

Phase I Study of STX 64 (667 Coumate) in Breast Cancer Patients: The First Study of a Steroid Sulfatase Inhibitor

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Abstract Purpose: Inhibition of steroid sulfatase (STS), the enzyme responsible for the hydrolysis of steroid sulfates, represents a potential novel treatment for postmenopausal women with hormone-dependent breast cancer. Estrone and DHEA are formed by this sulfatase pathway and can be converted to steroids (estradiol and androstenediol, respectively), which have potent estrogenic properties.

Experimental Design: STX64 (667 Coumate), a tricyclic coumarin-based sulfamate that irreversibly inhibits STS activity, was selected for entry into the first phase I trial of a STS inhibitor in postmenopausal women with breast cancer. STX64 was administered orally (nine patients at 5 mg and five patients at 20 mg) as an initial dose followed 1 week later by 3 × 2 weekly cycles, with each cycle comprising daily dosing for 5 days followed by 9 days off treatment. Blood and tumor tissue samples were collected for the assessment of STS activity and serum was obtained for steroid hormone measurements before and after treatment.

Results: The median inhibition of STS activity by STX64 was 98% in peripheral blood lymphocytes (PBL) and 99% in breast tumor tissue at the end of the 5-day dosing period. As expected, serum concentrations of estrone, estradiol, androstenediol, and DHEA all decreased significantly from pretreatment levels. Unexpectedly, androstenedione and testosterone concentrations also decreased. Four patients, all of whom had previously progressed on aromatase inhibitors, showed evidence of stable disease for 2.75 to 7 months. The drug was well tolerated with only minor drug-related adverse events recorded.

Conclusion: STX64 is a potent, well-tolerated STS inhibitor. It inhibits STS activity in PBLs and tumor tissues and causes significant decreases in serum concentrations of steroids with estrogenic properties.

The realization that the steroid sulfatase (STS) enzyme has an important role in regulating the formation of steroids with estrogenic properties stimulated research to develop potent STS inhibitors for breast cancer therapy (1–3). In postmenopausal

women, in whom the incidence of breast cancer is highest, estrogens are produced almost exclusively by the peripheral conversion of androstenedione to estrone (E₁) by the aromatase enzyme complex (Fig. 1). Current therapies for hormone-dependent breast cancer in postmenopausal women include antiestrogens, which block the action of estrogens, or aromatase inhibitors that inhibit the conversion of androstenedione to E₁ (4, 5). The development of these drugs has led to major advances in the treatment of breast cancer. Aromatase inhibitors have recently been shown to have increased efficacy compared with tamoxifen in increasing disease-free survival and reducing the incidence of contralateral breast cancer (6, 7). However, the use of aromatase inhibitors has, thus far, not resulted in any significant increase in overall survival compared with that achieved with tamoxifen. Unfortunately, in spite of these advances, breast cancer in many women will progress, although their tumors still possess estrogen receptors (ER) on relapse (8).

Inhibition of STS represents a novel approach to block the formation of steroids with potent estrogenic properties. Much of the E₁ formed from androstenedione by aromatase is converted to E₁ sulfate (E₁S) by sulfotransferases (9). Plasma concentrations of estrogen sulfates are much higher than for unconjugated estrogens and, furthermore, their half-life in blood (10–12 hours) is considerably longer than for unconjugated estrogens (20–30 minutes; refs. 10, 11). Thus,

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Received 9/13/05; revised 11/21/05; accepted 12/19/05.

Grant support: Sterix Ltd. (a member of the Ipsen Group) and Cancer Research UK (R.C. Coombes).

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doi:10.1158/1078-0432.CCR-05-1996

