Dose escalation of tamoxifen in patients with low endoxifen level: evidence for therapeutic drug monitoring - The TADE Study.

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Running head: Tamoxifen dose escalation and therapeutic drug monitoring

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Chris Liddle – Holds patents (not related to this paper) with University of Sydney and the Salk Institute.
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Translational Relevance

Endoxifen level relies on CYP2D6 but CYP2D6 genotype does not consistently correlate with breast cancer outcome. There is evidence that low endoxifen level is associated with an impaired disease-free survival. We studied 122 breast cancer patients treated with tamoxifen and increased the tamoxifen dose in those with endoxifen level of <30nM. Concomitant medications, CYP2D6 genotype, compliance and absorption, and unknown factors contributed to low endoxifen. We found that endoxifen level increased regardless of CYP2D6 genotype category. The best predictor for reaching potentially therapeutic target levels of endoxifen with tamoxifen dose escalation was the baseline endoxifen level, and not CYP2D6 genotype. Hot flashes did not predict endoxifen level. Clinical trials are needed to confirm an association between low endoxifen and inferior outcome. Our data suggests that tamoxifen dose escalation in such trials should be by measuring endoxifen level alone without reference to CYP2D6 genotype or hot flashes.
Abstract

Purpose: Endoxifen is the major mediator of tamoxifen effect and endoxifen levels <15nM may be associated with increased risk of breast cancer recurrence. We increased tamoxifen dose in breast cancer patients with low endoxifen levels and assessed the influence of various parameters on reaching 15nM and 30nM endoxifen levels.

Experimental design: Tamoxifen dose was increased in those with endoxifen levels below 30nM. Toxicity, including hot flash score, was measured. CYP2D6 metabolizer status was classified as ultra-rapid (UM), extensive (EM), intermediate (IM) or poor (PM) based genotype of somatic DNA.

Results: Dosage was escalated in 68 of 122 participants. On 20mg tamoxifen, 24% had endoxifen levels below 15nM and this reduced to 6% following dose escalation. In over 50% of cases there was no identified cause for low endoxifen. Low baseline endoxifen level, and not CYP2D6 metabolizer status, independently predicted reaching threshold targets for both the 15nM and 30nM targets (p = 0.04 and 0.003 respectively). The 15nM target was reached in all UM/EM and IM patients, 63% of PM patients, and 58% of those with baseline endoxifen of <10nM. There was no correlation between hot flash score and genotype or any tamoxifen metabolite level including endoxifen (R=0.07).

Conclusion: Low endoxifen on standard dose tamoxifen was the only independent predictor of failure to achieve potentially therapeutic levels. Trials examining tamoxifen dose escalation and breast cancer outcome should be guided by endoxifen levels alone, without reference to CYP2D6 genotype or presence of hot flashes.
INTRODUCTION

Tamoxifen has been a treatment for breast cancer for over 35 years, essentially becoming the first biologically targeted anti-cancer agent following discovery of its target, the estrogen receptor (ER) (1). Its major impact has been in long-term adjuvant therapy for ER positive breast cancer, showing a 50% reduction in annual recurrence rate and a 30% reduction in breast cancer mortality following five years of treatment (2).

Tamoxifen is weakly anti-estrogenic with its effect largely dependent on formation of 4-OH-N-desmethyltamoxifen (endoxifen) by the enzyme cytochrome P450 2D6 (CYP2D6) (3, 4). An association between CYP2D6 genotype and low endoxifen levels has been repeatedly demonstrated, (5-7) although no association between genotype and clinical outcome has been seen in post-analyses of large adjuvant trials, (8-10) implying that even patients with impaired endoxifen production may have adequate levels for anticancer effect. However, some studies have performed CYP2D6 genotyping on tumor rather than germline DNA which can lead to inaccuracies due to somatic chromosomal abnormalities in tumor samples (11). Moreover, endoxifen levels have not been directly measured in clinical outcome studies but rather inferred from CYP2D6 genotype which belies the impact of other enzymes involved in tamoxifen metabolism such as CYP3A4, CYP2C9/19, UDP-glucuronosyltransferases and sulfotransferases and non-genetic factors such as adherence and absorption, and concomitant medications that inhibit CYP2D6 (12-14). A recent population pharmacokinetic modeling study using dextromethorphan-derived metabolic phenotype in tamoxifen-treated patients, suggests that variation in activity of CYP3A, in addition to CYP2D6, appears to considerably influence endoxifen levels (15).
There is now increasing evidence that systemic exposure to endoxifen is important for anti-cancer effect (16-20). In-vitro and xenograft models have shown that breast cancer cell proliferation is endoxifen concentration dependent (16, 17, 20). Two large retrospective studies have shown a correlation between endoxifen level and disease-free survival in patients with early breast cancer treated with tamoxifen (18, 19). Using quintile-group analyses both studies showed that endoxifen trough levels below approximately 15nM had worse outcome compared to those with higher levels in the adjuvant setting.

