The Molecular Biology of RU486. Is There a Role for Antiprogesterins in the Treatment of Breast Cancer?*

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I. Introduction

ENDOCRINE therapy used either prophylactically or therapeutically for the treatment of locally advanced or metastatic breast cancers offers many advantages to patients whose tumors contain functional estrogen and progesterone receptors (ER and PR). The range of treatments defined as endocrine include surgical ablation of endocrine glands, administration of pharmacological doses of steroid hormones, chemical blockade of steroid hormone biosynthesis, and inhibition of endogenous steroid hormone action at the tumor with synthetic antagonists. The last of these approaches is the most widely used, making the antiestrogen tamoxifen the preferred first-line therapeutic agent for treatment of hormone-dependent metastatic breast cancer. The widespread use of tamoxifen reflects its efficacy and low toxicity, and the fact that it makes good physiological sense to block the local proliferative effects of estrogens directly at the breast. But are estrogens the only hormones with a proliferative impact on the breast and on breast cancers? This review focuses on evidence that progesterone also has proliferative actions in the breast; on the role of synthetic progestins in breast cancer treatment; on the molecular biology of progesterone antagonists; and on the preliminary data showing that progesterone antagonists may be powerful new tools for the management of metastatic breast cancer, because they block the local effects of endogenous progesterone on breast cell proliferation. The reader is also referred to the excellent general review on progestin regulation of cell proliferation by Clarke and Sutherland (1). The structures of the agonists and antagonists discussed in this review are shown in Fig. 1.

II. Progesterone and the Normal Breast

Conventional wisdom holds that the mechanisms by which estradiol and progesterone regulate the proliferation and differentiation of uterine epithelial cells apply equally to the breast. This is probably inaccurate (2-4). In the uterus, estrogens are clearly mitogenic, and addition of progesterone to the estrogenized endometrium leads to the appearance of a secretory pattern characterized by cells engaged in protein synthesis rather than cell division (5). That is, in the uterus, estradiol is a proliferative hormone; progesterone is a differentiating hormone. For this reason the unopposed actions of estradiol are considered to be tumorigenic in the uterus, while the risk of endometrial hyperplasia and cancer is lowered when estrogens are combined with progestins. In fact, the combined regimen may even be protective since a decrease in endometrial cancers has been reported in women prescribed combined estrogens and progestins, compared to women receiving no treatment (Ref. 6 and references therein).
However, considerable evidence has now accrued to suggest that in the epithelium of the breast, progesterone has a different influence. That, like estradiol, progesterone in the breast has a strong proliferative effect. Studies in support of this come both from experimental models and from normal cycling women. Both the proliferation of normal mammary epithelium in virgin mice and the lobular-alveolar development of mammary tissues in pregnant mice require progesterone (7,8). A fundamental difference in the actions of estradiol and progesterone in the breast is that the latter stimulates DNA synthesis, not only in the epithelium of the terminal bud, but also in the ductal epithelium (9). The stimulating effects of progesterone on the development of mammary gland buds can be inhibited by progesterone antagonists (10).

Data from normal human mammary cells have been more difficult to obtain and are often equivocal. Compared to the increase caused by estradiol treatment (11.3-fold), progesterone treatment only marginally (2.0-fold) increases the mitotic index of normal human breast ductal epithelium maintained in intact athymic nude mice (11). In fact, Mauvais-Jarvis and colleagues (12,13) concluded, using primary cultures of epithelial cells from normal human mammary gland, that while estradiol treatment stimulates growth, progestins inhibit growth. Their data are difficult to interpret, however, since the experiments using estradiol were done with cells growing in minimally supplemented medium, while the progestin treatment studies were done with cells in optimally supplemented medium, and any progestin growth-stimulatory effect might have been masked. In contrast to the sparse and conflicting in vitro data are studies of the mitotic rate in breast epithelial cells during the normal menstrual cycle and in women taking oral contraceptives. These data show that the highest thymidine labeling indices occur during the progestin-dominated, secretory phase of the menstrual cycle. Both the estrogen and the progestin components of oral contraceptives increase the thymidine labeling index with progestin-only formulations exhibiting high activity (2–4). The investigators conclude that it is difficult to sustain the idea that progestins are protective in the breast (3). It would seem that more work must be done to understand the actions of progestins in the normal breast, but that clinical decisions based on an inappropriate uterine model system are unjustified (14).

III. Progesterone and Breast Cancer

A discussion of the role of progestins in breast cancer must distinguish between their effects on carcinogenesis and their role in regulating proliferation of established cancers.

