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Blocking 17β-hydroxysteroid dehydrogenase type 1 in endometrial cancer: a potential novel endocrine therapeutic approach

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Abstract

The enzyme type 1 17β-hydroxysteroid dehydrogenase (17β-HSD-1), responsible for generating active 17β-estradiol (E2) from low-active estrone (E1), is overexpressed in endometrial cancer (EC), thus implicating an increased intra-tissue generation of E2 in this estrogen-dependent condition. In this study, we explored the possibility of inhibiting 17β-HSD-1 and impairing the generation of E2 from E1 in EC using in vitro, in vivo, and ex vivo models. We generated EC cell lines derived from the well-differentiated endometrial adenocarcinoma Ishikawa cell line and expressing levels of 17β-HSD-1 similar to human tissues. In these cells, HPLC analysis showed that 17β-HSD-1 activity could be blocked by a specific 17β-HSD-1 inhibitor. In vitro, E1 administration elicited colony formation similar to E2, and this was impaired by 17β-HSD-1 inhibition. In vivo, tumors grafted on the chicken chorioallantoic membrane (CAM) demonstrated that E1 upregulated the expression of the estrogen responsive cyclin A similar to E2, which was impaired by 17β-HSD-1 inhibition. Neither in vitro nor in vivo effects of E1 were observed using 17β-HSD-1-negative cells (negative control). Using a patient cohort of 52 primary ECs, we demonstrated the presence of 17β-HSD-1 enzyme activity (ex vivo in tumor tissues, as measured by HPLC), which was inhibited by over 90% in more than 45% of ECs using the 17β-HSD-1 inhibitor. Since drug treatment is generally indicated for metastatic/recurrent and not primary tumor, we next demonstrated the mRNA expression of the potential drug target, 178-HSD-1, in metastatic lesions using a second cohort of 37 EC patients. In conclusion, 17β-HSD-1 inhibition efficiently blocks the generation of E2 from E1 using various EC models. Further preclinical investigations and 17β-HSD-1 inhibitor development to make candidate compounds suitable for the first human studies are awaited.

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Keywords: 17β-hydroxysteroid dehydrogenase type 1; endometrial cancer; estrone; 17β-estradiol; estrogen metabolism

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Conflict of interest statement: Pasi Koskimies is an employee of Forendo Pharma Ltd. No additional conflicts of interest are declared.

Introduction

Endometrial cancer (EC) is the most frequent gynecological malignancy in the Western world [1]. About 80% of all cases are estrogen-dependent type-1 endometroid endometrial carcinoma, characterized by low-grade histology and hormone receptor expression. Most of these patients present with complaints of abnormal uterine bleeding, are diagnosed at early stage, and have a favorable outcome after surgery (hysterectomy with bilateral salpingo-oophorectomy). Yet advanced-stage or recurrent EC patients have limited treatment options. Obesity, early menarche, nulliparity, late menopause, and use of tamoxifen are associated with estrogen...
exposure (systemic) and are risk factors for EC, especially type 1 [1,2]. In addition, local estrogen synthesis through 17β-hydroxysteroid dehydrogenases (17β-HSDs) also contributes significantly to estrogen exposure in endometrial cells [3,4]. Reductive 17β-HSDs catalyze the conversion of the low-active estrone (E1) to active 17β-estradiol (E2), whereas oxidizing 17β-HSDs catalyze the oxidation of E2 to E1 [3,5].

In a previous study, we showed that 17β-HSD reductive activity was increased in EC versus both normal tissue adjacent to the tumor and healthy post-menopausal endometrium. This was mainly due to the reductive 17β-HSD type 1, whose mRNA was upregulated in EC versus controls [3].

Based on these results, we hypothesized that 17β-HSD type 1 could be of therapeutic value in EC. In the present study, a well-characterized 17β-HSD type 1 inhibitor (FP4643) was tested using in vitro and in vivo models based on the Ishikawa endometrial cancer cell line (modified to express 17β-HSD-1) and ex vivo on patient specimens.

Materials and methods

Ethical statement

Procedures were conducted in accordance with ethical standards, national guidelines, and international guidelines according to the Declaration of Helsinki and were approved by the local ethics authority (METC 14-4-003).

Ishikawa cell line, plasmids, and stable transfection

The estrogen receptor (ERα) and progesterone receptor (PR)-positive and estrogen-dependent human endometrial adenocarcinoma cell line Ishikawa (ECACC; Sigma-Aldrich, Zwijndrecht, The Netherlands) was routinely cultured in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with sodium pyruvate, L-glutamine, penicillin–streptomycin, and 10% fetal bovine serum at 37°C with 5% CO₂ in humidified air. Ishikawa cells do not express 17β-HSD types 1 or 2 and are devoid of 17β-HSD reductive and oxidative activity (results not shown).

