Antiprogestins in gynecological diseases

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Abstract

Antiprogestins constitute a group of compounds, developed since the early 1980s, that bind progesterone receptors with different affinities. The first clinical uses for antiprogestins were in reproductive medicine, e.g., menstrual regulation, emergency contraception, and termination of early pregnancies. These initial applications, however, belied the capacity for these compounds to interfere with cell growth. Within the context of gynecological diseases, antiprogestins can block the growth of and kill gynecological-related cancer cells, such as those originating in the breast, ovary, endometrium, and cervix. They can also interrupt the excessive growth of cells giving rise to benign gynecological diseases such as endometriosis and leiomyomata (uterine fibroids). In this article, we present a review of the literature providing support for the antigrowth activity that antiprogestins impose on cells in various gynecological diseases. We also provide a summary of the cellular and molecular mechanisms reported for these compounds that lead to cell growth inhibition and death. The preclinical knowledge gained during the past few years provides robust evidence to encourage the use of antiprogestins in order to alleviate the burden of gynecological diseases, either as monotherapies or as adjuvants of other therapies with the perspective of allowing for long-term treatments with tolerable side effects. The key to the clinical success of antiprogestins in this field probably lies in selecting those patients who will benefit from this therapy. This can be achieved by defining the genetic makeup required – within each particular gynecological disease – for attaining an objective response to antiprogestin-driven growth inhibition therapy.

Free Spanish abstract

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Introduction

Antiprogestins represent a family of compounds developed with the purpose of antagonizing the effect of progesterone on progesterone receptors (PR). Most derivatives are steroidal in nature and have mixed activities on the PR, ranging from pure antagonism to various degrees of agonistic effects contingent on the target tissue and the intracellular environment. Owing to these mixed activities on the PR, antiprogestins have been comprehensively categorized as PR modulators (PRMs). The degree of antagonistic or agonistic activity of the PRMs seems to depend on the balance among co-activators and co-repressors regulating the transcriptional activity of the PR, the intracellular molecular environment accounting for post-translational modifications, and the ratio of PR isoforms – i.e., PR-A vs PR-B, with PR-B having a strong transcriptional activation activity and PR-A being mostly transcriptionally inactive (Chabbert-Buffet et al. 2005, Hagan et al. 2012, Hagan & Lange 2014, Knutson & Lange 2014).

Paradoxically, the first compound with antiprogestin activity, originally termed RU-38486, was introduced to the scientific community in 1981 as a potent antiglucocorticoid agent (Philibert et al. 1981). RU-38486, or mifepristone, is a derivative of 19-nortestosterone with a dimethylaminophenyl moiety in position C11 that confers antagonistic properties. Its synthesis was part of an effort to develop an efficient antagonist against the glucocorticoid receptors (GR), which could be used to alleviate the consequences of excess glucocorticoid activity in patients with hypercortisolism (Baulieu 1997). During preclinical studies, it was rapidly discovered that mifepristone could cause termination of pregnancy (Spitz & Bardin 1993, Baulieu 1997). This outcome was attributed to the fact that the compound was equally potent in antagonizing PR and GR (Cadepond et al. 1997). The lack of discrimination by mifepristone between PR and GR was not surprising considering the similarities between the structures of both steroid hormone receptors (Baulieu 1991). Additionally, it has been shown that mifepristone can also bind androgen
receptors (ARs; Song et al. 2004), and, to further increase the complexity of the intracellular biochemistry of mifepristone, it has been recently shown that the steroid binds not only to the ligand-binding domain favoring repressor interaction and hindering receptor transactivation, but also to a second site representing the coactivator-binding domain of the ancestral 3-keto-steroid receptor, which is the ancestor of PR, GR, AR, as well as the mineralocorticoid receptor (Colucci & Ortlund 2013).

A large volume of studies explored the effect of mifepristone on different aspects of the mammalian reproductive axis. The contraceptive potential of mifepristone was extensively assessed in terms of its capacity to prevent ovulation, block implantation of a fertilized egg, and terminate early pregnancies (reviewed in Spitz et al. (1996)). The first human trial conducted with mifepristone in women with up to 8 weeks of amenorrhea led to termination of pregnancy in 80% of the cases (Herrmann et al. 1982). Shortly thereafter, it was shown that the efficacy of mifepristone abrogating early pregnancies by blocking PR was significantly enhanced when combined with a prostaglandin analog that potentiates uterine contractions (Bygdeman & Swahn 1985). Thus, the combination of mifepristone with a prostaglandin analog was adopted in many countries for medical termination of pregnancies at the first trimester (Spitz & Bardin 1993). In the USA, the combination of mifepristone and misoprostol was approved in 2000 for interrupting gestations of up to 49 days since the last menstrual period (Ellerton & Waldman 2001).

The antiglucocorticoid effect of mifepristone has been amply documented (reviewed in Agarwal (1996)), with the main application being the mitigation of the clinical manifestations of endogenous hypercortisolism (Nieman et al. 1985). After a successful multicenter trial (Fleseriu et al. 2012), mifepristone was approved in 2012 by the US Food and Drug Administration (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm292462.htm) to control hyperglycemia in patients with endogenous Cushing’s syndrome associated with type 2 diabetes. As mifepristone does not differentiate between PR and GR, a series of efforts were conducted to develop compounds capable of modulating either receptor without impinging on the other. The group of PRMs with a potent antiprogesterone activity and a minimal antiglucocorticoid effect includes ZK-98299 (onapristone), ZK-230211 (lonaprisan), CDB-2914 (ulipristal), CDB-4124 (telpaprion), ORG-31710, ORG-33628, and J-867 (asoprisnil) (reviewed in Chabbert-Buffet et al. (2005), Spitz (2006), and Lanari et al. (2012)), and, more recently, a 17-fluorinated steroid termed EC304 (Nickisch et al. 2013). Conversely, compounds with more antiglucocorticoid properties than antiprogestrone properties are also under development. They are termed GR modulators, are steroidal or non-steroidal in nature (Ray et al. 2007, Clark 2008, Belanoff et al. 2010, De Bosscher 2010, Gross et al. 2010), and are geared toward treating Cushing’s syndrome as well as other conditions in which excess GR activity needs to be depressed without affecting PR-mediated physiological processes. Examples of such conditions are psychotic depression (reviewed in Benagiano et al. (2008)), weight gain (Belanoff et al. 2010), and glaucoma associated with high-dose glucocorticoid therapy (reviewed in Kersey & Broadway (2006)).

Herein, we review the current evidence supporting the efficacy of antiprogestins in attenuating the proliferation of cells encompassing a spectrum of benign and malignant gynecological diseases characterized by excessive cell division. We further describe the molecular mechanisms demonstrated and proposed that explain the growth-inhibitory properties of antiprogestins in cells of different genetic backgrounds and tissues of origin. The antiproliferative properties of antiprogestins are not always justified by a mere blockage by the compounds of the transactivation activity of the PR. Consequently, the term ‘antiprogestins,’ when stating the antigrowth activity of the compounds, is misleading. Yet, we have used the term to permit the allocation, under a unique chemical framework, of all compounds mentioned in this article. Timely, when the mechanisms driving the antigrowth effect of these synthetic steroid derivatives become widely understood, a better descriptor to represent this family of compounds will certainly arise.

