OriginalArticle

Steroid Sulphatase (STS) Inhibitors: A New Therapeutic Intervention In Treatment Of Breast Cancer

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Abstract

Steroid sulphatase is an emerging drug target for the endocrine therapy of hormone-dependent diseases, catalysing oestrogen sulphate hydrolysis to oestrogen. The inhibition of STS may effectively reducethe availability of active hormones for cancer cells, causing a positive therapeutic effect. A number of potent STS inhibitors have nowbeen developed, one of which, Irosustat (STX64, 667Coumate, BN83495), remains the only one tohave completed phase I/II clinical trials against numerous indications (breast, prostate, endometrial).This review summarizes work leading to the therapeutic concept of sulphatase inhibition, clinical trials executed to date and new insights into the significance of STS inhibition in treatment of different cancers especially metastatic breast cancer.

Keywords: Breast cancer, Clinical trial, Irosustat, Steroid, Steroid sulphatase, STS inhibitors.

INTRODUCTION

Cancer is one of the leading causes of death worldwide. More than 19 million new instances of cancer and 9.9 million deaths from tumor-related causes were reported globally in 2020, according to estimates from the International Agency for Research on Cancer[1,2]. In addition, by 2040, the National Cancer Institute (NCI) anticipates that there will be 29.5 million new cases of cancer annually. According to the NCI, 39.5% of men and women will be diagnosed with this condition at some point in their lifetime [1,3]. Due to the rapid spread of aberrant cells that have the capacity to invade neighbouring tissues and kill them as well as infect other organs, cancer is a complex disease that can harm any organ in the body [4,5].Nearly 50% of all new occurrences of cancer are of the most prevalent forms, which include colorectal, prostate, and lung tumours as well as breast, lung, and bronchus tumours. In addition, cancer accounts for close to 50% of all fatalities, including lung and bronchus, colorectal, pancreatic, and breast malignancies.The projections for the year 2019 showed that roughly 270,000 and 175,000 patients, respectively, were diagnosed with breast and prostate tumours. On the other hand, more than 41,000 and 31,000 mortalities were reported for breast and prostate in the United States respectively [1,3]. It is well known that the majority of malignancies exhibit hormone need in the early stages (for example, more than 90% of instances of breast cancer exhibit hormone dependence at first) [1,6]. So, androgens and oestrogens, which are physiologically active hormones, are the primary factors that promote the growth of cancer, according to the World Health Organization (WHO). Given the aforementioned information, the application of medications that can successfully lower active hormone concentrations should form the cornerstone of contemporary therapy [7]. The development of hormone-dependent cancer medications has long focused on the hormone signalling system[8].Recent findings suggest that abnormalities in the sulphation/desuphation process could be the cause of a variety of pathologies[9], and as a result, Steroid Sulphatase (STS), an important enzyme involved in the steroidogenesis process, is emerging as a new and intriguing molecular target in the search for novel and efficient hormone-dependent cancer treatment approaches. The majority of cancer cases have STS activity (for instance, 90% of breast tumours exhibit STS expression) [10]. Additionally, it was found that in 87% of individuals who underwent testing, STS mRNA levels in malignant cells were higher than in normal breast tissues [11].