A. Progestin agonists and tumor induction

Progestin agonists have been shown to be carcinogenic or to increase the incidence of spontaneous mammary tumors in dogs and mice (15–19). In mice, results vary with the strain tested, suggesting the contribution of a genetic component; however, tumorigenic effects of progestins have been observed whether or not the strain harbors the mouse mammary tumor virus (MMTV). The importance of progesterone in carcinogen-induced rat mammary cancers is documented by the early reports of Huggins et al. (20–22), who showed that pregnancy promotes the growth of dimethylbenzanthracene (DMBA)-induced mammary tumors, and that administration of progesterone together with the carcinogen to intact rats accelerates the appearance of tumors, increases the number of tumors, and augments the growth rate of established tumors. The relationship between progestins and carcinogenesis is temporally complex. In general, progesterone administered simultaneously with, or after, the carcinogen enhances tumorigenesis, while progesterone

![Image of molecular structures](https://example.com/image.jpg)
given before the carcinogen inhibits tumorigenesis (Ref. 23 and references therein). Thus, the high progesterone levels associated with pregnancy can be protective if the hormone precedes administration of the carcinogen (24). Extrapolation of these experimental models to human disease is unclear since the only data available for the latter are epidemiological in nature and relate hormone use, particularly oral contraceptive use, to the risk of breast cancer. The trend toward increased risk with increased duration of hormone use appears repeatedly (25, 26), and a possible adverse effect of progestins appears to be likely (27, 28). This is discouraging when taken together with the likelihood that in the breast, unlike the uterus, progestins enhance proliferation during the menstrual cycle.

B. Progestin agonists and growth of established tumors

Carcinogen-induced rat mammary tumors are a major model for in vivo studies of progestin-regulated growth (23). After ovariectomy, progesterone alone is usually unsuccessful in preventing regression of established tumors. The rapid decrease of PR levels due to estrogen withdrawal is probably a critical factor (29). In intact animals, which more closely mimic the clinical situation, progestin agonists at moderate doses have been reported to promote tumor growth and to reverse the antitumor effects of tamoxifen (30). Thus, it is possible that endogenous circulating progesterone may enhance breast cancer growth. Enigmatically, progestins at higher pharmacological doses appear to be growth inhibitory (31). The molecular mechanisms responsible for the opposing actions of physiological and high-dose progestins remain unclear and are discussed later in this review.

In vitro cell culture models designed to assess the role of progestin agonists in tumor cell proliferation have generated contradictory results. Experiments can be cited in support of any argument, that progestins stimulate (32–34), inhibit (35–39), or have no effect (40) on growth. Explanations for the lack of a consensus are as varied as the results. Responses of cells in culture are critically dependent on the conditions in which they are grown. In a rich medium, where growth is optimized, further growth enhancement is difficult to demonstrate, while inhibitory stimuli may be exaggerated. In a deprived medium the reverse is true, although here, key cofactors may be lacking. There is no simple solution to these inherent problems. Couple this generic uncertainty with other variables including the use of different cell lines (35, 37, 40); heterogeneity and genetic instability even within the same cell lines (41, 42); a burgeoning list of factors other than estradiol and progestins that directly or indirectly modulate progestin sensitivity through regulation of PR levels (43–47); the possibility that progestin-sensitive cells can generate resistant subpopulations (48); and it is possible that no physiological consensus is likely to be forthcoming using these in vitro models. These models remain invaluable, however, for the analysis of molecular mechanisms of progestin actions, and to underscore the complexities inherent in tumor cell biology.

Where does this leave us on the critical issue of the use of progestin agonists in breast cancer treatment? Interestingly, here there is more agreement, but the data contradict the conclusion that physiological levels of progestins are growth stimulatory. Especially at high doses, progestins appear to be antiproliferative in breast cancers. A comprehensive review of the clinical literature (Ref. 49 and references therein and Refs. 50–55) shows that synthetic progestins, used at pharmacological doses for first- or second-line therapy, are as effective as tamoxifen in the treatment of advanced breast cancer. That is, approximately 30% of patients whose tumors are not screened for steroid receptors have an objective, positive response. Since in addition, progestins are well-tolerated and have a relatively low toxicity (56), their use in the treatment of advanced breast cancer is experiencing a resurgence (57, 58). However, the mechanisms underlying the actions of intermediate and high doses of progestin agonists in breast cancer regression remain unclear, when compared to their proliferative actions at physiological doses. While some studies suggest that PR-negative tumors respond just as well as do PR-positive tumors (implying that PR are not involved), others suggest that methodological problems produce false PR-negative values in responders (58), and that PR are indeed required to obtain a response to progestins. An interesting study, in which tamoxifen therapy was compared to therapy in which tamoxifen was alternated with medroxyprogesterone acetate (MPA) in ER-positive patients, showed a 40% response to tamoxifen alone vs. a 62% response with the alternating treatment (53). It is postulated that when tamoxifen is cycled, its agonist properties predominate, which increases PR levels thereby enhancing the efficacy of MPA. The same argument is made for enhancing the therapeutic efficacy of antiprogestins (see below). In general, tamoxifen inducibility of PR is considered to be a good indicator for a positive response to hormone therapy (51). Thus, while definitive data are still lacking, it is likely that positive responses to therapy with progestins in breast cancer are mediated by the PR in the tumors.

In order to consider mechanisms by which progestin agonists and antagonists regulate tumor cell growth, and how the hormone dose influences cellular responses, the following section will review recent advances in the molecular biology of PR especially as they apply to progesterone antagonists.
IV. Molecular Mechanisms of Progesterone Antagonists

Like all steroid hormones, progesterone and the synthetic progestins function by binding to intranuclear proteins, the PR, the presence of which specifies a progesterone target tissue and target cell. PR, when bound to ligand, control the transcription of genes, which in the breast include, among others, the genes for ER (59), for insulin receptors (37, 60), and for epidermal growth factor (EGF) receptors (61), as well as the genes for EGF and tranforming growth factor-α (62, 63). Thus, the proliferative effects of progesterone may in part be a reflection of its ability to modulate the levels of growth factors and their cognate receptors. The mechanisms by which this regulation is achieved and the structure of the PR proteins are complex and are described here with specific reference to human PR. For additional information the reader is referred to several recent reviews of the steroid receptor family of proteins (64-67).