To generate Ishikawa clones expressing 17β-HSD type 1, cells were subjected to two rounds of genetic modification. In the first round, the reporter gene luciferase was introduced. Lentiviral particles were produced as described previously [6]. Ishikawa cells were transduced with a construct harboring a CMV-driven firefly luciferase cDNA (Photinus pyralis) and fused by 2TA peptide to a green fluorescent protein (GFP from Pontellina plumata). Clones from single cells were retrieved using cloning cylinders and expanded. To avoid clone-specific effects, a number of lines originated from single clones were characterized for the expression of luciferase, GFP, and ERα, and for estrogen responsiveness (not shown). Two clones were selected for the second round of genetic modification (Ishi-M1 and Ishi-M3) and were stably transfected with the plasmid pCEP4 containing the 17β-HSD type 1 cDNA [7] or with the empty vector. Transfection was performed as previously described [3] using the jetPEI reagent (Q-Biogene, Heidelberg, Germany) and hygromycin-B (Invitrogen) for selection. Clones originating from single cells were isolated by serial dilutions in 96-well plates and microscopic examination. This procedure was repeated twice.

A number of clones were further thoroughly characterized by reporter gene assay, RT-qPCR, western blotting, enzyme activity, and growth rate analyses to confirm that they maintained reporter gene expression and cytological and molecular characteristics of the parental Ishikawa cell line. Clones Ishi-M1-HSD-B, Ishi-M3-HSD-A (17β-HSD type 1), and Ishi-M3-EV-C (empty vector control) were used for further experiments. A full description and characterization of these lines are given in the supplementary material, Supplementary materials and methods and Figures S1 and S2.

The authenticity of clones Ishi-M1-HSD-B and Ishi-M3-HSD-A was confirmed by short tandem repeat (STR) analysis.

Cell treatment in vitro with estrogens and inhibitor

Three days before and during experiments with steroid stimulations, cells were cultured in RPMI 1640 (without phenol red) containing sodium pyruvate and L-glutamine, and supplemented with 5% hormone-stripped serum (c.c.pro GmbH, Neustadt, Germany). Cells were treated with either 0.1 nM E1 (Sigma-Aldrich) or with a combination of 0.1 nM E1 with 500 nM 17β-HSD type 1 inhibitor FP4643 (Forendo Pharma Ltd, Turku, Finland). This inhibitor has been thoroughly described and characterized [8–11]. As a positive control, 0.1 nM E2 (Sigma-Aldrich) was used. Vehicle or compound FP4643 alone was considered to be negative control.

17β-HSD type 1 inhibitor – FP4643

Compound FP4643 is an estrene substrate at the 15th carbon position [3-hydroxy-15-(4-morpholin-4-yl-4-oxo-butyl)-estr-1,3,5(10)-trien-17-one], described by Messinger et al as compound 21 [11]. This compound has been thoroughly characterized by the manufacturer (Forendo Pharma Ltd) and subsequent studies (compound 3 in ref [10]) for being a potent and specific inhibitor of 17β-HSD type 1, with little affinity towards the other 17β-HSDs and ERα. This inhibitor has been previously thoroughly described and characterized using in vivo models [8,9].

Colony formation assay

Ishi-M1-HSD-B, Ishi-M3-HSD-A, and Ishi-M3-EV-C clones were seeded onto six-well plates at a density of 3000 cells per well. Treatments were performed...
in triplicate in each experiment. After incubation for 10–14 days, cells were washed with 100 mM phosphate buffer saline (PBS; pH 7), fixed with 3.7% paraformaldehyde for 10 min, and stained with 1% crystal violet (Millipore BV, Amsterdam, The Netherlands) for 30 min. Pictures were taken and the total colony area was determined using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Chicken chorioallantoic membrane (CAM) assay
The CAM assay, initially used in angiogenesis research, is becoming increasingly more popular in other research areas. CAM contains extracellular matrix (ECM) proteins, mimics the physiological cancer cell (micro)-environment [12], and is a suitable and versatile model in cancer biology and pharmacological research [13].

