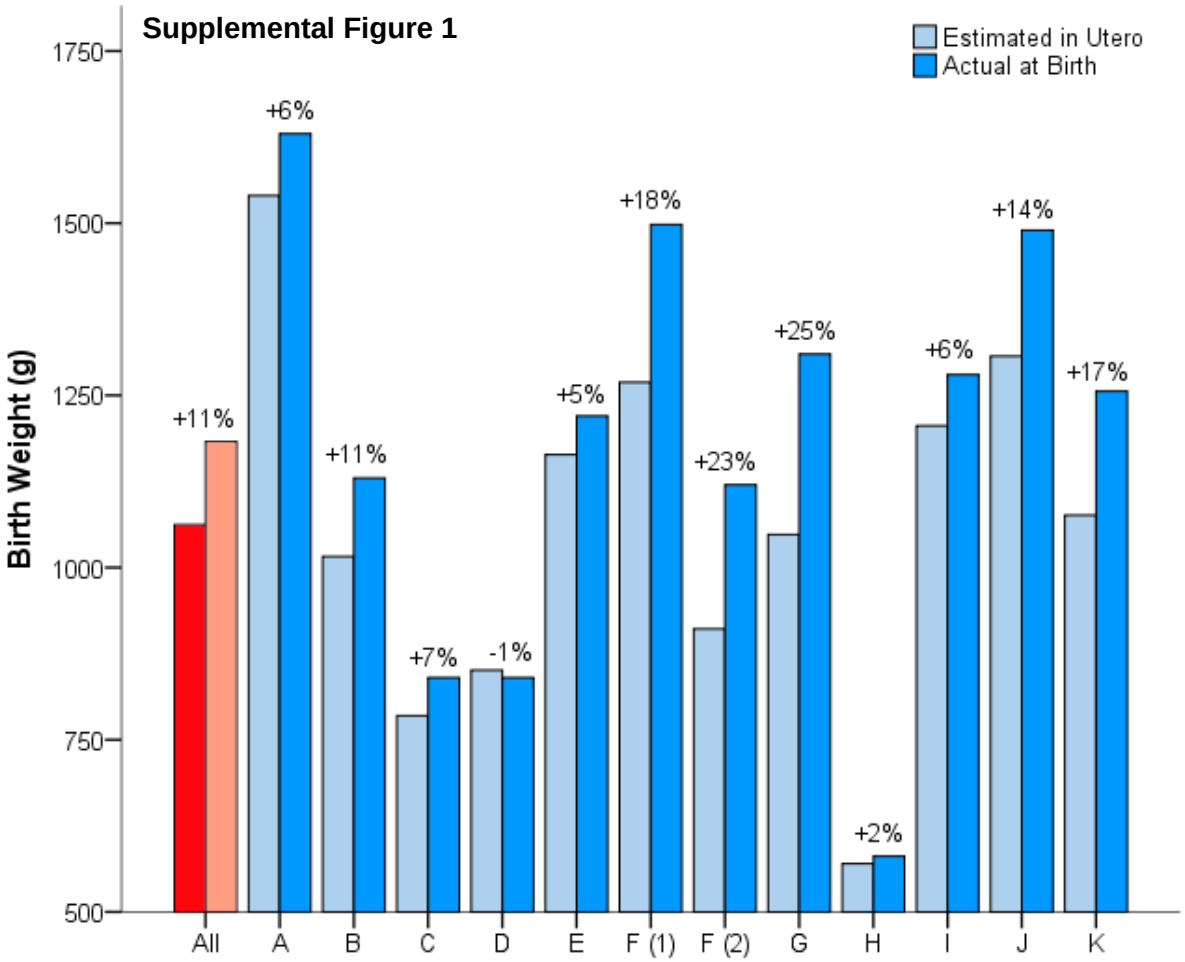
	Apheresis Group (n=11)	Control Group 1 (n=22)	Control Group 2 (n=22)
Maternal Age (years)	28 ± 6	32 ± 6	30 ± 5
White Race	11 (100.0)	20 (90.9)	22 (100.0)
SBP (mmHg)	161 ± 11	167 ± 12	129 ± 18
DBP (mmHg)	101 ± 9	104 ± 11	70 ± 13
sFlt-1 (pg/ml)	15837 ± 8126	14319 ± 7629	Not done
sFlt-1/PlGF Ratio	664 ± 546	501 ± 388	Not done

Table shows number (percentage) or mean ± SD

Abbreviations: BMI=body mass index; SBP=systolic blood pressure; DBP=diastolic blood pressure; sFlt-1=soluble Fms-like tyrosine kinase-1; PlGF=placental growth factor

Infant Birth Weights

Supplemental Figure 1. Estimated and actual birth weights for infants born to all patients. Fetal birth weight estimated *in utero* prior to the first treatment (g) and actual neonatal weight at birth (g) for 11 patients treated with apheresis. Percent difference between estimated and actual weights are shown for each patient. Average birth weight estimated *in utero* for all apheresis patients is shown as the pink bar and average actual neonatal weight at birth is shown as the red bar. Patient F is represented twice due to twins.

