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**Steroid sulfatase: A pivotal player in estrogen synthesis and metabolism**

Atul Purohit<sup>a\*</sup>, L.W. Lawrence Woo<sup>b</sup> and Barry V.L. Potter<sup>b</sup>

<sup>a</sup> Oncology Drug Discovery Group, Section of Investigative Medicine, Hammersmith Hospital, Imperial College London, London W12 0NN, UK

<sup>b</sup> Medicinal Chemistry, Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, UK

\* To whom correspondence should be addressed.

Tel. +44 208 383 4287

Fax +44 208 383 8320

Email: [a.purohit@imperial.ac.uk](mailto:a.purohit@imperial.ac.uk)

Abbreviations: Adione, androstenedione; Adiol, 5-androstenediol; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; E1, estrone; E2, estradiol; E1S, estrone sulfate; ER+, estrogen receptor positive; EST, estrone sulfotransferase; 17 $\beta$ -HSD1, 17 $\beta$ -hydroxysteroid dehydrogenase type 1; 17 $\beta$ -HSD2, 17 $\beta$ -hydroxysteroid dehydrogenase type 2; OATP-B, organic anion transporting polypeptide B; PBL, peripheral blood lymphocytes; PR+, progesterone receptor positive; STS, steroid sulfatase.

Key words: Steroid sulfatase inhibitors; androstenediol; DHEAS; estrogens; breast cancer; Irosustat; BN83495

**Abstract**

Steroid sulfatase plays a pivotal role in regulating the formation of biologically active steroids from inactive steroid sulfates. It is responsible for the hydrolysis of estrone sulfate and dehydroepiandrosterone sulfate to estrone and dehydroepiandrosterone, respectively, both of which can be subsequently reduced to steroids with estrogenic properties (i.e. estradiol and androstenediol) that can stimulate the growth of tumors in hormone-responsive tissues of the breast, endometrium and prostate. Hence, the action of steroid sulfatase is implicated in physiological processes and pathological conditions. It has been five years since our group last reviewed the important role of this enzyme in steroid synthesis and the progress made in the development of potent inhibitors of this important enzyme target. This timely review therefore concentrates on recent advances in steroid sulfatase research, and summarises the findings of clinical trials with Irosustat (BN83495), the only steroid sulfatase inhibitor that is being trialed in postmenopausal women with breast or endometrial cancer.

## Introduction

Breast cancer is the most commonly diagnosed neoplasm in women in the UK, causing over 45,000 new cases and 12,000 deaths annually (Office for National Statistics, 2009). In the 75% of invasive breast cancers that express the estrogen receptor (ER), estrogens are key promoters of tumorigenesis (Yager & Davidson, 2006). It is surprising, therefore, that more than two-thirds of breast cancers occur in postmenopausal women when the ovaries cease to produce estrogen (Pasqualini, 2004). However, despite the 90% reduction in plasma estradiol (E2) levels that occur with the menopause, the tissue concentrations of estrogens in normal breast tissue of pre- and post-menopausal women are comparable (Geisler, 2003; van Landeghem et al, 1985; Thijssen et al, 1986). This reflects extragonadal biosynthesis of estrogens, which occurs in a number of peripheral tissues, including not only breast, but also adipose tissue, muscle, skin and bone (Suzuki et al, 2005). These represent the main sites of estrogen synthesis beyond the menopause (Simpson et al, 1999), which is derived from the conversion of circulating precursor C19 steroids. These include the androgens, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEAS) and androstenedione (Adione), which are primarily of adrenal origin (Couzinet et al, 2001).

The importance of local breast tissue estradiol production in the pathogenesis of breast carcinoma is supported by numerous studies which have reported tumor tissue estrogen levels being 2-20 fold elevated when compared to corresponding plasma levels (van Landeghem et al, 1985; Vermeulen et al, 1986; Pasqualini et al, 1996). In fact, it has been demonstrated that in postmenopausal women, intratumoral estradiol levels are 2-3 fold higher than in areas considered as morphologically normal

(Bonney et al, 1983; Chetrite et al, 2000). This suggests an augmented local tumoral biosynthesis and accumulation of these hormones which is thought to play an important role in the development of hormone-dependent breast cancer. Using in vitro approaches, a number of authors have confirmed the ability of human breast and its neoplasms to locally synthesize estrogens (Perel et al, 1980; Miller et al, 1982; Adams & Li, 1975; Varela & Dao, 1978) and in fact, Yue and co-workers demonstrated that in situ estradiol synthesis predominates over uptake from plasma in the maintenance of elevated intratumoral hormone concentrations (Yue et al, 1998a, 1998b). Although there is overwhelming evidence for intracrine mechanisms of estrogen synthesis and action, the issue of quantitative contribution of local synthesis of estrogenic steroids versus uptake from circulation, remains controversial (Haynes et al, 2010). These authors found a significantly higher concentration of E2 in tumors versus normal tissue which correlated significantly with ER+ status. Other alternative factors which can influence intra-tumoral E2 disposition, such as EST, STS and 17 $\beta$ -HSD1/7, cannot be excluded. However, the improvement in the sensitivity of the assay procedures may explain the discrepancy between the findings of Lønning et al.(2009) and those reported by Thijssen et al. (1987). Clearly, components of both uptake and local synthesis are physiologically important (Yue et al. 1998a).

### **Expression of enzymes involved in synthesis of estrogenic steroids**

A number of estrogen metabolizing enzymes are involved in the local synthesis of estrogens in breast tumors from circulating inactive steroid precursors (Figure 1.) Synthesis of steroids with estrogenic activity occurs via 2 main pathways: the aromatase and steroid sulfatase (STS) pathways. In the former, androstenedione

(Adione) is converted to estrone (E1) by aromatase (Miller et al, 1982). The latter pathway utilizes inactive sulfated steroids, including estrone sulfate (E1S), as precursors. E1S is the predominant estrogen found in the circulation of postmenopausal women (Santner et al, 1984). Although biologically inactive, its long half-life (Ruder et al, 1972) and high serum concentrations (Noel et al, 1981; Pasqualini, 2004) is thought to create a large reservoir which is utilized by steroid sulfatase (STS) for the formation of E1 by desulfonation. Of note, E1S, carrying a net negative charge, is hydrophilic and unable to cross cell membranes. It is thought that its uptake into cells involves specific transporter proteins, and the organic anion transport polypeptide B (OATP-B), has received particular attention in this respect. OATP-B (OATP2B1, *SLCO2B1*) has been reported to be expressed in both normal and neoplastic breast tissue (Al Sarakbi et al, 2006; Alcorn et al, 2002; Pizzagalli et al, 2003; Wlcek et al, 2008). Additionally, immunoreactivity of human liver-specific transporter LST-2 (OATP8, *SLCO1B3*) was reported to be a potent prognostic factor in human breast cancer (Muto et al., 2007). Therefore, breast tumors express the necessary machinery for the uptake and conversion of sulfated steroids into biologically active estrogens. Estrone sulfotransferase (EST), a member of the superfamily of steroid sulfotransferases, is also expressed in breast tissue and opposes the action of STS by sulfating E1 to E1S (Sasano et al, 2009). E1, formed either through the aromatase or sulfatase pathways, is subsequently reduced to the biologically potent estradiol (E2), by 17 $\beta$ -hydroxysteroid dehydrogenase type 1 (17 $\beta$ -HSD1). Oxidation (inactivation) of E2 to E1 is carried out by 17 $\beta$ HSD type 2 (17 $\beta$ HSD2) (Figure 1).