The CAM protocol has been described earlier [13,14]. In brief, fertilized white leghorn chicken eggs were incubated at 37°C with 45% humidity for 3 days and tilted every 30 min. To expose the CAM, a 1.5 cm² hatch was made in the eggshell on embryonic day 4 (E4). On E7, the CAM was irritated with lens paper and 0.5 cm (diameter) sterile silicone rings were placed directly onto the CAM. Next, tumor grafts were seeded by suspending two million cells (Ishi-M1-HSD-B, Ishi-M3-HSD-A or Ishi-M3-EV-C) in 25 μl of Matrigel (Corning, New York, USA). Two grafts were made per egg. From E8 to E13, the CAMs were treated topically daily (by dropping the treatment on the top of the CAM) with no stimulation (vehicle alone), 1.0 nM E2, 0.1 nM E1, E1 (0.1 nM) plus FP4643 (2500 nM or as indicated) or FP4643 alone. The vehicle consisted of 0.9% NaCl, 25% ethanol, and 1% DMSO. Treatments were applied on the CAM as 40 μl solutions (containing 500X concentrated stimuli) and final concentrations were calculated based on the total volume of the egg (e.g. approximately 18–20 ml per egg). On E14, the tumor grafts with adjacent CAM were harvested, fixed with formalin for 30 min, and embedded in paraffin for immunohistochemical analyses.

Histology, cytology, and immunohistochemical analyses
Four-micrometer-thick sections of formalin-fixed, paraffin-embedded (FFPE) CAM tissue were cut and then stained using hematoxylin and eosin (Sigma-Aldrich) for evaluation of the histology. For immunohistochemical analyses, slides were baked for 20 min at 80°C on a heat plate, deparaffinized and then rehydrated. Next, endogenous peroxidase activity was quenched with H₂O₂ (3% in methanol) and heat-induced antigen retrieval was performed in Tris-EDTA buffer, pH 9. Non-specific antibody binding was blocked using 5% goat serum for cyclin A, 17β-HSD-1, and PR detection, followed by overnight incubation at 4°C with the following antibodies: sc-23900 for Ki-67 (1:50), sc-751 for cyclin A (1:500), B-30 for PR (1:50; all from Santa Cruz Biotechnologies, Heidelberg, Germany); EP1682-Y for 17β-HSD-1 (1:100; Epitomics, CA, USA); and 1D5 for ER-α (1:100; Dako, Glostrup, Denmark). To visualize antibody binding, an avidin/biotin-based peroxidase system (Vectastain ABC-kit, Vector Laboratories, Peterborough, UK) and diaminobenzidine (DAB; Dako) were used according to the manufacturers’ recommendations. Sections were counterstained with hematoxylin (Merck, Darmstadt, Germany). The Allred method with slight modifications [3] was used to quantify the immunoreactivity by three independent observers (KvK, GFJK, and AR), who were blind to each other’s results; staining intensity (I) was graded from 0 to 3 and percentage of positive cells (P) from 0 to 5. The final score was computed by multiplying I × P.

Patient specimens
Cohort 1
Tumor biopsies were obtained from 52 women undergoing hysterectomy for uterine cancer (47) or endometrial hyperplasia (5) and hospitalized at Maastricht University Medical Centre between 2008 and 2015. Clinical characteristics and surgical and follow-up care are described in the supplementary material, Table S1. Biopsies were immediately frozen in liquid nitrogen and kept at ~80°C for further processing. None of the patients had used hormonal medication within the last 6 months prior to surgery. The study protocol was approved by the local ethical committee (protocol No METC 14-4-003) and the harvesting of pathology material was approved by the Maastricht Pathology Tissue Collection Committee (protocol No MPTC 2012-03).

Cohort 2
A second patient cohort consisting of a subpopulation of 37 patients with metastatic uterine cancer from a population-based series prospectively collected in Hordaland county in Norway from 2001 to 2014 was used for mRNA analyses of 17β-HSD-1. The patient cohort and gene expression analyses have been previously described [15].

Frozen tissue processing
Frozen biopsies were processed for further analyses (RNA isolation and ex vivo 17β-HSD-1 activity measurement and inhibition) as described previously [3,8]. In brief, 10-μm-thick cryo-sections were cut, and ten sections were used for RNA isolation by immediate homogenization in TRIZol reagent (Invitrogen); ten sections were used for enzyme activity by homogenization in MPER buffer (Promea, Mannheim, Germany) just prior to use. The presence of endometrial cancer in the biopsies was confirmed by histology that was performed at the start, the end, and approximately at every 100 μm thickness of the material cut for lysate preparation. The histology and tumor grading were determined by an expert gynecopathologist (LK).
17β-HSD enzyme activity and inhibition ex vivo (cell-free assays)

