Antiprogestins in breast cancer treatment: are we ready?

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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.

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...
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 (E2) 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 E2 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

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**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 (Boonyaratanaokornkit & 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 (Faiivre & 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, Afpuppe
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[a]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, E2, 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 E2 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, buselgin 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 E2 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, cytolyis, 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 (Faiivre & 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 E2-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 E2-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 E2-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. 2006).
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

E2-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. 1995). 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 E2 followed by MPA (Liang et al. 2007). Additionally, previous studies have shown that E2 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. E2, ACTH, cortisol, and androstenedione serum levels were increased in all patients. The authors suggested that the increase in E2 may be due to aromatization of androstenedione, and therefore, they proposed a combinatorial treatment of MFP and TAM to counteract the effects of E2.

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 E2 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 E2 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 E2 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 10⁴ times lower than those that
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 E2 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, or with 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 DSIP, 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.

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