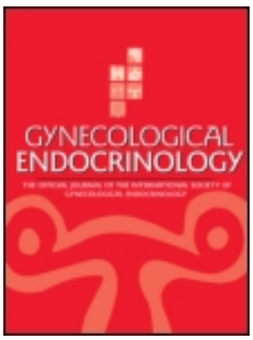

Gynecological Endocrinology

ISSN: 0951-3590 (Print) 1473-0766 (Online) Journal homepage: http://www.tandfonline.com/loi/igye20

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To cite this article: J. R. Pasqualini, C. Ebert & G. S. Chetrite (2001) Biological effects of progestins in breast cancer, Gynecological Endocrinology, 15:sup6, 44-52, DOI: 10.1080/gye.15.s6.44.52

To link to this article: http://dx.doi.org/10.1080/gye.15.s6.44.52

Published online: 18 Jun 2012.

Gynecol Endocrinol 2001;15(Suppl 6):44–52

Biological effects of progestins in breast cancer

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Key words: BREAST CANCER, ESTROGENS, PROGESTINS

ABSTRACT

The action of progestins is derived from many factors: structure, affinity for the progesterone receptor or for other steroid receptors, the target tissue considered, the biological response, the experimental conditions, the dose and metabolic transformation. The proliferative response to progestins in human breast cancer cells is contradictory: some progestins inhibit, others stimulate, have no effect at all, or have a dual action. For instance, medroxyprogesterone acetate has a stimulatory effect on breast cancer cells after a short period of treatment, but this effect becomes inhibitory when treatment is prolonged. It has been demonstrated that, in hormone-dependent breast cancer cells, various progestins (nomegestrol acetate, medrogestone, promegestone) are potent sulfatase inhibitory agents. The progestins can also involve the inhibition of the mRNA expression of this enzyme. In another series of studies it was also demonstrated that some progestins are very active in inhibiting 17β-hydroxysteroid dehydrogenase for the conversion of estrone to estradiol. More recently it was observed that the progestins promegestone and medrogestone stimulate sulfotransferase for the formation of estrogen sulfates. Consequently, the action of progestins in blocking estradiol formation via sulfatase, or in stimulating the effect on sulfotransferase activity, can open interesting and new possibilities in clinical applications in breast cancer.

INTRODUCTION

Progestins can be defined as substances that, like the natural progesterone, can transform an endometrium primed by estrogens into secretory status (Clauberg test). In general, progestins down-regulate target tissue estrogen receptors (ERs) and stimulate pathways of estrogen metabolism. Progestins exert their progestational activity by binding to the progesterone receptor (PR) (form A, the most active, and form B, the less active) and may also interact with other steroid hormone receptors (androgen, glucocorticoid, mineralocorticoid and estrogen).

Progestins can have important effects in other tissues besides the endometrium, including the breast, liver, bone and brain. The biological responses of progestins cover a very large domain: lipids, carbohydrates, proteins, water and electrolyte regulation, hemostasis and fibrinolysis, as well as cardiovascular and immunological systems. The action of progestins depends upon the administration route (e.g. oral, vaginal) as well as the dose used.

In recent years there has been an extraordinary development in the synthesis of new progestins. The synthetic progestins possess the structure of steroids. At present, more than 100 are available and a large variety are commercialized in different countries.

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The action of a progestin depends on many factors: its structure, affinity for the PR or other steroid receptors, the target tissue considered, the biological response, the experimental conditions, the dose and metabolic transformation.

CLASSIFICATION OF PROGESTINS

Progestins can be classified into various groups on the basis of their chemical structure and their distinctive biological effects. Table 1 indicates the different structural groups, including: pregnane, 19-nor-progesterone, 17α-hydroxy-nor-progesterone, androstane and estrane derivatives, the different progestins belonging to the group, their commercial name and the corresponding pharmaceutical firms.