Methods were as described previously [3,8,16]. In brief, fresh homogenates prepared in MPER buffer (see frozen tissue processing) were cleared from debris (centrifugation) and the protein concentration was determined (BC Assay Protein Quantitation Kit; Uptima-Interchim, Montlucon, France). Reactions (0.5 ml final volume in 50 mM KH₂PO₄, pH 7.4 and supplemented with 3.3 mM magnesium chloride) were carried out at 37°C for 24 h using 10–40 μg of protein lysates, 10 nmol E1 (in the presence or absence of 0.5 μM FP4643), 2.0 mM NADP⁺, and the NADPH-regeneration system based on glucose-6-phosphate (3.3 mM) plus glucose-6-phosphate dehydrogenase (2.5 U/ml). Steroids were derivatized with a fluorescent moiety, measured by HPLC (LC-10AD), and analyzed using Labsolutions software (Shimadzu, Kyoto, Japan).

mRNA expression levels

Patient cohort 1

mRNA levels for ERα (ESR1) and 17β-HSD-1 (HSD17B1) were measured by RT-qPCR, normalized to three reference genes, and expressed as relative values (supplementary material, Supplementary materials and methods). For ESR1, a threshold for positivity in each biopsy was determined based on the detection of ERα by immunohistochemistry/fluorescence in the same sample, which was performed on 12 biopsies analyzed in a previous study [3] that were common to the present investigation. Type 1 17β-HSD (HSD17B1) mRNA levels were clustered in two quantiles, one with low expression (n = 17) and one with high expression (n = 16; RNA was not available for 19 samples).

Type 1 17β-HSD enzyme activity is expressed as nmol E2/mg protein and showed a good correlation ($r^2 = 0.8$) with the enzyme activities measured in a previous
Inhibition of 17β-HSD type 1 impairs E2 synthesis from E1

Human endometrial adenocarcinoma Ishikawa cells, well-differentiated and estrogen-dependent but devoid of any 17β-HSD reductive or oxidative activity, were genetically modified to express 17β-HSD-1. Two 17β-HSD-1-positive clones (Ishi-M1-HSD-B and Ishi-M3-HSD-A) and one empty vector clone (Ishi-M3-EV-C, negative control) were selected for our studies. Selected clones maintained all major features of the parental Ishikawa cells (a full description of their generation and characterization is given in the supplementary material, Supplementary materials and methods and Figures S1 and S2). The estrogen sensitivity of these cells was also confirmed in nude-mouse xenografts (results not shown). Expression data for Ishi-M3-HSD-A and Ishi-M1-HSD-B, respectively; supplementary material, Figure S2C). The specific 17β-HSD-1 inhibitor FP4643 was used to determine whether the formation of E2 could be impaired. Both Ishi-M3-HSD-A and Ishi-M1-HSD-B clones showed dose-dependent inhibition of E2 formation by FP4643 (cell-free assay; Figure 1B). Inhibition of 80% was reached at a concentration of 100–200 nM and of over 90% with a concentration of 500 nM, and the IC50 for FP4643 was estimated to be 50 nM.

In these experiments, a high concentration of FP4643 was used (2.5 μM) in order to prevent poor solubility,
FP4643 inhibits the upregulation of cyclin A induced by E1 in vivo using the chicken chorioallantoic membrane (CAM) assay. (A) Top image: histology of the induced tumors (hematoxylin and eosin staining). Tumor is indicated by black arrowheads (Tu). At the top right, a macroscopic image of a CAM assay at sacrifice is shown, with the two tumor grafts indicated by white arrowheads (Tu). Middle image: enlargement of the black-squared inset. Several blood vessels grow in the tumor grafts. Lower image: enlargement of the red-framed region. Red arrowheads indicate mitotic figures, whereas green arrowheads show apoptotic cells. These are representative images obtained from the clone Ishi-M1-HSD-B, vehicle treatment. No major histological differences were observed between treatments/clones. Scale bar in the middle image = 100 μm. (B) Representative immunohistochemistry images (clone Ishi-M1-HSD-B, vehicle treatment) for ERα, PR, 17β-HSD type 1, and cyclin A. Scale bars = 40 μm. (C) Immunohistochemical staining intensity of cyclin A computed using a modified Allred system (range 0–15 [3]) for Ishi-M1-HSD-B and Ishi-M3-HSD-A upon the indicated treatment: control (vehicle); E2 0.1 nM; E1 0.1 nM; E1 0.1 nM plus FP4643 2.5 μM, and inhibitor alone. Statistical test: ANOVA; *p < 0.05 was considered significant. #p < 0.05 compared with control; *p < 0.05 compared with E1 (post hoc Bonferroni test). The number of samples in this experiment is indicated above the x-axis (n). The results were reproduced in one independent experiment (not shown).

