Preeclampsia is a placentaally induced hypertensive disorder of pregnancy that is associated with substantial morbidity and mortality to mothers and fetuses. Clinical manifestations of preterm preeclampsia result from excess circulating soluble vascular endothelial growth factor receptor FLT1 (sFLT1 or sVEGFR1) of placental origin. Here we identify short interfering RNAs (siRNAs) that selectively silence the three sFLT1 mRNA isoforms primarily responsible for placental overexpression of sFLT1 without reducing levels of full-length FLTI mRNA. Full chemical stabilization in the context of hydrophobic modifications enabled productive siRNA accumulation in the placenta (up to 7% of injected dose) and reduced circulating sFLT1 in pregnant mice (up to 50%). In a baboon preeclampsia model, a single dose of siRNAs suppressed sFLT1 overexpression and clinical signs of preeclampsia. Our results demonstrate RNAi-based extrapneumonic modulation of gene expression with nonformulated siRNAs in nonhuman primates and establish a path toward a new treatment paradigm for patients with preterm preeclampsia.

Preeclampsia complicates 5–10% of all human pregnancies and causes nearly 76,000 maternal and 500,000 infant deaths globally each year. In the United States alone, preeclampsia is responsible for 100,000 premature births and 10,500 infant deaths annually, with aggregated costs of ~6.5-billion dollars to the health care system (http://www.preeclampsia.org). Women with preeclampsia rapidly develop hypertension, often with proteinuria, usually after the 20th week of pregnancy. Maternal complications include kidney injury, HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome, seizure, stroke and death. Consequences for the fetus can be grave, ranging from intrauterine growth restriction to hypoxia-induced neurologic injury (for example, cerebral palsy) to death. Currently the only treatment option for women with preeclampsia is to deliver the fetus—regardless of its gestational age—when the mother’s symptoms become too severe. Thus, preeclampsia represents a substantial public health concern with an unmet clinical need.

Epidemiological and experimental studies indicate that the maternal signs and symptoms of preeclampsia that presents preterm are caused by abnormally high serum levels of sFLT1 proteins — secreted antiangiogenic proteins that correspond to the extracellular domain of vascular endothelial growth factor receptor 1 (VEGFR1, also known as full-length FLTI). sFLT1 proteins scavenge circulating VEGF and placental growth factor and attenuate VEGF signaling by membrane-bound VEGF receptor FLTI. Circulating sFLT1 levels rapidly decrease after delivery of the placenta, and maternal preeclampsia signs usually resolve within 48 to 72 h, consistent with the placental origin of sFLT1. Serum sFLT1 levels are considered a diagnostic and prognostic marker of preeclampsia. Moreover, lowering sFLT1 and other circulating factors by apheresis—i.e., filtering maternal blood through a dextran sulfate column—can control both blood pressure and proteinuria and extend preeclamptic pregnancies. Thus, sFLT1 is a biologically validated and clinically promising therapeutic target for preeclampsia.

Short interfering RNA (siRNA) therapeutics are an emerging class of drugs that target nucleic acids. The most clinically advanced siRNA technology comprises fully chemically stabilized siRNAs conjugated to N-acetylgalactosamine (GalNAc), which drives safe, efficient siRNA delivery to and long-lasting (6- to 9-month) silencing activity in liver. Whereas the utility of GalNAc chemistry is limited to hepatocytes, hydrophobic conjugates (for example, cholesterol) enable nonselective siRNA distribution to a range of tissues, favoring tissues with high blood flow and fenestrated endothelium. These tissue characteristics are shared by the placenta, suggesting that fully chemically modified, cholesterol-conjugated siRNAs might also accumulate in placenta and allow selective silencing of sFLT1.
As a first step toward developing siRNAs that selectively silence sFLT1 but not full-length FLT1, we recently analyzed the transcriptional profiles and 3′ ends of sFLT1 and FLT1 mRNAs in normal and preeclamptic placentas. sFLT1 and FLT1 mRNAs are predominantly expressed in the placenta, with sFLT1 mRNA isoforms comprising as much as 97% of placental FLT1 mRNAs. Use of alternative polyadenylation sites in intron 13 and exon 15 gives rise to three dominant sFLT1 mRNA isoforms—sFLT1-i13 short, sFLT1-i13 long and sFLT1-e15a (Fig. 1a,b)—which are all upregulated in preeclamptic placentas (Supplementary Fig. 1). The unique 3′ ends of the sFLT1 isoforms offer an opportunity to selectively target sFLT1.

Here we identify siRNAs that selectively silence sFLT1 mRNAs without affecting full-length FLT1. Full chemical stabilization and cholesterol conjugation allow systemically delivered siRNAs to accumulate in mouse and baboon placentas, where they silence sFLT1 mRNA. Finally, we show that sFLT1-targeting siRNAs lower circulating sFLT1 protein and alleviate hypertension and proteinuria in a baboon model of preeclampsia. Taken together, these results provide a strong foundation for future development of an siRNA-based therapeutic for preeclampsia.

RESULTS

Screen to identify siRNAs selectively silencing i13 and e15 sFLT1 mRNA isoforms

Three sFLT1 mRNA isoforms—sFLT1-i13 short, sFLT1-i13 long and sFLT1-e15a—predominantly contribute to the expression of sFLT1 protein, which circulates at high levels in patients with preterm preeclampsia. Thus all three isoforms need to be targeted to productively decrease the levels of circulating sFLT1 protein across the spectrum of preeclampsia. The sFLT1-i13 long and sFLT1-e15a mRNA isoforms contain 435 and 567 unique nucleotides, respectively, not present in full-length FLT1 mRNA, allowing selective targeting. We designed a panel of 26 siRNAs targeting essentially all possible regions in the two sFLT1-i13 isoforms (sFLT1-i13 long includes the sequence of sFLT1-i13 short) and 21 siRNAs targeting sFLT1-e15a. The selection was favored, to the extent possible, to comply with conventional siRNA design parameters that account for G+C content, specificity and low frequency of complementary seed sites in the genome, and thermodynamic bias. Design criteria also favored siRNAs that target sites with perfect homology in nonhuman primates, to enable evaluation in a baboon preeclampsia model (see below).

For initial in vitro screening, we synthesized hydrophobically modified asymmetric siRNAs (hsiRNAs), which can be added directly to the cell medium, without lipid formulation. hsiRNAs are partially chemically modified for improved stability and composed of a 20-nucleotide (nt) guide strand base-paired to a 15-nt passenger strand, leaving a fully phosphorothioate-modified single-stranded end of the passenger strand is conjugated to cholesterol, which promotes membrane intercalation and rapid, efficient internalization by all cell types, including primary trophoblasts. All sequences and chemical modification patterns used in this study are shown in Supplementary Table 1.

