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:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA.

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** 4100047645

6. **Project Number and Title of Research Project:** 3 - Role of UGT2B7 Genotype in Patient Response to Tamoxifen

7. **Start and End Date of Research Project:** 5/1/2009 – 6/30/2010

8. **Name of Principal Investigator for the Research Project:** Leah Cream, MD

9. **Research Project Expenses**.

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

   $ 177,362

   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
Cream	Principal Investigator	2.5%	$4,147
Brucklacher	Core Facility Senior Gene Expression Analyst	50%	$52,956
Bixler	Core Facility Gene Expression Analyst	25%	$23,350
Lazarus	Co-Investigator	1%	$3,923
Smith	Co-Investigator	2%	$3,936
Sun	Post Doc	10%	$5,004
Giambrone	Research Tech	17%	$5,004

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
None		

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
None		

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:_)		$	$

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

Yes_____ No____x_____

If yes, please describe your plans:

I plan to apply for additional internal and external funding. However right now my plans are on hold, since my co-investigator who did the lab based part of the research left to go to another institution. I need to find a new translational researcher to partner with.

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

We plan to do another study with a larger cohort of patients so that we will have enough patients with polymorphisms to extract meaningful statistical data. We are also conducting a similar study with aromatase inhibitors—we have used what we learned from the current trial to design this new trial.

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__x_____ No_____

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

    Because of this study, we have enrolled more breast cancer patients in clinical trials than we had previously. As a result, more breast cancer patients in Pennsylvania have had improved access to the latest new experimental therapies that biomedical research has to offer.

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_____ No____x_____

If yes, please describe the collaborations:

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 agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). 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.

The specific aims of this project were to investigate whether the UGT2B7 codon 268 SNP is associated with:

1. Incidence/intensity of hot flashes in TAM-treated women.
2. Altered TAM metabolite profiles in TAM-treated women.

Summary

1. We did not find that polymorphisms in glucoronidation (UGTB7 codon SNP) are associated with any differences in the intensity or frequency of hot flashes in tam treated women.(data attached)
2. We did not find that polymorphisms of UGBT2B7 codon 268 SNP affected levels of tamoxifen metabolites.

For localized breast cancer, systemic therapy can decrease recurrence and improve overall survival. Systemic therapy is used in combination with surgery and radiation. It includes cytotoxic chemotherapy, biologic agents, and hormonal therapy such as a selective estrogen receptor modulator (tamoxifen) (TAM) or an aromatase inhibitor (anastrazole, exemestane, letrozole). Hormonal therapy has drastically changed the landscape of breast cancer treatment, but despite this, women continue to die of hormone positive breast cancer.

Patients whose tumors express the estrogen (E2) or progesterone receptor on pathologic immunohistochemistry review are candidates for hormonal therapy (in addition to cytotoxic chemotherapy and/or biologic agents). These patients are classified as "hormone positive" (ER+). Hormone therapy is used as both adjuvant therapy and chemoprevention; and its use is supported by several large clinical trials. A pivotal NSABP trial showed that adding TAM to standard therapy improved disease-free survival by about 50% in ER+ patients (1). TAM binds to the estrogen receptor and acts as an antagonist; therefore it is effective in both pre and post-menopausal women. TAM functions by blocking signaling from the E2 receptor and decreasing proliferation or allowing apoptosis of cells. In the ATAC trial, TAM was compared to the aromatase inhibitor, anastrazole, and this demonstrated that anastrazole was superior to TAM in prolonging disease-free survival in postmenopausal ER+ patients (2). Aromatase inhibitors prevent only the peripheral conversion into estrogen by aromatase and are only active in post-menopausal women. Based on this data, tamoxifen is standard adjuvant hormonal therapy for premenopausal women and anastrazole is standard therapy for postmenopausal women.

Several other groups of patients in the PSCI are also treated with TAM. For patients with ductal carcinoma in situ (DCIS), the standard of care is TAM. In addition, patients are also prescribed

TAM if they are high risk for developing breast cancer (based on Gail model risk assessment). The NSABP prevention trial, published in 1998, showed that TAM decreased the chance of developing invasive cancer in high-risk patients (eg, lobular carcinoma in situ or atypical ductal hyperplasia, strong family history) by 50% (3). The STAR trial corroborated this by showing that raloxifene or TAM when given to high-risk postmenopausal women decreases the chance of developing invasive cancer by 50% (4).

Unfortunately, TAM is not effective in preventing all recurrences of ER+ breast cancer. Some E2 receptor-positive cancers seem to be resistant to TAM or develop resistance during or after treatment; we suspect one mechanism of resistance may be related to polymorphisms in TAM metabolism.

Furthermore, TAM causes varying degrees of toxicity. In addition to its anti-estrogenic properties which have been related to menopausal symptoms including hot flashes and vaginal dryness (5), TAM also has partial estrogen-agonistic effects that may be linked to reduced risk for ischemic heart disease and osteoporosis (6,7) but may also increase the risk for endometrial cancer (8,9) and venous thromboembolism (10).

One of the mechanisms that contributes to variability in toxicity is that patients with a low-activity or knock-out polymorphism in the CYP2D6 phase I metabolizing enzyme gene are less able to activate TAM to its active metabolites, 4-hydroxy-TAM and endoxifen. TAM is extensively metabolized *in vivo* by cytochrome (CYP) P450 enzymes into several metabolites having variable potencies towards the estrogen receptor. The hydroxylated metabolites, 4-hydroxy (OH)-TAM and 4-OH-*N*-desmethylTAM (endoxifen), exhibit up to 100 times the anti-estrogenic activity of TAM itself and are abundant in the serum of TAM-treated women (11-15)

Another likely mechanism that contributes to variability to patient response to TAM treatment is due to genetic variation in the major mode of phase II metabolism of TAM, the glucuronidation pathway, a metabolic pathway that results in the effective elimination of TAM and its active metabolites from the body. Non-CYP450-mediated conjugation pathways also appear to be highly important in terms of TAM's overall metabolism and activity profile. While both sulfonation and glucuronidation can occur for hydroxylated TAM metabolites, perhaps the most important route of elimination and detoxification of TAM and its metabolites is by glucuronidation (Figure 1). While TAM is excreted predominantly through the bile, a process largely facilitated by TAM conjugation to glucuronic acid during the glucuronidation process (16), TAM glucuronides have been identified in the urine of TAM-treated patients (17). Most of the 4-OH-TAM and endoxifen found in the bile of TAM-treated patients was as a glucuronide conjugate (16, 18) and 4-OH-TAM and TAM are glucuronidated with very high activity by HLMs (see Preliminary Results). The fact that TAM metabolites are found in their unconjugated form in feces is likely due to β-glucuronidase-catalyzed deglucuronidation within the micro-flora of the small intestine (16). TAM glucuronide conjugates have also been identified in the serum of TAM treated patients (16, 18), and it has been suggested that glucuronidation within target tissues like the adipose tissue of the breast may also be important in terms of TAM metabolism and overall TAM activity (19). Unlike the relatively large body of literature describing CYP-induced TAM metabolism, limited previous studies have been performed examining the glucuronidation pathways involved in TAM metabolism and the potential role of variant UGTs in individual response to TAM treatment.

