1. Executive Summary

In 1977, tamoxifen was approved by the FDA for the treatment of metastatic breast cancer. Subsequently the drug received approval for adjuvant treatment of breast cancer, ductal carcinoma in situ, and for the reduction in breast cancer incidence in high risk women. Available evidence indicates that patients whose tumors are estrogen receptor (ER) positive are more likely to benefit from tamoxifen treatment compared to women who are not ER-positive. The recommended daily dose of tamoxifen is 20-40 mg.

Tamoxifen is a non-steroidal agent with potent anti-estrogenic effect in animal and in vitro models. This pharmacologic property is related to the drug's ability to compete with estrogen for estrogen receptors in breast tissues and to inhibit the stimulatory effect of estrogen for tumor growth.(1) Tamoxifen is metabolized by a number of cytochrome P-450 enzymes, such as, CYP2D6, CYP3A4, CYP2C9, CYP2C19, and CYP2B6 to active metabolites, N-desmethyl tamoxifen and 4-hydroxytamoxifen.(1) N-desmethyl tamoxifen is further metabolized to endoxifen by CYP2D6. Endoxifen has 100-fold greater affinity for the estrogen receptor and is 30-100 fold more potent than tamoxifen in suppressing estrogen-dependent cell proliferation. Endoxifen is considered an entity responsible for significant pharmacologic effect of tamoxifen. The enzyme CYP2D6 is a polymorphic enzyme with a number of variant alleles that are deficient or overactive in enzyme activity.   Patients with deficient alleles likely have lower exposure to endoxifen and have compromised clinical effect.

Recent publication in the Journal of Clinical Oncology by Goetz et al.(2) indicated that in tamoxifen-treated breast cancer patients, women with a variant allele of cytochrome P-450 2D6 enzyme, CYP2D6*4/*4 genotype, have a higher risk of disease relapse demonstrated by relapse-free time and disease-free survival. An updated analysis of the data presented at the Annual American Society of Clinical Oncology Meeting in June 2006 showed that the 2-year relapse-free survival was 68% in patients who are poor metabolizers of CYP2D6 and patients on strong inhibitors of CYP2D6 compared to 98% in patients who are extensive metabolizers of CYP2D6.(3)

The estimated new cases of breast cancer in women is 212,920 and the estimated death from breast cancer is approximately 40,970.(4) Two-thirds of the newly diagnosed breast cancer patients are ER positive and are candidates for hormonal therapy.   Tamoxifen is a likely choice of treatment either in the early stage or in the advanced stage for both pre- and post-menopausal women. Although tamoxifen has been approved for more than 25 years, recent understanding of the tamoxifen pharmacogenetics allows for optimizing risk-benefit of this therapy for patients with certain genetic disposition. The objective of this advisory committee meeting is to obtain the committee's recommendations on the following:

a) the scientific evidence demonstrates the role of CYP2D6 and endoxifen in tamoxifen treatment for breast cancer

b) the clinical evidence demonstrate that postmenopausal women who are CYP2D6 poor metabolizer are at increased risk for beast cancer recurrence following tamoxifen adjuvant treatment.

2. Background

Tamoxifen Indications

Tamoxifen (NOLVADEX) is a selective estrogen receptor modulator that is used for treatment and prevention of ER-positive breast cancer. According to the approved labeling, the antiestrogenic effects may be related to the ability of tamoxifen to compete with estrogen for binding sites in target tissues such as breast. There are many metabolites of tamoxifen, the pharmacologic activity of which have not been well characterized. N-desmethyl tamoxifen has been considered to be the major metabolite and has a biological activity similar to tamoxifen. 4-hydroxytamoxifen has been identified as a minor metabolite. In clinical use, there is variability in clinical efficacy as well as adverse effects, the most frequent being hot flashes.