To simplify the screening process, we generated luciferase-based reporters in which the sFLT1-i13 and sFLT1-e15a unique sequence regions were cloned downstream of the Renilla luciferase coding strand. We then assayed the ability of each hsiRNA to reduce Renilla luciferase activity as a proxy for productive silencing of sFLT1-i13 and sFLT1-e15a. Primary screens identified several functional hsiRNAs that efficiently silenced sFLT1-i13 or sFLT1-e15a mRNA isoforms (Fig. 1c,d; see Online Methods). Based on dose–response studies of all functional hsiRNAs, we chose the top two hsiRNAs from each primary screen as lead configurations: hsiRNA\textsuperscript{sFLT1-i13-2283} and hsiRNA\textsuperscript{sFLT1-e15a-2519} for silencing of sFLT1-i13, and hsiRNA\textsuperscript{sFLT1-i13-2519} for silencing sFLT1-e15a. The half-maximal inhibitory concentrations (IC\textsubscript{50}) of selected compounds ranged from 50 to 90 nM (passive uptake), and their sequences and target positions are shown in Figure 1e–g.

To confirm that lead hsiRNAs efficiently silence sFLT1 mRNA in a cell type relevant to preeclampsia, we used QuantiGene 2.0 assays that allowed specific and direct detection of human sFLT1-i13 and sFLT1-e15a mRNAs in human primary cytotrophoblasts, the placental cell type responsible for sFLT1 overexpression. Identified hsiRNAs silenced sFLT1 mRNAs in cytotrophoblasts, with IC\textsubscript{50} values comparable to those found in the reporter assay (50 to 70 nM; Fig. 1e,h). Moreover, productive silencing of sFLT1 mRNA translated to a significant reduction in the amount of sFLT1 protein secreted by cytotrophoblasts into the cell medium (Fig. 1j). hsiRNA\textsuperscript{sFLT1-i13-2283} target sequences are conserved in humans, baboons, and mouse; hsiRNA\textsuperscript{sFLT1-e15a-2519} target sequences are conserved in baboons and humans. We therefore selected these compounds for all subsequent studies.

hsiRNA\textsuperscript{sFLT1-i13-2283} and hsiRNA\textsuperscript{sFLT1-e15a-2519} efficiently modulate expression of major sFLT1 mRNA isoforms without effect on full length FLT1 mRNA

Successful therapeutic reduction of circulating sFLT1 in preeclampsia using hsiRNAs will require simultaneous suppression of all three sFLT1 (i13 short and long and e15a) mRNA isoforms (Fig. 1a,b and Supplementary Fig. 1). We therefore tested whether a mixture of isoform-specific hsiRNAs could be used to silence sFLT1-i13 and sFLT1-e15a mRNAs simultaneously. Using cytotrophoblasts and WM-115 cells (human melanoma cells that express sFLT1-i13 and sFLT1-e15a mRNAs), we confirmed that hsiRNA\textsuperscript{sFLT1-i13-2283} specifically silenced the sFLT1-i13 isoform and that hsiRNA\textsuperscript{sFLT1-e15a-2519} specifically silenced the sFLT1-e15a isoform (Fig. 2a,b and Supplementary Fig. 2). An equimolar mixture of the two (hsiRNA\textsuperscript{sFLT1-i13-2283/2519}) efficiently silenced both sFLT1-i13 and sFLT1-e15a isoforms (Fig. 2a,b). Neither the individual hsiRNAs nor the hsiRNA mixture affected the levels of full-length FLT1 mRNA (Fig. 2c). Of note, the hsiRNA\textsuperscript{sFLT1-i13-2283/2519} mixture was more potent than individual hsiRNAs, with the effect being more pronounced for the sFLT1-i13 isoform. We have confirmed this phenomenon in several independent experiments.

Efficient in vivo delivery of fully chemically modified, cholesterol-conjugated hsiRNA\textsuperscript{sFLT1-2283} to the placental labyrinth by systemic administration

Productive systemic delivery of nonformulated hsiRNA to placenta had not been previously demonstrated. Although the partially modified siRNAs used for original screening could be taken up by cytotrophoblasts in vitro, these siRNAs failed to accumulate to appreciable levels in placenta after systemic administration (Supplementary Fig. 3). We recently showed, however, that full chemical stabilization is essential for systemic, conjugate-mediated siRNA delivery. We therefore synthesized fully chemically modified variants of lead compounds, in which all sugars were modified using an alternating 2′-flouro and 2′,O-methyl modification pattern. In addition, all 5′- and 3′-terminal linkages were phosphorothioate-modified, providing additional protection against exonucleases (Fig. 3a). To visualize the
extent of delivery to tissues in vivo, we labeled the passenger strand of hsiRNA\textsuperscript{FLT1-2283} with Cy3 fluorescent dye.

We injected Cy3-labeled hsiRNA\textsuperscript{FLT1-2283} into pregnant mice at gestational day 15, collected tissues 24 h after injection, and examined the tissue distribution of labeled hsiRNA by fluorescence microscopy. hsiRNA\textsuperscript{FLT1-2283} accumulated mainly in liver, kidneys, spleen and placental labyrinth (Fig. 3b), consistent with their high degree of endothelial fenestration and blood flow. The placental labyrinth

Figure 1 Development of hydrophobically modified, chemically stabilized hsiRNA compounds against sFLT1. (a) Schematic representation of exon–intron structure of sFLT1-i13 and sFLT1-e15a mRNAs. (b) sFLT1-i13 and sFLT1-e15a unique sequence regions. Locations of lead siRNA target sites are indicated. Stop codons are shown in red. (c,d) Results of a screen for hsiRNAs (1.5 μM) that silence sFLT1-i13 and sFLT1-e15a using luciferase-based reporters in HeLa cells. siRNA numbers correspond to the 5' position of the siRNA target site in the mRNA. UNT, untreated control; NTC, nontargeting control. (n = 3, mean ± s.d.). Red squares indicate lead compounds selected for further evaluation. (e) siRNAs targeting sFLT1-i13 and sFLT1-e15a. (f,g) Dose–response curves of lead hsiRNA\textsuperscript{FLT1} silencing of sFLT1-i13 and sFLT1-e15a luciferase reporters in HeLa cells. M represents the molar concentration of hsiRNA. (h,i) Dose–response curves of lead hsiRNA\textsuperscript{FLT1} silencing of sFLT1-i13 and sFLT1-e15a mRNAs in cytotrophoblast (CTB) cells. (n = 3, mean ± s.d.). (j) sFLT1 protein levels produced by CTB cells treated with hsiRNA\textsuperscript{FLT1-2283} or hsiRNA\textsuperscript{NTC}, sFLT1 protein was measured by ELISA (n = 3, mean ± s.d.) (**P < 0.01, *P < 0.05; ns, not significant, one-way ANOVA).
functions as a maternal–placental barrier and the site of nutrient transport between the maternal and fetal blood (Fig. 3b). Consistent with the barrier function of the labyrinth, Cy3-labeled hsiRNA \textit{sFLT1}-2283 was detected only in placenta (Fig. 3c). The labyrinth constitutes most of the placental disk, where fetal capillaries are supported by connective tissue and surrounded by trophoblast cells—mononuclear sinusoidal trophoblast giant cells and two layers of multinucleated syncytiotrophoblast cells. Notably, we observed efficient delivery of Cy3-labeled hsiRNA \textit{sFLT1}-2283 to the placental labyrinth, with no detectable uptake by other cell types in the decidua, the uterine endometrium that forms the maternal part of the placenta. Histological examination showed no obvious pathology associated with oligonucleotide delivery.