Although TAM exists in both a *trans* and a *cis* configuration, the *trans* isomer possesses the most potent anti-estrogenic properties (20, 21). Therefore, the *trans* isomer of TAM is the pharmaceutically manufactured form of TAM used in the treatment/prevention of breast cancer. Several enzymes including CYP3A4 and CYP2D6 appear to be the principal P450 enzymes involved in the metabolism of TAM to its active metabolites (22-26). DMT is the primary metabolite of TAM as determined in assays with human liver microsomes [HLM; (24)]; the levels of DMT versus 4-OH-TAM formation in assays of TAM with HLM suggest that they may account for ~92 and 7% of primary TAM oxidation, respectively. These data are consistent with the fact that the levels of steady-state plasma DMT are, on average, greater than 70-times the levels of 4-OH-TAM in the serum of TAM-treated patients (27, 28). These data are also consistent with the observation that the steady state plasma levels of endoxifen are, on average, ~6-fold the levels observed for 4-OH-TAM in TAM-treated subjects (24, 28, 29), consistent with the fact that endoxifen may be formed by both DMT and 4-OH-TAM. Endoxifen and 4-OH-TAM exhibit the same relative levels of anti-estrogenic activity as determined by inhibition experiments using cell proliferation assays (28, 30), the ability to inhibit expression of β-estradiol-induced ER-dependent target genes (8, 30), to inhibit global estrogen-dependent gene expression (31), and in direct binding assays with ER [α and β; (30)]. Both endoxifen and 4-OH-TAM exhibit up to 100-fold the levels of anti-estrogenic activity as TAM and other TAM metabolites including DMT (6, 8, 28, 30-33). Therefore, endoxifen, and to a lesser extent 4-OH-TAM, may be the major contributors to TAM's anti-estrogenic properties.

The potential association between genetic polymorphisms in enzymes responsible for the metabolism of TAM and (i) TAM metabolism phenotype, or (ii) patient response to TAM treatment has been investigated in several studies. Large inter-individual variability in endoxifen plasma concentrations have been reported (34, 35). This variability was in part explained by CYP2D6 status since the use of antidepressants, which act to inhibit CYP2D6, was associated with significantly smaller reductions in plasma endoxifen levels in TAM-treated patients with a variant CYP2D6 genotype (34, 35). A deletion polymorphism in CYP2D6 is associated with higher risk of relapse in TAM-treated women (33). Together, these data suggest that the levels of circulating active TAM metabolites may differ between individuals based upon metabolizing enzyme genotype. Since UGT2B15 was reported to exhibit glucuronidation activity towards 4-OH-TAM, the role of the UGT2B15 codon 85 SNP and patient response to TAM was examined in a retrospective study of breast cancer patients (12). In this study, the high-activity $UGT2B15^{85Tyr}$ variant was associated with an increased risk of breast cancer, although this result was not significant (p=0.11). This is consistent with the fact that we have shown that UGT2B15 is not active against the *trans* isomers of 4-OH-TAM and endoxifen (see below), which are the primary anti-estrogenic metabolite isomers of TAM. Although these data support the idea that the rapid elimination of TAM or active TAM metabolites prevents maximal drug efficacy, it is obvious that that there is much to learn regarding glucuronidation and its role in TAM metabolism and the role of UGT pharmacogenetics on TAM therapeutics.

Study Results

This clinical trial received institutional review board approval in March 2009 and accrued 47 patients. The serum samples were used to characterize the effect of pharmacogenetics of functional SNPs in UGT2B7and CYP2D6 on TAM metabolism profiles in patients treated with tamoxifen (TAM).

The initial step was to study TAM metabolism vs. metabolic profiles (Figure 1). The procedure used for this analysis was developed and performed in the laboratory of Dr. Philip Lazarus. TAM, N-desmethyltamoxifen (NDT), *trans*-4-hydroxy (OH)-TAM, *cis*-4-OH-TAM, *trans*-endoxifen, *cis*-endoxifen, and venlafaxine were purchased from Sigma-Aldrich (St Louis, Missouri, USA) and used as standards for methods development. TAM-d5, NDT-d5, *trans*-4-OH-TAM-d5, endoxifen-d5 (1:1 *trans*/*cis* isomer), and venlafaxine-d6 were purchased from Toronto Research Chemicals (North York, Ontario, Canada) and were used as internal standards. This procedure involved the following experimental milestones:

1. *Preparation of calibration curve*

Preparation of standards for examination in plasma from healthy, untreated subjects. Calibration samples were prepared freshly by spiking 5 ul standard working solution to 300 ul control human plasma. Take 100 ul plasma with spiked standards and add 300 ul internal standard working solution in acetonitrile to extract the compounds. The mixture was vortexed and centrifuged at 16,100 × g for 10 min at 4 °C. The supernatant was dried by vacuum and was then resuspended in 50 ul acetonitrile and 50 ul buffer (5 mmol/L NH_4Ac, pH 7.5). The 100 ul sample was transferred to UPLC autosampler vial, and 5 ul was loaded onto UPLC/MS/MS.

Linearity and detection limit. Calibration curves were derived using the peak area ratio of standard compound *vs*. corresponding internal standard. Eight non-zero calibration standards were assessed through the linear least squares regression calculation with a weighting factor of 1/X. The detection limit was defined by the signal/noise > 3~5. A daily linear calibration curve was prepared with each clinical sample batch to be analyzed.

Clinical sample preparation. After thawing on ice, a 100 ul aliquot of plasma sample was transferred to 1.5 ml polypropylene tube and added to 300 ul acetonitrile with internal standards. The preparation procedure was the same as that of standard samples in control plasma. Every sample was prepared and analyzed in triplicate independently.

Results

We developed a sample pretreatment method to extract TAM metabolites from plasma and eliminate matrix including lipids and proteins. We also developed analytical methods to determine TAM metabolite concentrations in plasma by UPLC/MS/MS.

2. *Development and optimization of analytical methods for determination of TAM metabolites in plasma by UPLC/MS/MS.*

Standards representing the major metabolites of TAM, including *trans*-4-OH-TAM, *cis*-4-OH-TAM, *trans*-endoxifen, *cis*-endoxifen, NDT and TAM, are easily separated and detected when added to plasma and processed in a manner identical to that utilized for plasma samples from TAM-treated patients. In addition, venlafaxine standard (venlafaxine is used as an anti-depressant in virtually all of the TAM-treated patients) was well-separated from TAM metabolites in our UPLC/MS/MS methodology. Data not shown.

3. *Calibration curve and linearity*

The reported concentrations of TAM metabolites in patient plasma are within the TAM metabolite standard linear range analyzed in our developed method. The calibration curves shown for each TAM metabolite in exhibit a correlation coefficient of $r > 0.999$ in all cases, and are within the reliable criteria for quantification of TAM metabolites in clinical plasma samples.

4. *Determination of TAM metabolites in plasma from untreated healthy controls by UPLC/MS/MS.*

As shown in Fig. 2, the detection limit of *trans*- and *cis*-4-OH-TAM is about 0.5 ng/ml, while the detection limit of *trans*- and *cis*-endoxifen is about 1 ng/ml as determined by signal to noise ratio > 3~5. The reported concentrations of 4-OH-TAM and endoxifen in the plasma of patients treated with TAM are higher than the detection limits for all metabolites. For NDT and TAM, the lowest concentrations in the calibration curve demonstrated clear peaks, and the signal to noise ratio >> 5. Clear peaks with a slightly lower signal:noise ratio were also observed for 4-OH-TAM and endoxifen isomers. Thus, our method is valid for the determination of TAM metabolites in plasma.

5. *Determination of TAM metabolites in plasma from subjects treated with TAM by UPLC/MS/MS.*

As shown in Fig. 3, clear peaks corresponding to *trans*-4-OH-TAM (1.65 ng/ml), *cis*-endoxifen (2.09 ng/ml), *trans*-endoxifen (10.95 ng/ml), NDT (128.14 ng/ml), and TAM (103.75 ng/ml) could be detected and quantified for each of the major plasma TAM metabolites in plasma from a patient treated with TAM. In addition, two additional peaks likely corresponding to the *cis* and *trans* isomers of TAM-N-oxide (based on molecular weight) are also clearly detected; this will be verified using purchased internal standards. The levels of each metabolite are within range of previously reported plasma levels for patients treated with TAM. *cis*-4-OH-TAM was not detected in this individual's plasma; perhaps the levels of this metabolite were too low for detection in this individual.

This is the first report where the isomers of 4-OH-TAM and endoxifen have been separated and quantified. This is important since it is the *trans* isomers of these metabolites that exhibit the majority of the anti-estrogenic activity of TAM, with their respective *cis* isomers postulated to contribute an estrogen agonist activity.