PROGESTINS AND BREAST CANCER PROLIFERATION

In normal breast

Despite the fact that the development of the human breast starts during fetal life, the breast is one of the few organs of the body that is not differentiated at birth. Its growth, with lobule formation, occurs during puberty, but the development and differentiation are completed only at the end of puberty and regress in the postmenopausal period. During the menstrual cycle, the volume and morphology of the breast depend on the fluctuations of gonadal steroids (mainly estrogens and progesterone). Maximal epithelial mitosis is found between 22 and 26 days of the cycle, which corresponds to the high serum levels of estradiol

Table 1 Classification of progestins, their commercial name and pharmaceutical firms

Structure	*Progestin*	*Commercial name*	*Laboratory*
Pregnane derivatives			
Progesterone and derivatives	progesterone	Utrogestan	Besins-Iscovesco
	medrogestone	Colprone-5	Wyeth France
Retroprogesterones	dydrogesterone	Duphaston 10	Solvay
17α-Hydroxyprogesterone derivatives	medroxyprogesterone acetate	Depo-provera	Upjohn
	hydroxyprogesterone caproate	Pharlon	Schering
	megestrol acetate	Megace	B.M.S.
	chlormadinone acetate	Luteran	Solymes
	cyproterone acetate	Androcur	Schering
19-nor-Progesterone derivatives	demegestone	Lutionex	Roussel
	promegestone	Surgestone	Cassenne
	trimegestone	*	HMR/Wyeth
17α-Hydroxy-nor-progesterone derivatives	gestonerone caproate	Depostat	Schering
	nomegestrol acetate	Lutenyl	Theramex
Androstane and estrane derivatives			
19-nor-Testosterone derivatives	norethisterone	Norluten	S.K.F.
	norethisterone acetate	Primolut-Nor	Schering
	norethisterone enanthate	Noristerat	Schering
	lynestrenol	Exluton, Orgametril	Organon
	ethynodiol diacetate	Lutometrodiol	Searle
	norethynodrel	*	Searle
	tibolone	Livial	Organon
	levonorgestrel	Microval	Wyeth-Byla
	gestodene	*	Schering
	desogestrel	Varnoline	Organon
	dienogest	*	Jenapharm
	norgestrienone	Ogyline	Roussel/HMR
	gestrinone	Dimetriose	Roussel/HMR
	norgestimate	*	Ortho

*These progestins are applicable only when associated with estrogens in combined progestative contraceptive pills and are not marketed on their own

and progesterone[1]. During pregnancy, it is suggested that the elevated values of circulating progesterone are responsible for the induction of lobular–alveolar development, in order to prepare the breast for lactation[2].

A possible 'direct effect' of progesterone was extensively explored in *in vitro* studies using organ culture, transplantation of normal human breast to nude mice or primary cell culture. The data on the effect of progesterone in breast epithelial proliferation are contradictory. It has been found that progesterone increases DNA synthesis in normal breast epithelium in organ culture[3]. However, other studies have shown that progesterone either decreases or has no effect on the proliferation of normal mammary epithelium explanted into nude mice[4,5]. Using normal epithelial cells of human breast, it was demonstrated that the progestin promegestone (R-5020) decreased cell proliferation[6].

Progestins can inhibit[6] or have no effect upon[4,7] the stimulatory effect provoked by estradiol. The present information clearly demonstrates that, in normal breast, gonadal steroids can act directly on the epithelium, but a possible paracrine mechanism involving stromal factors is to be explored.

In breast cancer

Data on the effects of progestins on cell proliferation in *in vivo* studies in patients with breast cancer are very limited. Most were observed after a combined treatment of estrogens plus progestins. In a study after administration of progesterone alone in patients with breast cancer, Jones and Russo[8] found a decrease in four out of six tumors; however, in the other two tumors there was stimulation of growth. The same authors reported that, in a combined treatment of estradiol plus progesterone, growth increased in four of seven cases at low doses, but treatment with 10- to 100-fold higher concentrations of both hormones invariably led to a decrease in proliferation.

The most important information on the effect of progestins in breast cancer was explored with isolated models: cell lines, organ culture or transplantation of breast cancer cells in nude mice. The data are contradictory; it was reported that progestins could inhibit[9–13], stimulate[14–18], or have no effect on the proliferation of breast cancer cell lines[19].

Figure 1 shows that T-47D breast cancer cells are inhibited by the progestin nomegestrol acetate[9], while Figure 2 indicates a stimulatory effect by gestodene and 3-ketodesogestrel[15].

