REVIEW

Endocrine-Related Cancer (2012) **19** R35–R50

Antiprogestins in breast cancer treatment: are we ready?

Claudia Lanari, Victoria Wargon, Paola Rojas and Alfredo A Molinolo[1]

Instituto de Biología y Medicina Experimental (IBYME-CONICET), Buenos Aires, Argentina
[1]Oral and Pharyngeal Cancer Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, Maryland 20892-4340, USA

(Correspondence should be addressed to A A Molinolo; Email: amolinol@mail.nih.gov)

Abstract

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in females worldwide. It is accepted that breast cancer is not a single disease, but instead constitutes a spectrum of tumor subtypes with distinct cellular origins, somatic changes, and etiologies. Molecular gene expression studies have divided breast cancer into several categories, i.e. basal-like, ErbB2 enriched, normal breast-like (adipose tissue gene signature), luminal subtype A, luminal subtype B, and claudin-low. Chances are that as our knowledge increases, each of these types will also be subclassified. More than 66% of breast carcinomas express estrogen receptor alpha (ERα) and respond to antiestrogen therapies. Most of these ER+ tumors also express progesterone receptors (PRs), the expression of which has been considered as a reliable marker of a functional ER. In this paper we will review the evidence suggesting that PRs are valid targets for breast cancer therapy. Experimental data suggest that both PR isoforms (A and B) have different roles in breast cancer cell growth, and antiprogestins have already been clinically used in patients who have failed to other therapies. We hypothesize that antiprogestin therapy may be suitable for patients with high levels of PR-A. This paper will go over the experimental evidence of our laboratory and others supporting the use of antiprogestins in selected breast cancer patients.

Endocrine-Related Cancer (2012) **19** R35–R50

Introduction

Breast cancer is the most frequently diagnosed malignant neoplasia and is a leading cause of cancer death in females worldwide. Breast cancer ranks second overall in cancer mortality (10.9%) and accounts for 23% (1.38 million) of new cancer diagnoses and 14% (458 400) of total cancer deaths (Jemal *et al*. 2011). Breast cancer is not a single disease but instead constitutes a spectrum of lesions with distinct cellular origins, somatic changes, and etiologies. Gene expression studies have divided breast cancer into several categories, i.e. basal-like, ErbB2-enriched, normal breast-like (adipose tissue gene signature), luminal subtype A, luminal subtype B, and claudin-low (Prat *et al*. 2010). More than 70% of breast carcinomas express estrogen receptor alpha (ERα) and respond to antiestrogen therapies. These carcinomas may also express progesterone receptors (PRs), which are a reliable marker of functional ERs (Kastner *et al*. 1990, Petz & Nardulli 2000). In this paper, we will review the evidence that PRs are valid targets for breast cancer therapy. We hypothesize that antiprogestin therapy is a valid therapeutic approach for patients with high levels of the PR-A isoform. We will discuss available clinical data and experimental evidence from our laboratory and others that support the therapeutic use of antiprogestins in a subset of breast cancer patients.

Breast cancer and hormones

The bulk of the evidence regarding breast cancer etiology points to estrogens as the major etiological factors (Santen *et al*. 2009). Available experimental and epidemiological evidence, as reviewed in recent papers (Aupperlee *et al*. 2005, Horwitz 2008, Lange *et al*. 2008), have also implicated the PR in breast carcinogenesis. Furthermore, the Women Health Initiative study (Women's Health 2002) and the Million Women Study (Beral 2003) reported an increase in breast cancer risk

DOI: 10.1530/ERC-11-0378

Online version via http://www.endocrinology-journals.org

in women undergoing therapy with estrogen plus a progestin, such as medroxyprogesterone acetate (MPA). These results were later confirmed in other studies (Chlebowski *et al*. 2003, 2010).

More than 70% of breast cancers express ERs and PRs, and are thus susceptible to adjuvant endocrine therapy. This adjuvant therapy is designed to target the ERs using antiestrogens (Jordan 2008), such as tamoxifen (TAM; Jordan 1990) or Fulvestrant (Faslodex, ICI 182 780, AstraZeneca, Cheshire, UK; Dauvois *et al*. 1993), or by inhibiting the endogenous synthesis of 17β-estradiol (E_2) using aromatase inhibitors (Brodie *et al*. 1986). Nevertheless, some of these tumors fail to respond from the very beginning (constitutive-resistant tumors), while others may acquire hormone resistance (McGuire 1975, Jordan 2008).

Because E_2 regulates the expression of the PR (Kastner *et al*. 1990, Petz & Nardulli 2000, Petz *et al*. 2002, Schultz *et al*. 2003) and because there is ample evidence linking progestin to breast cancer pathogenesis, it is reasonable to utilize inhibition of the PRs as a rational target for the management of breast cancer (Moore 2004).

Progesterone receptors

The PR is a member of the steroid–thyroid hormone–retinoid receptor superfamily of ligand-activated nuclear transcription factors (Evans 1988, Kastner *et al*. 1990). Upon progesterone binding, the receptor undergoes a series of conformational changes, dimerizes, and translocates to the nucleus, where it interacts with specific DNA sequences (progesterone receptor elements (PREs)) in the promoter regions of target genes (Edwards *et al*. 1995, Lange *et al*. 2008). These transcriptional effects may also be mediated by PRE-independent actions through protein–protein interactions between the PR and other sequence-specific transcription factors (Leonhardt *et al*. 2003). The PR, like all transcription factors, localizes to the nuclear compartment. It has also been described to be located in the cytoplasm and at the cell membrane (Bottino *et al*. 2011), where it triggers nongenomic or membrane-initiated signaling pathways. PR target genes encode for a wide range of proteins that control or modulate crucial cellular functions, such as cell growth, apoptosis, transcription, steroid, and lipid metabolism (Li & O'Malley 2003). Two PR isoforms have been described: isoform B (PR-B), which is 933 amino acids long in humans with a molecular weight (MW) of 116 kDa; and isoform A (PR-A), which lacks 164 amino acids at the N-terminus but is otherwise identical to isoform B (MW: 94 kDa; Fig. 1A). They are transcribed from two different promoters of the same gene on human chromosome 11 q22–q23 (Kastner *et al*. 1990) or on chromosome 9 in mice (band A1). The presence of CpG islands in both PR promoters indicates that both isoforms may be silenced by CpG island methylation (Vasilatos *et al*. 2009). In mice, the isoforms have a MW of 115 and 83 kDa respectively (Schneider *et al*. 1991). When PR-A and PR-B are present in equimolar amounts in wild-type

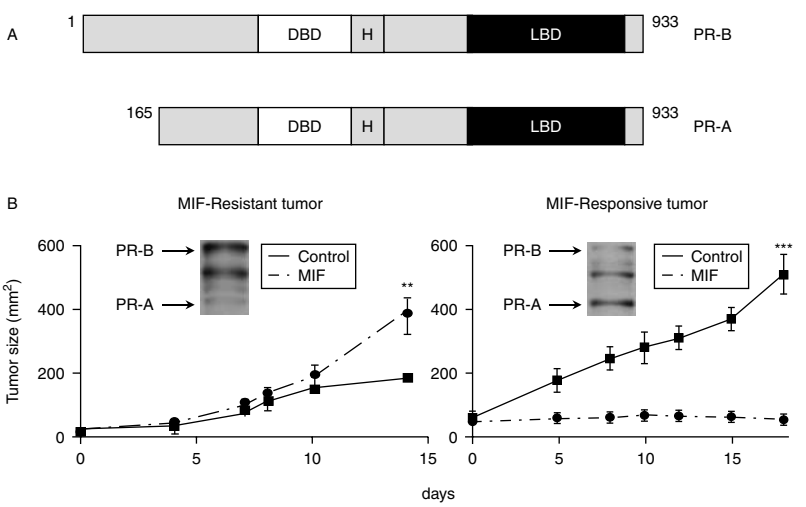

Figure 1 Different PR-B and PR-A ratios in MIF-resistant and MIF-responsive mammary carcinomas. (A) Schematic representation of the two PR isoforms. DBD, DNA binding domain; H, hinge; LBD, Ligand binding domain – PR-A lacks the first 164 amino acids. (B) Representative growth curves of the patterns of MIF responsiveness of tumors with high levels of PR-B (left) or higher levels of PR-A (right). The insets show representative western blots of each tumor.

PR-positive cells or are transiently coexpressed in PR-negative cells, they dimerize and bind to DNA as three species: A/A and B/B homodimers and A/B heterodimers. Posttranscriptional modifications of the PR include phosphorylation, acetylation, sumoylation, and ubiquitination (Dressing & Lange 2009, Hagan *et al*. 2009). Although some sites might be basally phosphorylated, most are phosphorylated by ligand-dependent or ligand-independent mechanisms. Phosphorylation affects the ability of the PRs to interact with the promoters on their target genes, the subsequent transcriptional activation of these genes as well as their ability to interact with other proteins (Takimoto *et al*. 1996, Lange *et al*. 2000). The *PR* (*PGR*) is an estrogen-regulated gene (Horwitz *et al*. 1978, Kastner *et al*. 1990). ER may regulate PR (A or B isoform) by acting on estrogen responsive elements (EREs) or ERE half sites located at great distances up or downstream of the promoters (Carroll *et al*. 2006, Birney *et al*. 2007).

Many of the studies on PRs, including the cloning of the human receptor, were done using T47D cells, a human breast cancer cell line overexpressing both PR isoforms (Keydar *et al*. 1979). Other important information comes from genetically modified mice overexpressing either PR-A (Shyamala *et al*. 1998) or PR-B and from mice lacking one or both of the isoforms (Lydon *et al*. 1995, Conneely & Lydon 2000). It has been shown in these knockout models that the PR isoforms have different roles *in vivo*. PR-B mediates the proliferative effects of progesterone in the mammary gland (Conneely *et al*. 2003, Mulac-Jericevic *et al*. 2003), whereas PR-A is more important in maintaining ovarian and uterine functions. PR-B has been regarded as a much stronger transcriptional activator than PR-A. The latter can act as a ligand repressor of other steroid receptors, including PR-B, ER, androgen receptors, glucocorticoid receptors, or mineralocorticoid receptors, in a cell- and promoter-dependent manner (Boonyaratanakornkit & Edwards 2007).