The relevance and interplay between aromatase, STS, OATP-B and 17 $\beta$ HSD1 to facilitate local synthesis of estrogenic steroids within breast and other tissues, is

detailed in several excellent reviews (Nussbaumer & Billich, 2005; Ghosh, 2007; Buono & Cosma, 2010; Lin et al, 2010; Bojarova & Williams, 2008; Suzuki et al, 2009).

Studies examining the expression, immunoreactivity and/or activity of the above proteins have revealed their importance in breast carcinoma. For example, intratumoral aromatase and STS mRNA levels have been shown to be significantly elevated when compared to adjacent non-malignant tissues (Utsumi et al, 1996; Miki et al, 2007; Utsumi et al, 2000; Honma et al, 2006). Indeed, STS activity has been reported to be 10-200 fold higher than that of aromatase in both pre- and postmenopausal breast cancer patients (Pasqualini et al, 1996; Chetrite et al, 2000; Santner et al, 1984). Additionally, STS immunoreactivity is detected more frequently in breast carcinomas (59-88% Suzuki et al, 2003; Tsunoda et al, 2006; Saeki et al, 1999) and compares well with that reported by others: 40-66.7% (Yamamoto et al 2003; Esteban et al, 1992; Santen et al, 1994; Lu et al, 1996; Shenton et al, 1998; Brodie et al, 2001) and it has been estimated that this pathway may be responsible for 10-fold greater intratumoral estradiol production (Santen et al, 1986). STS has also been associated with a number of clinicopathological parameters in breast cancer patients such as tumor size, risk of recurrence, poor prognosis, reduced disease free survival and disease progression, whereas the data for aromatase is less conclusive (Silva et al, 1989; Lipton et al, 1992; Miyoshi et al, 2003; Suzuki et al, 2003, Utsumi et al, 1999; Yoshimura et al, 2004)

The literature also suggests a trend towards elevated OATP-B expression in malignant breast tissue, and higher mRNA levels of this transporter have been linked with increasing tumor grade (Al Sarakbi et al, 2006). Although not necessarily over-

expressed in breast cancer, several immunohistochemical studies have reported 17 $\beta$ -HSD1 expression in approximately 50 to 60% of breast neoplasms (Poutanen et al, 1992; Sasano et al, 1996; Suzuki et al, 2000). Additionally, 17 $\beta$ -HSD1 gene amplification and expression have been associated with poor prognosis (Gunnarsson et al, 2008; Oduwole et al, 2004). By contrast, 17 $\beta$ -HSD2 mRNA or immunoreactivity is frequently not detected in breast carcinomas (Suzuki et al, 2000; Gunnarsson et al, 2001), and low expression, in conjunction with a high levels of 17 $\beta$ -HSD1, has prognostic significance and is associated with higher rates of recurrence in ER positive patients (Gunnarsson et al, 2001; Gunnarsson et al, 2005). From these studies it is clear that 17 $\beta$ -HSD2, by inactivating E2, protects against tumor progression in normal breast tissue.

In recent years, the expression of other isoforms of 17 $\beta$ -HSD such as 17 $\beta$ -HSD7 and 17 $\beta$ -HSD12 has been described in breast cancer tissue and cell lines (Haynes et al, 2010; Day et al, 2009; Shehu et al, 2011). The relative contribution of each of these isoforms to intra-tumoral E2 synthesis remains to be determined. The enzyme kinetic activities of each of these isoforms has not been independently determined. Selective knockdown with siRNA will further highlight the contribution of each isoform. In addition, in contrast to 17 $\beta$ -HSD1/2, the prognostic significance of these recent isoforms remains to be assessed.

## Therapeutic relevance of steroid sulfatase

### (a) Androstenediol: the underappreciated product of the steroid sulfatase pathway

Apart from E1, the STS pathway is also responsible for the production of another steroid with estrogenic properties, namely androstenediol (Adiol). DHEAS, secreted exclusively by the adrenal cortex (Panjari & Davis, 2007), is converted to DHEA by STS, which can subsequently be reduced to Adiol by  $17\beta$ -HSD1. Adiol, although an androgen, can bind to the estrogen receptor, and has been shown to stimulate the proliferation of a number of ER-positive breast cancer cells in an ER-dependent manner (Poulin & Labrie, 1986; Aspinall et al, 2004). Despite its lower affinity for the ER (Poulin & Labrie, 1986), the 100-fold higher circulating concentrations of this hormone have led some to speculate that it may have equipotent estrogenic properties to E2 (Spinola et al, 1986). In vivo rodent models of carcinogen induced mammary carcinomas have demonstrated the ability of Adiol to stimulate tumor growth, even in the presence of aromatase inhibitors, confirming that this hormone does not need to be further aromatized to reveal its estrogenic effects (Dauvois & Labrie, 1989). Billich and colleagues demonstrated for the first time that inhibition of STS blocked DHEAS-stimulated growth of MCF-7 breast cancer cells, an effect which was not reproduced by concurrent treatment with aromatase inhibitors (Billich et al, 2000). This confirms that the STS pathway is responsible for the production of the estrogenic compound Adiol from DHEAS, and that this occurs in an aromatase-independent fashion. This is of clinical significance because in postmenopausal breast cancer patients treated with aromatase inhibitors, unrestrained production of Adiol can occur via the STS pathway and may promote tumor progression. Furthermore, Masamura et al (1995) reported that