A. Progesterone receptors

Complementary DNAs for the chicken PR were cloned independently by Jeltsch et al. (68) and Conneely et al. (69) in 1986, and for the human PR by Misrahi et al. (70) in 1987. Structure/function analyses of the encoded proteins followed quickly. PR belong to the steroid/thyroid hormone receptor superfamily of ligand-activated DNA binding proteins which are composed of a number of independently functioning domains required for nuclear localization, ligand binding, DNA binding, dimerization, and transcriptional activation. Additional sites on the receptors are responsible for their interaction with one or more accessory proteins including heat shock proteins, and several of the serine residues are covalently modified by phosphorylation. Not all of these domains have been definitively mapped, and the functional regions exhibit some overlap, but in general the N terminus is involved in transactivation and contains several phosphorylation sites; the DNA-binding domain is centrally positioned and may have a weak dimerization function; and ligand-binding activity is restricted to the C terminus, which also contains structural features required for protein-protein interactions plus a second transcriptional activation domain (64-67, 71).

The single-copy human PR gene encodes at least nine messenger RNA species ranging in size from 2.5–11.4 kilobases (72, 73). The nine messages direct the synthesis of at least two (74–76), and possibly three (77), structurally related receptor proteins. The two major protein species, the B- and A receptors, were originally described by O'Malley and co-workers (78, 79) in the chick oviduct. Subsequent studies with the breast cancer cell line T47D showed that human PR also exist as two isoforms, 116 kilodalton (kDa) B receptors and N-terminally truncated 94 kDa A receptors (74–76, 80). While A receptors were originally thought to be produced by a proteolytic artifact (81), it is now clear that the amino-truncated receptors, at least in chickens and humans, are a naturally synthesized form (76, 82–85). In human endometrial carcinoma (86) and breast cancer cell lines (74, 75), the two receptor isoforms are expressed in approximately equimolar amounts. It is not known whether this quantitative relationship between the two isoforms is maintained in all human target tissues and tumors, and the mechanisms for their differential regulation are not known, but at least two of the nine mRNA species lack the translation initiation site for B receptors and can therefore encode only A receptors (77, 84). These messages arise by transcription from an internal promoter in the human PR gene (84). Five other message species can potentially encode both receptor isoforms, synthesized by alternate translation initiation from two in-frame AUG codons. In theory, use of the upstream codon generates the B receptors and use of the downstream codon generates the A receptors, but it is not known whether initiation at the downstream site actually occurs in intact cells (85). Interestingly, two additional message species have been mapped that lack both the A and B translation start sites and would encode neither of the two receptor isoforms. These messages do, however, contain downstream translation start sites that if utilized would generate a protein with a partial DNA-binding domain, and complete hormone binding domain and dimerization sequences (77). This "C receptor," when reconstructed by site-directed mutagenesis and expressed in PR-negative cells, binds the synthetic progestin R5020 with the same affinity as the A and B receptors (K. B. Horwitz, unpublished). Expression of such an N-terminally truncated protein in breast cancer cells would lead to a functionally impaired but PR-positive phenotype. The existence of such variant forms of receptors, not only for progesterone, but also for estrogen, may in part explain the recurrent descriptions of receptor-positive, but hormone-resistant breast cancers.

PR are unique among steroid receptors in having two naturally occurring hormone binding forms, and this structural feature may have important functional implications with respect to antagonist action (discussed below). Originally, the unliganded A and B isoforms were thought to be subunits of a larger heteromeric 8S receptor (87). This is not the case; instead, each receptor species forms an independent 8S heteromeric complex on sucrose gradients (88–90) by binding to as many as five non-hormone-binding proteins including two molecules of the 90 kDa heat shock protein (hsp) and one molecule of hsp 70 (91–94). In this oligomeric state, the receptor proteins are basally phosphorylated and unable to bind DNA, but they are maintained in a stable conformation...
that allows them to bind to progestins. Hormone binding to the 8S receptor form activates a rapid series of changes, the exact sequential order of which is still unknown. Within minutes, if not seconds after hormone binding, the receptors become hyperphosphorylated (95-97), and several of the associated proteins, but not hsp 70, dissociate, leading to generation of a faster sedimenting 4S receptor form (93, 94, 98). Two 4S receptor molecules dimerize (98) and then bind to DNA at specific transcription enhancer sites called progesterone response elements (PREs) (Refs. 64-67 and references therein). Since both homo- and heterodimers can form between the A and B isoforms, three possible classes of receptor dimers (A:A, A:B, B:B) can bind to a PRE, each having a potentially different transcription regulatory capacity (71, 98). This diversity in the repertoire of responses to a hormone sets progesterone apart from the regulatory properties to an antagonist, providing molecular explanations for a PR-positive but hormone-resistant state.