The overall aim of this study was to determine whether a substantial increase in endoxifen level could be achieved by tamoxifen dose escalation in individuals with low endoxifen level, and to examine the impact of genetic and other factors, such as concomitant medications, on achieving potentially therapeutic levels.

MATERIALS AND METHODS

Patients

Participants had hormone-receptor positive breast cancer and a minimum 8 weeks of tamoxifen at a dose of 20mg per day (pd) as part of routine management. Other inclusion criteria included Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy >6 months, and adequate renal and hepatic function. Patients with a history of thrombosis, uncontrolled medical conditions and those receiving concurrent chemotherapy or radiotherapy were excluded. Concomitant use of CYP2D6 inhibitor medications was permitted.
The study protocol was approved by the Human Research Ethics Committee of the Cancer Institute New South Wales. All participants gave written informed consent. The study was registered at ClinicalTrials.gov NCT01075802.

**Study design**

**Definition of the study target endoxifen level**

At the commencement of the study, the target level of endoxifen was derived as the mean serum Z-endoxifen (endoxifen) in an initial series of 32 women recruited. This empirical approach was to ensure that an adequate number of patients entered the dose escalation phase of the study, whilst avoiding dose increase in individuals who had relatively high baseline endoxifen. In this sub-group, the mean endoxifen level was 30.6nM and the study target level was set at 30nM for dose escalation purposes. During data analysis, a second target of 15nM was specified based on studies published in the interim suggesting the importance of this threshold (18, 19).

**Dose escalation protocol**

After a minimum of eight-weeks of tamoxifen 20mg pd, serum levels of tamoxifen, endoxifen and other metabolites were measured. Participants with endoxifen levels below 30nM had tamoxifen dosage increased according to the schedule outlined in Figure 1 every eight weeks up to a maximum dose of 60mg pd; the target endoxifen level was reached; or withdrawal from the study. The upper dosage limit of 60mg was chosen due to limited safety data on tamoxifen in excess of 40mg pd. In a proportion of participants (65/122, 53%), baseline serum tamoxifen and metabolite levels were measured on two occasions prior to implementation of the study protocol. For this subgroup, assignment to dose escalation was based on initial endoxifen level with the second baseline level included in analysis.
Clinical data collection

Data were collected on breast cancer history, concomitant medications, menstrual history and ethnicity. Ethnicity was defined according to the country of origin or ethnic group of the patient’s four grandparents (21). CYP2D6 inhibition category of concomitant medications was allocated according to the Flockhart, Indiana University website (22).

Participant-reported hot flash scores were derived monthly from both the frequency and severity of flashes over a one-week period using the Loprinzi method (23). Non-vasomotor toxicities were also recorded at each visit and for six months after study completion.