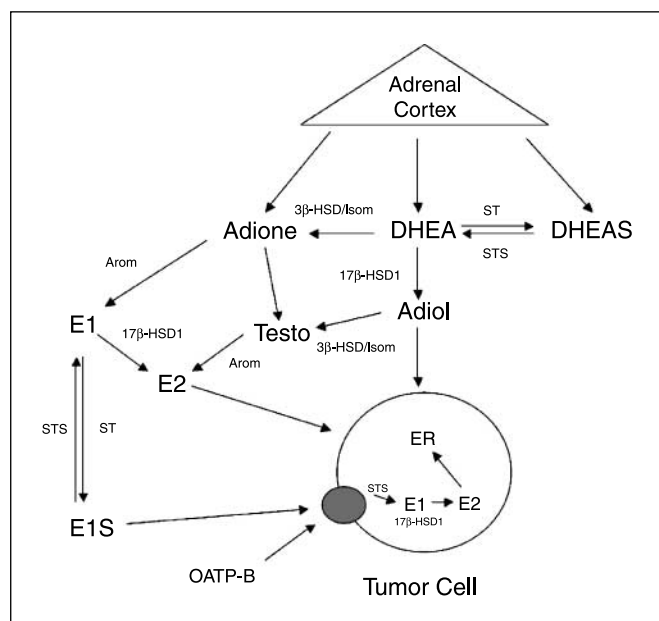


Fig. 1. Origin of steroids with estrogenic properties in postmenopausal women. DHEAS and androstenedione (*Adione*) can be secreted by the adrenal cortex. Androstenedione is the substrate for the synthesis of E_1 by aromatase (*Arom*), which can be reduced to E_2 by 17β -hydroxysteroid dehydrogenase type 1 (17β -HSD1). DHEA can be reduced to androstenediol (*Adiol*) by 17β -hydroxysteroid dehydrogenase type 1, which can give rise to testosterone (*Testo*); the latter steroid is also formed from androstenedione. Much of the E_1 formed from androstenedione can be converted to E_1S by steroid sulfotransferases (*ST*), but can be reactivated by STS. E_1S can also be taken up directly into breast tissues by organic anion transporter polypeptide B (*OATP-B*), where it can be converted to a biologically active estrogen. Both E_2 and androstenediol can bind to and activate the ER. The conversion of DHEA to androstenedione and androstenediol to testosterone is mediated by 3β -hydroxysteroid dehydrogenase/isomerase.

estrogen sulfates may act as a reservoir for the formation of estrogens via the action of STS (12). There is now convincing evidence that the uptake of E_1S , an organic anion, into malignant breast tissues is facilitated by a specific organic anion transporter, organic anion transporter polypeptide B (13). As high STS activity is detected in most breast tumors, the uptake of E_1S by organic anion transporter polypeptide B into breast tumors, where it is hydrolyzed, may account for the high concentrations of estrogens found in these tumors (14).

Expression of STS mRNA in malignant breast tissue is much higher than in the normal breast and also considerably higher than aromatase mRNA expression (15). Three studies have now confirmed that expression of STS mRNA in ER-positive breast tumors is an independent prognostic indicator in predicting relapse-free survival, with higher levels of expression being associated with a poor prognosis (16–18). This contrasts with measurements of aromatase mRNA expression in breast tumors, which is of no prognostic value (17). Although STS mRNA expression is detected in most breast tumors, only 75% of tumors from postmenopausal women express aromatase mRNA (18). The higher levels of STS mRNA expression than aromatase mRNA expression suggest that the STS pathway may be more important than the aromatase route for intratumor estrogen synthesis, as previously indicated from activity studies (19). STS is located within malignant breast epithelial cells and STS immunoreactivity is correlated with

tumor size (20). Thus, inhibition of STS activity, especially in patients expressing a high level of STS mRNA, could have therapeutic potential.

A further impetus to the development of STS inhibitors was the knowledge that the production of another steroid with potent estrogenic properties, androstenediol, might be blocked by a STS inhibitor. Androstenediol, although an androgen, can bind to the ER and stimulate the *in vitro* growth of ER-positive breast cancer cells or carcinogen-induced mammary tumors in rodents (21, 22). Most androstenediol in postmenopausal women originates from DHEA after its formation from DHEA sulfate (DHEAS; ref. 23). As there is only one STS, which hydrolyses both aryl (E_1S) and alkyl (DHEAS) steroid sulfates, inhibition of STS should lead to a reduction in both E_1 and androstenediol production (24).

Evidence to support an important role for DHEAS as a substrate for androstenediol was recently obtained when it was shown that its ability to stimulate the proliferation of MCF-7 (ER+) breast cancer cells could be blocked with an antiestrogen or STS inhibitor, but not an aromatase inhibitor (25). Clinical evidence for DHEAS in having a role in supporting the growth of breast tumors in postmenopausal women has originated from a study in which serum concentrations of this steroid were measured in women receiving third-generation aromatase inhibitor therapy (26). Although estrogen concentrations were below the limit of assay detection in all patients, those progressing while receiving aromatase inhibitor therapy were found to have significantly higher serum DHEAS concentrations than those with stable disease. As aromatase inhibitors do not block androstenediol formation, this finding suggests that its production from DHEAS, while patients are receiving aromatase inhibitor therapy, may have a role in promoting tumor progression.

Research carried out over the last decade has identified a number of potent STS inhibitors most of which have an aryl sulfamate ester as their active pharmacophore (1, 27). STX64 is a tricyclic coumarin sulfamate (Fig. 2A) that was designed as an orally active, nonsteroid-based, irreversible STS inhibitor (28).