In T47D cells engineered to express only PR-A (T47D-YA) or PR-B (T47D-YB; Sartorius *et al*. 1994), PR-B controls the majority of the progesterone-regulated genes (~65% of the genes); 4% are regulated by PR-A, and 25% are regulated by both (Richer *et al*. 2002). When PR-A was expressed in PR-null T47D cell models it appeared to regulate a greater number of genes in the absence of added progesterone or progestins relative to forced PR-B expression (Jacobsen *et al*. 2002). However, most of these experiments have been performed in cells forced to express either PR isoform. In normal human tissue there is a balanced expression of both PR isoforms suggesting that heterodimers PR-A–PR-B are responsible for gene expression in normal tissue. This has been extensively reviewed (Scarpin *et al*. 2009), and it has been suggested that a lack of balance of PR isoforms may play a role in influencing cells' transcriptional program.

PR-A is a much more stable PR isoform than PR-B (Faivre & Lange 2007), and it is frequently overexpressed in breast cancer (Graham *et al*. 1995, 2005) usually due to increased transcriptional activity of PR-B that leads to its downregulation (Mote *et al*. 2007). Interestingly, a high ratio of PR-A/PR-B has been associated with poorer outcome in patients undergoing hormonal therapy (Hopp *et al*. 2004). Therefore, evaluation of the PR isoform ratio may be important in breast cancer prognosis and therapeutic decisions.

Antiprogestins

Selective modulators of PRs (SPRM) are classified into three groups. With type I SPRMs, such as onapristone (ONA; ZK 98299; Leonhardt *et al*. 2003), an antagonist-bound PR does not bind to DNA. With type II SPRMs, such as mifepristone (MFP; RU-486), the complex does bind to DNA. Interestingly, type II SPRMs act as agonists if the cells are stimulated with activators of the cAMP/PKA pathway; however, this effect occurs in a PR-B tissue- and species-specific manner. PRs bound to type III modulators bind DNA and have a purely antagonistic effect, even in the presence of activated PKA. This class of SPRMs includes lonaprisan (ZK 230211; Afhuppe *et al*. 2009).

MFP was the first PR antagonist developed for human use. At very low concentrations MFP may behave as an agonist through nongenomic mechanisms (Bottino *et al*. 2011). A similar agonist effect is observed when PR-B is activated by PKA (Beck *et al*. 1993), but this does not occur when it binds to PR-A (Meyer *et al*. 1990). MFP induces PR dimerization and DNA binding with an affinity higher than that of progesterone, the natural ligand (DeMarzo *et al*. 1991, Skafar 1991). The inhibitory effect of MFP is related to its ability to recruit corepressors (Jackson *et al*. 1997). Additionally, MFP has antiglucocorticoid effects, albeit at concentrations much higher than those needed for its antiprogestin activity (Gaillard *et al*. 1984). ONA, which also displays antiglucocorticoid effects at higher concentrations, was discontinued due to hepatotoxicity (Robertson *et al*. 1999).

Lonaprisan, a latest generation antiprogestin (Afhuppe *et al*. 2009, 2010), has low antiglucocorticoid activity and no effect on PKA-activated PR-B (Chwalisz *et al*. 2000, Fuhrmann *et al*. 2000, Afhuppe

et al. 2010). Breast cancer patients are now being recruited for a phase I/II clinical trial of this compound (http://clinicaltrials.gov/ct2/show/NCT00555919).

Aglepristone (RU534), an antiprogestin approved for veterinary use (Galac *et al*. 2004), binds the PR with a high affinity and the GR with lower affinity (Polisca *et al*. 2010). Clinically, aglepristone is indicated for pyometra, pregnancy control, and vaginal fibromas in dogs, and for the treatment of fibroadenomatous mammary hyperplasias in cats (Muphung *et al*. 2009).

Other antiprogestins under development are Org 31710 and Org 31806 from Organon (Oss, The Netherlands), as well as CDB-2914 and CDB-4124 (Contraceptive Development Branch (CDB)) from the National Institute of Child Health and Human Development. Like MFP, both CDBs have 11 alpha substitutions, but in contrast to MFP, they are derivatives of 19-norprogesterone. Additionally, their antiglucocorticoid activity is less than that of MFP (Hild *et al*. 2000, Attardi *et al*. 2002, 2004).

Other SPRMs with mixed agonistic and antagonistic activity include asoprisnil (J867) and its derivatives. These compounds were developed to have ideal SPRM activity, such that they would act both as agonists in the ovaries and as antagonists in the mammary gland and uterus (Chwalisz *et al*. 2005).

Antiprogestins in mammary glands

Data on the effects of antiprogestins on the normal human mammary gland are sparse. Inhibition of cell proliferation was observed in aspirates of mammary glands from postmenopausal women with leiomyomas treated with MFP (50 mg/every other day) for 3 months (Engman *et al*. 2008).

In experimental animals, antiprogestins may induce differentiation by increasing the levels of mammary-derived growth inhibitor (Li *et al*. 1995). In mice, MFP (12 μM/kg in sesame oil) induced activation of the PR in luminal cells to an even greater degree than did the pure agonist R5020 (Han *et al*. 2007). In BALB/c female mice, daily doses of MFP (10 mg/kg) for 1 week reverted MPA-induced branching; however, it resulted in duct differentiation when administered alone (Cerliani *et al*. 2010). It has also been reported that MFP is unable to revert mammary hyperplasia in PR-A transgenic mice (Simian *et al*. 2009) or in FGF2-treated mice (Cerliani *et al*. 2010).

Antiprogestins in breast cancer models

Rats

All of these studies were performed in animals treated with 7,12-dimethylbenz[α]anthracene (DMBA) or *N*-methyl nitrosourea (MNU). In DMBA-treated animals, MFP (10 mg/kg per day for 3 weeks) delayed tumor development (Bakker *et al*. 1987) and inhibited tumor growth. Antiprogestin treatment increased the levels of LH, E_2, prolactin, and progesterone but did not alter the levels of FSH, ACTH, or corticosterone.

MFP (10 mg/kg per day) and TAM (400 μg/kg per day), in combination, induced regression of DMBA-induced mammary tumors (Klijn *et al*. 1989). Two explanations were put forward to explain the increased efficacy resulting from this combined therapy. First, this improved effect could be due to the increase in PR expression induced by TAM (Horwitz 1987) allowing for a better response to MFP. Alternatively, TAM may have negated the effects of high E_2 levels induced by MFP. In this model, ONA was more efficacious than MFP at the same doses (Michna *et al*. 1989), although both drugs increased differentiation. Ovariectomy induced complete regression but did not affect differentiation. The SPRMs Orgs 31710 and 31806 were more effective than MFP when administered *per os* (p.o.; Bakker *et al*. 1990); the responses were observed in combination with LHRH agonists, buserelin or goserelin (Bakker *et al*. 1989). Similar results were obtained with Org 31710 in combination with Org 33628. This antiprogestin was given p.o. and was more effective than MFP (Kloosterboer *et al*. 2000).

The results were comparable when MNU was used as a chemical carcinogen, instead of DMBA, using s.c. antiprogestin doses of 10 mg/kg per day (Michna *et al*. 1989). In contrast to s.c. administration, there were no increases in ACTH levels or the weights of the uterus, adrenals, and ovaries when MFP, Org 31710, or Org 33628 were administered p.o. (Klijn *et al*. 1994). Treatment with TAM increased PR expression. In contrast, administration of MFP alone induced down-regulation of the PR, and the combination of TAM and MFP inhibited the expression of both the ER and the PR.

Additive effects of ONA and TAM were reported in DMBA and MNU rat models (Nishino *et al*. 2009). TAM, at a concentration of 6 mg/kg per day, was more efficacious than when it was administered at a dose of 10 mg/kg per day. Earlier studies had demonstrated that the combination of TAM and ONA treatment at doses of 5 mg/kg per day was more effective than either monotherapy, an effect attributed to decreased circulating progesterone levels observed in animals in the combination treatment group (Nishino *et al*. 2009).

More recently it has been shown that CDB-4124 also suppressed, in a dose-dependent manner, MNU-induced mammary carcinogenesis in rats. CDB-4124 was administered by gavage for 24 months (20–

200 mg/kg per day) or in 3 or 30 mg pellets implanted 6 days after MNU treatment (Wiehle *et al*. 2011).

Mice

ONA or MFP treatment (1 or 10 mg/kg per day) initiated 1 day posttransplantation inhibited both tumor take and the stimulatory effects of E_2 and MPA in the MXT mouse model of breast cancer (Michna *et al*. 1989). ONA proved to be better than MFP at inhibiting cell proliferation at the 10 mg/kg per day dosage. Tumor regression was associated with necrosis, cytolysis, and decreased PR expression. Ovariectomy completely inhibited PR expression (Bakker *et al*. 1989). No significant anti-glucocorticoid effects were seen, and no changes in adrenal gland weight were measured (Schneider *et al*. 1991). Dexamethasone failed to rescue the inhibitory effects of MFP (Bardon *et al*. 1985). An increase in uterine, ovary, and pituitary weight was observed in antiprogestin-treated mice. Histopathological analyses of the uterus and vagina indicated an estrogenic effect, probably due to low estrogen levels (Michna *et al*. 1989). Similarly, we demonstrated that BALB/c mice, treated with antisense PR (asPR) oligonucleotides, showed continuous estrous (Lamb *et al*. 2005).

Genetically modified mice

Nulliparous mice null for BRCA1/p53 developed mammary hyperplasias that express high levels of PR, and eventually progressed to develop adenocarcinomas. MFP (35 mg, 60-day releasing pellets) treatment prevented the induction of either hyperplasia or carcinoma. These authors proposed the use of MFP to prevent breast cancer in BRCA+ women (Poole *et al*. 2006). Interestingly, in normal breast tissues of women with a germline pathogenic mutation in one of the BRCA genes, an increase in PR-A expression has been reported (Mote *et al*. 2004).

Studies on breast cancer cell lines

A growth-modulatory role for progestins in human breast cancer cells remains controversial. Progestins stimulate or inhibit cell proliferation depending on the concentrations and the experimental conditions used. Moreover, while progestins were shown to exert a biphasic effect on breast cancer cells growing on plastic dishes (2D; reviewed in Clarke & Sutherland (1990)), they (MPA or progesterone) were clearly proliferative when these same cells grew in soft agar (Faivre & Lange 2007) or in 3D culture systems (reviewed in Mote *et al*. (2007)), suggesting that modulation of cell polarity/architecture is also required to define progestin-induced cell fate.