The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) conducted overviews of systemic adjuvant therapy for early breast cancer in 1985, 1990, and in 1995. In 1998, 10-year outcome data were reported for 36,689 patients in 55 randomized trials using doses of 20-40 mg/day for 1 to 5+ years. Among women with ER-positive or unknown breast cancer and positive nodes who received about 5 years of treatment, overall survival at 10 years was 61.4% for tamoxifen compared to 50.5% for control. The recurrence-free rate was 59.7% for tamoxifen versus 44.5% for control. Among women with ER positive or unknown breast cancer and **negative** nodes who received about 5 years of treatment, overall survival at 10 years was 78.9% for tamoxifen compared to 73.3% for control. The recurrence-free rate was 79.2% for tamoxifen versus 64.3% for control. The control arm included no adjuvant therapy or chemotherapy without tamoxifen. A prospective, double-blind, randomized study (NSABP B-14) compared tamoxifen to placebo in women with axillary node-negative, ER positive breast cancer. After five years of treatment, there was a significant improvement in disease-free survival in women receiving tamoxifen. There are other clinical trials that established the effectiveness of tamoxifen therapy in adjuvant breast cancer.(1) The incidence of contralateral breast cancer is reduced in breast cancer patients receiving tamoxifen compared to placebo. About five-year tamoxifen treatment reduced the contralateral breast cancer rate from 7.6 per 1,000 patients in the control group compared with 3.9 per 1,000 patients in the tamoxifen group. The incidence of invasive breast cancer was reduced by 43% among patients with ductal carcinoma in situ (DCIS) receiving 5 years of tamoxifen therapy compared to placebo. The Breast Cancer Prevention Trial (BCPT) demonstrated that the incidence of invasive breast cancer was reduced by 44% among women who received tamoxifen compared with placebo.

Recent *in vitro* studies have identified endoxifen (4-hydroxy-N-desmethyl-tamoxifen) as a potent active metabolite of tamoxifen. The formation of endoxifen is mediated by

CYP2D6. Clinical studies suggest formation of endoxifen is reduced in patients who are CYP2D6 PMs or who are taking potent CYP2D6 inhibitors. A recently published clinical study suggested that post-menopausal ER-positive women who are CYP2D6 PMs taking endoxifen for adjuvant treatment of breast cancer have significantly reduced disease-free or relapse-free survival compared to EMs. This summary will review the *in vitro* and clinical studies that support that finding.

In Vitro Pharmacologic Activity of Endoxifen

In vitro studies suggest that the pharmacologic activity and potency of endoxifen and 4-hydroxy tamoxifen are similar,(5),(6) with approximately 100-fold greater affinity for estrogen receptors than does tamoxifen and 30-100 fold more potency than tamoxifen in suppressing estrogen-dependent cell proliferation. These data support a role for the desired pharmacologic activity of tamoxifen being attributed to these primary and secondary metabolites.

In Vitro Tamoxifen Metabolism

The primary and secondary routes of metabolism of tamoxifen have recently been evaluated *in vitro*, and CYP3A and CYP2D6 are identified as the major enzymes involved. (7) Endoxifen is a metabolite of 4-hydroxytamoxifen (mediated by CYP3A) and of N-desmethyltamoxifen (mediated by CYP2D6), and CYP2D6 is thought to be the principal route of formation of both 4-hydroxytamoxifen and endoxifen.
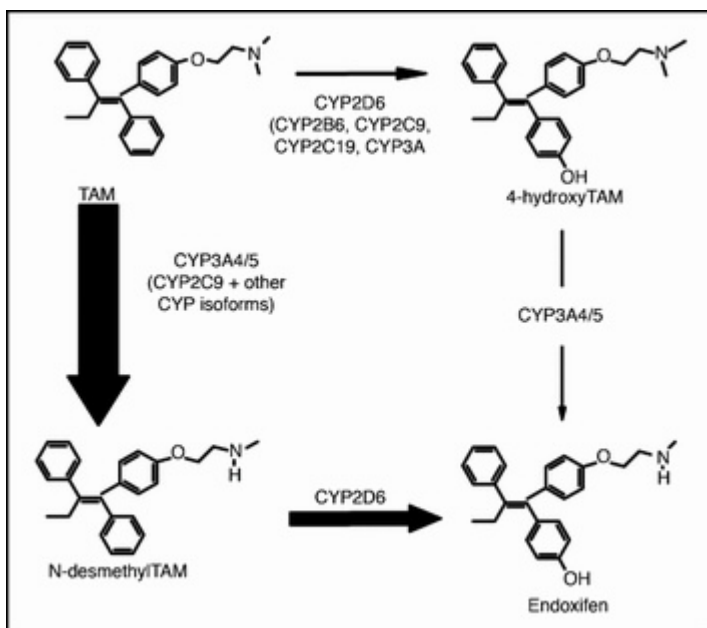
Fig 1.