To quantify the accumulation of hsiRNA in placenta and other tissues, we used a recently developed peptide nucleic acid (PNA)-based hybridization assay to directly measure guide strand levels\textsuperscript{33,36}. Consistent with histological examination, fully chemically stabilized, cholesterol-conjugated hsiRNAs accumulated in maternal liver (\textasciitilde 200 ng/mg), kidney (\textasciitilde 50 ng/mg), spleen (\textasciitilde 50 ng/mg) and placenta (10 to 20 ng/mg), but not in other fetal tissues (<0.05 ng/mg, detection limit) (Fig. 3d,e). Notably, placental accumulation of hsiRNA was similar whether administered by subcutaneous or intravenous injection (Fig. 3b,d,e). As much as 7% of injected hsiRNA \textit{sFLT1}-2283 accumulated in placenta, and the concentration of hsiRNA in the placenta was \textasciitilde 20 ng/mg, which was sufficient to induce productive silencing (Fig. 3d,e).

\textbf{In vivo silencing of placental-derived \textit{sFLT1} does not affect pup survival or ability to thrive}

Mice express only the \textit{sFlt1}-113 isoform\textsuperscript{40}. Thus, we used hsiRNA \textit{sFLT1}-2283, which selectively silences \textit{sFlt1}-113, to test whether placental accumulation of hsiRNA results in productive \textit{sFlt1}-113 silencing. We injected 20 mg/kg hsiRNA \textit{sFLT1}-2283 in phosphate-buffered saline (PBS) intravenously into pregnant mice at gestational days 14 and 15 (Fig. 4a). Control animals were treated with PBS or a nontargeting control (NTC) hsiRNA\textsubscript{NTC} of identical chemical configuration (conjugated to cholesterol and with a 2′-O-methyl, 2′-fluoro alternating modification pattern, or hsiRNA\textit{sFLT1}-2283 lacking the cholesterol conjugate (NoC-siRNA\textit{sFLT1}-2283); \textit{Supplementary Table 1}).

At gestational day 19, we measured \textit{sFlt1} mRNA levels in placenta. We observed a \textasciitilde 40% reduction of \textit{sFlt1} mRNA in placentas of mice treated with hsiRNA\textit{sFLT1}-2283 (\textit{P} < 0.001, one-way ANOVA, relative to both PBS and hsiRNA\textsubscript{NTC}; Fig. 4b). The hsiRNA\textsubscript{NTC} was inactive, showing that \textit{sFlt1} silencing is specific to hsiRNA\textit{sFLT1}-2283. NoC-siRNA\textit{sFLT1}-2283 failed to accumulate in placenta (Fig. 4c) and consequently failed to silence \textit{sFlt1} mRNA (Fig. 4b), suggesting that full chemical stabilization and the cholesterol moiety are both required for placental delivery and silencing activity. We found no evidence for silencing of \textit{sFlt1} in fetal liver (data not shown), consistent with the histological and quantitative observations that hsiRNA\textit{sFLT1}-2283 did not accumulate in fetal tissues other than placenta (Fig. 3c,d,e).

Consistent with significant accumulation of hsiRNA\textit{sFLT1}-2283 in liver and kidney (Fig. 3d,e), we also observed productive silencing of \textit{sFlt1} mRNA in both of these tissues (\textit{Supplementary Fig. 4}). In pregnant mice, however, >95% of circulating \textit{sFlt1} protein originates from placenta\textsuperscript{40–42}. Thus, extraplacental silencing does not measurably contribute to reduced serum levels of \textit{sFlt1} in mice treated with hsiRNA\textit{sFLT1}-2283.

In a parallel study, we treated pregnant mice (at gestational days 14 and 15) with hsiRNA\textit{sFLT1}-2283 or PBS and collected blood samples at three time points during the third trimester to measure serum levels of \textit{sFlt1} protein by ELISA. In PBS-treated control mice, \textit{sFlt1} protein levels increased by about \textasciitilde 50% from day 14 to day 17 (near term). As expected, this increase was suppressed in mice treated with hsiRNA\textit{sFLT1}-2283 (Fig. 4d) as evidenced by blood sampling at gestational days 10, 15, 17 and 19 from mice injected with PBS or hsiRNA\textit{sFLT1}-2283. At each time point, mice treated with hsiRNA\textit{sFLT1}-2283 had lower levels of circulating \textit{sFlt1} than did mice treated with PBS (\textit{P} < 0.001, two-way ANOVA; Fig. 4d,j) or hsiRNA\textsubscript{NTC} (\textit{P} < 0.05, two-way ANOVA; Fig. 4j). Silencing of \textit{sFlt1} (up to 50%) or treating mice with hsiRNA\textsubscript{NTC} did not affect the number of newborn pups, their weight at birth, or their ability to thrive (\textit{Supplementary Fig. 5a–e}). Moreover, levels of the maternal liver transaminases ALT and AST were normal (Fig. 4e), indicating that, at the doses tested, hsiRNA\textit{sFLT1}-2283 treatment does not generate obvious adverse effects.

To confirm that the observed modulation of \textit{sFlt1} expression is indeed due to RNAi-based cleavage of \textit{sFlt1} mRNA, we purified total mRNA from placentas treated with hsiRNA\textit{sFLT1}-2283, hsiRNA\textsubscript{NTC} or PBS. The purified RNA was subjected to 5′-RACE-PCR amplification. The expected product size was observed only in hsiRNA\textit{sFLT1}-2283-treated samples, and sequencing confirmed the expected cleavage site (\textit{Supplementary Fig. 6}). Taken together, these data show that systemic administration of hsiRNA\textit{sFLT1}-2283 leads to efficient RNAi-based silencing of \textit{sFlt1} mRNA in the placenta and reduces the level of circulating \textit{sFlt1} protein without affecting the health of the mother or her pups.
Our goal was to test whether the mixture of isoform-specific siRNAs effectively silences all three sFLT1 isoforms in a well-characterized nonhuman primate model of preeclampsia. Before doing so, we measured the efficacy and safety of the hsiRNA\textsuperscript{sFLT1}-2283/2519 mixture in pregnant mice. An equimolar mixture of hsiRNA\textsuperscript{sFLT1}-2283/2519 injected into pregnant mice efficiently reduced sFlt1 mRNA in placenta (40%, \(P < 0.01\), one-way ANOVA) and other maternal tissues (Fig. 4f). hsiRNA\textsuperscript{sFLT1}-2283 and hsiRNA\textsuperscript{sFLT1}-2519 accumulated to nearly identical levels in the placenta, with no detectable transfer to the fetus (Fig. 4g). hsiRNA\textsuperscript{sFLT1}-2283/2519 administration reduced circulating sFlt1 protein levels by day 17 (\(P < 0.001\), two-way ANOVA) but did not affect pup viability, weight, or ability to thrive (data not shown), and maternal liver enzymes remained normal (Fig. 4h,i). The observed reduction in sFlt1 was sequence specific, as hsiRNA\textsuperscript{NTC}, compound with identical chemical configuration but not targeting sFlt1, had no impact on sFlt1 protein expression and was indistinguishable from PBS (Fig. 4j), with no observable effect on liver enzymes (Fig. 4k). As expected, hsiRNA\textsuperscript{NTC} treatment had no impact on number of pups or ability to thrive (Supplementary Fig. 5d,e), confirming that this chemical scaffold was well tolerated at dose used.

