Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 01/01/09-12/31/09

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl A. Richards, MBA

4. **Grant Contact Person's Telephone Number:** 412-641-8932

5. **Grant ME Number or SAP Number:** 4100047639

6. **Project Number and Title of Research Project:** Project 05 - Soluble KIT Receptor in the Pathogenesis of Preeclampsia

7. **Start and End Date of Research Project:** 01/01/09-12/31/09

8. **Name of Principal Investigator for the Research Project:** Carl A. Hubel, PhD

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $ 158,537.24

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Hubel	Associate Professor	40%	$43,391.48
Plymire	Research Associate	50%	$17,865.48

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____x_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____x_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___x_____ No_____

If yes, please describe your plans:
The plan is to submit a National Institutes of Health Exploratory/Developmental Research Grant Award (R21) application in October 2010.

12. **Future of Research Project.** What are the future plans for this research project?

We will work toward an initial peer-reviewed publication and submission of an R21 application to NIH. To ensure this, we plan to obtain further evidence that the enzyme-linked immunoassay (ELISA) for soluble c-kit (sc-kit) is accurately measuring sc-kit released from human mast cells in culture. On February 17, 2010 we received an aliquot of HMC-1 cells (human mast cell line; Mayo Clinc Reference #MMV-88-049) as a kind gift from Joseph H. Butterfield, M.D., Mayo Clinic. HMC-1 is a unique human cell line that can serve as a resource for mast cell proteins including soluble receptors. HMC-1 cells lack a functional IgE receptor and have mutations in the coding sequence of the c-Kit proto oncogene. The HMC-1 cells, with and without small interfering (si)RNA blockade of c-kit synthesis, will be used to validate the ELISA assays. Once this and a few other assay validation tests are accomplished we feel that we will have the necessary material for submission of a manuscript entitled "Reduced Concentration of Soluble c-Kit Receptor in the Maternal Circulation: an Early Marker for Preeclampsia".

We will use the HMC-1 cells to study circulating and placenta-derived factors that may regulate shedding of c-kit from the cell surface in vitro. The R21 will be designed to further study the regulation and biological effects of c-kit, including effects on endothelial progenitor cells, of sc-kit in cells in culture and in pregnant mice.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____x_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

    Yes_____ No_____x_____

    If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

    Yes_____ No____x_____

    If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. **Collaboration, business and community involvement.**

    16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

    Yes____x_____ No_____

    If yes, please describe the collaborations:

To facilitate our research, we recently received a mast cell line, HMC-1,that secrete measureable quantities of sc-kit, from J.H. Butterfield, MD, Mayo Clinic, Rochester, MN. I have corresponded with him directly regarding the maintenance and use of these cells, and

will also do so regarding interpretation of data. I am optimistic that this will lead to more formal research collaboration with his research group in the future.

16(B) Did the research project result in commercial development of any research products?

Yes_____ No_____x_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____x_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Research Plan:

The Project will begin to investigate the potential importance of the soluble receptor *sc-kit* (CD117, also known as stem cell factor soluble receptor, SCFsR) in preeclampsia, potentially the third dysregulated, angiogenic soluble receptor contributing to this pregnancy disorder.

Hypothesis 1. Maternal plasma *sc-kit* concentrations are reduced at mid-pregnancy in women who subsequently develop preeclampsia compared to women whose pregnancies will remain uncomplicated.
Specific Aim 1: A nested, case-control comparison will be conducted, comprised of 60 nulliparous women with uncomplicated normotensive pregnancies who delivered appropriate weight for gestational age (AGA) neonates and 30 nulliparous women who developed preeclampsia, matched (2:1) for gestational age at blood sampling (venipuncture) and race. Concentrations of *sc-kit* and SCF (kit ligand) will be compared longitudinally using archived maternal plasma samples obtained prior to clinically evident preeclampsia at mean gestational ages of 8 weeks (range 6-12 weeks), 18 weeks (range 15-24), 29 weeks (range 27-34), and predelivery at the time of diagnosis of/hospital admission for preeclampsia (mean 34.4 weeks, range 27-41 weeks). Umbilical cord (fetal) blood samples will also be obtained after delivery and compared for SCF and sc-kit levels.

Hypothesis 2: Lower median plasma *sc-kit* concentrations will remain evident 18 months postpartum in women with a history of preeclampsia compared to women with a history of normal pregnancy, consistent with constitutional differences between these groups of women or lasting effects of preeclampsia. Circulating *sc-kit* will correlate inversely with measures of insulin resistance, blood pressure, body mass index and family history of cardiovascular disease.

Specific Aim 2: Plasma *sc-kit* and SCF concentrations will be measured in relation to fasting glucose and insulin concentrations in women with a history of preeclampsia (n = 30) and women with a history of normal pregnancy (n=30), 6 to 24 months post first pregnancy. Correlation of *sc-kit* and SCF with insulin resistance, body mass index and cardiovascular variables will be assessed.

Hypothesis 3. Relative to normal pregnancy placentas, placentas from pregnancies complicated with preeclampsia express lower protein levels of *kit* (both 140-kDa membrane *c-kit* and 100-kDa *sc-kit*) and SCF, and secrete lower quantities of *sc-kit* and SCF in culture media in an hypoxia-insensitive fashion.

Specific Aim 3: Imbalances in placenta-derived angiogenic growth factors have been implicated in the pathogenesis of preeclampsia. Expression levels of *kit* (both 140-kDa membrane *c-kit* and 100-kDa *sc-kit*) and SCF will be compared in third trimester archived placental biopsies from

preeclamptic and normal pregnancies. We will also ask if third trimester preeclampsia placental villous tissue in fresh explant culture show significant reductions in SCF and *kit* protein mass, and secrete less SCF and *sc-kit* into the culture medium compared to normal pregnancy placental tissue. Experiments will also be performed to determine whether concentrations of *kit* in villous explant culture and explant conditioned media are reduced under low oxygen partial pressure (2% oxygen) compared to standard culture conditions (21% oxygen).

Research Completed:

Specific Aim 1:
We have found that the number of circulating EPCs increases with advancing normal pregnancy but not in women with preeclampsia. Circulating *sc-kit* may serve as a surrogate measure of *sc-kit* (CD117)- positive endothelial progenitor cells and *sc-kit* is known to mediate the mobilization of these cells from bone marrow to the circulation. Given these potential relationships, we sought to test the hypotheses 1) that women with preeclampsia are distinguished by abnormally low concentrations of the soluble receptor *sc-kit* in maternal or umbilical cord (fetal) blood compared to both controls with uncomplicated (normotensive) pregnancies and women with just gestational hypertension without proteinuria or other hallmark signs of preeclampsia, and 2) that maternal plasma *sc-kit* concentrations are reduced early in pregnancy (~19 weeks' gestation) in women who subsequently develop preeclampsia compared to women whose pregnancies remain uncomplicated.

Methods, Specific Aim 1:

Overview of study subjects and sample processing:
We used archived plasma samples from the Preeclampsia Program Project (National Institutes of Health P01 HD030367) longitudinal and cross-sectional studies of pregnant women enrolled at their first prenatal visit and followed through the immediate postpartum period. These women delivered at Magee-Womens Hospital within the years 1997–2004, and were recruited from clinics and private practices (University of Pittsburgh IRB #0404159). Thus, all participants provided written informed consent for use of their samples and de-identified clinical data under the umbrella of the Preeclampsia Program Project and related ancillary studies by the same investigators. A jury of clinicians met monthly to determine pregnancy diagnosis of all the women. Criteria for gestational hypertension were an absolute blood pressure of > 140 mmHg systolic and/or > 90 mmHg diastolic. Proteinuria was defined as > 300 mg of protein in a 24-hour urine collection, or > 2+ on a voided or > 1+ on a catheterized random urine sample, or a random urine protein/creatinine ratio of > 0.3. Hyperuricemia was defined as > 1 standard deviation above normal for the given gestational age [at term > 5.5 mg/dL (3.3 mM)].

Preeclampsia is defined as: 1) new onset hypertension arising after 20 weeks' gestation, plus 2) manifesting proteinuria, plus 3) manifesting hyperuricemia, with reversal of hypertension and proteinuria postpartum. Gestational hypertensives met the blood pressure criteria but lacked significant proteinuria and hyperuricemia. Uncomplicated pregnancy controls were normotensive, without proteinuria or hyperuricemia throughout gestation, and delivered of healthy babies at term. All participants were without illicit drug use, and had no history of renal or vascular disease.

Maternal and umbilical cord (fetal) nonfasting plasma samples were collected 10 mL sterile tubes containing 4 mmol/L potassium-ethylenediaminetetraacetic acid (EDTA) and processed within 2 hours of collection. The plasma was stored in aliquots at -80°C without thaw until further analysis.