The plasma venlafaxine concentration (1.94 ng/ml) was lower than the lowest point screened in our calibration curve, but a clear peak with a signal/noise >>5 was still detected in this individual. We will re-perform the venlafaxine calibration curve using lower concentrations (3-fold that of the detection limit) to assure that we are within range of detecting this agent in patient plasma and that it is not confounding our TAM metabolite analysis for each individual.

The optimized sample preparation and analytical methods have now been established for the measurement of TAM metabolites in plasma of patients treated with TAM.

The second part of the laboratory analysis involved genotyping CYP2D6 and UGTB7. Genotyping of known functional SNPs in UGT2B7 and CYP2D6 using lymphocytes from the same blood specimens used for metabolite analysis was performed.

Studies performed in Dr. Lazarus's laboratory have fully elucidated the enzymes responsible for the glucuronidation of TAM as well as 4-hydroxy-TAM and endoxifen, with one of the major enzymes responsible for this activity being UGT2B7. Dr. Lazarus and co-workers have also demonstrated that a common (50% allelic fre-quency) single nucleotide polymorphism (SNP) at codon 268 of the UGT2B7 gene, resulting in a histidine>tyrosine amino acid change, results in 2-5-fold decreased activity against active TAM metabolites in vitro and a ~40% decrease in overall glucuronidation in human liver. This decrease is similar to that observed for the UGT1A1*28 SNP and glucuronidation of the major active metabolite of the chemotherapeutic agent irinotecan, a SNP that has been approved by the Food and Drug Administration as a genetic test for determining dosage and use of irinotecan in cancer patients.

Therefore, we tested whether the glucuronidation phase II metabolic pathway is an important factor in breast cancer recurrence and toxicity associated with TAM treatment and that the UGT2B7 codon 268 SNP may play a key role in determining overall patient response to TAM. By understanding the role of phase II metabolism of TAM we hope to be able to individualize drug dosing in order to maximize efficacy and minimize toxicity. In addition, these studies should enable us to better assess whether patients are better suited for treatment with TAM or an aromatase inhibitor. The effect of these studies on human health is enormous since there are over 200,000 new breast cancers a year in the US, the majority of which are hormone positive, and millions of women in the U.S. and around the world are currently taking TAM.

While glucuronidation greatly enhances the elimination of substrates from the body by increasing hydrophilicity, glucuronidation is also usually associated with deactivation of that substrate. This is particularly important with respect to target tissue glucuronidation pathways (eg, within the breast). To confirm that glucuronidation of active TAM metabolites actually deactivates TAM metabolites, we measured the ability of glucuronidated TAM metabolites to inhibit the stimulatory effects of E_2 on the expression of a representative E_2-inducible gene – the progesterone receptor (PGR).

There was a significant reduction of E_2 inducibility of PGR mRNA in MCF-7 cells as determined by real-time PCR by both the *trans* and *cis* isomers of 4-OH-TAM and endoxifen using TAM metabolite concentrations that ranged from 1/ 100x - 10x that observed in the serum of women treated with the standard dose of 20 mg TAM. This effect was not observed for any of the glucuronidated conjugates of these TAM metabolites at any of the concentrations used, suggesting that, in addition to increasing excretion via the liver, glucuronidation eliminates the anti-estrogenic effects of active TAM metabolites in target tissues like the breast.

Our group has fully characterized the UGTs involved in the glucuronidation of TAM and its active metabolites. All of the known human UGTs were individually overexpressed in HEK293 cells as previously described (36) and homogenates were prepared from each cell line for analysis of glucuronidating activities against both the *trans* and *cis* isomers of 4-OH-TAM and endoxifen. The amount of UGT1A or UGT2B protein expression in each line was quantitatively determined by Western blot analysis and densitometry, and normalized to levels of β-actin as a

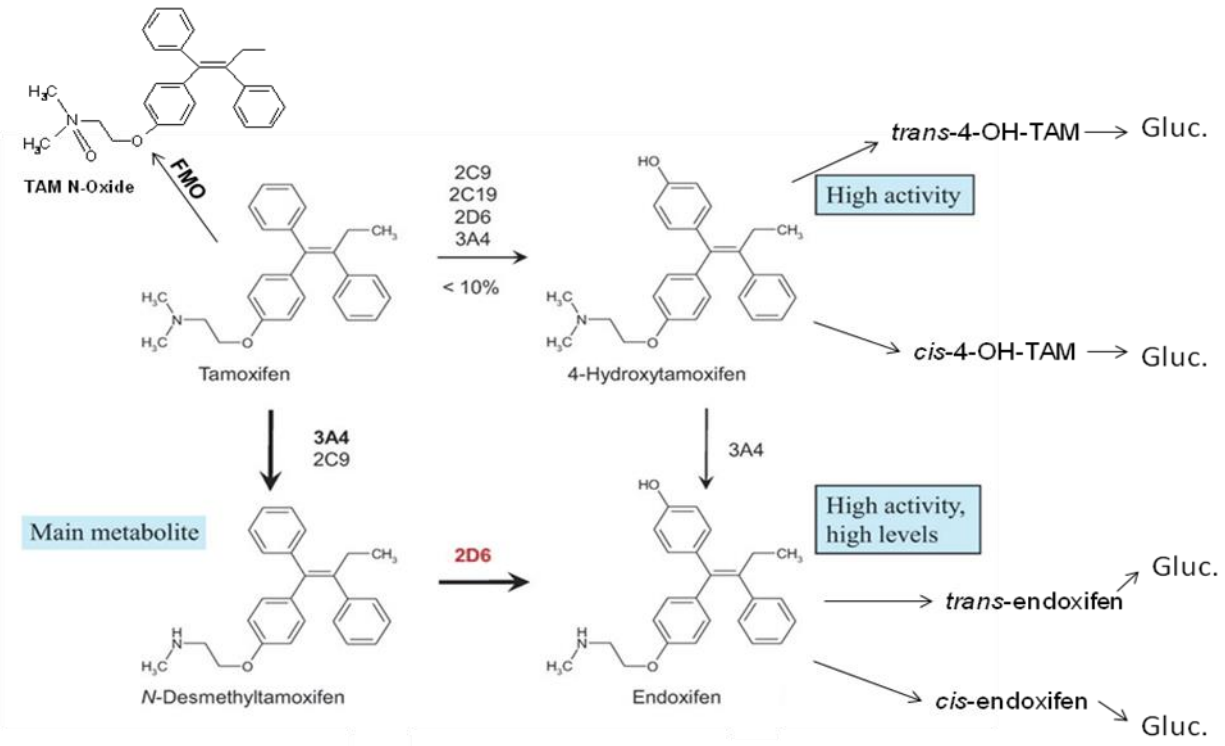
loading control. Varying amounts of human UGT1A and UGT2B7 standard protein were used for quantification.

In studies examining the *O*-glucuronidation of TAM metabolites (TAM itself does not get *O*-glucuronidated due to the absence of an available hydroxyl group), UGTs 1A8, 2B7 and 1A10 showed the high-est overall activities against *trans*-4-OH-TAM as determined by V_{max}/K_m, with UGT2B7 exhibiting the highest binding affinity and lowest K_m. A similar pattern was observed for endoxifen isomers, with the highest overall glucuronidating activities as determined by kinetic analysis (V_{max}/K_m) for UGTs 1A10>1A8>2B7>1A1>1A3> 1A7>1A9 ~2B15~ 2B17 for *trans*-endoxifen. Therefore, UGTs 2B7, 1A10, & 1A8 exhibit the highest overall activities against active TAM metabolites. Of these, only UGT2B7 is hepatic, and it is also expressed in breast (32). Therefore, it is likely to be a major contributor to the glucuronidation and elimination of TAM metabolites

UGT2B7 contains a highly prevalent SNP at codon 268 resulting in a His>Tyr change. We have investigated the effect of this SNP on UGT2B7 glucuronidation activity against 4-OH-TAM and endoxifen. The $UGT2B7^{268Tyr}$ isoform was associated with a 2.2-5.0-fold decreased activity against active TAM metabolites *in vitro* and a ~40% decrease in activity in human liver (results not shown), suggesting that this SNP could potentially be an important genetic factor for overall response to treatment with TAM.