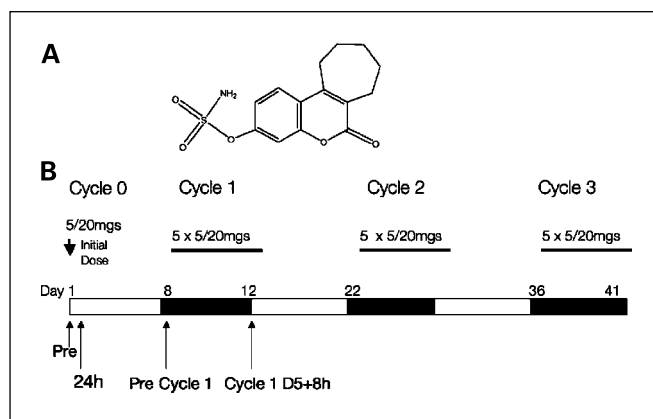


Fig. 2. A, structure of STX64 (667 Coumate). B, phase I trial dosing schedule. Patients received an initial 5 or 20 mg dose of STX64 followed by 3 × 2 weekly cycles with daily dosing for 5 days followed by 9 days off therapy. Arrows, times of blood sample collection to monitor STS inhibition in PBLs and steroid concentrations. Pre, before treatment; 24h, twenty-four hours after initial dose; Pre Cycle 1, before start of cycle 1; D5+8h, 8 hours after the administration of the dose on day 5 of the first cycle.

STX64 causes regression of E₁S-stimulated, nitrosomethylurea-induced mammary tumors in ovariectomized rats (29). STX64, in common with other aryl sulfamates, is transported in erythrocytes by binding reversibly to carbonic anhydrase II (30). After oral administration, it undergoes hepatic transit in erythrocytes without undergoing first-pass metabolism. Its oral bioavailability in rats is >90% (31). STX64 had an acceptable toxicologic profile and was selected as the first STS inhibitor to be tested in postmenopausal women with breast cancer to inhibit the production of steroids with estrogenic properties that are formed by the sulfatase pathway.

Patients and Methods

Patients were eligible for this study if they were postmenopausal, were over 18 years old, had been amenorrheic for at least 12 months (or had a previous ovariectomy), and had histologically diagnosed ER-positive breast cancer that was either locally advanced or metastatic. All patients had to have previously received at least one form of systemic treatment, either endocrine therapy or chemotherapy for breast cancer. Demographic data are summarized in Table 1. The patients had a predicted life expectancy of at least 3 months and a WHO performance status of 0 to 2. All patients had adequate hematologic function (neutrophils $>1.5 \times 10^9/L$, hemoglobin >9.0 g/dL, platelets $>100 \times 10^9/L$, and partial thromboplastin time and prothrombin time $<1.5 \times$ upper limit of normal), renal function (calculated creatinine clearance >50 mL/min, with the exception of one patient), and liver function (serum bilirubin $<1.5 \times$ upper limit of normal, alanine aminotransferase and/or aspartate aminotransferase $<2.5 \times$ upper limit of normal, unless due to tumor in which case up to $5 \times$ upper limit of normal was permissible). Exclusion criteria included radiotherapy, endocrine therapy, immunotherapy, chemotherapy, or major surgery in the 4 weeks before treatment with STX 64, current extensive nonmalignant skin disease, any continuing toxic manifestations of previous treatment, except alopecia or grade 1 peripheral neuropathy. Patients at high risk from nonmalignant systemic disease or positive for hepatitis B, hepatitis C, or HIV, or receiving concurrent treatment with warfarin, hormone replacement therapy, corticosteroids, or bisphosphonates were also excluded.

Trial design and study protocol. A two-center, single-arm, open-label, dose-escalation phase I study was designed. The protocol was approved by the ethics committees of Charing Cross Hospital, London and Belfast City Hospital, Belfast, where the study was done, as well as the Cancer Research UK internal ethics review committee. Patients gave written informed consent in accordance with the Declaration of Helsinki, and the trial was conducted to ICH Good Clinical Practice guidelines monitored by Cancer Research UK. Laboratory studies were conducted according to Good Laboratory Practice guidelines and the advice of a Good Laboratory Practice advisor. STX64 was administered to nine patients at the 5 mg dose and five patients at the 20 mg dose, which was given orally in 7.5% v/v cyclodextrin freeze-dried powder, reconstituted in water before administration. STX64 was given as an initial dose (cycle 0) followed 1 week later by 3×2 weekly cycles (cycles 1-3) with each cycle consisting of daily dosing for 5 days followed by 9 days off treatment (Fig. 2B). Patients were kept in hospital overnight for monitoring after the initial dose. A patient was considered evaluable for response if she had received four cycles of treatment (cycles 0-3) but was evaluable for toxicity if more than one cycle was completed. It was intended to continue treatment for 4 cycles until study completion, disease progression, unacceptable toxic effects, or withdrawal of consent. Patients whose disease was stable at the end of cycle 3 were considered for extended dosing.