ER positive breast cancer cells become sensitized under very low estrogen exposure. Taken together, this could translate to potentiation of the estrogenic effects of Adiol in patients on aromatase inhibitors in whom E2 levels are virtually undetectable. Further support for the role of STS in utilization of DHEAS in human adipose tissue has been recently reported by Dalla Valle et al (2006). These investigators found tissue-specific transcripts and activity of STS in human adipose tissue and corresponding expression of OATPs B, D and E. In line with this, uptake plus desulfation of  $^3\text{H}$ -DHEAS could be measured, whereas sulfotransferase expression was not found. It is interesting to speculate whether excessive fat may therefore provide an important source of estrogenic Adiol. This provides further rationale and motivation for the development of STS inhibitors (reviewed by Woo, L.W et al in this issue, 2010; Reed et al, 2005)

Although much in vitro and in vivo evidence exists regarding the role of DHEAS-derived estrogenic hormones in supporting breast cancer progression, there are few studies investigating their importance in women. A raised serum DHEAS has been demonstrated in postmenopausal women with breast cancer when compared to controls (Aspinall et al, 2003), suggesting that increased adrenal secretion of this androgen may have a role in the pathogenesis of breast cancer, possibly due to its conversion to Adiol. Higher concentrations of DHEAS and DHEA have also been associated with an increased risk of breast cancer in postmenopausal women, especially in ER+/PR+ cases (Key et al, 2002; Dorgan et al, 1997; Missmer et al, 2004; Morris et al, 2001).

At this junction, it is important to emphasise that the affinities of the substrates for the aromatase or STS are very different. For example, the  $K_m$  of androstenedione for aromatase is 8-10 nM whereas that of E1S or DHEAS for STS is 7-14  $\mu\text{M}$ . The

numerical value of  $K_m$  is important as it establishes an approximate value for the intracellular level of the substrate (Segal 1975). There is no physiological sense in maintaining a substrate concentration much lower or much higher than the  $K_m$  as the catalytic potential of the enzyme would be wasted or the enzyme would function inefficiently. The median plasma concentrations of androstenedione and DHEAS in postmenopausal women in our study were 2-4 nM and 1-2  $\mu$ M, respectively (Stanway et al., 2006). These are proportional to the corresponding  $K_m$  values, although the tissue concentrations can be much higher. The physiological relevance of the substrate affinities (i.e.  $K_m$  values) and the substrate concentrations is highlighted by Santner et al. (1984). These authors reported that comparison of STS with aromatase activity in human tumors at physiological levels of substrate revealed that 10 times more E1 was formed from the STS pathway than the aromatase pathway. This finding would hold true even more in those tumors where STS mRNA expression is significantly increased.

#### b) Resistance to endocrine therapy

A common problem with the available endocrine therapies is that of acquired resistance (Musgrove & Sutherland, 2009). There is a growing body of evidence to suggest that hormonal adaptations, and the emergence of alternative intratumoral estrogen production pathways, may contribute to this. Indeed, studies in breast cancer patients treated in the adjuvant setting with aromatase inhibitors or tamoxifen have demonstrated that those with elevated DHEAS were associated with disease progression (Calhoun et al, 2003; Morris et al, 2001). It is tempting to speculate that this may be due to increased synthesis of Andiol, although this was not investigated

directly in these studies. DHEAS levels have also been reported to increase equally with 12 months of adjuvant tamoxifen and letrozole treatment, again supporting the notion of a compensatory increase in the production of this adrenal androgen to overcome the endocrine blockade (Rossi et al, 2009).

Very recently, in elegant studies carried out by Chanplakorn et al (2010), it has been reported that neoadjuvant treatment with exemestane caused a significant increment in intratumoral  $17\beta$ -HSD1 and STS immunoreactivity, suggesting an upregulation of estrogen synthesizing enzymes in response to estrogen depletion. Exemestane was given daily at 25mg/day for 16-24 weeks to 116 Japanese postmenopausal patients with primary invasive ductal carcinoma. Status of STS, EST,  $17\beta$ -HSD1, ER, PR, Her2 and Ki67 in 49 pre- and post-exemestane specimens was evaluated by immunohistochemistry. A significant increase in STS and  $17\beta$ -HSD1 immunohistological scoring following AI neoadjuvant therapy was demonstrated for the first time. The authors hypothesise that this increase in STS and  $17\beta$ -HSD1 may be a compensatory response of the breast tumors to estrogen depletion, particularly as the significant increment was only detected in the group associated with decreased Ki67 labelling index. The same researchers also demonstrated increased intratumoral  $5\alpha$ -dihydrotestosterone (DHT) concentration and  $17\beta$ -HSD2 expression following exemestane therapy (Takagi et al, 2010). The important role of androgens in apocrine breast cancer is reviewed by Suzuki et al (2010).

In vitro, cancer cells exposed to a long-term E2-deprived environment, adapted by up-regulation of signalling pathways involving ER $\alpha$ , HER-2/neu, EGFR and IGFR. These pathways signal through MAPK, PI3K and mTOR and the cross-talk between these

pathways is believed to drive proliferation. These pathways have also been shown to be activated in in vivo models of development of resistance to aromatase inhibitors by the group of Angela Brodie. A combination of trastuzumab with letrozole is found to be superior to the aromatase inhibitor alone in these xenograft models (reviewed in: Santen et al. 2009).

### **Lessons learnt from clinical trials with enzyme inhibitors**

Many clinical trials have now been carried out with the third-generation aromatase inhibitors but so far only one phase I trial with a STS inhibitor has been conducted (Smith & Dowsett, 2003; Coombes et al. 2004; Stanway et al. 2006). Measurement of serum oestrogen concentrations by RIA, employed as a surrogate marker of the effectiveness of AIs, is not straight forward and in some early studies it was difficult to detect the real effects of aromatase inhibitors from such measurements. More recently, very sensitive RIAs have been developed involving solvent extraction and chromatographic separation of oestrogens prior to RIA (Lonning & Ekse, 1995; Lonning et al, 1997). However, a 'gold standard' GC-MS/MS method has now been developed for the measurement of serum oestrogens (Sundaram et al, 2003). Using highly sensitive RIAs, there is no doubt that in most cases, plasma or serum levels of E1 and E2 are suppressed to below the limits of quantitation of the assays by third-generation aromatase inhibitors (Geisler et al, 2002). For E1S, while levels has been found to be suppressed by >98% by third-generation aromatase inhibitors, the geometric mean E1S concentrations after 16 weeks treatment with letrozole was 3.9pmol/l (Geisler et al, 2008). As most patients treated with aromatase inhibitors will eventually progress, it is possible that the low levels of E1S still detectable may

contribute to tumor cells becoming resistant to this form of therapy. Although these plasma levels of E1S are very low it is now well documented that breast cancer cells, grown in an estrogen-deprived environment, can become sensitive to extremely low estrogen concentrations (Masamura et al, 1995).