Once we had the tamoxifen metabolite data and the genotype data, we correlated the metabolite levels according to CYP2D6 genotype (figure 4a). We were able to show that with 2 wild type alleles of CYP2D6, levels of tam metabolites were higher than in patients with polymorphisms (both homogeneous and heterogeneous mutations) (Fig 4b). We then did a similar process with UGT alleles. Unlike with CYP2D6, we did not find a correlation between UGT2B7 genotype and tam metabolite levels. Hot flash frequency and intensity did not correlate with either genotype. Therefore genotype of UGTB27 does not predict toxicity from tam (hot flashes) (figure 5and 6).

Fig. 1 Schematic of TAM metabolites



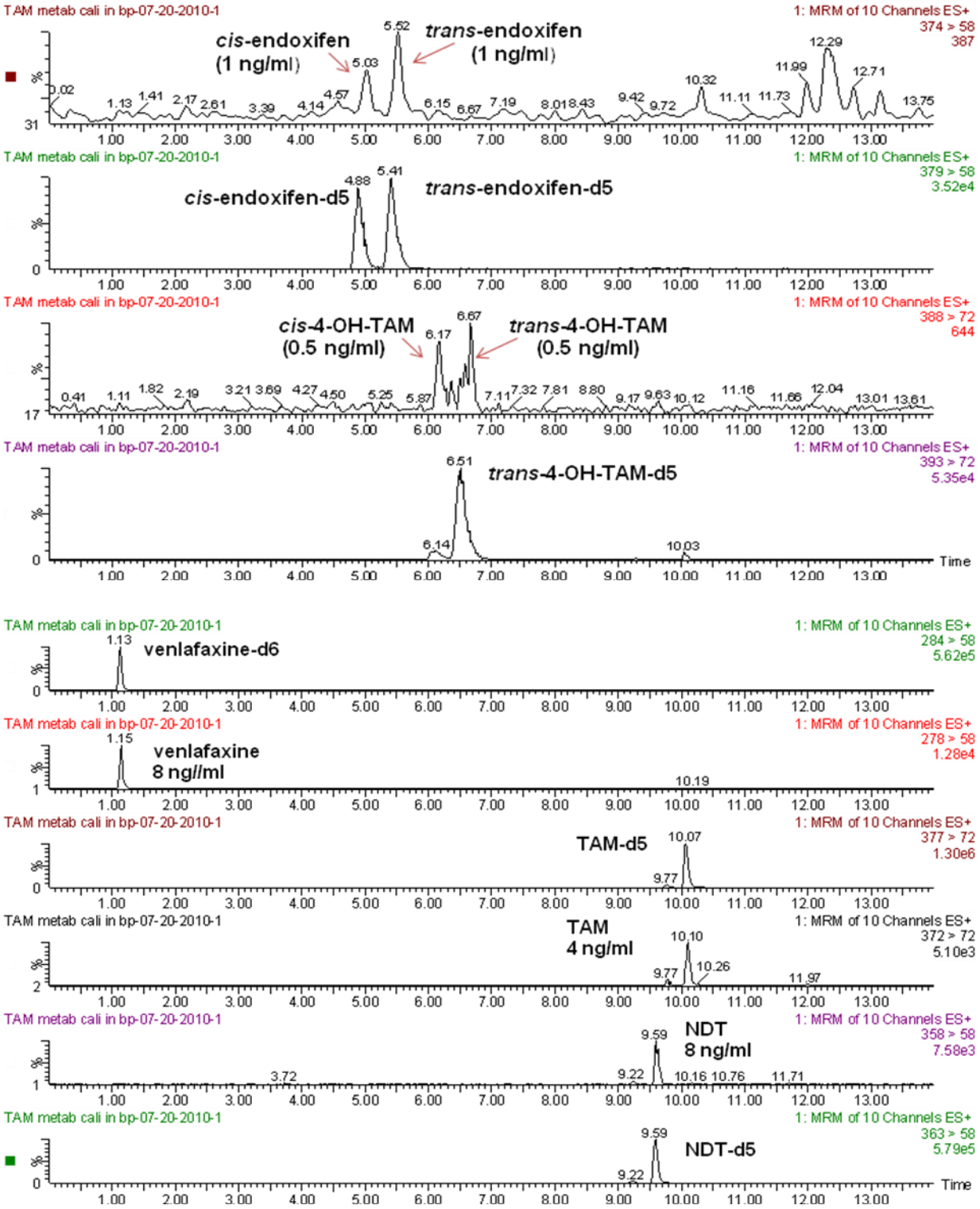


Fig. 2. Representative UPLC/MS/MS MRM chromatograms of TAM metabolites and venlaxifine in plasma from untreated subjects at the lowest concentration of the calibration curve for each compound.

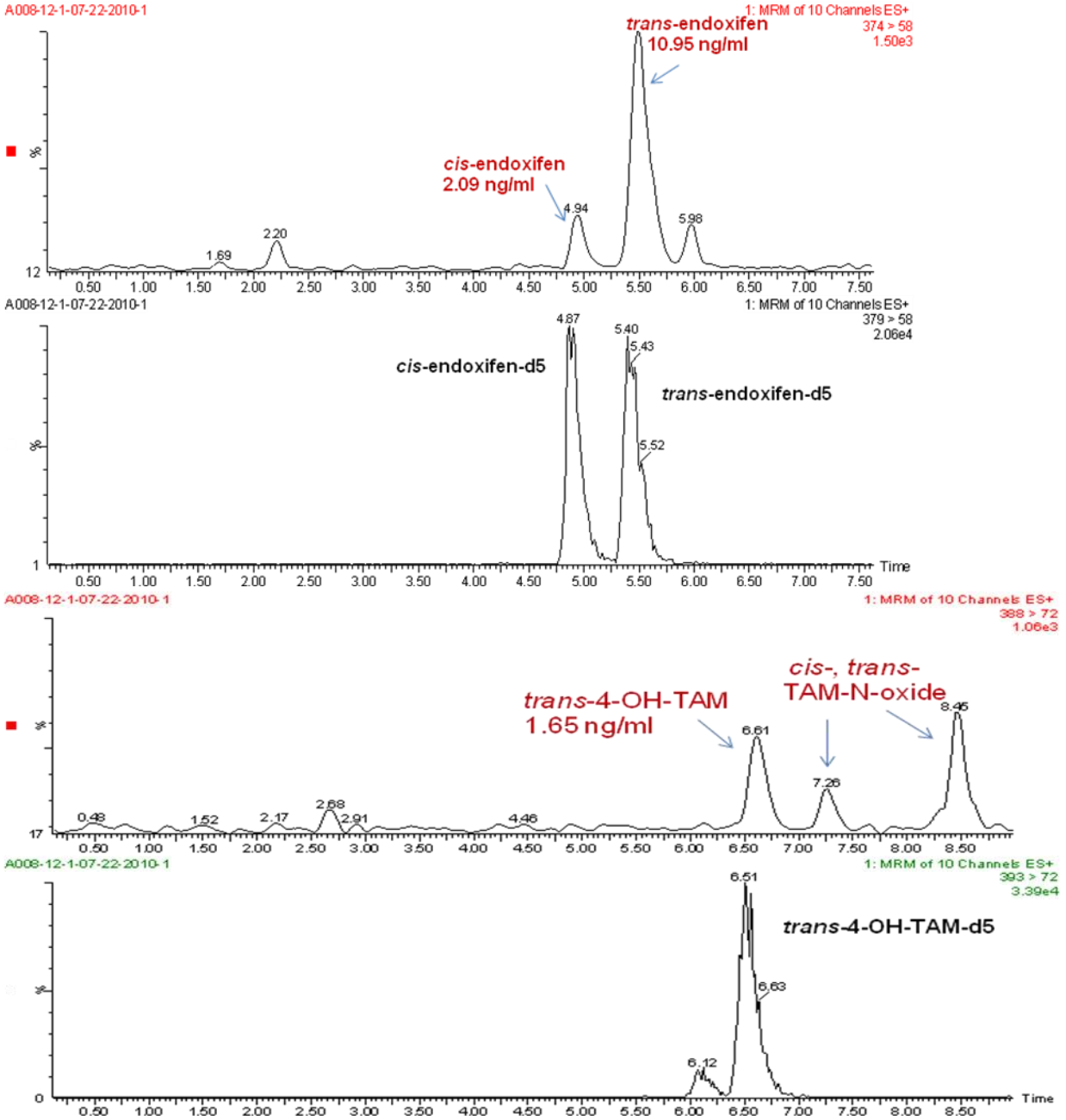


Fig. 3. Representative UPLC/MS/MS MRM chromatograms of TAM metabolites in plasma from a single patient treated with TAM