Breast cancer

Breast cancer is second only to lung cancer as the most common cause of cancer-related death in women (Siegel et al. 2013). Estrogen and progesterone have both been involved in breast carcinogenesis and progression (reviewed in Knutson & Lange (2014)). While antiestrogen therapy reached clinical application with the use of blockers of estrogen receptors (ER; e.g., tamoxifen and fulvestrant) or inhibitors of estradiol (E2) synthesis known as aromatase inhibitors ( exemestane, anastrozole, and letrozole) (reviewed in Journe et al. (2008)), the antagonism of progesterone activity has not yet reached clinical practice. Nevertheless, evidence accumulated over the past 20 years suggests that antiprogesterone therapy for breast cancer has a large potential to be soon included within the armamentarium of approaches to treat breast cancer (reviewed in Horwitz (1992), Lanari et al. (2012), Giulianelli et al. (2013), Knutson & Lange (2014), and Muti (2014)). For instance, as monotherapy, mifepristone was demonstrated to block the proliferation of breast cancer cells carrying PR and ER, such as T-47D and MCF-7 cells in the absence or presence of estrogens (Bardon et al. 1985, Horwitz 1985, Gill et al. 1987, Musgrove et al. 1997). However, because mifepristone also blocked the proliferation of ER-negative/PR-negative MDA-MB-231 breast cancer cells (Liang et al. 2003), the relevance of PR as a mediator of the
antiproliferative action of mifepristone in breast cancer cells is controversial. Contrasting this large volume of evidence for an inhibitory effect of mifepristone on breast cancer cell growth, growth stimulation by mifepristone in T-47D and MCF-7 cells was also timely reported (Bowden et al. 1989, Jeng et al. 1993), suggesting that the concentration of mifepristone used, and the presence or absence of progesterone and E2 in the culture media to name some variables, may justify such different outcomes in otherwise similar cell lines.

Mifepristone also had an additive interaction leading to the death of MCF-7 cancer cells when coupled with antiestrogens such as tamoxifen (El Etreyb et al. 1998) or 4-hydroxytamoxifen (Schoenlein et al. 2007); it also inhibited the growth of and killed MCF-7 cells in combination with the Chk1 inhibitor, 7-hydroxystauorosporine (Yokoyama et al. 2000), and the aromatase inhibitor anastrozole (Gil et al. 2013). Mifepristone also interrupted the proliferation of MCF-7 sublines that were made resistant to 4-hydroxytamoxifen (Gaddy et al. 2004). More recently, mifepristone pretreatment has been reported to potentiate the toxicity of paclitaxel in mammary fat pad xenografts of ER-negative/PR-negative/GR-positive MDA-MB-231 cells (Skor et al. 2013).

Growth inhibition of PR-positive T-47D cells was reported with other antiprogestins, such as onapristone (Classen et al. 1993), ORG-31710 (Musgrove et al. 1997), lonaprisan (Afhupe et al. 2010, Busia et al. 2011), telapristone (Gupta et al. 2013a), and EC304 (Nickisch et al. 2013).

Valuable information on the anti-breast-cancer effect of antiprogestins was generated from mouse models of breast cancer. For instance, mice genetically engineered to lack expression of p53 and BRCA1 tumor suppressors in their mammary glands develop spontaneous aggressive PR-overexpressing breast tumors, which can be prevented by the administration of mifepristone (Poole et al. 2006). Breast tumors induced by continuous administration of medroxyprogesterone acetate (MPA) in BALB/c mice and expressing a high PR-A to PR-B ratio respond to antiprogestins mifepristone, onapristone, or lonaprisan with inhibition of tumor growth and apoptosis (Montecchia et al. 1999, Helguero et al. 2003, Simian et al. 2006, Wargon et al. 2009). The response of these tumors to mifepristone is associated with an increase in tumor stroma and microvasculature, which allows better access to other chemotherapeutic agents such as paclitaxel or doxorubicin when provided in nanoparticle formulations, leading to a better therapeutic outcome (i.e., further growth inhibition than that caused by paclitaxel or doxorubicin alone) (Sequeira et al. 2014). In mammary gland tumors induced in rats by 7,12-dimethylbenzanthracene (DMBA), concomitant administration of mifepristone significantly delayed tumor development (Bakker et al. 1987), whereas administration of mifepristone to animals with pre-established DMBA-induced tumors abrogated tumor progression. This latter effect was additive to tamoxifen, leading to further growth inhibition (Bakker et al. 1989). DMBA-induced mammary tumors in rats were also significantly inhibited by onapristone, ulipristal, and ORG-31710 (Michna et al. 1989, Kloosterboer et al. 2000, Wiehle et al. 2007).

In patients with breast cancer, clinical trials with antiprogestins have had only partial responses. In one study, 200 mg mifepristone given daily for 3 months in tamoxifen-resistant breast tumors generated a positive response in 18% of patients (Romieu et al. 1987). In another study in patients with metastatic breast cancer resistant to tamoxifen, daily doses of 200–400 mg mifepristone led to an objective response in seven out of 11 patients (Klijn et al. 1989), whereas in another study with 28 patients treated with daily doses of 200 mg mifepristone, only three patients showed a response (Perrault et al. 1996). In a clinical trial using onapristone, 75% of patients responded to the treatment with an objective response; yet, the compound prompted liver toxicity, which discouraged further use (Robertson et al. 1999). A recent clinical trial with lonaprisan has reported limited efficacy in advanced stage IV, PR-positive, HER2-negative, metastatic breast cancer (Jonat et al. 2013). In summary, the apparent lack of robust objective responses reported in patients with breast cancer when receiving antiprogestins has been attributed to the lack of patient stratification according to their molecular profile, in particular the proportion of expression of PR-A relative to PR-B (Lanari et al. 2012, Giulianielli et al. 2013), and the lack of assessment of PR target gene signatures in responders vs non-responders (Hagan & Lange 2014, Knutson & Lange 2014). As breast cancer comprises four major subtypes with specific molecular drivers and histological characteristics (Cancer Genome Atlas Network 2012), new clinical trials should be tailored only to select groups of patients whose tumor genetics and PR target gene signatures make them suitable candidates to likely benefit from antiprogesterone therapy.

In terms of breast cancer prevention, antiprogestin therapy also has potential application. For instance, when antiprogestin mifepristone was provided to premenopausal women before a scheduled hysterectomy as a consequence of a leiomyoma, needle biopsies from mammary tissue clearly demonstrated a reduction in epithelial cell proliferation, suggesting the chemopreventive nature of the drug (Engman et al. 2008).

**Ovarian cancer**

Ovarian cancer is the most lethal disease of the female reproductive tract. Its 5-year survival below 50% has not changed for the past 30 years, indicating the need for new therapeutic interventions (reviewed in Vaughan et al. 2011, Modugno et al. 2012, Romero & Bast 2012, and Coleman et al. 2013). The first reported
effect of antiprogestins in ovarian cancer was in 1996 when it was demonstrated that mifepristone efficiently blocked the growth of A2780 and OVCAR-3 cells in vitro (Rose & Barnea 1996). Thereafter, it was reported that mifepristone potentiated the toxicity of cisplatin against COC1 ovarian cancer cells (Qin & Wang 2002, Li et al. 2003), and that onapristone and mifepristone inhibited cell growth and the synthesis of DNA in ML5 and ML10 human ovarian cystadenoma cells, as well as in HOC-7 and OVCAR-3 ovarian cancer cells (Zhou et al. 2002). The OVCAR-3 cell growth block by mifepristone was further confirmed in 2006 (Fauvet et al. 2006). Using various ovarian cancer cell lines of different genetic backgrounds, our laboratory additionally found that mifepristone blocked cell growth in vitro and demonstrated its efficacy in vivo at doses of 0.5 or 1 mg/day in mice carrying ovarian cancer xenografts (Goyeneche et al. 2007). We observed that when used at concentrations likely achievable in the clinic (i.e., pharmacological), mifepristone, ulipristal, and ORG-31710 all had cytostatic effects, with cells returning to the cell cycle upon drug removal; however, if used at supra-pharmacological doses, the antiprogestins, instead, killed the cells (Goyeneche et al. 2007, 2012). We further reported that adding antiprogestin mifepristone following a platinum agent potentiates platinum lethality and improves overall treatment efficacy (Freeburg et al. 2009a), and that resistance to platinum and/or paclitaxel does not affect the sensitivity of ovarian cancer cells to antiprogestin-mediated cytotoxicity (Freeburg et al. 2009b, Gamarra-Luques et al. 2014). The repopulation of ovarian cancer cells that escaped platinum or platinum/paclitaxel therapy was also blocked by chronic presence of antiprogestin mifepristone (Freeburg et al. 2009a, Gamarra-Luques et al. 2012), providing evidence for a long-term use of antiprogestins as antirepopulation therapy from a minority of cells that escaped otherwise effective chemotherapies (Telleria 2013).