The primary end points of the study were to assess the dose of STX 64 needed for inhibition of STS activity in PBLs $>90\%$ at the cycle 1 day 5 + 8 hours time point (Fig. 2B), and to assess its tolerability profile. The

Table 1. Patient characteristics

Characteristics	5 mg dose (n = 9)	20 mg dose (n = 5)
Age (y): median (range)	65 (51-82)	53 (38-67)
WHO performance status		
0	1	3
1	5	2
2	3	0
Disease-free interval		
Median (range)	5 y 3 mo (0-11 y 8 mo)	3 y 1 mo (2 y 3 mo-8 y 6 mo)
Target lesions at study entry		
Liver metastases	6	0
Metastatic nodes	2	2
Other metastases	3	0
Local recurrence	1	2
Lung metastases	1	1
Regional nodes	1	1
Skin metastases	2	0
Primary tumor	1	0
Previous endocrine therapy		
Tamoxifen	9	5
Anastrozole	8	5
Exemestane	4	0
Formestane	2	1
Rogletimide	1	0
Fulvestrant	3	0
Progestogens	2	0
Number of prior chemotherapy regimens		
Median (range)	2 (1-6)	2 (2-3)

secondary end points included pharmacokinetic analyses (to be reported in full elsewhere), measurement of serum steroid concentrations following STX 64 administration, determination of the dose of STX 64 needed to inhibit intratumoral STS, and establishing if there was any antitumor activity.

Full clinical assessments were done before treatment start, every 2 weeks during treatment, and at the end of the trial. In patients with radiologically assessable disease, radiology was done before treatment start and at the end of the four cycles of treatment. For patients who received extended dosing, radiological assessments were done every 3 months. All radiology was reported according to Response Evaluation Criteria in Solid Tumors (32). Adverse events were recorded daily in diaries and graded using the National Cancer Institute Common Toxicity Criteria Version 2.0.

STS activity and serum steroid concentration measurements. Blood samples were collected for the assessment of STS activity in peripheral blood lymphocytes (PBL) as a biomarker to monitor the extent and duration of STS inhibition, using CPT vacutainers (Becton Dickinson, Franklin Lakes, NJ). Blood was also collected for the measurement of serum steroid concentrations, with samples for assays being collected before treatment, 24 hours after the initial dose, before the start of cycle 1, and 8 hours after the administration of the dose on day 5 of the first cycle (Fig. 2B). These time points for blood sample collection were selected as the optimal times to monitor the effects of STX64 on STS activity and serum steroid concentrations. Blood samples were also taken for pharmacokinetic measurements, with further samples also being taken during cycles 2 and 3 to monitor patient safety.

Core biopsies (2.5-30 mg) from primary or metastatic lesions were also obtained from four patients at the 5 mg dose and three patients at

the 20 mg dose before the initial dose and 8 hours after the administration of the first cycle to monitor the extent of inhibition within tumors.

PBLs isolated from 8 mL blood were used to measure STS activity for monitoring the extent and duration of enzyme inhibition. After centrifugation ($1,500 \times g$ at 22°C for 30 minutes), isolated PBLs were washed with PBS ($5 \text{ mL} \times 2$) to yield a final volume of 1.2 mL and stored at -20°C . The STS was solubilized before assaying, using Triton X-100/PBS (0.2%). Aliquots of the solubilized enzyme were used for the measurement of STS activity and also for determination of the protein concentration. STS activity of the solubilized enzyme from PBLs was assayed using a physiologic substrate concentration of $[6,7\text{-}^3\text{H}]\text{E}_1\text{S}$ (2-3 nmol/L, 46-57 Ci/mmol, Perkin-Elmer Life Sciences, Wellesley, MA) over a 20-hour period, using $[4\text{-}^{14}\text{C}]\text{E}_1$ to monitor procedural losses (33). For some patients, STS activity was also measured in core-biopsy samples taken from the primary or metastatic lesions. For this, tissue was transported on solid carbon dioxide and stored at -20°C until assayed. Samples were homogenized in PBS sucrose (0.25 mol/L)/Triton X-100 (0.2%) to yield a supernatant containing solubilized enzyme after centrifugation (Eppendorf, 4,000 rpm, 5 minutes). Aliquots of the supernatant were used for the assay of STS activity as described for PBLs. The assay of STS activity was linear over 2 to 24 hours and 9 to 12 μg protein. Intraassay coefficient of variations were $<15\%$. The Bradford protein assay (34) was used to measure protein concentrations in PBL and tumor preparations, with STS activity results being expressed as fmol $\text{E}_1/\mu\text{g}$ protein/20 hours.

Steroid concentrations. Concentrations of DHEAS and DHEA were assayed using kits obtained from DSL (Webster, TX) according to the instructions of the manufacturer. Androstenediol concentrations were measured by RIA after organic solvent extraction and Celite column partition chromatography (35). Concentrations of E_1 , estradiol (E_2), androstenedione, testosterone, and E_1S were measured using a gas chromatographic/tandem mass spectrometric assay by SFBC Taylor (Princeton, NJ; refs. 36, 37). The limits of quantitation for E_1 and E_2 were 6 and 2.3 pmol/L, respectively. Intraassay and interassay coefficients of variation for these assays were $<15\%$.

