REVIEW

Steroid sulfatase inhibitors for estrogen- and androgen-dependent cancers

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Abstract

Estrogens and androgens are instrumental in the maturation of many hormone-dependent cancers. Consequently, the enzymes involved in their synthesis are cancer therapy targets. One such enzyme, steroid sulfatase (STS), hydrolyses estrone sulfate, and dehydroepiandrosterone sulfate to estrone and dehydroepiandrosterone respectively. These are the precursors to the formation of biologically active estradiol and androstenediol. This review focuses on three aspects of STS inhibitors: 1) chemical development, 2) biological activity, and 3) clinical trials. The aim is to discuss the importance of estrogens and androgens in many cancers, the developmental history of STS inhibitor synthesis, the potency of these compounds *in vitro* and

in vivo and where we currently stand in regards to clinical trials for these drugs. STS inhibitors are likely to play an important future role in the treatment of hormone-dependent cancers. Novel *in vivo* models have been developed that allow pre-clinical testing of inhibitors and the identification of lead clinical candidates. Phase I/II clinical trials in postmenopausal women with breast cancer have been completed and other trials in patients with hormone-dependent prostate and endometrial cancer are currently active. Potent STS inhibitors should become therapeutically valuable in hormone-dependent cancers and other non-oncological conditions.

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Introduction

The aromatase and steroid sulfatase (STS) enzymes are involved in the synthesis and regulation of physiologically active sex steroids, the estrogens and androgens. They play important roles in a plethora of normal pathological conditions. However, it is their oncogenic activities and cancer cell mitogenesis that are of particular interest to researchers and a full understanding of how these enzymes act has, and will, continue to reap therapeutic rewards. A large percentage of human cancers, most notably breast, prostate, and endometrial, initially rely on steroid production for growth: only later in development do they usually become steroid-independent with the exception of prostate cancer which can remain androgen dependent even at late-stage (Knudsen & Penning 2010, de Bono *et al.* 2011). Consequently, inhibitors capable of manipulating steroid production have potential benefits to many patients suffering from the early onset of a wide range of malignancies.

The past few decades have seen cancer research focus on new ways of limiting estrogen availability/activity. Hugely successful anti-estrogen therapies, such as tamoxifen treatment, block the estrogen receptor (ER), whereas aromatase inhibitors (AI), such as letrozole, stop the synthesis of estrone

(E₁) from androstenedione thereby restricting the formation of biologically active estradiol (E₂). Unfortunately, clinical studies involving breast cancer patients suggest that the majority of women undergoing these treatments will eventually have progressing disease despite having tumors that remain ER positive (ER+) upon relapse (Taylor *et al.* 1982).

Another enzyme, STS, also plays a pivotal role in steroid synthesis and recent significant advances have been made on the development and use of STS inhibitors in treating hormone-dependent cancers (Reed *et al.* 2005). This review aims to examine the importance of STS in estrogen and androgen production, which chemicals lead to effective STS inhibitors, and how these STS inhibitors should become clinically beneficial to patients.

The synthesis of sex steroids in cancer

Estrogen metabolism

Since the groundbreaking work of Lacassagne in 1932 demonstrated that the administration of estrogens to mice increases the incidence of mammary cancer, there is now an agreed consensus that sex hormones are involved in the

development of neoplasias (Lacassagne 1955, Soto & Sonnenschein 2010). The maturation of tumors in hormone-dependent tissues, such as mammary, ovarian and endometrial, is supported by estrogens (James & Reed 1980, Bernstein & Ross 1993). Interestingly, the greatest risk and highest incidence of breast cancer occurs in postmenopausal women – i.e. when ovarian estrogen production has ceased (MacDonald *et al.* 1978, Reed *et al.* 1979). However, biologically active estrogens are still readily synthesized by the peripheral conversion of androstenedione (Adione) to E_1 , a reaction regulated by the aromatase enzyme complex (see Fig. 1). In postmenopausal women, the production rates for E_1 and E_2 are ~ 40 and $6 \mu\text{g/day}$ respectively (Reed & Murray 1979). The majority of these mainly hydrophobic estrogens can be further converted to estrogen sulfate (E_1S) by the action of estrogen sulfotransferase and phenol sulfotransferase, rendering them hydrophilic (Hobkirk 1993, Falany & Falany 1996, Falany *et al.* 2002, Stott 2002). However, it must be remembered that as E_1S has a very low affinity for the ER, it is, in effect, biologically inactive. Circulating concentrations of E_1S are much higher than that of the unconjugated estrogens (Noel *et al.* 1981, Pasqualini 1989) and because they are capable of binding to albumin they