Despite the evidence of antiprogestins being efficient in blocking ovarian cancer cell growth, clinical studies on the subject have been very limited. In 2000, in a phase II clinical trial, 34 patients with recurrent ovarian cancer no longer responsive to cisplatin–paclitaxel chemotherapy were treated with 200 mg oral mifepristone in courses of 28 days. Nine patients had a response to mifepristone showing a decrease in tumor size by at least 50%, or a 50% decline in antigen CA-125 used to assess disease recurrence (Rocereto et al. 2000). However, a second phase II clinical trial including 24 patients with advanced ovarian cancer that recurred from standard chemotherapy within 6 months showed that only one patient had an objective response to a 28-day course of 200 mg mifepristone given daily (Rocereto et al. 2010). This clinical evidence is highly limited in terms of the number of patients, the lack of predictive biomarkers of response, and the fact that the studies consider ovarian cancer as a single disease.

There is growing consensus that ovarian cancer is a very heterogeneous disease, not only from the histological standpoint, but also genetically and within their different histological subtypes (Cancer Genome Atlas Research Network 2011, Vaughan et al. 2011). For instance, the value of patient stratification by disease subtype has been recently evidenced in a study by the Ovarian Tumor Tissue Analysis (OTTA) consortium including almost 3000 women worldwide with invasive epithelial ovarian cancer (Sieh et al. 2013). The study provided a significant positive association between expression of PR and survival advantage in patients with high-grade serous and endometrial ovarian cancers, but not in patients with mucinous, clear cell, or low-grade serous ovarian carcinomas. Then, it became clear that new clinical studies should be conducted stratifying patients by disease subtype and PR expression status, with the overall goal of defining the genetic backgrounds of the ovarian cancers that would likely respond to antiprogestin therapy. Moreover, clinical studies using antiprogestins other than mifepristone are required. Finally, the dynamic of expression of PR in the ovarian cancer cells and the cells encompassing the accompanying tumor stroma is crucially needed in order to define whether direct or paracrine cancer–stroma interactions explain the antiproliferative actions of antiprogestins.

Endometrial cancer

Endometrial carcinoma is a frequent malignancy of the female reproductive track (reviewed in SGO Clinical Practice Endometrial Cancer Working Group et al. (2014a,b)). Information on the putative therapeutic benefit of antiprogestins for these patients, however, is scarce. Mifepristone was shown to bind estrogen-independent PR in the human endometrial cell line IK-90 and prevent the growth inhibition induced by the synthetic progestin R5020 (Terakawa et al. 1988). By contrast, mifepristone blocked growth and promoted cell death in EM-42 endometrial cells established from a benign endometrium (Han & Sidell 2003). Similarly, in the well-differentiated human endometrial adenocarcinoma Ishikawa cells, which express a functional PR (Lessey et al. 1996), mifepristone blocked their growth (Li et al. 2005, Navo et al. 2008, Moe et al. 2009). Clinically achievable doses of mifepristone also inhibited the growth of three endometrial cancer cell lines (Hec-1A, LEK, and RLI9-5-2), while inducing a decline in the abundance of GR (Schneider et al. 1998).

Cervical cancer

Cervical cancer is highly frequent worldwide and its development almost always is associated with previous infection with human papillomavirus (HPV; reviewed in Haie-Meder et al. (2010), and Meijer & Snijders (2014)).
In the cervical adenocarcinoma cell line C4-1, mifepristone sensitized the cells to the toxicity of gamma irradiation by reversing dexamethasone-induced HPV E6/E7 mRNA expression, p53 inhibition, and survival effects, all opposing the deleterious effects of radiation therapy (Kamradt et al. 2000). Mifepristone also blocked the growth of HeLa cervical adenocarcinoma cells in vitro and in vivo, synergizing with cisplatin-induced toxicity (Juraudo et al. 2009). More recently, a reversal of the resistance to mitomycin-C by mifepristone has been described in HeLa cells (Chen et al. 2014).

Leiomyoma

Uterine leiomyoma, also known as uterine fibroid, represents a benign tumor of the smooth muscle cells of the myometrium that relies on estrogen and progesterone to grow (Murphy & Castellano 1994, Shimomura et al. 1998, Yoshida et al. 2010). The utilization of PRMs to interrupt leiomyoma growth has been quite successful. For instance, patients receiving 25 or 50 mg mifepristone daily for 3 months exhibited a decrease in the size of the leiomyomata by 50% after concluding the treatment and without displaying any significant side effect (Murphy et al. 1993, 1995). Mifepristone reduced growth and ameliorated the symptoms in premenopausal women with large leiomyomata (Eisinger et al. 2003). Women with leiomyoma, who were treated with low doses of mifepristone for 6 months, showed a significant reduction in their uterine volume, bleeding, and pain, which resulted in an increase in their overall quality of life (Eisinger et al. 2009). In another study, 30 women with uterine leiomyomata scheduled for surgery received 50 mg mifepristone for 3 months before the operation and showed a significant reduction in the leiomyoma volume concurrently with reduced bleeding (Engman et al. 2009). A meta-analysis of 11 clinical trials performed using treatment with mifepristone ranging from 2.5 to 25 mg/day for 3–6 months concluded that the antiprogestin was globally effective, resulting in the decline of the volume of the uterus and the leiomyoma, as well as the alleviation of leiomyoma symptoms, including blood loss, pelvic pain, and pressure, without any evidence of causing endometrial hyperplasia or atypia (Shen et al. 2013). Finally, a phase II clinical trial performed in women with symptomatic uterine leiomyomata reported that vaginal mifepristone given at a dose of 10 mg daily for 3 months was a safe and effective way of controlling bleeding and reducing the volume of the fibroids (Yerushalmi et al. 2014).

Ulipristal also was shown to be effective in reducing the number of viable primary cultured leiomyoma cells in a dose-dependent manner (Xu et al. 2005). The reduced antiglucocorticoid effect of ulipristal, when compared with mifepristone, might be beneficial for long-term treatment schedules. For instance, in a randomized controlled trial, administration of ulipristal for 3 months led to a reduction in the volume of the tumors and improved the quality of life without any serious side effects; these would have included the lack of antiglucocorticoid effects and uterine hyperplasia observed, sometimes, as a consequence of prolonged mifepristone activity that precludes estrogenic effects from being counteracted by progesterone (Levens et al. 2008). A clinical trial comparing ulipristal given at doses of either 5 or 10 mg daily to patients with symptomatic uterine fibroids before surgery against a once-a-month injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate used as a standard of care demonstrated similarities with the approaches in controlling uterine bleeding, with the advantage that ulipristal-treated patients were less likely to have hot flashes, very commonly induced by GnRH agonists due to suppression of E2 (Donnez et al. 2012).