STEROID SULPHATASE (STS) AND THEIR ROLE IN BIOSYNTHETIC OF STEROIDS WITH OESTROGENIC AND ANDROGENIC PROPERTIES

STS is one among the 15 sulphatases found in humans [1]. The STS gene encodes this protein, which has 587 amino acid residues. The fact that STS is present everywhere in the body is directly related to its participation in a number of physiological and pathological processes [12]. The fallopian tubes, testicles, ovaries, adrenal glands, brain, foetal lung, endometrium, aorta, kidneys, bones, placenta, and breasts are the principal sites of localization of this enzyme [13]. Androstenedione (A4) and dehydroepiandrosterone (DHEA), two androgen precursor hormones produced in the adrenal cortex, are converted to produce oestrogens and androgens (**Fig.1**). Most DHEA is carried in the circulation in its sulphated form, DHEA-sulphate (DHEA-S), which improves solubility and serves as a circulating reservoir for the production of downstream active oestrogen and androgens in peripheral tissues. Consequently, the primary pathway in the synthesis of all active androgens and oestrogens is STS desulphation of DHEA-S to DHEA. For androgens, 3-hydroxysteroid dehydrogenase (HSD)/isomerase can further metabolise DHEA to A4, which can subsequently be transformed to testosterone by 17 hydroxysteroid dehydrogenase type-3. Both A4 and testosterone can be converted into the oestrogens estrone (E1) and oestradiol (E2) through the process of aromatization [14]. Oestrone [E1] and dehydroepiandrosterone [DHEA] are produced when steroid sulphates, such as oestrone sulphate [E1S] and dehydroepiandrosterone sulphate [DHEAS], are hydrolyzed by STS [15]. E1 and DHEA can later be converted into bioactive oestrogens and androgens (such as E2 and Adiol, respectively), which are in charge of promoting the growth of hormone-dependent cancer cells [16]. Given the aforementioned information, STS is an exceedingly desirable biological target for the creation of hormone-dependent cancer therapeutics since it is crucial to the development of breast cancer tumorigenesis [1].