Serum tamoxifen and metabolites

Blood sampling for drug level measurements was performed 24 hours after the last tamoxifen dose as far as practicable and after at least eight weeks of stable tamoxifen dose to ensure steady state levels. Specimens were collected in an EDTA tube and immediately centrifuged for 10 minutes at 3000 rpm and serum separated, and then frozen at minus 80°C, protected from light. Levels of tamoxifen and metabolites were measured by reverse phase liquid chromatography coupled with mass spectrometry. Analysis was performed using an adaptation of a previously described method.(24) Metabolites measured were tamoxifen, N-desmethyltamoxifen (NDMT), 4-hydroxy tamoxifen (4OHT), 4’-hydroxy tamoxifen (4’OHT), Z-endoxifen (endoxifen) and 4’-OH N-desmethyltamoxifen (Z’-endoxifen). Only endoxifen and not Z’-endoxifen was used in the statistical analysis. Chromatographic separations were carried out with a Waters 2695 pump equipped with an autoinjector. The analytes were separated on a Synergi 4μ Hydro-RP 80Å 150x2.00mm Column (Phenomenex, Torrance, CA, USA.) The mobile phase consisted of solvent A (water), solvent B...
(acetonitrile), and solvent C (35 mM ammonium formate, pH 3.5) delivered as a gradient: 0-10 min for solvent B, 40-70%; 10-14 min for solvent B, 70 80%; and 14-17 min for solvent B, 80-40%, with 10% solvent C, at a constant flow rate of 0.25 ml/min. The high performance liquid chromatography was coupled with a Waters ZQ quadruple mass detector via an electrospray ionization interface operating in the positive ion mode. Quantitative determination of tamoxifen and its metabolites was performed by time scheduled single ion recordings using (M + H)+ ions. Poor tamoxifen adherence or absorption was defined as a baseline trough level of <150 nM (19).

**CYP2D6 genotyping**

Whole blood was collected for DNA extraction to assess CYP2D6 genotype. DNA was extracted from whole blood using Promega Maxwell DNA extraction kits. CYP2D6 genotyping was performed by a single base extension assay (iplex) that was analyzed on a Sequenom Massarray MALDI-TOF mass spectrometer (Sequenom, San Diego, USA). Copy number variants of CYP2D6 were determined by quantitative real-time PCR using an ABI7900 (Applied Biosystems, Foster City, USA). Genotyping was provided by a commercial provider (Healthscope Pathology), which is accredited by the National Association of Testing Authorities of Australia and adheres to the Quality Assurance Program of the College of American Pathologists. Two DNA samples of known genotype were included in each run.

Each allele was defined as inactive (CYP2D6*3, *4, *5, *6, *7, *8, *14); partially active (CYP2D6*9, *10, *18, *41, *14B); active (CYP2D6 *1, *2) or duplicated (CYP2D6 *1xN, *2xN), corresponding to CYP2D6 activity scores of 0, 0.5, 1 and 2 respectively (25). Four genotype based metabolizer groups were initially designated as ultra-rapid (UM): total
activity score >2, extensive (EM): activity score 1.5 - 2, intermediate (IM): activity score 1 – 1.5 and poor (26); activity score ≤ 0.5. However, in the final analysis metabolizer categories were reassigned to align with 2014 Guidelines (25) with activity scores: UM >2, EM 1-2, IM 0.5 and PM 0.

Statistical analysis

Based on the original metabolizer categorization, it was estimated that the majority of the CYP2D6 extensive metabolizer (EM) participants, approximately 10% of intermediate metabolizer (IM) and 5% of the poor metabolizer (26) participants would achieve the 30nM target endoxifen level with a 20mg dosage of tamoxifen. A dose escalation would be considered worthwhile if the proportion of IM and PM groups reaching the endoxifen target could be increased to at least 50%. Based on Simon’s two stage design, with 90% power and 95% confidence, a sample size of at least 13 IM patients and seven PM patients would be sufficient to detect this difference. According to an estimated population distribution of 75% UM/EM, 20% IM and 5% PM, a total of 120 patients was considered sufficient to recruit the appropriate numbers of IM (at least 13) and PM (at least seven) to detect differences of clinical significance.

The Spearman rank correlation coefficient was utilized to assess the association between serum analytes and hot flash score. A two-samples T-test was used to compare CYP2D6 metabolizer and inhibitor categories with metabolite levels. Variables which potentially could impact on target endoxifen level were examined by multivariate analysis (linear and logistic regression). Variables were declared significant if the p-value was <0.05 in the model. As this is an exploratory study, no adjustments were made for multiple comparisons. Deviation from Hardy-Weinberg equilibrium was assessed using the Pearson's chi-squared test.
RESULTS

Cohort characteristics

The cohort comprised 122 individuals taking tamoxifen as treatment for breast cancer. Median length of tamoxifen treatment before study entry was 10.4 months (range 1-69). A single participant was taking 40mg tamoxifen at study entry and was excluded from baseline analysis but included in dose escalation data. Other cohort characteristics are summarized in Table 1. Patients who had tamoxifen dose escalated were younger (median 48 vs 53 yr, p=0.02), more likely to be premenopausal (72% vs 47%) and more likely to be PM (15% vs 0%).