In contrast to the problems associated with measuring aromatase activity in patients, the effects of STS inhibitors can be readily assessed. STS is present in peripheral blood lymphocytes (PBLs) and the extent and duration of STS inhibition can be readily determined by measuring its activity in these cells (Purohit et al, 1995). In the first ever phase I trial of Irosustat (STX64, BN83495), STS activity, as measured in PBLs, was suppressed by >95% at the 5 mg/day and 20 mg/day doses tested (Stanway et al, 2006). This level of STS inhibition was associated with moderate, but significant, reductions in the median concentrations of E1 (57-76%), E2 (38-39%) and testosterone (27-30%). In addition, the median concentration of the steroid with oestrogenic properties, Adiol, decreased by 70-74%. Unexpectedly, serum Adione median concentrations also decreased by 62-72% indicating that, at least in postmenopausal women, a significant proportion of this steroid is derived from the peripheral conversion of DHEAS. The results from the STX64 phase I trial therefore show that while median serum concentrations of Adiol, Adione and E1 all decreased by approximately 70%, the reductions for testosterone and E2 were less, at about 30%. Similar results were obtained in a second dose-escalation study of Irosustat in postmenopausal women with ER+ve metastatic breast cancer (Coombes et al, 2009). Patients were recruited into 5 sequential dose cohorts (1, 5, 20, 40 and 80mg). The optimum biological dose was determined to be 40mg. At this dose, Adiol concentrations decreased by 34-74% and E2 concentrations decreased by 7-27%.

Disease stabilization of 7-13 months was demonstrated in 3 of 14 patients who received >3 months treatment. Considering that the aromatase pathway of estrogen synthesis was not inhibited in these patients, the reduction in serum hormone concentrations and the disease stabilization obtained in some patients, were very encouraging. Currently, Phase II studies in women with endometrial cancer are in progress ([www.ipсен.com](http://www.ipсен.com)). Endometrial cancer is the most common gynecological malignancy with an unmet need for better therapy. When measured by validated mass spectrometry assays, circulating levels of E1, E2 and E1S were found to be significantly higher in women with endometrial cancer when compared with unaffected controls. Enhanced E2 synthesis in tumors was supported by increased expression of STS and 17 $\beta$ -HSD1 in peritumoral normal endometrium. The expression of these enzymes was significantly increased in tumors (Lepine et al, 2010) again highlighting the importance of this pathway for the synthesis of estrogenic steroids.

### **Summary and Future perspectives**

Steroid sulfates are now acknowledged to have an important role as prohormones for the formation of biological active steroids. In recent years, a wealth of evidence has emerged, particularly from the Japanese groups of H. Sasano, T. Suzuki and N. Harada, strongly supporting the important role of STS expression and immunoreactivity in breast cancer progression and in the development of resistance to endocrine therapy. Although much work has been carried out to characterize the expression of the main enzymes involved in the synthesis of estrogenic steroids breast and other cancers, most studies have investigated these genes in isolation. It is essential to study the simultaneous expression of these genes, and compare this to the level in normal tissue, in an attempt to improve our understanding of how these proteins are expressed in

concert and how their levels may be altered in the tumor microenvironment. Information from such studies may also facilitate the identification and selection of patients who are most likely to benefit from treatment with STS inhibitors. In addition, important structural information derived from the x-ray crystallographic studies of STS pioneered by D. Ghosh and co-workers, will aid to design novel inhibitors.

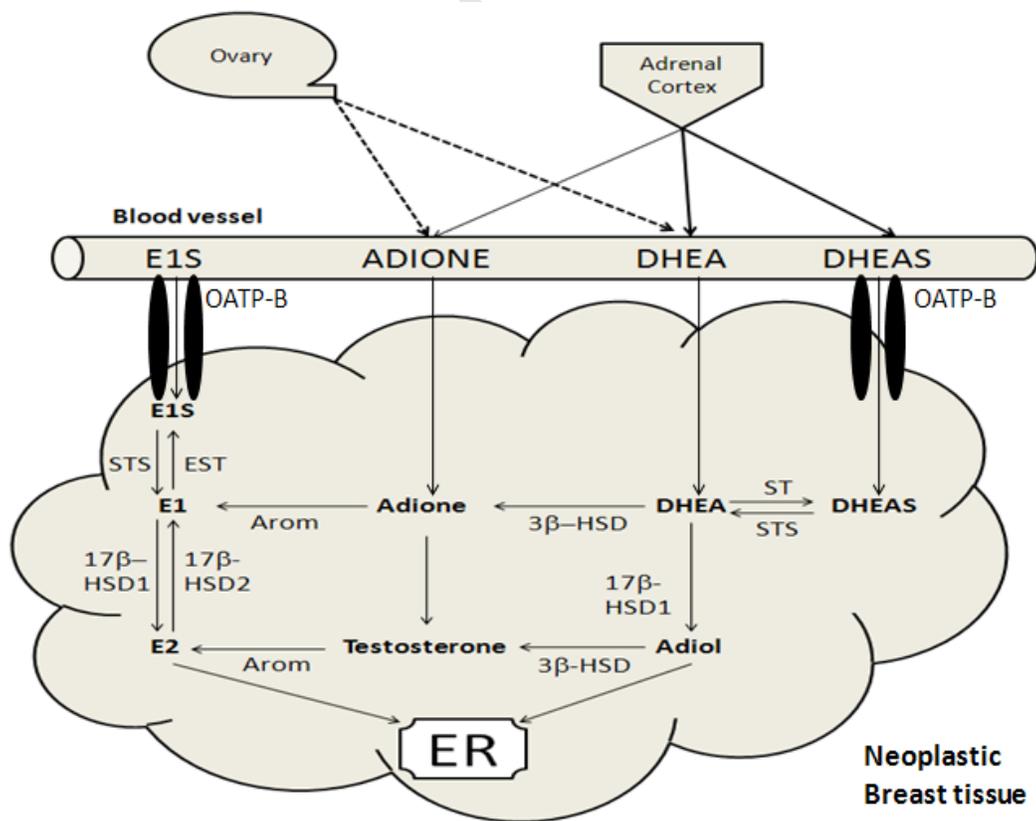
Currently, Phase I clinical trials of Irusustat are in progress in women with advanced breast or endometrial cancer. Additional phase II/III trials will be required to confirm whether STS inhibitors are to have a place in the armory against breast and other hormone-dependent cancers. Future trials of STS inhibitors in combination with aromatase inhibitors, or other agents including dual-target inhibitors (Woo et al, 2010), will be required to determine whether such combinations offer any advantage over the use of single-agent therapy. The trend in breast cancer therapy is towards personalised medicine. Therefore, patient enrichment by evaluation of the expression of aromatase, STS and other enzymes and receptors in tumor tissue by immunostaining and/or combined laser capture microdissection/qRT-PCR will be essential. These techniques have been successfully developed and thoroughly validated in the laboratories of Drs. Sasano and Harada (Sasano et al. 2009).