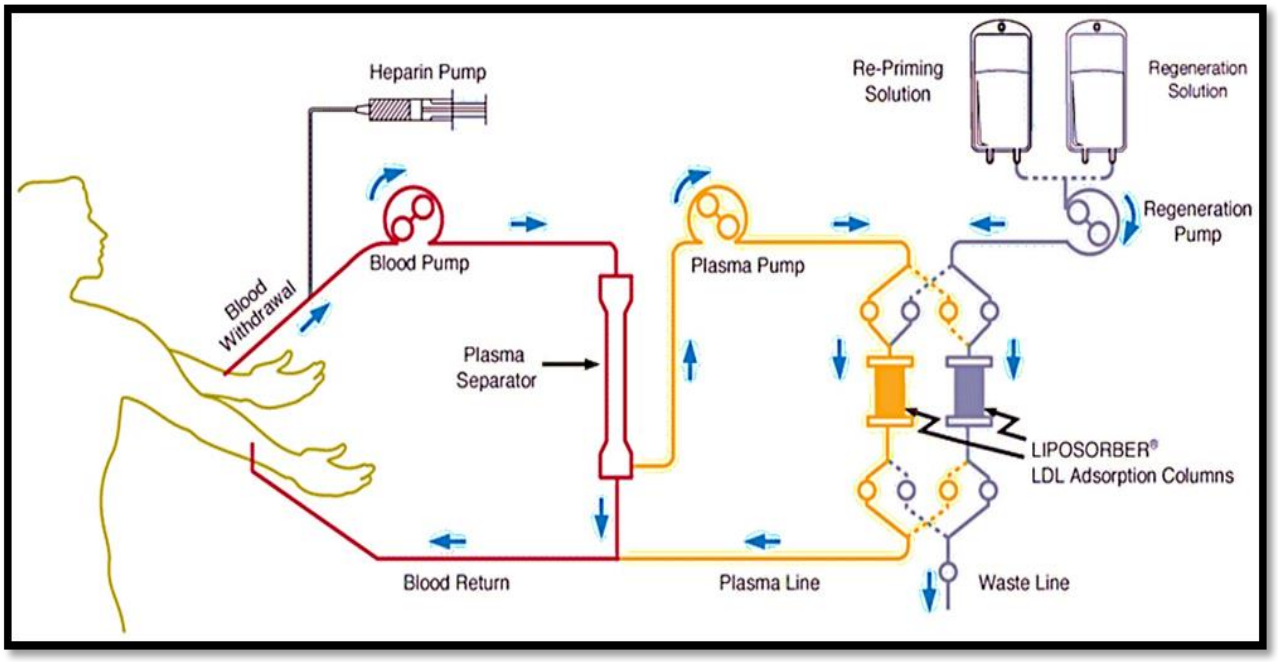


Supplemental METHODS

In vitro *removal of human sFlt-1 from spiked plasma by whole blood vs PSDS columns*

These *in vitro* experiments compare the efficiency of sFlt-1 removal using negatively charged dextran sulfate adsorption (DSA) columns configured to process either whole blood or plasma. In the former, unseparated whole blood was passed directly through the column, while in the latter (current device), an in-line plasma separator diverted only plasma to the DSA column (**Supplemental Figure 2**).

