CYP2D6: tamoxifen 2432-2434

appr. = approximately, CI = confidence interval, C_{ss} = steady state plasma concentration, CTCAE = common terminology criteria for adverse events, ER = oestrogen receptor, HR = hazard ratio, HR_{corr} = corrected hazard ratio, IM = intermediate metaboliser (gene dose 0.25-1) (decreased CYP2D6 enzyme activity), NDM = N-desmethyltamoxifen, NM = normal metaboliser (gene dose 1.25-2.5) (normal CYP2D6 enzyme activity), NS = non-significant, OR = odds ratio, OR_{corr} = corrected odds ratio, PM = poor metaboliser (gene dose 0) (absent CYP2D6 enzyme activity), PR = progesterone receptor, RR = relative risk, S = significant, SmPC = Summary of Product Characteristics, UM = ultra-rapid metaboliser (gene dose ≥ 2.75) (increased CYP2D6 enzyme activity)

Disclaimer: The Pharmacogenetics Working Group of the KNMP formulates the optimal recommendations for each phenotype group based on the available evidence. If this optimal recommendation cannot be followed due to practical restrictions, e.g. therapeutic drug monitoring or a lower dose is not available, then the health care professional should consider the next best option.

Brief summary and justification of choices:
Tamoxifen is converted in two steps to the active metabolite endoxifen (hydroxy-N-desmethyl-tamoxifen), which has an anti-oestrogenic effect that is 30-100x stronger than tamoxifen. One of these steps is catalysed by CYP2D6, the other by CYP3A4/5. Tamoxifen is further converted by CYP2D6 to the active metabolite 4-hydroxytamoxifen. This metabolite is as potent as endoxifen, but occurs at much lower concentrations.
Reduced CYP2D6 activity thereby results in a lower plasma concentration of the most potent metabolites of tamoxifen.
Reduced effectiveness of tamoxifen has a major impact. However, there is major heterogeneity among the studies, which produce contradictory results. The mechanism of action of tamoxifen is also not clear. It is not known whether a link exists - and if so which link that is - between the plasma concentration of tamoxifen and the active metabolites and the clinical outcome. However, Madlensky 2011 found a 30% increase in the occurrence of breast cancer-related measures of outcome for endoxifen concentrations below 5.97 ng/mL (Madlensky L et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clin Pharmacol Ther 2011;89:718-25). In addition to this, six out of eight meta-analyses found a deterioration in clinical outcomes in patients with variant alleles that result in reduced CYP2D6 activity (Chan 2022, Lu 2017, Jung 2014, Cronin Fenton 2014, Lum 2013, and Zeng 2013). The oldest meta-analysis (Seruga 2010) found no significant effect. Province 2014 found no significant effect for all patients combined, but did find a significant effect for a well-defined subgroup (post-menopausal patients with surgically removed, non-metastatic, invasive, oestrogen receptor-positive breast cancer, who received adjuvant monotherapy with tamoxifen 20 mg/day for an intended period of five years). Contrary to the results for the entire group, there was no heterogeneity among the studies for this subgroup.
For the reasons mentioned above, the KNMP Pharmacogenetics Working Group decided that this concerns a gene-drug interaction for IM and PM and that action is required, namely to consider an alternative or to increase the dose based on the endoxifen concentration (yes/yes-interactions).
For UM, a study with 48 UM+gene dose 2.5 found an increased use of symptom relieving drugs (antiemetics, anxiolytics or hot flash medications) after tamoxifen initiation, an increased risk of tamoxifen discontinuation in the first 0.5 year, and a higher risk of all cause and breast cancer specific mortality (He 2020). However, a study with 6 UM found a reduction in the risk of breast cancer recurrence with increasing gene dose, so a lower risk for UM than for NM (Schroth 2010). In addition, 4 studies suggest a small kinetic effect of the UM phenotype. In a study with 11 UM, the median endoxifen plasma concentration in UM did not differ significantly from that in NM (Khalaj 2019). A study with 4 UM found a 9% higher endoxifen plasma concentration in UM compared to NM+gene dose 1/0, but did not determine whether this difference was significant (Martinez de Dueñas 2014). A study with 5 UM found a 11% lower endoxifen plasma concentration in UM compared to NM, but a significant increase with increasing gene dose in logistic regression correcting for age (Gjerde 2008). A study with 4 UM found no significant difference in endoxifen plasma concentration in UM compared to NM (Lim 2007). Because of these contradictory data, the KNMP Pharmacogenetics Working Group decided that there is insufficient evidence for a negative effect of UM at the moment, so for the gene-drug interaction necessitating therapy adjustment for UM (yes/no-interaction).
An overview of the observed clinical and kinetic effects per phenotype is provided in the background information text of the gene-drug interactions in the KNMP Kennisbank. You may also have access to this background informa-

tion text via your pharmacy or physician electronic decision support system. More detailed substantiation of the recommendation for IM and PM is provided below.

Recommendations from other organisations

The guidelines of the European Society for Medical Oncology (ESMO) and American Society of Clinical Oncology (ASCO) state that genotyping should not be performed, as there is not enough evidence in support. However, these guidelines do not state what should be done if the genotype is known. The KNMP Pharmacogenetics Working Group considers the current evidence sufficient to recommend action for patients known to be IM or PM.

Possible alternatives

Aromatase inhibitors are an alternative for post-menopausal women. However, there is no alternative for pre-menopausal women. The same applies to post-menopausal patients who do not tolerate aromatase inhibitors.

The KNMP Pharmacogenetics Working Group asked oncologists about raloxifene and these oncologists indicated that they do not consider this to be an alternative that is at least as effective as tamoxifen.

Dose recommendations

As an alternative is not always an option, the possibility of increasing the dose based on the endoxifen concentration has been included in the recommendation.

IM: Welzen 2015 found a higher endoxifen concentration for 12 IM at a dose of 40 mg/day than for NM at a dose of 20 mg/day. Kiyotani 2012 found an endoxifen concentration for 27 IM at a dose of 40 mg/day that did not differ from the concentration for NM at a dose of 20 mg/day. Irvin 2011 found an endoxifen concentration for 31x IM + 20x gene dose 1.5 at a dose of 40 mg/day that did not differ from the concentration for gene dose 2+UM at a dose of 20 mg/day. None of the three studies found an increase in side effects as a result of the dose increase.

Barginear 2011 found an endoxifen concentration for IM that was 85% of the concentration for NM at a dose of 20 mg/day. When the dose was increased to 30 mg/day for 14 IM, the endoxifen concentration increased by 35% for gene dose 1 and by 53% for gene dose 0.5. So the resulting endoxifen concentration for IM at 30 mg/day was numerically higher than for NM at 20 mg/day.

Hertz 2016 found a 4-month dose increase from 20 mg/day to 40 mg/day for 254 IM+gene dose 1.25-1.5 to result in a 48% increase in the plasma concentration of endoxifen. The endoxifen plasma concentration after dose increase was not significantly different from that for NM at a dose of 20 mg/day anymore (numerically even somewhat higher). The endoxifen concentration was above the threshold of 5.9 ng/mL reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen both at a dose of 20 and 40 mg/day. The dose increase only had a modest effect on toxicity scores. Some adverse events occurred more often, some occurred less, but most did not differ significantly.

Dezentjé 2015 found a 2-month dose increase from 20 mg/day to 30-100 mg/day (mean 46 mg/day) for 12 IM to result in a 70% increase in the plasma concentration of endoxifen. The increased dose was calculated by multiplying 20 mg/day with the ratio of the median endoxifen concentration in a group of NM at 20 mg/day (33.7 nM) and the endoxifen concentration of the patient at 20 mg/day. Before dose escalation, the endoxifen concentration in 50% of the IM was below the threshold of 5.97 ng/ml (16.0 nM) reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen. Following dose escalation, the endoxifen concentration was above this threshold in all patients. The dose increase did not result in a significant increase in toxicity. In addition, no grade 3 or 4 toxicity was observed as a result of the dose escalation.

Khalaj 2019 found dose escalation from 20 mg/day to 30 or 40 mg/day for 17 IM+PM to result in an increase of the median plasma concentration of endoxifen from below 16 nM, which is considered the minimal effective plasma endoxifen concentration, to above 16 nM. However, the median plasma concentration remained numerically lower than that for gene dose 2 and gene dose 1.25-1.5 (both NM) on tamoxifen 20 mg/day (39% and 24% lower respectively after 8 months and 18% lower and 1.6% higher for the mean of month 4 and 8). The occurrence of some side effects increased as a result of dose escalation (bloating grade 2 and 3, irritability grade 2 and 3, sexual pain grade 2 and 3, vomiting grade 1 and 2), but the occurrence of other side effects did not (hot flushes, night sweat, sexual unwillingness, weight gain, cold sweat, mood swings, vaginal irritation, vaginal bleeding, vaginal dryness, dizziness, diarrhoea, plasma concentrations of liver enzymes, blood urea nitrogen, and plasma concentration of creatinine).

Buck 2022 found a geometric mean $AUC_{0\text{-}24h}$ of endoxifen corresponding to therapeutic concentrations (≥ 14-16 nM) in 7 IM on adjuvant tamoxifen therapy for 2 of whom the dose had been escalated from 20 mg/day to 40 mg/day due to endoxifen concentrations below this threshold.

Based on the abovementioned data, a dose increase by a factor of 1.5-2 is recommended for IM.

PM: Welzen 2015 found a numerically lower endoxifen concentration for 4 PM at a dose of 40 mg/day than for NM at a dose of 20 mg/day (significance not determined). Irvin 2011 found an endoxifen concentration for 2 PM at 40 mg/day that was significantly lower than the concentration for gene dose 2+UM at 20 mg/day. Martinez de Dueñas 2014 found no difference between the endoxifen concentration of 11 PM at 40 mg/day or 8 PM at 60 mg/day and NM+gene dose 1/0 at 20 mg/day. None of the three studies found an increase in side effects as a result of a dose increase.

Hertz 2016 found a 4-month dose increase from 20 mg/day to 40 mg/day for 26 PM to result in a 61% increase in the plasma concentration of endoxifen. However, the endoxifen plasma concentration after dose

increase was still lower than that for NM at a dose of 20 mg/day. In addition, the endoxifen concentration in PM was below the threshold of 5.9 ng/mL reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen at both a dose of 20 and 40 mg/day. The dose increase only had a modest effect on toxicity scores. Some adverse events occurred more often, some occurred less, but most did not differ significantly.

Dezentjé 2015 found a 2-month dose increase from 20 mg/day to 60-120 mg/day (mean 90 mg/day) for 12 PM to result in a 3.4-fold higher plasma concentration of endoxifen. The increased dose was calculated by multiplying 20 mg/day with the ratio of the median endoxifen concentration in a group of NM at 20 mg/day (33.7 nM) and the endoxifen concentration of the patient at 20 mg/day, but with a maximum of 120 mg/day. Before dose escalation, the endoxifen concentration in all PM was below the threshold of 5.97 ng/ml (16.0 nM) reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen. Following dose escalation, the endoxifen concentration was above this threshold in all patients. One of the PM stopped after approximately 2 weeks of dose-escalation, because of toxicity at tamoxifen 60 mg/day (grade 1 hot flashes and diarrhoea, grade 2 headache, dizziness, and fatigue). However, for the other PM the dose increase did not result in a significant increase in toxicity. In addition, no grade 3 or 4 toxicity was observed as a result of the dose escalation.

Khalaj 2019 found dose escalation from 20 mg/day to 30 or 40 mg/day for 17 IM+PM to result in an increase of the median plasma concentration of endoxifen from below 16 nM, which is considered the minimal effective plasma endoxifen concentration, to above 16 nM. However, the median plasma concentration remained numerically lower than that for gene dose 2 and gene dose 1.25-1.5 (both NM) on tamoxifen 20 mg/day (39% and 24% lower respectively after 8 months and 18% lower and 1.6% higher for the mean of month 4 and 8). The occurrence of some side effects increased as a result of dose escalation (bloating grade 2 and 3, irritability grade 2 and 3, sexual pain grade 2 and 3, vomiting grade 1 and 2), but the occurrence of other side effects did not (hot flushes, night sweat, sexual unwillingness, weight gain, cold sweat, mood swings, vaginal irritation, vaginal bleeding, vaginal dryness, dizziness, diarrhoea, plasma concentrations of liver enzymes, blood urea nitrogen, and plasma concentration of creatinine).

Buck 2022 found all 4 PM to still have endoxifen trough concentrations below the therapeutic threshold (14-16 nM) after dose escalation from 20 mg/day to 40 mg/day.

Blancas 2023 did not find a difference in clinical outcome of adjuvant tamoxifen therapy for 84 IM + 13 PM (and for 55 propensity score matched IM+PM) with PM on a temporary dose increase to 40 mg/day in months 5-8 and to 60 mg/day in months 9-12 compared to 123 NM+UM+gene dose 2.5 (or 55 propensity score matched NM+UM+gene dose 2.5) without temporary dose increase. However, because the study is relatively small and there was no control group, it is not clear if a difference would have been found if there would not have been temporary dose increases in PM.

Based on the above-mentioned data, a dose increase by a factor of 2-3 is recommended for PM.

Buck 2022 found the endoxifen trough concentrations in the 4 PM on tamoxifen 40 mg/day to increase to borderline therapeutic concentrations after co-treatment with probenecid 1000 mg 2 times daily during 2 weeks. This combination did not result in serious side effects. However, the KNMP Pharmacogenetics Working Group decided that, at the moment, there is not enough information on the advantages and disadvantages of this approach to recommend co-treatment with the CYP3A4 inducer and UGT inhibitor probenecid in PM.

Relevant groups

It is not possible to deduce from the available literature whether the genotype is important for all patients or only for certain groups of patients. For this reason, the recommendation was not limited to certain subgroups or treatment methods. As described below, the literature often provided contradictory results for certain subgroups, or there was no second publication to confirm this.

The meta-analysis by Province 2014 found a significant effect for post-menopausal patients with surgically removed, non-metastatic, invasive, oestrogen receptor-positive breast cancer, who received adjuvant monotherapy with tamoxifen 20 mg/day for an intended period of 5 years, but not for all patients combined. However, the meta-analysis by Cronin-Fenton 2014 found an effect in studies involving 20-80% pre-menopausal women, but not in studies involving 0-9% pre-menopausal women. There are very few data about the effect on pre-menopausal patients, the group for which no alternative endocrine therapy is available.

The meta-analysis by Zeng 2013 only found an effect with treatment over a 5-year period. This is partially supported by Province 2014. The latter study, however, did not separately examine the same group that received treatment for a period of less than 5 years.

The meta-analysis by Zeng 2013 found an effect for both monotherapy and combination therapy. This contradicts the study by Kiyotani 2010, which only found an effect for tamoxifen monotherapy and not for combination therapy.

Four studies (3 studies and 2 half studies) reported chemotherapy in (some of) the patients. A significant effect was found in 37.5% of these studies. This figure was 58% for the 6 studies (5 studies and 2 half studies) in which chemotherapy was excluded.

Of the selected studies, only 2 related to the treatment of metastatic breast cancer. However, the study by Lim 2007 used a lower dose of 20 mg/day instead of the standard dose of tamoxifen 40 mg/day. As the kinetic studies

demonstrate that the endoxifen concentrations for IM and PM are higher at a dose of 40 mg/day, the results of this study say little about the effectiveness of the normal therapy for IM and PM. The study by Lammers 2010 found no significant difference for IM versus NM. A higher risk of death was found for PM (HR = 2.09).
Of the 19 studies into adjuvant therapy and the meta-analyses included in the risk analysis, 3 of the studies and most of the meta-analyses (also) used doses higher than the standard dose of tamoxifen 20 mg/day. As the kinetic studies demonstrate that the endoxifen concentrations for IM and PM are also higher as the dose is increased, the results of these studies say little about the effectiveness of the normal therapy for IM and PM.
The meta-analysis by Zeng 2013 found an effect for studies involving Asian patients, but not for studies involving White patients. The fact that two studies without Hardy-Weinberg equilibrium in which DNA was isolated from tumour tissue (Rae 2012 and Regan 2012) were both studies in Whites may play a role in this. Jung 2014 indicates that exclusion of these studies from the meta-analysis results in a greater HR with a greater significance. Cronin-Fenton 2014 indicated that studies using DNA from blood exhibited a significant effect more often than studies using DNA from tumour tissue.

Recommendation concerning pre-emptive genotyping, including justification of choices:
The KNMP Pharmacogenetics Working Group considers genotyping of patients before starting tamoxifen to be beneficial for drug effectiveness. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection.
The clinical implication of the gene-drug interaction scores 7 out of the maximum of 10 points. Pre-emptive genotyping is considered to be essential for scores ranging from 6 to 10 points (see below and the clinical implication score tables at the end of this risk analysis). However, the KNMP Pharmacogenetics Working Group decided to downgrade this score, because results are conflicting. Some large studies showed a negative effect of genetically diminished CYP2D6 activity on tamoxifen effectiveness while other large studies showed no effect. Because the level of evidence supporting the clinical effect in the clinical implication score is only based on the number of studies showing an effect, the level of evidence in this score is overestimated in case of conflicting results. For this reason, the KNMP Pharmacogenetics Working Group decided that there is insufficient evidence that genotyping of CYP2D6 in patients planned to be started on tamoxifen is essential and downgraded the recommendation to beneficial.
The rationale for the (sub)scores on the clinical implication score is indicated below:
A decrease in overall survival has been observed in IM and PM patients and in one study also in UM patients (code F corresponding to CTCAE grade 5). This results in the maximum score of 2 points for the first criterion of the clinical implication score, the clinical effect associated with the gene-drug interaction (2 points for code F (CTCAE grade 5)).
Eleven studies and seven meta-analyses showed serious adverse events (code E or F corresponding to CTCAE grade 4 or 5) for PM and/or IM patients. This results in the maximum of 3 points for the second criterion of the clinical implication score, the level of evidence supporting the associated clinical effect grade ≥ 3 (3 points for at least three publications with level of evidence score ≥ 3).
The meta-analysis of Lum 2013 reported an increase in the risk of death, decrease of a surrogate endpoint for survival or non-fatal events (such as recurrence of cancer) for 1 or 2 variant alleles leading to reduced enzyme activity versus no variant alleles with HR = 1.22 (17 studies, 9,555 patients, >1088 events). 1088 events in 9555 patients corresponds to an incidence of 11.4%. An HR of 1.22 means that the incidence in patients with a variant allele should be 1.22 fold those of patients without a variant allele, which approximates 1.22 fold the incidence in the total population if the patients with 1 or 2 variant alleles would be a small minority compared to those without. So, the excess incidence of serious events in patients with 1 or 2 variants could be approximated as 0.22x11.4 = 2.5%. This is the approximate percentage of IM and PM in which serious events could be prevented by adjusting the therapy such that it becomes as effective as therapy in NM. In the Netherlands, the prevalence of IM+PM is estimated to be 43-47%. A prevalence of 43% would amount to an approximation of the possibility of prevention of serious events in 0.95% of treated patients by genotyping. This indicates that the number needed to genotype (NNG) to prevent one serious event is roughly estimated to be 105. This results in 1 out of the maximum of 3 points for the third criterion of the clinical implication score, the number needed to genotype (NNG) to prevent one clinical effect code ≥ D (grade ≥ 3) (1 point for 100 < NNG ≤ 1000).
The Dutch SmPC of tamoxifen mentions that CYP2D6 PM can exhibit a reduced response, but that the consequences of these findings for the treatment of CYP2D6 PM are not entirely clear yet. This results in 1 out of the maximum of 2 points for the fourth and last criterion of the clinical implication score, the pharmacogenetics information in the SmPC (1 point for at least one genotype/phenotype being mentioned in the SmPC, but not mentioned as a contra-indication and no recommendation to genotype).
While the KNMP Pharmacogenetics Working Group considers CYP2D6 genotyping to be beneficial for Dutch patients, 2 recent and very similar cost-effectiveness analyses in Chinese patients from the same investigators suggested genotype-guided therapy to be cost-effective (Wei 2020 Clin Drug Investig and Wei 2020 Pharmacogenomics).

The table below uses the KNMP definitions for NM, PM, IM and UM. As a result, the definitions of NM, PM, IM and UM in the table below can differ from the definitions used by the authors in the article.

<table>
<tr><th>Source</th><th>Code</th><th>Effect</th><th>Comments</th></tr>
<tr><td>ref. 1, adjuvant
Blancas I et al.
Early increase in tamoxifen dose in CYP2D6 poor metaboliser breast cancer patients and survival: A propensity score matching analysis.
Breast
2023;69:342-8.
PMID: 37011481.</td><td>3

genotype-guided therapy (PM temporarily on a higher dose):
IM+PM:
AA</td><td>220 patients with ER- and/or PR-positive breast cancer were treated with tamoxifen for 5 years. The tamoxifen dose was 20 mg/day for non-PM. PM initially received a dose of 20 mg/day for 4 months and subsequently a dose of 40 mg/day for 4 months, followed by 60 mg/day for another 4 months, and then the usual dose of 20 mg/day until completing 5 years of treatment. The mean follow-up period was 112.6 months. Propensity score matching was performed for 110 patients: patients were selected 1:1 in each comparison group considering the covariates age, tumour grade, nodal status, chemotherapy, radiotherapy, and tumour size. In the complete group and in the propensity score matched group there were no significant differences between NM+UM+gene dose 2.5 and IM+PM regarding the following covariates: age, histological grade, nodal status, tumour size, HER-2 status, Ki-67 expression, chemotherapy, and radiotherapy administration. Comedication affecting CYP2D6 was not excluded.

Genotyping:
complete group
- 4x UM+gene dose 2.5
- 119x NM
- 84x IM
- 13x PM

propensity score matched group
- 55x NM+UM+gene dose 2.5
- 55x IM+PM

Results:
<table>
<tr><td colspan="4">Results compared to NM+UM+gene dose 2.5:</td></tr>
<tr><td></td><td>group</td><td>IM+PM</td><td>value for NM+UM+gene dose 2.5</td></tr>
<tr><td rowspan="2">disease-free survival</td><td>complete</td><td>NS</td><td>84.6%, 136.6 months</td></tr>
<tr><td>matched</td><td>NS</td><td>80.0%, 133.9 months</td></tr>
<tr><td rowspan="2">overall survival</td><td>complete</td><td>NS</td><td>87.8%, 142.2 months</td></tr>
<tr><td>matched</td><td>NS</td><td>87.3%, 141.6 months</td></tr>
<tr><td colspan="4">In the complete group, results were also NS for PM versus IM versus NM versus UM+gene dose 2.5.</td></tr>
</table>
Note: Genotyping was with the AmpliChip 450 test. The following gene variants were found in this Spanish patient group: *2 through *6, *9, *10, *15, *17, *29, *31, *35, *41 and multiplication of *1 and *2. These are the most important gene variants in the Spanish population.</td><td>Author’s conclusion:
”An early increase in tamoxifen dose in PM patients is not associated with survival differences among CYP-2D6 phenotypes.”</td></tr>
<tr><td>ref. 2, kin
Buck SAJ et al.
Influence of probenecid on endoxifen systemic exposure in breast cancer patients on adjuvant tamoxifen treatment.
Ther Adv Med Oncol
2022;14:175883592</td><td>4</td><td>Probenecid 1000 mg twice daily was added during 14 days in 11 patients (7 IM and 4 PM) treated with steady state adjuvant tamoxifen monotherapy. The tamoxifen dose was the standard adjuvant dose of 20 mg/day in 5 IM and had been escalated to 40 mg/day due to endoxifen concentrations below the threshold of 14-16 nM in 2 IM and all 4 PM. Probenecid induces CYP3A4 and thereby the formation of endoxifen and inhibits UGT-enzymes and thereby the metabolic clearance of endoxifen.
Concomitant use of strong CYP3A4, CYP2D6, CYP2C9, CYP-</td><td>Author’s conclusion:
”Probenecid resulted in a clinically relevant increase of endoxifen concentrations in breast cancer patients treated with adjuvant</td></tr>
</table>

<table>
<tr>
<td>21081075.
PMID: 35321309.

ref. 2, continuation</td>
<td>change in tamoxifen pharmacokinetics by probenecid co-treatment: PM: A

change in tamoxifen pharmacokinetics by probenecid co-treatment: IM: AA</td>
<td>2C19, UGT and P-gp inhibitors or inducers, herbal or dietary supplements or other over-the-counter medication besides paracetamol, and of drugs which may show an increased systemic exposure when taken concomitantly with probenecid (e.g. methotrexate, penicillin, cephalosporin or chinolon antibiotics or NSAIDs), was excluded.
Assuming a standard deviation of the difference in endoxifen AUC_{0-24h} of 25% based on literature, a total of 11 patients were calculated to give a power of 90% power to detect a 25% difference (which was considered clinically relevant) between treatment with and without probenecid.
The endoxifen concentration threshold of 14-16 nM corresponds to an endoxifen AUC_{0-24h} higher than 336-384 nmol.h/L.