The antiproliferative effect of ulipristal on primary leiomyoma cells was also mimicked by asoprisnil (Chen et al. 2006). Both asoprisnil- and ulipristal-treated primary leiomyoma cells produced less extracellular matrix proteins, usually responsible for the fibrotic nature of the tumor, when compared with matched normal endometrial cells (Yoshida et al. 2010). A controlled clinical trial demonstrated that asoprisnil reduced the volume of leiomyoma, suppressed uterine bleeding, and improved patient's quality of life without causing hypoestrogenism (Chwalisz et al. 2007) and the consequent bone loss associated with other non-surgical treatment(s) that blocks the pituitary–ovarian axis (e.g., usage of GnRH agonists). Telapristone has also shown promise in inhibiting cell proliferation in primary cultures of uterine leiomyoma smooth muscle cells isolated from premenopausal women undergoing hysterectomy due to leiomyoma-associated symptomatology; this effect occurred without affecting the growth of control myometrial smooth muscle cells collected from adjacent corresponding uteri (Luo et al. 2010).

Leiomyosarcoma

Leiomyosarcoma is a rare malignant tumor from smooth muscle cells most commonly originating in the uterus. Some sporadic cases of low-grade uterine leiomyosarcoma have been reported as responding to mifepristone therapy. For instance, daily administration of 200 mg mifepristone in a patient with low-grade leiomyosarcoma with osteolytic metastasis showed a 5-year regression of the bone tumors (Baulieu 1997). Another patient with PR-positive, low-grade leiomyosarcoma displayed a 3-year regression response to 50–200 mg of mifepristone daily (Koivisto-Korander et al. 2007).
Endometriosis is a condition characterized by the presence of endometrial glands and stroma outside the uterus. It is a common cause of infertility and affects up to 17% of women of reproductive age (reviewed in Giudice & Kao (2004), Hansen & Eyster (2006), and Bernardi & Pavone (2013)). In 1991, the efficacy of mifepristone on this disease was first explored by administering a dose of 100 mg/day for 3 months to six cycling women diagnosed with endometriosis. The treatment showed improved relief of pelvic pain in all subjects (Kettel et al. 1991). A study by the same group confirmed the finding in another cohort of subjects 3 years later (Kettel et al. 1994), and the overall data were timely reviewed (Murphy & Castellano 1994). Further studies using 50 mg/day mifepristone concluded that endometriosis regressed by half after 6 months of treatment (Kettel et al. 1996).

In a rat model of experimental endometriosis generated by implanting endometrium into the peritoneal cavity, animals receiving mifepristone for 8 weeks did not show any blockage of disease progression (Tjaden et al. 1993). Onapristone, on the other hand, when tested in another model of surgically induced endometriosis in intact rats reduced the growth of endometrioid foci by 40% without affecting the proliferation of the eutopic endometrium (Stoeckemann et al. 1995). More recent studies with mifepristone have shown that when the drug was administered in slow-release pellets to rats, it was able to slow the growth of endometrial explants used as a model of endometriosis in vivo (Mei et al. 2010). Another recent study using a rat model of surgically induced endometriosis has provided evidence that ulipristal, given as oral daily doses of 0.1 mg for 2 months, reduced the size of endometrioid foci by at least 50% and was associated with a decline in the number of cells showing expression of the proliferation marker Ki67 (Huniaidi et al. 2013). Finally, in a study on monkeys with surgical induction of endometriosis, mifepristone caused thinning of the pelvic endometrioid lesions similar to that caused by GnRH agonists, yet with the benefit of not causing hypoestrogenism and the consequent bone loss (Grow et al. 1996).

Antiprogesterin-induced cell cycle arrest

A large number of studies support the concept that, when antiprogestins block cell growth, one key mechanism involved is the arrest of the cell cycle. Such arrest mostly occurs in the G1 phase and involves upregulation of cyclin-dependent kinase (Cdk) inhibitors p21cip1 and/or p27kip1 and their re-localization to the nuclear compartment and inhibition of the G1/S kinase Cdk2, thus not allowing DNA synthesis and cell division to proceed further.

The tumor-promoting capacity of progesterone, the role of PR, and the effect of antiprogestins on mammary tumor development were in part drawn from a series of studies using a mouse model in which chronic exposure of female BALB/c mice to MPA induces ductal mammary adenocarcinoma (reviewed in Lanari et al. (2009)). In vivo, in these MPA-induced tumors, daily treatment with mifepristone or onapristone led to tumor growth retardation in association with increased expression of p21cip1 (Peters et al. 2001). Furthermore, lung and axillary metastases caused by the MPA-induced tumor line termed C7-2-H1, which expresses high levels of PR and ER, underwent complete regression when the animals received estrogens in combination with mifepristone; a reduction in metastatic growth was associated with increased expression of p21cip1 and p27kip1 (Vanzulli et al. 2005). In vitro, in two metastatic cell lines originated from MPA-induced mouse ductal mammary adenocarcinomas, mifepristone or onapristone reduced the number of mitosis and cell growth, while increasing expression of p21cip1, p27kip1, and p53 (Vanzulli et al. 2002). Concordant with these data from mice, in DMBA-induced experimental mammary tumors in rats, treatment with antiprogestins induced accumulation of cells in the G0/G1 phase of the cell cycle with a concomitant reduction in cells transiting the S and G2/M phases (Michna et al. 1992). This outcome is consistent with a phenotype of differentiation as the number of mitotically active cells is reduced in association with a cell phenotype resembling that of non-proliferative secretory cells (Michna et al. 1989).

In human MCF-7 breast cancer cells, mifepristone showed synergistic cytotoxicity with 4-hydroxytamoxifen in association with downregulation of retinoblastoma (Rb) tumor suppressor (Schoenlein et al. 2007). When treated only with mifepristone, MCF-7 cells were arrested at the G1 phase of the cell cycle (Fjellidal et al. 2010). In T-47D and BT-474 breast cancer cells, mifepristone and ORG-31710 reduced the number of cells transiting the S phase and increased the abundance of cells including prostate (El Etreby et al. 2000, Liang et al. 2002), meningioma (Matsuda et al. 1994, Grunberg et al. 2006), glioblastoma (Pinski et al. 1993), osteosarcoma (Tieszen et al. 2011), and gastric adenocarcinoma (Li et al. 2004a).

Mechanisms of growth inhibition driven by antiprogestins

The molecular mechanisms triggered by antiprogestin(s) while blocking cell growth are multiple and not yet fully understood. In the following section, we describe the molecules and pathways that either directly or indirectly have been reported to be involved in antiprogestin-induced cell growth inhibition. The section is not limited to the gynecological diseases described previously: it expands on mechanisms uncovered while antiprogestins block the growth of non-gynecological-related cancer...
of hypo-phosphorylated (inactive) Rb, thus arresting the cells at the G1 phase of the cell cycle in association with an increase in p21cip1 expression and reducing cyclin E/Cdk associated kinase activity (presumably Cdk2) (Musgrove et al. 1997). Lonaprisan, on the other hand, also halted proliferation of T-47D breast cancer cells blocking the S phase entry induced by E2 (Alhuppe et al. 2010), inducing G1 cell cycle arrest, which required the upregulation of p21cip1 triggered by lonaprisan-bound PR to the p21cip1 promoter (Busia et al. 2011).

Endometrial Ishikawa cancer cells treated with mifepristone underwent cell cycle arrest with a decline in the proportion of cells transiting G2/M and an increase in cells in the S phase (Li et al. 2005); yet, other reports with the same cells indicate that cell cycle arrest occurred in G1 in a manner likely depending on the induction of p53 (Navo et al. 2008, Moe et al. 2009). Cdk2 activity by mifepristone involving upregulation of p21cip1 was also shown in Hec-1A endometrial cancer cells (Schneider et al. 1998).