Although this review has focused on the potential use of STS inhibitors for the treatment of hormone-dependent cancers, they could also have therapeutic efficacy in a number of other conditions that still remain to be explored. STS is ubiquitously distributed throughout the body and may have important roles in regulating the production of androgens in a number of skin conditions (Reed et al, 2008) and part of the immune response (Rook et al. 1994; Reed et al. 2003). In addition, little is known about the role of STS in normal male and female reproduction although there is

evidence that STS inhibitors could be effective in conditions such as endometriosis (Purohit et al. 2008). With the advent of potent STS inhibitors it will, for the first time, be possible to explore their therapeutic potential in a wide range of normal and abnormal conditions.

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## References

- Adams JB, Li K 1975 Biosynthesis of 17 $\beta$ -oestradiol in human breast carcinoma tissue and a novel method for its characterization. *Br J Cancer* 31:429-33
- Al Sarakbi W, Mokbel R, Salhab M, Jiang WG, Reed MJ, Mokbel K 2006 The role of STS and OATP-B mRNA expression in predicting the clinical outcome in human breast cancer. *Anticancer Res* 26:4985-4990
- Alcorn J, Lu X, Moscow JA, McNamara PJ 2002 Transporter gene expression in lactating and nonlactating human mammary epithelial cells using real-time reverse transcription-polymerase chain reaction. *J Pharmacol Exp Ther* 303:487-496
- Aspinall SR, Cook DB, Shenton BK, Griffiths AB, Griffith CD, Bliss RD, Lennard TW 2003 Serum adrenal androgens in women with primary operable breast cancer and their relationship with age and body mass index. *Breast* 12:63-71
- Aspinall SR, Stamp S, Davison A, Shenton BK, Lennard TW 2004 The proliferative effects of 5-androstene-3  $\beta$ ,17  $\beta$ -diol and 5  $\alpha$ -dihydrotestosterone on cell cycle analysis and cell proliferation in MCF7, T47D and MDAMB231 breast cancer cell lines. *J Steroid Biochem Mol Biol* 88:37-51
- Billich A, Nussbaumer P, Lehr P 2000 Stimulation of MCF-7 breast cancer cell proliferation by estrone sulfate and dehydroepiandrosterone sulfate: inhibition by novel non-steroidal steroid sulfatase inhibitors. *J Steroid Biochem Mol Biol* 73:225-235
- Bojarova P, Williams SJ 2008 Sulfotransferases, sulfatases and formylglycine-generating enzymes: a sulfation fascination. *Curr Opin Chem Biol* 12, 573-581
- Bonney RC, Reed MJ, Davidson K, Beranek PA, James VH 1983 The relationship between 17  $\beta$ -hydroxysteroid dehydrogenase activity and oestrogen concentrations in human breast tumours and in normal breast tissue. *Clin Endocrinol (Oxf)* 19:727-739
- Brodie AM, Lu Q, Long BJ, Fulton A, Chen T, Macpherson N, DeJong PC, Blankenstein MA, Nortier JW, Slee PH, van de Ven J, van Gorp JM, Elbers JR, Schipper ME, Blijham GH, Thijssen JH 2001 Aromatase and COX-2 expression in human breast cancers. *J Steroid Biochem Mol Biol* 79:41-47
- Buono M, Cosma MP 2010 Sulfatase activities towards the regulation of cell metabolism and signaling in mammals. *Cell Mol Life Sci* 67:769-780
- Calhoun K, Pommier R, Cheek J, Fletcher W, Toth-Fejel S 2003 The effect of high dehydroepiandrosterone sulfate levels on tamoxifen blockade and breast cancer progression. *Am J Surg* 185:411-415
- Chanplakorn N, Chanplakorn P, Suzuki T, Ono K, Chan MSM, Miki Y, Saji S, Ueno T, Toi M, Sasano H 2010 Increased estrogen sulfatase (STS) and 17 $\beta$ -hydroxysteroid dehydrogenase type 1 (17 $\beta$ -HSD1) following neoadjuvant aromatase inhibitor therapy in breast cancer patients. *Breast Cancer Res Treat* 120:639-648

Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR 2000 Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. *J Steroid Biochem Mol Biol* 72:23-27

Coombes R, Schmid P, Isambert N, Soulie P, Cardoso F, Besse-Hammer T, Lesimple T, Slosman D, Kornowski A, Fohanno V, Fumoleau 2009 A phase 1 dose escalation study of steroid sulfatase inhibitor BN83495/STX64 in postmenopausal women with ER positive breast cancer. *Cancer Res.* 69:Abstract number 4097

Coombes RC, Hall E, Gibson LJ Coombes RC, Hall E, Gibson LJ, Paridaens R, Jassem J, Delozier T, Jones SE, Alvarez I, Bertelli G, Ortmann O, Coates AS, Bajetta E, Dodwell D, Coleman RE, Fallowfield LJ, Mickiewicz E, Andersen J, Lønning PE, Cocconi G, Stewart A, Stuart N, Snowdon CF, Carpentieri M, Massimini G, Bliss JM, van de Velde C; Intergroup Exemestane Study 2004 A randomised trial of exemestane after two to three years of tamoxifen therapy in postmenopausal women with primary breast cancer. *New Eng J Med.* 350:1081-1092

Couzinet B, Meduri G, Lecce MG, Young J, Brailly S, Loosfelt H, Milgrom E, Schaison G 2001 The postmenopausal ovary is not a major androgen-producing gland. *J Clin Endocrinol Metab* 86:5060-5066

Dalla Valle L, Toffolo V, Nardi A, Fiore C, Bernante P, Di Liddo R, Parnigotto PP, Colombo L 2006 Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate uptake and intracrine steroid activations in human adipose tissue. *J. Endocrinol.* 190:129-139

Dauvois S, Labrie F 1989 Androstenedione and androst-5-ene-3 beta,17 beta-diol stimulate DMBA-induced rat mammary tumors--role of aromatase. *Breast Cancer Res Treat.*13:61-69

Day JM, Foster PA, Tutill HJ, Parsons MF, Newman SP, Chander SK, Allan GM, Lawrence HR, Vicker N, Potter BV, Reed MJ, Purohit A 2008 17beta-hydroxysteroid dehydrogenase Type 1, and not Type 12, is a target for endocrine therapy of hormone-dependent breast cancer. *Int J Cancer* 122:1931-40