While most sFLT1 expression is derived from the placenta, sFLT1 is involved in fine-tuning regulation of VEGF signaling, specifically important for macular and corneal vascularization\textsuperscript{44,45}. We did not find any appreciable levels of the drug in the eye, and it is unlikely to have any biological consequences in that regard (Supplementary Fig. 7).

We did not see any detectable levels of compound transfer to any additional fetal tissues by fluorescent microscopy (Fig. 3c) and PNA hybridization assay (Fig. 3d,e). Breast is another heavily vascularized tissue in which hydrophobic siRNAs may accumulate. We observed around 2.5 ng/mg of hsiRNA\textsuperscript{sFLT1} compounds in breast tissue of nursing mice after hsiRNA treatment. This compound concentration was less than one-fifth that in the placenta of the same animals. No appreciable compound transfer was observed in milk (<1 ng/µL), indicating that chances for substantial neonatal hsiRNA accumulation in the pup via breast milk is minimal (Supplementary Fig. 7).

Although our studies were not designed to formally evaluate pharmacokinetics, in our limited dataset, we were unable to detect
appreciable concentrations of the drug in breast tissue or in milk. This in contrast to the robust levels of drug in the placental tissue. Additional pharmacokinetic studies in nonhuman primates are needed before we can conclude that the risk of breast milk excretion is low.

Finally, 40% to 50% sFLT1 repression in placenta might influence placental vascularization. In a subset of mice injected with the hsiRNA\(^{-2283}\), we examined the placental tissue for vascular development and did not find any substantial vascular disruptions in the placental labyrinth (Supplementary Fig. 8\(^{40}\)). We can therefore conclude that injection of sFlt1 siRNA was therapeutic when injected during third trimester and at modest doses did not induce any notable vascular anomalies in the placenta. Although our studies do not show any obvious side effects in mothers or the fetuses, additional long-term safety studies will be needed before drawing definitive conclusion of the safety of this approach. Thus, co-delivery of hsiRNA\(^{-2283}\) and hsiRNA\(^{-2519}\) efficiently and safely reduced sFlt1 without affecting full-length Flt1 levels, recapitulating the pharmacological behavior observed with hsiRNA\(^{-2283}\) alone.

Single hsiRNA\(^{-2283}\)/\(^{-2519}\) injection efficiently modulates sFLT1 serum levels and reduces hypertension and proteinuria in a baboon model of preeclampsia

Placental morphology and physiology differ between humans and mice, limiting the value of rodent models in mimicking human pathology. Placental morphology, maternal physiology, gestation
length and the immune system of baboons (Papio hamadryas), however, better resemble those of humans. *P. hamadryas* expresses all three sFLT1 mRNA isoforms, and an accepted preeclampsia model has been developed. Thus, baboons represent a more accurate preclinical model in which to study efficacy, safety and toxicity of preeclampsia therapeutics. Induction of uteroplacental ischemia (UPI) in baboons via ligation of a single uterine artery at gestational day 133 (full term is 182 d) reduces placental blood flow by ~30%, causing a spike in sFLT1 levels and maternal hypertension and proteinuria, typical clinical manifestations of preeclampsia. Continuous telemetry recordings can accurately measure blood pressure throughout the experiment, allowing real-time assessment of even subtle changes to this key metric.

To assess the potential therapeutic benefit of the hsiRNA\(^{sFLT1, 2283/2519}\)-treated animals had lower blood pressure (BP, awake) plotted from telemetry recordings. (d) Proteinuria presented as spot protein/creatinine ratio.}

In hsiRNA\(^{sFLT1, 2283/2519}\)-treated UPI animals, we observed a potent reduction in circulating sFLT1 protein, as compared to UPI control animals (Fig. 5b), although the kinetics of sFLT1 reduction was somewhat variable. At ~2 weeks after injection in hsiRNA\(^{sFLT1, 2283/2519}\)-treated baboons, serum sFLT1 levels were reduced by >50% (P < 0.001, two-way ANOVA; UPI versus hsiRNA\(^{sFLT1}\)). (b) sFLT1 serum concentration measured by ELISA (Fig. 5c) and reduced proteinuria (Fig. 5d), consistent with potent reduction of sFLT1. Newborn baboon weights and centiles did not change significantly from control animals (Supplementary Fig. 9). However, we observed a trend toward lower birth weights in the offspring of baboons treated with hsiRNA\(^{sFLT1}\), likely suggesting a pharmacological effect related to reduction in systemic blood pressure. Additional dose ranging studies with larger sample sizes will be needed to definitively assess whether the reduction in birth weights are related to sFLT1 repression. Thus, a single injection of hsiRNA\(^{sFLT1, 2283/2519}\) potently reduced serum levels of sFLT1 and normalized blood pressure and proteinuria, common clinical manifestations of preeclampsia.

To assess clearance of hsiRNA\(^{sFLT1, 2283}\) and hsiRNA\(^{sFLT1, 2519}\) from blood and their accumulation in tissues, we collected six or seven placental biopsies and one kidney biopsy from each baboon, in addition to the blood samples noted above. hsiRNAs exhibited similar pharmacological behavior, consistent with the expectation that hsiRNA distribution and clearance properties are mainly defined by the oligonucleotide chemical architecture rather than by sequence. The kinetics of hsiRNA clearance from blood were similar in all three hsiRNA\(^{sFLT1, 2283/2519}\)-treated baboons: serum hsiRNA levels remained constant and ranged from 100 to 300 ng/ml during the first
hour. By 24 h, most of the hsiRNA had been cleared from blood, with levels dropping to ~1 ng/ml. Residual hsiRNA was detected in blood (<0.1 ng/ml) throughout the study. After day 1, hsiRNA levels in urine remained below 0.5 ng/ml, and they fell below the limit of detection after approximately 30 d (Supplementary Fig. 10). Placental hsiRNA levels remained generally constant throughout the 4- to 6-week study duration (1.6 ± 0.3 ng/mg for siRNA-sFLT1-2283 and 2.6 ± 0.9 ng/mg for hsiRNA-sFLT1-2519, mean ± s.d., n = 3; Supplementary Fig. 10). hsiRNA levels in kidney biopsies, collected at day 14, were comparable to placental levels (1.8 ± 0.9 ng/mg for hsiRNA-sFLT1-2283, and 2.7 ± 1.4 ng/mg for hsiRNA-sFLT1-2519). Thus, the three- to fourfold difference in hsiRNA accumulation between placenta and kidney that we observed in mice does not hold true in baboons. Moreover, these findings show that persistent low levels of hsiRNA in placenta are sufficient to maintain therapeutic silencing of sFLT1 in this well-accepted nonhuman primate model of preeclampsia.