The biological significance of two or more PR isoforms extends beyond breast cancer; there is evidence that, in the chicken, the two receptor isoforms are differentially expressed developmentally and differentially regulated by hormone treatment (100, 101). The two homodimers and the heterodimer may therefore differentially regulate transcription depending on the gene, the target cell, the hormonal milieu, or the developmental program, leading to a complex combinatorial response that is unique to the progestins.

B. Molecular mechanisms of progesterone antagonists

Synthetic hormone antagonists are exceptionally useful as both clinical and experimental tools. Their efficacy and low toxicity have made antiestrogens the drugs of choice for the treatment of advanced hormone-dependent breast cancer. Experimentally, these compounds have been crucial for unraveling the mechanisms of ER action. Antiprogestins have even greater potential, not only as experimental tools and as anticancer agents, but as midcycle contraceptives and implantation inhibitors and as abortifacients. By the end of the 1970s, more than a decade of computer-assisted design and testing of molecules likely to have antiprogestational activity had not led to any clinically or experimentally useful products. In 1981, however, Philbert et al. (102) announced the synthesis of RU38486 [17β-hydroxy-11β-(4-dimethylamino phenyl)-17α-(1-propynyl)estra-4,9-diene-3-one]. RU38486 was one of a large number of 11β-substituted steroid derivatives that were synthesized by scientists at Roussel-Uclaf exploiting their extensive experience in analyzing the structure/activity relationships of compounds that bind both glucocorticoid receptors (GR) and PR (103). The antiglucocorticoid activity of RU38486 was demonstrated first (102), and its antiprogestin and abortifacient properties in experimental animals were reported soon thereafter (104). Testing in humans followed rapidly, and it was shown that oral administration of RU38486 to women in preliminary clinical trials induced interruption of the luteal phase of the menstrual cycle and termination of early pregnancy (105). The mechanisms of action of RU38486 (renamed RU486 or mifepristone) have since been the subject of intense...
investigation (106). Using RU486 as a starting-point, scientists at Schering (107) reported the synthesis of a second generation of antagonists with potent antiprogestational activity but weaker antiglucocorticoid activity than RU486 (108). These compounds hold much promise for long-term clinical use, as preliminary studies suggest.

In theory, an antagonist can act by blocking agonist activity at any of the multiple stages between its entry into the target cell and first interaction with the receptors, through its role in terminating the transcriptional signal. The effects of antiprogestins at each of these stages are reviewed below.

1. Affinity for inactive PR. RU486 binds to the PR of rodents and humans with a dissociation constant (Kd) of approximately $2 \times 10^{-9}$ M, roughly equivalent to the affinity of the synthetic progestin R5020 for PR (109, 110), but tighter than the affinity of the natural hormone, progesterone. Interestingly, unlike progesterone, RU486 does not bind to chicken or hamster PR (106), which suggests that there are subtle differences in the contact sites of agonists and antagonists at the hormone binding domain of the receptors. As in R5020-bound PR, unactivated RU486-bound PR complexes sediment at 8S on sucrose density gradients (111, 112). RU486 absorbs UV at approximately 300 nm, and this wavelength can be used to covalently cross-link the drug to PR in intact cells. This method was used to show that, like R5020, low concentrations of [3H]RU486 bind to both the A receptors and the B receptors of human PR (110). In intact breast cancer cells, R5020 and RU486 have identical affinity for PR, and low concentrations (6 to 8 nM) of RU486 rapidly deplete more than 95% of hormone-unoccupied PR and activate them to a state in which they are tightly bound to DNA and require high concentrations of salt for their extraction (110). Thus, it was clear soon after its development that RU486 promotes binding of PR to DNA in intact cells. Like other synthetic progestins, RU486 is not metabolized in intact breast cancer cells (113), allowing its activity to be prolonged. Consequently, it chronically suppresses receptor replenishment and lowers the levels of inactive 8S PR available for ligand binding (but see Section IV.B.6 below). In this fashion RU486 resembles long-acting synthetic agonists, so that this property is unlikely to be involved in its antagonist actions.

2. PR activation. Initial studies by Rauch et al. (114) suggested that while RU486-bound PR complexes were activation-deficient and had lower affinity for DNA than agonist-bound complexes, these differences were modest and could not fully explain its antagonist properties. These investigators suggested that the major defect was at the transcriptional step. The receptor activation step that follows hormone binding is unclearly defined; it appears to be related to rapid dissociation of the multiple accessory proteins, including hsp 90, from PR (92–94, 98), and to the equally rapid hormone-dependent phosphorylation of PR (95–97). The two reactions occur in a still undetermined order. The dissociation of accessory proteins is readily measured on sucrose density gradients as a decrease in the sedimentation coefficient. Mullick and Katzenellenbogen (115) first showed that while activated R5020-receptor complexes sediment at approximately 4S, the RU486-bound receptors sediment as a prominent 6S species as well as a 4S species; the heavier species is likely to be due to persistent receptor oligomerization with other proteins (116, 117). The suggestion that RU486 traps hetero-oligomeric 8S PR in an inactive state, thereby preventing their interaction with DNA (112), is not supported by in vivo data (71).