Results:
<table>
<tr><td colspan="4">Changes due to probenecid addition:</td></tr>
<tr><td></td><td>phenotype</td><td></td><td>value before probenecid addition</td></tr>
<tr><td>geometric mean AUC_{0-24h} endoxifen</td><td>IM+PM</td><td>x 1.26 (S)</td><td>402 nmol.h/L</td></tr>
<tr><td></td><td>PM</td><td>x 1.41 (S)
All PM had endoxifen trough concentrations below the therapeutic threshold before probenecid. These concentrations increased to borderline therapeutic concentrations after co-treatment with probenecid.</td><td>287 nmol.h/L</td></tr>
<tr><td></td><td>IM</td><td>trend for an increase (p = 0.09) (NS)</td><td>487 nmol.h/L</td></tr>
<tr><td>geometric mean AUC_{0-24h} tamoxifen</td><td>IM+PM</td><td>x 0.60 (S)</td><td>8844 nmol.h/L</td></tr>
<tr><td>ratio geometric mean AUC_{0-24h} endoxifen/tamoxifen</td><td>IM+PM</td><td>x 2.10 (S)</td><td>0.05</td></tr>
<tr><td>ratio geometric mean AUC_{0-24h} N-desmethyl-tamoxifen/tamoxifen</td><td>IM+PM</td><td>x 1.36 (S)</td><td>2.39</td></tr>
<tr><td>ratio geometric mean AUC_{0-24h} endoxifen/4-hydroxy-tamoxifen</td><td>IM+PM</td><td>x 1.43 (S)</td><td>3.97</td></tr>
<tr><td>ratio geometric mean AUC_{0-24h} 4-hydroxy-tamoxifen/tamoxifen</td><td>IM+PM</td><td>x 1.47 (S)</td><td>0.01</td></tr>
<tr><td>ratio geometric mean AUC_{0-24h} endoxifen/N-</td><td>IM+PM</td><td>x 1.55 (S)</td><td>0.02</td></tr>
</table>
</td>
<td>tamoxifen. This combination therapy could provide a solution for patients with a CYP2D6-poor metabolizer phenotype or endoxifen concentrations below the threshold despite earlier tamoxifen dose.”</td>
</tr>
</table>

ref. 2, continuation		desmethyl-tamoxifen Observed adverse events during combination treatment were relatively mild. There were no severe or serious adverse events (CTCAE grade ≥ 3) observed. Probenecid treatment-related adverse effects included hypokalaemia grade 2 (n = 1), neutropenia grade 2 (n = 1), nausea grade 1 (n = 4), headache grade 1 (n = 3 versus n = 1 before probenecid), dizziness grade 1 (n = 1), increased creatinine grade 1 (n = 2), and leukopenia grade 1 (n =1 versus n =1 before probenecid). Except for muscle cramps grade 1, which occurred three times more often (n = 3 versus n = 1), tamoxifen-related adverse events (including hot flashes grade 1, n =3 both before and with probenecid) did not increase during combination therapy, compared to monotherapy. Note: Genotyping was with the Infiniti or Quantstudio test. Both tests determine the most important gene variants in this Dutch population.	
ref. 3, adjuvant Chan CWH et al. Association between genetic polymorphisms in cytochrome P450 enzymes and survivals in women with breast cancer receiving adjuvant endocrine therapy: a systematic review and meta-analysis. Expert Rev Mol Med 2022;24:e1. PMID: 34991754.	3	Meta-analyses of 22 studies investigating the association between CYP2D6 gene variants on survival outcome in breast cancer patients treated with adjuvant tamoxifen. 8 studies were included in the meta-analysis investigating overall survival, 9 in the meta-analysis investigating disease-free survival, 4 in the meta-analysis investigating relapse-free survival and comparing IM+PM to NM+UM, and 6 in the meta-analysis investigating relapse-free survival and comparing homozygote variant (PM+IM) versus (homozygote wild type + heterozygote variant) (NM+gene dose 1/0). Based on assessment using the Effective Public Health Practice Project (EPHPP), 1 of the included studies was rated as strong (i.e. high quality) (1 of the 4 included studies in the smallest relapse-free survival meta-analysis), 6 as moderate (i.e. moderate quality) (2 of the 8 included studies in the overall survival meta-analysis, 2 of the 9 studies in the disease-free survival meta-analyse, and 1 of the 4 studies and 1 of the 6 studies in the relapse-free survival meta-analyses) and 15 as weak (i.e. low quality) (6 of the 8 included studies in the overall survival meta-analysis, 7 of the 9 studies in the disease-free survival meta-analyse, and 2 of the 4 studies and 5 of the 6 studies in the relapse-free survival meta-analyses). The EPHPP rates six components including selection bias, study design, confounders, blinding, data collection methods, and withdrawals and drop-outs, as either strong, moderate or weak according to a standardised guide and dictionary. A study is overall rated strong if none of the six components is rated weak. Moderate rating indicates that one of the six components is rated weak. The study is rated as weak if two or more of the six components are rated weak. Of the 22 studies in the meta-analyses, 11 were also included in our risk analysis separately (Park 2012, Regan 2012, Thompson 2011, Abraham 2010, Kiyotani 2010, Lammers 2010, Schroth 2009, Goetz 2007, Schroth 2007, Goetz 2005, and Nowell 2005). Of the studies in this meta-analysis, 11 were also included in the meta-analysis by Zeng 2013 (Nowell 2005, Newman 2008, Okishiro 2009, Schroth 2009, Abraham 2010, Kiyotani 2010, Thompson 2011, Park 2012, Regan 2012, Sukasem 2012, and Goetz 2013), 10 in the meta-analysis by Cronin-Fenton 2014 (Nowell 2005, Newman 2008, Okishiro 2009, Schroth	Author's conclusion: "Meta-analyses were performed on CYP2D6 studies ... . The results of meta-analyses demonstrated that shorter overall survival, disease-free survival and relapse-free survival were found in the patients with decreased metabolisers when compared to normal metabolisers."

<table>
<tr>
<td>ref. 3, continuation</td>
<td>IM+PM: F</td>
<td>2009, Abraham 2010, Kiyotani 2010, Thompson 2011, Regan 2012, Sukasem 2012, and Goetz 2013), 8 in the meta-analysis by Jung 2014 (Newman 2008, Okishiro 2009, Schroth 2009, Kiyotani 2010, Thompson 2011, Park 2012, Regan 2012, and Sukasem 2012), 7 in the meta-analysis of Lum 2013 (Nowell 2005, Schroth 2007, Schroth 2009, Abraham 2010, Lammers 2010, Thompson 2011, and Regan 2012), 6 in the meta-analysis of Seruga 2010 (Nowell 2005, Newman 2008, Okishiro 2009, Schroth 2009, Kiyotani 2010, and the data from Thompson 2011), 3 in the meta-analysis of Province 2014 (Goetz 2005, Schroth 2009, and Kiyotani 2010), and 2 in the meta-analysis by Lu 2017 (Kiyotani 2008 and Sukasem 2012).
Meta-analyses were performed with a random-effects model in case of significant heterogeneity between the studies and with a fixed-effect model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised.
Publication bias analysis was not performed.

Results:
<table>
<tr><td colspan="3">Results for IM+PM or homozygote variant carriers:</td></tr>
<tr><td></td><td>comparison</td><td></td></tr>
<tr><td>less overall survival</td><td>IM+PM compared to NM</td><td>HR = 1.30 (95% CI: 1.08-1.57) (S)</td></tr>
<tr><td>less disease-free survival</td><td>IM+PM compared to NM</td><td>HR = 1.52 (95% CI: 1.26-1.83) (S)</td></tr>
<tr><td rowspan="2">less relapse-free survival</td><td>IM+PM compared to NM+UM</td><td>NS</td></tr>
<tr><td>IM+PM compared to NM+gene dose 1/0</td><td>trend for a reduction in survival (p = 0.08) (NS)</td></tr>
<tr><td colspan="3">There was significant heterogeneity between the studies for both relapse-free survival comparisons.
There was no significant heterogeneity between the studies for the overall and disease-free survival comparisons.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 4, adjuvant
He W et al.
CYP2D6 genotype predicts tamoxifen discontinuation and prognosis in patients with breast cancer.
J Clin Oncol 2020;38:548-57.
PMID: 31800347.</td>
<td>4</td>
<td>1309 breast cancer patients were treated with tamoxifen. Of the tumours for which this was determined, 83% was positive for the progesterone receptor and 7.5% for human epidermal growth factor 2 (HER2). No information on the oestrogen receptor is provided. 62% of the patients with known menopausal status was postmenopausal. Median follow-up was 10.4 years (range 1.1-13.4 years).
Use of symptom-relieving drugs was defined as filling at least one prescription of the corresponding drugs within 90 days of tamoxifen initiation. Women who used the corresponding symptom-relieving drugs within 90 days before tamoxifen initiation were excluded from the analyses.
Tamoxifen discontinuation was defined as having any interval between 2 consecutive tamoxifen dispenses exceeding 180 days during the follow-up.
Co-medication with moderate to potent CYP2D6 inhibitors was excluded, as was switching from tamoxifen to aromatase inhibitors.
Hazard ratios were corrected for age (25-50, 50-60 and ≥ 60 years), menopausal status, hormone replacement therapy (never or ever use), family history of cancer, education (≤ 12 years, > 12 years, other), body mass index (< 25, 25-30 and ≥ 30 kg/m^2), cigarette smoking (never or ever), Charison comor-</td>
<td>Author’s conclusion:
”Both poor and ultrarapid CYP2D6 metabolizers of tamoxifen have a worse prognosis for breast cancer compared with normal metabolizers after receiving a standard dose of tamoxifen. This U-shaped association might call for individualized tamoxifen dosage.”</td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td>PM: F
IM: F
UM: F</td>
<td>bidity index (0, 1 or ≥ 2), parity (0, 1-2 or ≥ 3), tumour size (< 20 or ≥ 20 mm), lymph node involvement, progesterone receptor status, HER2 status, cancer grade (I, II or III), chemotherapy, and radiotherapy. None of these possible confounders differed significantly between the genotype groups at baseline.

Genotyping:
- 645x NM
- 503x IM
- 113x PM
- 48x UM+gene dose 2.5

Results:
<table>
<tr><td colspan="6">Results compared to NM:</td></tr>
<tr><td colspan="2"></td><td>PM</td><td>IM</td><td>UM</td><td>value for NM</td></tr>
<tr><td rowspan="4">use of symptom relieving drugs after tamoxifen initiation</td><td>antiemetics</td><td>NS</td><td>NS</td><td>appr. x 28 (S)</td><td>appr. 0.2%</td></tr>
<tr><td>anxiolytics</td><td>NS</td><td>NS</td><td>appr. x 4.2 (S)</td><td>appr. 1.7%</td></tr>
<tr><td>hot flash medications</td><td>NS</td><td>NS</td><td>appr. x 4.8 (S)</td><td>appr. 2.3%</td></tr>
<tr><td>analgesics</td><td>NS</td><td>NS</td><td>NS</td><td>appr. 7.7%</td></tr>
<tr><td rowspan="3">tamoxifen discontinuation</td><td>first 0.5 year</td><td>NS</td><td>NS</td><td>HR_{corr} = 2.06 (95% CI 1.11-3.82)</td><td></td></tr>
<tr><td>0.5-5 years</td><td>NS</td><td>NS</td><td></td><td></td></tr>
<tr><td colspan="5">Use of symptom relieving drugs (antiemetics, anxiolytics or hot flash medications) was associated with tamoxifen discontinuation within 0.5 year.</td></tr>
<tr><td rowspan="3">mortality, all patients</td><td>all cause</td><td>HR_{corr} = 2.59 (95% CI 1.39-4.83)</td><td>HR_{corr} = 1.86 (95% CI 1.20-2.87)</td><td>HR_{corr} = 4.92 (95% CI 2.27-10.64)</td><td>6.0%</td></tr>
<tr><td>breast cancer specific</td><td>HR_{corr} = 2.59 (95% CI 1.01-6.57)</td><td>NS</td><td>HR_{corr} = 4.52 (95% CI 1.42-14.37)</td><td>2.6%</td></tr>
<tr><td colspan="5">Tamoxifen discontinuation was associated with breast cancer mortality.</td></tr>
<tr><td rowspan="2">mortality, postmenopausal patients</td><td>all cause</td><td>HR_{corr} = 2.44 (95% CI 1.17-5.10)</td><td>NS</td><td>HR_{corr} = 4.86 (95% CI 1.76-13.45)</td><td>8.4%</td></tr>
<tr><td>breast cancer specific</td><td>NS</td><td>NS</td><td>NS</td><td>2.6%</td></tr>
<tr><td>mortality, pre-</td><td>all cause</td><td>HR_{corr} = 6.63</td><td>HR_{corr} = 3.14</td><td>HR_{corr} = 6.24</td><td>3.0%</td></tr>
</table>
</td>
<td></td>
</tr>
</table>

<table>
<tr>
<td>ref. 4, continuation</td>
<td></td>
<td>
<table>
<tr><td>meno-pausal patients</td><td></td><td>(95% CI 1.74-25.22)</td><td>(95% CI 1.20-8.19)</td><td>(95% CI 1.57-24.75)</td><td></td></tr>
<tr><td></td><td>breast cancer specific</td><td>HR_{corr} = 4.86 (95% CI 1.22-19.31)</td><td>NS</td><td>HR_{corr} = 4.48 (95% CI 1.16-17.27)</td><td>3.0%</td></tr>
</table>
Note: Genotyping was for *2, *2A, *3 through *10, *14, *17, *29, *35, *41A and gene multiplication. These are the most important gene variants in this Swedish population. All variants, except *4, *6 and *10, were in Hardy-Weinberg equilibrium. *8 and *14 were not found in this patient group.
</td>
<td></td>
</tr>
<tr>
<td>ref. 5, kinetics
Khalaj Z et al.
Clinical trial: CYP-2D6 related dose escalation of tamoxifen in breast cancer patients with Iranian ethnic background resulted in increased concentrations of tamoxifen and its metabolites.
Front Pharmacol 2019;10:530.
PMID: 31178724.</td>
<td>4

PM: A
IM: A
UM: AA</td>
<td>
134 breast cancer patients, including 27x gene dose 0.5-1, and 5x gene dose 0-0.5, were treated with tamoxifen 20 mg/day for at least 4 months. 15 patients (12 NM and 3 UM) with a tamoxifen plasma concentration <100 nM, and 2 patients with ambiguous gene multiplication analysis were excluded from the tamoxifen metabolite quantifications, because these low plasma concentrations implied that these patients had missed at least one daily dose. For 15 of the patients with gene dose 0.5-1 and two of the patients with gene dose 0-0.5, the dose was escalated to 30 mg/day and 40 mg/day respectively during 8 months. The other patients continued on 20 mg/day.
Co-medication with known CYP2D6 inhibitors, and reduced liver and kidney function were excluded.

Genotyping:
- 68x gene dose 2 (actually 65x gene dose 2, 2x gene dose 2.5 or 2 ((1/*41)xN), and 1x gene dose 1 or 2 ((*1/*4)xN))
- 21x gene dose 1.25-1.5
- 23x gene dose 1 (gene dose 1/0)
- 4x gene dose 0.5-1 (2x gene dose 0.5/0.5, 1x gene dose 0.5/0.25, and 1x gene dose 0.25/0.25)
- 5x PM+IM (4x PM, and 1x gene dose 0.5/0)
- 11x UM
- 2x genotype unknown

Results:
<table>
<tr><td colspan="7">Median plasma concentration (in nM) compared to gene dose 2:</td></tr>
<tr><td></td><td>PM+ IM</td><td>gene dose 0.5-1</td><td>gene dose 1</td><td>gene dose 1.25-1.5</td><td>UM</td><td>value for gene dose 2</td></tr>
<tr><td>tamoxifen</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>356</td></tr>
<tr><td></td><td colspan="5">NS for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM</td><td></td></tr>
<tr><td>desmethyl-tamoxifen</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>NS</td><td>580</td></tr>
<tr><td></td><td colspan="5">NS for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM</td><td></td></tr>
<tr><td>Z-endoxifen</td><td>x 0.28 (S)</td><td>x 0.55 (NS)</td><td>x 0.55 (S)</td><td>x 0.94 (NS)</td><td>x 0.91 (NS)</td><td>30.6</td></tr>
</table>
</td>
<td>Author’s conclusion:
”We show the feasibility of dose escalation of tamoxifen in breast cancer patients with compromised CYP2D6 activity and Iranian ethnic background to increase the plasma concentrations of (Z)-endoxifen.”</td>
</tr>
</table>

ref. 5, continuation				S for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM				
				The value for IM+PM is below 16 nM, which is considered the minimal effective plasma endoxifen concentration. The median plasma concentration for gene dose 0.5-1 and gene dose 1 is 16.8 nM.				
			E-endoxifen	x 0.53 (NS)	x 0.14 (NS)	x 0.25 (S)	x 0.28 (NS)	x 0.72 (NS)	3.7
				S for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM				
			Z+E-endoxifen	x 0.23 (S)	x 0.46 (NS)	x 0.56 (S)	x 0.80 (NS)	x 0.92 (NS)	38.3
				S for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM				
				The value for IM+PM is below 16 nM, which is considered the minimal effective plasma endoxifen concentration. The median plasma concentration for gene dose 0.5-1 and gene dose 1 is 17.7 nM and 21.6 nM respectively.				
			4-hydroxytamoxifen	x 0.53 (NS)	x 0.79 (NS)	x 0.63 (NS)	x 0.99 (NS)	x 0.94 (NS)	5.3
				S for the trend PM+IM versus gene dose 0.5-1 versus gene dose 1 versus gene dose 1.25-1.5 versus gene dose 2 versus UM				

Results after 4 and 8 months of dose-escalation (to 30 or 40 mg/day) for 17 patients with gene dose 0-1 compared to the same patients before dose escalation and (for adverse events) also to the other patients (all without dose escalation):

	8 months	4 months	value before dose escalation	value for group without dose escalation
Median plasma concentrations (in nM)				
tamoxifen	x 1.9 (S)	x 1.8 (S)	346	
	S			
desmethyltamoxifen	x 2.0 (S)	x 2.1 (S)	629	
	S			
Z-endoxifen	x 1.6 (S)	x 1.7 (NS)	14.9	
	S			
	Dose increase rai-			

ref. 5, continuation			ses the median plasma concentration from below 16 nM, which is considered the minimal effective plasma endoxifen concentration, to above 16 nM. However, the median plasma concentration remains numerically lower than that for gene dose 2 and gene dose 1.25-1.5 (both NM) on tamoxifen 20 mg/day (21% and 17% lower respectively).			
		E-endoxifen	x 0.6 (S)	x 5.5 (NS)	0.6	
			S			
		Z+E-endoxifen	x 2.0 (S)	x 3.3 (S)	11.9	
			S			
			Dose increase raises the median plasma concentration from below 16 nM, which is considered the minimal effective plasma endoxifen concentration, to above 16 nM. However, the median plasma concentration remains numerically lower than that for gene dose 2 and gene dose 1.25-1.5 (both NM) on tamoxifen 20 mg/day (39% and 24% lower respectively after 8 months and 18% lower and 1.6% higher for the mean of month 4 and 8).			
		4-hydroxytamoxifen	x 2.0 (S)	x 1.8 (NS)	3.4	
			S			
		% of patients with adverse events				
		hot flushes	grade 0	NS	12%	11%
			grade 1	NS	24%	29%
			grade 2	NS	35%	34%
			grade 3	NS	29%	26%
		night sweat	grade 0	NS	41%	38%
			grade 1	NS	23%	23%
			grade 2	NS	18%	21%
			grade 3	NS	18%	18%

ref. 5, continuation	IM+PM on 30-40 mg versus NM on 20 mg: C	sexual unwillingness	grade 0	NS		35%	38%
			grade 1	NS		18%	22%
			grade 2	NS		24%	22%
			grade 3	NS		24%	18%
		weight gain	grade 0	NS		47%	45%
			grade 1	NS		23%	29%
			grade 2	NS		12%	10%
			grade 3	NS		18%	16%
		cold sweat	grade 0	NS		53%	50%
			grade 1	NS		18%	17%
			grade 2	NS		18%	19%
			grade 3	NS		12%	14%
		bloating	grade 0	x 0.71	x 0.57	41%	39%
			grade 1	x 1.0	x 0.75	23%	29%
			grade 2	x 1.7	x 2.0	18%	19%
			grade 3	x 1.0	x 1.3	18%	13%
				S for group without dose escalation versus before dose escalation versus month 4 versus month 8			
		irritability	grade 0	x 1.3	x 1.0	18%	21%
			grade 1	x 0.14	x 0.29	41%	43%
			grade 2	x 1.4	x 1.2	29%	26%
			grade 3	x 2.5	x 3.0	12%	10%
				S for group without dose escalation versus before dose escalation versus month 4 versus month 8			
		mood swings	grade 0	NS		12%	12%
			grade 1	NS		53%	57%
			grade 2	NS		23%	22%
			grade 3	NS		12%	9%
		sexual pain	grade 0	x 0.75	x 0.75	47%	49%
			grade 1	x 0.38	x 0.63	47%	38%
			grade 2	x 3.1[a]	x 1.5[a]	0%	8%
			grade 3	x 4.0	x 4.0	6%	5%
				S for group without dose escalation versus before dose escalation versus month 4 versus month 8			
				[a] Compared to the group without dose escalation, because the value before dose escalation was 0%.			
		vomiting	grade 0	x 0.67	x 0.80	88%	86%
			grade 1	x 3.0	x 2.0	12%	10%
			grade 2	x 1.7[a]	x 1.7[a]	0%	4%
			grade 3	x 1.0	x 1.0	0%	0%
				S for group without dose escalation versus before dose escalation versus			

<table>
<tr>
<td>ref. 5, continuation</td>
<td></td>
<td>
<table>
<tr><td></td><td></td><td>month 4 versus month 8</td><td></td><td></td></tr>
<tr><td></td><td></td><td>[a] Compared to the group without dose escalation, because the value before dose escalation was 0%.</td><td></td><td></td></tr>
<tr><td colspan="2">vaginal irritation</td><td rowspan="8">NS</td><td rowspan="8"></td><td rowspan="8"></td></tr>
<tr><td colspan="2">vaginal bleeding</td></tr>
<tr><td colspan="2">vaginal dryness</td></tr>
<tr><td colspan="2">dizziness</td></tr>
<tr><td colspan="2">diarrhoea</td></tr>
<tr><td colspan="2">plasma concentrations of liver enzymes</td></tr>
<tr><td colspan="2">blood urea nitrogen</td></tr>
<tr><td colspan="2">plasma concentration of creatinine</td></tr>
<tr><td colspan="5">Adherence to therapy</td></tr>
<tr><td colspan="3">Adherence to therapy in patients with dose escalation was entirely satisfactory, with 47.1% not missing any tamoxifen doses, 29.4% missing 1-5 doses, 17.6% missing 6-10 doses, and 5.9% (1 patient) missing more than 10 doses.</td><td></td><td></td></tr>
</table>

Note: Genotyping was for *2, *4 through *6, *10, *17, *41 and gene multiplication. These are the most important gene variants in Middle East populations, like this Iranian patient group. All variants were in Hardy-Weinberg equilibrium.
</td>
<td></td>
</tr>
<tr>
<td>ref. 6, adjuvant
Brooks JD et al. CYP2D6 phenotype, tamoxifen, and risk of contralateral breast cancer in the WECARE Study. Breast Cancer Res 2018;20:149. PMID: 30526633.</td>
<td>3</td>
<td>1514 breast cancer cases who developed contralateral breast cancer were compared to 2203 controls who did not develop contralateral breast cancer. 1250 patients were treated with tamoxifen for their first primary breast cancer, 465 cases and 785 controls. 84% of the these patients had oestrogen receptor (ER) positive breast cancer and 69% was premenopausal at first diagnosis. All patients had their first breast cancer prior to age 55 years. Matching was based on year of birth, year of diagnosis, cancer registry region, and case/ethnicity. 825 patients were matched to one control. 689 patients were matched to two controls, such that two members of each case-control trio had received radiation treatment for the first breast cancer. Data were derived from an interview by telephone, medical records, pathology reports, and hospital charts.
Co-medication with CYP2D6 inhibitors was not excluded.
Rate ratios (relative risks) were corrected for age at first diagnosis, menopausal status and age at menopause 2 years before first diagnosis, histology, stage, and oestrogen receptor status of first diagnosis, first-degree family history of breast cancer, chemotherapy for first breast cancer, radiation treatment for first breast cancer, hormonal therapy other than tamoxifen for first breast cancer, number of full-term pregnancies before first diagnosis, and age at menarche. In addition, a correction term was used for the way of matching of patients with two controls.