**DISCUSSION**

Here we show that a single dose of hsiRNAs mediating silencing of sFLT1 provided therapeutic benefit in animal models of preeclampsia without adverse consequences to the developing fetus. Currently, iatrogenic birth often occurring preterm remains the only clinically accepted treatment for preeclampsia when maternal signs and symptoms become life threatening. Therapeutic strategies aimed at reducing circulating sFLT1 are being pursued as a way to treat and prevent severe preeclampsia. Circulating sFLT1 can be removed from the maternal bloodstream by apheresis, thereby prolonging preeclamptic pregnancy, but this procedure is still in early-stage clinical trials. Moreover, apheresis is expensive and requires infrastructure that is only available in developed countries.

To validate RNAi-based placental modulation of sFLT1 expression as a therapeutic approach, we chose a baboon model of preeclampsia that mimics many features of the human disease, including upregulation of placental sFLT1, a well-validated therapeutic target in preeclampsia. Placental growth factor was recently used as a competitive inhibitor of sFLT1 in this baboon model of preeclampsia, reducing hypertension and proteinuria with safe progression to birth. However, this approach does not target sFLT1 at its source and requires multiple protein injections. In contrast, our approach reduces sFLT1 in the placenta after a single injection. Physiological and anatomical similarities between humans and baboons make baboons an appropriate animal model for studying preeclampsia. Proof-of-concept studies in pregnant baboons therefore represent a key step toward clinical trials in humans.

Advances in siRNA-based therapeutics, specifically in the area of liver diseases, recently resulted from two conceptual breakthroughs: extreme chemical stabilization of oligonucleotides and optimization of a trimeric GalNAc conjugate that drives selective uptake by hepatocytes. Fully chemically modified siRNAs provide unprecedented durability and sustained silencing activity—e.g., up to 9 months duration of effect after a single injection. The hydrophobic cholesterol conjugate supports siRNA delivery to a range of tissues, including liver, kidney, bone marrow, spleen, muscle and placenta (this study and refs. 23, 25–27, 35, 36, 56–58). In mice, cholesterol-conjugated siRNA predominantly accumulated in liver and kidney, but up to 7% of the injected dose accumulated in the placenta. The cholesterol moiety was essential for siRNA accumulation in placenta. Although the cholesterol conjugate does not provide selective delivery to placenta, delivery to a range of tissues allows functionally selective silencing when the intended target is a gene overexpressed in only one of those tissues. In the case of preeclampsia, sFLT1 is specifically overexpressed in placenta.

sFLT1 represents an exceptional target for siRNA-based therapeutics. Despite substantial effort, attempts to develop small molecules and antibodies that selectively downregulate sFLT1 have failed—perhaps not surprisingly, since sFLT1 protein sequences are almost identical to the extracellular domain of full-length FLT1. Recently, a nanoparticle-formulated siRNA that nonspecifically targets sFLT1 and full-length Flt1, with a ~1 mg/kg dose, was found to improve outcomes in a rat model of preeclampsia. Nevertheless, rodents do not express the predominant isoform of sFLT1 mRNA made in humans, and systemic delivery of therapeutic siRNAs that target full-length FLT1 could be a liability. Our approach, despite higher dosage, therefore takes advantage of FLT1 alternative polyadenylation, which creates a unique sequence space in sFLT1 mRNA isoforms that can be selectively targeted by siRNAs.

Our data suggest that a single injection of anti-sFLT1 hsiRNAs might suppress sFLT1 levels for several weeks, potentially extending pregnancy duration. In preeclampsia, extension of pregnancy by 2–4 weeks is of clinical significance, especially when the disease occurs preterm. The desired level of sFLT1 silencing is only 30% to 40% (ref. 18), with a higher degree of silencing being potentially disadvantageous owing to reduced uterine blood flow as a consequence of reduced blood pressure. We found that a 10 mg/kg dose produced >50% silencing in baboons, so dosing needs to be carefully titrated to tune sFLT1 silencing to the desired level. A strong relationship exists between hsiRNA dosing and the level and duration of target silencing.

Notably, the initial phases of hsiRNA clearance were different in baboons and mice. In mice, >99% of hsiRNA was cleared within an hour after injection, but in baboons, hsiRNA blood levels remained constant during the first hour after injection (i.e., around 100 to 200 ng/ml at 15, 30 and 60 min). The rapid clearance of oligonucleotides in rodents compared to nonhuman primates has been previously reported for other classes of oligonucleotides and might reflect the higher metabolic rate of rodents. Careful pharmacological studies are needed to define an optimal chemical composition, clinical dose and schedule to achieve the ideal level and duration of sFLT1 silencing.

Clinical utility also depends on an acceptable overall toxicity and safety profile. Complete knockdown of sFLT1 in trophoblasts with a lentivirally mediated siRNA approach has been associated with decidual hemorrhage. However, at the doses tested (10 to 40 mg/kg), sFLT1 protein was reduced by 50%, which was not associated with adverse effects on blood chemistry; inflammatory markers; or pup viability, weight, vigor or placental vascularization. Notably, our data indicate that hsiRNAs administered during pregnancy are not transmitted to the fetal tissues other than placenta, minimizing concerns that sFLT1 silencing in the fetus would negatively affect fetal development or pup viability.

Preeclampsia is a disease with unmet medical need in both developed and underdeveloped countries. We show that siRNA-mediated silencing of sFLT1 mRNAs represents a viable path toward development of a preeclampsia therapeutic, particularly when the disease presents preterm. In dry form, siRNAs can be stored at ambient temperature for extended periods of time, they are relatively simple to manufacture, and they can be administered by subcutaneous injection. Thus, siRNA therapeutics potentially hold promise to address the needs of patients with preterm preeclampsia independently of economic status.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.
ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

A.A.T. managed the project, performed most of the experiments and drafted the manuscript, M.J.M., S.A.K. and A.K. conceived the study and led the project. M.R.H., D.E.M. and L.R. synthesized all compounds and developed and optimized protocols for gram-scale synthesis. A.H.C. and B.M.D.C.G. helped with in vivo studies. R.A.H. performed RNA assay analysis. A.-A. P. performed polyadenylation site sequence analysis and an initial siRNA screen. J.F.A. helped with the siRNA screen. A.L. performed primary cytotrophoblast isolations, ELISAs, toxicity studies and mouse pregnancy studies. Z.K.Z. performed placenta vascular immunohistochemistry. A.H., A.M., S.P., J.L. and R.O. developed the baboon preeclampsia model and performed all baboon experiments. A.A.T., A.K., M.J.M., S.A.K. and A.H. wrote the manuscript.

COMPETING INTERESTS

A.K. discloses ownership of stock in RXi Pharmaceuticals and Advirna. S.A.K. is a consultant to Thermofisher Scientific and owns stock in Aggamin Therapeutics. M.J.M. is employed by Moderna Therapeutics.