The distribution of participants between CYP2D6 metabolizer categories was two UM (1.6%), 103 EM (84.4%), 9 IM (7.4%) and 8 PM (6.6%; Supplemental table S1). This distribution was substantially influenced by post hoc application of genotype / phenotype category definitions described in recent guidelines, (25) with the effect of substantially increasing the EM category and reducing IM and PM subgroups. There was no significant deviation from Hardy-Weinberg equilibrium (chi-square = 2.01, p= 0.16). Allele frequency of the study subjects compared to recently published results for selected racial groups is shown in Supplemental table S2.

Baseline endoxifen was associated with indicators of CYP2D6 activity

The mean levels of tamoxifen and endoxifen in 121 patients taking 20mg tamoxifen at baseline were; tamoxifen 326.8 nM (range 76-905.2nM, interquartile range 188.9nM), and endoxifen 25.8 nM (3.1–59.1 nM, interquartile range 20.8nM), indicating a 12-fold variation in baseline tamoxifen and 20-fold variation in endoxifen levels across the cohort.
Baseline endoxifen was significantly lower in CYP2D6 PM individuals (mean 9.4nM, SD 5.3) compared with UM/EM individuals (mean 27.8nM, SD 13.5; p<0.001). There was no significant difference between baseline endoxifen in IM (mean 15.6nM, SD 8.5) and UM/EM individuals. One UM patient had a baseline endoxifen level of 15.3nM (tamoxifen level 333.5nM; all patient mean 326nM), was not on CYP2D6 inhibitors, but did not undergo tamoxifen dose escalation. After controlling for CYP2D6 genotype, there were no significant differences in the mean endoxifen concentration between ethnic groups (data not shown).

Endoxifen levels were not different between participants taking weak or minimal CYP2D6 inhibitor medications compared with no CYP2D6 inhibitors (p=0.5). However, five participants taking moderate or strong CYP2D6 inhibitors had lower levels of endoxifen compared to others (p=0.003) (Supplementary table 2).

**Low baseline endoxifen can be attributed to multiple factors**

Twenty-nine participants had baseline endoxifen level <15nM. Fourteen (48%) had an identifiable cause: seven patients were PM, four were on moderate or strong CYP2D6 inhibitors, and six had poor tamoxifen adherence/absorption (baseline tamoxifen <150 nM). One patient was both PM and on a moderate inhibitor, and one patient had all three factors present. Fifteen (52%) had no obvious cause for low endoxifen level.

**Endoxifen levels were increased by tamoxifen dose escalation**

Seventy-eight participants (63%) entered the dose escalation phase of the study, including one with a baseline endoxifen level of 30.09 nM. Nine patients withdrew before dose escalation. Overall, eighteen participants (23.7%) withdrew from the study due to treatment
related effects (N=9), social reasons (N=3), cessation of tamoxifen by clinician (N=2), relocated overseas (N=2), enrolment in another clinical trial (N=1) and progressive disease (N=1). The maximum dose reached by patients allocated to dose escalation but withdrew before target endoxifen or 60mg/d tamoxifen dose was reached was 20mg in eight, 30/40mg in seven and 50mg in three participants.

Following tamoxifen dose escalation, an endoxifen level of >15nM was achieved in 117/122 individuals (96%) compared with 93/122 (76%) at baseline. Overall, 93/122 (76%) achieved the 30nM threshold with dose escalation compared with 42/122 (34%) at baseline. Of 68 dose-escalated individuals, 65 (96%) and 47 (69%) achieved the 15nM and 30nM endoxifen targets respectively (Table 2, Figure 2). The effect of dose escalation on levels of tamoxifen and other metabolites is shown in Supplemental Figure 1.