Supplemental Figure 2: Configuration of the LIPOSORBER® LA-15 System for plasma separation and apheresis



The LIPOSORBER® LA-15 System (http://www.kaneka-med.jp) is comprised of a tubing set, a hollow fiber plasma separator (SULFLUX® KP-05) and two single use dextran sulfate-adsorption columns (LA-15). A computer-controlled machine (MA-03) controls the entire apheresis procedure. The patient's blood is withdrawn via venous access and enters the plasma separator. As whole blood is pumped through the hollow fibers in the plasma separator, separated plasma exits from the plasma outlet. The remaining blood elements (red and white blood cells and platelets) exit from the blood outlet. Cell-free plasma is pumped into one of the

two adsorption columns, adsorbing the positively-charged sFlt-1 protein. sFlt-1-depleted plasma exits the column, passes through a membrane filter and is recombined with the blood cells. Recombined blood and plasma flows through a built-in blood warmer and is returned to the patient via a second venous access. When the first column has completed adsorbing sFlt-1, the computer-regulated machine automatically switches the plasma flow to the second column to continue sFlt-1 adsorption. The first column is regenerated using 5% Sodium Chloride Injection USP, eluting the adsorbed sFlt-1. Once elution is completed and flushed through waste lines, the column is completely reprimed for the next cycle of adsorption, allowing continuous apheresis. No additional fluids are given to the patient (except in the case of hypotension as defined in the protocol) during column switchovers; only treated plasma is returned. A typical procedure takes ~2 hours.

In these experiments, 50 ml of human amniotic fluid rich in endogenous sFlt-1 and its various isoforms was spiked into two units of discarded human blood, and the entire volume of unseparated or separated blood was passed through the respective columns in the apheresis circuit. For these studies, sFlt-1 concentrations were measured pre- and post-passage through the columns using an automated sFlt-1 assay (inter- and intra-assay coefficients of variation <3%; optimal range 10-85,000 pg/ml; Elecsys sFlt-1 Assay, Roche Diagnostics, Germany).[1] As shown in **Supplemental Table 4**, the PSDS column removed 23% more sFlt-1 after three passages than was previously reported for the whole blood column.[2] Given this improved efficiency, as well as evidence that DL-75 and LA-15 may have differential effects in activation of cytokines and chemokines,[3] we chose the PSDS configuration to perform apheresis in women with very preterm preeclampsia.

Supplemental Table 4. *In vitro* removal of sFlt-1 using two different columns (LA-15, plasma separation vs. DL-75, whole blood system)

	sFlt-1 (pg/ml)	% Reduction
LIPOSORBER® LA-15 (Kaneka)		
Baseline (pre)	3791	
Passage 1 (post)	2225	41.3
Passage 2 (post)	1110	70.7
Passage 3 (post)	376	**90.8**
DL-75 (Kaneka) DSA*		
Baseline (pre)	4576	-
Passage 1 (post)	2273	50.3
Passage 2 (post)	1611	64.8
Passage 3 (post)	1185	**74.1**

*As previously reported by Thadhani et al.[2]

