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Review

# Estradiol measurement in translational studies of breast cancer

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ABSTRACT

Plasma estrogen measurement with use of radioimmunoassays has been instrumental in the development of aromatase inhibitors for endocrine therapy of postmenopausal breast cancer. However, due to low plasma estrogen concentrations in postmenopausal women, direct radioimmunoassays lack the sensitivity required. While certain laboratories have developed highly sensitive assays for research purposes revealing plasma estrogen suppression consistent with results from tracer studies, such assays are time and labor-consuming due to need for pre-analytical chromatographic purification, sample concentration and sometimes conversion of precursors to products. While novel chromatographic methods involving mass spectrometry analysis are likely to replace such radioimmunoassays in the future, so far a limited number of laboratories have developed suitable assays with a detection limit (around 1 pM) that is required for analyzing plasma estrogen levels in patients during treatment with potent aromatase inhibitors.


**Contents**



## 1. Introduction

While the history of endocrine therapy in breast cancer started more than a century ago, the scientific rationale arose with the discovery of [1] and confirmation of the predictive value of [2] the estrogen receptor (today known as ERα). In parallel, introduction of what was known at that time as an adrenotoxic antiepileptic, aminoglutethimide, in an attempt to achieve a "medical adrenalectomy" [3], indirectly led to introduction of aromatase inhibition as a successful endocrine therapy for postmenopausal women. While clinically efficient [4], subsequent studies by the Hershey group revealed sustained androgen production, despite estrogen suppression [5], a question solved when tracer studies [6] revealed that aminoglutethimide directly inhibited peripheral aromatization of androstenedione into estrone ($E_1$). The discovery that aminoglutethimide executed its anti-tumor efficacy through aromatase inhibition had profound influence on subsequent development of endocrine therapy for breast cancer. While effective, aminoglutethimide treatment was associated with substantial side effects [7], for which reason much efforts were placed on developing novel, less toxic agents (see Ref. [8]). Importantly, the successful implementation of aromatase inhibition as a major endocrine for breast cancer, from which more than a million breast cancer women around the world currently are benefitting, had not been possible without these careful translational studies applying sensitive radioimmunoassay's, in concert with tracer studies, to define the

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pharmacological mechanisms of action of aminoglutethimide and, thus, aromatase inhibition as a useful endocrine treatment option in breast cancer.

## 2. Tracer studies

While tracer studies and plasma estrogen assessment in concert confirmed aminoglutethimide to act as a potent aromatase inhibitor *in vivo* [9], plasma estrogen radioimmunoassay's lacked the sensitivity to detect estrogen suppression >90%, which was what to expect based on the tracer results. Thus, direct assessment of *in vivo* aromatase inhibition by tracer studies were considered to be the "gold standard". With new aromatase inhibitor compounds in development, in collaboration with Professor Mitch Dowsett and his team, we initiated a program for *in vivo* assessment of aromatase inhibition. Using an HPLC method to separate estrogen metabolites [10], we developed an assay allowing assessment of *in vivo* aromatase inhibition with an average detection limit >99.1% [11–18]. The results are depicted in Table 1; in brief, while most compounds (the so-called 1st and 2nd generation compounds) caused *in vivo* aromatase inhibition <90%, the three 3rd generation compounds; the steroidal inhibitor exemestane, as well as the non-steroidal compounds anastrozole and letrozole, each caused on average >98% aromatase inhibition. Most importantly; these endocrine results were paralleled by clinical findings; while the 1st and 2nd generation compounds in general revealed clinical efficacy similar to tamoxifen [8], the three 3rd generation compounds revealed superiority, also with respect to clinical efficacy, and are today used as routine endocrine therapy for postmenopausal women in the adjuvant setting [19].

## 3. Plasma estrogen measurements in relation to treatment with aromatase inhibitors

The results from *in vivo* tracer studies, in concert with the results from large randomized studies, underline the imperative of maximal aromatase inhibition for optimal clinical efficacy. Thus, while randomized studies revealed superiority for third-generation aromatase inhibitors as compared to tamoxifen [19], in contrast first-and second-generation compounds were found of similar efficacy, but not superior, as compared to conventional therapy [8,20]. Tracer studies however are laborious and expensive to conduct and may be applied to small patient groups only. Thus, there is a need for simpler methods, like plasma estrogen measurement.