Genotyping:
genotype group number of patients</td>
<td>Author’s conclusion:
”This study suggests that the CYP2D6 phenotype may contribute to some of the observed variability in the impact of tamoxifen treatment for a first breast cancer on risk of developing contralateral breast cancer.”</td>
</tr>
</table>

ref. 6, continuation	PM: E IM: AA			tamoxifen treated	not tamoxifen treated			
			gene dose 2	493	944			
			gene dose 1.25-1.5	206	397			
			gene dose 1 (gene dose 1/0)	363	753			
			gene dose 0.5-1 (gene dose 0.5/0.5, gene dose 0.5/0.25, and gene dose 0.25/0.25)	43	62			
			gene dose 0.25-0.5 (gene dose 0.5/0 or gene dose 0.25/0)	92	156			
			PM	53	155			
		Results:						
		Rate ratios (95% CI) of contralateral breast cancer for tamoxifen treatment of the first breast cancer compared to no tamoxifen treatment of the first breast cancer:						
				gene dose or phenotype				
				PM	0.25-0.5	0.5-1	1	1.25-1.5	2
			all patients	1.17 (NS)	1.08 (NS)	0.64 (NS)	0.55 (0.40 – 0.74)	0.45 (0.30 – 0.68)	0.81 (0.62 – 1.06) (NS, but trend for S)
				1.18 (NS)	0.95 (NS)		0.63 (0.51-0.78)		
				Risk ratios differed significantly between the gene doses (S), but not between PM, IM (gene dose 0.25-0.5 plus 0.5-1) and NM+ gene dose 1 (NS), although a trend was present (p = 0.09).					
			ER-positive first breast cancer	1.85 (NS)	0.51 (NS)	0.54 (NS)	0.54 (0.33 - 0.89)	0.38 (0.20 - 0.74)	1.06 (NS)
				1.85 (NS)	0.55 (NS)		0.69 (0.50-0.97)		
				Risk ratios differed significantly between the gene doses (S), but not between PM, IM (gene dose 0.25-0.5 plus 0.5-1) and NM+ gene dose 1 (NS).					
			premeno-pausal at first breast cancer	1.06 (NS)	0.77 (NS)	0.64 (NS)	0.51 (0.33 - 0.79)	0.47 (0.28 - 0.79)	0.87 (NS)
				1.06 (NS)	0.76 (NS)		0.65 (0.49-0.85)		
				Risk ratios did not differ significantly between the gene doses and between PM, IM (gene dose 0.25-0.5 plus 0.5-1) and NM+gene dose 1 (NS).					
			Non-Hispanic Whites	1.10 (NS)	1.10 (NS)	0.56 (NS)	0.56 (0.41 , 0.78)	0.46 (0.29 , 0.71)	0.88 (NS)

<table>
<tr>
<td>ref. 6, continuation</td>
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<table>
<tr><td></td><td>1.11 (NS)</td><td>0.96 (NS)</td><td>0.66 (0.53, 0.83)</td></tr>
<tr><td></td><td colspan="3">Risk ratios differed significantly between the gene doses (S), but not between PM, IM (gene dose 0.25-0.5 plus 0.5-1) and NM+ gene dose 1 (NS).</td></tr>
</table>

Note: Genotyping was for *2 through *6,*9, *10, and *41. Except for gene multiplication, these are the most important gene variants in the populations included in this patient group from the USA, Canada and Denmark. Only *4 and *9 did not deviate from Hardy-Weinberg equilibrium in the controls.
</td>
<td></td>
</tr>
<tr>
<td>ref. 7, adjuvant
Lu J et al.
The effect of CYP-2D6 *10 polymorphism on adjuvant tamoxifen in Asian breast cancer patients: a meta-analysis.
Onco Targets Ther 2017;10:5429-37.
PMID: 29180876.</td>
<td>4</td>
<td>Meta-analysis of retrospective cohort studies in Asian female breast cancer patients treated with 20 mg/day adjuvant tamo--xifen for 2-5 years. Only studies with more than 30 patients and more than 4 points on the 8-point Newcastle-Ottawa scale, indicating high quality, were included. Included studies scored 5-7 points on the Newcastle-Ottawa scale.
For the comparison of IM (*10/*10) versus NM (*1/*10+*1/*1), 4 studies with a total of 538 patients were included for disease free survival, 2 studies for overall survival, and 5 studies with a total of 382 patients for breast cancer recurrence.
For the comparison of *10/*10 versus *1/*10, 2 studies with a total of 145 patients were included for disease free survival and 1 study for overall survival,
For the comparison of *10/*10 versus *1/*1, 4 studies with a total of 450 patients were included for disease free survival, 3 studies for overall survival, and 5 studies with a total of 382 patients for breast cancer recurrence.
Because the frequency of *10 in these Asian patients was approximately 50%, approximately 25% of the patients in the studies was IM (*10/*10). For overall survival, the included studies were not mentioned, so the total number of patients in the meta-analyses could not be calculated.
None of the 11-13 studies in the meta-analyses were also included in our risk analysis separately.
Of the studies in this meta-analysis, 4 were also included in the meta-analysis by Cronin-Fenton 2014 (Xu 2008, Sirachainan 2012, Sukasem 2012, and Teh 2012), 3 in the meta-analysis by Zeng 2013 (Xu 2008, Sukasem 2012, and Teh 2012), 2 in the meta-analysis by Jung 2014 (Xu 2008 and Sukasem 2012), 1 in the meta-analysis of Seruga 2010 (Xu 2008), 0 in the meta-analysis of Lum 2013, while none were reported to be included in the meta-analysis of Province 2014.
Meta-analyses were performed with a random-effects model in case of significant heterogeneity between the studies and with a fixed-effect model in case of low heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent and the data extraction was standardised.
Publication bias analysis was performed for all comparisons except for overall survival in *10/*10 versus *1/*10 for which only one study was available. In addition, Eggert's test could not be performed for 2 comparisons for which only two studies were available (disease free survival in *10/*10 versus *1/*10, and overall survival in IM versus NM).

Results:
<table>
<tr><td colspan="4">Results for *10/*10 (IM):</td></tr>
<tr><td></td><td colspan="3">compared to</td></tr>
<tr><td></td><td>*1/*10+*1/*1</td><td>*1/*10</td><td>*1/*1</td></tr>
</table>
</td>
<td>Author's conclusion:
"In conclusion, our meta-analysis suggests that significant association of *10/*10 (TT) genotype with poorer disease-free survival and recurrence exists in female Asian breast cancer patients with tamoxifen 20 mg/day adjuvant treatment."</td>
</tr>
</table>

<table>
<tr>
<td>ref. 7, continuation</td>
<td>IM: E</td>
<td>
<table>
<tr><td></td><td>(NM)</td><td></td><td></td></tr>
<tr><td>less disease free survival</td><td>HR = 2.19 (95% CI: 1.07-4.50) (S)</td><td>HR = 2.03 (95% CI: 1.41-2.93) (S)</td><td>HR = 1.79 (95% CI: 1.14-2.80) (S)</td></tr>
<tr><td>overall survival</td><td>NS</td><td>NS</td><td>NS</td></tr>
<tr><td>breast cancer recurrence</td><td>OR = 3.69 (95% CI: 2.13-6.41) (S)</td><td></td><td>OR = 4.07 (95% CI: 1.88-8.80) (S)</td></tr>
<tr><td colspan="4">There was significant heterogeneity between the studies for the following comparisons:
- disease-free survival in IM versus NM
- overall survival in *10/*10 versus *1/*1
There was no significant heterogeneity between the studies for the other comparisons.
No meta-analysis was performed for overall survival in *10/*10 versus *1/*10 because there was only one study.</td></tr>
<tr><td colspan="4">The authors claim that there are no indications for publication bias for any of the comparisons and that for the Egger’s test p > 0.05 for all comparisons. However, the tables mention p = 0.012 for the Egger’s test of disease-free survival in IM versus NM. It is not known whether the claim of the authors or the p-value of 0.012 is wrong.
In addition, Egger’s test could not be performed for the following comparisons, because there were only 2 studies in the meta-analysis:
- disease free survival in *10/*10 versus *1/*10
- overall survival in IM versus NM
No meta-analysis was performed for overall survival in *10/*10 versus *1/*10 because there was only one study.</td></tr>
<tr><td colspan="4">Sensitivity analyses showed that omitting each individual study from all the analyses did not affect the pooled odds ratios significantly, and no substantial change was detected.</td></tr>
</table>
</td>
<td></td>
</tr>
<tr>
<td>ref. 8, kinetics
Hertz DL et al. Tamoxifen dose escalation in patients with diminished CYP2D6 activity normalizes endoxifen concentrations without increasing toxicity. Oncologist 2016;21:795-803. PMID: 27226358.</td>
<td>4</td>
<td>The effect on toxicity of 4 months of genotype-guided tamoxifen treatment (increase of the dose from 20 mg/day to 40 mg/day for (IM+gene dose 1.25-1.5) and PM, no dose change in NM and UM) was analysed in 421 patients. Patients were derived from Irvin 2011 (120 patients with endoxifen concentration data being available for 89) and from a new expansion-cohort of 380 patients (with endoxifen concentrations available for 353 patients at baseline and for 302 patients after 4 months of genotype-guided treatment). Kinetic data were only analysed in the expansion-cohort.
All patients used tamoxifen 20 mg/day for at least 4 months before start of genotype-guided dosing.
Quality-of-life (patient-reported toxicity relevant to breast cancer endocrine therapy) was assessed with the Functional Assessment of Cancer Therapy-Breast, including the Endocrine Subscale (FACT-B [ES]), and the Breast Cancer Prevention Trial Menopausal Symptom Scale (BCPT-MSS) at the time of consent and after 4 months of genotype-guided treatment. FACT-B [ES] assesses quality-of-life for the past 7 days and BCPT-MSS in the past 4 weeks. For all FACT summary scores, higher scores indicate better quality of life; however, for individual items on the endocrine symptom subscale, higher scores indicate worse symptoms. For BCPT-MSS higher scores on both summary and individual items indicate worse symptoms. For both FACT and BCPT, the maximum score per individual item is 4.</td>
<td>Author’s conclusion:
”Differences in endoxifen concentration during treatment can be eliminated by doubling the tamoxifen dose in IM patients, without an appreciable effect on quality of life.”</td>
</tr>
</table>

ref. 8, continuation

At baseline, patients reported decent overall quality of life (FACT-B scores ranging from 81-83% of the maximum score) and relatively minor decreases in quality of life as a result of their endocrine therapy (FACT [ES] scores ranging from 82-85% of the maximum score).
Reduced liver- and kidney function were excluded in all patients. Co-medication with moderate or strong CYP2D6 inhibitors was excluded in the expansion cohort. Co-medication with strong CYP2D6 inhibitors was excluded in Irvin 2011 cohort, but co-medication with (es)citalopram and venlafaxine was not.

Genotyping (based on the distribution in the total group for the toxicity analysis):

toxicity analysis	endoxifen analysis	
	baseline	genotype-guided treatment
- 141x NM+UM	- 119x NM	- 106x NM
- 254x IM+gene dose 1.25-1.5	- 212x IM+gene dose 1.25-1.5	- 179x IM+gene dose 1.25-1.5
- 26x PM	- 17x PM	- 13x PM
	- 5x UM	- 4x UM

Results:

Results compared to NM+UM (baseline toxicity), to baseline (toxicity after 4 months of genotype-guided treatment), or to NM (endoxifen concentration):

	PM	IM + gene dose 1.25-1.5	UM	value for NM+UM or NM
Baseline toxicity scores (tamoxifen 20 mg/day for all)				
PM: A IM: A FACT-B vaginal bleeding	x 3.78	x 1.22		0.09
	S for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			
FACT-B vaginal dryness	x 0.72	x 0.80		1.06
	S for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			
BCPT-MSS vaginal dryness	NS for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			0.98
FACT-B vomiting	x 0	x 0		0.03
	S for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			
BCPT-MSS vomiting	NS for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			0.04
PM: AA[#] IM: AA[#] FACT-B breast tenderness	x 0.42	x 0.78		0.74
	S for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			
49 other FACT-B or BCPT-MSS individual toxicities, subscales. and total scores	NS for PM versus (IM+gene dose 1.25-1.5) versus NM+UM			
Changes in toxicity scores during genotype-guided treatment (tamoxifen 20 mg/day for NM+UM, and 40 mg/day for PM and IM+gene dose 1.25-1.5) (with an increase in FACT summary scores indicating less toxicity)				
FACT, breast cancer subscale	NS	0.67 (S)	0.09 (NS)	

ref. 8, continuation		FACT-B trial outcome index	NS	0.85 (S)	-0.17(NS)	
	genotype -guided dosing: PM: AA[#] IM: AA[#]	BCPT, hot flushes summary score	NS	NS	-0.14 (S)	
		BCPT, vaginal problems summary score	-0.33 (S)	NS	0.00 (NS)	
		BCPT, arm problems summary score	NS	-0.10 (S)	-0.02 (NS)	
	genotype -guided dosing: IM: A	FACT-B night sweats	NS	NS	0.18 (S)	
		BCPT-MSS night sweats	NS	NS	0.20 (S)	
		FACT-B vaginal discharge	NS	0.12 (S)	-0.09 (NS)	
		FACT-B pain/ discomfort with intercourse	-0.55 (S)	NS	0.02 (NS)	
		BCPT-MSS pain/ discomfort with intercourse	-0.48 (S)	NS	0.00 (NS)	
		FACT-B mood swings	-0.30 (S)	NS	0.00 (NS)	
		FACT-B irritable	NS	-0.13 (S)	-0.01 (NS)	
	genotype -guided dosing: PM: A	BCPT-MSS diffi-culty with bladder control when laugh/cry	0.42 (S)	NS	0.08 (NS)	
		BCPT-MSS easily distracted	0.42 (S)	NS	0.16 (S)	
		BCPT-MSS decreased range of motion in arm on surgery side	NS	-0.19 (S)	-0.02 (NS)	
		39 other FACT-B or BCPT-MSS individual toxici-ties, subscales. and total scores	NS	NS	NS	
		Endoxifen concentration				
	PM: A IM: A	tamoxifen 20 mg/day for all	x 0.34 (S)	x 0.71 (S)	x 0.84 (NS)	10.0 ng/ mL
	genotype -guided dosing: PM: A IM: AA	tamoxifen 20 mg/day for NM and UM, and 40 mg/day for PM and IM+gene dose 1.25-1.5	x 0.59 (S)	x 1.15 (trend for an increase (p = 0.08) (NS)	x 1.65 (S)	9.30 ng/ mL
		Doubling the dose resulted in a 48% rise in endoxifen con-centrations in (IM+gene dose 1.25-1.5) and a 61% rise in PM.				
		For (IM+gene dose 1.25-1.5), the endoxifen concentration was above the threshold of 5.9 ng/mL reported to be requi-red for adequate lowering of breast cancer recurrence risk by tamoxifen both at a dose of 20 and 40 mg/day. For PM, the endoxifen concentration was below this thres-hold at both doses.				

<table>
<tr><td>ref. 8, continuation</td><td></td><td>The endoxifen concentration estimates or statistical comparisons were not meaningfully altered by excluding the 51 patients who had baseline endoxifen concentration data (tamoxifen 20 mg/day for all) but did not have genotype-guided treatment concentration data.

Note: Genotyping was for *2 through *11, *15, *17, *19, *20, *29, *35, *36, *40, *41, and gene multiplication of *1, *2, *4, *17, and *41. These are the most important gene variants in this population from the USA.
Note: Gene multiplications of *17 and *41 were considered to have the same gene dose (0.5) as the not-multiplicated alleles.</td><td></td></tr>
<tr><td>ref. 9, kinetics
Dezentjé VO et al. CYP2D6 genotype- and endoxifen-guided tamoxifen dose escalation increases endoxifen serum concentrations without increasing side effects. Breast Cancer Res Treat 2015;153:583-90. PMID: 26369533.</td><td>3

genotype - and endoxifen-guided dose increase: PM: C (1)

genotype - and endoxifen-guided dose increase: IM: A PM: A</td><td>In 12 IM and 12 PM treated with tamoxifen 20 mg/day, tamoxifen dose was escalated for a period of 2 months. The escalated dose was equal to 20 mg/day multiplied by the ratio of the median endoxifen serum concentration in 292 NM on tamoxifen 20 mg/day (33.7 nM) and the endoxifen serum concentration of the patient on tamoxifen 20 mg/day, but with a maximum of 120 mg/day. The mean escalated tamoxifen dose was 46 mg/day (range 30-100 mg/day) for IM and 90 mg/day (range 60-120 mg/day) for PM. One of the PM stopped after approximately 2 weeks of dose-escalation, because of toxicity at tamoxifen 60 mg/day (grade 1 hot flashes and diarrhoea, grade 2 headache, dizziness, and fatigue). For another PM, endoxifen concentration on the escalated dose of 90 mg/day was not available.
Serum trough concentrations were determined.
Co-medication with CYP2D6 inhibitors was not excluded. One of the IM used venlafaxine and one of the PM paroxetine.

Results:
Results after 2 months of dose-escalation (to 30-100 mg/day (mean 46 mg/day) for IM and to 60-120 mg/day (mean 90 mg/day) for PM) compared to before dose escalation (20 mg/day):
<table>
<tr><td></td><td></td><td></td><td>value before dose escalation</td></tr>
<tr><td>Serum concentrations (in nM)</td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">endoxifen</td><td>IM</td><td>x 1.7 (S)</td><td>17.8 nM</td></tr>
<tr><td>PM</td><td>x 3.4 (S)</td><td>8.0 nM</td></tr>
<tr><td colspan="3">Before dose escalation, the endoxifen concentration in all PM and 50% of the IM was below the threshold of 5.97 ng/ml (16.0 nM) reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen.
Following dose escalation, the endoxifen concentration was above this threshold in all patients.</td></tr>
<tr><td rowspan="2">tamoxifen</td><td>IM</td><td>x 1.9 (S)</td><td>271 nM</td></tr>
<tr><td>PM</td><td>x 3.7 (S)</td><td>313 nM</td></tr>
<tr><td rowspan="2">4-hydroxytamoxifen</td><td>IM</td><td>x 1.9 (S)</td><td>4.1 nM</td></tr>
<tr><td>PM</td><td>x 3.5 (S)</td><td>3.4 nM</td></tr>
<tr><td rowspan="2">N-desmethyltamoxifen</td><td>IM</td><td>x 2.2 (S)</td><td>610 nM</td></tr>
<tr><td>PM</td><td>x 4.0 (S)</td><td>809 nM</td></tr>
<tr><td colspan="4">% of patient with adverse events</td></tr>
</table></td><td>Author’s conclusion:
”Tamoxifen dose escalation in CYP-2D6 poor and intermediate metabolizers significantly increased endoxifen concentrations without increasing side effects. In intermediate metabolizers, dose escalation increased endoxifen to levels comparable with those observed in normal metabolizers. In poor metabolizers, the mean endoxifen level increased from 24 to 81% of the mean concentration in normal metabolizers. In all patients, the endoxifen threshold of 5.97 ng/ml (=16.0 nM) reported by Madlensky et al. was reached following dose escalation.”</td></tr>
</table>

ref. 9, continuation		hot flushes grade ≥ 2	IM	NS	8%
			PM	NS	0%
			all	NS	4%
		headache grade ≥ 1	all	NS	13%
		dizziness grade ≥ 1	all	NS	4%
		nausea grade ≥ 1	all	NS	4%
		alopecia grade ≥ 1	IM	NS	8%
			PM	NS	18%
			all	NS	13%
		vaginal discharge grade ≥ 1	all	NS	9%
		vaginal dryness grade ≥ 1	all	NS	9%
		fatigue grade ≥ 1	IM	NS	25%
			PM	NS	18%
			all	NS	22%
		ocular adverse events grade ≥ 1	all	NS	0%
		musculoskeletal adverse events grade ≥ 1	all	NS	17%
		No grade 3 or 4 toxicity was observed as a result of the dose escalation: only one patient already had grade 3 hot flashes at baseline. Only a nonsignificant increase in grade 1 fatigue and grade 1 alopecia was observed.			
		One patient using tamoxifen 50 mg/day experienced a bothersome grade 2 tendinitis of one of her fingers. On ECG, the QTc in one patient using 100 mg tamoxifen was slightly prolonged at 2 months (464 ms vs. 435 ms at baseline) but normalized 2 weeks after returning to the 20 mg dose (QTc = 436 ms). In 4 patients, grade 1 hot flashes disappeared during dose escalation. In two of these patients, grade 1 hot flashes reappeared 1 month after returning to the normal dose. Nearly all side effects that increased during tamoxifen escalation returned to baseline values 1 month after cessation of the escalation.			
	IM on increased dose: AA PM on increased dose: A	Endoxifen serum concentration in IM and PM on escalated tamoxifen doses compared to the median value for NM on tamoxifen 20 mg/day:			
					median value for NM on tamoxifen 20 mg/day
		IM on tamoxifen 30-100 mg/day (mean 46 mg/day)	x 0.90 (NS)		33.7 nM
		PM on tamoxifen 60-120 mg/day (mean 90 mg/day)	x 0.81 (S)		
		In all IM and PM patients, the endoxifen threshold of 5.97 ng/ml (16.0 nM) reported to be required for adequate lowering of breast cancer recurrence risk by tamoxifen was reached following dose escalation.			
		Note: Genotyping was for 33 alleles, including 7 gene duplication alleles. This includes the most important gene variants in this Dutch/Belgium population.			

ref. 9, continuation			

ref. 10, kinetics
Welzen ME et al.
The effect of tamoxifen dose increment in patients with impaired CYP2D6 activity.
Ther Drug Monit 2015;37:501-7.
PubMed PMID: 26192892.
	3
	
	PM: A
	IM: AA
		42 breast cancer patients who used tamoxifen 20 mg/day for at least 4 weeks. The phenotype was determined based on the genotype and the co-medication. Users of a CYP2D6 inhibitor were assigned the PM phenotype, regardless of their genotype.
		Plasma concentrations were measured before and 4 weeks after increase of the dose to 40 mg/day for 12 phenotypically IM and 4 phenotypically PM. Side effects were measured using the Functional Assessment of Cancer Therapy - Endocrine Symptom Subscale (FACT-ESS-19).
		