Inclusion and Exclusion Criteria and Study Schema

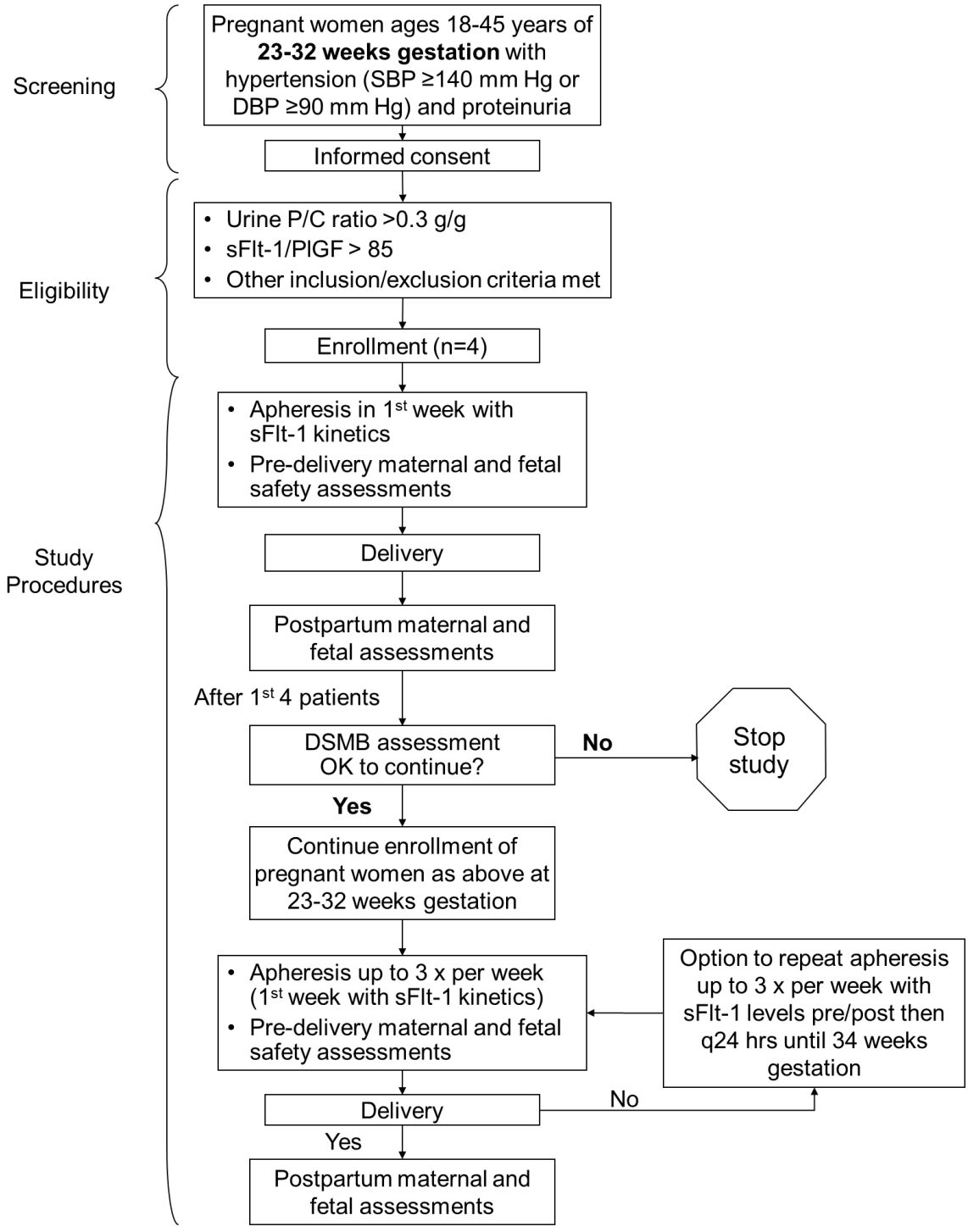
See **Supplemental Table 5** below for complete inclusion/exclusion criteria and **Supplemental Figure 3** for study schema.

Supplemental Table 5. Inclusion and Exclusion Criteria

Inclusion Criteria	a. Signed informed consent in a pregnant woman ages 18 to 45 years hospitalized for pre-term preeclampsia. b. Pre-term preeclampsia defined by systolic BP ≥140 mmHg or ≥90 mmHg diastolic ≥23 weeks of gestation or ≤32 weeks of gestation, in a woman with previously normal BP, and new onset proteinuria (protein/creatinine ratio >0.3 g/g). c. sFlt-1/PlGF ratio >85 (blood levels of sFlt-1 and PlGF determined using CE-approved Roche Diagnostics assays).
Standard Exclusion Criteria	a. Taking any form of angiotensin cascade blocker b. History or diagnosis of pre-existing chronic hypertension (first 4 patients only) c. History of cardiac impairments including uncontrolled arrhythmia, unstable angina, decompensated congestive heart failure or valvular disease d. History or diagnosis of chronic renal disease e. Patients receiving therapeutic anticoagulation therapy prior to study entry f. Anticipated immediate delivery within 24 hours g. Signs or history of cerebral nervous system dysfunction, including seizures, cerebral edema (previously confirmed by CT-scan or MRI) h. History of thyroid disease i. History of liver abnormalities j. Pulmonary edema k. Thrombocytopenia (platelet count < 10^9/L) l. Anemia – hemoglobin < 80 g/L m. Evidence of “reverse Doppler” flow on umbilical Doppler n. Placenta previa o. Multiple pregnancy (first 4 patients only) p. History of placental abruption q. Pre-term labor r. Active hepatitis B, C, or tuberculosis infection or HIV positive status s. Any condition that the investigator deems a risk to the patient or fetus

	in completing the study t. Any condition which in the opinion of the investigator would necessitate delivery in the next 24 hours.
Exclusion Criteria Based on Fetal Characteristics	a. Trisomy b. Biophysical profile (BPP) < 6 c. Amniotic fluid index (AFI) < 5 cm d. Estimated fetal weight (EFW) < 5th percentile for gestational age (IUGR).

Supplemental Figure 3: Study Schema Abbreviations: p:c=protein:creatinine; PlGF=placental growth factor; sFlt-1=soluble FMS-like tyrosine kinase; SBP=systolic blood pressure; DBP=diastolic blood pressure; DSMB=Data Safety Monitoring Board.