In addition to the loss of accessory proteins, PR activation may be regulated by phosphorylation (95–97). Human PR are phosphorylated at serine residues at the time of their synthesis, and in the absence of progestins this basal phosphorylation continues for several hours. However, progestin binding to the receptors rapidly promotes additional serine phosphorylation concomitant with receptor activation and is accompanied by structural changes that alter the mobility of human PR on electrophoretic gels (118, 119). The function of this hormone-dependent PR phosphorylation is still unknown, but it may play a role in the release of hsp proteins, in receptor dimerization, in DNA binding, and in regulating the functional state of PR while they are bound to the transcription complex. Since a subset of the hormone-dependent phosphorylation precedes DNA binding, and the remainder is DNA-dependent (K. B. Horwitz, unpublished), it is likely that phosphorylation of human PR subserves more than one function. Of interest is the fact that compared to agonists like R5020, binding of the antagonist RU486 results in hyperphosphorylation of the PR (118, 119), so that this stage of receptor activation is, if anything, enhanced by the antagonist. It remains to be determined whether appropriate or inappropriate serine residues are the targets of this intensive RU486-dependent phosphorylation.

3. DNA binding. Whether RU486-bound PR complexes have altered DNA binding capacity when compared to agonist-bound PR complexes remains a matter of contention, partially due to the lack of consensus for any hormone requirement! This issue has been addressed by in vivo studies, by in vitro DNA binding studies, and most recently by cell-free transcription studies, with often contradictory conclusions. The following example illustrates the methodological conundrum for glucocorticoids: In consecutive articles published in Nature, Becker et al. (120) concluded that, based on in vivo
genomic footprinting analysis, GR interactions with a glucocorticoid response element (GRE) require the presence of hormone, and Willmann and Beato (121) concluded that based on in vitro DNase I footprinting, hormone-free GR bind specifically to a GRE.

There is little question that progesterone is required to activate PR in intact cells, and that this is accompanied by increased affinity of receptors for chromatin/DNA as assessed by the concentration of salt required to extract the receptors from cells or nuclei [for a caveat see recent studies by O'Malley and collaborators (122)]. Hormone-free receptors can be extracted with little or no salt in buffers; after hormone treatment at least 0.3 M salt is required. When PR are bound by RU486 in vivo, the receptors also require high salt concentrations for their extraction, suggesting that the antagonist indeed promotes PR binding to DNA (110). That both B and A receptors can bind DNA when occupied by RU486 was first demonstrated by their high salt requirement for extraction after in situ photoaffinity labeling (110). These in vivo assays cannot, however, distinguish between PR that are bound to nonspecific DNA sequences and PR bound to specific PREs. Becker et al. (120) used in vivo protein DNA footprinting to show that GR binding to the GRE of the tyrosine amino transferase gene could be detected only after glucocorticoid treatment of hepatoma cells. Similar studies have not been done to analyze PR binding. Instead, in vitro kinetic methods have been used in an attempt to measure the affinity of receptors for DNA under varying conditions of ligand occupancy. Using crude receptors, Milgrom and his collaborators (114) showed that RU486-PR complexes bind to nonspecific DNA with a slightly lower affinity than do R5020-PR complexes, but that a ligand was required for any PR/DNA interactions. The affinity differences between R5020- and RU486-bound PR were insufficient to explain antagonist action, but it appeared that hormone occupancy can indeed modulate binding, even to nonspecific DNA. It was therefore surprising that in a later study (123), this same group found that PR, whether hormone-free or bound to an agonist or antagonist, interacted with a similar affinity and protected the same DNA regions on three fragments of the uteroglobin gene. A major difference between the two studies was that instead of the crude PR used in the earlier study, the later study used immunopurified PR, which may have lost a component necessary to demonstrate the hormone effect. It has been suggested that this repressor is hsp 90. Alternatively, it is possible that extensive purification irreversibly modifies the structure of receptors in a manner that mimics the structural changes associated with hormone binding. Thus, paradoxically, excessive purification may hinder in vitro study of the relationship between hormone occupancy and DNA binding, unless lost components are restored. This is clearly evident in more recent studies using in vitro transcription methods. O'Malley and his colleagues have developed such an assay using purified chick PR or crude human PR. The purified chick PR (124) lacks a hormonal requirement for transcription that the crude human PR (125) retains.

In addition to issues of receptor purification, accurate kinetic data obtained from in vitro DNA binding studies require that the reactants be at equilibrium conditions, and that many factors including pH, incubation times, salt concentrations, DNA and receptor concentrations, etc., be controlled. Differences among these parameters, plus differences among the hormones and DNAs tested, may account for the lack of consensus about hormone requirements, or about the kinetics of reactions. The greatest kinetic difference between agonist and antagonist regulation of PR binding to DNA was reported by Schauer et al. (126) who showed that in the presence of agonist and under equilibrium conditions, the on-rate of receptor binding to DNA was accelerated 2- to 5-fold, and the off-rate was accelerated 10- to 20-fold compared to ligand-free receptors. It is thus surprising that the actual DNA occupancy time of receptors was shortened by the agonist. The investigators postulated that this reduced occupancy time accelerated DNA scanning potential of PR, but it was apparent that these results were unexpected. In the same study, the kinetic values observed with RU486 occupancy were closer to those observed with ligand-free receptors. That is, both hormone free receptors and RU486 occupied receptors bind tighter to DNA than do agonist-bound receptors. This apparently tighter DNA binding of RU486-bound complexes compared to agonist-bound complexes (due to the slowed off-rate with RU486) had previously been observed by Bagchi et al. (127) using gel mobility shift assays. In contrast, ligand-free receptors fail to bind PREs when gel mobility shift methods are used (71, 127), results that differ from those observed using the kinetic equilibrium assays. Finally, while confirming that ligand is required for PR binding to DNA by gel mobility shift assay, Meyer et al. (71) found no differences with respect to specificity, affinity, or stability when comparing R5020 to RU486.