A key problem relates to low plasma estrogen levels in postmenopausal women, in particular when on aromatase inhibitor therapy. Taking into account plasma levels of estradiol ($E_2$), $E_1$ and estrone sulfate ($E_1S$) to be in the 15–20 pM, 70–80 pM and 4–500 pM ranges, respectively [21], the assays need sensitivity limits of a few pM to detect potential suppression >98%. Developing highly sensitive radioimmunoassays with a detection limit of about 1 pg/ml (3.7 pM), the Herhsey group revealed significant differences with respect to plasma estrogen suppression between the second-generation aromatase inhibitor CGS16949A [22] and letrozole [23]. Notably, as for both studies the difference in plasma estrogen suppression was corroborated by similar findings with respect to suppression of urinary estrogen secretion.

A problem related to use of $^{3}H$-labelled standards in radioimmunoassays relates to the limited specific activity of these standards (in the 50–160 mCi/mmol range). A higher specific activity may be achieved with use of $^{125}I$-labelled compounds (specific activity in the 2000 Ci/mmol range). The first sensitive $^{125}I$-based RIA for $E_2$ measurement in patients treated with aromatase inhibitors was developed by Professor Mitch Dowsett at the Royal Marsden Hospital [24] and subsequently used to measure plasma $E_2$ suppression with different aromatase inhibitors [25–30]. Some years later, learning this assay for $E_2$ measurement, we used the same $^{125}I$-$E_2$ standard and $E_2$ antibody developing a highly sensitive assay for plasma $E_1S$ measurement. The procedure involved taking the samples through multiple purification steps (Fig. 1), hydrolysis and finally conversion of unconjugated $E_1$ into $E_2$ [31].

Taking this approach further, we improved our assay, allowing $E_1$ as well as $E_1S$ to be converted into $E_2$, each steroid to be measured with the same $^{125}I$-$E_2$ assay (Table 2). The approach involved adding minor amounts of $^{3}H$ $E_2$ as well as $^{3}H$ $E_1$ and $^{3}H$ $E_1S$ for recovery standard including correction of the final results. As for this assay, we achieved a detection limit of 0.67 pM for $E_2$, 1.14 pM for $E_1$ and 0.55 pM for $E_1S$ [32], a significant improvement as compared to our earlier $^{3}H$-based methods [33]. Applying this assay to patients on treatment with anastrozole versus letrozole [18], we recorded a mean suppression of plasma $E_2$ of 92.8% versus 95.2%, for $E_1$ 96.3% versus 98.8%, and for $E_1S$ 95.3% versus 98.9%, respectively [34]. It should be noted however that, even with this sensitive assay, 5 out of 12 patients had plasma levels of $E_2$ below detection limit during anastrozole treatment; corresponding figures for letrozole was as high as 11 out of 12 [34].

Another interesting approach was taken by Dr. Klein and her team who used an ultrasensitive recombinant cell bioassay to measure estrogen levels in patients on treatment with letrozole [35]; this approach is discussed in detail in another paper in this issue.

A particular problem relates to plasma estrogen measurement for patients on steroidal aromatase inhibitors, such as exemestane.

**Table 1**
*In vivo* aromatase inhibition by different drugs evaluated in the clinical setting.

| Drug | Dose | Mean inhibition | References |
|---|---|---|---|
| *First/second generation compounds* | | | |
| Aminoglutethimide (AG) | 1000 mg daily | 90.6% | [13] |
| Roglethimide | 400/800/1600 mg daily | 50.6%/63.5%/73.8% | [13] |
| Fadrozole | 2 mg/4 mg daily | 82.4/92.6% | [11] |
| Formestane* | 125 mg/250 mg daily | 62.3%/70.0%**/57.3%*** | [14] |
| Formestane* | 250 mg/500 mg i.m./2 w | 84.8%/91.9% | [12] |
| Formestane* | 500 mg i.m./w | 91.3% | [15] |
| Formestane + AG | 500 mg i.m./w 1000 mg daily | 94.2% | [15] |
| *Third-generation compounds* | | | |
| Exemestane | 25 mg daily | 97.9% | [17] |
| Anastrozole | 1 mg daily | 96.7% | [16] |
| Anastrozole | 10 mg daily | 98.1% | [16] |
| Anastrozole | 1 mg daily | 97.3% | [18] |
| Letrozole | 2.5 mg daily | >99.1% | [18] |

* Formestane = 4-hydroxyandrostenedione.
** Administered as 125 mg b.i.d.
*** Administered as 250 mg once daily.