Cross-sectional maternal study methods:
Women with preeclampsia develop the disease and deliver at an earlier gestational age on average compared to controls or women with gestational hypertension without proteinuria. We therefore group- matched women with preeclampsia and women with gestational hypertension to separate control groups according to the gestational age at the time of venipuncture. Thus, we compared soluble sc-kit and SCF) in third trimester plasma samples from 26 women with preeclampsia (mean ± SD gestational weeks at venipuncture: 34.1 ± 3.0) matched to 27 controls (34.6 ± 2.7 weeks; P=0.49) and separately compared 12 women with gestational hypertension (39.1 ± 1.9 weeks) matched to a different set of 10 controls (39.1 ± 1.9 weeks; P=0.99). Plasma sc-kit, SCF, placental growth factor (PlGF, a pro-antiogenic factor decreased in preeclampsia) and soluble fms-like tyrosine kinase-1 (sFlt-1, an anti-angiogenic factor increased in preeclampsia) were each measured in duplicate by enzyme-linked immunoassays (ELISA) purchased from R&D Systems. The human quality control samples available from R&D Systems were used.

To corroborate the ELISA results, we performed immunoprecipitation of sc-kit followed by Western analysis of sc-kit in pools of plasma of women with normal pregnancy and women with preeclampsia. Two pools of plasma (200 µL each, pooled from n=4women with preeclampsia and n=4 women with normal prengnacy) were diluted to 0.5 ml with sterile PBS and mixed with equal volume of 2X buffer, in the presence of protease and phosphatase inhibitors. To this 1ml sample, we added 20 µL of protein A/G sepharose to pre-clear any non-specific proteins. The protein A/G agarose was then removed by centrifugation. Ten µL of the human specific anti c-kit (SCF-R; CD117, c-kit) antibody (R&D Systems) was added to the supernantant and incubated overnight at 4 C. These samples were further incubated for an additional 30 min with proteinA/G sepharose and the immune complexes were captured and washed by centrifugation. The immunoprecipitated proteins were solubilized in 1X Laemmli buffer and subjected to electrophoresis followed by Western analysis using three different antibodies: 1) the same R&D Systems antibody used in the prevous immunoprecipitation step, 2) a c-kit antibody from Santa Cruz biotechnology and 3) a c-kit antibody from eBioscience.

Cross-sectional umbilical cord (fetal) blood study methods:
Concentrations of SCF are reportedly significantly higher in fetal cord blood and neonatal blood than in the maternal blood, and strikingly high SCF mRNA expression and moderate c-kit mRNA expression is found in human umbilical vein (fetal) endothelial cells. This suggests that endothelial cells are an important source of SCF in cord blood (perhaps during development of the fetoplacental vasculature and blood flow). If kit/SCF dysregulation exists in the fetoplacental circulation, this might be of pathophysiologic significance. We therefore measured SCF and sc-kit in cord blood samples (plasma) that were available from a subset of the cross-sectional patients (n=19 preeclamptic pregnancies and n= 22 control pregnancies).

Longitudinal case-control study methods:
Sc-kit concentrations were measured in a 2:1 (case:control) study of gestational age-matched maternal plasma samples collected at mid-pregnancy from 26 women who later developed preeclampsia and from 52 normotensive controls with uncomplicated outcome; third trimester samples were obtained during preeclampsia from 24 of the 26 cases and 46 of the 52 controls. Plasma sc-kit was measured by ELISA in duplicate (R&D Systems).

Longitudinal sc-kit changes during uncomplicated pregnancy, methods:
Our data using gestational age-matched maternal plasma samples at mid-pregnancy and 3rd trimester, from women who later developed preeclampsia compared to uncomplicated (normotensive) pregnancy controls, suggested a decline in sc-kit concentrations with advancing gestation. To demonstrate this directly, we assayed longitudinal samples collected at 4 gestational time points (weeks 5-10, 16-19, 27-30, and 40-42 (pre-delivery)) from 9 controls with uncomplicated pregnancy outcome. Plasma sc-kit, PlGF and sFlt-1 were measured in duplicate by separate ELISAs (R&D Systems), on single plates.

Statistical analyses:
Cross sectional and longitudinal case-control data were analyzed by Student's unpaired t-test, or by Mann-Whitney U test for skewed variables. Longitudinal data across normal pregnancy were analyzed by repeated measures ANOVA and Student-Newman-Keuls post-hoc pairwise comparisons. Relationships among continuous variables were assessed by Spearman rank correlation.

Results, Specific Aim 1:

Cross-sectional maternal study, results:
As shown in Table 1, patients with preeclampsia delivered earlier ($p<0.01$), had babies with lower birth weights ($p<0.01$) and birth weight centiles ($p<0.01$), and by definition had higher blood pressure at admission to labor and delivery ($p<0.001$). By definition, blood pressures were higher in gestational hypertensives compared to controls (Table 2).

Concentrations of sc-kit were significantly lower in women with preeclampsia compared to controls ($P<0.001$; Figure 1, Table 1) whereas no difference in sc-kit was observed between gestational hypertensives and their controls ($P=0.84$) (Figure 1, Table 2). Levels of sc-kit were marginally lower in the controls for gestational hypertension than controls for preeclampsia ($P=0.07$; Figure 1), likely reflecting the later gestational age of the former control group. In contrast to sc-kit, no group differences were observed for plasma SCF except, surprisingly, for lower levels of SCF in women with gestational hypertension compared to their controls ($P<0.04$; Figure 2).

The ELISA results were verified by immunoprecipitation analysis. The antibodies gave similar profiles. The R&D antibody profile is shown in Figure 3, showing fainter bands in preeclampsia samples. Note: soluble c-kit molecular weight has previously been estimated as ~90 kilodaltons (kd).

Concentrations of sc-kit are reportedly lower in non-pregnant women with systemic lupus erythematosus compared to healthy controls, partly attributable to the corticosteroid treatment that these patients receive. In our study, 15 of 26 women with preeclampsia and 1 control received injections of betamethasone prior to blood sampling. The 11 preeclamptics without betamethasone nevertheless had significantly lower plasma sc-kit concentrations than controls without betamethasone ($P<0.04$). This was despite the greater gestational age of preeclamptics who had not received betamethasone (probably reflecting milder disease of later onset) (mean 36.6 weeks) compared to preeclamptics who did receive the corticosteroid (mean 32.2 weeks), a difference ($P<0.0005$) that should have biased sc-kit levels upward in preeclamptics who had received betamethasone. Further supporting a negligible effect of betamethasone administration, sc-kit levels were not different between the subgroups of preeclamptics who did versus did not receive betamethasone ($P=0.34$). SCF levels were not different between these betamethosone +/- subgroups. No gestational hypertensives in our patient sample received betamethasone.

We looked for correlations of sc-kit or SCF with plasma concentrations of free placental growth factor (PlGF, a pro-angiogenic factor) and soluble fms-like tyrosine kinase-1 (sFlt-1; also known as soluble VEGF receptor-1, an anti-angiogenic factor increased in most women with preeclampsia that binds and thus neutralizes VEGF and PlGF). Except for a weak positive correlation of SCF with sFlt-1 ($r = 0.41$, $P<0.05$) among women with preeclampsia, no correlations were observed within any of the disease or control groups (including betamethasone +/ – subgroups). Consistent with several previous studies, sFlt-1 was significantly elevated and PlGF reduced in women with preeclampsia, but not women with gestational hypertension, compared to controls (Tables 1 and 2).

Cross-sectional umbilical cord (fetal) blood study results:
As with the maternal circulation, concentrations of sc-kit were significantly lower in umbilical cord (fetal) plasma from preeclamptic pregnancies (n=19) compared to control pregnancies (n=22) ($P<0.03$; Figure 4). As with the maternal values, cord concentrations of SCF did not differ between preeclampsia and control groups ($P=0.28$; Figure 5). Consistent with previous reports, however, fetal SCF concentrations were significantly higher in cord than maternal blood (compare Figures 4 and 5).

Longitudinal case-control study results:
Similar to the cross-sectional study subjects, longitudinal subjects who developed preeclampsia delivered earlier ($p<0.05$), had lower birth weight babies ($p<0.001$) and birth weight centiles ($p<0.01$), and by definition significantly higher blood pressure at admission to labor and delivery ($p<0.001$) (Table 3).

Plasma sc-kit concentrations were significantly reduced, both at 19 weeks (prior to clinically evident preeclampsia) (13%, $P<0.02$) and during the third trimester (during the clinical syndrome) (19%, $P< 0.005$) in women who developed preeclampsia compared to controls (Figure 6).

Soluble c-kit receptor levels declined significantly in both groups from 19 to 37 weeks (Table 3).