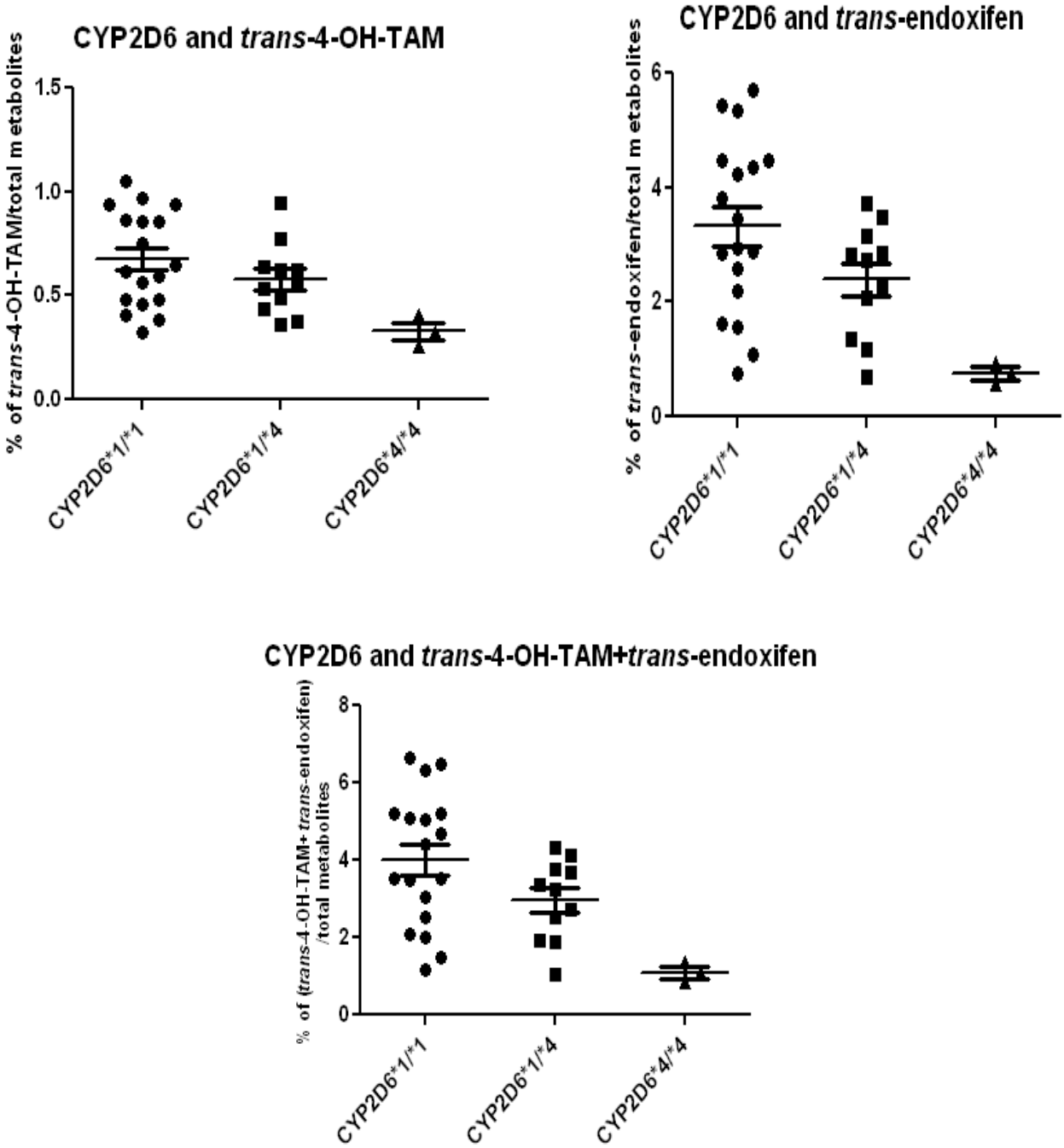


Figure 4a CYP2D6 Polymorphisms and endoxifen levels

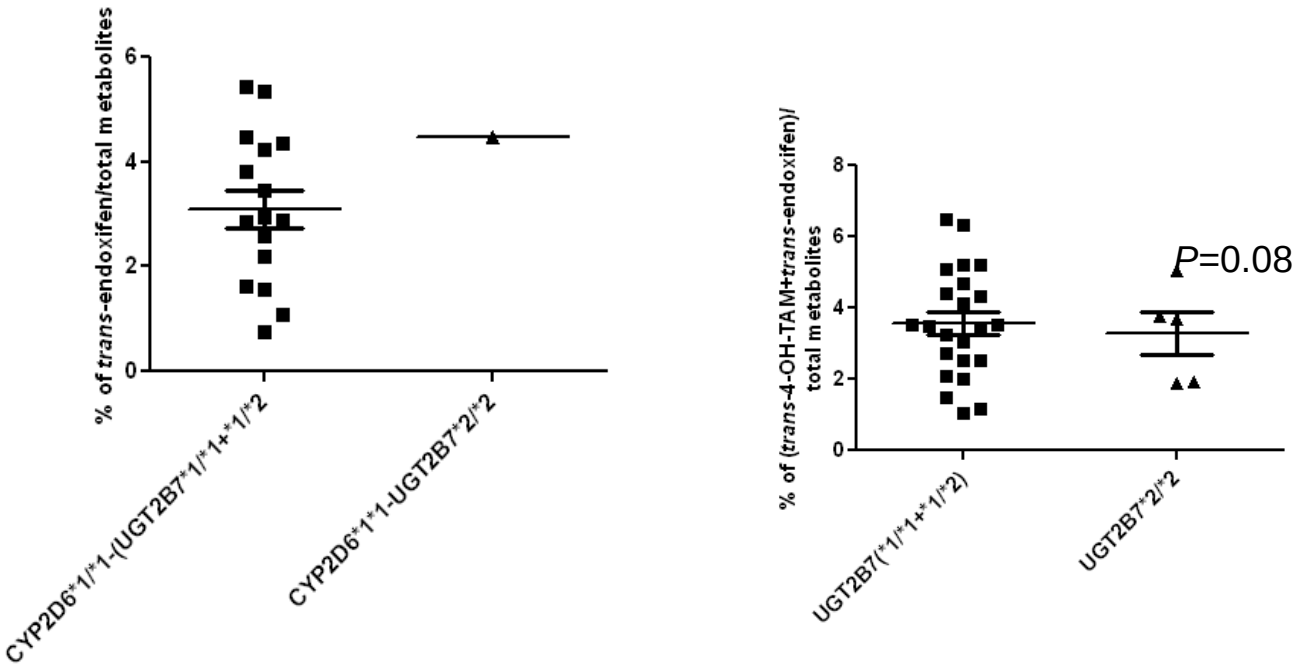
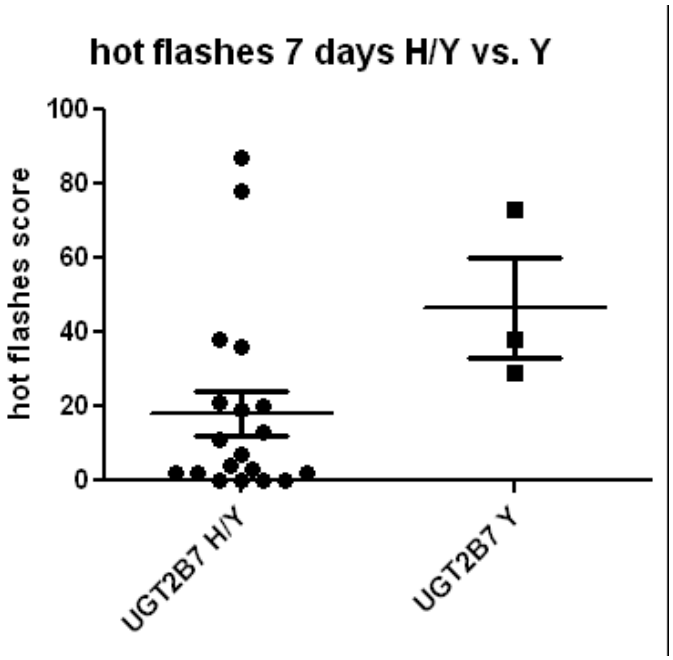