Fig 1. Selected transformation pathways of tamoxifen and the main CYP enzymes involved. The relative contribution of each pathway to the overall oxidation of tamoxifen is shown by the thickness of the arrow, and the principal P450 isoforms responsible are

highlighted in larger fonts.
From: Goetz: J Clin Oncol, Volume 23(36).December 20, 2005.9312-9318

Exposure to Metabolites *In Vivo* and the Role of CYP2D6

Following chronic administration of tamoxifen, the mean plasma concentration of endoxifen is approximately 5-10 fold higher than that of 4-hydroxytamoxifen.(8), (9) Based on the plasma concentrations of endoxifen relative to 4-hydroxytamoxifen and its similar pharmacologic activity *in vitro*, endoxifen appears to be a relevant active metabolite.

The results of several clinical studies in patients taking tamoxifen suggest an important role for CYP2D6 in the formation of (and exposure to) endoxifen. This has been studied by examining the role of pharmacogenomics as well as the role of CYP2D6 inhibitors.

Stearns et al. evaluated tamoxifen and its metabolites in plasma of 12 women of known CYP2D6 genotype with breast cancer who were taking adjuvant tamoxifen before and after 4 weeks of coadministered paroxetine, a potent inhibitor of CYP2D6, in the absence of other potent CYP2D6 inhibitors or SSRIs.(9) CYP2D6 genotype was determined for *1 (wild type) or *4, *6, and * 8 (variant alleles). The 5 women who had a variant allele had lower baseline endoxifen concentrations than women who had the wild type genotype (p = 0.002). Mean paroxetine-mediated reduction of endoxifen concentrations in the women who were WT for CYP2D6 was 64% (95% CI = 39-89%) and was 24% (95% CI=23-71%) in the women with a variant allele (p=0.03).

Jin et al. evaluated the effects of concomitant use of SSRIs (in 24 subjects) as well as candidate gene genotypes (in 80 subjects) on plasma concentrations of tamoxifen and its metabolites.(8) Genes that were analyzed were CYP2D6, CYP2C9, CYP3A5, and SULT1A1. CYP2D6 was the only enzyme for which a statistically significant association was seen. For CYP2D6, genotype was not statistically significantly associated with mean plasma concentrations of tamoxifen, 4-hydroxtamoxifen, or N-desmethyltamoxifen. However subjects who were CYP2D6 *1/*3, *1/*4, *1/*5, or *1/*6 (grouped together as WT/Vt) had mean endoxifen concentrations that were 55% (95% CI=16.9-147.4%) of those of subjects were homozygous for WT CYP2D6. Subjects were *4/*4 had mean endoxifen concentrations that were 26% (95% CI = 1.0-636.6%) of WT subjects. This suggests a role for CYP2D6 in endoxifen exposure as well as a gene-dose effect. Borges et al similarly a CYP2D6 gene-dose effect on endoxifen plasma concentrations in a prospective trial in 158 breast cancer patients taking tamoxifen.(10)

Jin et al. (4) also reported that among subjects who were Wt/Wt, mean endoxifen concentration for those using CYP2D6 inhibitors was 58% lower than for those not using CYP2D6 inhibitors (difference -52.8 nM, 95% CI -86.1 to -19.5 nM, p = .0025). There was also a statistically significant difference in endoxifen concentrations in the presence of CYP2D6 inhibitors for the Wt/Vt group. The decrease was seen primarily with paroxetine. (The 3 subjects in the *4/*4 group were not taking CYP2D6 inhibitors). These results support the role for CYP2D6 in the formation of endoxifen.

Role of CYP2D6 in Efficacy of Tamoxifen Therapy for Breast Cancer

Goetz et al have evaluated the role of polymorphisms in CYP2D6 and in CYP3A5 in clinical outcomes of post-menopausal women receiving tamoxifen as adjuvant therapy in ER-positive breast cancer.(2)   The primary objectives were to determine the relationship between genotype and relapse-free (RF) time, disease-free survival (DFS), and overall survival (OS). A secondary objective was to determine whether incidence of hot flashes differed with genotype. The women were participants in the tamoxifen-only arm of the North Central Cancer Treatment Group (NCCTG) randomized Phase III clinical trial to assess the value of adding 1 year of fluoxymestrone to 5 years of tamoxifen adjuvant therapy. DNA was extracted from paraffin-embedded tumor blocks and from living women show supplied buccal samples. Samples were genotyped for CYP2D6*4 and CYP2D6*6 polymorphisms and for CYP3A5*3. Of the 256 women enrolled in the tamoxifen-only arm, 223 paraffin- blocks were available for DNA extraction. No CYP2D6*6 variants were detected.