MCF-7 and T47D are the most widely used cell lines to study the effects of hormones and hormone antagonists. In MCF-7 cells, MFP inhibited PR-mediated cell proliferation (Bardon *et al*. 1985). Similarly, TAM or MFP at a concentration of 10 nM inhibited E_2-induced cell proliferation (Bakker *et al*. 1987). These experiments were performed using tissue culture media supplemented with 10% steroid-deficient (charcoal-stripped (ch)) human serum.

Different results have been reported by different laboratories using T47D cells. TAM or MFP specifically inhibit E_2-induced cell proliferation in T47D cells, clone 11, which are ER- and PR-positive (Horwitz *et al*. 1982). Other cell lines, similarly cultured, did not show this response (Bardon *et al*. 1985). It has been hypothesized that the inhibitory effect of MFP could be due to the fact that antagonist-bound receptors remain bound to DNA for longer periods of time, thus impeding PR recycling (Sheridan *et al*. 1988). Alternatively, the inhibitory effect caused by MFP could result from its antiestrogenic effects (Vignon *et al*. 1983) or because it may have a different affinity for the PR isoforms (Meyer *et al*. 1990). Furthermore, progestins also inhibited cell proliferation, and it has been suggested that their antiestrogenic actions were responsible for this inhibition. In both cases, entry into S phase was inhibited, and the cells were arrested in G0/G1 (Michna *et al*. 1990).

Other laboratories have reported different results on the inhibitory effects of MPA and MFP on E_2-induced cell proliferation. R5020 (Hissom & Moore 1987) and MFP (Bowden *et al*. 1989, Jeng *et al*. 1993), with the latter at micromolar concentrations, can stimulate the proliferation of T47D and MCF-7 cells. The estrogenic effect of MFP at these high concentrations was attributed to the short length of the group associated with the aromatic nucleus at position 11 β (Jeng *et al*. 1993). Type II antiprogestins, such as MFP, had similar or greater PR affinity than the agonist itself; however, the agonistic effect was inhibited at equimolar concentrations of both ligands, suggesting that there are different levels of regulation in addition to receptor binding. Mixed agonist–antagonist dimers of the PR did not bind to DNA (Edwards *et al*. 1995). MFP-bound PR was able to bind to DNA and with a greater affinity than the agonist-bound PR. In contrast, type I antagonists permitted PR dimerization; however, they bound DNA with a very low affinity, which suggests that different conformational changes are induced by different PR antagonists. T47D cells transfected with reporter genes (*MMTV-CAT*) clearly showed that when these cells are treated with analogs of cAMP, MFP exerts an agonistic effect (Beck *et al*. 1993, Sartorius

et al. 1993). In this experimental setting, ONA still behaved as an antagonist (Edwards *et al*. 1995). MFP treatment (100 nM) increased cell proliferation in T47D-YB cells and induced phosphorylation of ERK, which resulted in increased cyclin D1 expression via nongenomic mechanisms (Skildum *et al*. 2005). These conflicting results may have contributed to the decreased clinical interest in these drugs.

el Etreby *et al*. (2000) demonstrated that MFP and TAM cotreatment increased apoptosis levels (increase in DNA laddering, decrease in Bcl-2, PKC translocation, and increase of transforming growth factor β1 (TGFβ1)). The authors, however, used concentrations as high as 1 μM for TAM and 10 μM for MFP, making it impossible to distinguish between specific and nonspecific PR-mediated effects.

Similarly, Hyder *et al*. (1998) demonstrated that progestins stimulate the synthesis of vascular endothelial growth factor, which plays an important role in tumor angiogenesis. This effect was also blocked with micromolar concentrations of MFP in cells carrying p53 mutations, such as T47D and BT474 cells, but not in cells expressing wild-type p53, such as MCF-7 cells (Liang *et al*. 2005). A similar regulatory mechanism was shown for thrombospondin-1 (TSP-1; Hyder *et al*. 2009). Cytostasis and apoptosis (both the intrinsic and extrinsic pathways) were induced at micromolar MFP concentrations (Gaddy *et al*. 2004).

However, inhibition of progesterone-induced cell proliferation was already observed in MCF-7 cells using nanomolar MFP concentrations (Calaf 2006). A recent study demonstrated that lonaprisan (10 nM) induces apoptosis in T47D cells with a concomitant increase in p21 levels (Busia *et al*. 2011). While it is known that both progestins and antiprogestins increase the expression of p21 (Bottino *et al*. 2011), the induction by progestins is transient (Busia *et al*. 2011).

It has recently been suggested that all of the effects induced by MFP at micromolar concentrations are mediated through nongenomic mechanisms (Fjelldal *et al*. 2010). Moreover, Tieszen *et al*. (2011) showed an inhibition of cell proliferation using cells from nervous system, breast, prostate, ovary, and bone and the authors propose that the growth inhibition of cancer cells by MFP is not dependent upon the expression of classical PR. However, it is worth mentioning that all cell lines responded to the growth inhibitory effect of MFP with IC_{50s} ranging from ∼9 to 30 μM. We agree that these unspecific effects have nothing to do with the specific inhibition observed in breast cancer cells in which the inhibition occurs at concentrations compatible with the PR Kd.

Xenotransplants of human cell lines

E_2-induced proliferation of MCF-7 xenografts in athymic BALB/c mice was inhibited by MFP (50 mg/kg per day) or ONA (30 mg/kg per day) administered for 17 days (el Etreby *et al*. 1998). Combination treatment with TAM (15 mg; 60 days releasing pellet) increased this inhibitory effect. MFP (25 mg; 60 days releasing pellets) can prevent the growth of BT-474 and T47D xenografts in nude mice that had been previously treated with E_2 followed by MPA (Liang *et al*. 2007). Additionally, previous studies have shown that E_2 induces tumor regression, TAM inhibits tumor growth, ONA has no effect, and ZK 112993 (a different antiprogestin) significantly inhibits the growth of T61 human tumors that are maintained by serial transplants in nude mice (Schneider *et al*. 1990).

Antiprogestins in different experimental neoplasias

The variable inhibitory and stimulatory effects attributed to high concentrations of MFP in cells expressing the PR complicate the interpretation of the data from these different studies. Edwards *et al*. (1995) demonstrated that equimolar concentrations of agonists and antagonists exert inhibitory effects. It seems likely that MFP, at concentrations of 1 μM or higher, also induces nonspecific effects that may be masking PR-mediated actions. The same principle holds true in xenograft models. MFP (50 mg/kg per day) was shown to be inhibitory not only in MCF-7 cells but also in prostate (el Etreby *et al*. 2000) and ovarian cancer xenografts (Goyeneche *et al*. 2007). Lower concentrations of antiprogestins should be used if more specific effects are desired, as reported in the rat and mouse models. MFP may also be combined with chemotherapy due to its ability to inhibit multidrug-resistance proteins (Gruol *et al*. 1994, Lecureur *et al*. 1994).

MFP: clinical uses

MFP has been used for different obstetric indications, such as uterine ripening and intrauterine fetal death, at doses of 200 mg/day prior to the vacuum aspirate or in doses of 850–600 mg for 48 h with very low side effects compared to prostaglandins (Ulmann & Dubois 1988). MFP at a dose of 200 mg/12 h increased the percentage of women with spontaneous delivery. The first trial using MFP for abortion purposes was launched in 1981 (Herrman *et al*. 1982). Its use was advocated for different oncological applications,

including breast cancer, prostate cancer, cervical cancer, meningiomas, and leiomyosarcoma (Grunberg *et al*. 1991, 2006, Spitz *et al*. 2005, Engman *et al*. 2008, Check *et al*. 2010, Yoshida *et al*. 2010). Additionally, it has potential use in different psychiatric disorders, including depression and Alzheimer's; however, in these diseases the antiglucocorticoid function seems to be more important (Benagiano *et al*. 2008).

Antiprogestins in breast cancer treatment

Twelve years after the first description of the role of the PR in breast cancer (Horwitz & McGuire 1975), the first clinical trial to evaluate antiprogestin therapy in patients recruited 22 patients for a third-line study (Romieu *et al*. 1987). Each patient had TAM-resistant metastases and had failed to respond to previous chemotherapy and hormone therapies. All study patients were either postmenopausal or had been oophorectomized, and they were treated with 200 mg/day of MFP for 1–3 months. Treatment efficacy was evaluated according to clinical parameters and follow-up levels of carcinoembryonic antigen. There was an 18% response rate following 3 months of therapy. The long-term tolerance was good, and there was an increase in cortisol coupled with a slight decrease in potassium levels. The results of a second trial were reported in 1989 (Klijn *et al*. 1989). Eleven patients with metastases who had received TAM as a first-line therapy were treated with daily doses of 200–400 mg MFP p.o., regardless of their response to TAM; some patients received progestins after MFP as a third-line therapy. There was an objective response in one patient, six patients showed temporal stabilization, and four patients had progressive disease. E_2, ACTH, cortisol, and androstenedione serum levels were increased in all patients. The authors suggested that the increase in E_2 may be due to aromatization of androstenedione, and therefore, they proposed a combinatorial treatment of MFP and TAM to counteract the effects of E_2.

Results from a third study, in which 28 postmenopausal PR+ patients were recruited, were described in 1996 (Perrault *et al*. 1996). These patients were given 200 mg/day of MFP for more than 8 weeks (median: 12.4 weeks). Low-grade side effects were reported in most patients: 68% lethargy, 39% anorexia, 29% vomiting, 50% hot flashes, and 32% skin rash. Only three patients showed a partial response, which indicates a poor overall response rate to the therapy, especially considering that only PR+ patients were preselected. All patients were at advanced stages of their disease with metastases when the treatment was initiated.

A fourth clinical trial with ONA, initiated in 1995, accrued 30 breast cancer patients (Robertson *et al*. 1999). However, the trial had to be stopped while they were recruiting the 19th patient due to liver function test abnormalities. All 19 patients opted to continue with the trial. Two-thirds showed clinical signs of tumor regression: 56% showed partial response, and 11% had stable disease, percentages that are very similar to those obtained with TAM or progestin treatment. The authors emphasized that ONA did not increase circulating E_2 levels.