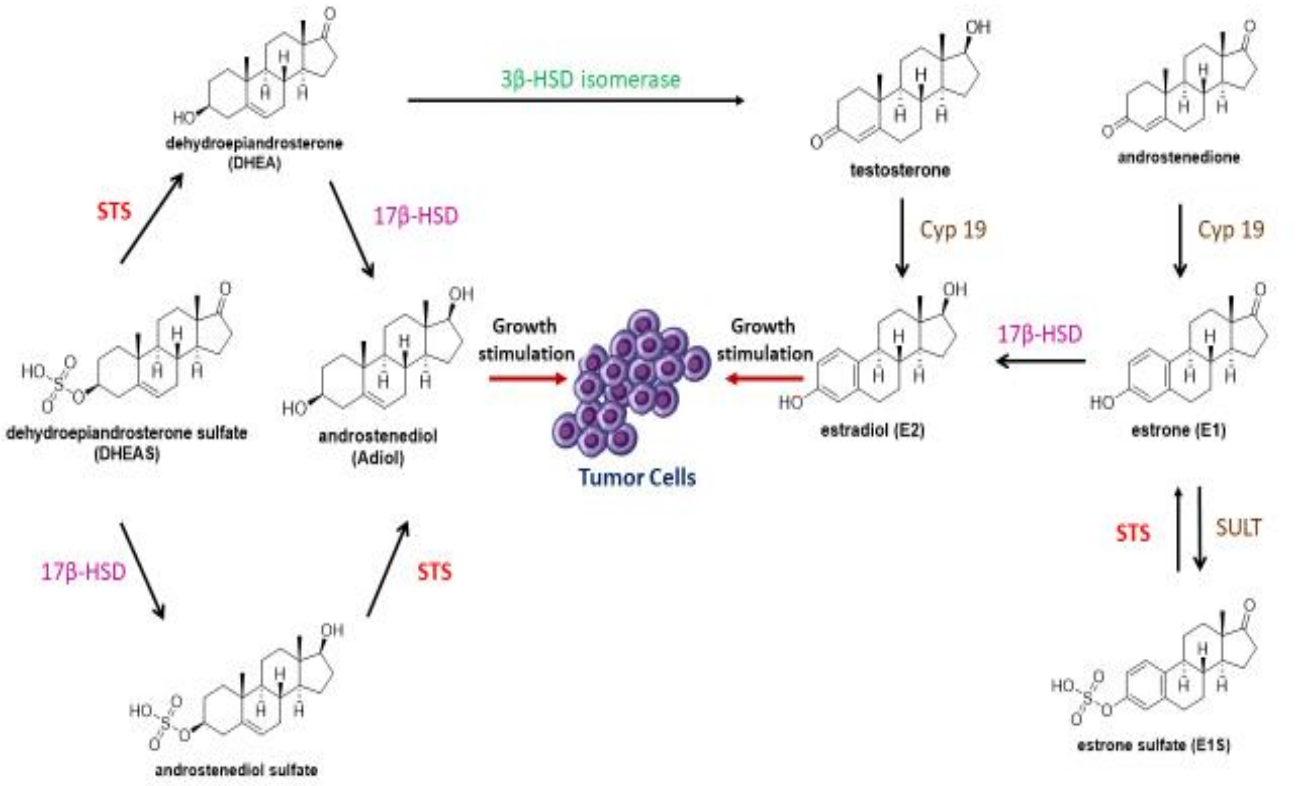

Fig.1. The biosynthesis pathway of estrogenic steroids

NEED FOR DEVELOPING STS INHIBITORS

E1S and DHEA-S are present in the blood at substantially higher quantities than their unconjugated counterparts [17]. In addition, steroid sulphates have a substantially longer half-life in blood (10–12 hours) than do unconjugated oestrogens due to their affinity for serum albumin (20–30 min)[18]. Strong evidence exists that MCF-7 breast cancer cells, LNCaP prostate cancer cells, and the majority of other cancer cells can hydrolyze steroid sulphates, such as E1S and DHEA-S, in vitro [19,20]. Since most hormone-dependent tumour homogenates include significant levels of STS activity, it has been assumed that, once within cells, steroid sulphates will be easily hydrolyzed to their unconjugated forms by STS [21]. When E1S is hydrolyzed, it can be converted to E2 by the enzyme 17-HSD1, which is found in the majority of hormone-dependent tumours and is overexpressed in several breast [22-24] and endometrial malignancies [25]. In addition to the formation of E2 from E1S, groups working on STS inhibitors have shown a great deal of interest in the potential contribution of adiol to the growth of tumours. Adiol, despite being an androgen, can bind to the ER and is well-documented for stimulating the growth of hormone-dependent breast cancer cells in vitro and carcinogen-induced mammary tumours in rodents [26-28]. Additionally, it has recently been demonstrated that adiol promotes proliferation of

LNCaP prostate cancer cells via activating the androgen receptor (AR)[29]. Therefore, inhibiting STS function may have a variety of impacts on the availability of different sex steroids, which may be very helpful for those with hormone-related diseases. For individuals whose disease has advanced or who have not responded to aromatase inhibitors, targeting STS in reference of hormone-dependent cancer, and in particular breast cancer, may be advantageous. According to studies, letrozole and other aromatase inhibitors cause breast cancer cell lines to upregulate STS expression and activity[30]. Therefore, although this has not yet been explicitly confirmed experimentally, it may be the case that STS expression is increased in breast tumours from patients treated with aromatase inhibitors. This shows that some hormone-dependent tumours may preferentially use the STS pathway to maintain E1 and then E2 generation by hydrolysis of circulating sulphated oestrogens despite loss of oestrogen synthesis through the androgen pathway [14]. Additionally, it is crucial to remember that methods to lower hormone synthesis and activity have been the cornerstone of many treatment regimens against endocrine disorder, which is another reason why discovering clinically effective STS inhibitors remains a major research aim. It follows that STS inhibition will also likely be a successful therapeutic strategy. In addition, many hormone-dependent tumours develop resistance to modern treatments. Resistance to aromatase inhibitors, both primary and secondary needed, is relatively prevalent and is seen to be a serious problem for effective treatment [31]. This makes way for complementary methods that totally obliterate hormone function and by means of which STS inhibition ought to have a clinical purpose. One strategy that needs more research is pairing STS inhibitors with established gold standard therapy regimens that include either oestrogen receptor inhibitors (like Tamoxifen) or aromatase inhibitors (Letrozole)[14].