		Phenotyping:
		- 19x NM (genotype NM)
		- 16x IM (genotype IM)
		- 7x PM (4x genotype PM, 1x genotype NM + escitalopram, 1x genotype NM + citalopram, 1x genotype NM + paroxetine)
		
		PM versus IM versus NM at 20 mg/day:
		- the median endoxifen plasma concentration decreased (4.0 versus 8.3 versus 11.4 ng/mL) (S for the trend and for PM versus NM)
		- no difference in median plasma concentrations of tamoxifen, N-desmethyltamoxifen and 4-hydroxytamoxifen (NS)
		- no difference in side effects (NS)
		
		Dose increase:
		- one of the patients was excluded from the dose increase due to multiple side effects at a dose of 20 mg/day
		- PM:
		  - dose increase was performed for 3 patients with genotype PM and one patient with genotype NM + escitalopram
		  - increase in the median plasma concentration of endoxifen by a factor of 2.05 (from 3.8 to 7.8 ng/mL) (S)
		  - no increase in side effects (NS)
		- IM:
		  - increase in the median plasma concentration of endoxifen by a factor of 1.83 (from 9.5 to 17.4 ng/mL) (S)
		  - no increase in side effects (NS)
		
		NOTE: Genotyping was performed for *1 to *11, *14, *15, *17, *19, *20, *29, *35, *36, *40, *41 and gene duplication.
			Authors' conclusion:
			"Raising the tamoxifen dose to 40 mg QD significantly increased endoxifen concentrations in IMs and PMs without increasing side effects. The tamoxifen dose increment in PMs was insufficient to reach endoxifen concentrations equal to those observed in NMs."

ref. 11, kinetics Martinez de Dueñas E et al. Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype. Breast 2014;23:400-6. PubMed PMID: 24685597.	4 PM: A IM: AA	The endoxifen concentration was determined in 111 breast cancer patients selected according to their genotype, who used tamoxifen 20 mg/day as adjuvant therapy for at least 4 months. Plasma concentrations for PM were measured before and 4 months after increase of the dose to 40 mg/day (n = 11) and subsequently to 60 mg/day (n = 8). CYP2D6 inhibiting comedication was excluded. Genotypes: - 80x "NM" (NM or gene dose 1/0) - 16x "IM" (gene dose 0.5 or gene dose 0.5/0.5) - 11x PM - 4x UM PM versus "IM" versus "NM" versus UM at 20 mg/day: - decrease in the endoxifen plasma concentration (2.33 versus 2.85 versus 11.26 versus 12.30 ng/mL) (S for PM	Authors' conclusion: "In CYP2D6 PM patients, increasing the standard tamoxifen dose two-fold or three-fold raises endoxifen concentrations to levels similar to those of patients with NM phenotype."

ref. 11, continuation	UM: AA PM on increased dose: AA	versus “NM”, significance not determined further (NS)) - decrease in the 4-hydroxy-tamoxifen plasma concentration (1.03 versus 1.21 versus 1.92 versus 1.35 ng/mL) (S for PM versus “NM”, significance not determined further (NS)) - no difference in plasma concentrations of tamoxifen (NS) Dose increase for PM: - increase to 40 mg/day: - increase in the plasma concentration of endoxifen by a factor of 3.60 (from 2.33 to 8.38 ng/mL) (S). The new plasma concentration of endoxifen does not differ from the concentration for “NM” at 20 mg/day (NS). - increase in the plasma concentration of 4-hydroxy-tamoxifen by a factor of 4.6 (from 1.0 to 4.6 ng/mL) (S). The new plasma concentration of 4-hydroxytamoxifen is 2.6x higher than the concentration for “NM” at 20 mg/day (1.8 ng/mL) (S). - no increase in the incidence of side effects (NS). One patient receiving 40 mg/day had to discontinue the treatment due to a uterine bleed caused by a hyperplastic endometrium polyp. - no increase in the number of hot flushes per day in comparison to “NM” at 20 mg/day (NS) - further increase to 60 mg/day: - non-significant increase in the average plasma concentration of endoxifen to 9.30 ng/mL (NS). The new plasma concentration of endoxifen does not differ from the concentration for “NM” at 20 mg/day (NS). - increase in the minimum of the measured plasma concentrations of endoxifen by a factor of 2.4 (from 2.00 to 4.80 ng/mL) (significance not determined (NS)) - non-significant increase in the plasma concentration of 4-hydroxytamoxifen to 5.4 ng/mL (NS). The new plasma concentration of 4-hydroxytamoxifen is 3x higher than the concentration for “NM” at 20 mg/day (1.8 ng/mL) (S). - no increase in the incidence of side effects (NS) - no increase in the number of hot flushes per day in comparison to “NM” at 20 mg/day (NS) NOTE: Genotyping was performed for *1 through *11, *14, *15, *17, *19, *20, *29, *35, *36, *40, *41 and gene duplication.	
ref. 12, adjuvant Province MA et al. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. Clin Pharmacol Ther 2014;95:216-27. PubMed PMID: 24060820.	3	Meta-analysis of 12 studies involving a total of 4,841 patients who were treated with tamoxifen. Subgroup 1 (n = 1,996) consisted of post-menopausal patients with surgically removed, non-metastatic, invasive, oestrogen receptor-positive breast cancer, who received adjuvant monotherapy with tamoxifen 20 mg/day for an intended period of 5 years. In addition, these patients received annual monitoring for recurrence of breast cancer and at least *4 was detected upon genotyping. Subgroup 2 (n=2,443) also consisted of pre-menopausal patients, patients with a different scheduled duration of tamoxifen and patients without annual monitoring. In addition to the published data sets, the meta-analysis also included unpublished data sets. A summary of at least 5 of the 12 studies has been included in this risk analysis (Goetz 2005; Wegman 2007 (expansion included in the meta-analysis); Schroth 2009, Kiyotani 2010 (only patients receiving monotherapy included in the meta-analysis) and Stingl 2010). Three studies in this meta-analysis were also included in the meta-analysis by Seruga 2010 (Wegman 2007, Schroth 2009	Authors’ conclusion: “Using strict eligibility requirements, CYP2D6 poor metabolizer status was associated with poorer invasive disease–free survival (IDFS). However, CYP2D6 status was not statistically significant when tamoxifen duration, menopausal status, and annual follow-up were not specified or when no exclu-

ref. 12, continuation	(IM+PM): E	and Kiyotani 2010). Meta-analyses were performed with a random-effects model. Prospective registration of the protocol was not mentioned, but the search and selection strategy was transparent and data extraction was standardised. All researchers known to have data, both published and unpublished data sets, were invited to submit them. A random-effects meta-analysis procedure was used to test for study heterogeneity (i.e., whether the 12 studies met the assumptions of the meta-analysis sufficiently so as to be combinable using that method). When the heterogeneity was not significant, the log-HRs were combined into a single, meta-analysis estimate of the effect of CYP2D6 on tamoxifen-treated recurrence and/or survival outcomes. A set of inclusion criteria of individual patients from the data sets for both meta-analyses, including oestrogen receptor positivity of the tumour, the absence of adjuvant chemotherapy and the absence of known additional adjuvant hormone treatment, was reported. There was no systematic quality assessment of the included data sets, but HRs were determined for the individual data sets with proportional-hazards models adjusting for relevant covariates. No submission bias analyses were performed. Results: - In subgroup 1, CYP2D6 polymorphisms increased the risk of recurrence of invasive disease (HR = 1.25; 95% CI: 1.06-1.47) and recurrence of breast cancer (HR = 1.27; 95% CI: 1.01-1.61) (S). There was no heterogeneity among the studies. - In subgroup 2 and in the total group, there was no significant effect of CYP2D6 polymorphisms on the risks of recurrence of invasive disease and recurrence of breast cancer (NS). The heterogeneity among the studies was almost significant.	sions were applied. Although CYP2D6 is a strong predictor of IDFS using strict inclusion criteria, because the results are not robust to inclusion criteria (these were not defined a priori), prospective studies are necessary to fully establish the value of CYP-2D6 genotyping in tamoxifen therapy.”
ref. 13, adjuvant Jung JA et al. Association between CYP2D6 genotypes and the clinical outcomes of adjuvant tamoxifen for breast cancer: a meta-analysis. Pharmacogenomics 2014;15:49-60. PubMed PMID: 24329190.	3 (IM+PM): E	Meta-analysis of 10 studies involving a total of 5,183 patients with early-stage hormone receptor-positive breast cancer who received tamoxifen 20 mg/day as post-operative, adjuvant therapy. Studies differed in the definition of the measure of outcome and in the genotype groups that were compared. A summary of 6 of the 10 studies in the meta-analysis was included in this risk analysis (Schroth 2009, Kiyotani 2010 (only patients receiving monotherapy were included in the meta-analysis), Thompson 2011, Rae 2012, Regan 2012 and Park 2012). Six studies in this meta-analysis were also included in the meta-analysis by Seruga 2010 (Newman 2008, Xu 2008, Okishiro 2009, Schroth 2009, Kiyotani 2010 and Thompson 2011). Prospective registration of the protocol was not mentioned, but the search and selection strategy was transparent and data extraction was standardised. Possible publication bias was analysed, but quality of the included studies was not. Results: - CYP2D6 polymorphisms increased the risk of cancer recurrence (HR = 1.60; 95% CI: 1.04-2.47) (S). Following exclusion of the studies by Rae 2012 and Regan 2012 that did not show Hardy-Weinberg equilibrium, the HR was 2.31 (95% CI: 1.3 - 3.54) (S). There was heterogeneity among the studies. The cause of this heterogeneity could not be found.	Authors’ conclusion: “Our present findings suggest that genetic polymorphisms of CYP2D6 may be important predictors of the clinical outcomes of adjuvant tamoxifen treatment for the patients with breast cancer. A large-scale, prospective, randomized, well-controlled trial is warranted to confirm our findings.”

		There were no indications of a publication bias.	
ref. 14 Cronin-Fenton DP et al. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. Future Oncol 2014;10:107-22. PubMed PMID: 24328412.	3 (IM+PM): E	Meta-analysis of 25 studies, total number of patients not reported. A summary of 11 of the 25 studies in the meta-analysis was included in this risk analysis (Nowell 2005, Wegman 2005, Wegman 2007, Schroth 2009, Abraham 2010, Kiyotani 2010, Stingl 2010, Lash 2011, Thompson 2011, Rae 2012 and Regan 2012). Nine of the 10 studies from the meta-analyses by Seruga 2010 and Jung 2014 were included. Meta-analyses were performed with a random-effects model. Prospective registration of the protocol was not mentioned and the method of data extraction was not specified. The search and selection strategy was transparent. Possible publication bias was analysed, but quality of the included studies was not systematically assessed. (one or two *4 or *10 alleles) versus *1/*1*:* - increased risk of breast cancer recurrence or death (HR = 1.38; 95% CI: 1.10-1.73) (S). With the exception of Schroth 2009 and Goetz 2013, the studies with the highest impact found an HR of around 1. The authors indicate that they believe this is also the case for the patients from Schroth 2009 in the repeated analysis over a longer period of time in Goetz 2013. There may have been some publication bias, particularly for positive studies, but also for negative studies. - no significant increase in the risk of cancer recurrence and death in 9 studies with 0-9% pre-menopausal women (NS), but an increase with HR = 1.54 (95% CI: 1.09-2.18) in 16 studies with 20-80% pre-menopausal women (S). The authors indicate that this points to the fact that the effect of variant alleles may be limited to pre-menopausal women, who have a much higher oestrogen concentration. (*4/*4 or *10/*10) versus *1/*1*:* - increased risk of breast cancer recurrence or death (HR = 2.08; 95% CI: 1.40-3.10) (S). Five studies with the strongest association studied the *10 allele. As the *4 allele results in a stronger reduction of enzyme activity, the authors would have expected the greatest effect when studying the *4 allele. There may have been some publication bias for both positive studies and negative studies. The authors indicated that studies that used tumour tissue as a DNA source generally found no risk or a reduced risk of breast cancer recurrence for patients with variant alleles of CYP2D6, whilst studies that used blood samples often found an increased risk. They also indicated that there was no Hardy-Weinberg equilibrium in the studies by Rae 2012 and Regan 2012. However, they themselves found a good concurrence between CYP2D6 genotypes that were determined using tumour and non-tumour samples and Hardy-Weinberg equilibrium also when using tumour tissue. The authors also indicated that studies with positive results often had significant fundamental and methodological limitations.	Authors' conclusion: "The evidence indicates that the effect of both drug-induced and/or gene-induced inhibition of CYP2D6 activity is probably null or small, or at most moderate in subjects carrying two reduced function alleles. Several issues remain unresolved, including the potential for stronger associations in premenopausal women."
ref. 15 Lum DW et al. CYP2D6 genotype and tamoxifen	3	Meta-analysis of 17 studies involving a total of 9,555 patients on tamoxifen. The studies differed in the definition of the measure of outcome and patient groups. A summary of 12 of the 17 studies in the meta-analysis was	Authors' conclusion: "Despite a weak association be-

response for breast cancer: a systematic review and meta-analysis. PLoS One 2013;8:e76648. PubMed PMID: 24098545. **ref. 15, continuation**	(IM+PM): E	included in this risk analysis (Nowell 2005, Wegman 2005, Gonzalez-Santiago 2007, Wegman 2007, Schroth 2007, Schroth 2009, Abraham 2010, Lammers 2010, Stingl 2010, Lash 2011, Thompson 2011 and Regan 2012). Six of the 10 studies from the meta-analyses by Seruga 2010, 4 of the 10 studies from the meta-analysis by Jung 2014 and 11 of the 25 studies from the meta-analysis by Cronin-Fenton 2014 were included. Meta-analyses were performed with a fixed- and random-effects models. Prospective registration of the protocol was not mentioned and the method of data extraction was not specified. The search and selection strategy was transparent. Possible publication bias was analysed, but quality of the included studies was not systematically assessed. Results: - no increase in the risk of death for 1 or 2 variant alleles versus no variant alleles (6 studies, 4,936 patients) (NS). The same applied for 1 variant allele and for 2 variant alleles versus no variant alleles (both 3 studies, both NS). - increase in the risk of death or reduction of a surrogate outcome for survival (such as progression or disease-free survival) for 1 or 2 variant alleles versus no variant alleles with HR = 1.34 (95% CI: 1.06-1.69) (10 studies, 6,721 patients) (S). There was no significant effect for 1 variant allele and for 2 variant alleles versus no variant alleles (4 and 3 studies, both NS). - increase in the risk of death, decrease of a surrogate endpoint for survival or non-fatal events (such as recurrence of cancer) for 1 or 2 variant alleles versus no variant alleles with HR = 1.22 (95% CI: 1.01-1.46) (17 studies, 9,555 patients, >1088 events) (S). There was no significant effect for 1 variant allele and for 2 variant alleles versus no variant alleles (9 and 8 studies, both NS). There were no indications for a publication bias or an excessively large influence of one study on the calculated HRs.	tween CYP2D6 genotype and surrogate endpoints for overall survival, we did not identify an association between CYP2D6 genotype and tamoxifen response for all-cause mortality or overall survival. The current evidence does not support the use of CYP2D6 genotyping to guide tamoxifen prescribing for the treatment of breast cancer.”
ref. 16, adjuvant Zeng Z et al. CYP2D6 polymorphisms influence tamoxifen treatment outcomes in breast cancer patients: a meta-analysis. Cancer Chemother Pharmacol 2013;72:287-303. PubMed PMID: 23712329.	3	Meta-analysis of 20 studies involving a total of 11,701 patients on tamoxifen. The studies differed in patient groups, therapy, the outcome that was used for disease-free survival and the phenotype groups that were compared. A summary of 11 of the 20 studies in the meta-analysis was included in this risk analysis (Nowell 2005, Wegman 2007, Schroth 2009, Abraham 2010, Kiyotani 2010, Stingl 2010, Lash 2011, Thompson 2011, Park 2012, Rae 2012 and Regan 2012). Nine of the 10 studies from the meta-analyses by Seruga 2010, all 10 studies from the meta-analysis by Jung 2014, 19 of the 25 studies from the meta-analysis by Cronin-Fenton 2014 and 10 of the 17 studies from the meta-analysis by Lum 2013 were included. Meta-analyses were performed with a random-effects model in case of significant heterogeneity between the studies and with a fixed-effects model in the absence of significant heterogeneity between the studies. This indicates that the statistical method was chosen afterwards. The search and selection strategy was transparent, but the method of data extraction was not specified. Potential publication bias was assessed with a funnel plot and the Egger’s test, but only for all studies, not for the subgroups.	Authors’ conclusion: “CYP2D6 polymorphisms may influence tamoxifen treatment outcomes of disease-free survival in breast cancer patients.”

ref. 16, continuation	(IM+PM): F	Quality of the included studies was assessed according to a set of predetermined criteria (Thakkinstian A et al. A method for meta-analysis of molecular association studies. Stat Med 2005;24:1291-306). 9 of the included studies were of high quality (scoring 11-14 of the maximum of 15 points). The other 11 were of low quality (scoring 4-9 of the maximum of 15 points). Results for CYP2D6 alleles with reduced activity: - decrease in disease-free survival with HR = 1.37 (95% CI: 1.12-1.69) (19 studies, 11,616 patients) (S). There was major heterogeneity among the studies, primarily caused by differences in ethnicity. Exclusion of the 5 studies with the greatest heterogeneity resulted in a significant reduction (HR = 1.17; 95% CI: 1.00-1.37, p = 0.047) without significant heterogeneity. None of the individual studies had an excessively large effect on the calculated HRs. The decrease was significant for Asian patients (HR = 3.29; 95% CI: 1.64-6.63) (8 studies, 1,708 patients, major heterogeneity among the studies) (S), but not for White patients (10 studies, 9,743 patients) (NS). The decrease was significant for 5-year treatment (HR = 1.59; 95% CI: 1.14-2.22) (11 studies, 4,780 patients, major heterogeneity among the studies) (S), but not for a shorter duration of treatment (6 studies, 3,429 patients) (NS). The decrease was significant for both tamoxifen monotherapy and in combination with chemotherapy (HR = 1.44; 95% CI: 1.01-2.06; 7 studies with 3,477 patients and HR = 1.35; 95% CI: 1.04-1.76; 12 studies with 8,139 patients respectively). In both cases, there was major heterogeneity among the studies. The decrease was significant for 1 variant allele versus no variant allele (HR = 1.65; 95% CI: 1.04-2.64) (7 studies, 3,770 patients, significant heterogeneity among the studies) (S). The decrease was not significant following exclusion of the three studies that contributed most to the heterogeneity (NS). None of the individual studies had an excessively large effect on the calculated HRs. There was no significant decrease for the other comparisons between phenotype groups (2 variant alleles versus no variant alleles (5 studies, 3,627 patients), 1 or 2 variant alleles versus no variant alleles (7 studies, 8,454 patients), 2 variant alleles versus 1 or no variant alleles (4 studies, 4,479 patients), 2 *10 alleles versus 1 or no *10 alleles (2 studies, 325 patients) and 2 studies with a different comparison) (NS). There were indications for publication bias for the comparison of 1 variant allele versus no variant alleles and for 2 variant alleles versus 1 or no variant alleles. - increase in the risk of death with HR = 1.25 (95% CI: 1.03-1.50) (4 studies, 4,730 patients) (S). There was no heterogeneity among the studies. The increase was not significant for the individual comparisons between phenotype groups. There were no indications for publication bias.	

ref. 17, adjuvant Regan MM et al. CYP2D6 genotype and tamoxifen response in post-menopausal women with endocrine-responsive breast cancer: the breast international group 1-98 trial. J Natl Cancer Inst 2012;104:441-51. PubMed PMID: 22395644.	3 IM: AA “PM”: AA	1,243 post-menopausal patients with operable, invasive breast cancer received tamoxifen 20 mg/day for 5 years as adjuvant therapy. Tumours were positive for oestrogen and/or progesterone receptor. Of these, 973 patients had not received any previous (neo-)adjuvant chemotherapy and 270 patients had. Co-medication was not known. The CYP2D6 genotype was not associated with premature termination of the treatment. Tumour genotyping (also see NOTE 2 below): - 777x “NM” (gene dose 2) - 354x IM + NM (approx. 250x IM (gene dose 0.5-1) + approx. 104x NM (*1/*41, gene dose 1.5)) - 112x “PM” (IM + gene dose 1.5) versus “gene dose 2”: - no difference in time to recurrence of breast cancer in either the group without previous chemotherapy or the group with previous chemotherapy (NS) - factor 1.17 increase in the percentage of patients with hot flushes as a side effect (from 42% to 49%; HR_{corr} = 1.23, 95% CI: 1.05-1.43) (S) in a group of 1,706 patients without previous chemotherapy who used tamoxifen for 2 years - no difference in the percentage of patients with hot flushes as a side effect in a group of 487 patients with previous chemotherapy who used tamoxifen for 2 years (NS) “PM” versus “gene dose 2”: - non-significant increase in the time to recurrence of breast cancer in both the group without previous chemotherapy and the group with previous chemotherapy (NS) - non-significant increase by a factor of 1.14 in the percentage of patients with hot flushes as an adverse drug reaction (from 42% to 48%; HR_{corr} = 1.24, 95% CI: 0.96-1.59) (NS) in a group of 1,706 patients without previous chemotherapy who used tamoxifen for 2 years - no difference in the percentage of patients with hot flushes as a side effect in a group of 487 patients with previous chemotherapy who used tamoxifen for 2 years (NS) Similar results were obtained when categorised according to the *4 allele only. NOTE 1: Alleles *3, *4, *6, *7 and *41 were genotyped. NOTE 2: Pharoah PD et al., Nakamura Y et al and Stanton V Jr (J Natl Cancer Inst 2012;104:1263-6) indicate that the genotyping was incorrect. The frequency of the genotypes was not in Hardy-Weinberg equilibrium. The percentage PM for the most important allele *4 was a factor 2.4 higher than expected for the allele frequency that was found. This means that 58% of the “PM” are probably not PM. A possible explanation for this is that deletions often occur in tumours. In this case the presence of only the *4 allele could mean either that the patient is *4/*4 or that the tumour is haploid for CYP2D6. The same problem occurs with the determination of the other homozygous genotypes, such as *1/*1. Nakamura et al. and Stanton indicate that the excess of *4/*4 corresponds to CYP-2D6 deletions in approximately 32-33% of the tumours. The authors responded that Hardy-Weinberg equilibrium does not apply to CYP2D6, because this also has a deletion as polymorphism (*5) and duplications. Furthermore, they indicated that tumour tissue samples always also contain non-tumour	Authors’ conclusion: “CYP2D6 phenotypes of reduced enzyme activity were not associated with worse disease control but were associated with increased hot flushes, contrary to the hypothesis. The results of this study do not support using the presence or absence of hot flushes or the pharmacogenetic testing of CYP2D6 to determine whether to treat postmenopausal breast cancer patients with tamoxifen.”