have an ~ 9 h half-life in blood, significantly longer than either E_1 or E_2 (Ruder *et al.* 1972). Therefore, these factors suggest that, as E_1S is available for extended periods of time in the plasma, it may act as a central reservoir for the formation of biologically active estrogens via the actions of tissue STS (Reed & Purohit 1993, 1994, Reed *et al.* 1996).

Although postmenopausal women have low circulating levels of E_1 and E_2 , there is now significant evidence indicating these estrogens are found in much higher concentrations in normal and malignant breast tissue (Bonney *et al.* 1983, Van Landeghem *et al.* 1985). In fact, there can be as much as a tenfold increase in E_1 and E_2 levels, as well as an increase in E_1S and E_2 sulfate (E_2S), in cancerous breast tissue compared with those found in circulation (Pasqualini *et al.* 1989). However, the source of intratumoral estrogen is still somewhat controversial. Either there is an uptake of circulating estrogens that then bind to the ER or there is *in situ* synthesis from various estrogen precursors. Although both these theories are possible, the fact that both ER-positive and ER-negative cancers exhibit similar estrogen concentrations implies that regional synthesis makes a significant contribution to tissue tumor estrogen levels (Fishman *et al.* 1977, Edery *et al.* 1981).

Figure 1 The origin of estrogenic steroids. Estrone sulfate (E_1S) is found circulating at high concentrations. STS, upregulated in many hormone-dependent cancers, causes peripheral tissue conversion of E_1S to estrone (E_1). E_1 is then reduced to estradiol (E_2) by 17β-HSD type 1, binds to the estrogen receptor (ER) and causes cell proliferation. AROM, aromatase; ST, sulfotransferase; STS, sulfatase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; ER, estrogen receptor; DHEA, dehydroepiandrosterone; DHEA-ST, dehydroepiandrosterone sulfotransferase.

from age-matched controls and were significantly higher in patients with a poor prognosis (Giton *et al.* 2008). Although recently a Phase I clinical trial using an STS inhibitor (667 COUMATE, also known as STX64, BN83495, and Irosustat) in patients with castrate-resistant prostate cancer has been initiated in North America (Geisler *et al.* 2011), it remains to be fully investigated whether an STS inhibitor would be an effective treatment for this malignancy.

STS in hormone-dependent cancers

Three enzymes are primarily responsible for E_2 synthesis in tissue: aromatase converts androstene to E_1 , 17β -hydroxysteroid dehydrogenase type 1 (17β -HSD-1) reduces E_1 to E_2 , and STS is responsible for hydrolyzing several sulfated steroids such as E_1 S, DHEAS, and cholesterol sulfate (Reed *et al.* 2005). A growing body of evidence now demonstrates the importance of STS in human cancers.

As mentioned above, breast tumor tissue of postmenopausal women can have as much as ten times the estrogen levels compared with plasma samples from the same patients (Ruder *et al.* 1972). This hyperestrogenic state is most likely caused by the fact that STS activity is at least 50 times greater in both pre- and postmenopausal breast tumors compared with normal breast tissue (Pasqualini *et al.* 1996). STS expression is detected in 90% of breast tumors (Lipton *et al.* 1992, Evans *et al.* 1994), whereas aromatase expression is only found in 60–70% (Lonning *et al.* 1990, Evans *et al.* 1993) and that STS activity in breast tumors is significantly higher than that of the aromatase complex (James *et al.* 1987). This increased STS activity could result in a tenfold greater amount of E_1 originating via the sulfatase route than via the aromatase pathway (Santen *et al.* 1984). In addition, real-time RT-PCR experiments have demonstrated that STS mRNA expression in malignant breast tissue is significantly higher than in normal tissue (Utsumi *et al.* 2000), which is consistent with increased STS activity observed in this tissue. Clinical studies have now shown that STS mRNA expression may be a predictor of recurrence in breast cancer patients (Utsumi *et al.* 1999) and that this association and prognosis is applied only to ER+ tumors (Miyoshi *et al.* 2003). Interestingly, the tissue of breast cancer patients treated with AI has shown elevated STS activity (Sasano *et al.* 2009, Chanplakorn *et al.* 2010, Suzuki *et al.* 2011), a result that has clear implications for the continued development of STS inhibitors. Furthermore, STS activity has also been detected in 97% of ovarian cancer specimens examined (Chura *et al.* 2009). Importantly, this study demonstrated that the median progression-free survival time was significantly longer in patients who had low STS activity compared with those with high STS activity.