Longitudinal soluble receptor changes during uncomplicated pregnancy, results:
Clinical characteristics of patients with uncomplicated pregnancy who were sampled at 4 gestational stages (who were different individuals from those in other study groups) are shown in Table 4. Figure 7 shows that sc-kit concentrations decreased progressively over the course of uncomplicated pregnancy. Significant decreases occurred at the 16-19-, 27-30- and 40-42-week compared to the 5-10 week intervals, and between the 16-19 and 40-42 weeks' gestation intervals. Figure 8 confirms previous reports that plasma PlGF increases during the first 30 weeks of gestation and then declines remarkably at term. Significant differences were found between the apparent apex at weeks' 27-30 and gestational weeks 5-10, 16-19 and 40-42. In contrast to sc-kit, sFlt-1 concentrations rose over the course of gestation, except for an apparent plateau at 27-30 weeks (Figure 9). Significant correlations between sc-kit and PlGF were observed at gestational ages 16-19 (r= 0.70, P=0.03) and 27-30 (r=0.65, P=0.05) but no other correlations of the three plasma variables within any of the other time points were observed. We are not aware of previous reports suggesting a relationship of these two angiogenic factors.

ELISA assay validation results:
ELISA of sc-kit (SCFsR) and SCF: The intra-assay coefficients of variation for sc-kit and SCF in our pregnancy plasma samples, respectively, were 5.1 and 5.9%. We further validated the ELISAs for use in pregnancy samples by performing sample dilution tests on separate pools of preeclampsia and normal pregnancy plasma (n=6 patient samples per pool) as done previously for sFlt-1 and PlGF. Samples were diluted with appropriate ELISA calibrator diluent and then assayed. Percent observed/expected values of 1:2, 1:4 and 1:8 dilutions, of both analytes, approximated 100%, ranging from 91 to 114%. Sc-kit levels in pools of normal pregnancy plasma were not appreciably affected by the addition of 2 ng/mL human recombinant SCF (R&D Systems, Minnesota). Given that sc-kit levels decline with advancing gestation whereas sFlt-1 rises, we also tested whether sFlt-1 interferes with the sc-kit ELISA. A pool of normal 3rd trimester pregnancy plasma (n=4 patients), with endogenous sc-kit concentration of 11.0 ng/mL, was spiked (in two separate assays) with 0, 5000, 25,000 and 50,000 pg/mL human recombinant sFlt-1 (R&D Systems). Measured sc-kit concentrations in the spiked samples were 84, 100, and 86% of the unspiked sample, respectively, indicating no diminution in sc-kit values with increasing sFlt-1. We also performed spike-recovers tests on pregnancy plasma, comparing unspiked plasma with the same sample in which the concentration of sc-kit had been doubled by addition of recombinant sc-kit (R&D Systems). Recovery of spike ranged from 78.0 to 98.8 percent. Furthermore, we showed that 3 freeze-thaw cycles had no appreciable effect on measured plasma sc-kit levels.

We measured sc-kit using an ELISA from Cell Sciences (Cat # 850.700.192), using 9 control an 9 preeclampsia 3rd trimester cross-sectional samples previously used for measurement of sc-kit by the R&D ELISA. The Cell Sciences ELISA is claimed by the manufacturer to recognize both natural and recombinant human soluble c-kit, but with much lower reported ranges. The correlation between the to ELISA kits, although positive, was poor (r= 0.2). Therefore, we plan to examine what distinguishes these two assays.

Specific Aim 2:
We explored whether deficits in circulating *sc-kit* persist (or reemerge) 1 year after pregnancy in primiparous women with a history of preeclampsia compared to women with a history of uncomplicated pregnancy.

Methods: Study participants had given permission to be contacted for additional preeclampsia-related research studies upon consenting to participate in the Preeclampsia Program Project with IRB approval. The women enrolled in this study comprised a cross section of women recruited from the University's prenatal outpatient clinic, which serves a mostly moderate- to low-income, racially diverse population. Subjects underwent a history and physical examination and a urine pregnancy test. Blood was collected on the morning after an overnight fast. Soluble-kit and SCF was measured in the banked plasma from 21 primiparous women with a history of preeclampsia, 28 primiparous women with a history of normal pregnancy (both on average 1 year postpartum) and from 23 nulligravid/never pregnant women. No women were lactating, and all were nonsmokers. There were insufficient numbers of pregnancy samples available from these women for comparison of values during and after pregnancy. Sc-kit and SCF concentrations were estimated in duplicate using ELISA based assays purchased from R&D Systems. Human sc-kit quality control samples available from R&D Systems were used. Data were analyzed by One Way Analysis of Variance with Bonferroni correction (normally distributed variables) or by Kruskal-Wallis test with Dunn's post hoc test (if variables were significantly skewed).

Results: The clinical characteristics and soluble receptor concentrations of the patient groups are presented in Table 5. Mean diastolic blood pressures in postpartum women with prior preeclampsia were slightly but significantly elevated compared to women who had never been pregnant ($P<0.05$). Mean systolic blood pressure was higher in former preeclamptics compared to the other two groups ($P>0.05$). Blood pressure increases in women with a history of preeclampsia have been reported by our group and other research groups, and may relate to the heightened risk of cardiovascular disease that these women face. Body mass index (BMI) did not differ between the postpartum prior preeclamptic (mean 29.2 kg/m^2) and normal pregnancy (28.1 kg/m^2) groups, but the latter groups had higher BMI's compared to the nulligravidas (23.5 kg/m^2) ($P<0.05$). This is due to weight retention from pregnancy. Mean resting heart rate, age, and days after delivery did not differ. Contrary to hypothesis, there were no between-group differences in sc-kit ($P=0.283$; Table 5). There were also no differences in SCF ($P= 0.81$; Table 5). There were no significant effects of self-reported oral contraceptives use (yes/no) on sc-kit or SCF levels within any of the patient groups.

Interestingly, there was a significant negative correlation of sc-kit with systolic blood pressure ($r=-0.47$, $P<0.04$) and with body mass index ($r=-0.45$, $P< 0.05$). These relationships have also been observed in a published study of obese, insulin resistant men. Therefore, completion of Aim 2 will include measurements of fasting insulin and glucose in freezer banked plasma samples from our cohort of postpartum and never-pregnant women.

Specific Aim 3:
We attempted to measure soluble c-kit receptor in conditioned media from human umbilical vein endothelial cells (HUVEC, Lonza, Inc) in culture.

Methods: Passage 3 HUVEC were grown to ~75% confluence. The T75 culture plates were washed once with phosphate buffered saline (with calcium), and the cells refreshed by addition of phenol red-free endothelial basal medium (EBM; Cambrex Bio Science, Walkersville, MD) supplemented with EGM-2 SingleQuot supplements plus either 5% v/v or 0.1 % fetal bovine serum. Cells were maintained at 37ºC in an atmosphere of 5% CO_2 for a total of 48 or 72 hours and then the conditioned media were harvested. Conditioned media was removed at the end of the time period, spun down to remove cells, and stored in a 15ml conical tube at -20C. On the day of the assay, media was thawed and concentrated 16-fold using Amicon Ultracel centrfugal filters with 30,000 molecular weight cut-off (cat# UFC803024). Sc-kit was measured by R&D ELISA on both concentrated and non-concentrated samples. A second series of experiments was performed in which conditioned media (EGM-2 plus 5% fetal bovine serum) were harvested after 4 hours treatment with phorbol ester (Sigma) [0ng/ml], [1ng/ml], [10ng/ml], and [100ng/ml], a manipulation reported to result in cleavage of membrane c-kit and thus production of sc-kit.

Results: Unfortunately, none of the sc-kit values were appreciably above the detection threshold for the assay, and there were no detectable trends in the data.

We processed a material transfer agreement, and last week obtained an aliquot of HMC-1 cells (human mast cell line; Mayo Clinc Reference #:MMV-88-049). These cells were a kind gift from Joseph H. Butterfield, M.D., Mayo Clinic. HMC-1 is a one-of-a kind human cell line that can serve as a resource for mast cell proteins including soluble receptors. The HMC-1 line was derived from a patient with mast cell leukemia and it is reportedly comprised of immature mast cells. HMC-1 cells lack a functional IgE receptor and have mutations in the coding sequence of the c-Kit proto oncogene, thus reportedly producing large quantities of sc-kit (Cruz AC et al. J Biol Chem 2004; 279:5612-5620). We are in the process of expanding these cells in culture. We recently became aware of data suggesting that the placenta is not a major source of circulating sc-kit compared to endothelial cells or mast cells. The HMC-1 cells should be valuable, with and without siRNA blockade of c-kit synthesis, in validating an ELISA assays, and to study circulating and placenta-derived factors from women with preeclampsia that may regulate shedding of c-kit from the cell surface of these HMC-1 cells in vitro.

Overall conclusions:
Concentrations of the soluble form of the c-kit receptor, *sc-kit (soluble CD117;* stem cell factor soluble receptor, SCFsR) are reported to be altered outside the setting of pregnancy in patients with hematologic malignancies, central nervous system germinoma, gastrointestinal tumors, atopic dermatitis, systemic lupus erythematosus, graft-versus-host disease, and in obese individuals with insulin resistance.