Statistical analysis. Results were analyzed using two statistical software packages: SPSS version 12 (SPSS, Inc., Chicago, IL) and SAS version 8.02 (SAS Institute, Cary, NC). Nonparametric methods of analysis were used to accommodate the skewed distributions and the outlier values in analyte levels found for some patients. Descriptive statistics are reported as medians and interquartile ranges. A two-factor nonparametric approach to longitudinal analysis of changes over time with the effects of dose was used using techniques implemented in SAS macros developed by Brunner et al. (38). The exact two-sided Wilcoxon signed rank test was used for pairwise comparison of time points, whereas the exact two-sided Mann-Whitney U tests were used to compare the effects of two doses at each time point.

Results

Patient characteristics. Fourteen patients were entered into the trial between April 2003 and August 2004. Results from the study were analyzed in April 2005. Of the 14 patients recruited for the study (nine patients at 5 mg and five patients at 20 mg), eight patients completed the study (i.e., received all 16 doses of STX64) and, of these, three went on to receive extended dosing with STX64. The remaining six patients were withdrawn from the study due to progressive disease. One patient was withdrawn after the initial dose due to a non-drug-related serious adverse event. For one patient, tumor, but no blood samples were collected, making 12 patients evaluable for steroid concentration analysis and STS activity assessments in PBLs. However, for two of these patients, their PBLs were contaminated with erythrocytes, precluding measurements of

protein levels, although it was still possible to determine the percentage of STS inhibition in these cells.

STS inhibition. STS activity was almost completely inhibited in PBLs and tumor tissues by STX64 (Fig. 3A and B). Overall, PBL STS activity was reduced from 4.7 fmol $\text{E}_1/\mu\text{g}$ protein/20 h (2.0-6.6 fmol $\text{E}_1/\mu\text{g}$ protein/20 h) to 0.09 fmol $\text{E}_1/\mu\text{g}$ protein/20 h (0.03-0.17 fmol $\text{E}_1/\mu\text{g}$ protein/20 h) for 10 patients at the 5 and 20 mg doses for whom it was possible to measure PBL protein concentrations ($P = 0.002$; Table 2). For all patients, including two whose samples were contaminated with erythrocytes, STS activity was reduced by 98% (95-99%) at the day 5 + 8 hours time point. Thus, almost complete inhibition of PBL STS activity was achieved in all patients. At the 24-hour time point, median inhibition at both dose levels was 96% (94-98%) with some recovery of STS activity in PBLs (20%, 14-49%) occurring after the initial dose before the start of cycle 1 (Fig. 4A and B). For tumor samples, the median inhibition of STS activity at the 5 and 20 mg doses was 99% ($P = 0.016$) but only 78% inhibition was achieved in one patient at the 5 mg dose level (Fig. 3B). Three patients went on to receive extended dosing: one patient each at the 5 and 20 mg doses received six additional cycles, whereas one at the 20 mg dose had 12 cycles of extended treatment. In these patients, analyses of PBLs for STS activity showed continued reduction by $>90\%$ at the end of cycles where this was monitored.

Steroid concentrations. Nonparametric longitudinal analyses showed significant decreases in serum steroid concentrations during treatment, with the exception of DHEAS and E_1S (Table 2; Fig. 4A and B). As there were no significant differences after dosing with the 5 or 20 mg dose levels, results from both groups were combined for statistical significance analysis. Inhibition of STS activity should result in an increase in the ratios of steroid sulfates to their nonsulfated counterparts. The ratios of DHEAS/DHEA increased at both dose levels, with the median ratio increasing to 320% ($P = 0.000$) at the day 5 + 8 hours time point. Ratios of $\text{E}_1\text{S}/\text{E}_1$ also increased, with the median increase being 266% ($P = 0.000$) at the same time point. In general, levels of unconjugated steroids reflected the changes resulting from

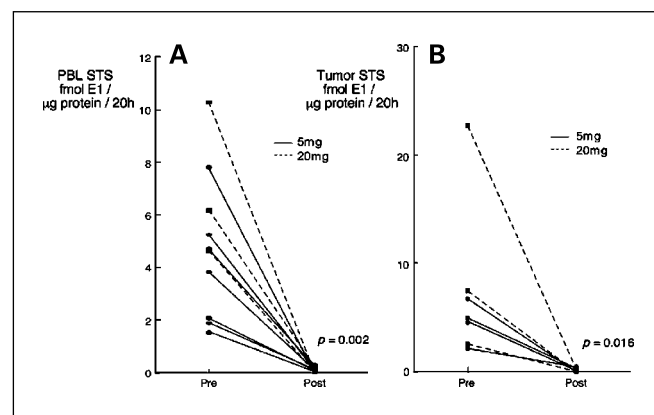


Fig. 3. Inhibition of STS activity in (A) PBLs in samples collected before treatment (Pre) with 5 or 20 mg STX64 and on day 5 + 8 hours of the first treatment cycle (Post), and in (B) tumor tissue collected before treatment with STX64 and on day 5 + 8 hours of the first treatment cycle. STS activity was assayed by a radiometric method using $[^3\text{H}]\text{E}_1\text{S}$ as the substrate. Values are for individual patients. Comparison of STS activity before and after treatment was carried out using the Wilcoxon signed rank test.