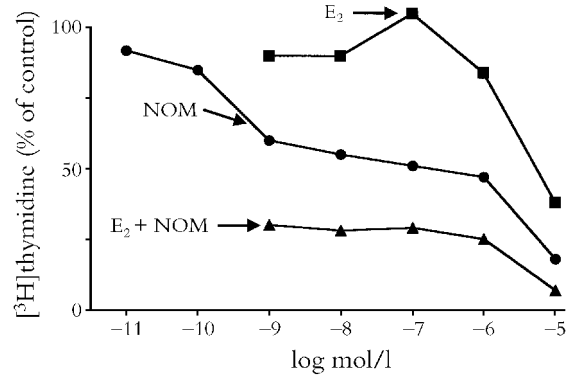

Figure 1 Effect of nomegestrol acetate (NOM) and estradiol (E_2) on the growth of T-47D cells: concentration–response curves. Exponentially growing T-47D cells were plated in 24- or 48-well dishes in DMEM medium containing 5% fetal calf serum in the presence of insulin. After 1 day, cells were treated with NOM alone, E_2 alone, or NOM + 5×10^{-9} mol/l E_2. Media were replaced 3 days later, and after an additional 3-day period, cells were processed for [^{3}H]thymidine incorporation assay. Results are expressed as percentage of control [^{3}H]thymidine values (without NOM and without E_2). Each point represents the mean of five determinations. From reference 9.

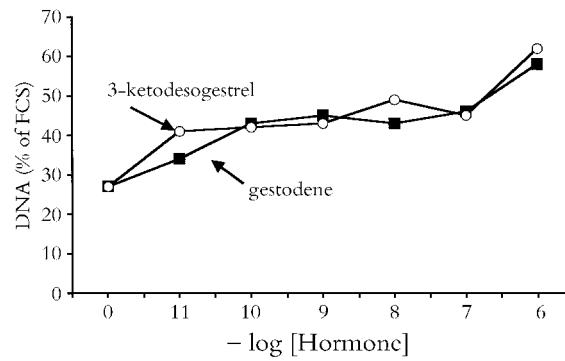

Figure 2 The effect of progestins on the proliferation of T-47D cells. Various concentrations of gestodene and 3-ketodesogestrel were added to the cells. After 4 days the total amount of DNA/well was determined as a measure of proliferation relative to the stimulation by 10% fetal calf serum (FCS). Each point represents the combined data of two independent experiments with determinations in triplicate. From reference 15

In a recent study it was demonstrated that medrogestone, a synthetic pregnane derivative used in the treatment of pathological deficiency of the natural progesterone, had an inhibitory effect on the proliferation of the hormone-dependent T-47D breast cancer cells (unpublished data) (see Figure 3).

The structure of the progestins is an important parameter since, in addition to their progestogenic activity, they can possess estrogenic, androgenic and glucomineralocorticoid properties[20–22]. It was observed that 19-nor-progestins (such as those derived from testosterone: norethindrone, norgestrel or norethynodrel) possess a weak estrogenic activity and can stimulate (at 10^{-6} mol/l) the proliferation of ER-positive but not ER-negative breast cancer cells[14]. Interesting data were obtained with nomegestrol acetate (Lutenyl®), a 19-nor-progestin derivative. This compound does not possess estrogenic activity and is exclusively antiproliferative in MCF-7 and T-47D cells. It was postulated that the estrogenic activity was determined mainly by the 17α-hydroxyl group associated with estrogenic progestins, rather than by the absence of a methyl group at the C_{19} position[23].

Some progestins can have a biphasic effect on breast cell proliferation; for instance, medroxyprogesterone acetate (MPA) increases its proliferative activity in T-47D cells after 24–48 h of treatment followed by an inhibition of this proliferative effect after 72 h[24].

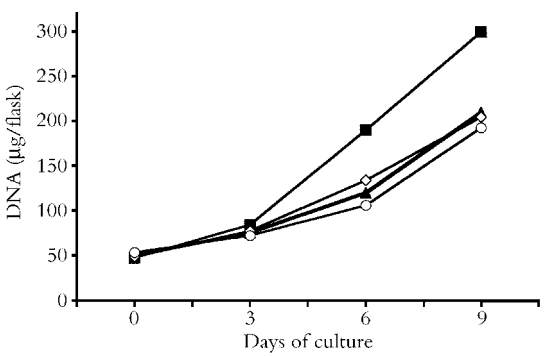

Figure 3 Comparative effects of estradiol 5×10^{-9} mol/l (squares) and medrogestone on the proliferation of T-47D breast cancer cells. T-47D cells were grown in MEM culture medium plus 5% dextran-coated charcoal fetal calf serum int he absence (control, triangles) or the presence of medrogestone (5×10^{-7} mol/l, diamonds; or 5×10^{-5} mol/l, circles). DNA content was evaluated after 3, 6 or 9 days of culture. Data are expressed as the mean of two independent experiments in triplicate