Klijn *et al*. (2000) reviewed these four studies together with unpublished results from a fifth study. There are no other published clinical results for breast cancer treatment using antiprogestins. However, two clinical trials are currently recruiting for preoperative evaluation of antiprogestins in early stage breast cancer (ClinicalTrials.gov Identifier: NCT01138553, testing MFP, and NCT00555919, Schering, testing lonaprisan).

MFP for the treatment of other neoplasias

MFP (200 mg/day for 2–31 months) has been used to treat meningiomas. Five out of 13 tumors responded after 1 year, with some showing signs of regression within 2–3 months (Grunberg *et al*. 1991). A later study by the same authors showed less promising results; however, the lack of serious side effects still merited the use of MFP (Spitz *et al*. 2005, Grunberg *et al*. 2006). They proposed to combine MFP and dexamethasone treatment during the first 2 weeks to avoid the antiglucocorticoid effects of MFP.

In 2008, a clinical trial with MFP (50 mg/every other day) in leiomyomas showed low levels of E_2 and progesterone and slightly higher concentrations of testosterone and androstenedione (Engman *et al*. 2008). Other SPRMs, such as asoprisnil and CDB-2914, were used for the treatment of nonsurgical leiomyomas (Yoshida *et al*. 2010); their therapeutic effects may be attributed to their agonistic properties.

More recently, two papers have reported on the effects of MFP (200 mg/day) in patients with thymic epithelial cell carcinoma, transitional cell carcinoma of the renal pelvis, leiomyosarcoma, colon adenocarcinoma, pancreatic adenocarcinoma, and malignant fibrous histiocytoma (Check *et al*. 2010). Improvements and pain relief were observed in all patients. The nonspecific effects of MFP in these diseases may be related to the increased activation and recruitment of NK cells, which also express the PR (Arruvito *et al*. 2008).

Contributions of the MPA murine breast cancer model

We developed an experimental model of breast cancer with continuous administration of MPA to female BALB/c mice (Lanari *et al*. 1986, Molinolo *et al*. 1987). The main features of this tumor model were recently reviewed (Lanari *et al*. 2009). Briefly, most tumors that develop in the mice are luminal ductal mammary carcinomas that express high levels of both ERs and PRs. The tumors metastasize to regional lymph nodes and the lungs and are maintained by serial syngeneic transplants (Lanari *et al*. 1989). Initially, all behave in a progestin-dependent manner, but after a few passages, progestin-independent (PI) variants may emerge. These PI variants still retain high levels of ERs and PRs, and they grow similarly in ovariectomized or nonovariectomized mice (Lanari *et al*. 1989, Kordon *et al*. 1990). Hormone-dependent tumors only grow in animals treated with MPA; however, FGF2 (Giulianelli *et al*. 2008, Cerliani *et al*. 2010), tumor necrosis factor α (TNFα; Rivas *et al*. 2008), or 8-Cl-cAMP (Actis *et al*. 1995) may replace MPA to stimulate tumor growth *in vivo*.

PI-responsive tumors regress with MFP, ONA, or lonaprisan treatment at daily doses of 10 mg/kg (Montecchia *et al*. 1999, Helguero *et al*. 2003, Wargon *et al*. 2009) or with aglepristone treatment at a dose of 3 mg/week (V Wargon, M Riggio, V Novaro & C Lanari (2011), unpublished data). The role of the PR in the antiprogestin-induced effect was confirmed using asPR oligonucleotides to knockdown PR expression *in vivo* (Lamb *et al*. 2005). These tumors may also regress with E_2 treatment (0.5–5 mg pellets), almost as well as with antiprogestin treatment. Additionally, tumor growth was inhibited by TAM treatment. Some PI tumors are resistant to these treatments, but they still express hormone receptors. We have demonstrated that constitutively resistant tumors show PR-A silencing due to methylation of the PR-A promoter. Similarly, it has recently been reported that the PR-A promoter is significantly methylated in TAM-resistant patients with poor outcome (Pathiraja *et al*. 2011).

Using selective pressure, we have been able to derive antiprogestin-resistant variants from antiprogestin-sensitive PI tumors. Interestingly, PR-A is downregulated in both constitutive (Helguero *et al*. 2003) and acquired antiprogestin-resistant carcinomas (Wargon *et al*. 2009). Upon estrogen or TAM treatment, tumors with acquired resistance may revert to the antiprogestin responsive phenotype (Wargon *et al*. 2009). In constitutive resistant tumors, however, cotreatment with demethylating agents to increase PR-A expression is necessary for reacquisition of antiprogestin responsiveness (Wargon *et al*. 2011).

C4-PI is one of the PI-responsive variants and C4-2-PI is the constitutive-resistant variant (Lanari *et al*. 2009), both originated from C4-HD. C4-PI tumors are completely inhibited by MFP (Fig. 1), and these tumors have higher levels of PR-A than PR-B (inset). Conversely, C4-2-PI shows higher levels of PR-B than PR-A, and is stimulated by MFP; an effect that seems to be unique for this tumor, because in other constitutive variants, MFP-treated tumors behaved in a manner similar to the controls. In C4-PI tumors treated with MFP an early upshift of the PR-A band is observed in western blots (Wargon *et al*. 2009). After 24 h of treatment both isoforms are downregulated (Lamb *et al*. 2005).

Although the mechanism by which MFP modulates tumor growth depending on the prevailing isoform expressed has not yet been elucidated, it is possible that PR-A homodimers or heterodimers activated by MFP can recruit corepressors instead of coactivators at the promoter regions of key pro-survival genes. Along this line we have recently showed that while both MPA and MFP at 10 nM concentrations can increase STAT5 or MYC expression in C4-PI cells, only MPA was able to increase CCND1 expression (Bottino *et al*. 2011).

We used a dose of 10 mg/kg per day for all antiprogestins or a 6 mg pellet of MFP, but inhibitory effects were also achieved at 1 mg/kg per day. All animals treated with MFP or asRP showed a continuous estrous cycle. The fact that the systemic actions of asPR were similar to those of antiprogestins clearly indicates that this is an indirect effect due to a pure antiprogestin effect.

In primary cultures of responsive tumors, we showed that 1–100 nM concentrations of MFP, ONA, or lonaprisan inhibited MPA-induced or FGF2-induced cell proliferation (Dran *et al*. 1995, Lamb *et al*. 1999). As reported by others (Edwards *et al*. 1995), inhibitory effects were observed when using equimolar concentrations of agonists and antagonists.

Another interesting observation was that MFP inhibited cell proliferation, while it increased ERK phosphorylation. This led us to hypothesize that the nongenomic actions or membrane-initiated effects of progestin and antiprogestins may occur at lower concentrations than those needed to elicit genomic effects. Furthermore, if MFP stimulated ERK through nongenomic mechanisms, then the proliferative effects should be observed at low MFP concentrations. In fact, we demonstrated that very low concentrations of MFP (10^{-12} M) were able to stimulate cell proliferation. *In vivo*, concentrations 10^4 times lower than those that

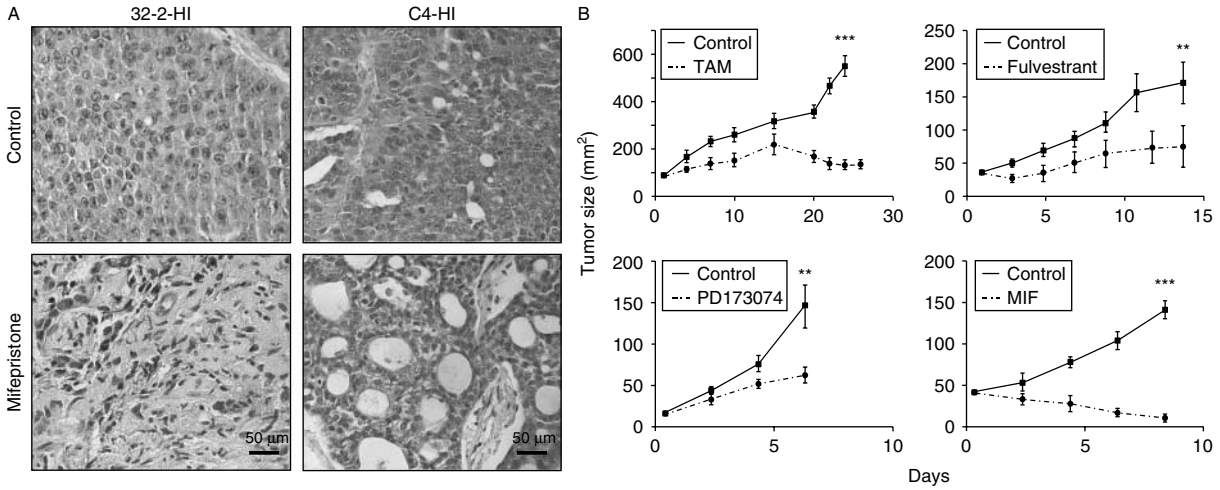

Figure 2 MIF-induced tumor regression. (A) MIF induces an increase in apoptosis and cytostasis, which is associated with a concomitant increase of the stromal compartment (left). In other tumors, MIF induces differentiation (right). (B) C4-PI tumors treated with TAM, Fulvestrant, or with an FGFR inhibitor PD 173074 show an inhibition of tumor growth ($P<0.01$) MIF induced complete regression ($P<0.001$).

exerted growth inhibitory effects stimulated C4-PI growth (Bottino *et al*. 2011). These results underscore the relevance of evaluating the PR isoform prior to administering an antiprogestin to breast cancer patients and indicate that concentrations high enough to induce a genomic response are the ones indicated for therapeutic purposes.

Antiprogestin-induced tumor regression

Tumor regression induced by antiprogestins or E_2 is a complex phenomenon involving stromal–parenchymal interactions. Increased cytostasis and apoptosis are the hallmarks of hormone-induced regression. The early events consist of increases in p21, p27, and p53 expression followed by a later decrease in hormone receptor expression (Vanzulli *et al*. 2002, 2005). This suggests that the decrease in hormone receptor expression is not the primary event that triggers regression. Certain tumors also show an increase in differentiation (Wargon *et al*. 2009); in these cases, there is a less evident increase in apoptosis. The stromal tissue shows signs of activation, including the translocation of β-catenin to the nucleus in carcinoma-associated fibroblasts and an increase in laminin, collagen I, and collagen IV deposited in the interstitial space between the tumor cells. This is also associated with increases in metalloproteases 2 and 9 (Simian *et al*. 2006). In Fig. 2A (left), we show a representative image of a 32-2-PI tumor following MFP treatment. This is a poorly differentiated adenocarcinoma with few connective tissue strands (control). After treatment, the tumor regresses, and the epithelial component is replaced by dense connective tissue with few remaining epithelial clusters. C4-PI is a moderately differentiated adenocarcinoma (Fig. 2A, right). Following MFP treatment, an increase in differentiation with numerous glandular structures is observed. In Fig. 2B, we show growth curves of C4-PI treated with TAM, Fulvestrant, an FGFR inhibitor (PD 173074) or MFP. This experiment provides evidence that targeting the PR is an effective therapeutic approach in these tumors. It is possible that all other treatments, in combination with MFP, may delay the onset of hormone resistance.