Table 2. Median and quartile ranges for serum steroid concentrations and STS activity in PBLs and tumor samples pretreatment and postdosing (cycle 1, day 5 + 8 hours)

Steroid/enzyme (unit)		Pretreatment median (quartile range)	Cycle 1, day 5 +8 h; median (quartile range)	P
DHEAS (nmol/L)	5 mg	825 (294-1,215)	834 (453-1,745)	NS
	20 mg	1,794 (1,268-3,033)	2,153 (1,255-5,144)	
DHEA (nmol/L)	5 mg	11.6 (7.6-16.8)	6.8 (4.7-9.8)	0.001
	20 mg	23.1 (18.9-35.3)	8.9 (7.2-15.5)	
DHEAS/DHEA	5 mg	80 (39-129)	275 (59-410)	0.000
	20 mg	93 (79-116)	359 (201-431)	
Androstenedione (pmol/L)	5 mg	2,045 (932-3,462)	778 (555-1,501)	0.007
	20 mg	4,432 (2,014-8,428)	1,242 (883-2,305)	
Androstenediol (pmol/L)	5 mg	643 (413-1,908)	193 (70-495)	0.001
	20 mg	2,353 (1,944-2,835)	619 (427-1,030)	
Testosterone (pmol/L)	5 mg	285 (236-659)	200 (89-345)	0.010
	20 mg	604 (595-954)	441 (262-659)	
E ₁ S (pmol/L)	5 mg	556 (368-1,274)	601 (362-1,884)	NS
	20 mg	901 (467-1,305)	952 (285-1,711)	
E ₁ (pmol/L)	5 mg	129 (81-164)	31 (29-66)	0.000
	20 mg	134 (69-177)	57 (28-117)	
E ₁ S/E ₁	5 mg	6.0 (5-9)	15 (12-33)	0.000
	20 mg	9.0 (6-12)	15 (9-30)	
E ₂ (pmol/L)	5 mg	11.7 (8.0-19.4)	7.1 (4.5-11.6)	0.001
	20 mg	35.0 (16.2-39.7)	21.7 (5.3-31.5)	
STS activity PBLs (fmol E ₁ /μg/20 h)	5 mg	3.83 (1.86-5.25)	0.09 (0.03-0.14)	0.002
	20 mg	6.18 (4.62-8.24)	0.08 (0.03-0.17)	
STS activity tumor (fmol E ₁ /μg/20 h)	5 mg + 20 mg*	4.85 (3.53-7.12)	0.06 (0.04-0.29)	0.016

NOTE: Results are shown for the 5 and 20 mg dose levels separately. P values are shown for combined doses that were calculated using the Wilcoxon signed rank test. Abbreviation: NS, not significant.
* Combined results are shown for tumor STS due to the small number of samples.

inhibition of STS activity. A decrease after the initial dose was followed by some recovery before the start of dosing for 5 days, after which a further decrease in concentrations was detected (Fig. 4A and B).

An important part of the first phase I trial of a STS inhibitor was to assess the effects of this novel therapy on serum E₁ and E₂ concentrations. As shown in Fig. 4A and B, median concentrations of E₁ at the 5 and 20 mg doses decreased significantly by 55% and 42%, respectively, with E₂ concentrations at these doses decreasing by 47% and 41%, respectively, at the cycle 1, day 5 + 8 hours time point. Although the precycle 0 concentrations of E₁ were similar in patients recruited for the 5 and 20 mg doses, concentrations of E₂ were somewhat higher in the group receiving 20 mg (35.0, 16.2-39.7 pmol/L) than for patients receiving 5 mg (11.7, 8.0-19.4 pmol/L). A similar difference in pretreatment E₂ concentrations for patients receiving different doses of letrozole has been previously reported (39).

Inhibition of the hydrolysis of DHEAS resulted in significant decreases in the serum concentrations of DHEA (Fig. 4A and B) with the median value reduced by 52%. Unexpectedly, the concentrations of the precursors, androstenedione and testosterone, for E₁ and E₂ synthesis by aromatase, also decreased significantly by 63% and 46%, respectively. The maximum decrease in androstenedione concentrations observed was 86%. A major impetus for the

development of STS inhibitors was the need to block the production of androstenediol in postmenopausal women, which is mainly derived from DHEAS. Serum androstenediol concentrations decreased by up to 90% with the median decrease at both dose levels being 72%. Pretreatment concentrations of DHEAS, androstenedione, and testosterone were higher in patients receiving the 20 mg dose of STX64, for which there is no apparent explanation apart from the lower median age of the patients receiving the 20 mg dose.