In ovarian cancer cells, mifepristone used at concentrations likely to be achieved in vivo induced G1 cell cycle arrest and inhibition of synthesis of DNA as measured by BrdU incorporation (Goyeneche et al. 2007). In agreement, inhibition of DNA synthesis as measured by [3H]-thymidine incorporation was observed after mifepristone treatment in cultured macrophages (Roberts et al. 1995). We also observed that mifepristone-treated cells had low expression of E2F transcription factor required for S phase transit and reduced activity of Cdk2 required for Rb hyper-phosphorylation and consequent activation (Goyeneche et al. 2007). A decline in Cdk2 activity by ORG-31710 associated with increased p21cip1 was also reported in T-47D breast cancer cells (Musgrove et al. 1997).

Cdk2 activity is necessary to promote S phase entry (Conradie et al. 2010). As such, Cdk2 triggers the transition in the cell cycle from G1 by stimulating histone gene transcription (Zhao et al. 2000). To be active and available, Cdk2 should bind to cyclin E, be allocated in the nuclear compartment, and not be bound to the Cdk inhibitors p21cip1 and p27kip1 (Lents et al. 2002, Brown et al. 2004, Conradie et al. 2010). Thus, promoting p21cip1/p27kip1 upregulation and favoring their nuclear localization, antiprogestins promote the decline in Cdk2 nuclear activity and, consequently, the progression of the cell cycle. We have shown in ovarian cancer cells that mifepristone, ORG-31710, ulipristal, telapristone, 17α-hydroxy CDB-4124, and CDB-4453 (a demethylated derivative of CDB-4124) all increase p21cip1 and p27kip1 (Goyeneche et al. 2012, Gamarra-Luques et al. 2014). We have also established that, with a potency of mifepristone > ORG-31710 > ulipristal, these antiprogestins increased p21cip1 and p27kip1 levels in the nuclear compartment while reducing cyclin E levels, consequently leading to an abrupt reduction in the nuclear activity of Cdk2 (Goyeneche et al. 2007, 2012). Reduction in the activity of Cdk2 by antiprogestins is relevant from a therapeutic standpoint because Cdk2 is often upregulated in ovarian cancer cells (Sui et al. 2001) and has been shown to be a valuable targetable molecule in ovarian (Etemadmoghadam et al. 2013) and breast (Achille et al. 2012), as well as other human cancer cells (Molenaar et al. 2009, Long et al. 2010). It is feasible that antiprogestins contribute to the recalibration of the activity of Cdk2 to that of normal cells.

Another cell fate phenotype that was reported to be associated with cell cycle arrest induced by antiprogestins is cellular senescence. Cellular senescence is a cell fate program described as permanent cell cycle arrest but with a very active and unique secretion phenotype termed senescence-associated secretory phenotype (SASP; reviewed in Perez-Mancera et al. (2014)). We have shown in LNCaP prostate cancer cells that exposure to mifepristone for 3 days caused a permanent cell cycle arrest that was not reversible upon the removal of the drug, yet was not associated with cell death. Instead, cells remained alive but irreversibly arrested, expressing the senescence marker, senescence-associated beta-galactosidase (SA-β-gal). This phenotype was not evident, however, in other cancer cells treated similarly, such as SKOV-3 (ovarian), U87MG (glioblastoma), or MDA-MB-231 (breast) (Brandhagen et al. 2013), suggesting that the senescence program requires a particular genetic underpinning. LNCaP cells, for instance, but not the other cells studied, express the tumor suppressor CDKN2A (p16INK4), which is a critical mediator of the senescence program (Alcorta et al. 1996). Alternatively, LNCaP cells express AR (Tieszen et al. 2011), which also bind mifepristone (Song et al. 2004), suggesting that AR may be mediators of mifepristone-induced senescence. Similarly, a senescence-like phenotype was reported in T-47D breast cancer cells exposed to lonaprisan (Busia et al. 2011). These data are highly relevant as pro-senescence therapy in cancer is undergoing intense scrutiny (Nardella et al. 2011).

**Antiprogestin-induced cell death**

When antiprogestins are used at high enough concentrations or for prolonged periods of time, cells that initially arrest in the cell cycle trigger their own death. A large body of evidence indicates that antiprogestin-induced cell death is associated with the following: i) morphological features of apoptosis; ii) downregulation of antiapoptotic Bcl2 family members (e.g. Bcl2 and BclX); and inhibitors of apoptotic proteins (e.g. XIAP); iii) upregulation of pro-apoptotic Bcl2 family members (e.g., Bax); iv) nuclear and DNA fragmentation; and v) downstream activation of caspase 3. For instance, in ovarian cancer, micromolar concentrations of
mifepristone, ORG-31710, ulipristal, telapristone, 17α-hydroxy CDB-4124, and CDB-4453 caused cell death with morphological features of apoptosis, accumulation of fragmented hypodiploid DNA, and activation of the executor of apoptosis, caspase 3 (Goyeneche et al. 2012, Gamarra-Luques et al. 2014). Cleavage of poly(adenosine 5′-diphosphate-ribose) polymerase (PARP), a substrate for active caspase 3, was observed in ovarian cancer cells after exposure to mifepristone, ORG-31710, and ulipristal. Ulipristal, however, at the same time upregulated PARP, a phenomenon that has been previously shown in cultured human uterine leiomyoma cells (Xu et al. 2005). In addition, ulipristal upregulated antiapoptotic proteins XIAP and Bcl2, yet cell death ensued with less effectiveness than that caused by mifepristone or ORG-31710, in which XIAP and Bcl2 were downregulated (Goyeneche et al. 2012).

In cultured human uterine leiomyoma cells, ulipristal was shown to downregulate the antiapoptotic protein Bcl2 (Xu et al. 2005). In the cholangiocarcinoma cell line FRH-0201, mifepristone blocked growth, induced apoptosis, and upregulated pro-apoptotic Bax with a simultaneous downregulation of Bcl2 (Sun et al. 2012). In endometrial HEC-1A and Ishikawa cancer cells, Bcl2 levels decreased in response to mifepristone in association with the increase in tumor suppressor p53 (Navo et al. 2008). An increase in pro-apoptotic Bax and FAS ligand, and a concomitant decrease in antiapoptotic Bcl2 and activation of caspase 3, was also observed in Ishikawa cells upon treatment with mifepristone (Li et al. 2005). Potentiation of apoptotic cell death was observed after exposing Ishikawa endometrial cancer cells to the combined treatment of mifepristone and progesterone (Moe et al. 2009). An increase in Bax levels and a decrease in Bcl2 levels were also observed in endometrial Hec-1A, KLE, and RL95-2 when treated with doses of mifepristone that caused apoptosis (Schneider et al. 1998). In the endometrial cell line EM42, mifepristone stimulated the activity of the transcription factor nuclear factor kappa B (NFkB) and induced apoptosis mediated by the induction of pro-apoptotic Bax and downregulation of antiapoptotic Bcl2, in a NFkB-dependent manner (Han & Sidell 2003). In HeLa cervical adenocarcinoma cells resistant to mitomycin C, mifepristone increased BAX expression while decreasing expression of BCL2 (Chen et al. 2014). In prostate cancer cells, presence of mifepristone sensitized the cells to apoptosis induced by TNFα-related apoptosis inducing ligand (TRAIL) by promoting activation of caspase 8 and truncation of pro-apoptotic Bcl2 family member Bid (Eid et al. 2002). Finally, in human SGC-7901 gastric adenocarcinoma cells, mifepristone blocked cell proliferation and induced morphological features of apoptosis in a dose-dependent manner in association with downregulation of pro-survival Bcl2 and increased caspase 3 activity (Li et al. 2004a).