CYP2D6 was amplified in 190 patients and there were 13 patients (6.8%) with *4/*4, 40 patients with wt/*4, and 137 patients with wt/wt/. CYP3A5*3 was amplified in 205 patients. No difference by CYP3A5*3 genotype (A/A vs A/G vs G/G) was found for RF-time, DFS, or OS. Women with CYP2D6 *4/*4 had significantly worse RF-time and DFS but not OS compared to women with normal alleles. Using a Cox model adjusted for nodal status and tumor size, women with the CYP2D6 *4/*4 genotype still tended to have worse RF-time and DFS.

Table 3. Unadjusted and Adjusted Hazard Ratios and Corresponding 95% CI and P Values Comparing Patients With the CYP2D6*4/*4 Genotype With the wt/wt or *4/wt Genotypes

	Unadjusted			Adjusted*		
	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P
Relapse-free time	2.71	1.15 to 6.41	.023	1.85	0.76 to 4.52	.176
Disease-free survival	2.44	1.22 to 4.90	.012	1.86	0.91 to 3.82	.089
Overall survival	1.73	0.79 to 3.76	.169	1.12	0.50 to 2.50	.780

NOTE. Hazard ratios for CYP2D6*4 *4/*4 relative to *4/wt and wt/wt are shown.
*A Cox model including nodal status and tumor size was used to estimate the adjusted hazard ratios.
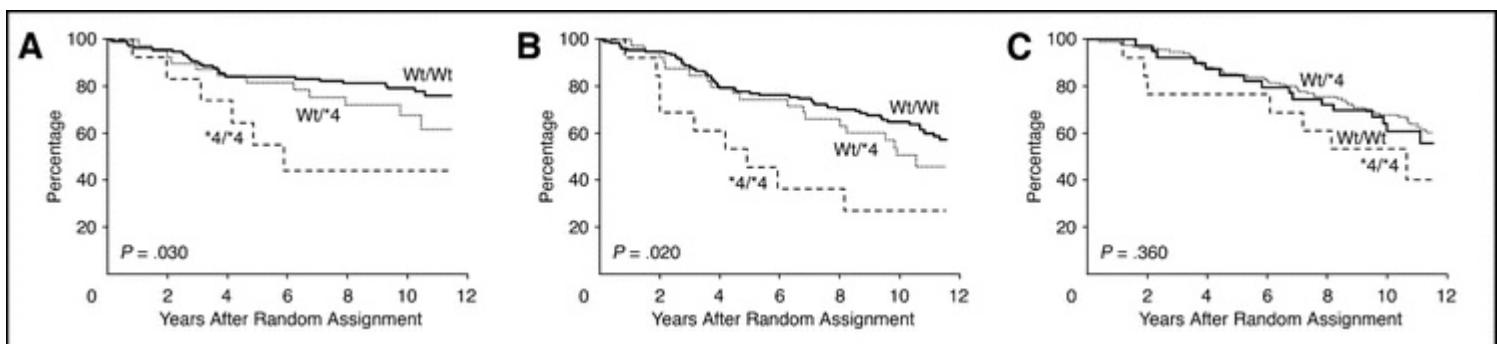

Fig 2. Kaplan-Meier estimates of (A) relapse-free time, (B) disease-free survival, and (C) overall survival for patients with the CYP2D6*4 genotype.
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The investigators of this study analyzed the data based on the metabolizer status. The poor metabolizer (PM) included patients who had CYP2D6*4/*4 genotype and patients on strong CYP2D6 inhibitors for a median duration of 2 to 3 years. The extensive metabolizers (EM) were patient with normal CYP2D6*1 alleles. The time to breast cancer recurrence and 2 year relapse free survival was significantly lower in PMs compared with EMs. A multivariate Cox regression model including tumor size, nodal status, and tumor grade showed a significant difference in the clinical outcome between the PMs and EMs.(3)