Conclusion

The clinical and experimental data reviewed herein strongly suggest that antiprogestins have a potential to be used in combination with TAM in a subgroup of breast cancer patients. We have demonstrated in experimental models that only tumors with levels of PR-A higher than those of PR-B can be specifically targeted with this therapy. The challenge is to determine in human breast cancer samples which are the patients who match this criterion. At the moment western blot is the adequate tool to quantify PR isoform ratios. However, we should still look for potential biomarkers to be used in immunohistochemistry associated with high expression of PR-A. Genes that are upregulated by progesterone treatment in T47D-YA cells, such as *BCL-XL, ERRalpha1, HEF1*, or *DSIPI*, may be excellent candidates to start working with (Richer *et al*. 2002).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Funding

Dr Molinolo is supported by the Intramural Research Program, NIDCR, NIH.

Acknowledgements

We are very grateful to the Laboratorios Craveri, Buenos Aires, for providing MPA depot and to Bayer Schering Pharma AG for ZK230211. We also wish to thank the Avon Foundation for AACR travel awards, the UICC for the ICRETT fellowships awarded to fellows from our labs and Fundación Sales, CONICET and Agencia de Promoción Científica y Tecnológica from Argentina for funding.

References

Actis AM, Caruso SP & Levin E 1995 Opposite effect of a cAMP analogue on tumoral growth related to hormone dependence of a murine mammary tumor. *Cancer Letters* **96** 81–85. (doi:10.1016/0304-3835(95)03908-F)

Afhuppe W, Sommer A, Muller J, Schwede W, Fuhrmann U & Moller C 2009 Global gene expression profiling of progesterone receptor modulators in T47D cells provides a new classification system. *Journal of Steroid Biochemistry and Molecular Biology* **113** 105–115. (doi:10.1016/j.jsbmb.2008.11.015)

Afhuppe W, Beekman JM, Otto C, Korr D, Hoffmann J, Fuhrmann U & Moller C 2010 *In vitro* characterization of ZK 230211 – a type III progesterone receptor antagonist with enhanced antiproliferative properties. *Journal of Steroid Biochemistry and Molecular Biology* **119** 45–55. (doi:10.1016/j.jsbmb.2009.12.011)

Arruvito L, Giulianelli S, Flores AC, Paladino N, Barboza M, Lanari C & Fainboim L 2008 NK cells expressing a progesterone receptor are susceptible to progesterone-induced apoptosis. *Journal of Immunology* **180** 5746–5753.

Attardi BJ, Burgenson J, Hild SA, Reel JR & Blye RP 2002 CDB-4124 and its putative monodemethylated metabolite, CDB-4453, are potent antiprogestins with reduced antiglucocorticoid activity: *in vitro* comparison to mifepristone and CDB-2914. *Molecular and Cellular Endocrinology* **188** 111–123. (doi:10.1016/S0303-7207(01)00743-2)

Attardi BJ, Burgenson J, Hild SA & Reel JR 2004 *In vitro* antiprogestational/antiglucocorticoid activity and progestin and glucocorticoid receptor binding of the putative metabolites and synthetic derivatives of CDB-2914, CDB-4124, and mifepristone. *Journal of Steroid Biochemistry and Molecular Biology* **88** 277–288. (doi:10.1016/j.jsbmb.2003.12.004)

Aupperlee M, Kariagina A, Osuch J & Haslam SZ 2005 Progestins and breast cancer. *Breast Disease* **24** 37–57.

Bakker GH, Setyono-Han B, Henkelman MS, de Jong FH, Lamberts SW, van der Schoot P & Klijn JG 1987 Comparison of the actions of the antiprogestin mifepristone (RU486), the progestin megestrol acetate, the LHRH analog buserelin, and ovariectomy in treatment of rat mammary tumors. *Cancer Treatment Reports* **71** 1021–1027.

Bakker GH, Setyono-Han B, Portengen H, de Jong FH, Foekens JA & Klijn JG 1989 Endocrine and antitumor effects of combined treatment with an antiprogestin and antiestrogen or luteinizing hormone-releasing hormone agonist in female rats bearing mammary tumors. *Endocrinology* **125** 1593–1598. (doi:10.1210/endo-125-3-1593)

Bakker GH, Setyono-Han B, Portengen H, de Jong FH, Foekens JA & Klijn JG 1990 Treatment of breast cancer with different antiprogestins: preclinical and clinical studies. *Journal of Steroid Biochemistry and Molecular Biology* **37** 789–794. (doi:10.1016/0960-0760(90)90421-G)

Bardon S, Vignon F, Chalbos D & Rochefort H 1985 RU486, a progestin and glucocorticoid antagonist, inhibits the growth of breast cancer cells via the progesterone receptor. *Journal of Clinical Endocrinology and Metabolism* **60** 692–697. (doi:10.1210/jcem-60-4-692)

Beck CA, Weigel NL, Moyer ML, Nordeen SK & Edwards DP 1993 The progesterone antagonist RU486 acquires agonist activity upon stimulation of cAMP signaling pathways. *PNAS* **90** 4441–4445. (doi:10.1073/pnas.90.10.4441)

Benagiano G, Bastianelli C & Farris M 2008 Selective progesterone receptor modulators 3: use in oncology, endocrinology and psychiatry. *Expert Opinion on Pharmacotherapy* **9** 2487–2496. (doi:10.1517/14656566.9.14.2487)

Beral V 2003 Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet* **362** 419–427. (doi:10.1016/S0140-6736(03)14596-5)

Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE *et al.* 2007 Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447** 799–816. (doi:10.1038/nature05874)

Boonyaratanakornkit V & Edwards DP 2007 Receptor mechanisms mediating non-genomic actions of sex steroids. *Seminars in Reproductive Medicine* **25** 139–153. (doi:10.1055/s-2007-973427)

Bottino MC, Cerliani JP, Rojas P, Giulianelli S, Soldati R, Mondillo C, Gorostiaga MA, Pignataro OP, Calvo JC, Gutkind JS *et al.* 2011 Classical membrane progesterone receptors in murine mammary carcinomas: agonistic effects of progestins and RU-486 mediating rapid non-genomic effects. *Breast Cancer Research and Treatment* **126** 621–636. (doi:10.1007/s10549-010-0971-3)

Bowden RT, Hissom JR & Moore MR 1989 Growth stimulation of T47D human breast cancer cells by the anti-progestin RU486. *Endocrinology* **124** 2642–2644. (doi:10.1210/endo-124-5-2642)

Brodie AM, Wing LY, Goss P, Dowsett M & Coombes RC 1986 Aromatase inhibitors and the treatment of breast cancer. *Journal of Steroid Biochemistry* **24** 91–97. (doi:10.1016/0022-4731(86)90037-3)

Busia L, Faus H, Hoffmann J & Haendler B 2011 The antiprogestin Lonaprisan inhibits breast cancer cell proliferation by inducing p21 expression. *Molecular and Cellular Endocrinology* **333** 37–46. (doi:10.1016/j.mce.2010.11.034)

Calaf GM 2006 Susceptibility of human breast epithelial cells *in vitro* to hormones and drugs. *International Journal of Oncology* **28** 285–295.

Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoute J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF *et al.* 2006 Genome-wide analysis of estrogen receptor binding sites. *Nature Genetics* **38** 1289–1297. (doi:10.1038/ng1901)

Cerliani JP, Giulianelli S, Sahores A, Wargon V, Gongora A, Baldi A, Molinolo A, Lamb CE & Lanari C 2010 Mifepristone inhibits MPA-and FGF2-induced mammary tumor growth but not FGF2-induced mammary hyperplasia. *Medicina* **70** 529–532.

Check JH, Dix E, Cohen R, Check D & Wilson C 2010 Efficacy of the progesterone receptor antagonist mifepristone for palliative therapy of patients with a variety of advanced cancer types. *Anticancer Research* **30** 623–628.

Chlebowski RT, Hendrix SL, Langer RD, Stefanick ML, Gass M, Lane D, Rodabough RJ, Gilligan MA, Cyr MG, Thomson CA *et al.* 2003 Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. *Journal of the American Medical Association* **289** 3243–3253. (doi:10.1001/jama.289.24.3243)

Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH, Manson JE, Stefanick ML, Ockene J, Sarto GE *et al.* 2010 Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. *Journal of the American Medical Association* **304** 1684–1692. (doi:10.1001/jama.2010.1500)

Chwalisz K, Brenner RM, Fuhrmann UU, Hess-Stumpp H & Elger W 2000 Antiproliferative effects of progesterone antagonists and progesterone receptor modulators on the endometrium. *Steroids* **65** 741–751. (doi:10.1016/S0039-128X(00)00190-2)

Chwalisz K, Perez MC, Demanno D, Winkel C, Schubert G & Elger W 2005 Selective progesterone receptor modulator development and use in the treatment of leiomyomata and endometriosis. *Endocrine Reviews* **26** 423–438. (doi:10.1210/er.2005-0001)

Clarke CL & Sutherland RL 1990 Progestin regulation of cellular proliferation. *Endocrine Reviews* **11** 266–301. (doi:10.1210/edrv-11-2-266)

Conneely OM & Lydon JP 2000 Progesterone receptors in reproduction: functional impact of the A and B isoforms. *Steroids* **65** 571–577. (doi:10.1016/S0039-128X(00)00115-X)

Conneely OM, Jericevic BM & Lydon JP 2003 Progesterone receptors in mammary gland development and tumorigenesis. *Journal of Mammary Gland Biology and Neoplasia* **8** 205–214. (doi:10.1023/A:1025952924864)

Dauvois S, White R & Parker MG 1993 The antiestrogen ICI 182 780 disrupts estrogen receptor nucleocytoplasmic shuttling. *Journal of Cell Science* **106** 1377–1388.