Newly developed cell-free assays reaffirm the hormone and DNA binding requirements for receptor-mediated transcription (124, 125, 128). Of interest is the report by Bagchi et al. (125) that nuclear PR, salt-extracted from T47D breast cancer cells grown in the absence of hormone, are in a 4S form and dissociated from hsp 90. While these criteria characterize "activated" receptors, these 4S PR complexes nevertheless require hormone for DNA binding and transcriptional activation, suggesting that hsp 90 dissociation is necessary but not sufficient to generate receptors capable of binding DNA. Other
nuclear factors or receptor modifications are likely to regulate DNA binding of the 4S complexes, with phosphorylation being a leading candidate.

Finally, it is important to recall the danger of deriving generalized conclusions about the effects of antagonists on PR/DNA binding when only one antagonist is tested. Recently, Klein-Hitpass et al. (129) compared RU486 and three newer Schering antiprogestins with regard to the DNA binding properties of PR by gel mobility shift assay. They found that while three of the antiprogestins promoted DNA binding, one, ZK98299, did not induce binding of PR to PREs, and in competition assays, blocked the DNA binding of RU486-bound PR complexes. The investigators postulate that there are at least two types of antiprogestins: type I that block PR binding to PREs; and type II that promote PR binding to PREs but impede transcription, echoing a general mechanism of antagonist action proposed by Guiochon-Mantel et al. (130) based on studies of antiglucocorticoids and anti-estrogens.

To summarize, in vitro analysis of PR binding to DNA for the purposes of assessing the function of agonists and antagonists remains complex, and the data obtained may depend on variables in assay conditions that are difficult to control. Critical among them is the structural status of the PR. Warming and salt exposure are among the factors known to artifactually activate receptors in vitro; the former is often included in binding reactions, and the latter is commonly used to extract or elute receptors. When artificial activation is minimized, it appears certain that hormone is required to generate the DNA binding form of PR. It is also clear that the most widely tested antagonist, RU486, also promotes PR binding to DNA, but that not all antiprogestins do so. However, because equally convincing studies have yielded contradictory conclusions, it is still not clear whether antagonist-bound receptors bind to DNA with kinetics that differ from those of agonist-bound receptors.

4. Dimerization. The palindromic structure of PREs, the dyad symmetry shown by receptor DNA contact sites, the failure of PR to bind to half-palindromic PREs in vitro, and the leucine zipper/helix-loop-helix motif found in PR all suggest that receptors bind to DNA as dimers (64-67, 71, 131-133). Since human PR exist naturally as two size classes, the A and B receptors, homo- and heterodimers formed between them can be distinguished by differences in their mobility on nondenaturating gels. This interaction between monomers is activated by ligand binding and takes place in solution independent of DNA binding (98, 133). Similar to the agonists, RU486 promotes dimerization as shown by its ability to induce nuclear transfer of a nuclear localization-deficient mutant, when the mutant dimerizes to wild-type RU486-occupied PR (133). As with R5020, RU486 and the Schering antiprogestins, ZK98434 and ZK112993, promote both homo- and heterodimerization between forms A and B, excluding the possibility that antiprogestins induce only one dimeric form of PR as a mechanism for their action (129). Complexes formed between the PRE and the PR dimers migrate faster on nondenaturing gels when the receptors are occupied by RU486 (71, 117); these differences are likely to be due to altered conformation of the receptors at the hormone binding domain generated by the antagonist, or to differences in DNA bending produced by occupancy of the receptors. How this structural change is translated into an antagonist effect is still unknown. This finding, however, raises an interesting question: can dimerization occur if one PR monomer is bound by agonist and another by antagonist? Since the receptor dimerization site is probably located in the hormone-binding domain, conformational differences at the dimerization domain interface of the two monomers may be incompatible. Indeed, Meyer et al. (71) found that R5020 and RU486-occupied receptors are unable to bind to PREs as heterodimers, thereby introducing a novel mechanism for antagonist action. This is confirmed by in vitro kinetic studies showing that the slope of the competition curve for RU486 is different than the slopes for progesterone and R5020 at receptor concentrations that favor dimerization. This is consistent with a preference of the receptor to have RU486 occupy both sites of a dimer, rather than having progesterone and RU486 each occupy one of the two monomers (134). On the other hand, failure to promote formation of stable receptor dimers is the postulated mechanism for antagonism of the non-DNA binding antiprogestin ZK98299 (129).