The development of STS inhibitors: a brief history

Maltais & Poirier (2011) and Woo *et al.* (2011) have recently published comprehensive reviews covering the previous

research on STS inhibitor development and these works should be consulted for a thorough understanding of STS drug development pipelines. The section here intends to be a shorter historical overview of the attempts made to generate STS inhibitors and will primarily focus on the research performed by Sterix Ltd (London, UK) and Ipsen Ltd (Paris, France).

Efforts to reduce estrogen and androgen concentrations in patients with hormone-dependent cancers have led to the development of a variety of steroidal enzyme inhibitors. The most notable successes come from AIs, such as letrozole and anastrozole, both of which have proven extremely effective in the treatment of breast cancer.

There have been various approaches undertaken to design effective STS inhibitors and they generally create three different categories of compound: alternative substrates (including competitive reversible inhibitors), reversible inhibitors, and irreversible inhibitors. The very first class of STS inhibitor, alternative substrates, examined in the late 1980s, were a series of 2-(hydroxyphenyl) indole sulfates, one of which (see Fig. 3, compound 1) exhibited an IC_{50} of

Figure 3 Examples of STS inhibitors.

80 μM (Birnböck & von Angerer 1990). Since then a number of synthetic and naturally occurring steroids have been identified which have STS-inhibitory activity, the most potent, at 2 μM , being 5-androstene-3 β ,17 β -diol-3-sulfate (Fig. 3, compound 2, Evan *et al.* 1991). However, these alternative substrate STS inhibitors have significant pharmaceutical problems, as they are liable to break down *in vivo* forming unwanted estrogenic metabolites with ER binding affinities with mitogenic activity. Therefore, they are of little clinical value for the treatment of hormone-dependent cancers.

Consequently, the early 1990s saw a concerted effort by various groups to create a potent, reversible STS inhibitor whose breakdown products were unlikely to be estrogenic in nature. Initially, studies focused on the replacement of the sulfate group (OSO_3^-) of E_1S with a range of surrogates or mimics such as phosphate (Anderson *et al.* 1997), phosphonates (Duncan *et al.* 1993, Howarth *et al.* 1994, 2002, Purohit *et al.* 1994), sulfonates (Li *et al.* 1995, Howarth *et al.* 1997), sodium methylenesulfonate (Li *et al.* 1995), sulfonyl halides (Li *et al.* 1993), sulfonamide, and the methylenesulfonyl group (Dibbelt *et al.* 1994, Anderson *et al.* 1997). The majority of these compounds were competitive inhibitors; designed to compete with E_1S for the STS enzyme active site whilst remaining metabolically stable and not acting as a substrate. $\text{E}_1\text{-MTP}$ (Fig. 3, compound 3) was the first compound to be specifically synthesized and to show significant activity. From this, a series of related STS surrogates were designed which included estrone-3-*O*-sulfamate (Fig. 3, compound 4, EMATE).

It was EMATE that emerged as the lead compound in this series with an extremely potent STS inhibitor activity in MCF-7 cells (IC_{50} of 65 pM) (Purohit *et al.* 1995a, 1996a). Further studies demonstrated that EMATE was an irreversible inhibitor and was able to attenuate the growth of E_1S -stimulated nitrosomethylurea (NMU)-induced mammary tumors in ovariectomized rats (Purohit *et al.* 1995b). Surprisingly, and unfortunately, EMATE proved to be estrogenic in rodents, which ruled it out for further pre-clinical testing. However, its discovery led to the design of a range of non-estrogenic STS inhibitors *in vivo* that remain as equipotent as EMATE. Several groups have tested a number of 1–3 ringed non-steroidal based sulfamates (Li *et al.* 1996, Reed *et al.* 1996). Others have investigated a number of modifications made to the A- and D-rings of the steroid estrane nucleus in attempts to reduce the estrogenicity of this class of inhibitor (Li *et al.* 1998, Purohit *et al.* 1998). Importantly, all the compounds that have so far been tested and have similar potency as that of EMATE incorporate an aryl sulfamate ester, which is the active pharmacophore for these compounds (Woo *et al.* 1998).