17β-HSD type 1 inhibition impairs the synthesis of E2 in human EC biopsies ex vivo

To confirm that inhibition of 17β-HSD-1 as observed in vitro and in vivo is relevant in human tissue as well, 52 human primary EC biopsies were investigated. The patient characteristics are summarized in the supplementary material, Table S1 (cohort 1).

17β-HSD-1 enzyme activity was measured in 48 samples. Two specimens were excluded because histologic cell/tissue permeability, and pharmacokinetics hampering the inhibitor from reaching its target. Nevertheless, in a titration experiment, FP4643 efficiently blocked E1-induced estrogen activation (i.e. the induction of cyclin A and the proliferation marker Ki67) at a concentration as low as 100 nM (supplementary material, Figure S4B). The gender of the chick embryo (determined as described in the supplementary material, Supplementary materials and methods) did not influence either the expression of cyclin A or that of Ki67.

17β-HSD-1 enzyme activity, inhibition, and mRNA levels (available from 39 specimens) were next explored for any association with the patients’ clinical features (Table 2 and supplementary material, Tables S2 and S3). For these analyses, enzyme activity levels were clustered in tertiles (high, medium, low), whereas mRNA levels (available from 39 specimens) were next explored for any association with the patients’ clinical features (Table 2 and supplementary material, Tables S2 and S3). For these analyses, enzyme activity levels were clustered in tertiles (high, medium, low), whereas mRNA levels (available from 39 specimens) were next explored for any association with the patients’ clinical features (Table 2 and supplementary material, Tables S2 and S3). 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Table 1. Enzyme activity of 17β-HSD-1 and clinical characteristics of the patients (cohort 1)

<table>
<thead>
<tr>
<th>Patients’ clinical characteristics</th>
<th>17β-HSD-1 activity measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Histology of endometrium</td>
<td>BMI</td>
</tr>
<tr>
<td>Hyperplasia No atypia</td>
<td>Post 64 u</td>
</tr>
<tr>
<td>No atypia</td>
<td>Pre 49 22.15</td>
</tr>
<tr>
<td>Atypia</td>
<td>Post 79 26.53</td>
</tr>
<tr>
<td>No atypia</td>
<td>Post 63 u</td>
</tr>
</tbody>
</table>

Total overview:

<table>
<thead>
<tr>
<th>Grade 1 EC (n = 20)**</th>
<th>Mean inhibition: 83.6%</th>
<th>Specimens with over 90% inhibition: n = 9; 45.0%</th>
<th>Min–max inhibition: 18–100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 2 EC (n = 7)**</td>
<td>Mean inhibition: 81.7%</td>
<td>Specimens with over 90% inhibition: n = 3; 42.9%</td>
<td>Min–max inhibition: 42–100%</td>
</tr>
<tr>
<td>Grade 3 EC (n = 16)**</td>
<td>Mean inhibition: 80.2%</td>
<td>Specimens with over 90% inhibition: n = 7; 43.8%</td>
<td>Min–max inhibition: 54–100%</td>
</tr>
</tbody>
</table>

Note: ERα: estrogen receptor positivity was defined as described in the Materials and methods section.
FIGO stage according to FIGO 2009 classification.
Nodal staging: N* = lymph-node positivity; N− = lymph-node negativity.
Rec: presence of local or regional recurrence. SFU: short follow-up time, i.e. less than 24 months.
†This activity/protein was estimated because protein determination was outside the calibration values.
*Irrespective of the histology.
BMI = body mass index; LAI = lymph-angi-invasion; u = unknown; n.a. = not analyzed; – = not applicable.

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levels were clustered in 2-quantiles and inhibition in two groups (lower than 95% of E2 formation, higher than/equal to 95%). The prevalence of specimens with high enzyme activity tended to be greater among tissues with high mRNA expression levels (supplementary material, Figure S5), although there was no clear correlation between protein (activity) and mRNA, as shown previously [22] and suggestive of the presence of post-transcriptional events. As shown previously [22], biopsies of patients with advanced-stage disease tended to have higher levels of 17β-HSD-1 mRNA (supplementary material, Table S2). More frequently than ERα-negative specimens, ERα-positive samples had high 17β-HSD-1 levels (enzyme activity, Table 2; non-significant trend for the mRNA level, supplementary material, Table S2). No additional associations between patient characteristics and mRNA, activity or 17β-HSD-1 inhibition levels (supplementary material, Table S3) were observed.