The effects of progestins alone or in combination with estradiol on the proliferation of tumor cell lines are markedly different. A series of data indicates that progestins can stimulate cell proliferation in an estrogen-free environment and induce inhibition of cell growth when cells are cultivated in an estrogenic environment[25,26]. This observation could be explained by the well known fact that estradiol is necessary for inducing the PR[27]. However, Horwitz and Freidenberg[11] have shown that R-5020 can inhibit the growth of the ER-negative T-47Dco antiestrogen-resistant cell line, in which the two PR isoforms are constitutively expressed.

PROGESTINS AND THE MECHANISM OF CELL GROWTH

How can progestins act on cell proliferation? The process by which these compounds are involved in the regulation of cell growth includes steroid receptors, growth factors and their receptors, oncogenes, the cell cycle and metabolizing enzymes of estrogens.

Effect of steroid receptors

It has been demonstrated that progestins reduce the cellular level of ER mRNA by decreasing the transcription of the ER gene[28,29]. This effect is maximal 6 h after the cell treatment. It was also shown that progestins, such as nomegestrol acetate, promegestone (R-5020) and norethindrone acetate, decrease by 80% the level of PR in the hormone-dependent T-47D and MCF-7 breast cancer cells[9,30].

Effects on growth factors, growth factor receptors and oncogenes

The effects of progestins on growth factors and oncogenes in breast cancer cells are contradictory. Progestins can inhibit the mitogenic effects of insulin-like growth factors (IGFs) in breast cancer cells either by decreasing expression of IGF receptors or by increasing the level of IGF-binding protein-1, which inhibits the mitogenic effect of IGF-I[31–33]. However, in another series of studies it was reported that, in T-47D cells, progesterone could potentiate the stimulatory growth effects of

insulin by increasing the level of insulin-receptor expression[16].

Expression of proto-oncogenes, such as c-*myc*, c-*fos* and c-*jun*, is stimulated by progestins[13,34,35]. A sequence sharing a great deal of homology with the progesterone response element (PRE) was recently identified in the 5′ flanking region of the human c-*myc* gene. This positive progesterone regulatory region confers progestin responsiveness in PR-rich T-47D cells, but not in PR-negative MDA-MB-231 cells[36]. The expression of the tumor-suppressor protein p53 is also decreased by progestins in T-47D cells, possibly contributing to the stimulatory activity[37].

EFFECTS OF PROGESTINS ON THE ENZYMES INVOLVED IN ESTROGEN BIOSYNTHESIS IN NORMAL AND CANCEROUS BREAST CELLS

It has been demonstrated that breast tumors and mammary cancer cells possess the enzymatic systems necessary for the intratumoral biosynthesis of estrogens from precursor molecules present in the plasma. Two main enzymatic pathways are implicated in this process: the aromatase pathway, which converts androgens into estrogens[38,39], and the sulfatase pathway, which transforms estrone sulfate to estrone[40–42]. Estrone is then reduced to estradiol by reductive 17β-hydroxysteroid dehydrogenase (17β-HSD) activity. Data indicate that the activity of estrone sulfatase in breast tumors is 10- to 500-fold higher than aromatase activity[43,44].

Figure 4 gives a schematic representation of the enzymatic process involved in the formation and transformation of estrogens in breast cancer.

Effect of progestins on estrone sulfatase action in breast cancer cells

In breast cancer cells (MCF-7, T-47D), promegestone (R-5020), nomegestrol acetate, medrogestone, norethisterone, as well as danazol, at a range of concentrations between 5×10^{-7} and 5×10^{-6} mol/l decrease the sulfatase activity by 40–70%[45–50]. In the homogenates of breast cancer cells (MCF-7, T-47D), R-5020 is a competitive inhibitor of the sulfatase enzyme[51]. This progestin can also decrease the expression of sulfatase mRNA in both cells. A correlation with the reduction of the enzymatic activity has been observed[52].