Overall, each 10mg increase in tamoxifen dosage was associated with a mean 7.8nM (±10.25M) increase in endoxifen level. For PM individuals, the mean increase in serum endoxifen per 10mg increase in tamoxifen was 1.15nM (±1.57nM) lower than other categories, but this difference was not statistically significant (test for trend p=0.30)

**Impact of genotype, low baseline endoxifen and CYP2D6 inhibitors on target levels**

The 15nM level was achieved by 100% of the dose-escalated UM/EM and IM groups and 63% of the PM category (Table 2). None of the dose-escalated PM group reached the 30nM target compared with 71% of IM and 83% of UM/EM dose-escalated individuals. The only CYP2D6 EM participant who dose escalated to 60mg without achieving the 30nM endoxifen target was taking a strong CYP2D6 inhibitor (fluoxetine). Across the cohort, endoxifen levels
at highest tamoxifen dosage were higher for UM/EM compared to IM and (p=0.04) PM individuals (p<0.001) (Table 2).

In those with baseline endoxifen levels <10nM, 7/12 (58%) and 2/12 (17%) reached the 15nM and 30nM targets respectively. Five of 12 were CYP2D6 PM and 3 were on moderate or strong CYP2D6 inhibitors (one participant had both factors). The patient with the lowest baseline endoxifen level (3.1nM) was CYP2D6 EM and on no inhibitors and had a serum tamoxifen level of 176nM (all patient mean tamoxifen was 326nM). In those with baseline endoxifen of 10-15nM, 12/12 (100%) and 5/12 (42%) reached the 15nM and 30nM targets (Table 2). Two of 12 were CYP2D6 PM and none were on moderate or strong CYP2D6 inhibitors.

Twenty eight of 68 patients had a change in concomitant medication during the tamoxifen dose escalation period and three of these involved a drug known to affect CYP2D6. In one EM patient, duloxetine (moderate inhibitor) was commenced after initial dose escalation with endoxifen levels (on tamoxifen dose) of 11.9nM (20mg), 17.3nM (40mg) and 30.6nM (50mg). One IM patient started venlafaxine (weak inhibitor) with endoxifen levels of 14.6 (20mg) and 31.8nM (40mg). A PM patient had fluoxetine (strong inhibitor) dose reduced with endoxifen levels 6.1nM (20mg), 10.2nM (40mg) and 20.9nM (60mg).

**Low baseline endoxifen predicts failure to achieve endoxifen targets**

Various parameters were examined for association with final endoxifen levels above 15nM and 30nM respectively (Table 3). In a univariate analysis, CYP2D6 PM was a predictor for reaching the 15nM and 30nM targets (p=0.01 and 0.001 respectively) but lost significance in the multivariate analysis for the 15nM target. PM was excluded from the multivariate
analysis for the 30nM target since no PM patients reached that target. In a multivariate analysis, the only independent predictor of achieving the 15nM and 30nM targets was low baseline endoxifen level (p = 0.04 and 0.003 respectively).

**Hot flashes and other toxicity**

On standard dose 20mg tamoxifen, 104/122 (85%) of participants reported hot flashes. The mean hot flash score for all participants on 20mg tamoxifen was 52.1 +/- 16.5 (SEM). There was no association between baseline endoxifen level and hot flash score when analyzed as continuous variables (Spearman rank correlation coefficient R=0.07, Figure 3A). *CYP2D6* genotype was not associated with the presence or severity of hot flashes (data not shown).

There was no association between hot flash score and endoxifen levels at maximum tamoxifen dosage (R=0.005, Figure 3B) and increase in tamoxifen dosage was not associated with an increase in hot flash score (Figure 3C). There were no episodes of venous or arterial thromboembolism during or within 6 months of the study period.

**DISCUSSION**

This study was commenced in March 2010 and since then other studies have shown that tamoxifen dose escalation can lead to an increase in endoxifen level (27-30). However, our study is notable since dose escalation occurred regardless of *CYP2D6* genotype, and medications that were known to inhibit *CYP2D6* metabolism were not an exclusion criteria allowing independent assessment of these factors.