The special relevance of recent decades draws attention to the practical therapeutic advancement of STS inhibitors that were discovered (e.g. E2MATE, Irosustat [also known as 667-COUMATE, STX64 and BN-83495]) (**Fig.2)**. Irosustat proved to beextremely promising STS inhibitor without having in vitro andin vivo oestrogenic properties. Irosustat has recently shown clinical advantages in early breast cancer and ER-positive advanced endometrial cancer treatments. (E2MATE), was clinically evaluated (phase II clinical trials) in hormone replacement therapy as an oestradiol prodrug. Furthermore, E2MATE is still being considered as a drug candidate for hormone dependent endometriosis therapy [1].

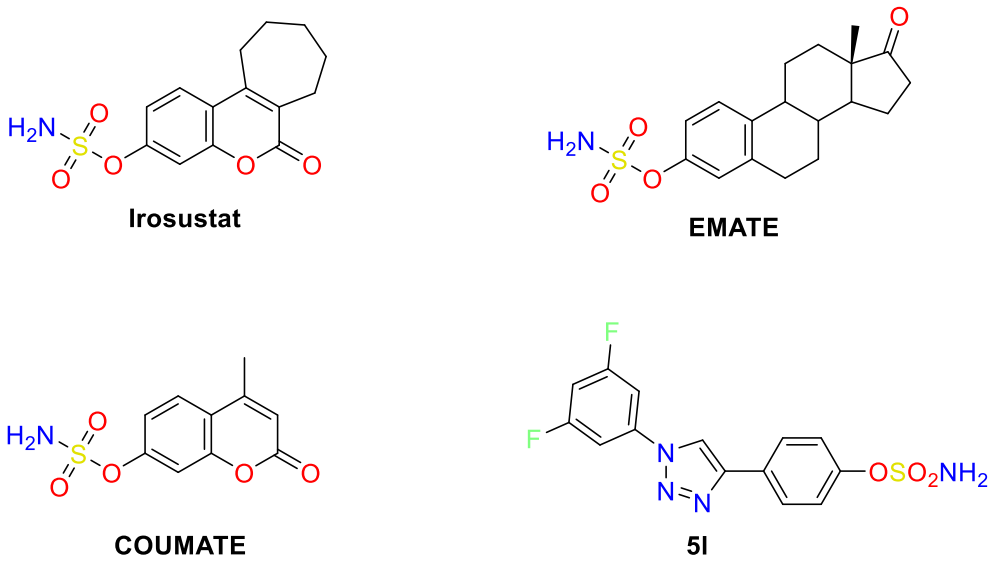

Fig. 2. Chemical structure of STS inhibitors

SIGNIFICANCE OF STS INHIBITORS IN BREAST AND GYNAECOLOGICAL CANCERS

Several studies have deliberated the expression and/or activity of STS in hormone-dependent malignancies using either reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry (IHC), or radiolabelled assay methods. In line with the high levels of STS activity identified in cancerous breast tissues, it was shown that the expression of STS mRNA was much higher in malignant breast tissue when compared to normal breast tissue [32,33]. High levels of expression are linked to a poor prognosis, and STS expression is also a standalone prognostic factor in predicting relapse-free survival [34-37]. Elevated STS expression in breast tumours, however, was strongly related with a much lower incidence of relapse and distant metastasis, leading to an improved prognosis in those cohorts, according to a second study on Norwegian patients [38].STS activity has also been found to be highly active in endometrial cancer tissue, 12 times more active than in healthy endometrium [39]. In a study using IHC, no STS expression was found in tissue from normal endometrium, however it was found in 86% of cases from endometrial cancer tissue [40]. In contrast to normal endometrial controls, more recent research on patient endometrial cancer tissue have not discovered modifications in STS mRNA or protein expression [41,42]. Milewich and Porter discovered STS in cells obtained from ovarian tumours in the beginning for ovarian cancer[43]. STS was discovered to be present in 71% of ovarian clear cell adenomas using IHC labelling[44].Carlstrom et al. discovered large levels of STS activity in ovarian tumour tissues when investigating the capacity of ovarian cancer tissues to produce E2 from E1S. They also discovered a significant link