Type 1 17β-HSD is expressed in metastatic EC
Primary EC is normally treated surgically. In cases of recurrent or metastatic disease, endocrine therapy becomes a treatment option. Hence we wondered whether 17β-HSD-1 as a potential drug target was also expressed in metastatic lesions. A second cohort of 37 primary ECs and 48 corresponding metastatic lesions (cohort 2) with available mRNA expression data from micro-array analysis [15] was used to investigate the expression of 17β-HSD-1 in paired primary (continuous gray line in Figure 3) and metastatic lesions (bars in Figure 3). 17β-HSD-1 expression was detected in all metastases and the level was higher than the lowest level measured in primary EC (dashed line in Figure 3). In over 50% of the cases (21/37), at least one metastasis had higher 17β-HSD-1 expression than the corresponding primary EC (Figure 3). There was no correlation between 17β-HSD-1 levels in primary tumor and metastases and no association with ERα status or with histology or grading (Figure 3 and supplementary material, Table S4).

Discussion
Local estrogen overexposure in EC is determined, at least in part, by an increased level of the enzyme 17β-HSD-1 [3]. Hence, blocking 17β-HSD-1 can be a potential novel endocrine therapeutic strategy. In the present study, we demonstrated that 17β-HSD-1 inhibition efficiently blocks the synthesis of active E2 using models of EC in vitro and in vivo. First, we genetically modified the well-differentiated endometrioid EC cell line Ishikawa to generate lines expressing 17β-HSD-1 at levels that are comparable to those observed in patients. Using these cells, we demonstrated that the 17β-HSD-1 inhibitor FP4643 blocks the synthesis of E2 from E1 in cell-free assays. Since this assay is devoid of cell membranes and intracellular signaling pathways, we demonstrated that FP4643 also blocks the conversion of E1 to E2 in a complete-cell contest by showing that treatment with FP4643 impairs E1-induced estrogen signaling activation in vitro as assessed by colony formation assay. Next, we confirmed that FP4643 blocks E1-induced estrogen signaling in vivo using the CAM model, demonstrating the potential of this compound in a more complex whole organism setting. The relevance of the inhibition of 17β-HSD-1 by FP4643 was further demonstrated using lysates of human primary EC biopsy samples (ex vivo) as well as in metastatic disease, a condition that is currently an indicator for hormonal treatment.

Although no compound directed against 17β-HSD-1 has reached the clinical phase yet, good results in various in vivo disease models have been demonstrated. These include human endometrial hyperplasia [9] and breast cancer [23] models in mice and endometriosis models in primates [24] and in human endometriotic lesions ex vivo [8].

Local synthesis, activation, and deactivation of estrogens, but also androgens and progesterone, occur in the endometrium and in several other peripheral tissues. Such hormone metabolism is termed ‘intracrinology’ and is increasingly more considered as part of the endocrine control [4]. Imbalances of intracrinological pathways lead to hormone-dependent pathologies. Increased local estrogen generation caused by deregulated 17β-HSD-1/17β-HSD-2 balance, controlling the interconversions of E1 and E2, is implicated in endometriosis [8,25–27], endometrial cancer [3], and non-gynecological conditions as well [28]. Estrogen overexposure can also be caused by imbalances of other intracrine enzymes, such as aromatase (reported to be increased in endometriosis [26,29,30], EC [31] or infiltrative patients [32]) or the sulfatase pathway that controls the cell influx/outflow of sulfated steroids [33,34].

Local steroid metabolism and intracrinology offer opportunities for novel drug targets and prognosis markers in hormone-dependent conditions such as EC [15,22,35–39]. At the same time, however, intracrine networks are complex; can convert adrenal steroid precursors into androgens and estrogens; and can interconvert androgens, estrogens, active and inactive compounds, and protective and cancer-promoting compounds. In addition, several intracrine enzymes have promiscuous and redundant activities; thus, blocking one step can be rescued by other compensating reactions. Therefore, it is not surprising that, so far, attempts to use inhibitors of intracrine enzymes for patient care have not confirmed the expectations generated in the preclinical phase. For instance, promising STS inhibitors in preclinical EC models [37] had inferior results compared with progestogens (standard endocrine care for EC) in a phase II trial on EC patients with advanced-stage disease [40]. The use of various aromatase inhibitors was attempted in a number of phase II trials on advanced-stage EC, with little efficacy [41–43].