5. Transcription. The ultimate effect of antiprogestins is to modify transcription of genes regulated by progesterone through PR. While it is usually assumed that antagonists inhibit the effects of agonists, this is not always the case since antagonists often have agonist actions as well. The paradoxical effects of steroid hormone antagonists are well-known (see below), but the mechanisms that underlie them are poorly understood. That antagonists regulate the transcription of specific genes, rather than having global, nonspecific effects, is certain (114), and it is also likely that whether agonist or antagonist effects predominate depends on complex interacting factors that include the antagonist concentration, the specific target gene, other transcriptional regulators of that gene, and the cellular environment in which that gene is active. Studies to unravel this complexity are still in the preliminary stages. While it is clear that progestins and antiprogestins regulate the expression of a variety of proteins in intact breast cells, transcriptional regulation
of PRE-containing genes by PR has not been analyzed in situ. Instead, two structurally different model systems have been used: the palindromic PRE of the tyrosine amino transferase (TAT) promoter (137) and the complex promoter of the MMTV long terminal repeat which has, in addition to one distal palindromic PRE, a cluster of three tandemly arrayed (138-141) proximal PRE half-sites.

The first model, the PRE of the TAT gene, consists of two symmetrical half-sites, each of which may be occupied by one molecule of the PR dimer (131, 135). This type of dimeric binding has been characterized as cooperative for GR, i.e. that (using a DNA-binding domain fragment) occupancy of one half-site by one monomer enhances binding to the second half-site by another monomer (131). This DNA binding cooperativity is difficult to reconcile with the fact that receptor monomers dimerize in solution. Assuming that only the preformed dimer binds DNA, one would have to postulate that when one monomer of the pair encounters the first PRE half-site, the other monomer is in close physical proximity and binds more efficiently to the second half-site. On the other hand, since dimers and monomers are likely to be in equilibrium in solution (141), it may be the monomeric form that accounts for the binding cooperativity seen in the in vitro assays. The receptor concentration used in these assays controls the state of the reactants at equilibrium with more dilute receptor concentrations favoring a monomeric state (134); these concentrations are rarely carefully controlled. When two PREs (PRE$_2$) are tandemly linked, occupancy of one PRE by receptor dimers increases the binding affinity of additional receptor dimers to the second PRE by as much as 100-fold depending on the distance between the PREs (136, 137). This probably involves protein-protein contacts between dimers, with looping of the intervening DNA (142). Cooperative binding of PR to PREs leads to synergistic induction of a coupled reporter gene (136, 137).

Transcriptional studies with the TAT PRE use either single (71, 135) or tandem copies (124). Assuming that R5020/RU486 PR heterodimers do not form (71), a single PRE should give clearly interpretable results when RU486 function is analyzed since both monomers would be occupied by the same hormone. But what happens in the tandem PRE$_2$ system when one of the dyads is occupied by a progestin-bound homodimer, and the other dyad is bound by an RU486-bound homodimer? The answer to this is unknown. Two studies using the TAT PRE$_2$ linked to the thymidine kinase and/or ovalbumin promoter, and using human PR in cell-free transcription assays, suggest that like the agonist R5020, the antagonist RU486 stimulates transcription (124, 125); that is, RU486 acts like a weak agonist having 25-30% of the activity of R5020. The effects of combining R5020 and RU486 were not tested in these studies, but Strähle et al. (135) showed that RU486 was able to inhibit an R5020 stimulatory effect using the same PRE-promoter constructs in transient transfection experiments. Unfortunately, in this study the agonist effects of RU486 alone were not tested. Clearly, even in this simple system, a considerable amount of work remains to be done in order to understand the different agonist and antagonist actions of RU486. Another observation that requires explanation is that the agonist actions of RU486 using the TAT PRE$_2$ can be blocked by the type I antagonist ZK98299 (129).

The second model used for transcription studies is the MMTV-long terminal repeat which may behave primarily as a PRE in mammary cells (143). By analogy with calculations of GR binding to the MMTV promoter (141), it is likely that one PR dimer interacts with the distal palindromic PRE. Additionally, four to six PR monomers bind the three proximal half-sites so that at full occupancy six to eight PR molecules are associated with the MMTV promoter. The distal and proximal PREs act synergistically to stimulate transcription (144, 145), and mutations in any of the four PREs reduce significantly the overall hormone inducibility of the MMTV promoter (144, 145). The complexity is obvious. How many RU486 bound PR molecules would it take to modify progesterone-stimulated transcription from this promoter? What is the functional difference between PR monomer and dimer binding sites?

Several studies using both stable and transient transfection models show that, when acting at the MMTV promoter, RU486 is a pure antagonist with no apparent agonist activity. In kinetic analyses Guiochon-Mantel et al. (130) calculate that when 6% of PR molecules are occupied by RU486, they can inhibit 50% of stimulatory PR activity (in this case the constitutive activity from a mutant PR). They propose that since all four PREs of the MMTV promoter are necessary for optimal transcriptional activity, partial occupancy of one PRE by a PR-RU486 complex is inhibitory, even if the other sites are occupied by stimulatory PR complexes. These conclusions are consistent with other studies using MMTV (117, 148), which show that lower concentrations of RU486 than R5020 are needed to shut down the agonist effects of R5020, despite the fact that both hormones bind to human PR with similar affinities.