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1. Stevens, W. et al. Short-term costs of preeclampsia to the United States health care system. Am. J. Obstet. Gynecol. 217, 237–248.e216 (2017).
2. Maynard, S.E. et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Invest. 113, 649–658 (2003).
3. Levine, R.J. et al. Circulating angiogenic factors and the risk of preeclampsia. N. Engl. J. Med. 350, 672–683 (2004).
4. Heydarian, M. et al. Placental-specific sFLT-1 e15a protein is increased in maternal plasma in preeclampsia has a uterine origin. J. Med. Chem. 54, 3673–3677 (2011).
5. Redman, C.W. & Sargent, I.L. Latest advances in understanding preeclampsia. J. Med. Chem. 57, 1648–1657 (2014).
6. Silencing myostatin using cholesterol-conjugated siRNAs induces muscle hypertrophy in vivo. Mol. Ther. Nucleic Acids 46, 3579–3594 (2018).
7. Chaiworapongsa, T. et al. Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma is a uterine origin. J. Am. Soc. Nephrol. 27, 903–913 (2016).
8. Sela, S. et al. A novel human-specific soluble vascular endothelial growth factor receptor 1: cell-type-specific splicing and implications to vascular endothelial growth factor homeostasis. Circ. Res. 102, 1566–1574 (2008).
9. Ahmad, S. & Ahmed, A. Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. Circ. Res. 95, 884–891 (2004).
10. Redman, C.W. & Sargent, I.L. Latest advances in understanding preeclampsia. Science 308, 1592–1594 (2005).
11. Vorová, S. et al. Induction of antagonistic soluble decoy receptor tyrosine kinases by intronic polyA activation. Mol. Cell 43, 927–939 (2011).
12. Rajkumar, A. et al. Transcriptionally active syncytiotrophoblasts in the maternal circulation may contribute to circulating soluble fms-like tyrosine kinase 1 in preeclampsia. Hypertension 59, 256–264 (2012).
13. Makris, A. et al. Uteroplacental ischemia results in proteinuric hypertension and elevated sFlt-1. Kidney Int. 71, 977–984 (2007).
14. Armbati, B.K. et al. Conical avascularity is due to soluble VEGF receptor-1. Nature 443, 993–997 (2006).
15. Lertkiatmongkol, P., Liao, D., Mei, H., Hu, Y. & Newman, P.J. Endothelial functions of single-stranded RNAs use RNAi to potently and allele-selectively inhibit mutant huntingtin expression. Cell 150, 740–750 (2016).
16. Nair, J.K. et al. Impact of enhanced metabolic stability on pharmacokinetics and pharmacodynamics of GalNAc-siRNA conjugates. Nucleic Acids Res. 45, 10969–10977 (2017).
17. Redman, C.W. & Sargent, I.L. Latest advances in understanding preeclampsia. J. Med. Chem. 54, 3673–3677 (2011).
18. Sela, S. et al. Angiogenic factors and the risk of adverse outcomes in women with suspected preeclampsia. Circulation 125, 911–919 (2012).
19. Chaiworapongsa, T. et al. Plasma concentrations of angiogenic/anti-angiogenic factors have prognostic value in women presenting with suspected preeclampsia to the obstetrical triage area: a prospective study. J. Matern. Neonatal Med. 27, 132–144 (2014).
20. Khvorova, A. & Watts, J.K. The chemical evolution of oligonucleotide therapeutics of clinical utility. Nat. Biotechnol. 35, 238–248 (2017).
53. Pasi, K.J. et al. Targeting of antithrombin in hemophilia A or B with RNAi therapy. N. Engl. J. Med. 377, 819–828 (2017).

54. Matsuda, S. et al. siRNA conjugates carrying sequentially assembled trivalent N-acetylgalactosamine linked through nucleosides elicit robust gene silencing in vivo in hepatocytes. ACS Chem. Biol. 10, 1181–1187 (2015).

55. Nair, J.K. et al. Multivalent N-acetylgalactosamine-conjugated siRNA localizes in hepatocytes and elicits robust RNAi-mediated gene silencing. J. Am. Chem. Soc. 136, 16958–16961 (2014).

56. Wolfrum, C. et al. Mechanisms and optimization of in vivo delivery of lipophilic siRNAs. Nat. Biotechnol. 25, 1149–1157 (2007).

57. Krützfeldt, J. et al. Silencing of microRNAs in vivo with ‘antagomirs’. Nature 438, 685–689 (2005).

58. Baerlocher, G.M., Burington, B. & Snyder, D.S. Telomerase inhibitor imetelstat in essential thrombocythemia and myelofibrosis. N. Engl. J. Med. 373, 2580 (2015).

59. Yu, J.J., Jia, J., Guo, X., Chen, R. & Feng, L. Modulating circulating sFlt1 in an animal model of preeclampsia using PAMAM nanoparticles for siRNA delivery. Placenta 58, 1–8 (2017).

60. Khankin, E.V., Mandala, M., Colton, I., Karumanchi, S.A. & Osol, G. Hemodynamic, vascular, and reproductive impact of FMS-like tyrosine kinase 1 (FLT1) blockade on the uteroplacental circulation during normal mouse pregnancy. Biol. Reprod. 86, 57 (2012).

61. Yu, R.Z. et al. Cross-species pharmacokinetic comparison from mouse to man of a second-generation antisense oligonucleotide, ISIS 301012, targeting human apolipoprotein B-100. Drug Metab. Dispos. 35, 460–468 (2007).

62. Geary, R.S., Norris, D., Yu, R. & Bennett, C.F. Pharmacokinetics, biodistribution and cell uptake of antisense oligonucleotides. Adv. Drug Deliv. Rev. 87, 46–51 (2015).

63. Savage, V.M. et al. Scaling of number, size, and metabolic rate of cells with body size in mammals. Proc. Natl. Acad. Sci. USA 104, 4718–4723 (2007).

64. Fan, X. et al. Endometrial VEGF induces placental sFlt1 and leads to pregnancy complications. J. Clin. Invest. 124, 4941–4952 (2014).
ONLINE METHODS

hsiRNA design. We designed and synthesized a panel of 47 hsiRNA compounds targeting the human sFLT1-i3 and e15a mRNA. These sequences span the unique sequence regions and were selected to comply with standard hsiRNA design parameters including assessment of G+C content, specificity and low seed complement frequency, elimination of sequences containing mRNA seeds, and examination of thermodynamic bias. (Supplementary Table 1).

Synthesis of oligonucleotides. Oligonucleotides were synthesized using standard and modified (2’-F, 2’-O-Me) phosphoramidite solid-phase synthesis conditions using a MerMade 12 (BioAutomation, Irving, Texas) and Expedite DNA/RNA synthesizer (ABI 8909). Unconjugated oligonucleotide strands were grown on controlled pore glass (CPG) functionalized with a long-chain alkyl amine and Unylinker terminus (Chemgenes, N-4000-10), and cholesterol-conjugated oligonucleotides were synthesized on modified solid support (Chemgenes, N-9166-05). Oligonucleotides were removed from CPG, deprotected, and purified by HPLC as described previously. Purified oligonucleotides were passed over a Hi-Trap cation exchange column to exchange the counterion with sodium.

