Update on CYP2D6 and Tamoxifen

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H&O What is CYP2D6? What is known about the relationship between CYP2D6 and tamoxifen?

MG CYP2D6 is a liver enzyme involved in the metabolism of many different xenobiotics. There is considerable variability in the activity of this enzyme, related mainly to genetic polymorphisms. Tamoxifen undergoes a series of metabolic steps, and importantly, CYP2D6 is a rate limiting enzyme involved in the bioactivation of tamoxifen into a more active metabolite called endoxifen.. Compared to tamoxifen, endoxifen has a significantly greater affinity for the estrogen receptor (ER) and a greater ability to inhibit cell proliferation. However, the differences between tamoxifen and its metabolites are much more complex, as recent data by Hawse and associates in PLoS One demonstrate that endoxifen-mediated recruitment of ERβ to known target genes differs from that of 4-hydroxy-tamoxifen (4HT) and ICI-182,780 (ICI) and that alterations in endoxifen concentrations dramatically altered the gene expression profiles of MCF7 cells, even in the presence of clinically relevant concentrations of tamoxifen and its metabolites.

It has been repeatedly observed that CYP2D6 enzyme activity (as predicted by CYP2D6 genetic polymorphisms) is associated with the plasma concentrations of endoxifen. As such, patients who are born with genetic alterations that lead to lower or absent CYP2D6 enzyme activity have lower concentrations of endoxifen, whereas those with genetic alterations associated with increased CYP2D6 enzyme activity have higher endoxifen concentrations. Therefore, the pharmacologic hypothesis is as follows: the rate at which tamoxifen is converted into the more active metabolite, endoxifen, is associated with the risk of breast cancer recurrence.

If you look at the available data regarding tamoxifen and CYP2D6, all reports published to date are derived from either retrospective studies or secondary analyses of prospective clinical trials. One of the original publications was a secondary analysis of a prospective North Central Cancer Treatment Group (NCCTG) clinical trial of approximately 200 patients with early-stage, ER-positive breast cancer. This study demonstrated that genetic variation in CYP2D6 was associated with the risk of recurrence or death among patients who received tamoxifen at a dose of 20 mg daily as mono-therapy for 5 years. Subsequently, larger studies have been performed, including a pooled analysis of the NCCTG 89-30-52 data along with a large group of tamoxifen treated patients from Europe. This latter study, was led by Dr. Hiltrud Brauch, and showed a very similar relationship between CYP2D6 genotype and disease free survival.

Following these early observations, other negative studies have been published. So there has been a need for validation in the setting of large trial that evaluated 5 years of adjuvant tamoxifen in postmenopausal women with ER positive breast cancer. Three clinical trials have reported CYP2D6 data thus far: the Breast International Group (BIG) 1-98, ATAC (Arimidex, Tamoxifen, Alone or in Combination), and the Austrian Breast and Colorectal Study Group (ABCSG) 8 trial. The BIG 1-98 and ATAC trials were published simultaneously last year, and reported no association between CYP2D6 and clinical outcome. However, multiple groups pointed out methodology flaws in these studies, and based on this, many researchers believe that no conclusions can be drawn from these studies. Specifically, the ATAC trial analyzed only 18% of the 3,000 patients enrolled onto the tamoxifen arm.. As expected, the clinical characteristics of the gen-
treated with tamoxifen for 5 years had recurrence of breast cancer, or died at a rate 2.5 times higher than women classified as extensive metabolizers. Women with intermediate levels of the CYP2D6 enzyme had rates of recurrence or death 1.7 times higher than CYP2D6 extensive metabolizers. Another important question that we were able to address in this study related to whether the recurrence rates were similar in women who were switched from a drug metabolized by CYP2D6 (tamoxifen) to a drug not metabolized by CYP2D6 (anastrozole). We observed that during the first 2 years, CYP2D6 poor metabolizers had an increased rate of recurrence of breast cancer, or death that was similar, regardless of which arm they were randomized to, as both arms received tamoxifen during the first 2 years. However, in arm B, once patients were switched to anastrozole, there was no longer an association between CYP2D6 genotype and the odds of recurrence/death. We therefore concluded that switching from tamoxifen to an aromatase inhibitor may be one reason for some of the discrepant results surrounding CYP2D6 and tamoxifen, as information on whether a patient took an aromatase inhibitor after tamoxifen is unlikely to be available in most prior studies.

