Steroid Sulfatase: A New Target for the Endocrine Therapy of Breast Cancer

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:

1. Discuss the role of steroid sulfatase in regulating estrogen production in postmenopausal women.
2. Describe the potential of steroid sulfatase inhibition in cancer therapy.
3. Discuss a potential new endocrine therapy for patients progressing on aromatase.

ABSTRACT
Inhibitors of steroid sulfatase are being developed as a novel therapy for hormone-dependent breast cancer in postmenopausal women. Data suggest that steroid sulfatase (STS) activity is much higher than aromatase activity in breast tumors and high levels of STS mRNA expression in tumors are associated with a poor prognosis. STS hydrolyzes steroid sulfates, such as estrone sulfate and dehydroepiandrosterone sulfate (DHEAS), to estrone and DHEA, which can be converted to steroids with potent estrogenic properties, that is, estradiol and androstenediol, respectively. Several potent irreversible STS inhibitors have now been identified, including STX64 (BN83495), a tricyclic sulfamate ester. This drug recently completed the first-ever trial of this new type of therapy in postmenopausal women with estrogen receptor–positive metastatic breast cancer. STX64, tested at 5-mg and 20-mg doses, was able to almost completely block STS activity in peripheral blood lymphocytes and tumor tissues. Inhibition of STS activity was associated with significant reductions in serum concentrations of androstenediol and estrogens. Unexpectedly, serum androstenedione concentrations also decreased by up to 86%, showing that this steroid, which is the main substrate for the aromatase in postmenopausal women, is derived mainly from the peripheral conversion of DHEAS. Of eight patients who completed therapy, five showed evidence of stable disease for up to 7.0 months. This new endocrine therapy offers considerable potential for the treatment of hormone-dependent breast cancer in postmenopausal women. The Oncologist 2007;12:370–374

Disclosure of potential conflicts of interest is found at the end of this article.

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INTRODUCTION
Breast cancer continues to be a major cause of death in European and American women, occurring most frequently in postmenopausal women. In this group of women, the production of estrogens, which have a crucial role in supporting the development and growth of breast tumors, is greatly reduced following the cessation of ovarian function. However, estrogens continue to be produced at a low level in postmenopausal women, mainly from the peripheral conversion of androstenedione to estrone. This reaction is mediated by the aromatase complex and takes place mainly in adipose tissue but also in normal and malignant breast tissues [1, 2]. In view of the important role that estrogens have in breast cancer, current therapies in postmenopausal women aim to either block its interaction with the estrogen receptor (ER), by use of an antiestrogen such as tamoxifen or an ER downregulator, or inhibit the conversion of androstenedione to estrone with an aromatase inhibitor (e.g., letrozole, anastrozole, exemestane) [3, 4]. Over the last decade, considerable research has been carried out into the synthesis of steroids with estrogenic properties, which has suggested that, in addition to aromatase, another enzyme, steroid sulfatase (STS), may also have a crucial role in regulating the synthesis of estrogenic steroids in breast cancer [5]. Potent STS inhibitors have now been developed, paving the way to use this new type of therapy for breast cancer.

STS
STS is the enzyme responsible for the hydrolysis of steroid sulfates to their unconjugated, biologically active forms. After the synthesis of estrone from androstenedione by aromatase, much of it is rapidly sulfated to estrone sulfate (E1S) by a number of sulfotransferases that are widely distributed throughout the body [6]. Plasma concentrations of E1S are 10–20 times higher than those of the unconjugated estrogens, estrone and estradiol [7]. Furthermore, the half-life of E1S in plasma (10–12 hours) is considerably longer than that for estrone and estradiol (20–30 minutes). E1S is therefore thought to act as a reservoir for the formation of active estrogens via the action of STS [8]. This enzyme converts E1S to estrone that is then reduced to the biologically active estrogen, estradiol, by 17β-HSD1, which is overexpressed in many breast tumors (Fig. 1).

STS also acts on other steroid sulfates, including dehydroepiandrosterone sulfate (DHEAS), which is secreted in large amounts (up to 20 mg per day) by the adrenal cortex [9]. STS converts DHEAS to DHEA, which can be reduced to androstenediol by 17β-HSD1. Androstenediol, although an androgen, can bind to the ER and stimulate the growth of hormone-dependent breast cancer cells in vitro and carcinogen-induced mammary tumors in rodents [10, 11]. Although its affinity for the ER is lower than that of estradiol, it has been suggested that, because of its much higher plasma and tissue concentrations, it may be equipotent to estradiol in postmenopausal women [12].

STS IN BREAST TUMORS
Research carried out some years ago initially revealed that STS activity was detectable in most breast tumors examined. STS activity was found to be present at a considerably higher level than aromatase and aromatase activity was only detectable in about 60% of tumors [13, 14]. Recently STS mRNA expression has been measured in breast tumors and shown to be at a much higher level than aromatase mRNA expression [15]. Such studies also revealed that STS mRNA expression is higher in malignant than in normal breast tissue. Several studies have suggested that high levels of STS mRNA expression in breast tumors are associated with a poor prognosis, whereas aromatase or 17β-HSD1 expression had no prognostic value [16, 17]. Thus, with the advent of potent STS inhibitors, the measurement of breast tumor STS mRNA expression may allow a patient enrichment strategy to be adopted to target patients who may particularly benefit from this therapy.