Pharmacokinetic analysis. Pharmacokinetic analysis revealed that there was good exposure to the drug as shown by the area under the plasma concentration curve after administration of the 5 mg [7,233 ± 608 (ng/mL).h, mean ± SD, n = 3] and 20 mg [16,323 ± 8,328 (ng/mL).h, mean ± SD, n = 5] doses. The apparent elimination half-lives, expressed as means ± SD after oral administration of the 5 and 20 mg doses, were 31.3 ± 6.0 and 28.5 ± 12.0 hours, respectively, indicating that it will be possible to use a once per day dosing schedule. The ratio of exposure of 667 Coumarin (the main metabolite) to 667 Coumate was very low (<0.026 in terms of ratio of area under the plasma concentration curve).

Clinical response and tolerability. Although the assessment of clinical response was not a primary end point of the study, it was assessed within 4 weeks of the end of cycle 3. Eight of the 14 patients received 4 cycles of treatment (cycles 0-3) and were evaluable for disease response according to Response

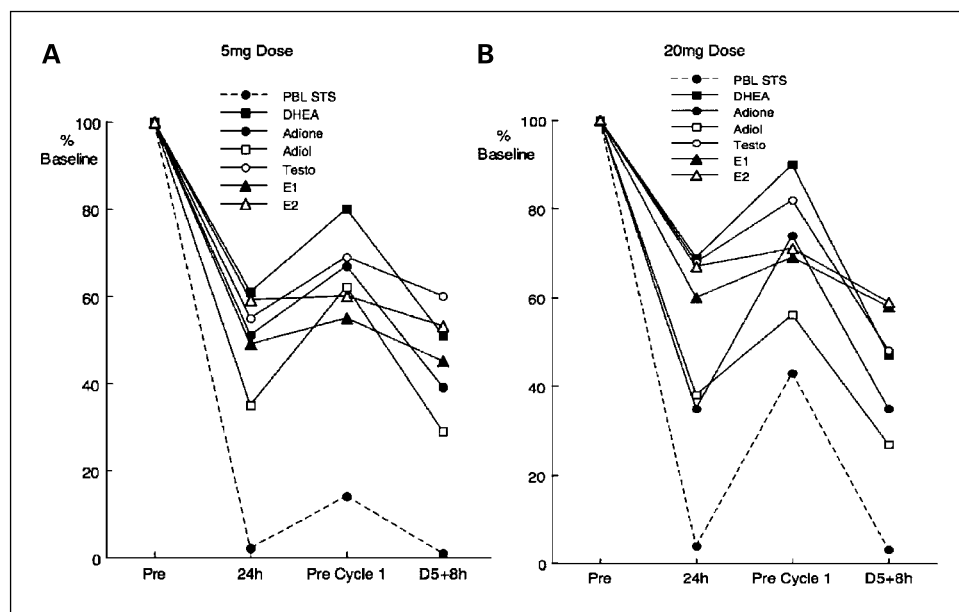


Fig. 4. Changes in STS activity in PBLs and serum steroid concentrations in blood samples taken before the start of treatment, 24 hours after the initial dose, before the start of cycle 1, and 8 hours after dosing on day 5 of cycle 1 in patients receiving the 5 mg (A) or 20 mg (B) dose of STX64. Points, median values for the percentage change from baseline.

Evaluation Criteria in Solid Tumors. However, one patient did not have her bone lesions examined, although her target lesions were assessed. Although the patients in this trial had been heavily pretreated with a minimum of first- and second-line therapies (Table 1), some patients, who had all previously received antiestrogen and aromatase inhibitor therapy, had stable disease as follows: Patient 1 had nonmeasurable inflammatory breast cancer that did not change for 6.25 months on STX 64 treatment. This patient had previously had a complete response to formestane and anastrozole and disease stabilization on fulvestrant. Patient 10 had lung metastases and pretracheal and subcarinal lymphadenopathy at study entry. She received six extended cycles of treatment with STX 64 after completing the trial and had stable disease for 6.5 months. She had previously progressed on anastrozole. Patient 13 had a local recurrence of her breast cancer with bone and skin metastases when enrolled on the study. Her disease stabilized for 2.5 months with STX 64 treatment. She previously had progressive disease on anastrozole. Patient 14 had lymph node metastases in the neck and mediastinum and lung nodules, which stabilized for 7 months during the phase I study. She had previously progressed on anastrozole and formestane. However, there was a further patient, number 5, who had a breast mass, lung and liver lesions stable for 2.75 months while on the study. Skeletal lesions were not assessed radiologically in this patient but she did not have worsening bone pain during treatment. This patient had previously had stable disease on exemestane and anastrozole.

The drug seemed to be well tolerated with only a few grade 1 or 2 adverse events recorded that were thought to be drug-related. The most frequent occurring event experienced by all patients was taste disturbance (maximum grade 2), which was thought to be related to the formulation of the drug (lyophilized from DMSO) and generally lasted for less than 12 hours except in one patient for whom it lasted for 8 days. Other adverse events recorded for some patients included fatigue, hot flushes, mood alterations, arthralgia, and head-

aches, with the maximum grade recorded for these events being 2. Blood and urine were regularly analyzed during the study but did not reveal any biochemical or hematologic changes that were thought to be drug related.