		cells without deletions.	
ref. 18, adjuvant Rae JM et al. CYP2D6 and UGT-2B7 genotype and risk of recurrence in tamoxifen-treated breast cancer patients. J Natl Cancer Inst 2012;104:452-60. PubMed PMID: 22395643.	3 PM: AA IM: AA	588 postmenopausal patients with operable, invasive breast cancer received tamoxifen as adjuvant therapy. Tamoxifen was given in the form of 20 mg tablets. Median follow-up was 10 years. 92.5% of the tumours were positive for oestrogen and/or progesterone receptor; 7.1% were not known. A total of 69.7% of the patients had previously received radiotherapy and 4.3% had received chemotherapy as adjuvant therapy. A total of 36.2% of the patients had previously received hormone replacement therapy. Severe menopausal symptoms were treated with progestogens for 3-6 months, or - if this failed - with hormone replacement therapy (<1% of the patients) or oestrogen creams (approx. 2%). Correction was performed for relevant co-medication by reducing the gene dose by 2 for a strong CYP2D6 inhibitor (3.2% of the patients) and by 1 for a moderate CYP2D6 inhibitor (2.2% of the patients). Tumour genotyping (also see NOTE 2 below): - 317x gene dose 2 - 51x gene dose 1.25-1.5 - 151x gene dose 0.5-1.0 - 31x gene dose 0.25-0.5 - 38x gene dose 0 (PM) PM versus gene dose 2: - no difference in the risk of recurrence of breast cancer at a metastatic site (NS) - no difference in the risk of recurrence of breast cancer (NS) - similar results were obtained after correction for tumour characteristics and age - similar results were obtained following incorporation of the effect of CYP2D6 inhibitors in the gene dose CYP2D6 gene doses: - no difference in the risk of recurrence of breast cancer at a metastatic site (NS) Similar results were obtained after correction for tumour characteristics and age. *4 heterozygote versus no *4: - no difference in the risk of recurrence of breast cancer at a metastatic site (NS) - no difference in the risk of recurrence of breast cancer (NS) *4 homozygote versus no *4: - no difference in the risk of recurrence of breast cancer at a metastatic site (NS) - no difference in the risk of recurrence of breast cancer (NS) NOTE 1: Genotyping was performed for *1, *2 to *4, *6, *10 and *41. NOTE 2: Stanton V Jr (J Natl Cancer Inst 2012;104: 1263-6) indicates that the frequency of the genotypes with *4 is not in Hardy-Weinberg equilibrium, although the authors in the article state that all the alleles are in Hardy-Weinberg equilibrium. The percentage PM for the most important allele *4 is a factor 1.8 higher than expected for the allele frequency that was found. The authors indicate that this is probably caused by the fact that *5 was not detected. However, this does not result in incorrect classification of PM and the incorrect classification of a few NM and IM does not cause any changes to the results.	Authors' conclusion: "CYP2D6 genotype showed no association with recurrence, which remained after adjustment for concomitant medication known to inhibit the CYP2D6 enzyme. Results do not support CYP2D6 genotyping in patients considering tamoxifen because it did not predict clinical benefit of adjuvant tamoxifen treatment among postmenopausal breast cancer patients."

ref. 19, adjuvant Park IH et al. Lack of any association between functionally significant CYP2D6 polymorphisms and clinical outcomes in early breast cancer patients receiving adjuvant tamoxifen treatment. Breast Cancer Res Treat 2012;131:455-61. PubMed PMID: 21437611.	3 IM: AA	716 patients with early operable, invasive breast cancer received tamoxifen 20 mg/day for more than 6 months (median 4.4 years) as adjuvant therapy. In 29.6% of the patients, tamoxifen was followed by treatment with an aromatase inhibitor. Median follow-up was 5.6 years. 178 patients came from the study by Lim et al., 2007. Tumours were positive for oestrogen and/or progesterone receptor. A total of 77.9% of the patients had previously received chemotherapy as (neo-)adjuvant therapy. Co-medication was not known. Genotyping: - 152x NM (gene dose 2; *1/*1) - 376x NM+IM (336x gene dose 1.25-1.5 (*1/*10, *1/*41) and 40x gene dose 1 (*1/*5)) - 188x IM+PM (147x gene dose 0.5-0.75 (*10/*10, *10/*41); 39x gene dose 0.25 (*5/*10); 2x gene dose 0 (*5/*5)) IM+PM versus (NM+IM + gene dose 2): - no difference in the risk of recurrence of breast cancer in the total group (NS) - no difference in the risk of recurrence of breast cancer in the group who received tamoxifen only (n=130) (NS) - no difference in the risk of recurrence of breast cancer in the group who received only chemotherapy and tamoxifen (n=374) (NS) - no difference in the risk of recurrence of breast cancer in the group who received chemotherapy, tamoxifen and aromatase inhibitor (n=184) (NS) - no recurrence of breast cancer in the group who received tamoxifen and aromatase inhibitor (n=28) - no difference in the risk of recurrence of breast cancer in high risk groups based on tumour size, lymph node status, absence of progesterone receptor, presence of HER2 and Ki67 > 15% respectively (NS) The latter three factors are associated with resistance to hormone therapy. *Kinetics (n =178, substudy by Lim et al., 2007)* NM+IM versus gene dose 2: - decrease in the plasma concentration of endoxifen by 20% (from 21.2 to 17.0 ng/mL) (S for the trend IM+PM, NM+IM, gene dose 2) - decrease in the plasma concentration of 4-hydroxytamoxifen by 14% (from 2.9 to 2.5 ng/mL) (S for the trend IM+PM, NM+IM, gene dose 2) IM+PM versus gene dose 2: - decrease in the plasma concentration of endoxifen by 54% (from 21.2 to 9.8 ng/mL) (S for the trend IM+PM, NM+IM, gene dose 2) - decrease in the plasma concentration of 4-hydroxytamoxifen by 45% (from 2.9 to 1.6 ng/mL) (S for the trend IM+PM, NM+IM, gene dose 2) NOTE: Genotyping was performed for *5, *10 and *41. These are the most common alleles in this population group (Asian).	Authors' conclusion: "Polymorphisms of CYP2D6 were not associated with clinical outcomes in early breast cancer patients receiving adjuvant tamoxifen treatment."
ref. 20, kinetics Kiyotani K et al. Dose-adjustment study of tamoxifen based on CYP2D6 genotypes in Japanese breast cancer	3	98 breast cancer patients who used tamoxifen 20 mg/day as adjuvant therapy for at least 4 weeks. Plasma concentrations were measured before and 8 weeks after increase of the dose to 30 mg/day for 30 *1 heterozygotes and up to 40 mg/day for 21 patients without *1. Comedication with SSRIs was excluded.	Authors' conclusion: "This study provides the evidence that dose adjustment is useful for the patients carry-

patients. Breast Cancer Res Treat 2012;131:137-45. PubMed PMID: 21947681. **ref. 20, continuation**	IM: A IM on increased dose: AA	Genotyping: - 24x gene dose 2 (*1/*1) - 40x gene dose 1.25 (*1/*10) - 27x gene dose 0.5-1 (5x *1/*5, 22x *10/*10) - 7x gene dose 0.25-0.5 (4x *5/*10, 1x *10/*21, 1x *10/*36-*36, 1x *21/*41) Results of dose increase: - *10/*10: increase in the plasma concentration of endoxifen by a factor of 1.69 when the dose was increased from 20 to 40 mg/day (from 9.3 to 15.8 ng/mL) (S) - *1/*10: increase in the plasma concentration of endoxifen by a factor of 1.41 when the dose was increased from 20 to 30 mg/day (from 15.9 to 22.4 ng/mL) (S) - following dose adjustment, there was no longer a significant difference in the plasma concentrations of endoxifen between *1/*1, *1/*10 and *10/*10 (19.7 versus 22.4 versus 15.8 ng/mL) (NS) - following dose adjustment, there was no longer a significant difference in the plasma concentrations of 4-hydroxytamoxifen between *1/*1, *1/*10 and *10/*10 (NS) - following dose adjustment, the plasma concentrations of tamoxifen and N-desmethyltamoxifen were higher for *1/*10 and *10/*10 than for *1/*1 (S) - dose adjustment did not result in higher ratios of endoxifen/tamoxifen and 4-hydroxytamoxifen/tamoxifen in *1/*10 and *10/*10 (NS) - for the group consisting of *10/*5, *10/*21 and *10/*36-*36, the plasma concentrations of endoxifen and 4-hydroxytamoxifen increased by a factor of 1.94 when the dose was increased from 20 to 40 mg/day (NS) - there were no differences in the incidence of side effects before and after dose increase (NS) - excessive sweating was less common in carriers of a variant allele and dose increase than in *1/*1 with the standard dose of tamoxifen (S for no *1; NS for *1 heterozygote) NOTE: Genotyping was performed for *4 to *6, *10, *14, *18, *21, *36, *41 and gene duplication.	ing CYP2D6*10 allele to maintain the effective endoxifen level."
ref. 21, kinetics Barginear MF et al. Increasing tamoxifen dose in breast cancer patients based on CYP2D6 genotypes and endoxifen levels: effect on active metabolite isomers and the antiestrogenic activity score. Clin Pharmacol Ther 2011;90:605-11. PubMed PMID: 21900890.	3	117 breast cancer patients who used tamoxifen 20 mg/day as adjuvant therapy for at least 90 days. Plasma concentrations were measured before and 60 days after increase of the dose to 30 mg/day for 2 PM and 22 patients with an endoxifen plasma concentration < 40 nmol/L. In this group, 1.7% of the patients used a strong CYP2D6 inhibitor, 1.7% used a moderate inhibitor, 6.0% used a weak inhibitor and 2.6% used two moderate inhibitors. The authors stated that comedication with inhibitors had no significant effect on the plasma concentrations of tamoxifen and endoxifen, but did not display any results. The patients for whom the dose was increased did not use any comedication. Genotyping: Dose increase: - 45x gene dose 2 - 4x gene dose 2 - 27x gene dose 1.5 - 4x gene dose 1.5 - 29x gene dose 1 - 9x gene dose 1 - 12x gene dose 0.5 - 5x gene dose 0.5 - 3x gene dose 0 - 2x gene dose 0 Results for 20 mg/day: - no difference in the plasma concentrations of tamoxifen and 4-hydroxytamoxifen among the gene doses (NS)	Authors' conclusion: "Increasing the tamoxifen dose in patients with low (i.e., <40 nmol/l) plasma endoxifen levels and/or CYP2D6 poor metabolizer activity can increase the concentration of the active tamoxifen metabolites. These results should support the initiation of larger randomized clinical trials based on the plasma active metabolite isomer concentrations as reflected by the antiestroge-

<table>
<tr>
<td>ref. 21, continuation</td>
<td>IM: A
PM: A</td>
<td>- increase in the plasma concentration of endoxifen with increasing gene dose (from 31 nmol/L for PM to 52 nmol/L for gene dose 2 and from 43 nmol/L for IM to 51 nmol/L for NM) (S)
- increase in the plasma concentration of Z-endoxifen with increasing dose (from 6 nmol/L for PM to 27 nmol/L for gene dose 2) (S)
- decrease in the plasma concentration of the less active isomer Z'-endoxifen with increasing gene dose (from 27 nmol/L for PM to 15 nmol/L for gene dose 2) (S)
- increase in the score for anti-oestrogenic activity - calculated based on the activity and concentrations - with increasing gene dose (increase by a factor of 2.7 from PM to gene dose 2) (S)
- for each gene dose there were patients with an endoxifen plasma concentration < 40 nmol/L

Percentage increase in the plasma concentration or score for anti-oestrogenic activity (AES) with dose increase from 20 to 30 mg/day:
<table>
<tr><th>gene dose</th><th>endo-xifen</th><th>4-OH-tam.</th><th>Z-en-doxi-fen</th><th>Z-4-OH-tam.</th><th>AES</th></tr>
<tr><td>2</td><td>76</td><td>129</td><td>200</td><td>100</td><td>186</td></tr>
<tr><td>1.5</td><td>44</td><td>36</td><td>80</td><td>67</td><td>77</td></tr>
<tr><td>1</td><td>35</td><td>20</td><td>67</td><td>50</td><td>60</td></tr>
<tr><td>0.5</td><td>53</td><td>60</td><td>186</td><td>200</td><td>157</td></tr>
<tr><td>0</td><td>27</td><td>56</td><td>29</td><td>0</td><td>36</td></tr>
</table>
- increase in the elevation of Z-endoxifen and Z'-endoxifen with increasing gene dose (S)
- no significant difference in increase in 4-hydroxytamoxifen per gene dose (NS)
- dose increase resulted in side effects in 5 of the 24 patients (hot flushes and sweating)

NOTE: Genotyping was performed for *2, *2A, *4 to *6, *9, *10, *17, *41 and gene duplication.</td>
<td>nic activity score, rather than on CYP2D6 or other pharmacogenetic variants, which may be inadequate predictors of required dosage."</td>
</tr>
<tr>
<td>ref. 22, kinetics
Irvin WJ Jr et al. Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced CYP2D6 metabolism: a multicenter study. J Clin Oncol 2011;29:3232-9. PubMed PMID: 21768473.</td>
<td>4

IM: A</td>
<td>89 breast cancer patients who used tamoxifen 20 mg/day for at least 4 months. Plasma concentrations were measured before and 4 months after increase of the dose to 40 mg/day for IM and PM. Co-medication with strong CYP2D6 inhibitors was excluded, co-medication with (es)citalopram and venlafaxine was not excluded.

Genotyping:
- 29x gene dose 2-3 (28x gene dose 2, 1x UM)
- 51x gene dose 0.25-1.5 (20x gene dose 1.25-1.5; 19x gene dose 1; 4x gene dose 0.5-1; 8x gene dose 0.25-0.5)
- 9x gene dose 0 (PM)

Gene dose 0.25-1.5:
- decrease in the median plasma concentration of endoxifen at 20 mg/day versus gene dose 2-3 by 43% (from 34.9 to 19.8 ng/mL) (S)
- increase in the median plasma concentration of endoxifen by a factor of 1.2 when the dose was increased from 20 to 40 mg/day (from 18.5 to 21.8 ng/mL) (S)
- increase in tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen and the ratio endoxifen/N-desmethyltamoxifen following dose increase (S)</td>
<td>Authors' conclusion:
"After the dose increase, there was no longer a significant difference in endoxifen concentrations between NM and IM patients; however, the PM endoxifen concentration was still significantly lower. This study demonstrates the feasibility of genotype-driven tamoxifen dosing and demonstrates that doubling the tamoxifen dose can increase endoxifen</td>
</tr>
</table>

ref. 22, continuation	PM: A	- no significant difference in median plasma concentration of endoxifen at 40 mg/day versus gene dose 2-3 at 20 mg/day (21.8 versus 29.2 ng/mL) (NS) PM: - decrease in the median plasma concentration of endoxifen at 20 mg/day versus gene dose 2-3 by 87% (from 34.9 to 4.6 ng/mL) (S) - increase in the median plasma concentration of endoxifen by a factor of 3.1 when the dose was increased from 20 to 40 mg/day (from 4.2 to 12.9 ng/mL) (S) - increase in the ratio endoxifen/ N-desmethyltamoxifen following dose increase (S), but not in tamoxifen, N-desmethyltamoxifen, 4-hydroxytamoxifen (NS) - lower median plasma concentration of endoxifen at 40 mg/day versus gene dose 2-3 at 20 mg/day (12.9 versus 29.2 ng/mL) (S) Gene dose 1.25-1.5: - no difference in the median plasma concentration of endoxifen at 20 mg/day versus gene dose 2-3 (from 20.2 to 34.9 ng/mL) (NS) Combination *1 and a null allele (gene dose 1): - decrease in the median plasma concentration of endoxifen at 20 mg/day versus gene dose 2-3 by 47% (from 34.9 to 18.5 ng/mL) (S) Side effects: - one patient experienced grade 3 vaginal bleeding (gene dose 0.25-1.5) and there were three patients with milder adverse drug reactions (nausea, cramping, joint pain) - there was no difference in hot flushes between the groups before or after dose increase. However, the authors indicate that at least 500 patients are required to find a 10% difference in hot flushes. NOTE: Genotyping was performed for *1 through *11, *15, *17, *19, *20, *29, *35, *36, *40, *41 and gene duplication.	concentrations in IM and PM patients.”
ref. 23, adjuvant Lash TL et al. CYP2D6 inhibition and breast cancer recurrence in a population-based study in Denmark. J Natl Cancer Inst 2011;103:489-500. PubMed PMID: 21325141.	3	Case-control study, in which 541 cases with recurrent or contralateral breast cancer were compared to 541 controls who did not have a recurrence of breast cancer after the same follow-up. Following surgery for early-stage breast cancer, all patients received adjuvant treatment with tamoxifen for 1 year or longer. In accordance with the Danish guidelines, they were treated with tamoxifen 30 mg/day. Participants in the international trial BIG 1-98 were treated with tamoxifen 20 mg/day. All tumours were positive for the oestrogen receptor. The cases and the accompanying controls had a similar date of breast cancer surgery (± 1 year) and the same menopausal status upon diagnosis (6.3% premenopausal), the same cancer stage and the same residential area. Maximum follow-up was 10 years (from 1 year after the diagnosis). 35% of the patients had previous radiotherapy and 12% had previous chemotherapy as adjuvant therapy. Comedication was unknown for patients who were diagnosed prior to 1995 (229 cases and 225 controls). Genotyping of the tumour was unsuccessful for 47 cases and 44 controls. As a result, the number of case-control pairs for which the effect of the genotype was determined decreased from 541 to 450-494. Results were corrected for time to recurrence/selection of the	Authors’ conclusion: “The association between CYP2D6 inhibition and recurrence in tamoxifen-treated patients is likely null or small.”

ref. 23, continuation	IM: AA PM: AA	control, menopausal status, stage, previous chemotherapy, previous radiotherapy and type of surgery. For correction of genotype for comedication, the following 5 "phenotypes" were distinguished: - NM phenotype (NM without CYP2D6 inhibitor) - slow NM phenotype (NM with strong CYP2D6 inhibitor for less than 30% of the time, or a moderate or weak CYP2D6 inhibitor) - fast IM phenotype (IM without CYP2D6 inhibitor or with CYP-2D6 inhibitor for less than 30% of the time) - slow IM phenotype (IM with moderate or weak CYP2D6 inhibitor for more than 30% of the time) - PM phenotype (PM or IM or NM with strong CYP2D6 inhibitor for more than 30% of the time) Cases versus controls: - no significant increase in the frequency of the *4 allele (from 22% to 24%) (NS) CYP2D6 genotypes and phenotypes: - the risk of cancer recurrence was not significantly increased for IM or PM or IM + PM (NS) Similar results were obtained for only those patients without previous chemotherapy. Quantitative bias analysis suggested similar results if other CYP2D6 alleles had been analysed in addition to *4. Similar results were also obtained for only those patients where the presence of the oestrogen receptor could be confirmed by a second, better assay (451 cases and 474 controls). - the risk of cancer recurrence was not significantly increased for any of the "phenotypes" versus the NM phenotype. NOTE: only *4 determined.	
ref. 24, adjuvant Thompson AM et al. Comprehensive CYP2D6 genotype and adherence affect outcome in breast cancer patients treated with tamoxifen monotherapy. Breast Cancer Res Treat 2011;125:279-87. PubMed PMID: 20809362.	4 IM + PM:	618 patients with oestrogen receptor-positive breast cancer received tamoxifen 20 mg/day as adjuvant treatment. The scheduled treatment duration was 5 years. In this group, 408 patients were post-menopausal and had received tamoxifen monotherapy. All co-variables were known for 543 patients, in particular all co-medication. Data about therapy compliance were known for 257 patients. 18.6% of the patients had previously received chemotherapy. 18.9% were premenopausal. The maximum follow-up was 12 years. A total of 7.6% of the patients were using a strong CYP2D6 inhibitor. For 133 patients, both fresh-frozen tumour tissue and blood were genotyped. The genotypes derived by both of these methods were identical. HR was corrected for lymph node status and tumour size. Tumour genotyping: - 243x gene dose 2 + UM (234x gene dose 2; 9x UM) - 341x gene dose 0.25-1.5 (126x gene dose 1.25-1.5; 171x gene dose 1; 13x gene dose 0.5-1; 31x gene dose 0.25-0.5) - 34x PM (gene dose 0) Gene dose 0-1.5 versus gene dose 2 + UM: - non-significantly increased risk of cancer recurrence for the entire group for which all co-variables were known (HR_{corr} = 1.52; 95% CI: 0.98-2.36) (NS) - increased risk of cancer recurrence for post-menopausal patients receiving tamoxifen monotherapy (HR_{corr} = 1.96;	Authors' conclusion: "For postmenopausal women on tamoxifen monotherapy, the HR for recurrence in patients with reduced functional alleles was 1.96 (CI 1.05–3.66, P = 0.036). However, RFS analysis limited to four common CYP2D6 allelic variants was no longer significant (P = 0.39). The effect of CYP2D6 genotype was increased by adjusting for adherence to tamoxifen therapy, but not significantly changed when adjusted for coadministration of

ref. 24, continuation	E PM: AA	95% CI: 1.05-3.66) (S) No significantly increased risk was found when this group was categorised into gene doses based on only the four most common alleles (*4, *5, *10, *41). - increased risk of cancer recurrence for patients with known therapy compliance (HR_{corr} = 2.57) (S) Categorisation of patients with therapy compliance < 80% in the group with gene dose 0-1.5 increased the calculated risk (HR_{corr} = 3.02; 95% CI: 1.07 - 8.47) (S). NM had a very low risk of recurrence of cancer after correction for therapy compliance. - categorisation of patients who used a strong CYP2D6 inhibitor in the group with gene dose 0-1.5 did not change the calculated HR PM: - there was no recurrence of cancer in the 27 PM for whom all co-variables were known This was probably due to the more favourable clinical characteristics in this group (tumour size < 2 cm significantly more often). NOTE: genotyping was performed for 33 alleles (including gene duplications).	potent inhibitors of CYP2D6. Comprehensive genotyping of CYP2D6 and adherence to tamoxifen therapy may be useful to identify breast cancer patients most likely to benefit from adjuvant tamoxifen.”
ref. 25, adjuvant Stingl JC et al. Impact of CYP2D6 *4 genotype on progression free survival in tamoxifen breast cancer treatment. Curr Med Res Opin 2010;26:2535-42. PubMed PMID: 20849243.	3 IM: AA PM: AA PM: E	493 patients with non-metastatic breast cancer received tamoxifen 20 mg/day as adjuvant therapy for > 6 months (average 3.4 years). In this group, 13.1% of the patients were treated with an aromatase inhibitor in addition to tamoxifen. 29.2% had received previous chemotherapy with anthracycline as adjuvant therapy. The average follow-up was 7 years. Tumours were positive for the oestrogen receptor. Comedication was not known. Genotyping: - 341x NM (*1/*1) - 124x IM (1/*4) - 28x PM (*4/*4) PM versus IM versus NM: - no significant differences in age at diagnosis, average duration of tamoxifen, tumour size, stage and lymph node status - no significant differences in the percentage of patients with recurrence of breast cancer (17.8% versus 19.4% versus 16.4%) (NS) - no significant differences in progression-free survival (5.6 versus 7.5 versus 8.6 years) (NS) The latter was also true for the 428 patients who did not receive an aromatase inhibitor. - regression analysis revealed that the CYP2D6 genotype was not a significant predictor of the time to progression (NS) - in the group of 144 patients who had previously received chemotherapy: - shorter time to tumour progression for PM than for IM + NM (S; HR = 4.0 (95% CI: 1.2-13.0) - no significant differences in progression-free survival between the three genotype groups (1.0 versus 6.3 versus 5.0 years) The patients who had received chemotherapy had a higher risk (more patients with tumour size > 2 cm, with positive lymph node status and with tumour stage ≥ 3, fewer patients older than 55 years). The time to tumour progression was	Authors’ conclusion: “While earlier data on CYP2D6 and tamoxifen excluded women with prior chemotherapy, the present analysis suggests that CYP2D6*4 genotype might be particularly crucial in this group of high-risk patients.”