Large-scale oligonucleotide synthesis was done on an AKTA Oligopilot 100 using standard protocols. Each synthesis was performed at the 200-µmol scale using Unylinker (ChemGenes, Wilmington, MA) for the antisense strand and a custom solid support containing a cholesterol-conjugated CPG for the sense strand. Phosphoramidites were prepared at 0.15 M concentrations for both the 2’-O-methyl (ChemGenes, Wilmington, MA) and 2’-fluoro (BioAutomation, Irving, Texas) amidites in acetonitrile. 5-(Benzylihydroxy)-1H-tetrazole was used as the activator at 0.25 M. Detryurations were performed using 3% dichloroacetic acid in CH₂Cl₂. Capping was done with non-THF-containing reagents consisting of 20% N-methylimidazole in acetonitrile as capping reagent A and 20% acetic anhydride, 30% 2,6-lutidine in acetonitrile as capping reagent B. Sulfurization was performed with 0.1 M DTT in acetonitrile for 3 min. Phosphoramidite coupling times were 8 min for all amidites used. All oligonucleotides were confirmed by HPLC analysis and mass spectrometry.

Cell culture. HeLa and WM-115 cells were purchased from ATCC (ATCC, Manassas, VA; CCL-2, CLR-165). HeLa cells were maintained in Dulbecco’s Modified Eagle’s Medium (Cellgro, Corning, NY; 10-013CV) supplemented with 10% FBS (Gibco, Carlsbad, CA; 26140) and 100 U/mL penicillin/streptomycin (Invitrogen, Carlsbad, CA; 15140) and grown at 37 °C in 5% CO₂. Cells were split every 2 to 5 days and discarded after 15 passages. WM-115 cells were maintained in Eagle’s Minimum Essential Medium (Gibco, Carlsbad, CA; 11095) supplemented with 2 mM glutamine, 1% nonessential amino acids, 1% sodium pyruvate, 10% FBS (all from Gibco) and 100 U/mL penicillin/streptomycin (Invitrogen) and grown at 37 °C in 5% CO₂. Cells were split every 2 to 4 d. CTBs were isolated from human placenta (under Institutional Review Board approval) using Unylinker (ChemGenes, Wilmington, MA) for the antisense strand and using a custom solid support containing a cholesterol-conjugated CPG for the sense strand. Direct delivery (passive uptake) of oligonucleotides.

Direct delivery (passive uptake) of oligonucleotides. For initial hsiRNA design, cDNA sequences corresponding to unique regions of human sFLT1-i3 and e15a mRNA were cloned into psiCheck-2 vector (Promega, Madison, WI; C8021) and a DualGlo luciferase assay system was used (Promega, Madison, WI; E2920) according to the manufacturer’s manual. Briefly, HeLa cells on a 10-cm dish were transfected with psiCheck-2-i13 or psiCheck-2-e15a plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA; 11668019) according to the manufacturer’s manual. Cells were plated 24 h later in DMEM containing 6% FBS at 10,000 cells per well in 96-well cell culture plates. hsiRNA oligo was diluted to twice the final concentration in OptiMEM (Carlsbad, CA; 31985-088), and 50 µL diluted hsiRNA was added to each well. Oligos were also assayed at 30% of each matched dose for in vitro dose response assays. To test hsiRNAs in CTB and WM-115 cells, cells were plated at 20,000–30,000 cells per well in 96-well plates in M199 or EMEM media containing 3% FBS and treated with hsiRNAs for 72 h.

ELISA and AST and ALT assays. ELISA for human and mouse sFLT1 was performed according to the manufacturer’s instructions (R&D Systems; Quantikine FLT1, MVR100). All assays were done in duplicate, and protein levels were calculated using a standard curve derived from known concentrations of the respective recombinant proteins. AST and ALT activities in mouse serum were measured according to the manufacturer’s instructions using colorimetric assay kits (Sigma; MAK055 and MAK052).

PNA hybridization assay. hsiRNA guide (antisense) strand accumulation in mouse tissues was quantified using a PNA hybridization assay, as described previously. Briefly, tissue punches were homogenized in MasterPure Tissue Lysis Solution (EpiCentre) with added protease K (2 mg/mL, Invitrogen) and homogenized using a TissueLyser II (Qiagen), using 100 µL of lysis solution per 10 mg tissue. Following lysis, sodium dodecyl sulfate (SDS) was precipitated with KCl (3 M) and cleared supernatant was hybridized to a Cy3-labeled PNA oligonucleotide fully complementary to the guide strand (PNABio). This mixture was analyzed by high-performance liquid chromatography (HPLC). Cy3-labeled peaks were integrated and plotted on an internal calibration curve. The mobile phase for HPLC was 50% water, 50% acetonitrile, 25 mM Tris-HCl (pH 8.5) and 1 mM ethylenediamine tetraacetate. The salt gradient was 0–800 mM NaClO₄.

Animals, efficacy and safety. Wild-type C57BL/6J or pregnant CD1 mice were purchased from Jackson Laboratory (Bar Harbor, ME). All animal procedures were approved by the University of Massachusetts Medical School Institutional Care and Use of Animals Committee (IACUC, protocol number A-2411) or the Beth Israel Deaconess Medical Center IACUC (protocol number 048-2017). Mice were 6 to 10 weeks old at the time of experiments. All animals were kept on a 12-h light/dark cycle in a pathogen-free facility, with food and water provided ad libitum.

Four 2.0-mm punches per side were made, three processed individually for the Quantigene 2.0 assay (Affymetrix, Q50011). For systemic...
administration of hsiRNA<sup>sFLT1</sup>, mice were injected with either phosphate-buffered saline (PBS) or different amounts of hsiRNA<sup>sFLT1</sup> or hsiRNA<sup>NTC</sup> resuspended in PBS, either through the tail vein or subcutaneously at the nape of the neck (interscapular). After treatment, mice were deeply anesthetized with 0.1% Avertin, and after cervical dislocation, tissues were collected and processed immediately or stored in RNAlater (Sigma, R0901) for later use.

5′-RACE-PCR analysis. Total RNA was isolated from placentas from mice treated with of hsiRNA<sup>sFLT1</sup>, hsiRNA<sup>NTC</sup> (2 × 20 mg/kg, IV) and PBS as described above, using TRizol reagent (Life Technologies). Five micrograms of isolated RNA was used for 5′-RACE analysis using GeneRacer kit (Life Technologies) without initial pretreatment as described before<sup>24</sup>. Total RNA samples were ligated to the GeneRacer RNA adaptor, and ligated RNA was used for reverse transcription using a gene-specific primer (GSP1: 5′-GCGTCCCC AGGTTGCTGTATAGCAGAA-3′). cDNA was amplified by PCR using GSP1 and GeneRacer 5′ primer (5′-CGACTGGAGCAGGACACTG-3′). Amplified PCR products were resolved and visualized on an agarose gel with ethidium bromide. Final PCR products were cloned into the pCR 4-TOPO vector (Invitrogen), and a specific cleavage site was confirmed by sequencing.