Dorgan JF, Stanczyk FZ, Longcope C, Stephenson HE,Jr, Chang L, Miller R, Franz C, Falk RT, Kahle L 1997 Relationship of serum dehydroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3 beta, 17 beta-diol to risk of breast cancer in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 6:177-181

Esteban JM, Warsi Z, Haniu M, Hall P, Shively JE, Chen S 1992 Detection of intratumoral aromatase in breast carcinomas. An immunohistochemical study with clinicopathologic correlation. *Am J Pathol* 140:337-343

Geisler J 2003 Breast cancer tissue estrogens and their manipulation with aromatase inhibitors and inactivators. *J Steroid Biochem Mol Biol* 86:245-253

Geisler J, Haynes B, Anker G, Dowsett M, Lonning PE 2002 Influence of letrozole and anastrozole on plasma estrogen levels in postmenopausal patients evaluated in a randomised cross-over study. *J Clin Oncol.* 20:751-757

Geisler J, Helle H, Ekse D, Duong NK, Evans DB, Nordbo Y, Aas T, Lonning PE 2008 Letrozole is superior to anastrozole in suppressing breast cancer tissue and plasma estrogen levels. *Clin Cancer Res*. 14:6330-6335

Ghosh D 2007 Human sulfatases: A structural perspective to catalysis. *Cell Mol Life Sci*. 64:2013-2022

Gunnarsson C, Hellqvist E, Stal O 2005 17beta-Hydroxysteroid dehydrogenases involved in local oestrogen synthesis have prognostic significance in breast cancer. *Br J Cancer* 92:547-552

Gunnarsson C, Jerevall PL, Hammar K, Olsson B, Nordenskjold B, Jansson A, Stal O 2008 Amplification of HSD17B1 has prognostic significance in postmenopausal breast cancer. *Breast Cancer Res Treat* 108:35-41

Gunnarsson C, Olsson BM, Stal O, Southeast Sweden Breast Cancer Group 2001 Abnormal expression of 17beta-hydroxysteroid dehydrogenases in breast cancer predicts late recurrence. *Cancer Res* 61:8448-8451

Haynes BP, Straume AH, Geisler J, A'Hern R, Helle H, Smith IE, Lonning PE, Dowsett M 2010 Intratumoral estrogen disposition in breast cancer. *Clin Cancer Res* 16:1790-1801

Honma N, Takubo K, Sawabe M, Arai T, Akiyama F, Sakamoto G, Utsumi T, Yoshimura N, Harada N 2006 Estrogen-metabolizing enzymes in breast cancers from women over the age of 80 years. *J Clin Endocrinol Metab* 91:607-613

Key T, Appleby P, Barnes I, Reeves G, Endogenous Hormones and Breast Cancer Collaborative Group 2002 Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 94:606-616

Lépine J, Audet-Walsh E, Grégoire J, Têtu B, Plante M, Ménard V, Ayotte P, Brisson J, Caron P, Villeneuve L, Bélanger A, Guillemette C 2010 Circulating estrogens in endometrial cancer cases and their relationship with tissular expression of key estrogen biosynthesis and metabolic pathways. *J Clin Endocrinol Metab*. 95:2689-98

Lin S, Chen J, Mazumdar M, Poirier D, Wang C, Azzi A, Zhou M 2010 Molecular therapy of breast cancer: progress and future directions. *Nat. Rev. Endocrinol*. 6:485-493

Lipton A, Santen RJ, Santner SJ, Harvey HA, Sanders SI, Matthews YL 1992 Prognostic value of breast cancer aromatase. *Cancer* 70:1951-1955

Lonning PE, Ekse D 1995 A sensitive assay for the measurement of estrone sulphate in patients on treatment with aromatase inhibitors. *J Steroid Biochem Mol Biol*. 55:409-412

- Lonning PE, Geisler J, Johannessen DC, Ekse D 1997 Plasma estrogen suppression with aromatase inhibitors evaluated by a novel sensitive assay for estrone sulphate. *J Steroid Biochem Mol Biol.* 61:255-260
- Lonning PE, Helle H, Duong NK, Ekse D, Aas T, Geisler J 2009 Tissue estradiol is selectively elevated in receptor positive breast cancers while tumour estrone is reduced independent of receptor status. *J Steroid Biochem Mol Biol.* 117:31-41
- Lu Q, Nakamura J, Savinov A, Yue W, Weisz J, Dabbs DJ, Wolz G, Brodie A 1996 Expression of aromatase protein and messenger ribonucleic acid in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancers. *Endocrinology* 137:3061-3068
- Masamura S, Santner SJ, Heitjan, Santen RJ 1995 Estrogen deprivation causes estradiol hypersensitivity in human breast cancer cells. *J Clin Endocrinol Metab.* 80:2918-2925
- Miki Y, Suzuki T, Tazawa C, Yamaguchi Y, Kitada K, Honma S, Moriya T, Hirakawa H, Evans DB, Hayashi S, Ohuchi N, Sasano H 2007 Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells. *Cancer Res* 67:3945-3954
- Miller WR, Hawkins RA, Forrest AP 1982 Significance of aromatase activity in human breast cancer. *Cancer Res* 42:3365s-3368s
- Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE 2004 Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. *J Natl Cancer Inst* 96:1856-1865
- Miyoshi Y, Ando A, Hasegawa S, Ishitobi M, Taguchi T, Tamaki Y, Noguchi S 2003 High expression of steroid sulfatase mRNA predicts poor prognosis in patients with estrogen receptor-positive breast cancer. *Clin Cancer Res* 9:2288-2293
- Morris KT, Toth-Fejel S, Schmidt J, Fletcher WS, Pommier RF 2001 High dehydroepiandrosterone-sulfate predicts breast cancer progression during new aromatase inhibitor therapy and stimulates breast cancer cell growth in tissue culture: a renewed role for adrenalectomy. *Surgery* 130:947-953
- Musgrove EA, Sutherland RL 2009 Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer* 9:631-643
- Muto M, Onogawa T, Suzuki T, Ishada T, Rikiyama T, Katayose Y, Ohuchi N, Sasano H, Abe T, Unno M 2007 Human liver-specific organic anion transporter-2 is a potent prognostic factor for human breast carcinoma. *Cancer Sci.* 98:1570-1576
- Noel CT, Reed MJ, Jacobs HJ, James VHT 1981 The plasma concentration of oestrone sulphate in postmenopausal women: lack of diurnal variation, effect of ovariectomy, age and weight. *J Steroid Biochem.* 14:1101-1105.