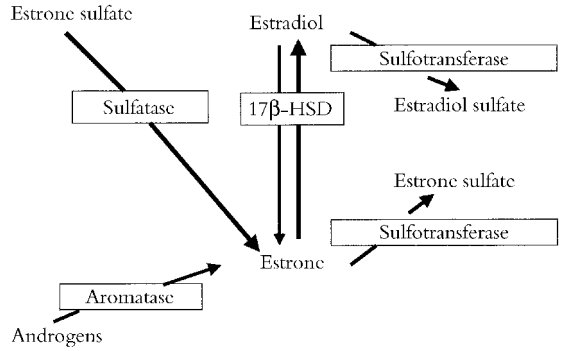

Figure 4 The enzymatic process involved in the formation and transformation of estrogens in human breast cancer. 17β-HSD, 17β-hydroxysteroid dehydrogenase

Effects of progestins on 17ß-hydroxysteroid dehydrogenase

17β-HSD catalyzes bidirectional reactions (oxidative activity: estradiol → estrone and reductive activity: estrone → estradiol) and can be involved in the inactivation or the synthesis of estradiol, respectively. It is now known that the enzyme 17β-HSD consists of a number of isoenzyme forms (up to ten) which express different activities, substrate specificities, regulations and localizations[53,54].

Interesting results show that nomegestrol acetate and medrogestone significantly reduce the reductive 17β-HSD activity in hormone-dependent breast cancer cells[45,47]. This effect is more intense with the PR-rich T-47D cells. Promegestone (R-5020) has no effect on this reductive activity but can increase the oxidative activity (estradiol → estrone) of 17β-HSD in T-47D cells[55,56].

Other authors have obtained an increase of both the reductive and the oxidative 17β-HSD activities with progesterone, MPA, levonorgestrel and norethisterone in MCF-7 cells[57,58]. MPA also stimulates these dual activities in ZR-75–1 cells, whereas Org 2058 increases the oxidative direction in T-47D cells[59,60].

Effects of progestins on sulfotransferases

Estrogen sulfates are water-soluble metabolites of unconjugated estrogens (estrone, estradiol)

which do not bind to the ER and have no estrogenic activity. Therefore, the increase of sulfotransferase activities can diminish the estrogenic stimulation. In breast cancer cells, several isoforms of this enzyme are present: estrogen sulfotransferase is specific for estrogens and acts at nanomolar concentrations; hydroxysteroid sulfotransferase and phenol sulfotransferase can also transform estrogens, but at micromolar concentrations[61,62].

Very little information is available on the regulation of estrogen sulfotransferase expression in breast cancer. Recent data have shown that, in hormone-dependent breast cancer cells (MCF-7, T-47D), low concentrations (5×10^{-7} mol/l) of promegestone (R-5020) can increase the enzyme activity, while higher concentrations (5×10^{-5} mol/l) decrease this activity. This dual effect is correlated with the mRNA expression of estrogen sulfotransferase, which is modulated by promegestone in a similar manner[63]. A stimulatory effect on sulfotransferase activity was also found with medrogestone in MCF-7 and T-47D cells[64].

CONCLUSIONS

Extensive information has been provided by various laboratories about the proliferative effect of different progestins on isolated models using breast cancer cells. These substances can have either an inhibitory or a stimulatory action.

However, interesting data were obtained concerning the action of various progestins (promegestone, nomegestrol acetate, medrogestone) on the inhibition of enzymes involved in the formation of estradiol in breast cancer cells. This includes the inhibitory effect on sulfatases and 17β-HSD.

More recently it was found that some progestins (promegestone, medrogestone) can stimulate sulfotransferase activity. This is an important point in the physiopathology of this disease, because it is well known that the estrogen sulfates are biologically inactive.

The utilization of various progestins in trials with breast cancer patients, showing an inhibitory effect on sulfatases and 17β-HSD, and a stimulatory effect on sulfotransferases, will provide a new possibility in the treatment of this disease.