**PR and antiprogestin-induced antiproliferation**

As several tumors of both gynecological and nongynecological origin are steroid hormone-dependent and express PR, antiprogestins have been investigated as potential anti-cancer therapeutic agents largely based on their capacity to modulate such receptors. However, the role of PR in the antiproliferative effect of antiprogestins is not without complexity and apparent discrepancies. For instance, mifepristone inhibited the growth of ER-negative/PR-negative MDA-MB-231 breast cancer cells (Liang et al. 2003). In another study, mifepristone showed an agonistic effect potentiating progesterone-mediated growth retardation and apoptosis (Moe et al. 2009). Such potentiation of cytotoxicity of progesterone by mifepristone was reported also in PR-positive MCF-7 breast cancer cells as well as in PR-negative C4-I cervical carcinoma cells, suggesting that the presence of PR may not be essential for the antigrowth properties of both, progesterone and mifepristone (Fjelldal et al. 2010).

In the aforementioned studies, however, mifepristone was utilized at micromolar concentrations, suggesting that at such doses the antigrowth effect may utilize a PR-independent mechanism as the concentrations needed to saturate intracellular PR are in the nanomolar range (Nardulli & Katzenellenbogen 1988).

The expression of PR in antiprogestin-responsive cancer cells is also controversial, probably as a consequence of the complex variables involved in PR actions, including the following: i) tissue-specific effects; ii) the presence of two isoforms (PR-A and PR-B) with distinct properties; iii) difficulties in tailoring the mRNA expression levels with that of protein receptor levels due to the scarcity of specific antibodies for each PR isoform; and iv) the differential kinetics of the isoforms, with PR-A being more stable than PR-B, because the latter undergoes post-translational modifications including phosphorylation, ubiquitination, acetylation, and SUMOylation, all contributing to its rapid turnover, and, probably, the difficulty in its detection. These factors contributing to the complexity in PR activity have been recently addressed in a comprehensive review (Hagan & Lange 2014).

In ovarian cancer, the majority of cell lines reported in the literature lack or show very low levels of the canonical PR (Hamilton et al. 1984, Keith Bechtel & Bonavida 2001, McDonnel & Murdock 2001, Akahira et al. 2002). Using an antibody that detected PR-A and PR-B isoforms in MCF-7 breast cancer cells, we reported that such receptor isoforms were not found in ovarian (SKOV-3 and OVCAR-3), breast (MDA-MB-231), prostate (LNCaP and PC-3), bone (U-2OS and SAOS-2), and meningioma (IOMM-Lee) cancer cell lines cultured under similar conditions (Tieszen et al. 2011). However, these cells lines, regardless of PR expression, responded to the growth inhibitory properties of micromolar doses of mifepristone. Furthermore, the abundance of PR-A...
and PR-B proteins in MCF-7 cells was highly reduced upon treatment with mifepristone, which retained its growth inhibition properties, discouraging the role of these nuclear receptors as mediators of the growth inhibitory effect of mifepristone. Similarly, in T-47D breast cancer cells made resistant to aromatase inhibitors, which express high levels of aromatase and grow in response to testosterone, telapristone caused growth arrest in association with downregulation of PR-B mRNA and protein levels (Gupta et al. 2013a).

The requirement for PR as a mediator of the antigrowth effect of antiprogestins, however, has been shown in other experimental models. For instance, in in vivo studies on mice with MPA-induced mammary carcinomas, antisense oligodeoxynucleotides against PR that leads to in vivo knockdown of the receptor caused inhibition of tumor growth similar to that of mifepristone (Lamb et al. 2005). In this model system, PR-A appears as a critical PR isoform conferring sensitivity to antiprogestins, as antiprogestin-resistant variants of the MPA-induced mammary tumors depict a heightened downregulation of PR-A when compared with antiprogestin-sensitive tumors (Wargon et al. 2009, Lanari et al. 2012). Using primary cultures of cells isolated from the MPA-induced mouse mammary carcinomas, mifepristone blocked MPA-induced growth at nanomolar concentrations (Lamb et al. 1999). More recently, it has been demonstrated that in these cells the antiprogestin increased tissue remodeling, which favored the efficacy of nanoparticle carrying chemotherapeutic agents (Sequeira et al. 2014). Notably, in this latter work, mifepristone-induced tissue remodeling involved the increase in the vascularity of the tumor and the increase in the ratio of stromal tissue to tumor tissue, indicating the tumor microenvironment as an evident target of antiprogestin therapy. From this mouse model of breast cancer, it has been suggested that tumors with levels of PR-A higher than PR-B should be the ones to be targeted with antiprogestin therapy (Lanari et al. 2012).

In human breast cancer cells, PR-B seems to be a critical determinant of the responsiveness to the antiproliferative effect of antiprogestins (reviewed in Knutson & Lange (2014)). The development of T-47D cells overexpressing either PR-A or PR-B led to the conclusion that, in the presence of mifepristone, there is inappropriate transactivation of PR-B but not of PR-A (Sartorius et al. 1994). In the presence of a ligand, PR-B is phosphorylated at Ser294 and translocated to the nucleus where it operates as a highly active transcription factor triggering gene expression encoding for proteins needed for cell cycle progression, proliferation, and survival (cyclin D1, Myc and Bcl2 respectively). However, when activated, PR-B has a very short half-life as phospho-Ser294-PR-B is recognized for degradation by the ubiquitin–proteasome system making activated PR-B difficult to detect by western blotting (Knutson & Lange 2014). Thus, in the studies described earlier using mifepristone as a growth inhibitor in human cancer cell lines, the fact that PR is undetectable by western blot (Tieszen et al. 2011) does not rule out its presence as, under the culture conditions used, it could have had a heightened turnover that did not allow for its detection with the antibodies utilized.

Early in 1987, experimental evidence using breast cancer cells suggested that the antihormone and antiproliferative activity of antiprogestin mifepristone are dissociated (Bardon et al. 1987). Our previous analysis of the literature has suggested that such statement is still valid and further studies need to be conducted to find out the role of PR in antiprogestin-mediated antiproliferative activity.

Membrane PR

The antitumor effect of antiprogestins may well be mediated by non-cognate PR, such as membrane PRs (mPRs, β, γ, δ, and ε; Thomas et al. 2007, Gellersen et al. 2009, Dressing et al. 2011). This hypothesis is supported by studies on Xenopus oocytes in which progesterone promotes germinal vesicle breakdown (GVBD) – an indicator of meiotic maturation – probably due to the interplay of cognate intracellular PR and mPR (Jørgensbøg Ben-Yehoshua et al. 2007). In this model system, antiprogestin mifepristone depicted a progesterone-like effect at micromolar concentrations (Sadler et al. 1985). When these mPRs were expressed in yeast, antiprogestin mifepristone also had an agonist effect (Smith et al. 2008). Curiously, Xenopus intracellular PR lacks the glycine residue considered essential for intracellular PR binding to mifepristone (Benhamou et al. 1992), suggesting that the membrane-linked but not the classical intracellular PR mediates the progesterone-like mifepristone-induced GVBD. Thus, effects of antiprogestins mediated via mPR might need to be differentiated from those controlled via the classical PR.

Membrane PRs have been shown in human breast biopsies, in cognate PR-positive MCF-7 and SKBR3 breast cancer cell lines, in cognate PR-negative MDA-MB-468 breast cancer cells, in HeLa cervical cancer cells, and in ovarian cancer biopsies (reviewed in Dressing et al. (2011)). Ovarian cancer cell lines (SKOV-3 and ES2) express the mRNA of the three mPRs, which regulate PKA, p38, and JNK signaling pathways (Dressing et al. 2011).

Another non-cognate PR, termed PR membrane component 1 (PGRMC1), was shown to increase in advanced ovarian cancer in association with an absence of the classical PR. Furthermore, it was shown that overexpression of PGRMC1 interfered with cisplatin-induced cytotoxicity, which suggests that PGRMC1 has a survival role in this particular gynecological cancer (Peluso et al. 2008).
In summary, the roles of mPR and PGRMC1 in antiprogestin-mediated antiproliferation in cells involved in gynecological diseases represent an attractive area of research that could lead to novel therapeutic interventions.