between ovarian tumour STS activity and serum E2 levels[45]. 97% of the ovarian cancer specimens evaluated had STS activity [46]. Importantly, in this investigation, patients with low STS activity relative to those with high activity had significantly longer median progression-free survival times. In fact, patients with high-grade serous ovarian cancer who had higher levels of the enzyme oestrogen sulphotransferase (SULT1E1), which sulphates E1 and E2 to render them inactive, had better prognoses[47]. This implies that STS inhibition may be a viable treatment option and that these patients may experience worse outcomes as a result of higher STS activity [12]. Despite these studies, it is still unclear whether human ovarian cancer exhibits higher STS activity than normal ovarian tissue; one study suggests there is no difference when assessed using IHC [12].

CLINICAL STUDIES OF STS INHIBITORS IN BREAST CANCER

Irosustat and E2MATE/J995 have been shown to have beneficial clinical effects [48], and the underlying academic research has supported these findings. Irosustat was given orally to 14 postmenopausal women with advanced breast cancer in three 2-weekly cycles of 5-day dosing and 9-day off treatment. The initial dose was given to 9 patients at 5 mg and 5 at 20 mg. The medication was found to be clinically effective, well tolerated, and had a pharmacokinetic profile acceptable for daily administration with just minor side effects. At the conclusion of the 5-day dosage period, the median STS activity inhibition was 98% in peripheral blood cells and 99% in breast tumour tissue from biopsies.Dehydroepiandrosterone (DHEA), androstenedione, and testosterone serum concentrations all reduced markedly from their pre-treatment levels as well as those of oestrogenic steroids, specifically androstenediol, oestrone, and oestradiol. Four patients who had previously progressed on Aromatase inhibitors (AI) showed signs of stable illness for 2.7–7 months, which was reassuring [48,49]. A three-part, open-label, multicenter, dosage escalation investigation of Irosustat in patients with ER+ breast cancer was carried out in a later phase I clinical research [50], employing a new solid tablet formulation to ascertain the best biological dose. The medicine was given orally once, followed by a 7-day observation period, a daily dose for 28 days, and an extension phase when treatment was maintained at the doctor's discretion if the patient appeared to benefit. In fifty patients, five Irosustat doses up to 80 mg were tested. All assessed individuals in the 5-, 20-, 40-, and 80-mg cohorts achieved 95% STS inhibition in peripheral blood mononuclear cells and associated endocrine suppression after 28 days of daily dosing. Since the maximum tolerable dose was not reached, the 40-mg dose was determined to be the best one. In the 40-mg cohort, 11.2 weeks represented the median time to progression. One month of treatment eliminated the erythematous skin infiltration in one patient that had been confirmed by biopsy. Five patients from this heavily pre-treated patient population (10%) remained progression-free for at least 24 weeks (33.1 weeks in one patient receiving 20 mg, 72.3, 28.4 and 27.1 weeks in three patients receiving 40 mg, and 30.7 weeks in one patient receiving 80 mg), potentially indicating drug activity. Disease stabilisation is frequently taken to be a reliable indicator of the effectiveness of a novel therapy, particularly endocrine therapy. Dry skin, the most prevalent adverse effect, was easily handled. The postmenopausal subjects in clinical trials, like the ones previously described, are typically already highly pre-treated, which may have activated different resistance pathways. The "IPET" research, a pre-surgical window-of-opportunity study to evaluate Irosustat for the first time in patients with ER+ early breast cancer, is one of the most recent clinical studies in breast cancer to be published[48, 51]. The purpose of this study was to evaluate Irosustat's impact on tumour cell proliferation in treatment-naive patients, as determined by levels of the tumour proliferation marker Ki67 and 3′-deoxy-3′-[18F] fluorothymidine (FLT) uptake monitored by positron emission tomography (PET) scanning (FLT-PET). Postmenopausal women with early-stage breast cancer who had not had treatment were chosen, and Irosustat was administered