Discussion

The results from this first phase I trial of a STS inhibitor have shown that it is possible to block PBL and tumor STS with STX64. The primary end point of the study was to determine the dose required to inhibit STS activity in PBLs by >90% at the cycle 0, 24 hours, and cycle 1, day 5 + 8 hours time points. Although this objective was achieved at the 5 mg dose, STS inhibition in one of four tumor samples at this dose did not reach the >90% level. At the 20 mg dose level, >90% inhibition of STS activity was achieved in PBLs and tumor samples. Inhibition of STS activity was associated with significant decreases in E₁ and E₂ concentrations at both dose levels. In the present study, a gas chromatographic/tandem mass spectrometric assay, recently shown to be more sensitive and specific than most immunoassay methods, was used to measure E₁ and E₂ concentrations. Results obtained for the measurement of E₂ in postmenopausal women using this method showed a good correlation ($r = 0.84$) with those measured using a recombinant ultrasensitive bioassay (37). For 8 of 12 patients, serum E₂ concentrations after five doses were <10 pmol/L and in the range reported for patients receiving third-generation aromatase inhibitors for 46 to 84 days (39, 40). It is anticipated that dosing for a longer period of time with STX64 will result in even greater decreases in serum E₁ and E₂ concentrations. A major factor contributing to the significant decrease in serum E₁ and E₂ concentrations was the finding that STS inhibition results in significant decreases in serum androstenedione and testosterone concentrations, which are the precursors for E₁ and E₂.

As androstenedione has generally been considered to be secreted directly from the adrenal cortex (41, 42), the finding that levels decreased by up to 86% as a result of inhibitor

therapy was unexpected. Although third-generation aromatase inhibitors reduce serum estrogen concentrations by >90%, they do not alter DHEAS or androstenedione levels (43). The reduction in serum androstenedione and testosterone levels resulting from STS inhibition raises important questions as to the origin of androgens in postmenopausal women. It suggests that, in these women, an important, if not the major, source of androstenedione is derived from the peripheral conversion of DHEAS rather than by its direct secretion from the adrenal cortex (see Fig. 1). It is known that *in situ* formation of E_1 from androstenedione makes an important contribution to the estrogen synthesized in most breast tumors (44). It is likely that the reduction in serum androstenedione concentrations available for uptake into tumors and aromatization to E_1 will contribute to the reduction in tissue levels of estrogens that should result from this new form of therapy.

STS inhibitors were developed not only to suppress the formation of E_1 from E_{1S} but also the formation of androstenediol, which in postmenopausal women is mainly derived from DHEAS. In addition to the suppression of estrogens, STS inhibitor therapy may offer the additional advantage over aromatase inhibitors of reducing androstenediol levels. Androstenediol, although an androgen, has potent estrogenic properties. Although its affinity for the ER is lower than that of E_2 , it has been suggested that, due to its much higher serum and tissue concentrations, it may be equipotent with E_2 in postmenopausal women (45). The reduction of up to 90% in serum androstenediol concentrations indicates that STS inhibition can effectively decrease its production.

Assessment of the clinical effectiveness of the drug, although not a primary end point, revealed that four patients had stable disease according to Response Evaluation Criteria in Solid Tumors. A further patient had evidence of stable disease in target lesions. Importantly, all of the patients showing evidence of stable disease had previously received first- and second-line endocrine therapies with some also receiving chemotherapy. In conclusion, STX64 almost completely blocks STS activity in PBLs and tumor tissue in postmenopausal women with breast cancer. STS inhibition is associated with significant decreases in serum E_1 , E_2 , and androstenediol concentrations and, unexpectedly, androstenedione and testosterone. The drug was well tolerated with only minor, drug-related, adverse events noted. The drug has an acceptable pharmacokinetic profile, indicating that it is suitable for daily dosing. Further phase I/II studies are planned to fully evaluate the efficacy of this novel form of endocrine therapy.

Acknowledgments

We thank Abdul Latif for the assistance with the RIAs; Samantha Weller at Charing Cross Hospital for help with data collection, sample processing, and patient dosing; Angela Morrison and Sonya McDowell at Belfast City Hospital for their help with data collection and patient dosing along with sample processing, respectively; Kathryn Cross and Katharine Bellenger at Cancer Research UK for their help with data monitoring and data management, respectively; Gavin Halbert and Steve Ford at the University of Strathclyde for their help with the formulation of STX 64; Dominic Blunt and John Lawson at Charing Cross and Belfast City Hospitals, respectively, for assistance in carrying out the radiological assessments; and Concepcion Peraire at Ipsen Pharma, Barcelona, for carrying out the pharmacokinetic analysis.

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Clin Cancer Res 2006;12:1585-1592.

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