Figure 4b CYP2D6 and UGBT2B7 Polymorphisms and endoxifen levels



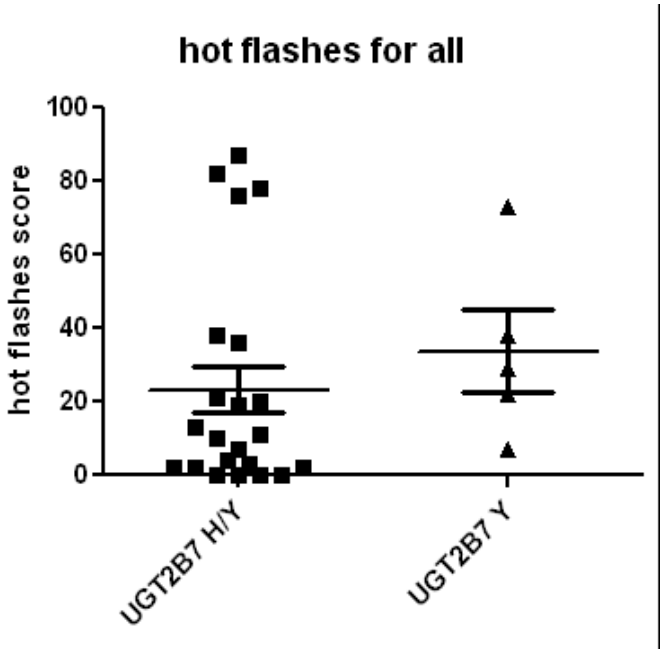


Fig 5: UGT2B7 Phenotype and Hotflashes

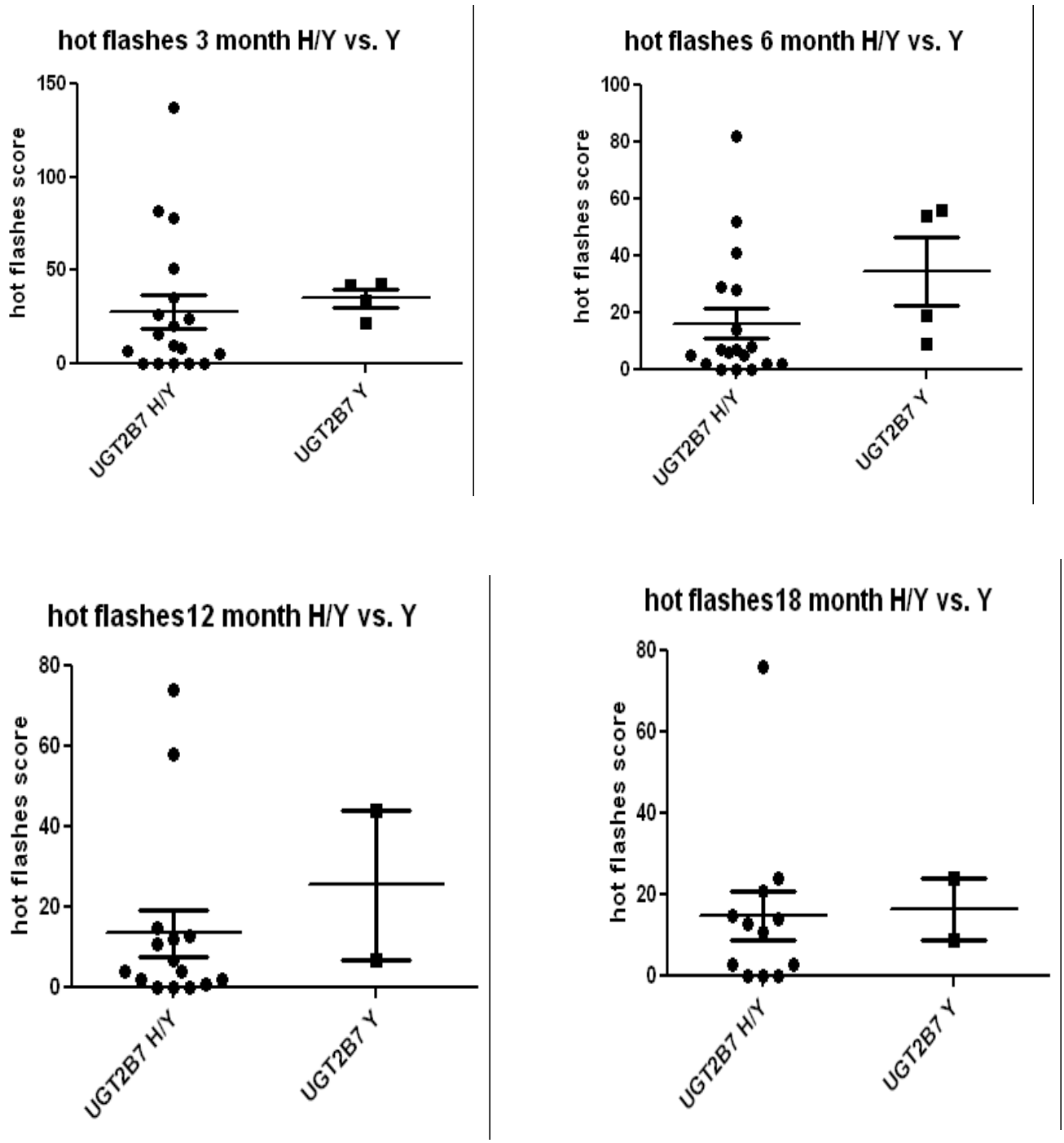


Fig 6: UGT2B7 Phenotype and Hotflashes at different time points

(1) References - If applicable, **insert references here:**

1. Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, et al. TAM for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J Natl Cancer Inst. 1998 Sep 16;90(18):1371-88.
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44. Westfall PH, Tobias R, Rom D, Wolfinger R, Hochberg Y. Multiple Comparisons and Multiple Tests using the SAS System, SAS Institute Inc.; 1999.

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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?

__x__Yes
_____No

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

__x__Yes
_____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?

___3__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?

___50__Number of subjects originally targeted to be included in the study
___47__Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

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

Gender:
_____Males
__47__Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
__47__Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
__47__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.)

Dauphin County

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 or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) 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. None				☐Submitted ☐Accepted ☐Published

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

Yes_____ No___x_____

If yes, please describe your plans:

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.