orally for at least two weeks at 40mg/day. The primary endpoint of FLT-PET imaging of tumours was changes in FLT uptake; secondary endpoints included safety and tolerability, changes in tumoural Ki67, circulating steroid hormone levels, and expression of steroidogenic enzymes. FLT-PET imaging of tumours was done at baseline and after drug treatment. Thirteen women were selected; ten of them began taking Irosustat for two weeks, and the remaining eight underwent additional FLTPET scans. Overall, the medication was well tolerated, and all side effects were of Grade 2. STS levels dropped in tumours with high baseline STS expression, while aromatase and 17-HSD types 1 and 2 levels also significantly decreased. One and three patients, respectively, reacted when the criterion of a response was drops of 20% in standardised uptake value or 30% in Ki67.Baseline STS expression was determined to be a potential biomarker of Irosustat sensitivity and may help with patient stratification in the future. Though in a very small patient sample, these data are significant since they are the first to show clinical activity of Irosustat in early breast cancer. Additionally encouraging, findings are largely equivalent to phase III data for the well-known medications exemestane and fulvestrant as well as to those from tamoxifen in the same context. Larger studies are now needed to build on these findings because patient recruitment in this pre-surgical single-centre cohort was difficult [48, 51]. The "IRIS" research was a recent clinical trial that was published in 2017 [52]. In patients with advanced breast cancer, the therapeutic benefit of adding an STS inhibitor in addition to a first-line AI was investigated in this multicenter, open-label, phase II trial. This was done in order to assess the combination's safety as well as to test the idea that combining Irosustat with an AI would further reduce E2 levels and have positive clinical effects. The study included postmenopausal women with ER+ locally progressed or metastatic breast cancer who had responded to a first-line aromatase inhibitor but were still advancing.The first-line AI was kept up while Irosustat oral 40 mg/day was introduced. Clinical benefit rate (CBR) was the main outcome, whereas safety, tolerability, and pharmacodynamics were the secondary outcomes. Local and centralised radiological evaluations determined that the study had achieved its pre-established success criterion. Four of the 27 recruited women stopped receiving treatment. According to local reporting, the CBR was 18.5% on intent to treat (ITT) basis, and by per-protocol analysis, it had increased to 21.7%. In both the ITT and per-protocol analyses, the median duration was 9.4 months and the median progression-free survival time was 2.7 months in those five patients who saw clinical benefit. Grade 2 side

effects were most frequently reported, with dry skin being the most prevalent, as one might anticipate. Fortunately, the inclusion of Irosustat to the conventional aromatase inhibitor medication led to clinical improvements.

CONCLUSION

Despite the development of many treatment strategies, cancerremains one of the most important medical problems that modernmedicine has to face. Considering that numerous carcinomasexhibit a hormone-dependent nature, it is rational to design agents that can block hormone biosynthesis processes. The administration of small molecules, exhibiting inhibitory activities against enzymes implicated in hormone biosynthesis may reduce the concentrations of these in tumour tissues and consequently may limit the oestrogenic stimulation of cancer cell growth. Currently, STS is recognised as an extremely promising molecular target in the development of effective agents with high therapeutic potential in the treatment of hormone-dependent cancers. To date, some of the reported compounds have been evaluated in clinical trials (e.g. Irosustat and E2MATE) and they seem to be very promising as drug candidates. However, none of them has reached pharmacies.Therefore, the identification and synthesisof novel compounds demonstrating STS inhibitory activity aswell as the evaluation of the biological influence and safety ofknown STS inhibitors are crucial for the development of effective treatment methods of hormone-dependent cancers.

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