**Growth factors and signaling pathways involved in antiprogestin-mediated growth inhibition**

Transforming growth factor beta 1 (TGFβ1) is induced by mifepristone and triggers apoptosis in LNCaP-C4 prostate cancer cells (Liang et al. 2002). In ER-negative/PR-negative MDA-MB-231 breast cancer cells, mifepristone and 4-hydroxytamoxifen potentiated one another’s effect in inducing apoptosis associated with DNA fragmentation and cytochrome c release from the mitochondrial compartment and activation of downstream executor of apoptosis, caspase 3, mediated via upregulation of TGFβ1 (Liang et al. 2003). Furthermore, MCF-7 and T-47D PR-positive breast cancer cell lines produce more TGFβ when incubated in the presence of onapristone in association with growth inhibition (Dannecker et al. 1996). Conversely, for insulin-like growth factor1 (IGF1), it was shown that it attenuates antiprogestin-mediated apoptosis in ER-positive breast cancer cells (Periyasamy-Thenadan et al. 2012). Ulipristal was shown to block the expression of fibronectin and VEGFA mRNA induced by activin A in cultured leiomyoma cells, thus blocking cell growth (Ciarmela et al. 2014) and the substantial angiogenesis required by these tumors to proliferate (Xu et al. 2006). In MPA-induced mouse mammary tumor line C4-HD, mifepristone was able to block the proliferation induced by bFGF alone or the combination bFGF/MPA (Lamb et al. 1999). Mifepristone blocked the secretion of IGF1 induced by progesterone and E2 in vivo explants of ER-positive/PR-positive breast cancers (Milewicz et al. 2005).

In MDA-MB-231 breast cancer cells transfected with PR, mifepristone arrested the cells in the G0/G1 phase of the cell cycle while activating p44/p42 MAPK (Lin et al. 2001). In another study, MAPK inhibitors and antiprogestins blocked the growth of BT-474 breast cancer cells upon induction with EGF and progesterins (Knutson et al. 2012). In cultured mouse cancer cells isolated from mouse mammary tumors induced by MPA, mifepristone, onapristone, and lonaprisan blocked proliferation induced by MPA or FGF2 while increasing phosphorylation of ERK via rapid mechanisms (reviewed in Lanari et al. (2012)). When cells from previous tumors were maintained in 3D cultures, lonaprisan induced cell death more efficiently in MPA-dependent cells having a low AKT activity, suggesting the survival role of the PI3K/Akt pathway in these cancer cells (Polo et al. 2010). In ovarian cancer cells cultured either in 2D or 3D, cytostatic doses of mifepristone caused synergistic lethality when combined with an inhibitor of the PI3K/Akt survival pathway, in association with downregulation of antiapoptotic proteins BCL2 and XIAP, and cleavage of PARP (Wempe et al. 2013).

Another pathway involved in antiprogestin-mediated growth inhibition is the Wnt pathway, which is critically involved in cancer development (reviewed in Veeck & Dahl (2012) and Gupta et al. (2013b)). For instance, Wnt1 was blocked by mifepristone in MCF-7 cells, while overexpression of Wnt1 prevented mifepristone-induced growth inhibition (Benad et al. 2011).

Mounting evidence demonstrates the role of cytoplasmic pro-proliferative protein kinases such as MARK, Ck2, and Cdk2 in controlling the phosphorylation status of classic PR (reviewed in Trevino & Weigel (2013)). Cdk2 is a cell cycle kinase critically important for the hyperphosphorylation of Rb, thus allowing the detachment of E2F transcription factor from Rb, and making E2F available to regulate the expression of genes driving DNA synthesis during the S phase (Conradie et al. 2010). Thus, small molecules are under development for the blockage of Cdk5, among them Cdk2, to treat cancer (reviewed in Esposito et al. (2013)). In human cells spanning many cancer types, we have shown that alongside blocking proliferation, mifepristone strongly inhibited the activity of Cdk2 (Tieszen et al. 2011). In ovarian cancer cells, we also reported that mifepristone promoted the upregulation of p21cip1 and p27kip1 and their association with Cdk2 in the nuclear compartment, thus blunting the activity of Cdk2 otherwise required to drive G1/S cell cycle progression (Goyeneche et al. 2007, 2012). Cdk2 is critically important to phosphorylate PR at Ser400, thus activating the transcriptional activity of PR in a ligand-independent manner during the cell cycle. As Cdk2 activity is blocked by p27kip1 overexpression (Pierson-Mullany & Lange 2004), we propose that mifepristone-induced cell cycle arrest in G1 is mediated by p27kip1-induced Cdk2 inhibition upstream of PR activation, thus preventing ligand-independent PR transcriptional activity required for cell cycle progression.

**Glucocorticoid receptors**

Except for the new generation of antiprogestins that bind GR with a much less affinity than PR, older antiprogestins such as mifepristone bind GR with a high affinity (Mao et al. 1992). Furthermore, GR are ubiquitously expressed in normal as well as cancer cells (Agarwal 1996). Our laboratory reported abundant expression of GR isoforms alpha (GRα) and beta (GRβ) as measured by western blot in ovarian, breast, prostate, bone, and brain cancer cells (Tieszen et al. 2011, Telleria & Goyeneche 2012). All such cell lines studied responded to mifepristone with growth inhibition, whereas the relative expression of GRα and GRβ was very variable, yet did not show any significant correlation with the growth inhibition potency of mifepristone (Tieszen et al. 2011). In OV2008 cancer cells, mifepristone, ORG-31710, and
ulipristal increased p21cip1 and p27kip1 and caused cell cycle arrest without major changes in the expression of the GR isoforms. Yet, under the same experimental conditions, equimolar concentrations of the GR agonist dexamethasone did not cause growth arrest or upregulation of p21cip1 and p27kip1, but blunted the expression of GR (Telleria & Goyeneche 2012). By contrast, in three endometrial cancer cell lines (Hec-1A, LEK, and RL95-2), mifepristone inhibited cell proliferation while causing a decline in the abundance of GR (Schneider et al. 1998).

Mostly, on GRα, mifepristone has an antagonistic activity; yet, it was shown to have an agonistic potency depending on the concentration of GR in the cell (Zhang et al. 2007). On the other hand, although GRβ has been considered a dominant negative regulator of GRα (Oakley et al. 1999, Yudt et al. 2003, Taniguchi et al. 2010), it was also reported to function, in the absence of GRα, as a receptor for mifepristone, leading to nuclear translocation and transcriptional activity (Lewis-Tuffin et al. 2007). All cancer cell lines we exposed to mifepristone undergo proliferation inhibition with IC50s – concentrations that reduce growth by 50% – ranging from 9 to 30 μM, and all cells had high expression of GRβ, yet variable abundances of GRα fluctuating from no expression (LNCaP) to high expression (SKOV-3 and MDA-MB-231) (Tieszen et al. 2011, Telleria & Goyeneche 2012). These evidences suggest to us that the role of mainly GRβ on antiprogestin-mediated cell growth inhibition deserves further exploration.