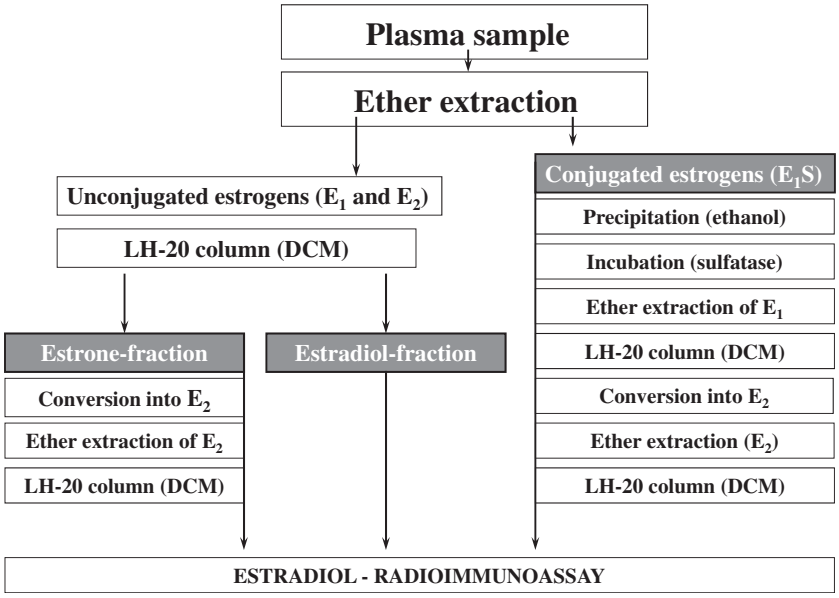


**Fig. 1.** Flow diagram depicting pre-purification steps required prior to determination of plasma estrogens with radioimmunoassay. Reproduced with permission from Ref. [32].

**Table 2**
Mean levels (ML), detection limits and isotope used for the radioimmuno-assays ($^{125}$I or $^{3}$H) with respect to different methods used at different time periods for measurement of postmenopausal estrogen levels in relation to aromatase inhibitor therapies in our laboratory.

| ML | $E_2$ 15 pM | $E_1$ 70 pM | $E_1S$ 400 pM | References |
|---|---|---|---|---|
| | $^{125}$I: 1.3 pM 8.7% | $^{3}$H: 5.3 pM 7.6% | $^{3}$H: 36.1 pM 9.0% | [33] |
| | | | $^{125}$I: 2.6 pM 0.7% | [31] |
| | $^{125}$I: 0.67 pM 4.4% | $^{125}$I: 1.14 pM 1.6% | $^{125}$I: 0.55 pM 0.14% | [32] |

Mean levels (ML) = consistent with expect based on steroid disposition [54,55].
The $^{125}$I radioimmunoassay was modified from original version by Dowsett and colleagues [24].

Due to potential interacting metabolites, samples collected from patients on treatment with such compounds need pre-purification with use of HPLC before radio immunoassaying [36].

Apart from differentiating between first/second generation aromatase inhibitors on the one hand as compared to the highly potent third-generation compounds on the other side, plasma estrogen measurements have been able to discriminate also between highly potent third-generation compounds like anastrozole and letrozole. Thus, two independent studies, both applying a cross-over design, have confirmed letrozole to be a more portent plasma estrogen suppressor as compared to anastrozole [30,34], consistent with tracer study findings [18]. Recently, data from the same studies have been analyzed with respect to body mass index (BMI). Conflicting data have challenged efficacy of aromatase inhibition for overweight/obese patients [37–39]; analyzing plasma estrogen levels with these sensitive radioimmunoassay's [40,41] revealed slightly higher plasma estrogen levels related to high BMI despite a similar degree of aromatase inhibition among overweight as compared to normal-weight individuals [41].