DeMarzo AM, Beck CA, Onate SA & Edwards DP 1991 Dimerization of mammalian progesterone receptors occurs in the absence of DNA and is related to the release of the 90-kDa heat shock protein. *PNAS* **88** 72–76. (doi:10.1073/pnas.88.1.72)

Dran G, Luthy IA, Molinolo AA, Montecchia F, Charreau EH, Pasqualini CD & Lanari C 1995 Effect of medroxyprogesterone acetate (MPA) and serum factors on cell proliferation in primary cultures of an MPA-induced mammary adenocarcinoma. *Breast Cancer Research and Treatment* **35** 173–186. (doi:10.1007/BF00668207)

Dressing GE & Lange CA 2009 Integrated actions of progesterone receptor and cell cycle machinery regulate breast cancer cell proliferation. *Steroids* **74** 573–576. (doi:10.1016/j.steroids.2008.12.001)

Edwards DP, Altmann M, DeMarzo A, Zhang Y, Weigel NL & Beck CA 1995 Progesterone receptor and the mechanism of action of progesterone antagonists. *Journal of Steroid Biochemistry and Molecular Biology* **53** 449–458. (doi:10.1016/0960-0760(95)00091-D)

Engman M, Skoog L, Soderqvist G & Gemzell-Danielsson K 2008 The effect of mifepristone on breast cell proliferation in premenopausal women evaluated through fine needle aspiration cytology. *Human Reproduction* **23** 2072–2079. (doi:10.1093/humrep/den228)

el Etreby MF, Liang Y, Wrenn RW & Schoenlein PV 1998 Additive effect of mifepristone and tamoxifen on apoptotic pathways in MCF-7 human breast cancer cells. *Breast Cancer Research and Treatment* **51** 149–168. (doi:10.1023/A:1006078032287)

el Etreby MF, Liang Y, Johnson MH & Lewis RW 2000 Antitumor activity of mifepristone in the human LNCaP, LNCaP-C4, and LNCaP-C4-2 prostate cancer models in nude mice. *Prostate* **42** 99–106. (doi:10.1002/(SICI)1097-0045(20000201)42:2<99::AID-PROS3>3.0.CO;2-I)

Evans RM 1988 The steroid and thyroid hormone receptor superfamily. *Science* **240** 889–895. (doi:10.1126/science.3283939)

Faivre EJ & Lange CA 2007 Progesterone receptors upregulate Wnt-1 to induce epidermal growth factor receptor transactivation and c-Src-dependent sustained activation of Erk1/2 mitogen-activated protein kinase in breast cancer cells. *Molecular and Cellular Biology* **27** 466–480. (doi:10.1128/MCB.01539-06)

Fjelldal R, Moe BT, Orbo A & Sager G 2010 MCF-7 cell apoptosis and cell cycle arrest: non-genomic effects of progesterone and mifepristone (RU-486). *Anticancer Research* **30** 4835–4840.

Fuhrmann U, Hess-Stumpp H, Cleve A, Neef G, Schwede W, Hoffmann J, Fritzemeier KH & Chwalisz K 2000 Synthesis and biological activity of a novel, highly potent progesterone receptor antagonist. *Journal of Medicinal Chemistry* **43** 5010–5016. (doi:10.1021/jm001000c)

Gaddy VT, Barrett JT, Delk JN, Kallab AM, Porter AG & Schoenlein PV 2004 Mifepristone induces growth arrest, caspase activation, and apoptosis of estrogen receptor-expressing, antiestrogen-resistant breast cancer cells. *Clinical Cancer Research* **10** 5215–5225. (doi:10.1158/1078-0432.CCR-03-0637)

Gaillard RC, Riondel A, Muller AF, Herrmann W & Baulieu EE 1984 RU 486: a steroid with antiglucocorticosteroid activity that only disinhibits the human pituitary–adrenal system at a specific time of day. *PNAS* **81** 3879–3882. (doi:10.1073/pnas.81.12.3879)

Galac S, Kooistra HS, Dieleman SJ, Cestnik V & Okkens AC 2004 Effects of aglepristone, a progesterone receptor antagonist, administered during the early luteal phase in non-pregnant bitches. *Theriogenology* **62** 494–500. (doi:10.1016/j.theriogenology.2003.11.004)

Giulianelli S, Cerliani JP, Lamb CA, Fabris VT, Bottino MC, Gorostiaga MA, Novaro V, Gongora A, Baldi A, Molinolo A *et al.* 2008 Carcinoma-associated fibroblasts activate progesterone receptors and induce hormone independent mammary tumor growth: a role for the FGF-2/FGFR-2 axis. *International Journal of Cancer* **123** 2518–2531. (doi:10.1002/ijc.23802)

Goyeneche AA, Caron RW & Telleria CM 2007 Mifepristone inhibits ovarian cancer cell growth *in vitro* and *in vivo*. *Clinical Cancer Research* **13** 3370–3379. (doi:10.1158/1078-0432.CCR-07-0164)

Graham JD, Yeates C, Balleine RL, Harvey SS, Milliken JS, Bilous AM & Clarke CL 1995 Characterization of progesterone receptor A and B expression in human breast cancer. *Cancer Research* **55** 5063–5068.

Graham JD, Yager ML, Hill HD, Byth K, O'Neill GM & Clarke CL 2005 Altered progesterone receptor isoform expression remodels progestin responsiveness of breast cancer cells. *Molecular Endocrinology* **19** 2713–2735. (doi:10.1210/me.2005-0126)

Grunberg SM, Weiss MH, Spitz IM, Ahmadi J, Sadun A, Russell CA, Lucci L & Stevenson LL 1991 Treatment of unresectable meningiomas with the antiprogesterone agent mifepristone. *Journal of Neurosurgery* **74** 861–866. (doi:10.3171/jns.1991.74.6.0861)

Grunberg SM, Weiss MH, Russell CA, Spitz IM, Ahmadi J, Sadun A & Sitruk-Ware R 2006 Long-term administration of mifepristone (RU486): clinical tolerance during extended treatment of meningioma. *Cancer Investigation* **24** 727–733. (doi:10.1080/07357900601062339)

Gruol DJ, Zee MC, Trotter J & Bourgeois S 1994 Reversal of multidrug resistance by RU 486. *Cancer Research* **54** 3088–3091.

Hagan CR, Faivre EJ & Lange CA 2009 Scaffolding actions of membrane-associated progesterone receptors. *Steroids* **74** 568–572. (doi:10.1016/j.steroids.2008.12.004)

Han SJ, Tsai SY, Tsai MJ & O'Malley BW 2007 Distinct temporal and spatial activities of RU486 on progesterone receptor function in reproductive organs of ovariectomized mice. *Endocrinology* **148** 2471–2486. (doi:10.1210/en.2006-1561)

Helguero LA, Viegas M, Asaithamby A, Shyamala G, Lanari C & Molinolo AA 2003 Progesterone receptor expression in medroxyprogesterone acetate-induced murine mammary carcinomas and response to endocrine treatment. *Breast Cancer Research and Treatment* **79** 379–390. (doi:10.1023/A:1024029826248)

Herrman W, Wyss R, Riondel A, Philibert D, Teutsch G, Sakiz E & Baulieu EE 1982 Effects of an anti-progestin steroid in women: interruption of the menstrual cycle or early pregnancy (author's transl). *Contraception Fertilité Sexualité* **10** 389–393.

Hild SA, Reel JR, Hoffman LH & Blye RP 2000 CDB-2914: anti-progestational/anti-glucocorticoid profile and post-coital anti-fertility activity in rats and rabbits. *Human Reproduction* **15** 822–829. (doi:10.1093/humrep/15.4.822)

Hissom JR & Moore MR 1987 Progestin effects on growth in the human breast cancer cell line T-47D – possible therapeutic implications. *Biochemical and Biophysical Research Communications* **145** 706–711. (doi:10.1016/0006-291X(87)91022-9)

Hopp TA, Weiss HL, Hilsenbeck SG, Cui Y, Allred DC, Horwitz KB & Fuqua SA 2004 Breast cancer patients with progesterone receptor PR-A-rich tumors have poorer disease-free survival rates. *Clinical Cancer Research* **10** 2751–2760. (doi:10.1158/1078-0432.CCR-03-0141)

Horwitz KB 1987 The structure and function of progesterone receptors in breast cancer. *Journal of Steroid Biochemistry* **27** 447–457. (doi:10.1016/0022-4731(87)90339-6)

Horwitz KB 2008 The Year in Basic Science: update of estrogen plus progestin therapy for menopausal hormone replacement implicating stem cells in the increased breast cancer risk. *Molecular Endocrinology* **22** 2743–2750. (doi:10.1210/me.2008-0291)

Horwitz KB & McGuire WL 1975 Predicting response to endocrine therapy in human breast cancer: a hypothesis. *Science* **189** 726–727. (doi:10.1126/science.168640)

Horwitz KB, Koseki Y & McGuire WL 1978 Estrogen control of progesterone receptor in human breast cancer: role of estradiol and antiestrogen. *Endocrinology* **103** 1742–1751. (doi:10.1210/endo-103-5-1742)

Horwitz KB, Mockus MB & Lessey BA 1982 Variant T47D human breast cancer cells with high progesterone-receptor levels despite estrogen and antiestrogen resistance. *Cell* **28** 633–642. (doi:10.1016/0092-8674(82)90218-5)

Hyder SM, Murthy L & Stancel GM 1998 Progestin regulation of vascular endothelial growth factor in human breast cancer cells. *Cancer Research* **58** 392–395.