Attempts to synthesize STS inhibitors without estrogenic properties also resulted in the generation of a group of non-steroidal sulfamates. From this, a series of coumarin sulfamates was synthesized, one of which, 4-methylcoumarin-7-*O*-sulfamate (Fig. 3, compound 5, COUMATE) inhibited STS activity in MCF-7 breast cancer cells by >90% at 10 μM

(Purohit *et al.* 1996b). Further research on a series of tricyclic coumarin sulfamates identified 667 COUMATE (Fig. 3, compound 6, also known as STX64 or BN83495) that was shown to be more potent at STS inhibition compared with EMATE with an IC_{50} of 8 nM in placental microsomes (Purohit *et al.* 1996a, 2000). However, unlike EMATE, 667 COUMATE does not possess the same estrogenic effects when tested *in vitro* and *in vivo* (Purohit *et al.* 2000, 2001). This suggested that this compound should be an excellent pre-clinical/clinical candidate and, indeed, it was the first STS inhibitor to enter clinical trials (see section ‘667 COUMATE clinical trial’).

There have also been studies on a series of 17 β -(*N*-alkylcarbamoyl)-estra-1,3,5(10)triene-3-*O*-sulfamate derivatives. This work identified a steroidal-based, selective STS inhibitor, 17-diisopropylcarbamoyl-1,3,5(10),16-estra-tetraen-3-yl sulfamate, also known as KW-2581 (Fig. 3, compound 7). This compound possesses an STS inhibitory IC_{50} of 4 nM (Ishida *et al.* 2007a) and 2.9 nM (Ishida *et al.* 2008) when biochemically assayed using crude enzyme isolates from recombinant Chinese hamster ovary cells. Despite its steroidal structure, KW-2581, when given orally, did not cause uterine growth in ovariectomized female rats, indicating non-estrogenic properties (Ishida *et al.* 2007a). Furthermore, it was able to inhibit the growth of implanted MCS-2 cells grown in hollow fibres *in vivo* when stimulated with s.c. injections of E_1S or Adiol. Therefore, this compound may be effective at inhibiting both estrogen and androgen stimulated carcinomas. Further studies by the same group demonstrated that KW-2581 was able to inhibit the growth of the hormone receptor-positive human breast cancer cell lines ZR-75-1 and BT-474 (Ishida *et al.* 2007b).

Second- and third-generation STS inhibitors

The development of second-generation STS inhibitors converged on exploring changes of the hydrophobic interactions in the region neighboring the D-ring of EMATE and resulted in several novel D-ring modified derivatives. These *N*-substituted piperidinedione derivatives led to the identification of two, potent, non-estrogenic STS inhibitors, *N*-(propyl) (Fig. 3, compound 8) and *N*-(1-pyridin-3-ylmethyl), which were shown to have exceptionally high potency, both having an IC_{50} value of 1 nM in human placental preparations (Fischer *et al.* 2003a). The *N*-unsubstituted derivatives have a similar STS inhibitory potency to EMATE with an IC_{50} of 20 nM, indicating that this six membered piperidinedione ring is an acceptable mimic of the D-ring of EMATE. Oral dosing at 10 mg/kg per day for 5 days with these compounds inhibited rat liver STS activity by 99% without estrogenic uterine effects (Fischer *et al.* 2003b). Further research on one of these compounds, now termed STX213 (Fig. 3, compound 8), showed it to have a far greater duration of activity at inhibiting rodent liver STS activity compared with 667 COUMATE (12 days compared with 4 days respectively) (Foster *et al.* 2006).

Why there are differences in the duration of STS inhibition by these compounds remains unknown, although three possible reasons have been suggested. 1) The binding affinities of STX213 and 667 COUMATE to erythrocyte carbonic anhydrase II may be different (Lloyd *et al.* 2005), 2) there is a difference in rates of metabolism or breakdown for the two compounds, and/or 3) the extent to which these compounds deposit in adipose tissues is dissimilar.