REFERENCES

1. Longacre TA, Bartow SA. A correlative morphologic study of human breast and endometrium in the menstrual cycle. *Am J Surg Pathol* 1986;10: 382–93
2. Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. *Physiol Rev* 1980;60:1049–106
3. van Bogaert LJ. Effect of hormone on human mammary duct *in vivo*. *Horm Metab Res* 1978;10: 337–40
4. McManus MJ, Welsch CW. The effect of estrogen, progesterone, thyroxine, and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. *Cancer* 1984;54:1920–7
5. Laidlaw IJ, Clarke RB, Howell A, *et al*. The proliferation of normal human breast tissue implanted into athymic nude mice is stimulated by estrogen but not progesterone. *Endocrinology* 1995;136: 164–71
6. Gompel A, Malet C, Spritzer P, *et al*. Progestin effect on cell proliferation and 17β-hydroxysteroid dehydrogenase activity in normal human breast cells in culture. *J Clin Endocrinol Metab* 1986;63: 1174–80
7. Longman SM, Buehring GC. Oral contraceptives and breast cancer. *In vitro* effect of contraceptive steroids on human mammary cell growth. *Cancer* 1987;59:281–7
8. Jones B, Russo J. Influence of steroid hormones on the growth fraction of human breast carcinomas. *Am J Clin Pathol* 1987;88:132–8
9. Botella J, Duranti E, Duc I, *et al*. Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and progesterone receptor content of T-47D human breast cancer cells. *J Steroid Biochem Mol Biol* 1994;50:41–7
10. Vignon F, Bardon S, Chalbos D, *et al*. Antiestrogenic effect of R-5020, a synthetic progestin in human breast cancer cells in culture. *J Clin Endocrinol Metab* 1983;56:1124–30
11. Horwitz KB, Freidenberg GR. Growth inhibition and increase of insulin receptors in antiestrogen-resistant T-47Dco human breast cancer cells by progestins: implication for endocrine therapies. *Cancer Res* 1985;45:167–73

12. Murphy LC, Dotzlaw H, Alkhalaf M, *et al*. Mechanisms of growth inhibition by antiestrogens and progestins in human breast and endometrial cancer cells. *J Steroid Biochem Mol Biol* 1992;43:117–21
13. Musgrove EA, Lee CS, Sutherland RL. Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor alpha, epidermal growth factor receptor, c-*fos* and c-*myc* genes. *Mol Cell Biol* 1991;11:5032–43
14. Jeng M-H, Parker CJ, Jordan VC. Estrogenic potential of progestins in oral contracteptives to stimulate human breast cancer cell proliferation. *Cancer Res* 1992;52:6539–46
15. Kalkhoven E, Kwakkenbos-Isbrücker L, de Laat SW, *et al*. Synthetic progestins induce proliferation of breast tumor cell lines via the progesterone or estrogen receptor. *Mol Cell Endocrinol* 1994;102: 45–52
16. Papa V, Reese CC, Brunetti A, *et al*. Progestins increase insulin receptor content and insulin stimulation of growth in human breast carcinoma cells. *Cancer Res* 1990;50:7858–62
17. Hissom JR, Moore MR. Progestin effects on growth in the human breast cancer cell line T-47D – possible therapeutic implications. *Biochem Biophys Res Commun* 1987;145:706–11
18. Catherino WH, Jeng M-H, Jordan VC. Norgestrel and gestodene stimulate breast cancer cell growth through an estrogen receptor mediated mechanism. *Br J Cancer* 1993;67:945–52
19. Schatz RW, Soto AM, Sonnenschein C. Effects of interaction between estradiol-17β and progesterone on the proliferation of cloned breast tumor cells (MCF-7 and T-47D). *J Cell Physiol* 1985;124:386–90