Hyder SM, Liang Y, Wu J & Welbern V 2009 Regulation of thrombospondin-1 by natural and synthetic progestins in human breast cancer cells. *Endocrine-Related Cancer* **16** 809–817. (doi:10.1677/ERC-08-0311)

Jackson TA, Richer JK, Bain DL, Takimoto GS, Tung L & Horwitz KB 1997 The partial agonist activity of antagonist-occupied steroid receptors is controlled by a novel hinge domain-binding coactivator L7/SPA and the corepressors N-CoR or SMRT. *Molecular Endocrinology* **11** 693–705. (doi:10.1210/me.11.6.693)

Jacobsen BM, Richer JK, Schittone SA & Horwitz KB 2002 New human breast cancer cells to study progesterone receptor isoform ratio effects and ligand-independent gene regulation. *Journal of Biological Chemistry* **277** 27793–27800. (doi:10.1074/jbc.M202584200)

Jemal A, Bray F, Center MM, Ferlay J, Ward E & Forman D 2011 Global cancer statistics. *CA: A Cancer Journal for Clinicians* **61** 69–90. (doi:10.3322/caac.20107)

Jeng MH, Langan-Fahey SM & Jordan VC 1993 Estrogenic actions of RU486 in hormone-responsive MCF-7 human breast cancer cells. *Endocrinology* **132** 2622–2630. (doi:10.1210/en.132.6.2622)

Jordan VC 1990 Long-term adjuvant tamoxifen therapy for breast cancer. *Breast Cancer Research and Treatment* **15** 125–136. (doi:10.1007/BF01806350)

Jordan VC 2008 The 38th David A. Karnofsky lecture: the paradoxical actions of estrogen in breast cancer – survival or death? *Journal of Clinical Oncology* **26** 3073–3082. (doi:10.1200/JCO.2008.17.5190)

Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H & Chambon P 1990 Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. *EMBO Journal* **9** 1603–1614.

Keydar I, Chen L, Karby S, Weiss FR, Delarea J, Radu M, Chaitcik S & Brenner HJ 1979 Establishment and characterization of a cell line of human breast carcinoma origin. *European Journal of Cancer* **15** 659–670. (doi:10.1016/0014-2964(79)90139-7)

Klijn JG, de Jong FH, Bakker GH, Lamberts SW, Rodenburg CJ & Alexieva-Figusch J 1989 Antiprogestins, a new form of endocrine therapy for human breast cancer. *Cancer Research* **49** 2851–2856.

Klijn JG, Setyono-Han B, Sander HJ, Lamberts SW, de Jong FH, Deckers GH & Foekens JA 1994 Pre-clinical and clinical treatment of breast cancer with antiprogestins. *Human Reproduction* **9** (Suppl 1) 181–189.

Klijn JG, Setyono-Han B & Foekens JA 2000 Progesterone antagonists and progesterone receptor modulators in the treatment of breast cancer. *Steroids* **65** 825–830. (doi:10.1016/S0039-128X(00)00195-1)

Kloosterboer HJ, Deckers GH, Schoonen WG, Hanssen RG, Rose UM, Verbost PM, Hsiu JG, Williams RF & Hodgen GD 2000 Preclinical experience with two selective progesterone receptor modulators on breast and endometrium. *Steroids* **65** 733–740. (doi:10.1016/S0039-128X(00)00189-6)

Kordon E, Lanari C, Meiss R, Charreau E & Pasqualini CD 1990 Hormone dependence of a mouse mammary tumor line induced *in vivo* by medroxyprogesterone acetate. *Breast Cancer Research and Treatment* **17** 33–43. (doi:10.1007/BF01812682)

Lamb C, Simian M, Molinolo A, Pazos P & Lanari C 1999 Regulation of cell growth of a progestin-dependent murine mammary carcinoma *in vitro*: progesterone receptor involvement in serum or growth factor-induced cell proliferation. *Journal of Steroid Biochemistry and Molecular Biology* **70** 133–142. (doi:10.1016/S0960-0760(99)00108-9)

Lamb CA, Helguero LA, Giulianelli S, Soldati R, Vanzulli SI, Molinolo A & Lanari C 2005 Antisense oligonucleotides targeting the progesterone receptor inhibit hormone-independent breast cancer growth in mice. *Breast Cancer Research and Treatment* **7** R1111–R1121.

Lanari C, Molinolo AA & Pasqualini CD 1986 Induction of mammary adenocarcinomas by medroxyprogesterone acetate in BALB/c female mice. *Cancer Letters* **33** 215–223. (doi:10.1016/0304-3835(86)90027-3)

Lanari C, Kordon E, Molinolo A, Pasqualini CD & Charreau EH 1989 Mammary adenocarcinomas induced by medroxyprogesterone acetate: hormone dependence and EGF receptors of BALB/c *in vivo* sublines. *International Journal of Cancer* **43** 845–850. (doi:10.1002/ijc.2910430518)

Lanari C, Lamb C, Fabris V, Helguero L, Soldati R, Bottino M, Giulianelli S, Cerliani J, Wargon V & Molinolo A 2009 The MPA mouse breast cancer model: evidence for a role of progesterone receptors in breast cancer. *Endocrine-Related Cancer* **16** 333–350. (doi:10.1677/ERC-08-0244)

Lange CA, Shen T & Horwitz KB 2000 Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *PNAS* **97** 1032–1037. (doi:10.1073/pnas.97.3.1032)

Lange CA, Sartorius CA, Abdel-Hafiz H, Spillman MA, Horwitz KB & Jacobsen BM 2008 Progesterone receptor action: translating studies in breast cancer models to clinical insights. *Advances in Experimental Medicine and Biology* **630** 94–111.

Lecureur V, Fardel O & Guillouzo A 1994 The antiprogestatin drug RU 486 potentiates doxorubicin cytotoxicity in multidrug resistant cells through inhibition of *P*-glycoprotein function. *FEBS Letters* **355** 187–191. (doi:10.1016/0014-5793(94)01186-9)

Leonhardt SA, Boonyaratanakornkit V & Edwards DP 2003 Progesterone receptor transcription and non-transcription signaling mechanisms. *Steroids* **68** 761–770. (doi:10.1016/S0039-128X(03)00129-6)

Li X & O'Malley BW 2003 Unfolding the action of progesterone receptors. *Journal of Biological Chemistry* **278** 39261–39264. (doi:10.1074/jbc.R300024200)

Li M, Spitzer E, Zschiesche W, Binas B, Parczyk K & Grosse R 1995 Antiprogestins inhibit growth and stimulate differentiation in the normal mammary gland. *Journal of Cellular Physiology* **164** 1–8. (doi:10.1002/jcp.1041640102)

Liang Y, Wu J, Stancel GM & Hyder SM 2005 p53-dependent inhibition of progestin-induced VEGF expression in human breast cancer cells. *Journal of Steroid Biochemistry and Molecular Biology* **93** 173–182. (doi:10.1016/j.jsbmb.2004.12.011)

Liang Y, Besch-Williford C, Brekken RA & Hyder SM 2007 Progestin-dependent progression of human breast tumor xenografts: a novel model for evaluating antitumor therapeutics. *Cancer Research* **67** 9929–9936. (doi:10.1158/0008-5472.CAN-07-1103)

Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM & O'Malley BW 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development* **9** 2266–2278. (doi:10.1101/gad.9.18.2266)

McGuire WL 1975 Endocrine therapy of breast cancer. *Annual Review of Medicine* **26** 353–363. (doi:10.1146/annurev.me.26.020175.002033)

Meyer ME, Pornon A, Ji JW, Bocquel MT, Chambon P & Gronemeyer H 1990 Agonistic and antagonistic activities of RU486 on the functions of the human progesterone receptor. *EMBO Journal* **9** 3923–3932.

Michna H, Schneider MR, Nishino Y & el Etreby MF 1989 Antitumor activity of the antiprogestins ZK 98.299 and RU 38.486 in hormone dependent rat and mouse mammary tumors: mechanistic studies. *Breast Cancer Research and Treatment* **14** 275–288. (doi:10.1007/BF01806299)

Michna H, Schneider M, Nishino Y, el Etreby MF & McGuire WL 1990 Progesterone antagonists block the growth of experimental mammary tumors in G0/G1. *Breast Cancer Research and Treatment* **17** 155–156. (doi:10.1007/BF01806296)

Molinolo AA, Lanari C, Charreau EH, Sanjuan N & Pasqualini CD 1987 Mouse mammary tumors induced by medroxyprogesterone acetate: immunohistochemistry and hormonal receptors. *Journal of the National Cancer Institute* **79** 1341–1350.

Montecchia MF, Lamb C, Molinolo AA, Luthy IA, Pazos P, Charreau E, Vanzulli S & Lanari C 1999 Progesterone receptor involvement in independent tumor growth in MPA-induced murine mammary adenocarcinomas. *Journal of Steroid Biochemistry and Molecular Biology* **68** 11–21. (doi:10.1016/S0960-0760(98)00166-6)

Moore MR 2004 A rationale for inhibiting progesterone-related pathways to combat breast cancer. *Current Cancer Drug Targets* **4** 183–189. (doi:10.2174/1568009043481515)

Mote PA, Leary JA, Avery KA, Sandelin K, Chenevix-Trench G, Kirk JA & Clarke CL 2004 Germ-line mutations in BRCA1 or BRCA2 in the normal breast are associated with altered expression of estrogen-responsive proteins and the predominance of progesterone receptor A. *Genes, Chromosomes & Cancer* **39** 236–248. (doi:10.1002/gcc.10321)

Mote PA, Graham JD & Clarke CL 2007 Progesterone receptor isoforms in normal and malignant breast. *Ernst Schering Foundation Symposium Proceedings* 77–107. (doi:10.1007/2789_2008_076)

Mulac-Jericevic B, Lydon JP, DeMayo FJ & Conneely OM 2003 Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *PNAS* **100** 9744–9749. (doi:10.1073/pnas.1732707100)

Muphung W, Rungsipipat A & Chatdarong K 2009 Effects of the anti-progestin aglepristone on the uterine tissue of cats administered medroxyprogesterone acetate. *Reproduction in Domestic Animals* **44** (Suppl 2) 204–207. (doi:10.1111/j.1439-0531.2009.01411.x)

Nishino T, Ishibashi K, Hirtreiter C & Nishino Y 2009 Potentiation of the antitumor effect of tamoxifen by combination with the antiprogestin onapristone. *Journal of Steroid Biochemistry and Molecular Biology* **116** 187–190. (doi:10.1016/j.jsbmb.2009.05.013)

Pathiraja TN, Shetty PB, Jelinek J, He R, Hartmaier R, Margossian AL, Hilsenbeck SG, Issa JP & Oesterreich S 2011 Progesterone receptor isoform-specific promoter methylation: association of PRA promoter methylation with worse outcome in breast cancer patients. *Clinical Cancer Research* **17** 4177–4186. (doi:10.1158/1078-0432.CCR-10-2950)

Perrault D, Eisenhauer EA, Pritchard KI, Panasci L, Norris B, Vandenberg T & Fisher B 1996 Phase II study of the progesterone antagonist mifepristone in patients with untreated metastatic breast carcinoma: a National Cancer Institute of Canada Clinical Trials Group study. *Journal of Clinical Oncology* **14** 2709–2712.