Dual-targeting STS inhibitors

As discussed previously in this review, there are two pathways for estrogen formation in postmenopausal women, i.e. the aromatase and sulfatase routes. It is apparent from these pathways that aromatase inhibition will fail to block Adiol production (see Fig. 1). This steroid possesses potent estrogenic properties and, consequently, it was proposed that the use of STS inhibitors in combination with AI could be a potentially fruitful strategy to maximize estrogen depletion.

Although it would be possible to administer aromatase and STS inhibitors separately, an alternative, and more pharmacologically favorable approach, would be to develop a single molecule with dual aromatase-sulfatase inhibitor (DASI) properties. Evidence suggests there are clinical advantages to using single-compound therapy compared with a multiple drug approach (Morphy & Rankovic 2005), avoiding as it does drug-drug interaction leading to more straightforward pre-clinical efficacious and toxicity dose testing. Furthermore, as tumors can develop resistance to single-targeted drugs, targeting a second enzyme may overcome or circumvent that resistance.

The initial chemical synthesis of a DASI compound took advantage of a number of flavonoids having aromatase-inhibitory activity (Kellis & Vickery 1984, Ibrahim & Abul-Hajj 1990). Sulfamoylation of this class of compound generated molecules with DASI properties. Therefore, sulfamoylation of 4'-hydroxy and 4',7-dihydroxyisoflavone to give 4'-mono- and 4',7-bis-sulfamates created compounds with STS-inhibitory activity *in vitro* and *in vivo*. Unfortunately, both these compounds were significantly less potent than EMATE (Purohit *et al.* 1999). However, these studies did confirm the proof of principle that DASI development was feasible by altering available AIs. Future work would focus on compounds with more potent aromatase-inhibitory activity properties than these isoflavones.

Consequently, studies have now been undertaken to sulfamoylate third-generation, non-steroidal, AIs such as letrozole and anastrozole (Purohit *et al.* 2003, Woo *et al.* 2003). These compounds accommodate a triazole ring that coordinates reversibly to the heme iron of the aromatase enzyme. This results in a class of compound that are reversible AIs, in contrast to the steroid-based inhibitors, such as exemestane, which act as irreversible inhibitors. This means that these DASI compounds, synthesized by including the STS inhibition pharmacophore, a phenol sulfamate ester, are irreversible STS inhibitors but reversible AIs.

Figure 4 Examples of dual-targeting STS inhibitors.

The first DASI to be created used the very potent and selective AI YM 511 (Fig. 4, compound 1) (Kudoh *et al.* 1995). Tested using JEG-3 choriocarcinoma cells, which possess aromatase and STS activity, YM 511 had an IC₅₀ value of 0.5 nM for aromatase inhibition but had no activity against STS (Woo *et al.* 2003). After sulfamoylation, a *p*-sulfamoyloxybenzyl derivative of YM 511, enhanced STS inhibition (IC₅₀=227 nM) with a reduction in aromatase inhibition (IC₅₀=100 nM). The *m*-bromo derivative of this compound significantly increased both aromatase and STS inhibition (IC₅₀ values: 0.82 and 39 nM respectively). When tested *in vivo*, using a pregnant mares serum gonadotrophin-stimulated ovarian aromatase rat model, this compound gave a 68% inhibition of aromatase and a 98% inhibition of STS activity. This supported the hypothesis that DASI compounds could offer considerable therapeutic benefit for the treatment of hormone-dependent cancer.

Further chemical synthesis of new DASI compounds has explored the *p*-sulfamoylated YM 511 series through introduction of substituents that are considered to be electron donating and/or electron-withdrawing at the positions ortho to the sulfamate group (Jackson *et al.* 2007). The *m*-sulfamoylated series of compounds has yielded interesting derivatives that bear a substituent at the para position of the phenyl ring. From this a potent DASI compound was identified, an *m*-sulfamate derivative (STX681, Fig. 4, compound 2) which was able to inhibit aromatase and sulfatase activity by 82 and 98%, respectively, when given at 10 mg/kg orally to female rats.

New DASI compounds continue to be developed. Most notably a series of letrozole-derived sulfamates, of vorozole-based sulfamates (Wood *et al.* 2011), and of bicyclic based on the AI 4-[(4-bromobenzyl)(4H-1,2,4-triazol-4-yl)amino]benzonitrile (Wood *et al.* 2010). All leads show significant *in vitro* activity against both enzyme targets in JEG-3 cells, but these compounds remain to be tested in pre-clinical models.

