Tamoxifen (tam) is a widely used endocrine therapy in the treatment of early and advanced stage breast cancer in women and men. It is a pro-drug having weak affinity with the estrogen receptor and needs to be converted to its main metabolite, endoxifen, to have full anti-cancer activity.

Cytotoxic 2D6 (CYP2D6) plays a major role in the metabolism of tamoxifen to endoxifen. It is genetically highly polymorphic and its activity influences profoundly the synthesis of endoxifen and potentially the efficacy of tamoxifen treatment.

Genotyping is currently the most widely used approach in studies and also in clinical practice to categorize patients as poor- (PM), intermediate- (IM), extensive- (EM) and ultra rapid-metabolizers (UM). Some clinicians already use genotyping in order to tailor the endocrine therapy of their patients. However, due to the inter-individual variations in concentrations of the active moiety due to genetic and non-genetic influences renders the predictive value of the test uncertain for an individual patient. A significant number of patients classified as EM or IM by genotyping have indeed relatively low endoxifen levels similar to PMs. This suggests that genotyping is probably not the optimal method for predicting endoxifen levels.

**Materials & Methods**

**Study design:**
- 2-4 months: Tamoxifen 20 mg/day
- Tumour and metabolites monitored:
  - CYP2D6-genotyping
  - CYP2D6 and CYP3A4S5-phenotyping

**Blood sampling:** Samples were centrifuged without delay. Cellular pellet was used for genetics analyses and plasma for determination of levels of tamoxifen and its metabolites by LC tandem MS and stored frozen (-80 °C).

**Tumour and Metabolites in plasma:**
- An ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) method was used for the simultaneous measurements of tamoxifen and its metabolites.

**Results**

57 patients were included in the principal test, 30 of them, with trough levels measured > 12 h post-dose, were eligible for these analyses.

- 7 patients were treated with CYP2D6 inhibitors (3 chloramphenicol, 1 escitalopram, 1 venlafaxine, 1 methadone, 1 paroxetine).

**Table 1: Patients characteristics**

<table>
<thead>
<tr>
<th>Age [mean (range)]</th>
<th>PM (n=26)</th>
<th>EM (n=26)</th>
<th>IM (n=26)</th>
<th>UM (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td>Caucasian</td>
<td>African</td>
<td>African</td>
<td>African</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>European</td>
<td>African</td>
<td>African</td>
<td>African</td>
</tr>
<tr>
<td>Menopausal Status</td>
<td>Premenopausal</td>
<td>Premenopausal</td>
<td>Premenopausal</td>
<td>Premenopausal</td>
</tr>
<tr>
<td>Endocrine Hypertrophy</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Concomitant drugs</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Treatment adherence</td>
<td>&gt; 95%</td>
<td>&gt; 95%</td>
<td>&gt; 95%</td>
<td>&gt; 95%</td>
</tr>
</tbody>
</table>

**Blood samples:**
- Samples were centrifuged without delay. Cellular pellet was used for genetics analyses and plasma for determination of levels of tamoxifen and its metabolites by LC tandem MS and stored frozen (-80 °C).

**Tumour and Metabolites in plasma:**
- An ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) method was used for the simultaneous measurements of tamoxifen and its metabolites.

**Efficacy criteria:**
- Breast cancer patients treated with tamoxifen for 4-6 months.
- No history of deep venous thrombosis or pulmonary embolism.
- No history of endometrial carcinoma.
- No known history of vaginal bleeding, endometriosis, endometrial hyperplasia, endometrial hypertrophy and/or polyps.
- No pregnancy or lactation in women.
- No known allergy to midazolam or dextromethorphan.

**Treatment adherence:**
- Each patient completed an anonymous questionnaire to report semi-quantitatively her treatment adherence.

**Statistical considerations:**
- Correlations between transformed trough levels of tamoxifen, its metabolites, and the ratio endoxifen/desmethyltamoxifen, endoxifen/desmethyltamoxifen/tamoxifen with CYP2D6 genotypes, phenotypes and CYP3A54 phenotypes were analyzed by univariate and multivariable linear regression analyses and analyses of variance.

**Conclusions**

- Our data confirm a significant correlation between plasma levels of endoxifen and CYP2D6 activity defined by genotyping or by phenotyping.
- Following the multivariate analyses, phenotyping appears slightly superior to genotyping in the prediction of endoxifen’s level.
- Due to the large overlap between EM and IM, the predictive value of genotyping seems uncertain for an individual patient.
- Our results are consistent with a potential superiority of monitoring the active metabolites themselves rather than genetic or phenotypic surrogates.

**References**