We have shown that, although PM *CYP2D6* genotype is associated with low endoxifen level, *CYP2D6* genotype alone is not a reliable predictor of endoxifen level especially in patients
undergoing tamoxifen dose escalation. It was clear that multiple factors contribute to low endoxifen level, including CYP2D6 genotype, concomitant CYP2D6 inhibitor use, and poor adherence or absorption. One or more of these factors were identified in 48% of those with endoxifen <15 nM. However, over 50% of low endoxifen subjects had no obvious cause identified. It is possible that variation in other non-CYP2D6 pathways may have also contributed to low endoxifen. These were not measured in this study but will be the subject of an extensive genotype analysis of drug metabolism and elimination genes in these patients. In a multi-variate analysis, low baseline endoxifen was the only predictor of failure to reach the 15nM, a level purported to be important for anti-cancer effect. We also showed no association between a patient’s experience of hot flashes and endoxifen levels on standard dose tamoxifen or following dose escalation. Overall, we conclude that direct measurement of endoxifen levels is more informative than inferring potential therapeutic endoxifen levels from CYP2D6 genotype, the use of CYP2D6 inhibitors, or by monitoring side effects such as hot flashes.

At the time of commencement of the study, there were few data available to indicate a therapeutic level for endoxifen. Hence, for the purposes of evaluating the effect of dose escalation, an empirical approach was taken to define the study target of 30nM. Since then, two studies have been published that indicate that an endoxifen level of approximately 15nM may be a critical level for anti-cancer effect. The Women’s Healthy Eating and Living (WHEL) Study of 1,370 patients with ER-positive early breast cancer reported that relapse rates were higher for patients in the lowest quintile of endoxifen levels corresponding to levels < 5.9 ng/mL (or 14.2nM). More recently, Saladores et al, have shown in 306 premenopausal women on adjuvant tamoxifen that those with low (<14 nM) compared with high (>35 nM) endoxifen concentrations were associated with shorter distant relapse-free
survival (19). *In vitro* and xenograft studies have indicated similar concentrations for endoxifen effect. Hawse et al. demonstrated that treatment of MCF7 breast cancer cells with 20nM endoxifen in combination with estrogen, tamoxifen and other metabolites had no cell cycle impact, whereas inclusion of 100nM endoxifen led to a reduction in S phase fraction (20). In a mouse MCF7 breast cancer cell xenograft model, Gong et al demonstrated dose dependent tumor growth inhibition by endoxifen with simulations suggesting 97% growth inhibition at 40nM compared with 83% at 15nM,(17) Overall, these data suggests that endoxifen levels below 15nM may be suboptimal for anti-cancer effect. In our study, tamoxifen dose escalation led to an endoxifen level of >15nM in 117/122 (96%) of patients compared to 93/122 (76%) on standard dose tamoxifen.

Our study did not seek to define an optimal therapeutic endoxifen concentration and we acknowledge that before monitoring of endoxifen levels in tamoxifen-treated patients can be accepted as standard of care, prospective studies are required to prove a link between endoxifen level and anti-cancer effect. Nonetheless, in our study, and consistent with other reports, 20% of individuals on standard dose tamoxifen have endoxifen levels below 15nM (28, 31). In approximately half of the cases of those with low endoxifen level (10% of the total population), no known cause was identified, including by CYP2D6 genotype measurement, raising the notion of therapeutic drug monitoring in this setting.

Our data show that the use of CYP2D6 genotype alone gives an imperfect guide to endoxifen level on standard dose tamoxifen and after dose escalation. Although the PM CYP2D6 genotype gave insight to low endoxifen levels, some PM individuals had baseline endoxifen > 15nM and others could achieve that level with tamoxifen dose escalation. Moreover, PM status accounts for less than 25% of patients with low endoxifen level. Overall, we did not
find a sub-group of genotype where endoxifen level could be inferred accurately especially in the setting of tamoxifen dose increase.