None

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_____ No___x_____

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:

b. Name of Inventor(s):

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

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_____ No____

If yes, indicate date patent was filed:

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___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

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___

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_____ No_____x___

If yes, please describe your plans:

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.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Lazarus, Philip	POSITION TITLE Professor and Chair, Department of Pharmaceutical Sciences Washington State University College of Medicine
eRA COMMONS USER NAME (credential, e.g., agency login) plazarus	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
McGill University, Montreal, Quebec, Canada	B.Sc.	06/81	Human Genetics
McGill University, Montreal, Quebec, Canada	Ph.D.	06/87	Experimental Medicine
McGill University, Montreal, Quebec, Canada	Postdoc	03/90	Biochemistry

A. Personal Statement

I was a Professor in the Department of Pharmacology at Penn State College of Medicine and Associate Director overseeing the Division of Population Sciences within the Penn State Cancer Institute when the studies outlined in this proposal were performed. I also served as Director of the Center for Pharmacogenetics at the Penn State Cancer Institute from 2009-2012 before moving on to the position of Chair of the Department of Pharmaceutical Sciences at Washington State University's College of Pharmacy. I supervised all genetic and biochemical aspects proposed in this collaborative study with Dr. Cream. I am an experienced molecular biologist/pharmacologist with basic training in biochemistry and epidemiology and have several years of experience in conducting pharmacogenetic and molecular epidemiologic studies. I have published and obtained significant previous funding to support studies examining the role of the UGT family of enzymes in cancer risk and in the pharmacogenetics of breast cancer agents, have over 120 publications, and have served on several study sections including the 2009 special emphasis panel reviewing applications for Centers for Pharmacogenetic Studies as well as being ad hoc on several Epidemiology of Cancer (EPIC) NIH review panels (permanent member beginning Oct 2013).

B. Positions and Honors

Positions

1990-1995 Associate Research Scientist, Div Pathol / Toxicol, American Health Foundation, Valhalla, NY

1992-1995 Assistant Professor (adjunct), Dept of Exper Pathol, New York Medical College, Valhalla, NY

1995-1999 Associate Professor (tenured, 1997), Dept of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia, PA

1996-2000 Associate Professor (adjunct), Dept of Otolaryngology, New York Eye & Ear Infirmary, NY, NY

1999-2001 Associate Professor, Dept's of Interdisc. Oncology, Biochemistry, U South Florida, Tampa, FL
2001-2003 Professor (tenured), Dept's of Interdisc. Oncology, Biochemistry, Pharmacology, Univ of South Florida, Tampa, FL
2002-2003 Program Leader, Cancer Epidemiol. & Prevention Program, Moffitt Cancer Center, Tampa, FL
2003-2011 Program Leader, Mol Epi & Cancer Control Program, Penn State Cancer Inst., Hershey, PA
2003-2012 Associate Director, Div of Population Sci Cancer Prev, Penn State Cancer Institute, Hershey PA
2009-2012 Director, Penn State Center for Pharmacogenetics
2004-2013 Professor (2°), Dept of Public Health Sciences, Penn State College of Medicine, Hershey, PA
2003-2013 Professor (tenured), Dept of Pharmacology, Penn State College of Medicine, Hershey, PA
2013-pres: Professor and Chair, Dept of Pharmaceutical Sciences, Washington State University, College of Pharmacy, Spokane, WA

Other Experience and Professional Memberships

American Association for Cancer Research (1986-present)
International Head and Neck Cancer Consortium (INHANCE; 1998-present)
International Lung Cancer Consortium (ILCCO; 1999 - present); Steering Committee, May 2012-present
American Society for Toxicology and Experimental therapeutics (FASEB; 2003-present)NIH Epidemiology of Cancer (EPIC) Study Section: June 2007, June 2012, Feb 2013; Perm Member: Oct 2013
NIH Special Emphasis Panel (Chair), ZRG1 DKUS-M: Oct 2011
NIH Pharmacogenetics Special Emphasis Panel UO1 [ZRG1 GGG-M (52)]: Nov 2009
NIH/NIEHS Superfund Applications: Nov 2005
NIH/NIDCR Special Emphasis Panel, RFA DE-04-003: Nov 2003
NIH/NIDCR R13; Scientific Conference Awards: June 2002
NIH Oral Biology & Medicine I Study Section: Feb 1999, Oct 2001

C. Selected Peer-reviewed Publications (selected from over 120 peer-reviewed publications)

Most relevant to the current application

1. Sun D, Chen G, Dellinger RW, Duncan K, Fang J-L, Lazarus P. Characterization of tamoxifen and 4-hydroxytamoxifen glucuronidation by human UGT1A4 variants. Breast Cancer Res 2006; 8(4):R50. PMCID: PMC1779459

2. Zheng Y, Sun D, Sharma AK, Amin S, Lazarus P. Elimination of anti-estrogenic effects of active tamoxifen metabolites by glucuronidation. Drug Metab Dispos 2007; 35:1942-8.

3. Sun D, Sharma AK, Blevins-Primeau AS, Dellinger R, Balliet R, Chen G, Boyiri T, Amin S, Lazarus P. Glucuronidation of active tamoxifen metabolites by the human UDP-glucuronosyltransferases (UGTs). Drug Metab Dispos 2007; 35:2006-14.

4. Blevins-Primeau AS, Sun D, Chen G, Sharma AK, Amin S, Lazarus P. Functional significance of polymorphic variants of UDP-glucuronosyltransferases (UGTs) active against tamoxifen metabolites. Cancer Res 2009; 69:1892–900. PMCID: PMC2683344 [Available on 2010/03/01]

5. Lazarus P, Blevins-Primeau A, Zheng Y, Sun D. Potential role of UGT pharmacogenetics in cancer treatment and prevention: Focus on tamoxifen. Steroid Enzymes and Cancer: Ann. N.Y. Acad. Sci. 2009; 1155: 99–111.

6. Lazarus P and Sun D. Potential role of UGT pharmacogenetics in cancer treatment and prevention: Focus on tamoxifen and aromatase inhibitors Drug Metabol Reviews .

7. Sun D, Chen G, Dellinger RW, Lazarus P. Characterization of 17-dihydroexemestane glucuronidation. Potential role of UGT2B17 deletion in exemestane pharmacogenetics. Pharmacogen Genomics 2010; 20:575-85.

8. Sun D, Jones NR, Manni A, Lazarus P. Characterization of raloxifene glucuronidation. Potential role of UGT1A8 genotype on raloxifene metabolism in vivo. Cancer Prev Res. (*accepted upon revision*).

Additional recent publications of importance to the field (in chronological order)

9. Wiener D, Fang J-L, Dosett N, Lazarus P. Correlation between UDP-glucuronosyl-transferase geno-types and NNAL glucuronidation phenotype in human liver microsomes. Cancer Res 2004; 64:1190-6.

10. Gallagher CJ, Muscat JE, Hicks AN, Zheng Y, Dyer A-M, Chase GA, Richie JP, Jr, Lazarus P. The UDP-glucuronosyltransferase 2B17 gene deletion polymorphism: Sex-specific association with urinary NNAL glucuronidation phenotype and risk for lung cancer. Cancer Epid Biomarkers Prev 2007 16:823-8

11. Chen G, Blevins-Primeau AS, Dellinger R, Muscat JE, Lazarus P. Glucuronidation of nicotine and cotinine by UGT2B10. Loss of function by the UGT2B10 codon 67 (Asp>Tyr) polymorphism. Cancer Res 2007; 67:9024-9.

12. Balliet RM, Chen G, Gallagher CJ, Dellinger RW, Sun D, Lazarus P. Characterization of UGTs active against suberoylanilide hydroxamic acid (SAHA) and association between SAHA glucuronidation activity phenotype with UGT genotype. Cancer Res 2009; 69:2981-2989. PMCID: PMC2694132

13. Chen G, Giambroni N, Dluzen D, Muscat JE, Berg A, Gallagher C, Lazarus P. Glucuronidation genotypes and nicotine metabolic phenotypes: importance of functional UGT2B10 and UGT2B17 polymorphisms. Cancer Res 2010; 70:7543-52. PMCID: PMC2998997

14. Jones NR, Sun, D, Freeman WM, Lazarus P. Quantification of UGT1A v1 and v2/v3 splice variant expression and correlation of UGT1A1 variant expression with glucuronidation activity. J Pharmacol Exper Ther. 2012 Jun 1. [Epub ahead of print].