Another strategy for dual-acting compounds is to attempt to combine STS inhibition with an anti-estrogenic component. *In vitro* studies have examined one such agent, SR 16157 (Fig. 4, compound 3), which is designed as a sulfamate-containing steroidal STS inhibitor that releases a selective ER α modulator (SERM) upon enzymic reaction with STS (Rasmussen *et al.* 2007). This approach, in theory, will have the potential to inhibit both estrogen biosynthesis and ER action. Investigations with this compound indicated it to have an STS inhibitory IC₅₀ of 100 nM in placental microsomes, slightly higher than EMATE (IC₅₀ = 20 nM). SR 16157 was able to significantly reduce the *in vitro* growth of MCF-7 breast cancer cells stimulated with E₂ and possessed ER binding properties, indicative of SERM activity. Recent *in vivo* toxicological testing of SR 16157 in female rats and beagle dogs demonstrated it possessing good pharmacokinetic and acceptable toxicological profiles (Rausch *et al.* 2011). Unfortunately, at present, there are no published reports of SR 16157's ability to inhibit hormone-dependent breast cancer in a pre-clinical setting and therefore much work still needs to be done before this compound enters clinic.

STS inhibitors and DASI compounds in pre-clinical tumor models

667 COUMATE and beyond

Danazol, a derivative of 17 α -ethinyl-testosterone, was the first compound to be shown to have STS-inhibitory activity *in vivo*. It was used in a rat model of endometriosis, causing an increase in the DHEAS to DHEA ratio, suggesting its action on STS (Carlstrom *et al.* 1984). The identification of EMATE as a potent, irreversible STS inhibitor *in vitro* led to studies to investigate its efficacy *in vivo*. Experiments demonstrated that 10 mg/kg given orally completely inhibited rat liver STS activity (Purohit *et al.* 2000). Furthermore, a single dose was found to inhibit STS activity in liver, brain, adrenal, uterine, and ovarian tissues for up to 3 days with only a 10–15% recovery being detected 7 days after dosing. As the half-life of STS activity is $\sim$ 3–4 days and EMATE is able to inhibit STS for longer than this, it is possible this compound forms a deposit in tissues prolonging its action. However, it is not known whether EMATE and sulfamate-related compounds possess long half-lives in blood.

The use of an NMU-induced mammary tumor model was the first attempt to block cancer growth by STS inhibition. In these studies, EMATE completely inhibited tumor STS activity and successfully blocked the growth of E₁S-stimulated

tumors in these ovariectomized rats (Purohit *et al.* 2000). Some studies have now shown that 2-methoxy derivatives of EMATE are also equipotent STS inhibitors *in vivo* (Raobaikady *et al.* 2003) whilst similar studies have assessed the *in vivo* potency of COUMATE and a series of tricyclic coumarin sulfamates, including 667 COUMATE. The two-ring coumarin sulfamate, COUMATE, at 10 mg/kg dosed daily, resulted in only an 85% inhibition of STS activity with full restoration of STS activity by 7 days (Purohit *et al.* 2006). Therefore, COUMATE is less active *in vivo* than EMATE. In the NMU-induced mammary tumor model, 667 COUMATE caused a significant regression of E₁S-stimulated tumor growth at 10 and 2 mg/kg. Experiments in rats further confirmed that 667 COUMATE was not estrogenic and was therefore selected for clinical trials (see below).

Recently, there has been the development of more specific clinically relevant tumor models to further evaluate STS inhibitors *in vivo*. One study examined the tumorigenicity of MCF-7 breast cancer cells overexpressing STS (MCF-7_{STS}) compared with wild types (MCF-7_{WT}) (James *et al.* 2001). This demonstrated that in ovariectomized nude mice given s.c. injections of E₁S these cells could produce small tumors. However, if E₂S injection was used as substrate then greater tumorigenicity and final growth volume was observed. Consequently, future STS inhibitor pre-clinical studies primarily used E₂S driven MCF-7 xenograft models.