**Antioxidation**

Early in 1994, it was reported that mifepristone, when used at micromolar doses, operates as an antioxidant and that such activity resided in the dimethylaminophenyl side chain of the molecule (Parthasarathy et al. 1994), which is present in most of the antiprogestins developed to date. In support of this action connected with the antiproliferative effect of mifepristone, two reports attributed such a mechanism in endometrial cells and macrophages (Roberts et al. 1995, Murphy et al. 2000). More recently, in a study in which the administration of 50 mg mifepristone every other day for 12 weeks before surgery led to leiomyoma volume reduction, when tissues were studied after surgery, it was clear that the glutathione pathway was the most clearly altered pathway. In particular, the antioxidant enzyme glutathione-S-transferase mu1 (GSTM1), reported to offer protection against free radicals and products of oxidation stress (Sharma et al. 2004), was significantly overexpressed among the good responders compared with the non-responders (Engman et al. 2013). The authors suggest that this enzyme might be important in the regulation of pathways leading to inhibition of cell cycle progression or to facilitate apoptosis. GSTM1 can be a potential molecular marker of objective response to mifepristone therapy. Furthermore, G1 arrest and p21cip1 upregulation were shown to be amplified in response to antioxidants in a p53-independent manner (Liu et al. 1999, Liberto & Cobrnik 2000). This mechanism can explain our results in which mifepristone blocked the growth of ovarian cancer cells regardless of their p53 expression background (Goyeneche et al. 2007, Freeburg et al. 2009b).

**Endoplasmic reticulum stress**

Cancer cells, when compared with non-cancer cells, operate with increased expression of endoplasmic reticulum stress-related proteins, a phenomenon termed ‘endoplasmic reticulum aggravation’ as a consequence of the environment within which cancer cells usually proliferate: reduced nutrients, acidosis, energy deficiency, and hypoxia (reviewed in Schonthal (2013)). First, in 2007, it was shown that asoprisnil triggered endoplasmic reticulum stress-induced apoptosis in cultured human uterine leiomyoma cells (Xu et al. 2007). Secondly, a serendipitous study carried out in 2010 reported that mifepristone induced endoplasmic reticulum stress in non-small cell lung carcinoma cells (Dioufa et al. 2010). Using genomic and proteomic screenings, we have recently reported that cytostatic concentrations of antiprogestin mifepristone trigger the unfolded protein response (UPR; Hapon et al. 2013). The UPR is a mechanism geared to compensate for the stress and to promote cell survival, but, if overwhelmed, it triggers a cell death pathway (Hetz 2012, Urna et al. 2013). We showed that the master chaperone involved in the UPR and associated with cell survival, glucose-regulated protein (GRP) of 78 KDa (GRP78) increased in response to mifepristone in a dose- and time-dependent manner, and independently of p53 tumor suppressor and sensitivity to chemotherapeutic agent cisplatin. In addition, we found the transcription factor C/EBP homologous protein (CHOP) to be highly upregulated, the induction of which is usually linked to cell death. We hypothesize that the UPR integrates the cytotoxicity of antiprogestins toward cancer cells when used as monotherapy or in combination therapies triggering, respectively, cell cycle arrest (cytostasis) or cell death (lethality) depending on the degree of cellular stress generated.

**Cytoskeleton, adhesion, migration, and invasion**

One key component of cancer metastasis is the detachment or de-adhesion of cancer cells from one tissue, migration, and invasion through the extracellular matrix, and re-adhesion to a nearby or distant location. Considering that progestins regulate metastasis-related molecules, it was proposed that antiprogestins could be relevant to fight metastatic diseases (Shi et al. 1994).
In breast cancer cells expressing PR-B, it was shown that mifepristone blocked the migration induced by IGF1 (Ibrahim et al. 2008). We have recently reported using SKOV-3 ovarian cancer cells, MDA-MB-231 breast cancer cells, U87MG glioblastoma cells, and LNCaP prostate cancer cells that a concentration of antiprogestin mifepristone sufficient to block cell proliferation caused changes in the cellular structure with cells developing a thin cytoplasm with neurite-like protrusions. Such changes were associated with redistribution of cytoskeletal actin fibers that mainly form non-adhesive membrane ruffles, which are sheet-like membrane folds that do not attach to the extracellular matrix, leading to a decline in the capacity of the cell to adhere to extracellular substrates (Brandhagen et al. 2013). This morphological phenomenon was associated with diminished cellular migration and invasion capacities toward extracellular matrix (A A Goyeneche, B N Brandhagen, R Srinivasan, and C M Telleria, unpublished observations). In the human gastric MKN-45 adenocarcinoma cells, mifepristone, in a dose-dependent manner, inhibited their adhesion to extracellular matrix and reduced migration through 8 μm pore size membrane filters; in vivo, 8 week treatment with mifepristone reduced the number of distant lung foci in nude mice carrying subcutaneous tumor xenografts (Li et al. 2004b). Consistent with these data, in Ishikawa endometrial cancer cells receiving mifepristone for 12 h, it was shown by RNA sequencing that mifepristone downregulated genes associated with cell–cell contact and adhesion (Tamm-Rosenstein et al. 2013). More recently, a monodemethylated metabolite of mifepristone termed metapristone (RU-42633) has been shown to block the adhesion of human colon cancer HT-29 cells to endothelial cells (Wang et al. 2014). These initial data provide the basis for further studies on the antimetastatic properties of antiprogestins.

**Antiprogestins in clinical trials for gynecological diseases**

Table 1 depicts the ongoing clinical trials registered in the public access database maintained by the US National Library of Medicine at the National Institutes of Health (NIH) (http://ClinicalTrials.gov). Of notice is the number of ongoing studies using mifepristone for conditions such as leiomyoma, breast, ovarian, and endometrial cancers; ulipristal for leiomyoma; and telapristone for endometriosis. The results of these trials will be essential in moving forward the utilization of antiprogestins as adjuvant treatment for gynecological diseases should they confirm their potentiality reflected in the preclinical and clinical studies detailed above. The progress made in the past years exploring the treatment of gynecological conditions such as endometriosis and leiomyoma has been remarkable; yet, the consequence has been of a less emphasis on treating gynecological cancers. However, as new information evolves on the pathogenesis of gynecological cancers and the mechanisms of action of available antiprogestins, there is optimism for quick developments in bringing these compounds to the clinic. Antiprogestins can ameliorate the signs and symptoms, and prevent (as monotherapy) or trigger chronic remission (as adjuvant to standard chemotherapeutic agents) of gynecological malignancies.

**Table 1** Current active interventional clinical trials using antiprogestins for gynecological diseases registered in ClinicalTrials.gov.

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aData obtained August 5, 2014.

**Concluding remarks**

Since the synthesis of mifepristone in 1981, much progress has been made in understanding the mechanisms whereby antiprogestins act at the tissue, cellular, and molecular levels in both normal cells and in cells with a derangement of their proliferation capacity. There has been a concurrent development of new compounds, major progress in understanding the biology of PR and GR isoforms, their cellular localization within the context of the molecular environment, and their involvement in driving cell cycle progression and cell death. As the basic molecular biology of steroidal compounds evolves, the applications of compounds designed to bind PR should be recalibrated continuously. For instance, as reviewed herein, we should exploit their potential for treating gynecological conditions related to unbalanced cell proliferation. The timeframe for their usage on such conditions, as well as their effective dosage, should be addressed to prevent undesired side effects. The analysis of the molecular genetics of the disease will be critical for identifying the cohort of individuals who more probably will benefit from antiprogestin treatment and, by doing so, prevent the inclusion of patients not likely to respond. Inappropriate inclusion of patients can highly contribute to derailing
the success of the clinical studies. With the current ongoing clinical trials, special attention should be given to patients that present an objective response to the treatment despite the fact that they may not represent a significant group within the trial. Such responders should guide the identification of the optimal conditions for objective responses of disease cells with respect to normal cells, such as the ratio of expression of PR-A/PR-B, levels of GRz and GRβ, intracellular signal transduction environment, oxidative stress, and excess or lack of critical cell cycle regulatory proteins to mention some. Thus, for instance, despite that the clinical trials for the usage of antiprogestin mifepristone against ovarian and breast cancers have not been as encouraging as originally envisioned, the knowledge gained on the molecular underpinning of such patients should lead to a better patient selection while more closely bridging basic and translational research.

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

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