To put these data into clinical context, if a clinician knows a patient has PM genotype, then it cannot be assumed that endoxifen level will be low. One of eight PM patients had baseline endoxifen levels above 15nM and PM patients had a 63% chance of achieving endoxifen levels above 15nM with dose escalation. Conversely, 16% (18/108) UM/EM and IM patients had endoxifen levels <15nM even when use of concomitant inhibitors was excluded. Those on moderate to strong CYP2D6 inhibitors had an 80% chance of having levels below 10nM. Importantly, if the baseline endoxifen level was <10nM, the patient had a 72% chance of substantial endoxifen increase with dose escalation, independent of genotype. In all situations the measurement of endoxifen takes into account known factors such as genotype, concomitant inhibitor, and adherence/absorption, as well as unidentified factors and could ensure that potentially effective levels are maintained, or help the clinician to decide whether an alternative to tamoxifen, such as an aromatase inhibitor, should be recommended.

Our study also examined the potential use of a clinical indicator – hot flashes – to infer endoxifen level and therefore potential anticancer effect. The potential links between inter-individual variations in tamoxifen metabolism, endoxifen level, vasomotor side effects and clinical outcome have remained contentious. There have been discordant reports relating metabolite levels to hot flashes, with one reporting an association (32), another an inverse correlation (33) and others an absence thereof (28, 34). We found no correlation between hot flash score and CYP2D6 genotype, any tamoxifen metabolite or specifically with endoxifen level in our patient group. Our data also do not support the measurement of endoxifen levels to investigate excessive hot flashes for patients taking tamoxifen (35). Although endoxifen
may be the most important metabolite for activity, it is not clear that it uniformly responsible for the rare but serious toxicities, including uterine cancer and deep vein thrombosis. Of note, the N-desmethyl and 4’-hydroxy metabolites increased by up to 4-fold with dose escalation. Little is known about the activity of 4’-hydroxy tamoxifen. It is predominantly produced by CYP2B6 and evidence by Ruenitz et al in 1982 indicates it may have significant estrogenic effects (36, 37). Safety of dose escalation should be investigated in larger numbers of patients to ensure no significant increase in these rare toxicities occur before this approach can considered on a routine basis.

Another potential solution to abrogating low endoxifen level is to increase tamoxifen dose unselectively in all patients. Arguments against this approach include difficulties in defining dose (40mg, 60mg or higher), and the fact that the majority of patients will experience very high endoxifen and other metabolite levels with resultant possible increased toxicity. Additionally, unselected dose escalation will not counter adherence issues.

It is clear from our data that endoxifen systemic exposure for patients on tamoxifen is complex and goes beyond CYP2D6 genotype alone. Important factors such as the concomitant use of inhibitors, adherence to tamoxifen dose, absorption and possibly other elimination and metabolism routes are involved (12-15). Moreover, patients with low endoxifen level can achieve significantly higher levels with escalation of tamoxifen dose regardless of CYP2D6 genotype. We also conclude that the severity of hot flashes is not associated with endoxifen exposure.

Our data indicate the best method for determining endoxifen exposure is to measure endoxifen trough level rather than implying a particular level using genetic or clinical...
measures. Together with other data, this study highlights the need to precisely define a clinically relevant endoxifen target concentration in prospective studies of tamoxifen dose adjustment with the primary outcome of efficacy. It is important that such studies are guided by direct measurement of circulating endoxifen levels.
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REFERENCES


Table 1 – Cohort characteristics

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<td>Moderate inhibitor</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Strong inhibitor</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>English/Irish/Scottish</td>
<td>61</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Continental European</td>
<td>23</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Asian</td>
<td>17</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Middle East</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Unknown</td>
<td>12</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

* Menopausal status was unknown in 3 patients

*CYP2D6* genotype: ultra-rapid (UM), extensive (EM), intermediate (IM) and poor metabolizers (PM).
Table 2: Achievement of 15 or 30nM Endoxifen targets according to baseline Endoxifen and CYP2D6 phenotype