Both 667 COUMATE (at 10 and 20 mg/kg, p.o.) and STX213 (at 10 mg/kg, p.o.) completely inhibited the growth of MCF-7_{STS} tumors and also significantly reduced MCF-7_{WT} tumor growth (Foster *et al.* 2006). At the end of 49 days' oral dosing, the liver and tumor STS activity in these animals was reduced by over 95%. During a recovery period, when the animals did not receive STS inhibitors, compounds retained a prolonged *in vivo* activity and they still attenuated growth. Further work has now demonstrated that STX213 is a more efficacious drug at inhibiting STS liver and tumor activity and at reducing MCF-7_{STS} xenograft development compared to 667 COUMATE. Given as a weekly oral dose at 1 mg/kg, STX213 blocked tumor STS activity and was able to reduce tumor development (Foster *et al.* 2008b). Similar results were also shown in a xenograft estrogen-driven mouse model of endometrial cancer (Foster *et al.* 2008c). Importantly, in both these models, STX213 completely inhibited tumor/liver STS activity and reduced circulating E₂ levels by >90%, again demonstrating its superiority compared with 667 COUMATE. Also in these studies, STX1938, a third-generation STS inhibitor, given weekly at 1 mg/kg, matched the effects observed by STX213.

KW-2581

KW-2581, a steroidal STS inhibitor, has also been tested in a range of *in vivo* tumor models. Studies demonstrate that KW-2581, given daily at an oral dose of 1 mg/kg, inhibits the growth of human breast cancer ZR-75-1 xenografts stimulated with E₁S in ovariectomized female nude mice

(Ishida *et al.* 2007a,b). Further work demonstrated the inhibitory effect of KW-2581 in E₁S-stimulated growth of NMU-induced mammary tumors in rats (Ishida *et al.* 2007a,b). With a daily oral dose of 0.3 and 1 mg/kg this compound inhibited the growth of 50% of the tumors, with lower doses of 0.1 and 0.03 mg/kg having limited effect. The tumor STS activity inversely correlated with the plasma concentration of KW-2581, indicating the importance of maintaining a high plasma concentration of this drug to maximize STS inhibition. These studies also elegantly show that tumors with an elevated STS activity had a greater final tumor volume. This was similar to data published by Foster *et al.* (2006) that demonstrated that tumor overexpression of the STS enzyme resulted in a larger tumor volume at the end of a 49 day study.

DASI

In a pre-clinical study to test the efficacy of the DASI compound STX681, MCF-7 cells overexpressing either the aromatase (MCF-7_{AROM}) or STS (MCF-7_{STS}) enzymes were inoculated into the flanks of ovariectomized nude mice (Foster *et al.* 2008a). Growth of MCF-7_{AROM} cells was stimulated with Adione and that of MCF-7_{STS} cells by E₂S. Results from this study convincingly demonstrated that while STX681 could inhibit the growth of tumor xenografts derived from both cell types, only letrozole inhibited that growth of MCF-7_{AROM} cells and only STX64 inhibited the growth of MCF-7_{STS} cells. Interestingly, the combination of letrozole and 667 COUMATE proved to be no more effective than STX681 alone at inhibiting circulating E₂ levels and the growth of both tumor types.

These studies on the excellent potency of STS inhibitors and DASIs *in vivo* and their effects on the reduction of circulating E₂ levels demonstrates the potential for these types of compounds for the treatment of hormone-dependent breast cancer. However, there are other hormone-dependent malignancies, notably endometriosis, which will benefit from the use of these drugs. Consequently, there is much work still to be done to elucidate the effects of these inhibitors in different *in vivo* models.

667 COUMATE clinical trial

The first ever STS inhibitor to be placed into a Phase I clinical trial in postmenopausal women with hormone-dependent breast cancer was 667 COUMATE (Stanway *et al.* 2006, 2007, Palmieri *et al.* 2011). Patients recruited onto the trial had all previously been given a range of endocrine therapies, mainly anti-estrogens and/or AIs, and some had also received chemo- and radiotherapy. Peripheral blood lymphocyte (PBL) STS activity was used as biomarker allowing measurement of the extent and duration of STS inhibition during the trial. This provided the primary endpoint of the study, to determine the dose of 667 COUMATE that inhibited STS activity by >90% in PBLs. Furthermore, the

ability of 667 COUMATE to inhibit STS in tumor samples obtained from some patients and its effect on serum androstenediol and estrogen concentrations was also measured.