Metabolizer Status and Clinical Outcome			
Endpoints	EM (n = 115)	PM (n = 16)	p value
10 yrs Relapse Free Time	82%	50%	0.019
Relapse-free Survival	98%	68%	0.009

Multivariate Cox Model			
Endpoints	Tumor Size (< 3 vs. ≥ 3)	Node Positive (yes vs. no)	CYP2D6 PM (yes vs. no)
Relapse-Free Survival	1.75 (1.04 – 2.94)	1.61 (0.98 – 2.65)	2.37 (1.15 – 4.89)
Disease-Free Survival	2.01 (1.26 – 3.21)	1.66 (1.05 – 2.63)	2.12 (1.07 – 4.19)

A recent correspondence in the Journal of Clinical Oncology reported by Bonanni et al. mentioned that a subgroup, genetic analysis of the Italian Chemoprevention Trial is in concordance with the concept that CYP2D6 has an important role in the metabolic activation of tamoxifen and suggests that women with the CYP2D6*4/*4 genotype may be less likely to benefit from tamoxifen.(11) The study conducted CYP2D6 genotype in 46 breast cancer patients treated with tamoxifen and 136 age-matched control group of women free of cancer.

Table 1. Genotype and Allele Frequencies for *CYP2D6 (*4)*

CYP2D6	Breast Cancer Patients No.	Breast Cancer Patients %	Controls No.	Controls %	P
All women					.015*
wt/wt	30	65	97	71	
wt/*4	12	26	38	28	
*4/*4	4	9	1	1	
Placebo					.27*
wt/wt	17	65	50	70	
wt/*4	8	31	21	30	
*4/*4	1	4	0	0	
Tamoxifen					.04*
wt/wt	13	65	47	72	
wt/*4	4	20	17	26	
*4/*4	3	15	1	2	

Abbreviation: wt, wild type.
*wt/wt or wt/*4 v *4/*4.

Nowell et al. studied the association of the genetic variation in CYP2D6, UGT2B15, and SULT1A1 enzymes with overall survival and recurrence of disease in breast cancer patients.(12) The study population comprised of a total of 162 patients who received tamoxifen and 175 who did not receive hormonal therapy for adjuvant treatment at the

Arkansas Cancer Research Center. The study didn't find any difference in the overall survival and the progression-free survival between patients with CYP2D6 genotype (CYP2D6*4/*4 plus wild type) and patients with wild type alleles. The study indicated that CYP2D6 genotype was not a prognostic factor related to the overall survival or the disease-free survival since genotype had no differential effect on the patients on placebo. The authors reported a non-significant trend towards increased recurrence of disease with increasing numbers of UGT2B15*2 allele (HR = 1.56, 95% CI 0.68-3.61 for UGT2B15*1/*2 and HR=2.27, 95% CI 0.82-6.28 for UGT2B15*2/*2, ptrend = 0.11).

Table 2. Overall and progression-free survival of breast cancer patients according to CYP2D6 Genotype

Genotype	Cases	Deaths	Person-years	Deaths/person-years	HR[a](95% CI)
Overall survival					
Tamoxifen	162	34			
Cyp2d6 wt/wt	114	27	593	0.046	1 (Ref)
Cyp2d6 *4/*4 + *4/wt	48	7	253	0.028	0.77 (0.32–1.81)
					ptrend = 0.51
No tamoxifen	175	66			
Cyp2d6 wt/wt	126	48	596	0.080	1 (Ref)
Cyp2d6 *4/*4 + *4/wt	49	18	286	0.063	0.79 (0.42–1.26)
					ptrend = 0.26
Progression-free survival[b]					
Tamoxifen	160	48			
Cyp2d6 wt/wt	112	38	524	0.073	1
Cyp2d6 *4/*4 + *4/wt	48	10	524	0.019	0.67 (0.33–1.35)
					ptrend = 0.19
No tamoxifen	166	71			
Cyp2d6 wt/wt	120	53	480	0.110	1
Cyp2d6 *4/*4 + *4/wt	46	18	240	0.075	0.69 (0.40–1.18)
					ptrend = 0.19

[a]HRs for fully adjusted model: age, stage with node status at diagnosis, race, ER status, and PR status.
[b]Eleven subjects were excluded because they were never disease-free.