Nussbaumer P, Billich A 2005 Steroid sulfatase inhibitors: Their potency in the therapy of breast cancer. *Curr Med Chem – Anticancer Agents*. 5:507-528

Oduwole OO, Li Y, Isomaa VV, Mantyniemi A, Pulkka AE, Soini Y, Vihko PT 2004 17beta-Hydroxysteroid Dehydrogenase Type 1 is an Independent Prognostic Marker in Breast Cancer. *Cancer Res* 64:7604-7609

Office for National Statistics 2009 Cancer Registration Statistics, England, 2007

Panjari M, Davis SR 2007 DHEA therapy for women: effect on sexual function and wellbeing. *Hum Reprod Update* 13:239-248

Pasqualini JR 2004 The selective estrogen enzyme modulators in breast cancer: a review. *Biochim Biophys Acta* 1654:123-143

Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M, Maloche C 1996 Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. *J Clin Endocrinol Metab* 81:1460-1464

Perel E, Wilkins D, Killinger DW 1980 The conversion of androstenedione to estrone, estradiol, and testosterone in breast tissue. *J Steroid Biochem*.13:89-94

Pizzagalli F, Varga Z, Huber RD, Folkers G, Meier PJ, St-Pierre MV 2003 Identification of steroid sulfate transport processes in the human mammary gland. *J Clin Endocrinol Metab* 88:3902-3912

Poulin R, Labrie F 1986 Stimulation of cell proliferation and estrogenic response by adrenal C19-delta 5-steroids in the ZR-75-1 human breast cancer cell line. *Cancer Res* 46:4933-4937

Poutanen M, Isomaa V, Lehto VP, Vihko R 1992 Immunological analysis of 17 beta-hydroxysteroid dehydrogenase in benign and malignant human breast tissue. *Int J Cancer*.50:386-390

Purohit A, Fusi L, Brosens J, Woo LWL, Potter BVL, Reed MJ 2008 Inhibition of steroid sulphatase activity in endometriotic implants by 667 Coumate: a potential new therapy. *Human Reprod*. 23:290-297

Purohit A, Williams GJ, Roberts CJ, Potter BVL, Reed MJ 1995 In vivo inhibition of oestrone sulphatase and dehydroepiandrosterone sulphatase by oestrone-3-O-sulphamate. *Int J Cancer* 63:106-111

Reed MJ, Purohit A, Singh A, Chander SK 2003 Androgenic modulation of the immune response. In *Menopause- the state of the art* pp 23-27 Ed HPG Schneider London: Parthenon Publishing Group

Reed MJ, Purohit A, Woo LW, Newman SP, Potter BV 2005 Steroid sulfatase: molecular biology, regulation, and inhibition. *Endocr Rev* 26:171-202

Reed MJ, Purohit A, Woo LWL, Potter BVL 2008 Steroid sulfatase inhibitors for the topical treatment of skin disorders. *Drugs Future* 33:597-606

Rook GAW, Hernandez-Pando R, Lightman S 1994 Hormones, peripherally activated prohormones and regulation of Th1/Th2 balance. *Immunol Today* 15:301-303

Rossi E, Morabito A, Di Rella F, Esposito G, Gravina A, Labonia V, Landi G, Nuzzo F, Pacilio C, De Maio E, De Maio M, Piccirillo MC, De Feo G, D'Aiuto G, Botti G, Chiodini P, Gallo C, Perrone F, de Matteis A 2009 Endocrine effects of adjuvant letrozole compared with tamoxifen in hormone-responsive postmenopausal patients with early breast cancer: the HOBEO trial. *J Clin Oncol.* 27:3192-3197

Ruder HJ, Loriaux L, Lipsett MB 1972 Estrone sulfate: production rate and metabolism in man. *J Clin Invest* 51:1020-1033

Saeki T, Takashima S, Sasaki H, Hanai N, Salomon DS 1999 Localization of Estrone Sulfatase in Human Breast Carcinomas. *Breast Cancer* 6:331-337

Santen RJ, Leszczynski D, Tilson-Mallet N, Feil PD, Wright C, Manni A, Santner SJ 1986 Enzymatic control of estrogen production in human breast cancer: relative significance of aromatase versus sulfatase pathways. *Ann N Y Acad Sci* 464:126-137

Santen RJ, Martel J, Hoagland M, Naftolin F, Roa L, Harada N, Hafer L, Zaino R, Santner SJ 1994 Stromal spindle cells contain aromatase in human breast tumors. *J Clin Endocrinol Metab* 79:627-632

Santen RJ, Brodie H, Simpson ER, Siiteri PK, Brodie A 2009 History of aromatase: Saga of an important biological mediator and therapeutic target. *Endocr Rev* 30:343-375

Santner SJ, Feil PD, Santen RJ 1984 In situ estrogen production via the estrone sulfatase pathway in breast tumors: relative importance versus the aromatase pathway. *J Clin Endocrinol Metab* 59:29-33

Sasano H, Frost AR, Saitoh R, Harada N, Poutanen M, Vihko R, Bulun SE, Silverberg SG, Nagura H 1996 Aromatase and 17 beta-hydroxysteroid dehydrogenase type 1 in human breast carcinoma. *J Clin Endocrinol Metab* 81:4042-4046

Sasano H, Nagasaki S, Miki Y, Suzuki T 2009 New developments in intracrinology of human breast cancer: estrogen sulfatase and sulfotransferase. *Ann N Y Acad Sci* 1155:76-79

Sasano H, Miki Y, Nagasaki S, Suzuki T 2009 In situ estrogen production and its regulation in human carcinoma: From endocrinology to intracrinology. *Pathol Int* 59, 777-789

Segel, I.H. 1975 *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*. John Wiley & Sons, New York. p34

Shehu A, Albarracin C, Devi SY, Luther K, Halperin J, Le J, Mao J, Duan RW, Frasor J, Gibori G 2011 The stimulation of HSD17B7 expression by estradiol provides a powerful feed-forward mechanism for estradiol biosynthesis in breast cancer cells. *Mol Endocrinol* 25:754-766

Shenton KC, Dowsett M, Lu Q, Brodie A, Sasano H, Sacks NP, Rowlands MG 1998 Comparison of biochemical aromatase activity with aromatase immunohistochemistry in human breast carcinomas. *Breast Cancer Res Treat* 49 Suppl 1:S101-7; discussion S109-119

Silva MC, Rowlands MG, Dowsett M, Gusterson B, McKinna JA, Fryatt I, Coombes RC 1989 Intratumoral aromatase as a prognostic factor in human breast carcinoma. *Cancer Res* 49:2588-2591