<table>
<thead>
<tr>
<th>Baseline Endoxifen (nM)</th>
<th>n</th>
<th>Number dose escalated</th>
<th>Mean baseline Endoxifen (nM)</th>
<th>Dose escalated subgroup</th>
<th>Mean highest Endoxifen (nM)</th>
<th>Reached 15nM target</th>
<th>Reached 30nM target</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>122</td>
<td>68</td>
<td>25.7*</td>
<td></td>
<td>34.4</td>
<td>65/68 (96%)</td>
<td>47/68 (69%)</td>
</tr>
<tr>
<td>0-10</td>
<td>14</td>
<td>12</td>
<td>6.7*</td>
<td></td>
<td>20.1</td>
<td>7/12 (58%)</td>
<td>2/12 (17%)</td>
</tr>
<tr>
<td>10-15</td>
<td>15</td>
<td>12</td>
<td>12.3</td>
<td></td>
<td>33.5</td>
<td>12/12 (100%)</td>
<td>5/12 (42%)</td>
</tr>
<tr>
<td>15-20</td>
<td>21</td>
<td>18</td>
<td>17.8</td>
<td></td>
<td>38.6</td>
<td>-</td>
<td>16/18 (89%)</td>
</tr>
<tr>
<td>20-30</td>
<td>30</td>
<td>24</td>
<td>24.2</td>
<td></td>
<td>38.7</td>
<td>-</td>
<td>20/26 (77%)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>42</td>
<td>1**</td>
<td>41.8</td>
<td></td>
<td>33.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CYP2D6 metabolizer category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>8</td>
<td>8</td>
<td>9.4</td>
<td></td>
<td>18.7</td>
<td>5/8 (63%)</td>
<td>0/8 (0%)</td>
</tr>
<tr>
<td>IM</td>
<td>9</td>
<td>7</td>
<td>15.6</td>
<td></td>
<td>26.6</td>
<td>7/7 (100%)</td>
<td>5/7 (71%)</td>
</tr>
<tr>
<td>UM/EM</td>
<td>105</td>
<td>53</td>
<td>27.8*</td>
<td></td>
<td>39.6</td>
<td>53/53 (100%)</td>
<td>43/53 (81%)</td>
</tr>
</tbody>
</table>

*Includes patient on 40mg/d tamoxifen at baseline
** Baseline endoxifen 30.09nM
Table 3: Multivariate analysis of the association between parameters and the achievement of endoxifen targets

<table>
<thead>
<tr>
<th>Parameter</th>
<th>15nM endoxifen target</th>
<th>30nM endoxifen target</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td>p value</td>
</tr>
<tr>
<td>Older Age</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>CYP2D6 inhibitor use</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>CYP2D6 IM</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>CYP2D6 PM</td>
<td>0.3</td>
<td>*</td>
</tr>
<tr>
<td>Low baseline endoxifen level</td>
<td>0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>Longer tamoxifen duration</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*PM could not be included in the multivariate analysis since no PM patient had endoxifen ≥30nM
Figure Legends

Figure 1: Consort diagram

Figure 2: Endoxifen level at baseline and after tamoxifen dose escalation according to CYP2D6 metabolizer category. PM – blue line; IM - green line; EM and UM – red line

Figure 3: A: Log hot flash score (measured by the Loprinzi instrument) plotted against endoxifen level (nM), demonstrating lack of correlation (R=0.07). B: Hot flash score according to endoxifen level at highest tamoxifen dose (R=0.005) C: Change in total hot flash score per patient with dose increment of tamoxifen (mg), showing no positive correlation.
Figure 1

Patients taking tamoxifen for ≥ 2 months

N= 23
<15 nM
Increase dose by 20mg/d

N= 53
15-29.9 nM
Increase dose by 10mg/d

N= 46
≥30 nM
No change in dose

Endoxifen level

Repeat endoxifen level after 8 weeks

Repeat dose escalation until endoxifen ≥30 nM, tamoxifen dose 60mg, or toxicity
Figure 3

(A) Scatter plot showing the relationship between log of hot flush score and endoxifen level (nM). The trend line indicates a negative correlation.

(B) Scatter plot showing the relationship between hot flash score (log scale) and endoxifen at highest tamoxifen dose per patient (nM). The coefficient of determination ($R^2$) is 0.00516.

(C) Line graph illustrating the change in hot flash score over the dosage of tamoxifen (mg). The graph shows a wide range of responses with some patients experiencing a decrease in hot flashes at higher doses.
Clinical Cancer Research

Dose escalation of tamoxifen in patients with low endoxifen level: evidence for therapeutic drug monitoring - The TADE Study.

Peter Fox, Rosemary Balleine, Clara Lee, et al.

Clin Cancer Res Published OnlineFirst February 4, 2016.

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