Secondary Outcome Measures

Secondary outcome measures included maternal proteinuria, blood pressure, and laboratory measures. Fetal assessments including Doppler ultrasound and cardiotocography (CTG) were performed during treatments by obstetricians. We also examined the following parameters of each infant: gestational age at delivery, body weight, body length and head circumference at birth, APGAR score (5 and 10 minutes), CRIB score,[4] cord blood pH, total neonatal ICU and hospital stay, duration of mechanical ventilation, administration of nasal continuous airway pressure (nCPAP), supplemental oxygen, and the doses of surfactant administered for early therapeutic treatment of respiratory distress syndrome (RDS). Neonatal treatment measures and short-term outcomes were assessed by neonatologists (B. Roth, U. Thome). Screening for retinopathy of prematurity (ROP) was carried out by a pediatric ophthalmologist.

Statistical Analysis

All statistical analyses were performed with SAS version 9.2. Two-tailed P-values <0.05 were considered to indicate statistical significance.

Apheresis Patients

Baseline and treatment characteristics of patients treated with apheresis are presented as raw values. Means and ranges are presented for percent changes in sFlt-1 levels, P/C ratios, and birthweights as well as the number days the pregnancy continued measured from the day of admission.

Neonatal Data (Apheresis Group vs Matched Controls)

We evaluated gestational age, birth weight, body length, head circumference at birth, cord blood pH, 5 and 10 minute APGAR score, CRIB score, total neonatal ICU and hospital lengths of stay, duration of mechanical ventilation, days of nCPAP and supplemental O_2 administration, and doses of surfactant administered for preterm infants born to women who underwent apheresis (n=12 from n=11 mothers). To assess whether treatments resulted in adverse consequences to the neonate, we then compared these data by matching (1:2) women who underwent apheresis to contemporaneous women with preterm preeclampsia who were not treated with apheresis (control group 1, n=22) and to contemporaneous women who delivered

early but for reasons other than preeclampsia (control group 2, n=22). Matching criteria were gender, gestational age ± 1 week, and birth weight ± 100 g. Each index patient was matched to control patients from the same center (Cologne or Leipzig), resulting in 11 matching groups. Therefore, each matching group consisted of one index patient, two patients from control group 1, and two patients from control group 2, forming a cluster. To account for matching effects and mixed effects, analysis with matching variables as fixed effects and clusters of matched preterm infants as random effects were adapted. Neonatal characteristics at birth and during follow-up were compared between groups using Mann-Whitney U tests.

Study approval

The study was coordinated by R. Thadhani (the Principal Investigator [PI]) at Massachusetts General Hospital (MGH; Clinicaltrials.gov NCT01404910) and approved by the Institutional Review Boards at the MGH and the University of Cologne (PI, T. Benzing). University of Leipzig (PI, H. Stepan) ceded review to the University of Cologne. Each patient signed written informed consent prior to any study procedures. A three member Data Safety Monitoring Board (DSMB) (Chair, C. Wanner, MD) reviewed the data from the 11 patients and their offspring. Kaneka Corporation generously provided funding but had no influence over study design, data collection, analysis, or interpretation of results.

REFERENCES CITED IN SUPPLEMENTAL MATERIALS

1. Verlohren S, Galindo A, Schlembach D, Zeisler H, Herraiz I, Moertl MG, Pape J, Dudenhausen JW, Denk B, Stepan H. An automated method for the determination of the sFlt-1/PIGF ratio in the assessment of preeclampsia. *Am J Obstet Gynecol.* Feb 2010;202(2):161 e161-161 e111.

2. Thadhani R, Kisner T, Hagmann H, Bossung V, Noack S, Schaarschmidt W, Jank A, Kribs A, Cornely OA, Kreyssig C, Hemphill L, Rigby AC, Khedkar S, Lindner TH, Mallmann P, Stepan H, Karumanchi SA, Benzing T. Pilot Study of Extracorporeal Removal of Soluble Fms-Like Tyrosine Kinase 1 in Preeclampsia. *Circulation.* Aug 1 2011;124(8):940-950.

3. Hovland A, Hardersen R, Sexton J, Mollnes TE, Lappegard KT. Different inflammatory responses induced by three LDL-lowering apheresis columns. *J Clin Apher.* 2009;24(6):247-253.

4. The International Neonatal Network. The CRIB (clinical risk index for babies) score: a tool for assessing initial neonatal risk and comparing performance of neonatal intensive care units. *Lancet.* Jul 24 1993;342(8865):193-198.