## 4. Tissue estrogen levels

Much interest has focused on issue estrogen levels since van Landeghem [42] and others three decades ago reported breast cancer tissue $E_2$ levels a magnitude higher as compared to plasma levels in postmenopausal women. Thus, issues have been raised with respect to local estrogen synthesis by aromatization [43] as well as de-conjugation of $E_1S$ [44]. Using our sensitive radioimmunoassay's on tissue samples following HPLC purification (Fig. 2), we were able to detect tissue levels of $E_2$ as well as $E_1$ and $E_1S$ with high degree of sensitivity [45]. Studying tumor tissue samples collected before and during treatment with anastrozole or letrozole [34,46], we confirmed effective tissue estrogen suppression with no evidence of "escape" for any single tumor. Further, studying tissue estrogens across benign and malignant breast tissue [21], we confirmed elevated tissue to plasma $E_2$ as well as $E_1$ gradients; as for benign tissue, the tissue to plasma ratio for $E_2$ and $E_1$ averaged about 2 and 5, respectively. As for $E_1S$, however, we found a tissue to plasma gradient averaging 0.1 only, contrasting previous findings obtained by others with use of a direct radioimmunoassay [47]. Interestingly, we confirmed elevated tumor $E_2$ levels in estrogen receptor positive but not in estrogen receptor negative tumors; these elevated levels were found positively correlated to transcriptional levels of the estrogen receptor as well as the reductive 17 hydroxy steroid dehydrogenase B7, but negatively correlated to the oxidative B2 and B12 dehydrogenases [48]. However, tissue $E_2$ and $E_1$ levels correlated even stronger to their corresponding plasma estrogen concentrations [21,48]. These findings are in agreement with the results from Professor Millers group revealing the bulk of tumor tissue estrogens to have a plasma origin [49] and the findings of Dunbier et al., reporting a strong correlation between postmenopausal plasma $E_2$ levels and tumor tissue expression of estrogen-regulated genes [50].

Based on these findings, we proposed a new hypothesis, explaining tissue to plasma hormone gradients based on physical–chemical properties for each individual compound [51]. Considering unconjugated $E_2$ and $E_1$, these compounds are highly lipophilic, explaining a high tissue to plasma concentration gradient. In contrast, $E_1S$ is a water-soluble conjugate. While the concentration of plasma $E_1S$ exceeds the concentration of circulating $E_1$ and $E_2$ by an average factor of 8 and 40, respectively [21], this is due to the fact that most unconjugated $E_2$ and $E_1$ are converted into $E_1S$ which, on the other hand, has a plasma clearance rate of only about 10% the clearance rate of the unconjugated estrogens [52,53]. Our hypothesis does not exclude local estrogen production; nor is it inconsistent

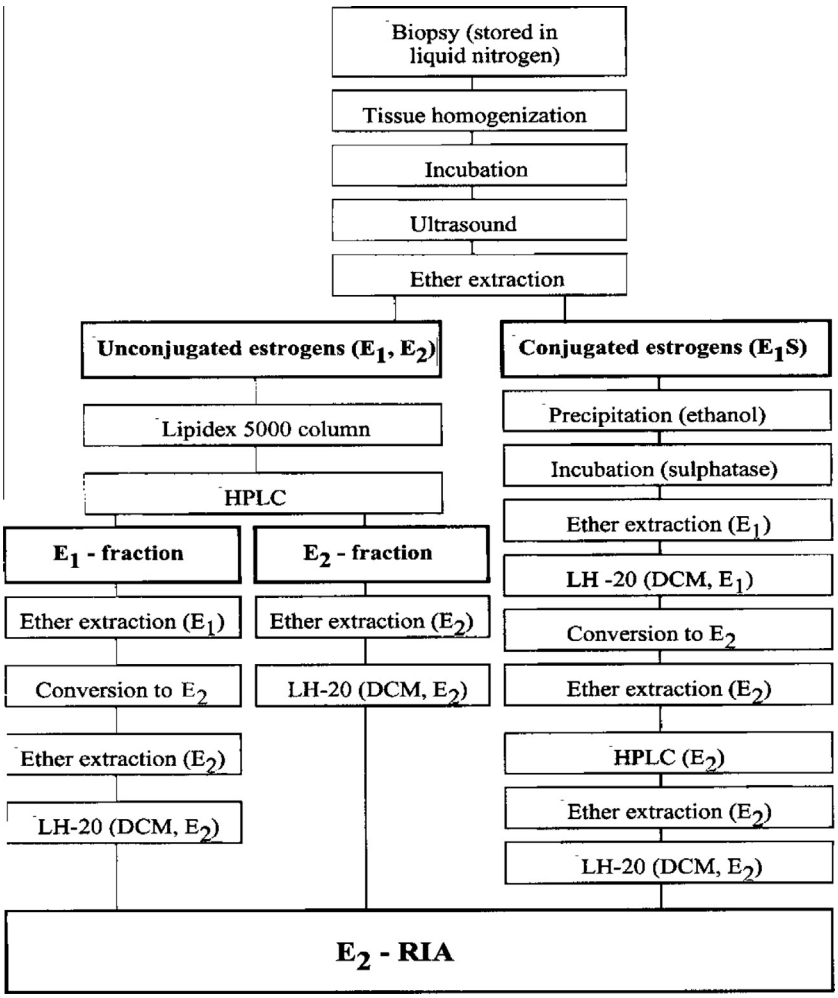


**Fig. 2.** Flow diagram depicting pre-purification steps required prior to determination of tissue estrogens with radioimmunoassay. Reproduced with permission from Ref. [45].