20. Markiewicz L, Hochberg RB, Gurpide E. Intrinsic estrogenicity of some progestagenic drugs. *J Steroid Biochem Mol Biol* 1992;41:53–8
21. Markiewicz L, Gurpide E. Estrogenic and progestagenic activities coexisting in steroidal drugs: quantitative evaluation by *in vitro* bioassays with human cells. *J Steroid Biochem Mol Biol* 1994; 48:89–94
22. Botella J, Porthe-Nibelle J, Paris J, *et al*. Interaction of new 19-nor progesterone derivatives with progestagen, mineralocorticoid and glucocorticoid cytosolic receptors. *J Pharmacol* 1986;17:699–706
23. Catherino WH, Jordan VC. Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. *J Steroid Biochem Mol Biol* 1995;55:239–46
24. Thuneke I, Schulte HM, Bamberger AM. Biphasic effect of medroxyprogesterone-acetate (MPA) treatment on proliferation and cyclin D1 gene transcription in T47D breast cancer cells. *Breast Cancer Res Treat* 2000;63:243–8
25. van der Berg B, Kalkhoven E, Isbrücker L, *et al*. Effects of progestins on the proliferation of oestrogen-dependent human breast cancer cells under growth factor-defined conditions. *J Steroid Biochem Mol Biol* 1992;42:457–69
26. Hissom JR, Bowden RT, Moore MR. Effects of progestins, estrogens and antihormones on growth and lactate dehydrogenase in the human breast cancer cell line T-47D. *Endocrinology* 1989;125: 418–23
27. Nardulli AM, Greene GL, O'Malley BW, *et al*. Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF-7 cells by estradiol: analysis of estrogen's effect on progesterone receptor synthesis and degradation. *Endocrinology* 1988;122:935–44
28. Alexander IE, Shine J, Sutherland RL. Progestin regulation of estrogen receptor messenger RNA in human breast cancer cells. *Mol Endocrinol* 1990;4: 821–8
29. Perez-Palacios G, Cerbon MA, Pasapera AM, *et al*. Mechanisms of hormonal and antihormonal action of contraceptive progestins at the molecular level. *J Steroid Biochem Mol Biol* 1992;41:479–85
30. Read LD, Snider CE, Miller JS, *et al*. Ligand-modulated regulation of progesterone receptor messenger ribonucleic acid and protein in human breast cancer cell lines. *Mol Endocrinol* 1988;2: 263–71
31. Goldfine ID, Papa V, Vignerie R, *et al*. Progestin regulation of insulin and insulin-like growth factor-I (IGF-I) receptors in cultured human breast cancer cells. *Breast Cancer Res Treat* 1992; 22:69–79
32. Papa V, Hartmann KKP, Rosenthal SM, *et al*. Progestins induce down-regulation of insulin-like growth factor (IGF-I) receptors in human breast cancer cells: potential autocrine role of IGF-II. *Mol Endocrinol* 1991;5:709–17
33. Yee D. The insulin-like growth factor system as target in breast cancer. *Breast Cancer Res Treat* 1994;32:85–95
34. Alkhalaf M, Murphy LC. Regulation of c-*jun* and *jun*-B by in T-47D human breast cancer cells. *Mol Endocrinol* 1992;6:1625–33
35. Wong MMSJ, Murphy LC. Differential regulation of c-*myc* by progestins and antiestrogens in T-47D human breast cancer cells. *J Steroid Biochem Mol Biol* 1991;39:39–44
36. Moore MR, Zhou J-L, Blankenship KA, *et al*. A sequence in the 5′ flanking region confers progestin responsiveness on the human c-*myc* gene. *J Steroid Biochem Mol Biol* 1997;62:243–52