To our knowledge we are the first to show that dysregulated soluble *c-kit* is associated with preeclampsia. Given the normal *sc-kit* concentrations in women with gestational hypertension without proteinuria, one can infer that the lower *sc-kit* in women with preeclampsia is not merely

a consequence of hypertension *per se.* These data are also consistent with the notion that preeclampsia and gestational hypertension (without proteinuria) are distinct syndromes with different pathophysiology.

The lack of correlation of *sc-kit* with sFlt-1 or PlGF suggests that *sc-kit* regulation is independent of sFlt-1 and PlGF (suggesting the possible utility of diagnostic or predictive rules that combine these variables). c-kit/SCF dysregulation exists in the fetoplacental circulation and thus may be of pathophysiologic significance. Low *sc-kit* has potential to impact the fetus and fetal side of the placenta.

Median plasma *sc-kit* concentrations were significantly lower weeks before the onset of preeclampsia, consistent with a potential causal role for *sc-kit* in the onset and progression of preeclampsia. These data thus support the principal hypothesis, that maternal plasma *sc-kit* concentrations would be reduced early in pregnancy in many women who subsequently develop preeclampsia compared to women whose pregnancies remain uncomplicated, heralding the development of preeclampsia.

The postpartum data suggest that the lower *sc-kit* evident associated with preeclampsia is a pregnancy-specific abnormality. Thus, *sc-kit* probably does not contribute to the increased risk of later-life cardiovascular disease in women with a history of preeclampsia.

There are currently no useful early pregnancy predictive tests for preeclampsia. Developing a low cost, high throughput assay for sc-kit for use in blood samples of pregnant women may facilitate such a predictive test.

The study of sc-kit metabolism in cultured cells in has proved more challenging but will continue with new strategies.

<u>Accepted abstracts citing Commonwealth of Pennsylvania support</u>:

1. <u>Hubel CA</u>, Rajakumar A, Plymire DA, Powers RW, Roberts JM, [2]David L. Lykins DL, Jeyabalan A, Edmunds LR. Reduced Concentration of Soluble c-Kit Receptor in the Maternal Circulation: an Early Marker for Preeclampsia. The 57th Annual Meeting of the Society for Gynecologic Investigation. Orlando, FL, March 2010. Poster presentation.

2. <u>Hubel CA</u> , Powers RW, Roberts JM, Edmunds LR, Plymire DA, Jeyabalan A Reduced Concentration of Soluble c-Kit Receptor in the Maternal Circulation Distinguishes Preeclampsia from Both Gestational Hypertension and Uncomplicated Pregnancy. The 57th Annual Meeting of the Society for Gynecologic Investigation. Orlando, FL, March 2010. Poster Presentation.

Table 1. Clinical and angiogenic factor data, cross-sectional preeclamptics and controls

	Normotensive Controls (n=27)	Preeclampsia (n=26)	P value
Age (years)	25.5 ± 4.3	28.8 ± 7.0	NS
BMI pre-pregnancy (kg/m^2)	25.7 ± 6.1	27.3 ± 4.8	NS
Gestational weeks at delivery	39.0 ± 1.9	35.0 ± 3.5	<0.01
Gestational weeks at venipuncture	34.6 ± 2.7	34.1 ± 3.0	NS
[#]Early pregnancy blood pressure:			
Systolic (mm Hg)	112 ± 8	118 ± 10	NS
Diastolic (mm Hg)	69 ± 6	70 ± 7	NS
Pre-delivery blood pressure:			
Systolic (mm Hg)	122 ± 10	155 ± 14	<0.001
Diastolic (mm Hg)	72 ± 9	96 ±8	<0.001
Uric acid (mg/dL)	NM	6.1 ± 1.2	
Birth weight (grams)	3368 ± 633	2120 ± 815	<0.01
Birth weight centile	56 ± 29	27 ± 20	<0.01
Cigarette smokers (%)	41% (11/27)	31% (8/26)	NS
Race (% black)	19% (5/27)	22% (4/26)	NS
Plasma angiogenic factors:			
sc-kit (ng/mL)	9.5 ± 2.2	7.2 ± 2.3	<0.001
SCF (pg/mL)	529 ± 115	579 ± 172	NS
sFlt-1 (pg/mL)	4949 ± 2816	25105 ±14146	<0.001
PlGF (pg/mL)	341 ± 285	59 ± 91	<0.001

Continuous variables are given as mean (standard deviation).
NS, not significant; NM, not measured; BMI, body mass index
[#]: average of gestational blood pressures recorded before 20 weeks of gestation

Table 2. Clinical and angiogenic factor data, cross-sectional gestational hypertensives and controls

	Normotensive Controls (n=10)	Gestational hypertensive (n=12)	P value
Age (years)	26.9 ± 6.0	28.9 ± 6.1	NS
BMI pre-pregnancy (kg/m^2)	24.8 ± 5.0	25.8 ± 7.3	NS
Gestational weeks at delivery	39.4 ± 1.6	39.9 ± 1.0	NS
Gestational weeks at venipuncture	39.1 ± 1.9	39.1 ± 1.9	NS
[#]Early pregnancy blood pressure:			
Systolic (mm Hg)	113 ± 9	117 ± 10	NS
Diastolic (mm Hg)	70 ± 5	69 ± 6	NS
Pre-delivery blood pressure:			
Systolic (mm Hg)	120 ± 9	143 ± 12	<0.01
Diastolic (mm Hg)	72 ± 7	86 ± 9	<0.01
Uric acid (mg/dL)	NM	4.8 ± 1.0	
Birth weight (grams)	3438 ± 622	3470 ± 373	NS
Birth weight centile	57 ± 32	55 ± 30	NS
Cigarette smokers (%)	20% (2/10)	31% (8/26)	NS
Race (% black)	10% (1/10)	22% (4/26)	NS
Plasma angiogenic factors:			
sc-kit (ng/mL)	8.2 ± 2.2	8.0 ± 1.4	NS
SCF (pg/mL)	768 ± 174	611 ± 100	<0.04
sFlt-1 (pg/mL)	9866 ± 11693	7410 ± 2726	NS
PlGF (pg/mL)	173 ± 203	132 ± 123	NS

Continuous variables are given as mean (standard deviation).
NS, not significant; NM, not measured; BMI, body mass index
[#]: average of gestational blood pressures recorded before 20 weeks of gestation

Table 3. Clinical and angiogenic factor data, longitudinal preeclamptics and controls

	Normotensive Controls (n=52)	Preeclampsia (n=26)	P value
Age (years)	22.7 ± 4.0	23.9 ± 6.1	NS
BMI pre-pregnancy (kg/m^2)	24.5 ± 6.0	26.2 ± 6.3	NS
Gestational weeks at delivery	40.0 ± 1.1	37.7 ± 3.6	<0.05
Gestational weeks at venipuncture	37.0 ± 4.2	37.3 ± 4.2	NS
[#]Early pregnancy blood pressure:			
Systolic (mm Hg)	113 ± 5	115 ± 8	NS
Diastolic (mm Hg)	67 ± 5	68 ± 6	NS
Pre-delivery blood pressure:			
Systolic (mm Hg)	120 ± 8	142 ± 14	<0.001
Diastolic (mm Hg)	72 ± 7	89 ±7	<0.01
Uric acid (mg/dL)	4.9 ± 1.3	6.6 ± 1.1	<0.05
Birth weight (grams)	3392 ± 439	2747 ± 793	<0.001
Birth weight centile	51 ± 27	27 ± 5	<0.01
Race (% black)	37% (19/52)	31% (8/26)	NS
Plasma angiogenic factors:			
mid-pregnancy sc-kit (ng/mL)	11.1 ± 2.3	9.7 ± 2.5	<0.02
3rd trimester sc-kit (ng/mL)	7.7 ± 1.8 (n=46)	6.2 ± 1.6 (n=24)	<0.005

Continuous variables are given as mean (standard deviation).
NS, not significant; NM, not measured; BMI, body mass index
[#]: average of gestational blood pressures recorded before 20 weeks of gestation

Table 4. Clinical Data of Longitudinal Normal Pregnancy Controls

	Normotensive Controls (n=9)
Age (years)	24.1 ± 6.1
BMI pre-pregnancy (kg/m^2)	26.1 ± 7.9
Gestational weeks at delivery	40.9 ± 0.6
Gestational weeks at venipuncture	
Sample 1	7.3 ± 1.8
Sample 2	17.7 ± 0.7
Sample 3	28.3 ± 0.8
Sample 4	40.8 ± 0.6
[#]Early pregnancy blood pressure:	
Systolic (mm Hg)	112 ± 6.8
Diastolic (mm Hg)	68 ± 6
Pre-delivery blood pressure:	
Systolic (mm Hg)	119 ± 17
Diastolic (mm Hg)	73 ± 4
Uric acid (mg/dL)	4.5 ± 0.9
Birth weight (grams)	3560 ± 540
Birth weight centile	51 ± 2
Race (% black)	33% (3/9)