with the finding that estrogen receptor expression as well as dehydrogenase activity to some degree may influence the ratio between $E_2$ and $E_1$ in breast cancer tissue. However, the finding of similar tissue to plasma estrogen gradients in pre- and postmenopausal women despite substantial differences with respect to plasma estrogen levels between these groups indicate tissue to plasma equilibrium to be a rapid event [51]. Thus, local synthesis would be of minor importance to local estrogen concentration, in as much as estrogens synthesized locally would be quickly buffered by the circulating pool of hormones.

## 5. Discussion

Radioimmunoassay of plasma and tissue estrogen levels, in concert with tracer studies, has contributed significantly to our understanding of estrogen disposition. This has been mandatory to development of aromatase inhibition, currently the major endocrine treatment option for postmenopausal breast cancer patients. In contrast, there have been many publications reporting plasma estrogen levels much higher than should be assumed based on theoretical calculations [54,55] and the need for caution with respect to critically interpreting the results in the literature cannot be overemphasized [55]. Further; recent studies applying radioimmunoassays have contributed to our understanding of estrogen disposition in general, including important topics like explaining elevated tissue to plasma estrogen levels.

Measurement of plasma estrogens in postmenopausal women, due to their low levels, are time- and labor-dependent methods, involving multiple steps such as chromatographic separation and conversion [55]. The topic becomes complicated in particular when measuring estrogen levels in patients on treatment with aromatase inhibitors. For example, patients on treatment with letrozole, the most potent aromatase inhibitor currently in clinical use, will frequently have estradiol levels below the level of detectability. In contrast, as estrone–sulfate circulates at much higher levels, this steroid is usually in the detectable range in women receiving aromatase inhibitor therapy. Due to its high plasma level, $E_1S$ has been considered a potential source for tissue estrogens through uptake and hydrolysis [56]. While $E_1S$ may easily be hydrolyzed into unconjugated hormones, its potential contribution to tissue estrogen levels, based on arguments raised above, may be questioned. Whether plasma $E_1S$ may be a significant contributor to tissue $E_2$ or not, plasma $E_1S$ may be a good proxy parameter for estrogen suppression with potent aromatase inhibitors as it exist at equilibrium with unconjugated $E_1$ and $E_2$ [21]. When considering tissue estrogen measurements, this becomes even more demanding, in general and requires pre-purification of samples by use of HPLC.

Taken together, while reliable radioimmunoassays are available and have played an important role in translational research, such methods are too time- and labor-demanding to be functional for routine purposes. While different liquid and gas chromatographic methods over the years gradually have improved and should be expected to replace use of radioimmunoassays for research and,

in particular, routine analysis in the future, it should be emphasized that, at this stage, there are only a few laboratories around the world with methods documented to have a detection limit allowing plasma estrogen assessment in patients on treatment with aromatase inhibitors. While such methods are expected to continuously improve, notably, they should be subject to the same strict criteria documenting sensitivity and specificity in the low concentration range with reproducibility similar to what has been shown with respect to the most sensitive radioimmunoassay's [55].