37. Hurd C, Khattree N, Alban P, *et al*. Hormonal regulation of the p53 tumor suppressor protein in T-47D human breast carcinoma cell line. *J Biol Chem* 1995;270:28507–10
38. Lipton A, Santner SJ, Santen RJ, *et al*. Aromatase activity in primary and metastatic human breast cancer. *Cancer* 1987;59:779–82
39. Perel E, Daniilescu D, Kharlip L, *et al*. Steroid modulation of aromatase activity in human cultured breast carcinoma cells. *J Steroid Biochem Mol Biol* 1988;29:393–9
40. Dao TL, Hayes C, Libby PR. Steroid sulfatase activities in human breast tumors. *Proc Soc Exp Biol Med* 1974;146:381–4
41. Pasqualini JR, Gelly C, Nguyen B-L, *et al*. Importance of estrogen sulfates in breast cancer. *J Steroid Biochem Mol Biol* 1989;34:155–63
42. Pasqualini JR, Gelly C, Lecerf F. Biological effects and morphological responses to estriol, estriol-3-sulfate, estriol-17-sulfate and tamoxifen in a tamoxifen-resistant cell line (R-27) derived from MCF-7 human breast cancer cells. *Eur J Cancer Clin Oncol* 1986;22:1495–501
43. 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
44. Pasqualini JR, Chetrite G, Blacker C, *et al*. Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer. *J Clin Endocrinol Metab* 1996;81:1460–4
45. Pasqualini JR, Ebert C, Chetrite G. Control of estradiol in human breast cancer. Effect of medrogestone on sulfatase, 17β-hydroxysteroid dehydrogenase and sulfotransferases activities in human breast cancer cells. In *Proceedings of the IV European Congress on Menopause*. Paris: Editions ESKA, 1998:625–33
46. Pasqualini JR, Varin C, Nguyen B-L. Effect of the progestagen R-5020 (promegestone) and of progesterone on the uptake and on the transformation of estrone sulfate in the MCF-7 and T-47D human mammary cancer cells: correlation with progesterone receptor levels. *Cancer Lett* 1992;66:55–60
47. Chetrite G, Paris J, Botella J, *et al*. Effect of nomegestrol acetate on estrone-sulfatase and 17β-hydroxysteroid dehydrogenase activities in human breast cancer cells. *J Steroid Biochem Mol Biol* 1996;58:525–31
48. Nguyen B-L, Fermé I, Chetrite G, *et al*. Action of danazol on the conversion of estrone sulfate to estradiol and on the sulfatase activity in the MCF-7, T-47D and MDA-MB-231 human mammary cancer cells. *J Steroid Biochem Mol Biol* 1993;46:17–23
49. Carlström K, Doberl A, Pousette A, *et al*. Inhibition of steroid sulfatase activity by danazol. *Acta Obstet Gynecol Scand* 1984;123(Suppl):107–11
50. Evans TRJ, Rowlands MG, Jarman M, *et al*. Inhibition of estrone sulfatase enzyme in human placenta and human breast carcinoma. *J Steroid Biochem Mol Biol* 1991;39:493–9
51. Chetrite G, Varin C, Delalonde L, *et al*. Effect of promegestone, tamoxifen, 4-hydroxytamoxifen and ICI 164,384 on the oestrone sulphatase activity of human breast cancer cells. *Anticancer Res* 1993; 13:931–4
52. Pasqualini JR, Maloche C, Maroni M, *et al*. Effect of the progestagen promegestone (R-5020) on mRNA of the oestrone sulphatase in the MCF-7 human mammary cancer cells. *Anticancer Res* 1994; 14:1589–94
53. Andersson S, Moghrabi N. Physiology and molecular genetics of 17β-hydroxysteroid dehydrogenases. *Steroids* 1997;62:143–7
54. Adamski J, Jakob FJ. A guide to 17β-hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* 2001;171:1–4
55. Nguyen B-L, Chetrite G, Pasqualini JR. Transformation of estrone and estradiol in hormone-dependent and hormone-independent human breast cancer cells. Effects of the antiestrogen ICI 164,384, danazol and promegestone (R-5020). *Breast Cancer Res Treat* 1995;34:139–46
56. Malet C, Vacca A, Kuttenn F, *et al*. 17β-Hydroxysteroid dehydrogenase (E2DH) activity in T47D cells. *J Steroid Biochem Mol Biol* 1991;39: 769–75
57. Adams EF, Coldham NG, James VHT. Steroidal regulation of oestradiol-17β-dehydrogenase activity of the human breast cancer cell line MCF-7. *J Endocrinol* 1988;118:149–54
58. Coldham NG, James VHT. A possible mechanism for increased breast cell proliferation by progestins through increased reductive 17β-hydroxysteroid dehydrogenase activity. *Int J Cancer* 1990;45:174–8
59. Couture P, Thériault C, Simard J, *et al*. Androgen receptor-mediated stimulation of 17β-hydroxysteroid dehydrogenase activity by dihydrotestosterone and medroxyprogesterone acetate in ZR-75–1 human breast cancer cells. *Endocrinology* 1993;132:179–85
60. Poutanen M, Isomaa V, Kainulainen K, *et al*. Progestin induction of 17β-hydroxysteroid dehydrogenase enzyme protein in the T-47D human breast cancer cell line. *Int J Cancer* 1990; 46:897–901
61. Anderson E, Howell A. Oestrogen sulphotransferases in malignant and normal human breast tissue. *Endocr Relat Cancer* 1995;2:227–33

62. Luu-The V, Bernier F, Dufort I. Steroid sulfotransferases. *J Endocrinol* 1996;150:587–97
63. Chetrite G, Le Nestour E, Pasqualini JR. Estrogen sulfotransferase activities and its mRNA in various breast cancer cell lines. Effect of the progestin, promegestone (R-5020). *J Steroid Biochem Mol Biol* 1998;66:295–302
64. Chetrite GS, Ebert C, Wright F, *et al*. Control of sulfatase and sulfotransferase activities by medrogestone in the hormone-dependent MCF-7 and T-47D human breast cancer cell lines. *J Steroid Biochem Mol Biol* 1998;70:39–45