In order to exploit the opportunities offered by the local steroid metabolism and by intracrinology, it is
Table 2. Enzyme level of 17β-HSD-1 and clinical characteristics of the patients (cohort 1)

<table>
<thead>
<tr>
<th>17β-HSD-1 activity level*</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD</td>
<td>69.9 ± 10.7</td>
<td>70.4 ± 10.9</td>
<td>68.6 ± 11.4</td>
<td>N.S.</td>
</tr>
<tr>
<td>Minimum - maximum age†</td>
<td>49–90</td>
<td>55–91</td>
<td>45–81</td>
<td></td>
</tr>
<tr>
<td>Mean BMI</td>
<td>26.6 ± 6.1</td>
<td>32.6 ± 10.8</td>
<td>30.0 ± 7.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>Minimum - maximum BMI</td>
<td>19.9–39.3</td>
<td>21.3–55.1</td>
<td>23.3–54.1</td>
<td></td>
</tr>
<tr>
<td>Mean CA-125†</td>
<td>147 ± 201</td>
<td>99.4 ± 165</td>
<td>53.4 ± 38.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>Minimum - maximum CA-125‡</td>
<td>24–379</td>
<td>9–346</td>
<td>15–104</td>
<td></td>
</tr>
<tr>
<td>No (%)</td>
<td>14 (30)</td>
<td>15 (33)</td>
<td>17 (37)</td>
<td>Fisher exact test</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>1 (2)</td>
<td>0</td>
<td>2 (4)</td>
<td>N.S.</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>13 (29)</td>
<td>14 (31)</td>
<td>15 (33)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 65</td>
<td>6 (13)</td>
<td>5 (11)</td>
<td>5 (11)</td>
<td>N.S.</td>
</tr>
<tr>
<td>≥ 65</td>
<td>8 (18)</td>
<td>9 (20)</td>
<td>12 (27)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
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<td>3 (8)</td>
<td>2 (5)</td>
<td>N.S.</td>
</tr>
<tr>
<td>25 ≤ BMI ≤ 30</td>
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<td>5 (13)</td>
<td>8 (21)</td>
<td></td>
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<tr>
<td>&gt; 30</td>
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<td>5 (13)</td>
<td>5 (13)</td>
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<tr>
<td>Unknown</td>
<td>3</td>
<td>2</td>
<td>2</td>
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</tr>
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<td>ERα expression§</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>9 (24)</td>
<td>10 (26)</td>
<td>7 (18)</td>
<td>0.02</td>
</tr>
<tr>
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<td>4 (11)</td>
<td>8 (21)</td>
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<tr>
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<td>5</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Histology of endometrium</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Hyperplasia</td>
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<td>1 (2)</td>
<td>N.S.</td>
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<td>Endometrioid EC</td>
<td>7 (15)</td>
<td>10 (22)</td>
<td>10 (22)</td>
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<tr>
<td>Non-endometrioid EC</td>
<td>4 (9)</td>
<td>5 (11)</td>
<td>6 (13)</td>
<td></td>
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<tr>
<td>Grading§</td>
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</tr>
<tr>
<td>Grade 1</td>
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<td>8 (19)</td>
<td>6 (14)</td>
<td>N.S.</td>
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<tr>
<td>Grade 2</td>
<td>3 (7)</td>
<td>1 (2)</td>
<td>3 (7)</td>
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<tr>
<td>Grade 3</td>
<td>2 (5)</td>
<td>6 (14)</td>
<td>7 (17)</td>
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<tr>
<td>FIGO Ia</td>
<td>5 (12)</td>
<td>6 (15)</td>
<td>2 (5)</td>
<td>0.09**</td>
</tr>
<tr>
<td>FIGO Ib</td>
<td>4 (10)</td>
<td>4 (10)</td>
<td>10 (24)</td>
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<tr>
<td>FIGO II, III or IV</td>
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<td>4 (10)</td>
<td>4 (10)</td>
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<tr>
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<td>Myometrium invasion¶</td>
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<td></td>
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<td>4 (10)</td>
<td>2 (5)</td>
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<tr>
<td>&lt; 1/2</td>
<td>4 (10)</td>
<td>4 (10)</td>
<td>7 (18)</td>
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<tr>
<td>&gt; 1/2</td>
<td>4 (10)</td>
<td>4 (10)</td>
<td>7 (18)</td>
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<td>3</td>
<td>1</td>
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<tr>
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<td>9 (24)</td>
<td>12 (32)</td>
<td>N.S.</td>
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<td>4 (11)</td>
<td>2 (5)</td>
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<td>2</td>
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<tr>
<td>17β-HSD-1 inhibition</td>
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</tr>
<tr>
<td>&lt; 95%</td>
<td>9 (20)</td>
<td>8 (17)</td>
<td>14 (30)</td>
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<tr>
<td>≥ 95%</td>
<td>5 (11)</td>
<td>7 (15)</td>
<td>3 (7)</td>
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<td>Parity</td>
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<td>7 (18)</td>
<td>15 (38)</td>
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<td>4</td>
<td>3</td>
<td>–</td>
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<tr>
<td>History of hypertension</td>
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</tr>
<tr>
<td>Negative</td>
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<td>8 (19)</td>
<td>11 (26)</td>
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</tr>
<tr>
<td>Positive</td>
<td>6 (14)</td>
<td>6 (14)</td>
<td>6 (14)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>1</td>
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</table>
Table 2. Continued