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## References

[1] Jensen EV, DeSombre ER, Jungblut PP. Estrogen receptors in hormone-responsive tissues and tumors. In: Wissler RW, Dao TL, Wood Jr S, editors. Endogenous factors influencing host-tumor balance. University of Chicago Press; 1967. p. 15–30.
[2] McGuire WL. Steroid receptors in human breast cancer. Cancer Res 1978;38:4289–91.
[3] Cash R, Brough AJ, Cohen MNP, Satoh PS. Aminoglutethimide (Elipten-Ciba) is an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial. J Clin Endocrinol Metab 1967;27:1239–48.
[4] Santen RJ, Lipton A, Kendall J. Successful medical adrenalectomy with aminoglutethimide. JAMA 1974;230:1661–5.
[5] Samojlik E, Veldhuis JD, Wells SA, Santen RJ. Preservation of androgen secretion during estrogen suppression with aminoglutethimide in the treatment of metastatic breast carcinoma. J Clin Invest 1980;65:602–12.
[6] Santen RJ, Santner S, Davis B, Veldhuis J, Samojlik E, Ruby E. Aminoglutethimide inhibits extraglandular estrogen production in postmenopausal women with breast carcinoma. J Clin Endocrinol Metab 1978;47:1257–65.
[7] Santen RJ, Worgul TJ, Lipton A, Harvey H, Boucher A. Aminoglutethimide as treatment of postmenopausal women with advanced breast carcinoma. Ann Intern Med 1982;96:94–101.
[8] Lønning PE. Aromatase inhibitors in breast cancer. End-Rel Cancer 2004;11:179–89.
[9] Santen RJ, Santner SJ, TilsenMallett N, Rosen HR, Samojlik E, Veldhuis JD. In vivo and in vitro pharmacological studies of aminoglutethimide as an aromatase inhibitor. Cancer Res 1982(42):3353s–9s.
[10] Lønning PE, Skulstad P, Sunde A, Thorsen T. Separation of urinary metabolites of radiolabelled estrogens in man by HPLC. J Steroid Biochem 1989;32:91–7.
[11] Lønning PE, Jacobs S, Jones A, Haynes B, Powles T, Dowsett M. The influence of CGS 16949A on peripheral aromatisation in breast cancer patients. Br J Cancer 1991;63:789–93.
[12] Jones AL, MacNeill F, Jacobs S, Lønning PE, Dowsett M, Powles TJ. The influence of intramuscular 4-hydroxyandrostenedione on peripheral aromatisation in breast cancer patients. Eur J Cancer 1992;28A:1712–6.
[13] MacNeill FA, Jones AL, Jacobs S, Lønning PE, Powles TJ, Dowsett M. The influence of aminoglutethimide and its analogue rogletimide on peripheral aromatisation in breast cancer. Br J Cancer 1992;66:692–7.
[14] MacNeill FA, Jacobs S, Dowsett M, Lønning PE, Powles TJ. The effects of oral 4-hydroxyandrostenedione on peripheral aromatisation in post-menopausal breast cancer patients. Cancer Chemother Pharmacol 1995;36:249–54.
[15] MacNeill FA, Jacobs S, Lønning PE, Powles TJ, Dowsett M. Combined treatment with 4-hydroxyandrostenedione and aminoglutethimide: effects on aromatase inhibition and oestrogen suppression. Br J Cancer 1994;69:1171–5.
[16] Geisler J, King N, Dowsett M, Ottestad L, Lundgren S, Walton P, Kormeset PO, Lønning PE. Influence of anastrozole (Arimidex), a selective, non-steroidal aromatase inhibitor, on in vivo aromatisation and plasma oestrogen levels in postmenopausal women with breast cancer. Br J Cancer 1996;74:1286–91.
[17] Geisler J, King N, Anker G, Ornati G, Di Salle E, Lønning PE, Dowsett M. In vivo inhibition of aromatization by exemestane, a novel irreversible aromatase inhibitor, in postmenopausal breast cancer patients. Clin Cancer Res 1998;4:2089–93.
[18] Geisler J, Haynes B, Anker G, Dowsett M, Lønning PE. Influence of letrozole (Femara) and anastrozole (Arimidex) on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over-designed study. J Clin Oncol 2002;20:751–7.
[19] Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J, Buyse M, Baum M, Buzdar A, Colleoni M, et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol 2010;28:509–18.
[20] Lundgren S, Gundersen S, Klepp R, Lønning PE, Lund E, Kvinnsland S. Megestrol acetate versus aminoglutethimide for metastatic breast cancer. Breast Cancer Res Treat 1989;14:201–6.
[21] Lonning PE, Helle H, Duong NK, Ekse D, Aas T, Geisler J. Tissue estradiol is selectively elevated in receptor positive breast cancers while tumour estrone is reduced independent of receptor status. J Steroid Biochem Mol Biol 2009;117:31–41.
[22] Santen RJ, Demers LM, Adlercreutz H, Harvey H, Santner S, Sanders S, Lipton A. Inhibition of aromatase with cgs 16949a in postmenopausal women. J Clin Endocrinol Metab 1989;68:99–106.
[23] Demers LM, Lipton A, Harvey HA, Kambic KB, Grossberg H, Brady C, Santen RJ. The efficacy of CGS 20267 in suppressing estrogen biosynthesis in patients with advanced stage breast cancer. J Steroid Biochem Mol Biol 1993;44:687–91.