Continuous variables are given as mean (standard deviation).
BMI, body mass index
[#] average of gestational blood pressures recorded before 20 weeks of gestation

Table 5. Clinical and soluble receptor data of groups of never-pregnant (nulligravid) and postpartum groups

	Nulligravidas (n=23)	Prior normal pregnant (n=28)	Prior preeclampsia (n=21)
Age (years)	26 ± 5	27 ± 6	28 ± 6
Body mass index (kg/m^2)	23.5 ± 3.6*	28.1 ± 7.5	29.2 ± 7.4
Resting heart rate (beats per minute)	72 ± 10	75 ± 10	75 ± 12
Days since delivery	NA	353 ± 132	384 ± 117
Blood pressure:			
Systolic (mm Hg)	108 ± 7	110 ± 7	119 ± 18ψ
Diastolic (mm Hg)	69 ± 7	71 ± 8	78 ± 13#
Oral contraceptives use (%)	0%	50% (14/28)	48% (10/21)
Race (% black)	17% (4/23)	29% (8/28)	43% (9/21)
Plasma soluble receptors:			
sc-Kit (ng/mL)	10.4 ± 3.1	9.3 ± 2.6	10.3 ± 2.4
Stem Cell Factor (SCF) (pg/mL)	588 ± 134	588 ± 87	567 ± 139

Continuous variables are given as mean (standard deviation).
NA, not applicable; NM, not measured
* P<0.05 vs. Prior preeclampsia
ψ P<0.05 vs. Nulligravid or Prior normal pregnant
# P<0.05 vs. Nulligravid

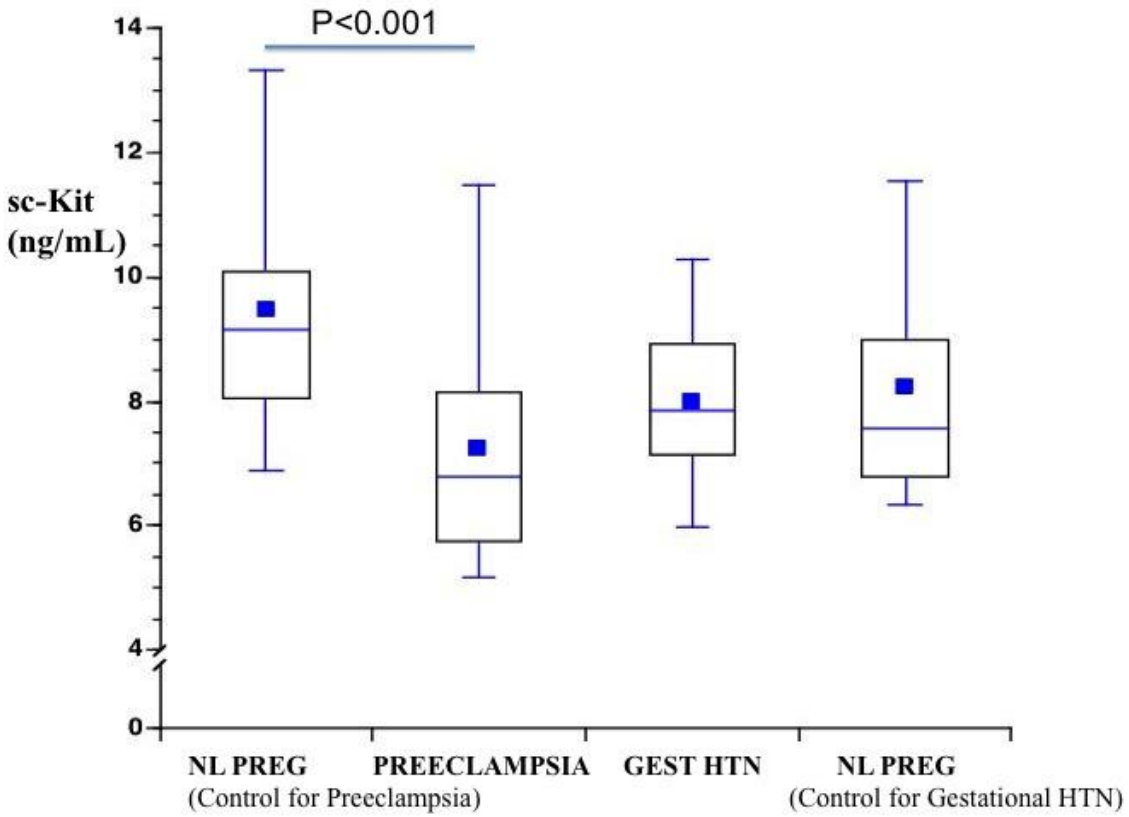

Figure 1. Box-plot of plasma sc-kit concentrations (y-axis) in women with preeclampsia compared to gestational age-matched uncomplicated pregnancy controls (Control for preeclampsia) and in women with gestational hypertension compared to gestational age-matched uncomplicated pregnancy controls (Control for Gestational HTN). The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Closed boxes denote the arithmetic mean. Significant difference is denoted by the horizontal line above the boxes.

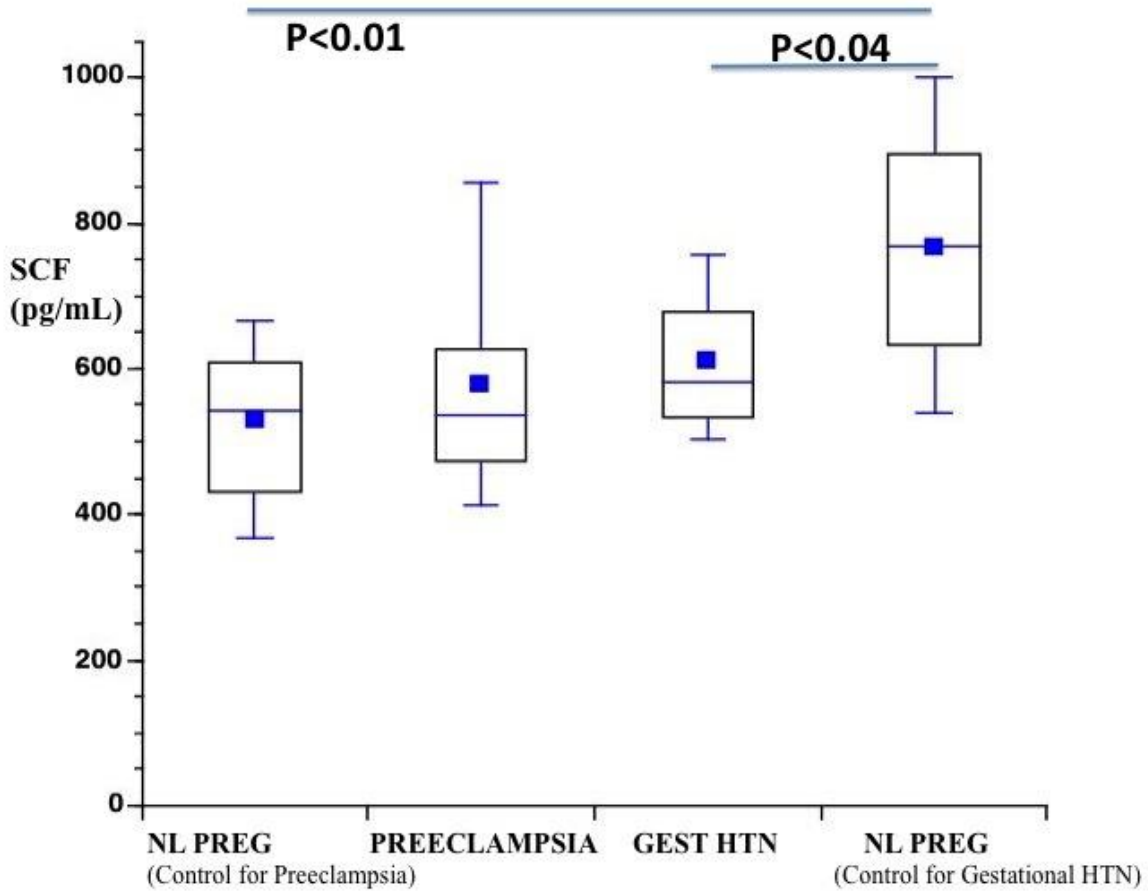

Figure 2. Box-plot of plasma stem cell factor (SCF) concentrations (y-axis) in women with preeclampsia compared to gestational age-matched uncomplicated pregnancy controls (Control for preeclampsia) and in women with gestational hypertension compared to gestational age-matched uncomplicated pregnancy controls (Control for Gestational HTN). The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Closed boxes denote the arithmetic mean. Significant differences are denoted by the horizontal lines above the boxes.