15. Bushey RT and Lazarus P. Identification and functional characterization of a novel UGT2A1 splice variant: Potential importance in tobacco-related cancer susceptibility. J Pharmacol Exper Ther. 2012; 343(3):712-24. PMID: 22984225

D. Research Support

Ongoing

5 R01 DE013158-11(Lazarus) 05/2007-04/2012
(0.30 cal mo)
NIH/NIDCR (competitively renewed once) (no-cost extension through 4/30/2013)
'UDP-Glucuronosyltransferase Genotype and Cancer Risk'
The major goal of this program project is to examine the role of UGTs in genetic susceptibility to cancer.

1R01 CA164366-01A1 (Lazarus) 08/2012-04/2017 (3.6 cal mo)
NIH/NCI
"Role of Pharmacogenetics on Exemestane Metabolism and Toxicity"
The goal of this study is to characterize the metabolism of the aromatase inhibitor, exemestane, and to explore the effects of polymorphisms that occur in enzymes that mediate exemestane metabolic reactions.

Completed (past three years)

Tobacco Formula Funding, Lazarus (PI & Leader, Proj. 1) 3/01/2005-12/31/2008
PA Department of Health
'UDP glucuronosyltransferases (UGTs): Role in cancer susceptibility and treatment'
The objective of this application is to bring together a group of investigators to generate sufficient preliminary data for the submission of a translational program project or SPORE grant application to the National Cancer Institute, focusing on the role of the UGT superfamily of enzymes in cancer susceptibility and treatment
Role: PI & Leader, Project 1

Tobacco Formula Funding (Spratt) 1/1/2006-6/31/2009
PA Department of Health
'DNA repair and susceptibility to tobacco-induced cancer'
The objective of this application is to better identify sub-groups within the population that exhibit deficient DNA repair and are at increased risk for cancer.
Role: Leader - Project 3, Investigator - Core

Non-Formula Tobacco Funding (Lazarus) 6/1/2007-5/31/2011
PA Department of Health/University of Pennsylvania (Whitehead)
'Gene-Environment Interactions for Colorectal Cancer in Northeast, PA'
The goal of this proposal is to examine the interaction between variants in genes involved in heterocyclic amine, nitrosamine and polycyclic aromatic hydrocarbon metabolism and environmental exposures like smoking and diet.

Non-Formula Tobacco Funding (Lazarus) 6/1/2007-5/31/2011
PA Department of Health/University of Pennsylvania (Whitehead)
'Center for the Study of Gene-Environment Interactions in Lung Cancer'
The goal of this proposal is to examine the interaction between variants in metabolic genes and environmental exposures and risk for lung cancer.

Dean's Feasibility Grant (Lazarus) 7/1/2007-6/30/2008
Penn State University College of Medicine
'Role of Glucuronidation Pharmacogenetics in Tamoxifen Treatment of Breast Cancer'
The goal of this proposal is to better examine the role of the UGT family of enzymes and their

genetics in patient response to Tamoxifen.

Dean's Feasibility Grant (Chen) 7/1/2008-6/30/2010
Penn State University College of Medicine
'Nicotine Metabolites in Urine as Potential Markers for Pathway Association Study of Lung Cancer Risk'
Role: Co-Principal Investigator

Grant (Muscat) 9/1/2008-8/31/2010
International Agency for Research on Cancer
'Genetics of Tobacco and Alcohol Related Cancer'
Role: Co-Investigator

PA Tobacco Settlement Fund (Cream) 1/1/2009-6/30/2010
CTSA Award
'Role of UGT2B7 Genotype in Patient Response to Tamoxifen'
The goal of this proposal is to examine the UGT2B7 codon 268 His>Tyr polymorphism on individual glucuronidation capacity of major active tamoxifen metabolites, and assess the effect of this polymorphism on tamoxifen-induced toxicities.
Role: Co-Principal Investigator

Contract W911QY-C-002 (Loughran / Lazarus) 3/1/2011-2/28/2013
Office of Naval Research
Center for Pharmacogenetics at Penn State University
Role: Director

Naval Health Research Center/NFGC Pilot Project (Muscat) 3/1/2011-2/28/2013
'Novel Rare SNPS and Lung Cancer'
Our overall goal in this proposal is to validate our methodology and provide preliminary data for NIH applications.
Role: Co-Principal Investigator

(Sun / Lazarus) 3/1/2011-2/28/2013
Naval Health Research Center/NFGC Pilot Project
'Potential Role of Pharmacogenetics in Breast Cancer Treatment by Exemestane'
The goal of this proposal is to characterize the major EXE metabolites in patients treated with EXE, determine their anti-aromatase activity, and to characterize the enzymes responsible for EXE metabolism.
Role: Co-Principal Investigator

(Omiecinski, Curtis J.) 3/1/2011-2/28/2013
Naval Health Research Center/NFGC Pilot Project
'Genetics of Microsomal Epoxide Hydrolase (EPHX1) as a Risk Modifier of Human Lung Cancer'
This study will provide highly informative data regarding the role of genetic variation in this important enzymatic system that contributes decisively in determining the balance between bioactivation and detoxication of PAHs with carcinogenic potential.
Role: Co-Investigator

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Cream, Leah vonReyn	Assistant Professor, Departments of Medicine Penn State College of Medicine
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YY/MM	FIELD OF STUDY
Carleton College, Northfield, Minnesota	B.A.	06/96	History
Dartmouth College, Hanover, New Hampshire	M.D.	05/00	Medicine

A. Personal Statement

As a clinical oncologist, my research focus is on the care of breast cancer patients. I am currently working with Phililp Lazarus, Ph.D., on a pharmacogenomic trial looking at how polymorphisms in glucuronidation of both tamoxifen and aromatase inhibitors affect metabolism, toxicity, and efficacy of these drugs. As an associate program director of the Hematology/Oncology Fellowship Program, I also work on research related to medical education. I am currently working with Jane Schubart, Ph.D. on developing a web based model to improve teaching primary care residents how to care for breast cancer survivors. I serve as a member of the Molecular Epidemiology and Cancer Control Program. I am beginning a collaboration with Dr. Wafik El-Deiry to analyze circulating tumor cells in my breast cancer patients to look at biomarkers of the tumor microenvironment and discover ways to adjust therapy based on the microrenvironment. In my breast cancer practice I evaluate 5 newly diagnosed breast cancer patients a week and see approximately 30 breast cancer patients on treatment and in follow-up.

B. Positions and Honors

Positions and Employment

2000-2003 Medical Residency, Internal Medicine, Penn State Milton S. Hershey Medical Center
2004-2007 Medical Fellowship, Hematology and Oncology, Penn State Milton S. Hershey Medical Center
2007-pres: Assistant Professor, Dept of Medicine, Milton S. Hershey Medical Center, Hershey, PA
2007-pres: Associate Director, Hematology/Oncology Fellowship Program
2011-pres: Vice Chair, Institutional Review Board

C. Selected Peer-reviewed Publications

Most relevant to current application

1. **von Reyn Cream L**, Ehmann WC, Rybka WB, Claxton DF. Sirolimus in unmanipulated haploidentical cell transplantation. Bone Marrow Transplant, Dec. 42(11): 765-6, 2008; Epub Aug 11, 2008. PMID: 18695659
2. Harvey HA, **Cream L**. Biology of bone metastases: causes and consequences. Clinical Breast Cancer 2007; 7 (1).

D. Research Support

Current Research Support

Pfizer, Cream (PI) 06/07/07 - present
Phase II Trial of sunitinib for refractory malignant ascites
The major goal of this study is to determine whether sunitinib, by blockade of vascular endothelial growth factor, decreases formation of malignant ascites.

CTSA Pilot Grant, Cream (PI) 01/09 – 01/11
An observational Study of Phase II metabolism on tamoxifen metabolites and toxicity
The major goal of this study is to determine whether polymorphisms in glucuronidation affects metabolism, efficacy, and toxicity of tamoxifen and aromatase inhibitions in breast cancer patients.

Completed Research Support
None