Simpson E, Rubin G, Clyne C, Robertson K, O'Donnell L, Davis S, Jones M 1999 Local estrogen biosynthesis in males and females. *Endocr Relat Cancer* 6:131-137

Smith IE, Dowsett M 2003 Aromatase inhibitors in breast cancer. *New Eng J Med*. 348:2431-2442

Spinola PG, Marchetti B, Labrie F 1986 Adrenal steroids stimulate growth and progesterone receptor levels in rat uterus and DMBA-induced mammary tumors. *Breast Cancer Res Treat* 8:241-248

Stanway SJ, Purohit A, Woo LW, Sufi S, Vigushin D, Ward R, Wilson RH, Stanczyk FZ, Dobbs N, Kulinskaya E, Elliott M, Potter BV, Reed MJ, Coombes RC 2006 Phase I study of STX 64 (667 Coumate) in breast cancer patients: the first study of a steroid sulfatase inhibitor. *Clin Cancer Res* 12:1585-1592

Sundaram B, Settlage JA, Ohorodnik SK, Taylor PA 2003 A combined GC/MS/MS and LC/MS/MS bioanalytical method for the quantification of estradiol, estrone, estrone sulfate, testosterone and androstenedione. 51st ASMS Conference on Mass Spectrometry and Allied Topics, Montreal, Canada

Susuki T, Miki Y, Takagi K, Hirakawa H, Moriya T, Ohuchi N, Sasano H 2010 Androgens in human breast carcinoma. *Med. Mol. Morphol.* 43:75-81

Suzuki M, Ishida H, Shiotsu Y, Nakata T, Akinaga S, Takashima S, Utsumi T, Saeki T, Harada N 2009 Expression level of enzymes related to in situ estrogen synthesis and clinicopathological parameters in breast cancer patients. *J Steroid Biochem Mol Biol.* 113:195-201

Suzuki T, Miki Y, Nakamura Y, Moriya T, Ito K, Ohuchi N, Sasano H 2005 Sex steroid-producing enzymes in human breast cancer. *Endocr Relat Cancer* 12:701-720

Suzuki T, Moriya T, Ariga N, Kaneko C, Kanazawa M, Sasano H 2000 17 $\beta$ -hydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters. *Br J Cancer* 82:518-523

Suzuki T, Nakata T, Miki Y, Kaneko C, Moriya T, Ishida T, Akinaga S, Hirakawa H, Kimura M, Sasano H 2003 Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. *Cancer Res* 63:2762-2770

Takagi K, Miki Y, Nagasaki S, Hirakawa H, Onodera Y, Akahira J, Ishida T, Watanabe M, Kimijima I, Hayashi S, Sasano H, Suzuki T 2010 Increased intratumoral androgens in human breast carcinoma following aromatase inhibitor exemestane treatment. *Endocrine – Related Cancer* 17:415-430

Thijssen JH, van Landeghem AA, Poortman J 1986 Uptake and concentration of steroid hormones in mammary tissues. *Ann N Y Acad Sci* 464:106-116

Thijssen JHH, Blankenstein MA, Miller WR, Milewicz A 1987 Estrogens in tissues: uptake from the peripheral circulation or local production. *Steroids* 50:297-306

Tsunoda Y, Shimizu Y, Tsunoda A, Takimoto M, Sakamoto MA, Kusano M 2006 Steroid sulfatase in breast carcinoma and change of serum estrogens levels after operation. *Acta Oncol* 45:584-589

Utsumi T, Harada N, Maruta M, Takagi Y 1996 Presence of alternatively spliced transcripts of aromatase gene in human breast cancer. *J Clin Endocrinol Metab* 81:2344-2349

Utsumi T, Yoshimura N, Takeuchi S, Ando J, Maruta M, Maeda K, Harada N 1999 Steroid sulfatase expression is an independent predictor of recurrence in human breast cancer. *Cancer Res* 59:377-381

Utsumi T, Yoshimura N, Takeuchi S, Maruta M, Maeda K, Harada N 2000 Elevated steroid sulfatase expression in breast cancers. *J Steroid Biochem Mol Biol* 73:141-145

van Landeghem AA, Poortman J, Nabuurs M, Thijssen JH 1985 Endogenous concentration and subcellular distribution of estrogens in normal and malignant human breast tissue. *Cancer Res* 45:2900-2906

Varela RM, Dao TL 1978 Estrogen synthesis and estradiol binding by human mammary tumors. *Cancer Res* 38:2429-2433

Vermeulen A, Deslypere JP, Paridaens R, Leclercq G, Roy F, Heuson JC 1986 Aromatase, 17 beta-hydroxysteroid dehydrogenase and intratumoral sex hormone concentrations in cancerous and normal glandular breast tissue in postmenopausal women. *Eur J Cancer Clin Oncol* 22:515-525

Wlcek K, Svoboda M, Thalhammer T, Sellner F, Krupitza G, Jaeger W 2008 Altered expression of organic anion transporter polypeptide (OATP) genes in human breast carcinoma. *Cancer Biol Ther* 7:1450-1455

Woo LWL, Jackson T, Putey A, Cozier G, Leonard P, Acharya KR, Chander SK, Purohit A, Reed MJ, Potter BVL 2010 Highly potent first examples of dual aromatase – steroid sulfatase inhibitors based on a biphenyl template. *J Med Chem*. 53:2155-2170

Yager JD, Davidson NE 2006 Estrogen carcinogenesis in breast cancer. *N Eng J Med* 354:270-282

Yamamoto Y, Yamashita J, Toi M, Muta M, Nagai S, Hanai N, Furuya A, Osawa Y, Saji S, Ogawa M 2003 Immunohistochemical analysis of estrone sulfatase and aromatase in human breast cancer tissues. *Oncol Rep* 10:791-796

Yoshimura N, Harada N, Bukholm I, Karsen R, Borresen-Dale AL, Kristensen VN 2004 Intratumoural mRNA expression of genes from the oestradiol metabolic pathway and clinical and histopathological parameters of breast cancer. *Breast Cancer Res* 6:R46-55

Yue W, Santner SJ, Masamura S, Wang JP, Demers LM, Hamilton C, Santen RJ 1998a Determinants of tissue estradiol levels and biologic responsiveness in breast tumors. *Breast Cancer Res Treat.* 49 Suppl 1:S1-7; discussion S33-37

Yue W, Wang JP, Hamilton CJ, Demers LM, Santen RJ 1998 In situ aromatization enhances breast tumor estradiol levels and cellular proliferation. *Cancer Res.* 1998b 58:927-32