667 COUMATE was administered orally, 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. The drug was well tolerated at 5 and 20 mg. PBL STS activity was inhibited by >90% with a dose of 5 mg and was almost completely abolished at 20 mg. Tumor tissue STS activity was also inhibited by >95% in the 20 mg treatment group. Surprisingly, investigation into the concentration of androstenedione, the main aromatase substrate in postmenopausal women, was also decreased by up to 86%. This suggests that androstenedione is derived from the peripheral conversion of DHEAS and not, as previously thought, by direct secretion from the adrenal cortex. As androstenedione can be converted to E₂ via reactions involving aromatase and 17β-HSD type 1, then reducing androstenedione concentrations may be an added advantage of STS inhibition. Final results from this trial were extremely encouraging. Five out of 14 patients showed evidence of stable disease and all of these five had previously been treated with, and progressed on, AI therapy.

Further, Phase I/II clinical trials on 667 COUMATE (now given the generic name Irosustat), performed by the pharmaceutical group Ipsen Ltd, did not repeat this initial success. In a Phase II endometrial cancer trial Irosustat did not reach the primary endpoints for patients to demonstrate stable disease for 6 months nor was it deemed that Irosustat would prove superior to megestrol acetate, a progesterone derivative commonly administered to patients with advanced endometrial cancer (Ipsen press release). Results from other Phase I/II trials, in metastatic breast and prostate cancer, involving Irosustat, remain to be published. Even if 667 COUMATE does not demonstrate encouraging outcomes in these trials, the future use of STS inhibitors as a treatment option remains positive in many estrogen- and androgen-driven malignancies. Furthermore, in the future, clinical trials combining STS inhibition with AI treatment in order to maximise estrogen deprivation should be seriously considered.

Future directions

It is now over 15 years since the first effective STS inhibitor, EMATE, was developed. During this time significant advances have been made on developing more potent, non-estrogenic compounds that have allowed a greater understanding of the importance of STS in cancer development. With the synthesis of potent second-generation inhibitors and new *in vivo* models, it is now possible to further explore the therapeutic potential of STS inhibitors. 667 COUMATE has shown some encouraging results in a Phase I clinical trial. It still remains to be seen whether a combinational approach of compounds with STS inhibitors, especially the DASI

concept, will prove effective. However, research in the past few years now strongly suggests that STS inhibitors should be effective in treating both estrogen- and androgen-dependent cancers and that this type of therapy will eventually be added to the arsenal of drugs available against a wide range of hormone-dependent cancers.

Overview

After the clinical success of anti-estrogenic compounds and AI, there still remains a significant interest in the development of new drugs that can target hormone-dependent cancers. STS inhibitors are now, after almost two decades of research, at a stage where further clinical trials will determine their therapeutic potential.

The first trial with 667 COUMATE in postmenopausal women showed promise, demonstrating that this drug is an effective STS inhibitor in humans. STS activity was almost completely blocked in PBLs and in most tumor samples that were investigated. However, despite almost complete STS activity inhibition, reductions in serum E_1 and E_2 concentrations remained moderate. Interestingly, serum Adione concentrations were also decreased, strongly indicating the potential effect of STS inhibitors on androgen-dependent cancers.

However, much remains to be done within this field. There are few animal models that have looked into the importance of androgens in various hormone-dependent cancers. One study by Kyowa using KW-2581 demonstrated that this compound could inhibit the ability of AdiolS to stimulate the *in vivo* growth of MCF-7 breast cancer cells overexpressing STS. As already detailed, a potential advantage of an STS inhibitor is its ability to block the formation of Adiol from DHEAS. *In vitro* Adiol has been shown to be a potent stimulator of hormone-dependent breast cancer cell growth and carcinogen-induced mammary tumors in rodents. However, definitive studies in this area remain to be done. The combinational therapeutic approach, e.g. STS inhibition with an anti-estrogen or the DASI concept, also remains to be fully investigated and pre-clinical/clinical trials in this area are the next logical step.

Therefore, it is essential to continue the development of relevant pre-clinical models and to carry out further clinical trials, not only for hormone-dependent cancers, but also in other non-oncological disorders, if the therapeutic potential of STS inhibition is to be medically appreciated.

Declaration of interest

Dr A P is a consultant for Ipsen Ltd. Dr P A F declares that he has no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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