ref. 25, continuation		significantly shorter for this group than for the patients without chemotherapy. NOTE: only *4 determined.	
ref. 26, metastasis Lammers LA et al. The impact of CYP2D6-predicted phenotype on tamoxifen treatment outcome in patients with metastatic breast cancer. Br J Cancer 2010;103:765-71. PubMed PMID: 20700120.	4 IM: AA PM: F	99 patients with metastatic breast cancer were treated with tamoxifen 40 mg/day until disease progression occurred (average 2.8 years). Tumours were positive for oestrogen and/or progesterone receptor. 74.5% of the patients had previously received radiotherapy and/or chemotherapy as adjuvant therapy. 48% had previously received radiotherapy and/or chemotherapy as treatment for metastatic breast cancer. Relevant co-medication was corrected by categorising patients who used CYP2D6 inhibitors for at least 6 months according to the predicted phenotype. There was no effect of previous treatment, ethnicity and number of metastases on the results. Genotyping: - 53x NM (45x gene dose 2; 6x gene dose 1.5; 2x gene dose 1.25) - 37x IM (31x gene dose 1; 5x gene dose 0.5; 1x gene dose 0.25) - 9x PM Phenotype categorisation: - 48x NM phenotype (NM without CYP2D6 inhibitor) - 38x IM phenotype (36x IM without CYP2D6 inhibitor; 2x NM with weak CYP2D6 inhibitor) - 13x PM phenotype (9x PM; 1x IM with strong CYP2D6 inhibitor; 3x NM with strong CYP2D6 inhibitor) IM phenotype versus NM phenotype: - no significant increase in time to death (“overall survival”) (NS) - no difference in time to disease progression (NS) PM phenotype versus NM phenotype: - decrease in time to death (“overall survival”) (S; HR = 2.09; 95% CI: 1.06 – 4.12) - no significant difference in time to disease progression (NS; HR = 1.69; 95% CI: 0.90 – 3.19) PM phenotype versus (NM+IM) phenotype: - decrease in the median time to death (“overall survival”) by 37% (from 7.9 years to 5.0 years) (S) - non-significant decrease in the median time to disease progression by 22% (from 1.8 years to 1.4 years) (NS) CYP2D6 genotype: - no significant difference in time to death (“overall survival”) (NS) - no significant difference in time to disease progression (NS) NOTE: Alleles *3 to *6, *10 and *41 were genotyped.	Authors’ conclusion: “CYP2D6 phenotype, defined as the combined effect of CYP2D6 genetic variation and concomitant use of CYP2D6-inhibiting medication, is an important predictor of treatment outcome in women who are receiving tamoxifen for metastatic breast cancer.”
ref. 27, adjuvant Schroth W et al. CYP2D6 polymorphisms as predictors of outcome in breast cancer patients treated with tamoxifen: expan-	3	This study involves a repeat analysis of the genotype of 492 patients from Schroth et al., 2009 using the AmpliChip Test. The median follow-up was 59.5 months. 1.4% of the patients was pre-menopausal and 1.2% were peri-menopausal. 82.1% had previously received radiotherapy. Based on non-published data, the percentage of users of strong CYP2D6 inhibitors was estimated at 1-2%. This can result in an underestimate of the actual size of the effect of the PM genotype on recurrence	Authors’ conclusion: “Approximately, one third of patients were misclassified based on a *4 analysis only, but inclusion of all

ded polymorphism coverage improves risk stratification. Clin Cancer Res 2010;16:4468-77. PubMed PMID: 20515869. **ref. 27, continuation**	PM: E UM: AA[#] IM: E	of breast cancer by 16%. Genotyping: - 6x gene dose 3 (UM) - 183x "gene dose 2" (182x gene dose 2; 1x gene dose 2.5) - 74x "gene dose 1.5" (63x gene dose 1.5; 10x gene dose 1.25; 1x gene dose 2) - 188x "IM" (147x gene dose 1 (140x combination of active and null allele; 7x two alleles with reduced activity other than *10); 3x gene dose 0.75 (2x *10 with another allele with reduced activity; 1x three *10-alleles); 34x gene dose 0.5; 4x gene dose 0.25)) - 41x PM PM versus "gene dose 2": - no significantly increased risk of recurrence of breast cancer if gene doses are based solely on *4 (HR = 1.33; p = 0.58) (NS) Genotyping based on *4 alone identifies 65.8% of the PM and results in "gene dose 2" being assigned incorrectly in 49% of cases. - no significantly increased risk of recurrence of breast cancer if gene doses are based solely on the alleles determined in Schrotz et al., 2009 (HR_{corr} = 2.12; 95% CI: 0.96-4.69) (NS) - increased risk of recurrence of breast cancer if gene doses are based on all alleles (HR_{corr} = 2.77; 95% CI: 1.31 - 5.89) (S) UM versus "gene dose 2" versus ("IM" + "gene dose 1.5") versus PM: - no significantly increased risk of recurrence of breast cancer with decreasing gene dose, if gene doses are based solely on the alleles determined in Schrotz et al., 2009 (p = 0.056) (NS) - increased risk of recurrence of breast cancer with decreasing gene dose, if gene doses are based on all alleles (p = 0.011) (S) NOTE: Genotyping was performed for 33 CYP2D6 alleles and for duplications. Alleles that occurred in the group were *2 to *7, *9, *10, *35. *41.	reduced-function alleles increased the PM-associated HR for recurrence from 1.33 (P = 0.58) to 2.87 (P = 0.006)."
ref. 28, adjuvant Seruga B et al. Cytochrome P450 2D6 and outcomes of adjuvant tamoxifen therapy: results of a meta-analysis. Breast Cancer Res Treat 2010;122:609-17. PubMed PMID: 20454926.	3	A meta-analysis of 10 studies into the effect of CYP2D6 genotype on the effectiveness of tamoxifen as adjuvant therapy for early-stage, invasive breast cancer. The total number of patients in the 10 studies is 3,205. The disease-free survival was determined for 3,120 patients and the overall survival was determined for 1,570 patients. 719 patients in 4 studies received tamoxifen 20 mg/day for 5 years. A total of 299 in 2 studies received tamoxifen 20-40 mg/day for 2 to 5 years. The tamoxifen dose was not known for 2,187 patients in 4 studies. A summary of 6 of the 10 studies was included in this risk analysis (Nowell 2005; Gonzalez-Santiago 2007; Wegman 2007; Schroth 2009; Thompson 2009 (an abstract containing the data of Thompson 2011), Kiyotani 2010 (only the 282 patients on tamoxifen monotherapy)). There was no correction for comedication. Meta-analyses were performed with a random-effects model, but prospective registration of the protocol was not mentioned. The search and selection strategy was transparent, although not shown in a flow diagram, and data extraction was standardised. Potential publication bias was assessed with funnel plots only.	Authors' conclusion: "Our present analysis suggests there is a potential detrimental effect of impaired metabolism of tamoxifen on the basis of CYP2D6 genotype or concomitant administration of tamoxifen and CYP2D6 drug inhibitors. However, the magnitude of this effect seems relatively small and may not be clinically relevant in all scenarios, espe-

ref. 28, continuation	IM + PM: AA	In addition, funnel plots were not shown. There were no indications for publication bias. Quality of the included studies was assessed using modified criteria for case-control studies developed by the US Preventive Services Task Force (Harris RP et al. Current methods of the US Preventive Services Task Force: a review of the process. Am J Prev Med 2001;20:21-35). Only the reference was mentioned, not the criteria themselves, nor the scores for these criteria for each study. 5 of the included studies were of poor quality, 3 of fair quality and 2 of high quality. Genotyping: - In 5 studies, gene dose 0-1.5 was compared to gene dose 2. Only *4 was determined in 4 of these studies. - In the other 5 studies, genotypes without *1 alleles (gene dose 0-1) were compared to genotypes with one or more *1 alleles (gene dose 1-2). Only *10 was determined in 2 of these studies. Results: - the two studies that were of good quality both found an increased risk of a shorter time until disease recurrence (one significant, the other not significant) and a non-significantly increased risk of a shorter overall survival for gene dose 0-1 versus gene dose 1-2 - the meta-analysis revealed a non-significantly increased risk of recurrence of breast cancer for the groups with low gene doses This was the case for both gene dose 0-1.5 and for gene dose 0-1. - there was no difference in the risk of death - there was no effect of CYP2D6 inhibitors on recurrence of breast cancer (2 studies, n = 3,621) - the CYP2D6 inhibitor paroxetine reduced the overall survival, but the CYP2D6 inhibitor fluoxetine did not (1 study, n = 2,430)	cially in women with a low-risk breast cancer. As compared to women with a low-risk disease, suboptimal or inefficient endocrine therapy can be associated with worse outcome in women with high-risk disease. In postmenopausal women with high-risk disease, upfront use of aromatase inhibitors is a reasonable alternative to tamoxifen, irrespective of CYP2D6 genotype. In premenopausal women, tamoxifen is still the gold standard.”
ref. 29, adjuvant Kiyotani K et al. Lessons for pharmacogenomics studies: association study between CYP2D6 genotype and tamoxifen response. Pharmacogenet Genomics 2010;20:565-8. PubMed PMID: 20574415.	3	449 patients with primary breast cancer received tamoxifen 20 mg/day for 5 years as adjuvant therapy. In this group, 282 patients received tamoxifen as monotherapy, 167 in combination with other therapies (55.7% chemotherapy; 20.4% gonadorelin agonists; 10.8% combination chemotherapy and gonadorelin agonists; 6.6% aromatase inhibitors and 6.6% other). The combination therapy group was more likely to be premenopausal (71.3% versus 43.6%), had larger tumours and was more likely to have positive lymph nodes (44.3% versus 17.0%). HRs were corrected for tumour size and lymph node status, but not for age. Tumours were positive for oestrogen and/or progesterone receptor. Comedication other than cancer therapy was unknown. Genotyping: - 131x “gene dose 2” (130x gene dose 2; 1x gene dose 3) - 222x *1 heterozygote (35x gene dose 1; 175x gene dose 1.25, 7x gene dose 1.5; 4x gene dose 2 (duplication of *10); 1x gene dose 2.25 (duplication *1)) - 96x no *1 (4x gene dose 0; 21x gene dose 0.25; 24x gene dose 0.5; 2x gene dose 0.75; 45x gene dose 1) (*1 heterozygote) versus “gene dose 2”: - increased risk of cancer recurrence in the monotherapy group (HR_{corr} = 4.44; 95% CI: 1.31 - 15.00) (S) - no significant difference in the risk of cancer recurrence in	Authors’ conclusion: “We earlier reported a significant association between the cytochrome P450 2D6 (CYP2D6) genotype and the clinical outcome in 282 Japanese breast cancer patients receiving tamoxifen monotherapy. We then studied 167 breast cancer patients who received tamoxifen-combined therapy to evaluate the effects of concomitant treatment on the association analysis and observed no significant association

ref. 29, continuation	IM + PM: E IM + PM: AA	the combination therapy group (NS) - similar results were obtained after analysis of subgroups based on lymph node status and tumour size (no *1) versus “gene dose 2”: - increased risk of cancer recurrence in the monotherapy group (HR_{corr} = 9.52; 95% CI: 2.79-32.45) (S) - no significant difference in the risk of cancer recurrence in the combination therapy group (NS) - similar results were obtained after analysis of subgroups based on lymph node status and tumour size NOTE: Genotyping was performed for *4, *6, *10, *14B, *18, *21, *36, *41 and gene duplication.	between CYP2D6 genotype and recurrence-free survival. When we carried out two subgroup analyses for nodal status and tumor size, we observed a positive association between the CYP2D6 genotype and the clinical outcome only in patients who received tamoxifen monotherapy. This study explained a part of the discrepancies among the reported results.”
ref. 30, adjuvant Abraham JE et al. CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. Breast Cancer Res 2010;12:R64. PubMed PMID: 20731819.	3 IM: AA IM + PM: AA	3,155 patients with invasive, non-metastatic breast cancer were treated with tamoxifen as adjuvant therapy. The treatment duration was unknown. The standard tamoxifen dose was 20 mg per day. A total of 63% of the patients (n = 1,989) had tumours that were positive for the oestrogen receptor. The oestrogen receptor was not determined for 30.4%. 19% of the patients were selected due to the fact that they were still alive in 1996 following a diagnosis of breast cancer between 1991 and 1996. The other patients were diagnosed after 1996. The percentage of premenopausal patients was not provided. 63% of the patients had previously undergone surgery. For 33% it was not known whether they had undergone surgery or not. 19% of the patients received chemotherapy; for 33% this was not known. The follow-up spanned an average of 6.0 years and a maximum of 10 years. 6.1% of the patients reported the use of CYP2D6 inhibitors at the moment of inclusion. For the individual alleles, the use of CYP2D6 inhibitors had no effect on the risk of death. This was not determined for the IM/PM groups. HRs were corrected for tumour grade, stage, chemotherapy, surgery, oestrogen receptor, tumour size and lymph node status. Correction for lymph node status was not performed for the IM/PM groups. Genotyping *4: - 1,950x no *4 (gene dose 1-2) - 978x *4 heterozygote (gene dose 0-1) - 130x PM (*4/*4) - 97x genotype unknown Risk of shorter time to death as a result of breast cancer in patients with oestrogen receptor-positive tumours (HR_{corr}): - no significantly increased risk per allele for *4 (NS) - no significantly increased risk per allele for the three other alleles with sufficient numbers of patients for calculation of HR_{corr} (*41, *6b/c and *9) (NS) - no significantly increased risk for (*4/*4 + *5/*5 + *6b-c/*6b-c + *9/*9 + *10/*10 + *41/*41) versus (*1/*1 and *1 heterozygote) (NS) - no significantly increased risk for carriers of a variant allele versus *1/*1 (NS) Similar results were obtained for overall survival.	Authors’ conclusion: “CYP2D6*6 may affect BCSS in tamoxifen-treated patients. However, the absence of an association with survival in more frequent variants, including CYP2D6*4, questions the validity of the reported association between CYP2D6 genotype and treatment response in breast cancer. Until larger, prospective studies confirming any associations are available, routine CYP2D6 genetic testing should not be used in the clinical setting.”

ref. 30, continuation		Similar non-corrected results were obtained for the subgroup of postmenopausal women with oestrogen receptor-positive tumours who did not receive chemotherapy. The same applies to the subgroup of premenopausal women. For *4, similar non-corrected results were found after exclusion of the group that was selected for survival of the first period after diagnosis. NOTE 1: Genotyping was performed for *4, *5, *6b/c, *9, *10, *41 and gene duplication. Only homozygotes could be determined for *5 and gene duplication. There was no Hardy-Weinberg equilibrium for *41. NOTE 2: The results obtained with SNP tagging were ignored. The reason for this is that this records general variation, irrespective of knowledge about the function. As a result, it is not known whether the SNP tags are associated with reduced CYP2D6 function.	
ref. 31, adjuvant Schroth W et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. JAMA 2009;302:1429-36. PubMed PMID: 19809024.	3 IM: E PM: E	1,325 patients with early-stage breast cancer received tamoxifen monotherapy as adjuvant therapy. The scheduled treatment duration was 5 years. The dose was not stated for some of the patients (treatment according to the standard hospital protocol in Germany). For the other patients (all postmenopausal), the dose was 20 mg/day. 350 patients were previously described in Schroth 2007, Goetz 2007 and Goetz 2005. None of the patients had previously received chemotherapy or another endocrine therapy. 58.0% had previously received radiotherapy; this was not known for 7.5%. 4.1% of the patients were premenopausal. Tumours were positive for oestrogen and/or progesterone receptor. The median follow-up was 6.3 years. DNA was isolated from blood (44.2%), fresh-frozen tumour tissue (7.4%) or tumour tissue fixed in formaldehyde or embedded in paraffin (48.4%). HRs were corrected for tumour size, lymph node status and tumour grade. Comedication was not known. Genotyping of tumour or blood: - 609x gene dose 2-3 - 637x gene dose 0.25-1.5 - 79x PM (gene dose 0) Gene dose 0.25-1.5 versus gene dose 2-3: - increased risk of recurrence of breast cancer (HR_{corr} = 1.40; 95% CI: 1.04-1.90) (increase from 14.9% to 20.9% of the patients after 9 years of follow-up) (S) PM versus gene dose 2-3: - increased risk of recurrence of breast cancer (HR_{corr} = 1.90; 95% CI: 1.10-3.28) (increase from 14.9% to 20.9% of the patients after 9 years of follow-up) (S) (PM + gene dose 0.25-1.5) versus gene dose 2-3: - increased risk of recurrence of breast cancer or death (HR_{corr} = 1.33; 95% CI: 1.06-1.68) (increase from 14.9% to 20.9% of the patients after 9 years of follow-up) (S) - increased risk of recurrence of breast cancer, occurrence of a different cancer or death (HR_{corr} = 1.29; 95% CI: 1.03-1.61) (increase from 14.9% to 20.9% of the patients after 9 years of follow-up) (S) - no significantly increased risk of death (NS) NOTE: Genotyping was performed for *3, *4, *5, *10, *41 and gene duplication. *5 and gene duplication could not be deter-	Authors' conclusion: "Among women with breast cancer treated with tamoxifen, there was an association between CYP2D6 variation and clinical outcomes, such that the presence of 2 functional CYP2D6 alleles was associated with better clinical outcomes and the presence of non-functional or reduced-function alleles with worse outcomes."

ref. 31, continuation		mined in fixed tumour tissue (48.4% of the patients). NOTE 2: Regan et al. (J Natl Cancer Inst 2012;104: 1266-7) indicate that the genotyping is incorrect. The frequency of the genotypes was not in Hardy-Weinberg equilibrium. For the most important allele *4, the percentage of PM was a factor of 1.3 higher than expected for the allele frequency that was found. This means that 25% of the "PM" are probably not PM.	
ref. 32, kinetics Gjerde J et al. Effects of CYP2D6 and SULT1A1 genotypes including SULT1A1 gene copy number on tamoxifen metabolism. Ann Oncol 2008;19:56-61.	3 PM: A IM: A UM: A	151 breast cancer patients who used tamoxifen 20 mg/day as adjuvant therapy for at least 80 days. Comedication was not known. Genotyping: - 86x NM - 49x IM (1x *1/*3, 43x *1/*4, 4x *1/*5, 1x *1/*6) - 11x PM (8x *4/*4, 3x *4/*5) - 5x UM (5x *1/*2x2) PM versus NM: - decrease in C_{ss} endoxifen from 52.3 to 36.7 ng/mL (by 30%, S for the trend) - decrease in C_{ss} 4-OH-tam from 5.8 to 5.1 ng/mL (by 12%, S for the trend) IM versus NM: - decrease in C_{ss} endoxifen from 52.3 to 49.6 ng/mL (by 5%, S for the trend) - decrease in C_{ss} 4-OH-tam from 5.8 to 5.7 ng/mL (by 2%, S for the trend) UM versus NM: - decrease in C_{ss} endoxifen from 52.3 to 46.3 ng/mL (by 11%, S for the trend increase (logistical regression with correction for age)) - increase in C_{ss} 4-OH-tam from 5.8 to 5.9 ng/mL (by 2%, S for the trend) NOTE: Genotyping was performed for *3 to *6 and gene duplication for *2 (approx. 20% of the UM patients).	Authors' conclusion: "CYP2D6 genotype influences conversion of tamoxifen to potent hydroxylated metabolites in a manner consistent with a gene-dose effect. Patients carrying CYP2D6 alleles with high-predicted enzymatic activity have high serum levels of 4OHtam and 4OHNDtam."
ref. 33, adjuvant Schroth W et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. J Clin Oncol 2007;25:5187-93.	3 IM: E	206 breast cancer patients received tamoxifen monotherapy as adjuvant treatment. Dose and duration of the treatment were not reported. Tumours were positive for the oestrogen receptor. The median post-operative follow-up was 71 months. Comedication with SSRIs was not known. Genotyping: - 118x NM+UM - 49x IM (gene dose 1/0) - 16x IM (gene doses 0.5/0.5, gene dose 0.75, gene dose 0.5 and gene dose 0.25) - 14x PM Patients with *4, *5, *10 and *41 alleles versus NM+UM: - recurrence of breast cancer was significantly more common (S, OR_{corr} = 1.86) - shorter time to recurrence of breast cancer (S, HR_{corr} = 2.24) - shorter time to recurrence of breast cancer or death (event-free survival) (S, HR_{corr} = 1.89) - shorter time to death (overall survival (NS, HR_{corr} = 1.73)) IM (gene dose 1/0) versus NM+UM: - increase in the prevalence of recurrence of breast cancer from 14% to 29% (S, OR = 2.37)	Authors' conclusion: "Because genetically determined, impaired tamoxifen metabolism results in worse treatment outcomes, genotyping for CYP2D6 alleles *4, *5, *10, and *41 can identify patients who will have little benefit from adjuvant tamoxifen therapy."

ref. 33, continuation	PM: E	- shorter time to recurrence of breast cancer (NS, HR_{corr} = 1.88) - shorter time to recurrence of breast cancer or death (NS, HR_{corr} = 1.68) IM (gene doses 0.5/0.5 and 0.25 through 0.75) versus NM+UM: - increase in the prevalence of recurrence of breast cancer from 14% to 31% (NS, OR = 2.70) IM versus NM+UM: - increase in the prevalence of recurrence of breast cancer from 14% to 29% (significance and OR not determined) PM versus NM+UM: - increase in the prevalence of recurrence of breast cancer from 14% to 36% (S, OR = 3.30) PM + IM (gene doses 0.5/0.5 and 0.25 through 0.75) versus NM+UM: - increase in the prevalence of recurrence of breast cancer from 14% to 33% (S, OR = 2.97) - shorter time to recurrence of breast cancer (NS, HR_{corr} = 1.63) - shorter time to recurrence of breast cancer or death (event-free survival) (S, HR_{corr} = 1.46) In 280 patients who were not treated with tamoxifen, no significant effect was found for the CYP2D6 genotype on the time to recurrence of breast cancer. NOTE: Genotyping was performed for *4, *5, *10, *41 and gene duplication.	

ref. 34, kinetics and metastasis
Lim HS et al. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. J Clin Oncol 2007;25:3837-45.
	4
	
	UM: AA
		202 Asian breast cancer patients who used tamoxifen 20 mg/day for more than 8 weeks. No relevant comedication.
		