[24] Dowsett M, Goss PE, Powles TJ, Hutchinson G, Brodie AMH, Jeffcoate SL, Coombes RC. Use of the aromatase inhibitor 4-hydroxyandrostenedione in postmenopausal breast cancer: optimization of therapeutic dose and route. Cancer Res 1987;47:1957–61.
[25] Dowsett M, Cunningham DC, Stein RC, Evans S, Dehennin L, Hedley A, Coombes RC. Dose-related endocrine effects and pharmacokinetics of oral and intramuscular 4-hydroxyandrostenedione in postmenopausal breast cancer patients. Cancer Res 1989;49:1306–12.
[26] Dowsett M, Stein RC, Mehta A, Coombes RC. Potency and selectivity of the non-steroidal aromatase inhibitor CGS 16949A in postmenopausal breast cancer patients. Clin Endocrinol 1990;32:623–34.
[27] Dowsett M, MacNeill F, Mehta A, Newton C, Haynes B, Jones A, Jarman M, Lonning P, Powles TJ, Coombes RC. Endocrine, pharmacokinetic and clinical studies of the aromatase inhibitor 3-ethyl-3-(4-pyridyl)piperidine-2,6-dione ('pyridoglutethimide') in postmenopausal breast cancer patients. Br J Cancer 1991;64:887–94.
[28] Dowsett M, Mehta A, King N, Smith IE, Powles TJ, Stein RC, Coombes RC. An endocrine and pharmacokinetic study of four oral doses of formestane in postmenopausal breast cancer patients. Eur J Cancer 1992;28:415–20.
[29] Iveson TJ, Smith IE, Ahern J, Smithers DA, Trunet PF, Dowsett M. Phase I study of the oral nonsteroidal aromatase inhibitor CGS 20267 in postmenopausal patients with advanced breast cancer. Cancer Res 1993;53:266–70.
[30] Dixon JM, Renshaw L, Young O, Murray J, Macaskill EJ, McHugh M, Folkerd E, Cameron DA, A'Hern RP, Dowsett M. Letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. J Clin Oncol 2008;26:1671–6.
[31] Lønning PE, Ekse D. A sensitive assay for measurement of plasma estrone sulphate in patients on treatment with aromatase inhibitors. J Steroid Biochem Mol Biol 1995;55:409–12.
[32] Geisler J, Ekse D, Helle H, Duong N, Lønning P. An optimised, highly sensitive radioimmunoassay for the simultaneous measurement of estrone, estradiol and estrone sulfate in the ultra-low range in human plasma samples. J Steroid Biochem Mol Biol 2008;109:90–5.
[33] Lonning PE, Helle SI, Johannessen DC, Adlercreutz H, Lien EA, Tally M, Ekse D, Fotsis T, Anker GB, Hall K. Relations between sex hormones, sex hormone binding globulin, insulin-like growth factor-I and insulin-like growth factor binding protein-1 in post-menopausal breast cancer patients. Clin Endocrinol (Oxf) 1995;42:23–30.
[34] Geisler J, Helle H, Ekse D, Duong NK, Evans DB, Nordbo Y, Aas T, Lønning PE. Letrozole is superior to anastrozole in suppressing breast cancer tissue and plasma estrogen levels. Clin Cancer Res 2008;14:6330–5.
[35] Klein KO, Demers LM, Santner SJ, Baron J, Cutler GBJ, Santen RJ. Use of ultrasensitive recombinant cell bioassay to measure estrogen levels in women with breast cancer receiving the aromatase inhibitor, letrozole. J Clin Endocrinol Metab 1995;80:2658–60.
[36] Johannessen DC, Engan T, Salle Ed, Zurlo MG, Paolini J, Ornati G, et al. Endocrine and clinical effects of exemestane (PNU 155971), a novel steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study. Clin Cancer Res 1997;3:1101–8.
[37] Pfeiler G, Konigsberg R, Fesl C, Mlineritsch B, Stoeger H, Singer CF, Postlberger S, Steger GG, Seifert M, Dubsky P, et al. Impact of body mass index on the efficacy of endocrine therapy in premenopausal patients with breast cancer: an analysis of the prospective ABCSG-12 trial. J Clin Oncol 2011;29:2653–9.
[38] Sestak I, Distler W, Forbes JF, Dowsett M, Howell A, Cuzick J. Effect of body mass index on recurrences in tamoxifen and anastrozole treated women: an exploratory analysis from the ATAC trial. J Clin Oncol 2010;28:3411–5.
[39] Ewertz M, Gray KP, Regan MM, Ejlertsen B, Price KN, Thurlimann B, Bonnefoi H, Forbes JF, Paridaens RJ, Rabaglio M, et al. Obesity and risk of recurrence or death after adjuvant endocrine therapy with letrozole or tamoxifen in the breast international group 1–98 trial. J Clin Oncol 2012;30:3967–75.
[40] Folkerd EJ, Dixon JM, Renshaw L, A'Hern RP, Dowsett M. Suppression of plasma estrogen levels by letrozole and anastrozole is related to body mass index in patients with breast cancer. J Clin Oncol 2012;30:2977–80.
[41] Lønning PE, Haynes B, Dowsett M. Relationship of body mass index with aromatisation and plasma and tissue estrogen levels in postmenopausal breast cancer patients treated with aromatase inhibitors. Eur J Cancer 2014;50:1055–64.
[42] van Landeghem AJJ, Poortman J, Nabuurs M, Thijssen JHH. Endogenous concentration and subcellular distribution of estrogens in normal and malignant breast tissue. Cancer Res 1985;45:2900–4.