STS INHIBITORS IN PROSTATE AND ENDOMETRIAL CANCERS
Although STS inhibitors were primarily developed for breast cancer therapy, they may also have a therapeutic role
in other hormone-dependent cancers, such as those occurring in the prostate and endometrium. STS activity is present in prostatic tissue and in LNCaP cells, which were derived from prostatic cancer tissue [18, 19]. In men with prostate cancer, castration results in only a 50% reduction in prostatic tissue concentrations of dihydrotestosterone [20]. It is thought that the remaining contribution originates from adrenal androgens, such as DHEAS, by an intracrine process within the prostate. Furthermore, therapies that aim to reduce prostatic testosterone concentrations (e.g., luteinizing hormone–releasing hormone agonists) do not reduce androstenediol concentrations. This androgen can activate a mutated form of the androgen receptor that is found in LNCaP cells and in many prostatic tumors [21]. Thus, inhibiting STS activity in men with prostate cancer may reduce not only the production of testosterone from DHEAS but also androstenediol. STS activity is increased in endometrial cancer tissue, so its inhibition may also have therapeutic potential, by reducing estrogen synthesis, in this hormone-dependent cancer [22].

STS INHIBITORS
Several potent irreversible STS inhibitors have now been identified, all of which have as their active pharmacophore an aryl ring to which a sulfamate ester is attached. The structure of the first-generation STS inhibitor STX64 (BN83495, also known as 667 Coumate) is shown in Figure 2. STX64 was shown to be a potent STS inhibitor in rodents and blocked the ability of E1S to stimulate the growth of carcinogen-induced mammary tumors in ovariectomized rats [23]. Second-generation STS inhibitors, such as the steroid-based STX213 (Fig. 2), have now been developed, which inhibit STS activity in rodents, after the administration of a single dose, for a much longer period of time than STX64 [24, 25]. These STS inhibitors are orally active with a high level of bioavailability. This results from their binding to carbonic anhydrase II in erythrocytes after absorption, which enables them to transit the liver without undergoing first-pass inactivation [26].

CLINICAL USE OF STX64
The first-ever phase I trial of an STS inhibitor in postmenopausal women with locally advanced or metastatic breast cancer was recently reported by our group [27]. All the patients in the trial had been heavily pretreated with endocrine therapies (antiestrogen and/or aromatase inhibitors) while some had also received chemo- or radiotherapy. The primary endpoint of the study was to determine the dose of STX64 that inhibited STS activity by >90% in peripheral blood lymphocytes (PBLs), which have a high level of STS activity. Secondary endpoints included an examination of the ability of the drug to inhibit STS in tumor samples obtained from some patients and its effect on serum androstenediol and estrogen concentrations.

The drug was administered orally, with patients receiving an initial dose (cycle 0) followed by 3 × 2 weekly cycles (cycles 1–3), with each cycle consisting of daily dosing for 5 days followed by 9 days off treatment. Patients in whom disease was stable at the end of cycle 3 were eligible for extended dosing. The drug was well tolerated, with only a few minor adverse events recorded. At a dose of 5 mg, >90% inhibition of STS activity in PBLs was achieved, and at the higher dose tested, 20 mg, almost complete inhibition of STS activity in PBLs and tumor tissue was detected (Fig. 3). Inhibition of STS activity was associated with significant reductions in serum androstenediol and estrogen concentrations. Unexpectedly, serum concentrations of androstenedione, the main substrate for aromatase in postmenopausal women, also decreased by up to 86%. This finding indicates that, in this group of women, androstenedione is derived mainly from the peripheral conversion of DHEAS and not, as previously thought, by direct secretion from the adrenal cortex. Of eight patients who completed cycles 0–3 of treatment, five showed evidence of stable disease for up to 7.0 months (range 2.5–7.0 months) by the Response Evaluation Criteria in Solid Tumors. Importantly, all of the patients showing evidence of stable disease had previously been treated with, and progressed on, aromatase inhibitor therapy. The therapy was well tolerated, although all patients experienced some taste disturbance that was thought to be related to the formulation of the drug.

SUMMARY
Research into the pathophysiological role of STS in breast cancer led to the development of potent STS inhibitors, one of which has now been tested in a phase I trial of postmeno-
pausal women with metastatic breast cancer. Results from that trial showed that STX64 almost completely blocked STS activity in PBLs and tumor tissues. Inhibition of STS activity was associated with significant decreases in not only serum concentrations of estrogens but also that of androstenediol, a steroid with potent estrogenic properties. Production of androstenediol is not blocked by aromatase inhibitors. Interestingly, this study also revealed that the main aromatase substrate in postmenopausal women, androstenedione, is derived mainly from DHEAS, rather than through direct secretion from the adrenal cortex.

Although all patients had been pretreated with endocrine agents, and in some cases chemotherapy, five of the eight patients who completed cycles 0–3 of their treatment showed evidence of stable disease. These encouraging results will be followed by further trials in women with breast cancer to define the optimal biological dosing schedule. A patient enrichment strategy will also be employed taking advantage of measurements of STS mRNA expression in tumors to select patients most likely to benefit from this new type of therapy. It will be particularly important to confirm that STS inhibitors can offer therapeutic benefit in patients who have progressed on antiestrogen and/or aromatase inhibitor therapies. It is anticipated that inhibition of STS activity will give rise to a significant clinical benefit and a favorable risk/benefit ratio in the setting of locally advanced or metastatic disease by offering a potential new additional option of endocrine therapy, initially in patients whose disease has progressed after prior aromatase inhibitor therapy. As this drug effectively blocks the production of androgens in addition to estrogens, it is also likely to be tested in other hormone-dependent tumors, such as prostate and endometrial cancer.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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