		Genotyping:
		- 64x no *10 (of which 51-55x *1/*1 (NM))
		- 89x heterozygous for *10 (of which 75-79x *1/*10 (NM))
		- 49x *10/*10 (IM)
		
		Results:
		- *10/*10 (IM) versus *10 heterozygote or no *10 respectively (both mainly NM):
		  - decrease in C_{ss} endoxifen from 18.1 and 19.9 respectively to 7.9 ng/mL (S by 56% and 60% respectively)
		  - decrease in C_{ss} 4-OH-tam from 2.5 and 2.8 respectively to 1.5 ng/mL (S by 40% and 46% respectively)
		- UM (heterozygous for *2xN, n=4) compared to the other genotypes:
		  - no significant difference in C_{ss} endoxifen and C_{ss} 4-OH-tam
		
		21 patients with metastatic breast cancer, of whom 12 from the above-mentioned group, received tamoxifen 20 mg/day for a median 9 months. Tumours were positive for the oestrogen or progesterone receptor. The median follow-up was 19.6 months.
		
		Genotyping:
		- 9x heterozygote or no *10 (mainly NM),
		- 12x *10/*10 (IM)
			Authors' conclusion:
			"Our study suggests that the CYP2D6*10/*10 genotype is a marker that is associated with lower steady-state plasma concentrations of tamoxifen active metabolites, that could lead to reduced clinical benefits in Asian breast cancer patients on tamoxifen."

ref. 34, continuation	IM: E	Results:
		- increase in the percentage *10/*10 (IM) versus the group with mainly non-metastatic breast cancer (57.1% versus 24.3%; S by 135%)
		- *10/*10 (IM) versus *10 heterozygote and no *10 (mainly NM):
		  - decrease in the percentage of stable disease for ≥ 24 weeks or with partial response from 100% to 50% (S by 50%)
		  - shorter time to disease progression from 21.8 to 5.03 months (S by 77%)
		  - univariate and multivariate Cox proportional hazard analysis confirms that the CYP2D6 genotype (*10/*10 versus other genotypes) is a significant variable for time to disease progression (HR = 3.69 and 3.68)
		
		NOTE: The most important variant alleles in Asians (*10, *5 and *2xN) were determined.	

ref. 35, adjuvant
Gonzalez-Santiago S et al.
CYP2D6*4 polymorphism as blood predictive biomarker of breast cancer relapse in patients receiving adjuvant tamoxifen.
J Clin Oncol 2007;25(18S):590.
	1
	
	IM + PM: E
		84 breast cancer patients received tamoxifen as adjuvant therapy (dose unknown). 98.7% of the tumours were positive for the oestrogen or progesterone receptor. Six patients (all *1/*4) were receiving CYP2D6 inhibitors as co-medication.
		
		Genotyping:
		- 48x *1/*1
		- 34x *1/*4
		- 2x *4/*4
		
		- (*1/*4 + *4/*4) versus *1/*1:
		  - a factor of 1.9 increase in the percentage of patients with recurrence of breast cancer (from 27% to 50%) (S)
		  - stronger association with recurrence of breast cancer (HR = 2.82; 95% CI: 1.0-7.9) (S)
		- patients with CYP2D6 inhibitors: recurrence of breast cancer in 50%
		
		NOTE: only *4 determined.
			Authors' conclusion:
			"Breast cancer patients with the CYP2D6 *4/*4 or wt/*4 genotype could have lower benefit of TAM adjuvant treatment and tend to have a higher risk of disease relapse. Pretreatment CYP2D6 genotype determination from blood sample could predicts TAM clinical outcomes and help to oncologist in treatment decision."

ref. 36, adjuvant Wegman P et al. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT-2B15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Res 2007;9:R7.	3 PM: AA IM: AA	677 postmenopausal breast cancer patients received tamoxifen 20 or 40 mg per day for 2 years, 5 years or an unspecified period as post-operative, adjuvant therapy. Tumours were positive for the oestrogen receptor. The median follow-up was 7.3 years. Comedication was unknown. Tumour genotyping (fresh-frozen): - 460x *1/*1 - 183x *1/*4 - 34x *4/*4 Risk of cancer recurrence: - lower for PM than NM (non-significant trend after correction for tumour stage, tumour size and lymph node status) (NS) - IM did not differ significantly from NM - (IM + PM) did not differ significantly from NM in the subgroups that were randomised to 2 and 5 years of tamoxifen respectively NOTE 1: only *4 determined. NOTE 2: Regan et al. (J Natl Cancer Inst 2012;104: 1266-7) indicate that the genotyping is incorrect. The frequency of the	Authors' conclusion: "The metabolism of tamoxifen is complex and the mechanisms responsible for the resistance are unlikely to be explained by a single polymorphism; instead it is a combination of several mechanisms."

ref. 36, continuation		genotypes was not in Hardy-Weinberg equilibrium. For *4, the percentage of PM was a factor of 1.5 higher than expected for the allele frequency that was found. This means that 32% of the "PM" are probably not PM.	
ref. 37, adjuvant Goetz MP et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. Breast Cancer Res Treat 2007;101:113-21.	4 PM: E IM: AA	180 post-menopausal breast cancer patients received tamoxifen 20 mg per day for 5 years as post-operative, adjuvant therapy. Tumours were positive for the oestrogen receptor. Comedication (involved 8 CYP2D6 inhibitors) was known for 171 patients. Correction for comedication was performed by including use of comedication in the determination of the "CYP2D6 phenotype". Tumour genotyping: - 124x *1/*1 - 40x *1/*4 - 13x *4/*4 - 3x unknown Phenotype categorisation: - 115x NM (*1/*1 without CYP2D6 inhibitor) - 40x IM (32x *1/*4 without CYP2D6 inhibitor; 8x *1/*1 with moderate inhibitor) - 16x PM (13x *4/*4; 1x *1/*1 with strong inhibitor; 1x unknown with strong inhibitor; 1x *1/*4 with strong inhibitor) - 9x unclassified (7x *1/*4 and co-medication unknown; 2x genotype unknown and moderate inhibitor) PM and IM versus NM phenotype: - increased risk of recurrence of breast cancer: PM: HR = 3.2 (S) IM: HR = 1.4 (NS) - increased risk of recurrence of breast cancer or death: PM: HR = 2.69 (S) IM: HR = 1.63 (NS) - increased risk of recurrence of breast cancer, occurrence of another cancer or death: PM: HR = 2.44 (S) IM: HR = 1.52 (NS) - increased risk of death: PM: HR = 2.0 (NS) IM: HR = 1.4 (NS) NOTE: Genotyping only for *4.	Authors' conclusion: "CYP2D6 metabolism, as measured by genetic variation and enzyme inhibition, is an independent predictor of breast cancer outcome in post-menopausal women receiving tamoxifen for early breast cancer. Determination of CYP2D6 genotype may be of value in selecting adjuvant hormonal therapy and it appears CYP2D6 inhibitors should be avoided in tamoxifen-treated women."
ref. 38, prophylaxis Bonanni B et al. Polymorphism in the CYP2D6 tamoxifen-metabolizing gene influences clinical effect but not hot flashes: data from the Italian Tamoxifen Trial. J Clin Oncol 2006;24:3708-9.	2 PM: E	2704 women with hysterectomy received tamoxifen 20 mg/day for 5 years, 20 of whom developed breast cancer. They were compared to 65 age-matched controls who had not developed breast cancer. Co-medication was unknown. Genotyping for *4 was performed using blood samples. - increase in the percentage *4/*4 in cases versus controls by a factor of 10 (from 1.5% to 15%) (S) - 3 women developed breast cancer: the tumour was negative for the oestrogen receptor in 2 cases and in 1 case it was positive All tumours were negative for the progesterone receptor. - all three women experienced hot flushes during tamoxifen therapy NOTE: frequency of *4/*4 in the population is low.	
ref. 39, kinetics Borges S et al.	4	158 breast cancer patients received tamoxifen 20 mg/day for 4 months. There were 94 without relevant co-medication.	Authors' conclusion:

Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clin Pharmacol Ther 2006;80:61-74. **ref. 39, continuation**	PM + IM: A	Genotyping: - 90x NM (62x gene dose 2; 28x gene dose 1.5) - 54x IM (6x gene dose 0.5; 48x gene dose 1) - 7x PM (gene dose 0) - 7x UM (gene dose ≥ 3) For patients without relevant co-medication, the following applies: - no fully functional allele: decrease versus NM + UM in the ratio of endoxifen/NDM (from 0.18 to 0.04, S) and decrease in C_{ss} endoxifen (from 88.6 to 21.9 nM, S) - 1 fully functional allele: decrease in the ratio of endoxifen/NDM versus NM + UM from 0.18 to 0.09, S and decrease in C_{ss} endoxifen from 88.6 to 64.2 nM, S. NOTE: Genotyping was performed for 33 CYP2D6 alleles (and for gene duplication).	'These data indicate that CYP2D6 genotype can explain part of the variability in the endoxifen plasma concentration and the endoxifen/NDM plasma ratio.' 'However, some variability in the endoxifen plasma concentration remains unexplained even after correction by CYP2D6 genotype and medication history.'
ref. 40, adjuvant Goetz MP et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. J Clin Oncol 2005;23:9312-8.	3 PM: AA	190 post-menopausal breast cancer patients received tamoxifen 20 mg per day for 5 years as adjuvant therapy. Tumours were positive for the oestrogen receptor. None of the women received adjuvant chemotherapy. Co-medication was not known. Tumour genotyping: - 137x NM (*1/*1) - 40x IM (*1/*4) - 13x PM (*4/*4) - PM: after correction for lymph node status and tumour size, the risk of recurrence of breast cancer and the risk of recurrence of breast cancer or death were non-significantly worse versus NM + IM (HR of 1.85 and 1.86 respectively). Without correction, both decreased (HR of 2.71 and 2.44 respectively, S). The risk of death was non-significantly worse, HR is 1.73. Hot flushes (moderate to severe) occurred in 0% of the PM patients and in 20% of the (NM + IM) patients. NOTE 1: Alleles *4 and *6 were genotyped. NOTE 2: Regan et al. (J Natl Cancer Inst 2012;104: 1266-7) indicate that the genotyping is incorrect. The frequency of the genotypes was not in Hardy-Weinberg equilibrium. The percentage PM for the most important allele *4 was a factor 2.4 higher than expected for the allele frequency that was found. This means that 58% of the "PM" are probably not PM. NOTE 3: for CYP3A5*3, there was no significant difference in relapse-free time, disease-free survival and overall survival.	Authors' conclusion: 'Nevertheless, these data suggest that CYP2D6 genetic variation is an important determinant of tamoxifen effect and that lower or absent CYP2D6 activity may increase the risk of tamoxifen treatment failure.' '…our findings suggest that the optimal biologically active dose of tamoxifen may differ with respect to interindividual variation in CYP-2D6.'
ref. 41, adjuvant Nowell SA et al. Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast Cancer Res Treat 2005;91:249-58.	3	162 breast cancer patients received tamoxifen as adjuvant therapy. Dose and duration of the treatment were not reported. It was also not reported whether all the tumours were positive for the oestrogen or progesterone receptor. It was reported that tamoxifen therapy is usually indicated for patients with oestrogen receptor-positive tumours. In this group of patients, 29% received tamoxifen only; 29% received tamoxifen and chemotherapy; 23% received tamoxifen and radiotherapy and 19% received tamoxifen, chemotherapy and radiotherapy. Co-medication was not known. Tumour genotyping: - 114x NM (*1/*1)	Authors' conclusion: 'When CYP2D6*4 was examined, there was no detectable influence of this genotype on overall survival or recurrence of disease in either the patients who received tamoxifen therapy or those

ref. 41, continuation	PM + IM: AA	- 48x (PM+IM) (*4/*4 or *4/*1) Results: - PM + IM: following correction for age, ethnicity, tumour stage and hormone receptor status, the risk of death versus NM was non-significantly reduced (HR = 0.77). The risk of recurrence of breast cancer was non-significantly reduced versus NM (HR 0.67). The number of deaths per person-years was non-significantly reduced versus NM. NOTE 1: Alleles *3, *4 and *6 were genotyped. Data were only analysed for *4. NOTE 2: for UGT2B15, no association was found between genotype and death or progression-free survival.	whose therapy did not include tamoxifen. It is interesting to note that in all subgroups, the CYP2D6*4 variant seemed to be associated with decreased risk of death or recurrence.'
ref. 42, kinetics Jin Y et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 2005;97:30-9.	3 PM: A IM: A	80 breast cancer patients received tamoxifen 20 mg/day for 4 months. In this group, 24 used CYP2D6 inhibitors as co-medication. Genotyping: - 48x NM - 29x IM - 3x PM Results: - PM: decrease in C_{ss} endoxifen from 78.0 to 20.0 nM (S by 74%), C_{ss} 4-OH-tam from 9.5 to 7.1 nM (NS by 25%), increase in C_{ss} N-des-tam from 653.4 to 664.1 nM (NS by 2%), decrease in C_{ss} tamoxifen from 372.5 to 288.9 nM (NS by 22%). - IM: decrease in C_{ss} endoxifen from 78.0 to 43.1 nM (S by 45%), C_{ss} 4-OH-tam from 9.5 to 8.3 nM (NS by 13%), increase in C_{ss} N-des-tam from 653.4 to 687.3 nM (NS by 4%), decrease in C_{ss} tamoxifen from 372.5 to 353.3 nM (NS by 5%). NOTE 1: Alleles *3 to *6 were genotyped. NOTE 2: no difference in C_{ss} tamoxifen or metabolites was found between the genotype groups for CYP2C9, CYP3A5 and SULT1A1.	
ref. 43, adjuvant Wegman P et al. Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. Breast Cancer Res 2005;7:R284-90.	3 PM + IM: AA[#]	76 postmenopausal breast cancer patients received tamoxifen 40 mg per day for 2 years as adjuvant therapy. Tamoxifen was given in combination with chemotherapy or radiotherapy. The patients had either lymph node metastases or a tumour size > 3 cm. All tumours were positive for the oestrogen receptor. The average follow-up was 10.7 years. Comedication was not known. Tumour genotyping (fresh-frozen): - 52x NM - 24x (IM+PM) Results: - the percentage of patients with recurrence of breast cancer was 48% for NM and 25% for IM + PM - IM + PM: lower risk of recurrence of breast cancer with tamoxifen than without tamoxifen (RR = 0.28; 95% CI: 0.11-0.74; following correction for age, lymph node status and tumour size) (S) - NM: lower risk of recurrence of breast cancer with tamoxifen than without tamoxifen (RR = 0.91) (NS)	Authors' conclusion: 'As shown in the present study, patients with at least one CYP2D6 *4 allele demonstrated better response to tamoxifen treatment than patients homozygous for the CYP-2D6*1 allele. Our results were obtained from a small number of patients, and therefore the association of the genotype and the benefit of tamoxifen treatment may be a

ref. 43, continuation		NOTE 1: Genotyping is for *4. NOTE 2: Regan et al. (J Natl Cancer Inst 2012;104: 1266-7) indicate that the genotyping is incorrect. The frequency of the genotypes was not in Hardy-Weinberg equilibrium. For all 112 patients on tamoxifen, the percentage of PM was a factor of 1.7 higher than expected for the allele frequency that was found. This means that 44% of the "PM" are probably not PM. NOTE 3: disease-free survival is significantly higher for homozygous SULT1A1*1 individuals than for carriers of SULT1A1*2.	coincidence.'
ref. 44 SmPC Tamoxifen Teva 03-04-24.	0 PM: E	Warning: The formation of the predominant active metabolite endoxifen occurs via the polymorphic CYP2D6 iso-enzyme. The literature reveals that CYP2D6 poor metabolisers have a reduced endoxifen plasma concentration. Endoxifen is one of the most important active metabolites of tamoxifen. Concomitant treatment with CYP2D6 inhibitors can result in reduced concentrations of the active metabolite endoxifen. Because of this, co-medication with strong CYP2D6 inhibitors (e.g. paroxetine, fluoxetine, quinidine, cinacalcet and bupropion) should be avoided as much as possible during tamoxifen treatment. Pharmacodynamic properties: The status of CYP2D6 polymorphism can be associated with variability in the clinical response to tamoxifen. Poor metabolisers can exhibit a reduced response. The consequences of these findings for the treatment of CYP2D6 poor metabolisers are not entirely clear yet. The available clinical data indicate that patients who are homozygous for non-functional CYP2D6 alleles can experience a reduced effect in tamoxifen treatment of breast cancer. The available studies were primarily performed on post-menopausal women. Pharmacokinetic properties: Tamoxifen is primarily metabolised by CYP3A4 to N-desmethyl-tamoxifen, which is then further metabolised by CYP2D6 to endoxifen, another active metabolite. In patients who are lacking the CYP2D6 enzyme, the endoxifen concentrations are approximately 75% lower than in patients with normal CYP2D6 activity. Administration of strong CYP2D6 inhibitors reduces the endoxifen exposure to a similar extent.	
ref. 45 SmPC Soltamox (tamoxifen citrate), USA, 08-04-19.	0	Pharmacokinetics: Metabolism: Endoxifen concentrations may differ among patients because of various CYP2D6 genotypes. Drug-drug Interactions, CYP2D6 inhibitors: Although concomitant administration of CYP2D6 inhibitors reduces the plasma concentration of endoxifen, a potent metabolite, the clinical significance is not well established. The mean steady-state endoxifen plasma concentration in patients taking CYP2D6 inhibitors was significantly reduced compared to those not taking concomitant CYP2D6 inhibitors (14.8 ± 10.6 versus 26.7 ± 15.4 ng/mL). The mean steady-state plasma concentration of endoxifen in CYP2D6 normal metabolizers who were not receiving CYP2D6 inhibitors was 31.4 ± 14.7 ng/mL compared to 8.8 ± 3.5 ng/mL in CYP2D6 normal metabolizers receiving potent CYP2D6 inhibitors (e.g., paroxetine, fluoxetine) with tamoxifen. The plasma levels of endoxifen in CYP2D6 normal metabolizers taking potent CYP2D6 inhibitors were similar to the levels observed in CYP2D6 poor metabolizers taking no CYP2D6 inhibitors (8.8 versus 7.2 ng/mL). Pharmacogenomics: The impact of CYP2D6 polymorphisms on the efficacy of tamoxifen is not well established. CYP2D6 poor metabolizers carrying two non-functional alleles	

ref. 45, continuation	PM: A	exhibit significantly lower endoxifen plasma concentrations compared to patients carrying one or more fully functional alleles of CYP2D6. In patients with estrogen receptor-positive breast cancer who were participating in the WHEL (Women's Health Eating and Living) Study (NCT00003787), the mean (SD) serum concentration of endoxifen was 22.8 (11.3), 15.9 (9.2), 8.1 (4.9) and 5.6 (3.8) ng/mL in 27 ultrarapid, 1,097 normal, 164 intermediate and 82 poor metabolizers ($p<0.001$), respectively. This finding is consistent with other published studies that report lower endoxifen concentrations in poor metabolizers compared to normal metabolizers.	

[#] There is a potential positive effect on survival for the PM or IM phenotype.

Risk group	IM patients with CYP2D6 inhibitors such as paroxetine or fluoxetine

Comments

- Only clinical studies with more than 1000 patients, meta-analyses of clinical studies, and genotype-guided studies were included for the period after 2012. Other studies did not contribute sufficiently to the burden of proof. A Japanese genotype-guided study was not included, because 53% of the patients receiving a genotype-guided dose increase were normal metabolisers (gene dose 1.25-1.5) (Tamura K et al. CYP2D6 genotype-guided tamoxifen dosing in hormone receptor-positive metastatic breast cancer (TARGET-1): a randomized, open-label, phase II study. J Clin Oncol 2020; 38:558-66. PMID: 31821071).
  For the period after February 2008, kinetic studies were only included if they contained a suggestion for a dose or therapeutic adjustment.
  Clinical studies were only included if they contained relevant endpoints for the treatment of cancer, such as recurrence of cancer or survival. All studies examining tamoxifen for the treatment of metastic breast cancer were included. This is the only registered indication for tamoxifen and the higher dose (40 mg/day) is also used here. For the studies involving adjuvant treatment, only studies with more than 400 patients were included. Studies examining tamoxifen as a prophylactic treatment were not included.
- <u>FDA guidelines</u>:
  - The American SmPC Soltamox (tamoxifen citrate) 08-04-19 states that there is a significant effect of CYP2D6 genotype on endoxifen levels, but the impact of CYP2D6 polymorphisms on the efficacy of tamoxifen is not well established.
  - Hartman AR, Helft P. Breast Cancer Res 2007;9:103 (comment):
    On 18 October 2006, the FDA Clinical Pharmacology Subcommittee unanimously decided that this new clinical evidence (reference Goetz, 2007) demonstrates that the CYP2D6 gene is an important predictor of tamoxifen effectiveness. Aromatase inhibitors are an alternative for post-menopausal women, with equal or better results. For these women, it is useful to determine (or report) the CYP2D6 genotype. There is no good alternative for pre-menopausal or peri-menopausal women.
  - Young D. Am J Health Syst Pharm 2006;63:2286, 2296 (news):
    The FDA committee recommends revised labelling of tamoxifen, so that prescribers are warned that patients with breast cancer who are poor metabolisers of this medicine have an increased risk of recurrence of the disease. This recommendation was not adopted by the FDA.
- <u>Other guidelines</u>:
  - Drögemöller BI et al. CYP2D6 as a treatment decision aid for ER-positive non-metastatic breast cancer patients: a systematic review with accompanying clinical practice guidelines. Breast Cancer Res Treat 2019;173:521-32. PMID: 30411242.
    The Canadian Pharmacogenomics Network for Drug Safety (CPNDS) Clinical Recommendations Group indicates that although conflicting literature exists, the majority of the current evidence points toward CYP2D6 genetic variation affecting survival outcomes after tamoxifen treatment.
    CPNDS indicates that evidence for the role of genetic variants on endoxifen levels has been provided by four CYP2D6-based tamoxifen dose-adjustment studies (Welzen 2015, Dezentjé 2015, Hertz 2016, and Kiyotani 2012). CPNDS indicates that these studies, which incorporated either an individualized dose escalation approach or a doubling in tamoxifen dose (from 20 to 40 mg/day) in PM and IM, consistently showed that:
    (i) baseline endoxifen levels were significantly lower in PM and IM when compared to NM;
    (ii) tamoxifen dose escalation in IM and PM significantly increased endoxifen levels, with endoxifen levels normalizing in IM in the majority of cases;
    (iii) the increase in tamoxifen dose did not increase short-term adverse events.
    The body of evidence for the guideline consisted of 38 articles, including one meta-analysis (Province 2014).