[43] Miller WR. Aromatase activity in breast tissue. J Steroid Biochem Mol Biol 1991;39:783–90.
[44] Santner SJ, Leszcynski D, Wright C, Manni A, Feil PD, Santen RJ. Estrone sulfate: a potential source of estradiol in human breast cancer tissue. Breast Cancer Res Treat 1986;7:35–44.
[45] Geisler J, Berntsen H, Lønning P. A novel HPLC-RIA method for the simultaneous detection of estrone, estradiol and estrone sulphate levels in breast cancer tissue. J Steroid Biochem Mol Biol 2000;72:259–64.
[46] Geisler J, Detre S, Berntsen H, Ottestad L, Lindtjørn B, Dowsett M, Lønning PE. Influence of neoadjuvant anastrozole (Arimidex) on intratumoral estrogen levels and proliferation markers in patients with locally advanced breast cancer. Clin Cancer Res 2001;7:1230–6.
[47] Chetrite GS, CortesPrieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Mol Biol 2000;72:23–7.
[48] Haynes BP, Straume AH, Geisler J, A'Hern R, Helle H, Smith IE, Lønning PE, Dowsett M. Intratumoral estrogen disposition in breast cancer. Clin Cancer Res 2010;16:1790–801.
[49] Larionov AA, Berstein LM, Miller WR. Local uptake and synthesis of oestrone in normal and malignant postmenopausal breast tissues. J Steroid Biochem Mol Biol 2002;81:57–64.
[50] Dunbier AK, Anderson H, Ghazoui Z, Folkerd EJ, A'Hern R, Crowder RJ, Hoog J, Smith IE, Osin P, Nerurkar A, et al. Relationship between plasma estradiol levels and estrogen-responsive gene expression in estrogen receptor-positive breast cancer in postmenopausal women. J Clin Oncol 2010;28:1161–7.
[51] Lonning PE, Haynes BP, Straume AH, Dunbier A, Helle H, Knappskog S, Dowsett M. Exploring breast cancer estrogen disposition: the basis for endocrine manipulation. Clin Cancer Res 2011;17:4948–58.
[52] Lønning PE, Kvinnsland S, Thorsen T, Ueland PM. Alterations in the metabolism of oestrogens during treatment with aminoglutethimide in breast cancer patients. Preliminary findings. Clin Pharmacokinet 1987;13:393–406.
[53] Lønning PE, Johannessen DC, Thorsen T. Alterations in the production rate and the metabolism of oestrone and oestrone sulphate in breast cancer patients treated with aminoglutethimide. Br J Cancer 1989;60:107–11.
[54] Lønning PE, Geisler J, Johannessen DC, Ekse D. Plasma estrogen suppression with aromatase inhibitors evaluated by a novel, sensitive assay for estrone sulphate. J Steroid Biochem Mol Biol 1997;61:255–60.
[55] Folkerd E, Lønning PE, Dowsett M. Interpreting plasma estrogen levels in breast cancer: caution needed. J Clin Oncol 2014;32:1396–400.
[56] Santner SJ, Feil PD, Santen RJ. *In situ* estrogen production via the estrone sulfatase pathway in breast tumors: relative importance *versus* the aromatase pathway. J Clin Endocrinol Metab 1984;59:29–33.