Immunohistochemistry. Paraffin sections (2 μm) of placental tissue were deparaffinized and rehydrated. Optimal staining was achieved with an anti-ogen retrieval method that was performed in 10 mM citric acid, pH 6.00 for 15 min. Endogenous peroxidases were quenched with 3% H<sub>2</sub>O<sub>2</sub> in ddH<sub>2</sub>O for 15 min. Sections were blocked with 2.5% normal horse serum at room temperature for 40 min and incubated 40 min with a 1:50 dilution of primary CD31 antibody (ab124432, Abcam, Cambridge, MA). Specific labeling was detected with an ImmPRESS HRP anti-rabbit IgG (peroxidase) polymer detection kit (MP-7401, Vector Laboratories, Burlingame, CA). The enzymatic reaction product was achieved by using VECTOR NovaRED Peroxidase (HRP) substrate to give a red-brown precipitate, and the sections were counterstained with hematoxylin, dehydrated, and mounted in Permount (Thermo Fisher Scientific, Atlanta, GA). Sections with no primary antibody were used as negative control slides.

Nonhuman primate model of preeclampsia. These experiments were approved by the Sydney Local Health District Animal Welfare Committee. We used a well-established baboon uterine artery ischemia model of preeclampsia that has been described previously<sup>41</sup>. This model is characterized by rapid rise in plasma sFLTI levels that correlate with the onset of hypertension following placental ischemia<sup>43</sup>. Female pregnant baboons (Papio hamadryas) from the National Baboon Colony, New South Wales, Australia, were provided food and water ad libitum. At the beginning of the protocol the animals were at 127 d of gestation of a normally 182-d gestation. Animals were anesthetized using ketamine infusion (0.2 mg kg<sup>−1</sup> min<sup>−1</sup>), with premedication with metoclopramide (5 mg intramuscularly) and clonazepam (intravenously at 0.01 mg/kg for seizure prophylaxis). All animals received benzylpenicillin and gentamicin intravenously at the time of surgery. They also received buprenorphine (0.02 mg/kg, intramuscularly) for analgesia, before immediately, after 8 h after and 16 h after the procedure. Pain was scored and further analgesia could be administered but was not required. All animals were acclimatized for several days and then a radiotelemeter was surgically inserted into a branch of the femoral artery and passed into the aorta to about the level of the renal arteries, to monitor intra-arterial blood pressure (PA-D70, DataSci, Minnesota, USA) as has been described previously<sup>13</sup>. After 1 week for recovery, uteroplacental ischemia (UPI) was induced experimentally. Briefly, animals underwent nondominant unilateral uterine artery complete ligation through a midline abdominal transperitoneal incision. The iliac vessels were visualized and the uterine artery identified at its point of origin from the internal iliac artery. The uterine artery was dissected and irrigated with 1% lidocaine solution to reduce arterial spasm. The vessel was then ligated with 4.0 silk sutures. Complete ligation was verified by performing repeat uterine artery duplex ultrasonography after the procedure that demonstrated no flow in the artery where previously flow had been demonstrated. The peritoneum, muscle and skin were closed in layers. After surgery, blood and urine samples were collected on days –6, 0, 3, 6, 10 and 14, as well as a chorial villus samples collected on around days 3, 6, 10 and 14 of UPI. The exact times of blood collection are shown in the figures. The variability in exact time of blood collection is due to logistical complications of working with a free-living baboon colony. hsiRNA<sup>sFLT1</sup> (equimolar mixture of hsiRNA<sup>sFLT1</sup>-2283 and hsiRNA<sup>sFLT1</sup>-2519 (20 mg/kg total) was injected intravenously within an hour of the UPI procedure. A timeline demonstrates when the procedures were performed (Fig. 5a). Renal biopsies were done as previously described<sup>26</sup>. All animals were allowed to deliver spontaneously (natural labor) and fetal weights measured at birth with the exception of animal 3 (which received hsiRNA therapy), which underwent emergency caesarean section for an unrelated condition (acute chorioamnionitis confirmed by histology) at gestational day 163 and was then euthanized. Data for controls were derived from sham surgery animals as described previously<sup>41</sup>.

Statistical analysis. Data were analyzed using GraphPad Prism 7 software (GraphPad Software, Inc., San Diego, CA). Concentration-dependent IC<sub>50</sub> curves were fitted using a log(inhibitor) versus response – variable slope (four parameters). The lower limit of the curve was set to zero, and the upper limit of the curve was set to 100. For each independent mouse experiment, the level of knockdown at each dose was normalized to the mean of the control group (PBS or untreated groups). In vivo data were analyzed using a Kruskal–Wallis one-way ANOVA with Dunn’s post hoc analysis or two-way ANOVA (time course of sFLTI serum concentration) as appropriate for experimental design. Differences in all comparisons were considered significant at P values less than 0.05.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. The data supporting the findings of this study are available from the corresponding authors upon reasonable request.

References:

65. Anderson, E., Boese, G., Khvorova, A. & Karpilow, J. Identifying siRNA-induced off-targets by microarray analysis. Methods Mol. Biol. 442, 45–63 (2008).

66. Osborn, M.F., et al. Guanabenz (Wytensin™) selectively enhances uptake and efficacy of hydrophobically modified siRNAs. Nucleic Acids Res. 43, 8664–8672 (2015).

67. Rajakumar, A., et al. Novel soluble Flt-1 isoforms in plasma and cultured placental explants from normotensive pregnant and preeclamptic women. Placenta 30, 25–34 (2009).

68. Coles, A.H., et al. A high-throughput method for direct detection of therapeutic oligonucleotide-induced gene silencing in vivo. Nucleic Acid Ther. 26, 86–92 (2016).

69. Nikan, M., et al. Docosahexaenoic acid conjugation enhances distribution and safety of siRNA upon local administration in mouse brain. Mol. Ther. Nucleic Acids 5, e344 (2016).
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- Experimental design

1. Sample size
   - Describe how sample size was determined. 
   - Power calculations done in IACUC protocol referenced in Online Methods section

2. Data exclusions
   - Describe any data exclusions. 
   - No data exclusions, no animal were excluded

3. Replication
   - Describe whether the experimental findings were reliably reproduced. 
   - All attempts of replication were successful

4. Randomization
   - Describe how samples/organisms/participants were allocated into experimental groups. 
   - No randomization

5. Blinding
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis. 
   - No blinding

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| Item | Status |
|------|--------|
| n/a  | Confirmed |
| ☒ ☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) | 
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| ☒ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons | 
| ☒ ☐ The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted | 
| ☒ ☐ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) | 
| ☒ ☐ Clearly defined error bars | 

See the web collection on statistics for biologists for further resources and guidance.
Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Data were analyzed using GraphPad Prism 7 software.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository [e.g. GitHub]. Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

Full chemical composition of oligonucleotides used was reported in Online Methods section and can be ordered from commercial sources.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

CD31 antibody (ab124432, Abcam, Cambridge, MA). Antibodies has been validated for their use as an endothelial marker in multiple studies in addition to validation statement on manufacturer’s website.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

HeLa, WM-115 were purchased from ATCC. CTB from S. A. Karumanchi as described in Online Methods

b. Describe the method of cell line authentication used.

No cells authentication were done

c. Report whether the cell lines were tested for mycoplasma contamination.

HeLa and WM-115 cells were mycoplasma tested.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by iCLAC, provide a scientific rationale for their use.

No commonly misidentified cells were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Strains reported in Online Method section

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human subjects