Five other meta-analyses were excluded because they included one or more studies that were included in the body of evidence separately. CPNDS indicates that 20 articles in the body of evidence (52.6%) reported at least one statistically significant association with CYP2D6 and tamoxifen survival outcomes, while 18 articles (47.4%) reported no statistically significant associations. CPNDS indicates that the type of CYP2D6 genotyping assay used was a significant confounder, with comprehensive genotyping panels being more likely to report a significant association with CYP2D6-survival outcome. CPNDS defines comprehensive genotyping as at least genotyping CYP2D6*3, *4, *5, *10 and *41 (at least CYP2D6*4, *5, *10 and *41 in Asians). CPNDS indicates that of the studies that used comprehensive genotyping (n = 13), only two studies did not report significant associations between CYP2D6 and tamoxifen survival outcomes. The two studies reporting non-significant associations had relatively small sample sizes (n = 92 and n = 106), which may have limited their power to detect statistically significant results. Because the type of genotyping is a confounder, CPNDS recommends comprehensive CYP2D6 genotyping.
CPNDS restricts its recommendations to non-metastatic ER-positive breast cancer patients.
The CPNDS recommendations for non-metastatic ER-positive breast cancer patients are as follows:

phenotype	therapeutic recommendation based on genotype[a]	strength of recommendation
UM+ gene dose 2.5	Use an aromatase inhibitor (with ovarian suppressor in premenopausal women) or tamoxifen 20 mg/day[b].	grade B – moderate[c]
NM	Use an aromatase inhibitor (with ovarian suppressor in premenopausal women) or tamoxifen 20 mg/day[b].	grade B – moderate[c]
IM	Use an aromatase inhibitor (with ovarian suppressor in premenopausal women). If aromatose inhibitors are contraindicated: use tamoxifen 40 mg/day[b].	grade B – moderate[c]
PM	Use an aromatase inhibitor (with ovarian suppressor in premenopausal women). If aromatose inhibitors are contraindicated: use tamoxifen 40 mg/day[b,d].	grade B – moderate[c]

[a]: CPNDS indicates that where feasible, by combining genotype-guided tamoxifen treatment with therapeutic drug monitoring (TDM), ER-positive non-metastatic breast cancer treatments can be further personalised. CPNDS recommends a dose increase in patients with endoxifen levels less than 6 ng/ml, and a switch to aromatase inhibitors if endoxifen levels remain less than 6 ng/ml despite dose increase.
[b]: In individuals receiving tamoxifen, moderate or strong CYP2D6 inhibitors (refer to the Flockhart P450 Drug Interaction Table for classification of CYP2D6 inhibitors) should be avoided.
[c]: The recommendation is based on reduced confidence scientific evidence and expert opinion; benefits are likely to outweigh risks.
[d]: Studies have shown that in patients with gene dose 0, although a dose change increases endoxifen levels, these levels do not completely normalise. Therefore, aromatase inhibitors may be a preferred treatment in these patients.

- Goetz MP et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 and tamoxifen therapy. Clin Pharmacol Ther 2018;103:770-7. PMID: 29385237.and change in CYP2D6 genotype to phenotype translation on the CPIC website in October 2019 (https://cpicpgx.org/guidelines/cpic-guideline-for-tamoxifen-based-on-cyp2d6-genotype/).
CPIC indicates that the literature review on CYP2D6 and tamoxifen was initiated because of conflicting data on the association between endoxifen concentrations and CYP2D6 polymorphisms with tamoxifen outcome.
CPIC indicates that initial and follow-up data demonstrated that CYP2D6 PMs had an ~2–3-fold higher risk of breast cancer recurrence (compared to CYP2D6 NMs) (Goetz 2005, Schroth 2007, and Schroth 2009) and led an FDA special emphasis panel to recommend a tamoxifen label change to incorporate data that CYP2D6 genotype was an important biomarker associated with tamoxifen efficacy (US Food and Drug Administration: summary minutes of the advisory committee pharmaceutical science, clinical pharmacology subcommittee, October 18-19, 2006. http://www.fda.gov/ohrms/dockets/ac/06/minutes/2006-4248m1.pdf). However, this label change was not implemented because of conflicting data from secondary analyses of 5-year tamoxifen prospective trials including ATAC, BIG1-98, and ABCSG8 (Rae 2012, Regan 2012, and Goetz MP et al. CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. Clin Cancer Res 2013;19:500-7). CPIC states that multiple other studies were summarized in a meta-analysis that demonstrated an association between CYP2D6 genotype and disease-free survival, but only in patients who received tamoxifen as adjuvant therapy at a dose of 20 mg/day for 5 years (Province 2014).
Regarding the role of measurement of endoxifen concentrations, CPIC indicates that a study identified an association between low endoxifen (lowest quintile) and recurrence (Madlensky L et al. Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. Clin Pharmacol Ther 2011;89:718-25). In addition, a separate study of premenopausal patients, demonstrated that patients with low endoxifen concentrations (<14 nM) exhibited a higher risk for distant relapse or death compared with those with high concentrations (>35 nM) (Saladores P et al. Tamoxifen metabolism predicts drug concentrations and outcome in

premenopausal patients with early breast cancer. Pharmacogenomics J 2015;15:84-94).
CPIC concludes from the literature review that there is substantial evidence linking the CYP2D6 genotype with phenotypic variability in endoxifen concentrations. The evidence was considered uniformly strong that PM have lower plasma endoxifen concentrations among patients taking adjuvant tamoxifen compared to NM, and that reduced CYP2D6 activity (gene dose 0 to 1) is associated with lower plasma endoxifen concentrations among patients taking adjuvant tamoxifen compared to normal CYP2D6 activity. CPIC indicates that CYP2D6 genotype explains 34–52% of the variability in absolute endoxifen concentrations (Schroth W et al. Improved prediction of endoxifen metabolism by CYP2D6 genotype in breast cancer patients treated with tamoxifen. Front Pharmacol 2017;8:582). Of particular note, for populations with a high frequency of the decreased function CYP2D6*10 allele, there was strong evidence that patients with CYP2D6 gene dose 0 to 1 had significantly lower plasma endoxifen concentrations compared to those with normal CYP2D6 activity (gene dose 1.5 and 2).
CPIC indicated that one prospective clinical study examined the association between CYP2D6 genotype and change in tumor Ki-67, a phenotype linked to drug efficacy, in patients with early-stage breast cancer receiving neoadjuvant tamoxifen. In this study, patients with *10/*10 and *5/*10 genotypes had significantly lower Ki-67 response compared to patients genotyped as *1/*1 (Zembutsu H et al. Significant effect of polymorphisms in CYP2D6 on response to tamoxifen therapy for breast cancer: a prospective multicenter study. Clin Cancer Res 2017;23:2019-26).
CPIC indicates that because of the extensive biological variability across the various clinical settings where tamoxifen is administered (prevention, ductal carcinoma in situ, premenopausal and postmenopausal adjuvant setting, and metastatic), the CPIC guideline focuses only on the role of CYP2D6 genotype in the adjuvant treatment of ER-positive breast cancer, using the endpoints of recurrence, recurrence-free survival, disease-free survival, distant relapse-free survival, breast cancer-specific survival, and overall survival. Based on the literature review, CPIC indicates that for the clinical endpoints of recurrence and event-free survival, the evidence was graded as moderate for the statements that PM have a higher risk of breast cancer recurrence or worse event-free survival. However, for the comparison of other metaboliser groups (IM, NM, and UM) and other clinical endpoints, the evidence was considered weak regarding an association between CYP2D6 metaboliser groups and clinical outcome.
CPIC indicates that, based on current evidence, UM and NM are expected to achieve therapeutic endoxifen concentrations after administration of tamoxifen and should receive the recommended standard of care doses of tamoxifen. In addition, CPIC indicates that PM and IM (including patients with a gene dose of 1.0) are expected to have lower endoxifen concentrations compared to NM and have a higher risk of breast cancer recurrence, and worse event-free survival compared to NM. For PM, CPIC indicates that a “strong” therapeutic recommendation was provided to recommend alternative hormonal therapy such as an aromatase inhibitor (AI) for postmenopausal women or AI along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype (Early Breast Cancer Trialists’ Collaborative Group. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015;386:1341-52, and Pagani O et al. Adjuvant exemestane with ovarian suppression in premenopausal breast cancer. N Engl J Med 2014;371:107-18) and based on knowledge that PM patients who switch from tamoxifen to anastrozole do not exhibit an increased risk of recurrence (Goetz MP et al. CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. Clin. Cancer Res 2013;19:500-7). Given that escalation of tamoxifen dose from 20-40 mg/day in PM significantly increases endoxifen concentrations (but not to concentrations achieved in NM (Hertz 2016)), the use of an AI (± ovarian function suppression) is recommended in this setting. Tamoxifen 40 mg/day can be considered for PM if there are contraindications to AI use. CPIC indicates that there are no clinical data that toremifene, another selective estrogen receptor modulator that also undergoes bioactivation (Kim J et al. Role and pharmacologic significance of cytochrome P-450 2D6 in oxidetive metabolism of toremifene and tamoxifen. Int J Cancer 2013;132:1475-85), should be substituted for tamoxifen based on CYP2D6 genotype.
For IM and *10/*10 or *10/decreased function allele, CPIC indicates that a “moderate” recommendation was made to consider use of an alternative hormonal therapy (i.e., aromatase inhibitor) for postmenopausal women or AI plus ovarian function suppression in premenopausal women. In addition, CPIC indicates that if AIs are contraindicated in IM, consideration can be given to the use of a higher FDA-approved dose of tamoxifen (40 mg/day), which is known to result in significantly higher endoxifen concentrations without an increase in toxicity (Hertz 2016). Based on extrapolation from evidence in *10 individuals, a similar recommendation applies to individuals who carry other decreased function alleles resulting in a gene dose of 1.0 but with an “optional” recommendation, given the paucity of data for this group.
CPIC indicates that, in general, prolonged overlap of tamoxifen with strong and moderate CYP2D6 inhibitors should be avoided in tamoxifen-treated patients (Hansten PD and Horn JR. Top 100 drug interactions 2017: a guide to patient management, 1st edn. (H&H Publications, Freeland, Washington, 2017)), whereas weak inhibitors are also contraindicated in IM.
The CPIC recommendations are as follows:

phenotype	activity score of subgroup	therapeutic recommendation	classification of recommendation
UM+ gene dose 2.5		Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	strong[a]
NM		Avoid moderate and strong CYP2D6 inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	strong[a]
IM	1	Consider hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype[b]. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day)[c]. Avoid CYP2D6 strong to weak inhibitors.	optional[d,e]
	0.25-0.75	Consider hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women, given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype[b]. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day)[c]. Avoid CYP2D6 strong to weak inhibitors.	moderate[f]
PM		Recommend alternative hormonal therapy such as an aromatase inhibitor for postmenopausal women or aromatase inhibitor along with ovarian function suppression in premenopausal women given that these approaches are superior to tamoxifen regardless of CYP2D6 genotype[b] and based on knowledge that CYP2D6 poor metabolizers switched from tamoxifen to anastrozole do not have an increased risk of recurrence[g]. Note, higher dose tamoxifen (40 mg/day) increases but does not normalize endoxifen concentrations and can be considered if there are contraindications to aromatase inhibitor therapy[c,h].	strong[a]

[a] Strong indicates that "The evidence is high quality and the desirable effects clearly outweigh the undesirable effects."
[b] Early Breast Cancer Trialists' Collaborative Group. Aromatase inhibitors versus tamoxifen in early breast cancer: patient-level meta-analysis of the randomised trials. Lancet 2015;386:1341-52.
[c] Hertz 2016.
[d] Optional indicates that the desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
[e] Those patients with genotype *10/*10 (gene dose 0.5) or gene dose 0.75 (*10 plus another reduced activity allele) are provided a "moderate" recommendation. In contrast, prescribing recommendations for those with gene dose 1 are graded as "optional" because the recommendations are primarily extrapolated from evidence generated from *10 patients (i.e., limited data for clinical outcomes and pharmacokinetics for this group).
[f] Moderate indicates that "There is a close or uncertain balance" as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.
[g] Goetz MP et al. CYP2D6 metabolism and patient outcome in the Austrian Breast and Colorectal Cancer Study Group trial (ABCSG) 8. Clin. Cancer Res 2013;19:500-7.
[h] Irvin 2011.

CPIC did not include meta-analyses in the body of evidence for the guideline.
On 26-9-2023, there was not a more recent version of the recommendations present on the CPIC-site.

- Cardoso F et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019;30:1194-220. PMID: 31161190.

The European Society for Medical Oncology (ESMO) indicatest hat the study of CYP2D6 polymorphisms as a decision aid regarding the use of adjuvant tamoxifen is not proven and should not be done outside a clinical trial (classification of recommendation: I E, i.e. level of evidence I (evidence from at least one large randomised, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomised trials without heterogeneity) and grade of recommendation E (strong evidence against efficacy or for adverse outcome, never recommended)).
Note: Regarding CYP2D6 inhibitors, ESMO indicates that patients on tamoxifen should be advised to avoid the use of strong and moderate CYP2D6 inhibitors (although there are no unequivocal data on their detrimental effects). If such drugs cannot be replaced, a switch to alternative treatment, i.e. aromatase inhibitors, should be considered (classification of recommendation: IV B, i.e. level of evidence IV (evidence from retrospective cohort studies or case–control studies) and grade of recommendation B (strong or moderate evidence for efficacy but with a limited clinical benefit, generally recommended)).

- Harris LN et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 2016;34:1134-50. PMID: 26858339.
  The American Society of Clinical Oncology (ASCO) indicates that the clinician should not use CYP2D6 polymorphisms to guide adjuvant endocrine therapy selection (type of recommendation: evidence based, evidence quality: intermediate, strength of recommendation: moderate).
  ASCO indicates that the ability of polymorphisms in CYP2D6 to predict tamoxifen benefit has been extensively studied (Province 2014, Goetz 2013, Regan 2012, and Rae 2012). The results of these pharmacogenomics studies have been controversial, with more recent studies being negative. At this point, data do not support the use of this marker to select patients who may or may not benefit from tamoxifen therapy.

- Other literature summaries:
  - Klein DJ et al. PharmGKB summary: tamoxifen pathway, pharmacokinetics. Pharmacogenet Genomics 2013;23:643-7.
    The authors indicate that it is likely that endoxifen is particularly responsible for the effect of tamoxifen. Endoxifen is 30-100x more potent than tamoxifen, the plasma concentration is 10x higher than that of 4-hydroxytamoxifen and it is the only metabolite of tamoxifen that stimulates the breakdown of oestrogen receptor-α.
    The results regarding the effect of gene variants on clinical outcomes of tamoxifen treatment are contradictory. Studies are heterogeneous and a lack of thorough genotyping and phenotyping can possibly play a role in the contradictory results. In addition, they list environmental factors, such as menopausal status or possible drug interactions with CYP2D6 inhibitors.

- Cost-effectiveness studies:
  - Wei X et al. Cost-effectiveness analysis of CYP2D6*10 pharmacogenetic testing to guide the adjuvant endocrine therapy for postmenopausal women with estrogen receptor positive early breast cancer in China. Clin Drug Investig 2020;40:25-32. PMID: 31559573.
    This cost-effectiveness analysis found CYP2D6*10 pharmacogenetic testing to be cost effective from the Chinese societal perspective for postmenopausal women with ER-positive early breast cancer receiving adjuvant treatment for a period of 5 years. In the genotype-guided therapy group, the total additional cost was US$17,966.95 and the total quality-adjusted life-years (QALY) gained was 3.582. Thus, the cost was US$5015.693 per QALY gained. Compared with a willingness-to-pay threshold of US$26,508/ QALY in China, the CYP2D6*10 testing is cost-effective in postmenopausal women with ER-positive breast cancer in China. Variation in input parameters showed that the model outcomes were quite stable. Non-genotype-guided therapy consisted of tamoxifen for all patients. Genotype-guided therapy consisted of tamoxifen for *1/*1 and *1/*10 (NM) and an aromatase inhibitor for *10/*10 (IM).
    Cost were calculated for a period of 2 years and from the Chinese societal perspective. So, direct medical cost (cost of drugs, inspection, examination, and administration) and direct non-medical costs (cost of work lost, transportation cost, and time cost for patients) were calculated. Input data were obtained from the public literature and from the National Cancer Center in China. Patients were assumed to be switched to an aromatase inhibitor in case of disease recurrence. Genotype-guided therapy was calculated to cost US$75,466.43 and provide 10.933 QALYs. Not-genotype-guided therapy was calculated to cost US$57,499.47 and provide 7.351 QALYs. Medical cost of disease-free survival state was US$4722.16, medical cost of recurrent disease state was US$5405.52, non-medical cost of disease-free survival state was US$286.13, non-medical cost of recurrent disease state was US$356.61, cost of tamoxifen was US$4.45/month, monthly cost of aromatase inhibitor was 30 times the monthly cost of tamoxifen, and genotyping cost was US$71.06.
    Variation of input parameters showed cost-effectiveness of genotype-guided therapy to be a robust result. At a willingness-to-pay threshold of US$15,904.8, the probability of being cost effective was 99.3% for genotype-guided therapy.
  - Wei X et al. CYP2D6*10 pharmacogenetic-guided SERM could be a cost-effective strategy in Chinese patients with hormone receptor-positive breast cancer. Pharmacogenomics 2020;21:43-53. PMID: 31769341.
    This cost-effectiveness analysis found CYP2D6*10 pharmacogenetic testing to be cost effective from the Chinese societal perspective for postmenopausal women with ER-positive early breast cancer receiving adjuvant treatment for a period of 5 years. Compared to tamoxifen for all patients, toremifene for all patients was calculated to cost US$5546.01139 per quality-adjusted life-years (QALY) gained and genotype-guided tamoxifen treatment US$5055.74221 per quality-adjusted life-years (QALY) gained. Considering a willingness-to-pay threshold of US$26,508/QALY in China, both genotype-guided tamoxifen treatment and toremifene for all are cost-effective in postmenopausal women with ER-positive breast cancer in China. Genotype-guided therapy consisted of tamoxifen for *1/*1 and *1/*10 (NM) and toremifene for *10/*10 (IM). Variation in input parameters showed the model outcomes to be very stable.
    Cost were calculated for a period of 30 years and from the Chinese societal perspective. So, direct medical cost and direct non-medical costs were calculated. Input data were obtained from the public literature. It was assumed that tamoxifen or toremifene were stopped in case of disease recurrence. The cost of tamoxifen for all, toremifene for all and genotype-guided tamoxifen was US$63,879.19, US$90,156.60 and US$95,021.41,

and the QALYs gained were 8.1588, 12.89687 and 13.85911, respectively. Medical cost of disease-free survival state was US$4722.16, medical cost of recurrent disease state was US$5405.52, non-medical cost of disease-free survival state was US$286.13, non-medical cost of recurrent disease state was US$356.61, cost of tamoxifen was US$4.45/month, cost of toremifene was US$20.00/month, and genotyping cost was US$ 71.06. The hazard ratios for disease free survival and overall survival were 0,91 and 1.02, respectively, for toremifene versus tamoxifen
Variation of input parameters showed cost-effectiveness of genotype-guided therapy to be a very robust result. At all variations, genotype-guided therapy remained below the willingness-to-pay threshold of US$ 26,508.

- Rae JM et al. Breast Cancer Research 2005:
Rae et al. state in a reaction to the reference Wegman, 2005 that the study has a high risk of selection bias due to the small number of patients from the original trial that were used for this study (10.5%). Furthermore, as expected, oestrogen-positive patients who are treated with tamoxifen result in improved outcomes, even if they have a CYP2D6*4 allele.

Date of literature search: 6 November 2024.

	Phenotype	Code	Gene-drug interaction	Action	Date
KNMP Pharmacogenetics Working Group decision	PM	4 F	Yes	Yes	27 January 2025
	IM	4 F	Yes	Yes	
	UM	4 F	Yes	No	

Mechanism:
The main conversion route of tamoxifen is by CYP3A4/5 to the relatively inactive N-desmethyltamoxifen. This is converted by CYP2D6 to endoxifen (hydroxy-N-desmethyltamoxifen), which has an anti-oestrogenic effect that is 30-100x stronger than tamoxifen. Tamoxifen is further converted by CYP2D6 to the active metabolite 4-hydroxytamoxifen. This metabolite is as potent as endoxifen, but occurs at much lower concentrations. CYP3A4/5 converts 4-hydroxytamoxifen further to endoxifen.
A CYP2D6 genetic polymorphism may cause a change in the plasma concentration of endoxifen in particular, but also of 4-hydroxytamoxifen.
The NVZA does not provide a therapeutic drug monitoring monograph for tamoxifen. In literature, the minimal effective plasma endoxifen concentration is considered to be 5.97 ng/ml (approximately 16 nM).

Clinical Implication Score:

Table 1: Definitions of the available Clinical Implication Scores

Potentially beneficial	PGx testing for this gene-drug pair is potentially beneficial. Genotyping can be considered on an individual patient basis. If, however, the genotype is available, the DPWG recommends adhering to the gene-drug guideline	0-2 +
Beneficial	PGx testing for this gene-drug pair is beneficial. It is advised to consider genotyping the patient before (or directly after) drug therapy has been initiated to guide drug and dose selection	3-5 +
Essential	PGx testing for this gene-drug pair is essential for drug safety or efficacy. Genotyping must be performed before drug therapy has been initiated to guide drug and dose selection	6-10 +

Table 2: Criteria on which the attribution of Clinical Implication Score is based

Clinical Implication Score Criteria	Possible Score	Given Score
Clinical effect associated with gene-drug interaction (drug- or diminished efficacy-induced)		
• CTCAE Grade 3 or 4 (clinical effect score D or E)	+	
• CTCAE Grade 5 (clinical effect score F)	++	++
Level of evidence supporting the associated clinical effect grade ≥ 3		
• One study with level of evidence score ≥ 3	+	
• Two studies with level of evidence score ≥ 3	++	
• Three or more studies with level of evidence score ≥ 3	+++	+++
Number needed to genotype (NNG) in the Dutch population to prevent one clinical effect grade ≥ 3		
• 100 < NNG ≤ 1000	+	+

• 10 < NNG ≤ 100 • NNG ≤ 10	++ +++	
PGx information in the Summary of Product Characteristics (SmPC) • At least one genotype/phenotype mentioned OR • Recommendation to genotype OR • At least one genotype/phenotype mentioned as a contra-indication in the corresponding section	 + ++ ++	 +
Total Score:	10+	7+
Corresponding Clinical Implication Score:		Essential
Score after taking additional considerations into account:		Beneficial