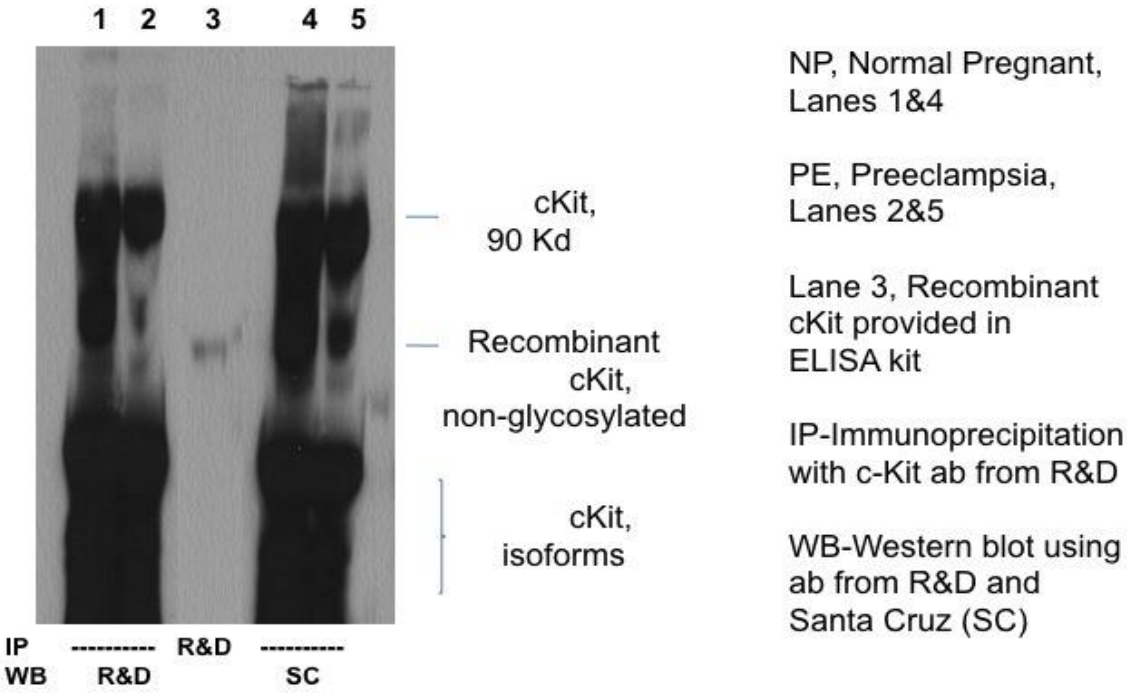

Figure 3. Immunoprecipitation analysis of sc-kit in pools of uncomplicated (normal pregnancy) control and preeclampsia plasma. Density of 90 kilodalton (Kd) c-kit band was substantially less in the normal pregnancy plasma (lanes 1 and 4) compared to preeclampsia (lanes 2 and 5).

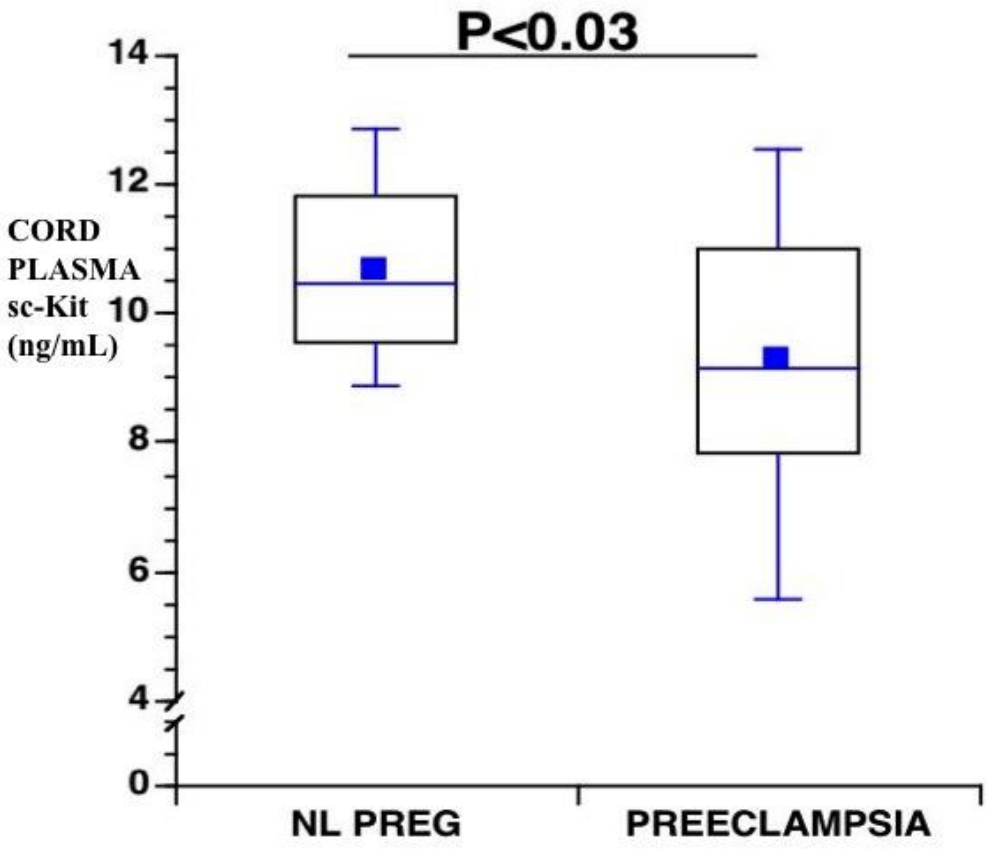

Figure 4. Box-plot of umbilical cord (fetal) plasma sc-kit concentration (y-axis) in preeclampsia compared to uncomplicated pregnancy control pregnancies (NL preg). The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Closed boxes denote the arithmetic mean. The significant difference is denoted by the horizontal line above the boxes.

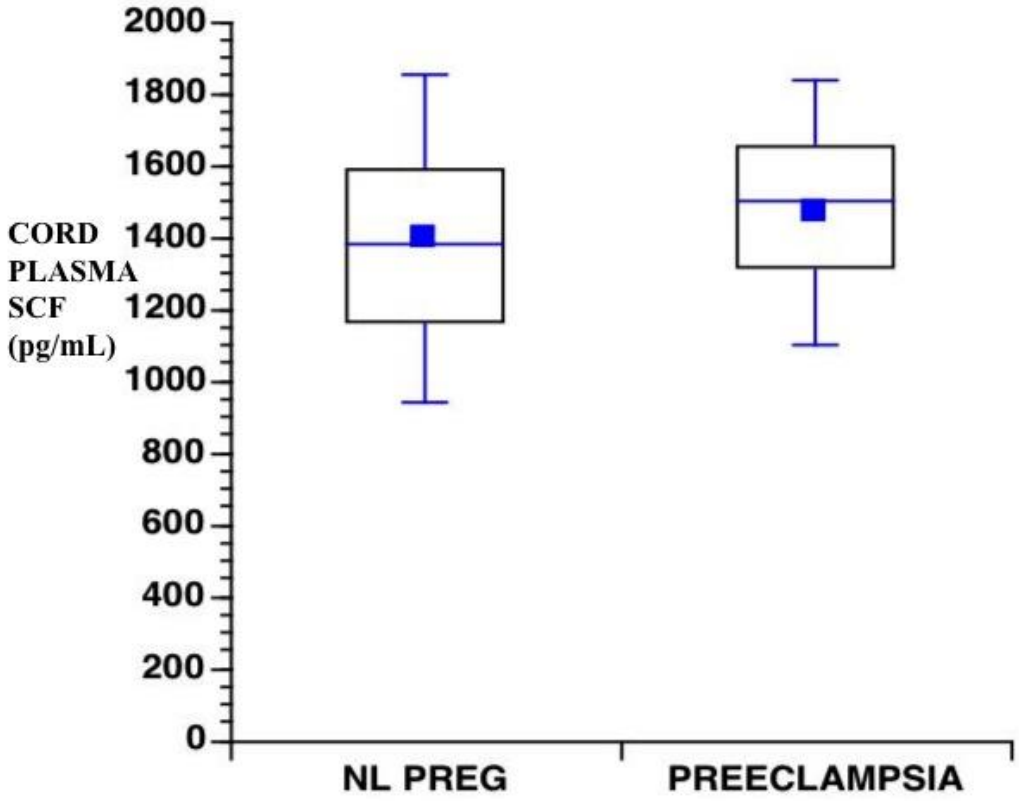

Figure 5. Box-plot of umbilical cord (fetal) plasma SCF concentration (y-axis) in preeclampsia compared to uncomplicated pregnancy control pregnancies (NL preg). The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Closed boxes denote the arithmetic mean.