Another major difference in the study design of the ABCSG 8 study (compared to the CYP2D6 analyses of ATAC and BIG 1-98) relates to the CYP2D6 genotyping protocol. In the BIG 1-98 study, investigators used tumor cores (designed for the development of breast tumor biomarkers) for DNA extraction. The problem with this approach is that tumor cells, by definition, have mutations, and there are published data demonstrating loss of heterozygosity involving chromosome 22 Q13, the location of the CYP2D6 gene. This can occur in up to 30% of ER-positive breast cancers. Therefore, the use of tumor tissue as a source of DNA is likely reason for the marked deviations from the Hardy-Weinberg equilibrium observed in the BIG 1-98 study. In the ABCSG 8 CYP2D6 analyses, we extracted DNA not just from the tumor, but from “normal” tissue surrounding the tumor as well to increase the probability that DNA representative of the germline would be present. Furthermore, with regard to the CYP2D6 PCR assay, we avoided amplification above the standard range of 35–40 cycles, so as to avoid nonspecific amplification. Because the most common and important CYP2D6 allele (CYP2D6 *4) was within HWE, we have confidence in our ability to make conclusions from our data.

H&O What are the current recommendations for breast cancer patients with decreased CYP2D6 metabolism or who are poor metabolizers of CYP2D6?

MG The National Comprehensive Cancer Network (NCCN) guidelines recognize the importance of CYP2D6 enzyme activity, and recommend that patients should avoid potent CYP2D6 inhibitors while taking tamoxifen. However, these guidelines do not recommend that patients be tested for CYP2D6 genetic polymorphisms (the most important reason for altered CYP2D6 enzyme activity).

Our approach at Mayo has been to test postmenopausal women with invasive ER-positive breast cancer who are being considered for adjuvant tamoxifen. If a patient is identified to be a CYP2D6 poor metabolizer, we advocate either starting on an aromatase inhibitor or switching to an aromatase inhibitor if she is already on tamoxifen, since these medications are approved by the US Food and Drug Administration (FDA) and are not metabolized by CYP2D6. Patients who are at low risk for recurrence and who are CYP2D6 poor metabolizers may be able to be treated with tamoxifen, but the benefit of tamoxifen (compared to placebo) is unknown for CYP2D6 poor metabolizers.
It should be noted that several prospective clinical studies are ongoing in this area. First, the Eastern Cooperative Group trial E3108 is a prospective, phase II, ongoing trial led by Dr. Vered Stearns that is examining whether CYP2D6 affects progression-free survival in patients with metastatic breast cancer treated with single-agent tamoxifen. However, given that this study is focusing on women with endocrine-refractory breast cancer, this study will not be able to answer the question of whether CYP2D6 metabolism is important in patients with hormonally responsive ER positive breast cancer. Because there are no data regarding CYP2D6 in this setting, we are supporting this study at our institution and are enrolling patients. Another European study, CYPTAMBRUT-2 study (NCT00965939) is enrolling hormonally naïve patients in the first line metastatic/neoadjuvant setting. Importantly, patients with hormone insensitive or aromatase-inhibitor refractory disease were excluded from inclusion in this study.

While these prospective studies are critically important, we believe that the research community needs to rigorously re-examine the available pharmacogenetic, pharmacokinetic, and pharmacodynamic data regarding CYP2D6 and tamoxifen, with a focus on the methodology and the quality of the data, in order to come up with guidelines regarding testing for CYP2D6 in the adjuvant setting.

**H&O** What are some ongoing efforts in this field?

**MG** Based on the extensive preclinical and clinical findings regarding the importance of tamoxifen metabolism, endoxifen is being developed as a primary drug for the treatment of ER-positive breast cancer. A collaborative effort between the Mayo Clinic and NCI has been ongoing for several years including the production of clinical-grade endoxifen hydrochloride, preclinical toxicology/pharmacology studies. More recently, phase I studies at Mayo (for women with ER positive breast cancer) and at NCI (for patients with hormonally responsive cancer) opened in 2011, and phase II studies are expected to begin later in 2013. Other companies are working to develop different formulations of endoxifen.

**H&O** What are the biggest remaining challenges?

**MG** As someone who works on biomarkers, and develops breast cancer drugs, the incredible heterogeneity of the outcome of the CYP2D6 tamoxifen studies (where only one gene and a few variants are evaluated) makes me take pause, given that the field is rapidly moving to evaluate many millions of variants across the entire host and tumor genome. The purpose is to identify genetic alterations “in real time” in order to make treatment decisions on an “n=1” basis. While this is incredibly exciting, we must learn from our past mistakes, and carefully address quality control and work to validate these new approaches so that we can confidently prescribe the right drug at the right dose for all of our patients.

**Suggested Readings**