Fritz et al. conducted a retrospective exploratory study to assess the relationship between microsomal epoxide hydrolase expression (mEH) and tamoxifen response in primary breast cancer.(13) The archival paraffin blocks of primary breast cancers from 179 patients (78 patients treated with tamoxifen and 101 patients not treated with tamoxifen) were assessed for mEH expression by immunohistochemistry. Expression of mEH correlated with poor disease outcome in all patients (p < 0.01; n = 179) and in patients receiving tamoxifen (p <0.01; n = 78) but not in patients not treated with tamoxifen. The authors concluded that mEH expression in primary breast cancer could be of predictive value for response to tamoxifen treatment and may be a novel independent prognostic factor for survival.

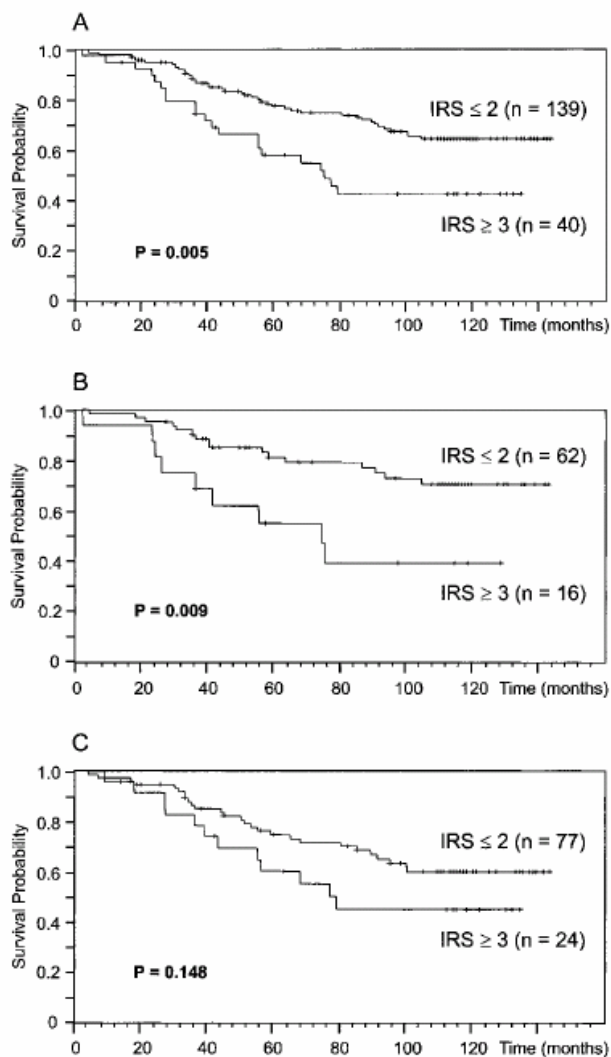In the figure below IRS is defined as immunoreactive score, a semiquantitative way of assessing mEH expression.

Fig 2. Relationship between mEH expression and overall survival. The Kaplan-Meier survival curves shown are for subgroups with low (immuno-reactive score index IRS ≤ 2) and high (IRS ≥ 3) mEH expression among (A) all patients under study (n = 179), (B) patients treated with tamoxifen (n = 78), and (C) patients not treated with tamoxifen (n = 101).

3. Conclusions

1. Endoxifen is a potent active metabolite of tamoxifen with steady state exposure 5-10 fold greater than the active metabolite, 4-hydroxytamoxifen.

2. *In vitro* studies demonstrate the primary role of CYP2D6 in the formation of endoxifen.

3. Patients taking potent CYP2D6 inhibitors such as paroxetine or fluoxetine with tamoxifen have a significant reduction in plasma concentrations of endoxifen in comparison to exposure after taking tamoxifen alone.

4. CYP2D6 genotype is significantly associated with steady state endoxifen plasma concentrations, and there is a gene-dose effect.

5. Post-menopausal ER-positive breast cancer patients taking tamoxifen as adjuvant therapy who are CYP2D6 *4/*4 (and therefore lack functional CYP2D6) and patients who are on potent CYP2D6 inhibitors have significantly decreased relapse-free survival and disease-free survival compared to patients who are CYP2D6 wt/*4 or CYP2D6 wt/wt.

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