Petz LN & Nardulli AM 2000 Sp1 binding sites and an estrogen response element half-site are involved in regulation of the human progesterone receptor A promoter. *Molecular Endocrinology* **14** 972–985. (doi:10.1210/me.14.7.972)

Petz LN, Ziegler YS, Loven MA & Nardulli AM 2002 Estrogen receptor alpha and activating protein-1 mediate estrogen responsiveness of the progesterone receptor gene in MCF-7 breast cancer cells. *Endocrinology* **143** 4583–4591. (doi:10.1210/en.2002-220369)

Polisca A, Scotti L, Orlandi R, Brecchia G, Maranesi M, Zerani M & Boiti C 2010 Aglepristone (RU534) administration to non-pregnant bitches in the mid-luteal phase induces early luteal regression. *Theriogenology* **74** 672–681. (doi:10.1016/j.theriogenology.2010.03.021)

Poole AJ, Li Y, Kim Y, Lin SC, Lee WH & Lee EY 2006 Prevention of Brca1-mediated mammary tumorigenesis in mice by a progesterone antagonist. *Science* **314** 1467–1470. (doi:10.1126/science.1130471)

Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X & Perou CM 2010 Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Research* **12** R68. (doi:10.1186/bcr2635)

Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM & Horwitz KB 2002 Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *Journal of Biological Chemistry* **277** 5209–5218. (doi:10.1074/jbc.M110090200)

Rivas MA, Carnevale RP, Proietti CJ, Rosemblit C, Beguelin W, Salatino M, Charreau EH, Frahm I, Sapia S, Brouckaert P *et al.* 2008 TNF alpha acting on TNFR1 promotes breast cancer growth via p42/P44 MAPK, JNK, Akt and NF-kappa B-dependent pathways. *Experimental Cell Research* **314** 509–529. (doi:10.1016/j.yexcr.2007.10.005)

Robertson JF, Willsher PC, Winterbottom L, Blamey RW & Thorpe S 1999 Onapristone, a progesterone receptor antagonist, as first-line therapy in primary breast cancer. *European Journal of Cancer* **35** 214–218. (doi:10.1016/S0959-8049(98)00388-8)

Romieu G, Maudelonde T, Ulmann A, Pujol H, Grenier J, Cavalie G, Khalaf S & Rochefort H 1987 The antiprogestin RU486 in advanced breast cancer: preliminary clinical trial. *Bulletin du Cancer* **74** 455–461.

Santen R, Cavalieri E, Rogan E, Russo J, Guttenplan J, Ingle J & Yue W 2009 Estrogen mediation of breast tumor formation involves estrogen receptor-dependent, as well as independent, genotoxic effects. *Annals of the New York Academy of Sciences* **1155** 132–140. (doi:10.1111/j.1749-6632.2008.03685.x)

Sartorius CA, Tung L, Takimoto GS & Horwitz KB 1993 Antagonist-occupied human progesterone receptors bound to DNA are functionally switched to transcriptional agonists by cAMP. *Journal of Biological Chemistry* **268** 9262–9266.

Sartorius CA, Groshong SD, Miller LA, Powell RL, Tung L, Takimoto GS & Horwitz KB 1994 New T47D breast cancer cell lines for the independent study of progesterone B- and A-receptors: only antiprogestin-occupied B-receptors are switched to transcriptional agonists by cAMP. *Cancer Research* **54** 3868–3877.

Scarpin KM, Graham JD, Mote PA & Clarke CL 2009 Progesterone action in human tissues: regulation by progesterone receptor (PR) isoform expression, nuclear positioning and coregulator expression. *Nuclear Receptor Signaling* **7** e009.

Schneider MR, Michna H, Nishino Y, Neef G & el Etreby MF 1990 Tumor-inhibiting potential of ZK 112.993, a new progesterone antagonist, in hormone-sensitive, experimental rodent and human mammary tumors. *Anticancer Research* **10** 683–687.

Schneider W, Ramachandran C, Satyaswaroop PG & Shyamala G 1991 Murine progesterone receptor exists predominantly as the 83-kilodalton 'A' form. *Journal of Steroid Biochemistry and Molecular Biology* **38** 285–291. (doi:10.1016/0960-0760(91)90099-Q)

Schultz JR, Petz LN & Nardulli AM 2003 Estrogen receptor alpha and Sp1 regulate progesterone receptor gene expression. *Molecular and Cellular Endocrinology* **201** 165–175. (doi:10.1016/S0303-7207(02)00415-X)

Sheridan PL, Krett NL, Gordon JA & Horwitz KB 1988 Human progesterone receptor transformation and nuclear down-regulation are independent of phosphorylation. *Molecular Endocrinology* **2** 1329–1342. (doi:10.1210/mend-2-12-1329)

Shyamala G, Yang X, Silberstein G, Barcellos-Hoff MH & Dale E 1998 Transgenic mice carrying an imbalance in the native ratio of A to B forms of progesterone receptor exhibit developmental abnormalities in mammary glands. *PNAS* **95** 696–701. (doi:10.1073/pnas.95.2.696)

Simian M, Molinolo A & Lanari C 2006 Involvement of matrix metalloproteinase activity in hormone-induced mammary tumor regression. *American Journal of Pathology* **168** 270–279. (doi:10.2353/ajpath.2006.050012)

Simian M, Bissell MJ, Barcellos-Hoff MH & Shyamala G 2009 Estrogen and progesterone receptors have distinct roles in the establishment of the hyperplastic phenotype in PR-A transgenic mice. *Breast Cancer Research* **11** R72. (doi:10.1186/bcr2408)

Skafar DF 1991 Differences in the binding mechanism of RU486 and progesterone to the progesterone receptor. *Biochemistry* **30** 10829–10832. (doi:10.1021/bi00109a003)

Skildum A, Faivre E & Lange CA 2005 Progesterone receptors induce cell cycle progression via activation of mitogen-activated protein kinases. *Molecular Endocrinology* **19** 327–339.

Spitz IM, Grunberg SM, Chabbert-Buffet N, Lindenberg T, Gelber H & Sitruk-Ware R 2005 Management of patients receiving long-term treatment with mifepristone. *Fertility and Sterility* **84** 1719–1726. (doi:10.1016/j.fertnstert.2005.05.056)

Takimoto GS, Hovland AR, Tasset DM, Melville MY, Tung L & Horwitz KB 1996 Role of phosphorylation on DNA binding and transcriptional functions of human progesterone receptors. *Journal of Biological Chemistry* **271** 13308–13316. (doi:10.1074/jbc.271.23.13308)

Tieszen CR, Goyeneche AA, Brandhagen BN, Ortbahn CT & Telleria CM 2011 Antiprogestin mifepristone inhibits the growth of cancer cells of reproductive and non-reproductive origin regardless of progesterone receptor expression. *BMC Cancer* **11** 207. (doi:10.1186/1471-2407-11-207)

Ulmann A & Dubois C 1988 Anti-progesterones in obstetrics, ectopic pregnancies and gynaecological malignancy. *Baillière's Clinical Obstetrics and Gynaecology* **2** 631–638. (doi:10.1016/S0950-3552(88)80049-X)

Vanzulli S, Efeyan A, Benavides F, Helguero L, Peters G, Shen J, Conti CJ, Lanari C & Molinolo A 2002 p21, p27 and p53 in estrogen and antiprogestin-induced tumor regression of experimental mouse mammary ductal carcinomas. *Carcinogenesis* **23** 749–757. (doi:10.1093/carcin/23.5.749)

Vanzulli SI, Soldati R, Meiss R, Colombo L, Molinolo AA & Lanari C 2005 Estrogen or antiprogestin treatment induces complete regression of pulmonary and axillary metastases in an experimental model of breast cancer progression. *Carcinogenesis* **26** 1055–1063. (doi:10.1093/carcin/bgi060)

Vasilatos SN, Broadwater G, Barry WT, Baker JC Jr, Lem S, Dietze EC, Bean GR, Bryson AD, Pilie PG, Goldenberg V *et al.* 2009 CpG island tumor suppressor promoter methylation in non-BRCA-associated early mammary carcinogenesis. *Cancer Epidemiology, Biomarkers & Prevention* **18** 901–914. (doi:10.1158/1055-9965.EPI-08-0875)

Vignon F, Bardon S, Chalbos D & Rochefort H 1983 Antiestrogenic effect of R5020, a synthetic progestin in human breast cancer cells in culture. *Journal of Clinical Endocrinology and Metabolism* **56** 1124–1130. (doi:10.1210/jcem-56-6-1124)

Wargon V, Helguero LA, Bolado J, Rojas P, Novaro V, Molinolo A & Lanari C 2009 Reversal of antiprogestin resistance and progesterone receptor isoform ratio in acquired resistant mammary carcinomas. *Breast Cancer Research and Treatment* **116** 449–460. (doi:10.1007/s10549-008-0150-y)

Wargon V, Fernandez SV, Goin M, Giulianelli S, Russo J & Lanari C 2011 Hypermethylation of the progesterone receptor A in constitutive antiprogestin-resistant mouse mammary carcinomas. *Breast Cancer Research and Treatment* **126** 319–332. (doi:10.1007/s10549-010-0908-x)

Wiehle R, Lantvit D, Yamada T & Christov K 2011 CDB-4124, a progesterone receptor modulator, inhibits mammary carcinogenesis by suppressing cell proliferation and inducing apoptosis. *Cancer Prevention Research* **4** 414–424. (doi:10.1158/1940-6207.CAPR-10-0244)

Women's Health 2002 Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *Journal of the American Medical Association* **288** 321–333. (doi:10.1001/jama.288.3.321)

Yoshida S, Ohara N, Xu Q, Chen W, Wang J, Nakabayashi K, Sasaki H, Morikawa A & Maruo T 2010 Cell-type specific actions of progesterone receptor modulators in the regulation of uterine leiomyoma growth. *Seminars in Reproductive Medicine* **28** 260–273. (doi:10.1055/s-0030-1251483)

Received in final form 8 February 2012
Accepted 14 February 2012
Made available online as an Accepted Preprint
20 February 2012