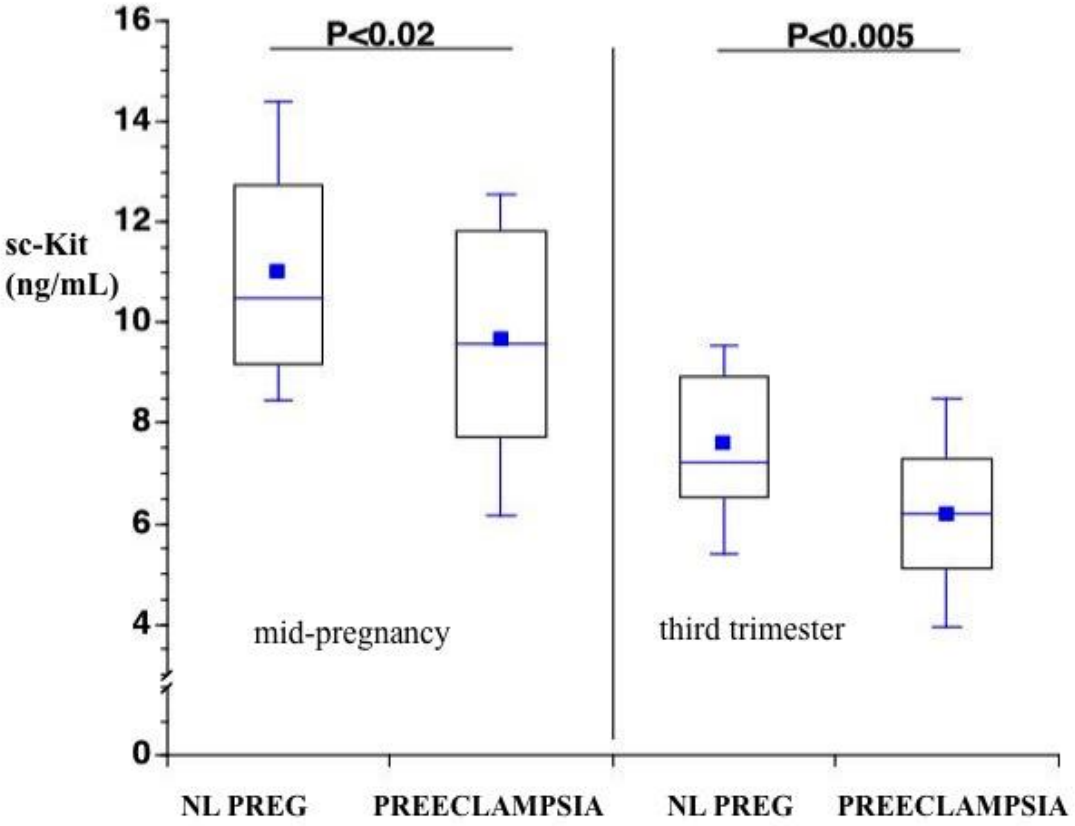

Figure 6. Box-plot of plasma sc-kit concentrations (y-axis) in gestational age-matched women with preeclampsia compared to uncomplicated pregnancy controls (NL Preg) at mid-pregnancy before preeclampsia (19 weeks) and during the 3rd trimester during preeclampsia. The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Closed boxes denote the arithmetic mean. Significant differences are denoted by the horizontal lines above the boxes.

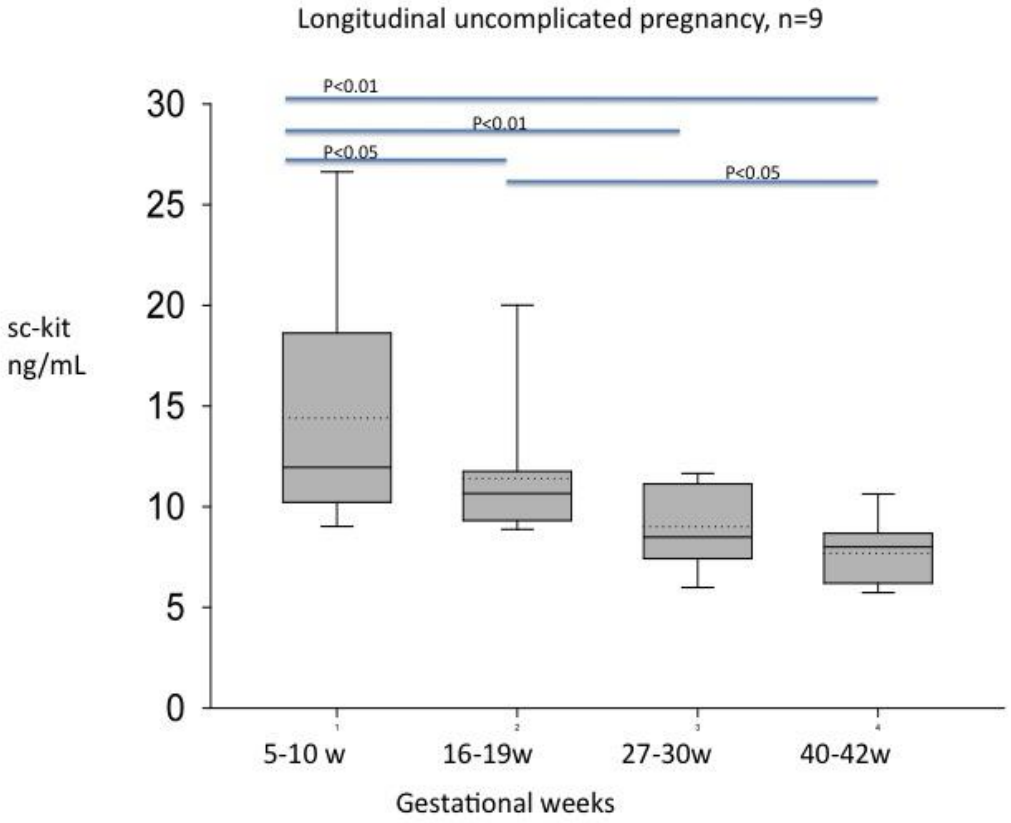

Figure 7. Box-plot of plasma sc-kit concentrations (y-axis) at successive gestational stages (x-axis) of uncomplicated pregnancy. The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Dotted line denotes the mean. Significant differences are denoted by the horizontal lines above the boxes.

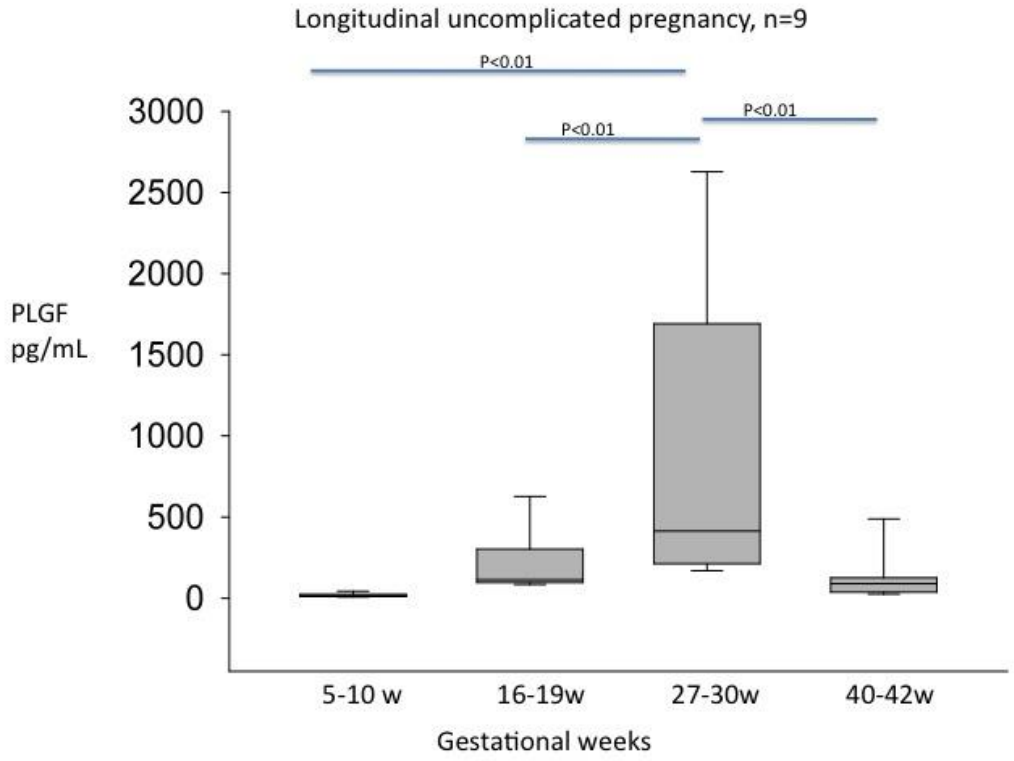

Figure 8. Box-plot of plasma placental growth factor concentrations at successive gestational stages of uncomplicated pregnancy. The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively. The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively. Significant differences are denoted by the horizontal lines above the boxes.