<table>
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<tr>
<th>History of type 2 diabetes</th>
<th>17β-HSD-1 activity level*</th>
<th>ANOVA</th>
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</thead>
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<tr>
<td></td>
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<td>Medium</td>
</tr>
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<td>12 (28)</td>
<td>10 (23)</td>
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<td>4 (9)</td>
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<tr>
<td>Unknown</td>
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<td>1</td>
</tr>
</tbody>
</table>

*17β-HSD-1 (reductive) activity was clustered in tertiles as described in the Materials and methods section.
†Age = age at diagnosis.
‡Pre-operative level of CA-125 (IE/ml).
§ERα positivity was defined on RT-qPCR data validated using immunohistochemistry as described in the Materials and methods section.
¶FIGO stage based on 2009 classification. Further characteristics (type of surgery, treatment, nodal staging, lymph-angio-invasion, CA-125 level) did not show any correlation with 17β-HSD-1 activity.
**FIGO Ia was compared with the rest (FIGO Ib and higher).
N.S. = non-significant.

![Diagram showing ER-α expression and 17β-HSD-1 mRNA levels in endometriod EC, serous EC, and other EC.](https://example.com/diagram)

**Figure 3.** 17β-HSD-1 mRNA levels in primary and the corresponding metastases. The mRNA level of 17β-HSD-1 in 37 primary ECs (grey bars) is plotted against the levels observed in the corresponding 47 metastatic lesions (black bars; grey bars when two metastases from the same patient were available). The dashed line indicates the minimum mRNA expression for 17β-HSD-1 seen in primary EC. All metastases have levels higher than this minimum. The expression of ERα based on immunohistochemistry as described earlier [15] is indicated at the top of the graph. The lower panel gives the numbers (and percentages) of metastatic lesions having lower or higher/equal 17β-HSD-1 mRNA levels than the corresponding primary ECs (the mRNA levels in primary EC are clustered in tertiles: low/medium/high). Additional patient characteristics are shown in the supplementary material, Table S4 and have been described previously [15]. The mRNA level for 17β-HSD-1 was derived from microarray analyses performed previously [15] and is outlined in the Materials and methods section. In the case of more than one metastasis corresponding to one primary EC, the metastatic lesion with the higher 17β-HSD-1 mRNA level was considered. The mRNA levels between the primary and metastasis were considered different when they deviated by more than ±0.03 arbitrary mRNA units (± 0.5% of the mRNA value). ERα expression was determined by immunohistochemistry as described previously [15]. Five samples were classified as carcinosarcomas; three were clear cell ECs; and one sample was classified as undifferentiated EC.

Fundamental to learn how to predict the therapeutic response of a patient to a drug, e.g. by assessing the presence of the drug target and the activation of relevant signaling pathways in the tissue intended to be treated (primary or metastatic lesion). This recommendation is being made for hormonal drugs in general [44]. Additionally, the development of dual/triple inhibitors, able to simultaneously target redundant intracrine pathways, is desirable. Several double STS-aromatase inhibitors are under investigations [34], and very recently, the first dual 17β-HSD-1/STS inhibitor was developed [45].

In conclusion, 17β-HSD-1 is a novel potential target for endocrine treatment in EC. Endocrine care in...
EC is currently prescribed in cases of advanced-stage or recurrent disease and in cases of fertility preservation. Since the efficacy of current endocrine care is limited due to the occurrence of drug insensitivity/resistance [1,46], novel endocrine and intracrine targets may allow alternating drug regimens targeting different pathways, thus diminishing the problem of drug resistance development.

Acknowledgements

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Author contributions statement

AR, GFJK, KMCC, BD, and RFPMK conceived and designed the study, AJG, MV, YJMS, KvK, MAS, DB, SX, GFJK, and KMCC acquired raw data. DB, SX, GFJK, KMCC, CK, HBS, MYB, RFPMK, PK, and LK analyzed and interpreted the data. AR, GFJK, and KMCC drafted the manuscript version of the study. All the authors were involved in critically revising the manuscript for important intellectual content and approved the final version to be published.

References


*Cited only in supplementary material.