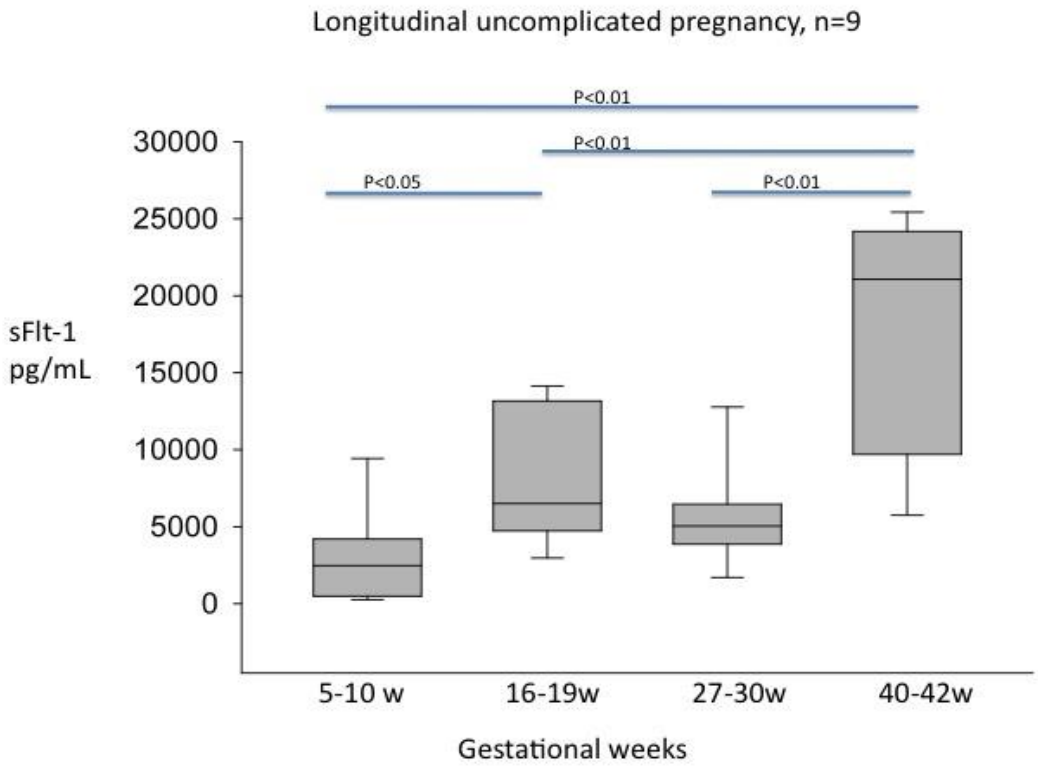

Figure. 9 Box-plot of plasma fms-like tyrosine kinase-1 (sFlt-1) concentrations at successive gestational stages of uncomplicated pregnancy. The top, bottom, and solid line through the interior of the box correspond to the 75th percentile, 25th percentile, and 50th percentile (median), respectively.
The whiskers (t bars) on the bottom and top denote the 10th percentile and 90th percentile, respectively.
Significant differences are denoted by the horizontal lines above the boxes.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____x_No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
____x_No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American

_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer

Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____x_____ No_____

If yes, please describe your plans:

I plan to submit a full-length manuscript, "Reduced Concentration of Soluble c-Kit Receptor in the Maternal Circulation: an Early Marker for Preeclampsia", to the Journal of Clinical Endocrinology and Metabolism, in May 2010.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project.

Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The etiology and pathogenesis of preeclampsia remains poorly understood and useful predictors of the disease are currently lacking. The study findings support the principal hypothesis that maternal plasma *sc-kit* concentrations are reduced early in pregnancy in many women who subsequently develop preeclampsia compared to women whose pregnancies remain uncomplicated, heralding the development of preeclampsia. These data may facilitate the development of methods for predicting or diagnosing preeclampsia or eclampsia in a pregnant subject, comprising detecting soluble *c-kit* in a blood, plasma, or serum sample obtained from the pregnant subject; wherein a significantly reduced concentration of soluble *c-kit* in the blood, plasma, or serum sample as compared to a control indicates that the pregnant subject will develop or has preeclampsia or eclampsia. It may also facilitate methods of distinguishing preeclampsia or eclampsia from gestational hypertension in a pregnant subject, comprising detecting soluble *c-kit* in a blood, plasma, or serum sample obtained from the pregnant subject; wherein a significantly reduced concentration of soluble *c-kit* in the blood, plasma, or serum sample as compared to a control indicates that the pregnant subject has preeclampsia or eclampsia and does not have gestational hypertension.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes__x_____ No_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

   Soluble CD117 (sc-kit) for diagnosis of preeclampsia and eclampsia

b. Name of Inventor(s):
   Carl A. Hubel, PhD
   Robin E. Gandley, PhD
   Augustine Rajakumar, PhD
   Janet M. Catov, PhD
   James M. Roberts, MD

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

This disclosure relates to methods of predicting and diagnosing preeclampsia and eclampsia in pregnant subjects.

Methods were provided for diagnosing or predicting preeclampsia or eclampsia in a pregnant subject. These methods include detecting soluble *c-kit* in a blood, plasma, or serum sample from the pregnant subject; wherein a significantly reduced concentration of soluble *c-kit* in the blood, plasma, or serum sample indicates that the subject has or will develop preeclampsia or eclampsia. In some examples, the concentration of *c-kit* is compared to a control, such as a standard value, the concentration in a sample from the same subject at an earlier point in pregnancy, or a sample from a subject known not to have preeclampsia or eclampsia. In other examples, the methods are utilized prior to detecting increased blood pressure or proteinuria in a pregnant subject.

In some embodiments, the methods disclosed can distinguish preeclampsia or eclampsia from gestational hypertension in a pregnant subject. These methods include detecting soluble *c-kit* in the blood, plasma, or serum sample; wherein a significantly reduced concentration of soluble *c-kit* in the blood, plasma, or serum sample indicates that the subject has or will develop preeclampsia or eclampsia and does not have gestational hypertension. In some examples, the concentration of soluble *c-kit* is compared to a control, such as a standard value, the concentration in a sample from the same subject at an earlier point in pregnancy, or a sample from a subject known not to have preeclampsia or eclampsia or known to have gestational hypertension.

This invention was made with support from the Pennsylvania Department of Health, Commonwealth Universal Research Enhancement (C.U.R.E.) Program.

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
   Yes___x___ No____

   If yes, indicate date patent was filed:

   November 30, 2009
   Pitt Ref. No. 02051
   Klarquist Ref. No. 8123-83984-01

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
   Yes_____ No___x_____
   If yes, indicate number of patent, title and date issued:

   Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No x__

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No___x

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes____x_____ No_____

If yes, please describe your plans:

We are trying to license the technology now.
Pharmaceutical companies have expressed interest in this technology.

Amy Phillips, Office of Enterprise Development, University of Pittsburgh, attended a pharma business development meeting in New York City on February 24, 2010. She had multiple meetings with companies that are interested in this technology; one unilateral confidentiality agreement has been executed at the time of this writing.

I expect to be approached by representatives of some these companies at the 57th Annual Meeting of the Society for Gynecologic Investigation, Orlando , FL, March 27, 2010. I am presenting two posters of my State-funded work at this meeting.

It is intended that the application will be issued as a patent once the claims are allowed by US Patent and Trademark Office (USPTO).

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Carl A. Hubel is Associate Professor of Obstetrics, Gynecology & Reproductive Sciences, with a secondary appointment in the Department of Environmental and Occupational Health, University of Pittsburgh. Dr. Hubel earned his Ph.D. degree from the University of Vermont, Department of Physiology and Biophysics, in 1988. Dr. Hubel's research has centered upon understanding the pathogenesis of the pregnancy disorder preeclampsia, and why women who have experienced preeclampsia are at heightened risk for developing heart disease in later life. This research has progressively provided evidence that dyslipidemia, oxidative stress, chronic inflammation and angiogenic imbalances interact to promote vascular dysfunction both during and after preeclampsia. Dr. Hubel is currently Program Director of

the NICHD-sponsored Program Project, “Mechanisms of Preeclampsia: Impact of Obesity”, and thus leads the country's largest program studying mechanisms of preeclampsia. One current focus of his research is to understand the role of “endothelial progenitor cells”, circulating angiogenic cells that play a crucial role in endothelial homeostasis, and associated circulating angiogenic soluble receptors in normal and preeclamptic pregnancies.