Finally, by comparing both the TAT PRE$_2$ and the MMTV model systems in one study, Meyer et al. (71) lend support to the findings that RU486 can be a partial agonist or a pure antagonist, depending on the context of the promoter and the simplicity or complexity of the PREs. They confirmed the agonist activity of RU486-occupied human PR when they are bound to PRE$_2$ in the context of a thymidine kinase promoter and their
lack of agonist activity when bound to the MMTV promoter. In addition, these investigators tested the functional capacity of the B and A isoforms of human PR separately with totally unexpected findings: only the B form of the receptors exhibited the agonist properties when bound by RU486. Since the A and B isoforms also had quite different inductive capacities when bound to R5020 (the A receptors had only 10% of the agonist activity of the B receptors on the MMTV promoter but 60% of B on the TAT PREG), the combinatorial possibilities for modulating transcriptional responsiveness (by type of ligand, by receptor isoform, by promoter, or by cell type) are staggering. Tora et al. (99) showed that the cell type into which receptors are transfected and tested strongly influences transcriptional capacity. Note also that the transcriptional actions of an AB heterodimer, likely to be the most abundant naturally occurring human PR form, remain unknown.

6. PR "processing" or down-regulation. A persistent transcriptional signal by hormone-receptor complexes cannot be tolerated by the cell, so mechanisms must exist to terminate it. Little is known about this final stage in receptor action. Receptor recycling and covalent modifications are among the mechanisms that have been proposed, but down-regulation may be the most common feature (146-153) associated with receptor inactivation. After agonist treatment, both DNA-bound ER and PR are "processed" or down-regulated, a stage that begins approximately 1 h after DNA binding and is complete 6-12 h later. Since this loss of DNA-bound receptors is not immediately accompanied by replenishment of unactivated receptors, total cellular receptor levels fall by 80-90%. The down-regulated receptors are restored only many hours (>24) later, presumably after synthesis of new receptors. The receptor replenishment time differs depending on the persistence of agonists within the cell (147-153).

While down-regulation has been documented for ER and PR bound to agonists, the scenario is entirely different for receptors bound to antagonists. Antagonist-bound receptor/DNA complexes down-regulate only partially or not at all; instead nuclear receptor levels remain elevated for hours, even days, after hormone treatment. In the specific case of RU486 in breast cancer cells, PR remain complexed to DNA for at least 48 h (72, 97, 118). The sustained DNA occupancy of RU486-bound PR may, at least in part, explain its antagonist actions. Is it possible that agonist effects of RU486 result from the initial contact between RU486-bound PR and DNA, and that antagonist effects follow from the subsequent prolonged DNA occupancy? Recall also that RT1486-PR complexes bound to DNA are hyperphosphorylated compared to their R5020 counterparts (97, 118). Nothing is known about the role of PR phosphorylation in initiating or sustaining the transcriptional signal, or in regulating receptor down-regulation. It is possible that PR structural changes associated with hyperphosphorylation prevent the down-regulatory machinery from being activated or from functioning.

In the case of ER, it is known that this structural block to down-regulation imparted by antagonist occupancy of the receptors is rapidly reversible. In intact breast cancer cells, ER occupied by the antiprogestin nafinoxidine bind persistently to DNA and are not down-regulated for at least 24 h. If, at 24 h, cells are switched to medium containing estradiol, the excess agonist exchanges with the antagonist on the DNA-bound receptors, and down-regulation is initiated and proceeds with a normal time course. The estradiol-substituted ER, which were transcriptionally silent when bound to antagonist, are rapidly reactivated; in fact, a superinduction of the transcriptional signal is seen (154). This is an interesting observation for which no molecular explanations are presently forthcoming. These studies also address persistent questions regarding the length of time that a ligand must occupy receptors after they bind to DNA. Is the function of the ligand then terminated and is it dispensible? The antagonist to agonist switching study suggests either that ligand occupancy persists or that conformational changes are permanently imprinted on the receptor by ligand binding and are preserved when the ligand dissociates. The imprints imparted by agonists and antagonists differ but are exchangeable.

V. Progesterone Antagonists and the Treatment of Breast Cancer

Because progesterin antagonists are relatively new compounds, and because of the political controversy that surrounds them, their promise and use in the treatment of breast cancer are just beginning to be evaluated. What is the rationale for their use? What is the explanation for the paradox that both antiprogestins and high-dose progesterin agonists inhibit growth of breast cancers?

A. Human breast cancer cell lines

Two PR-positive human breast cancer cell lines that are phenotypically different have served as the major models for studies of growth regulation by antiprogestins. The MCF-7 cell line is classically estrogen-responsive cells; the cells are ER-positive but have only low PR levels unless these are induced by estradiol (153). The T47D cell line (155) is more complex; it is genetically unstable (41, 42) and differs phenotypically among (34, 35, 37) and within laboratories, which leads to reported differences in response to hormone treatment. One major T47D subline, clone 11, is ER-positive and PR-positive,
and the cells respond to estradiol treatment by proliferating, a response that is tamoxifen-inhibitable (35). Another subline, T47Dco, is estrogen-resistant, and cell growth is neither accelerated by estradiol nor inhibited by tamoxifen (147). The response to antiprogestins is generally similar among these cell lines, but interpretation of the results differs. In general, RU486 inhibits the growth of both T47D cells and MCF-7 cells (110, 156–161). The antiproliferative effects are evident at low doses, and their magnitude correlates loosely with PR levels; T47D > estrogen primed MCF-7 > unprimed MCF-7 (156). That the antiproliferative effects are mediated by PR is also shown by the fact that in T47D cells growth inhibition is confined to progestins; other steroid hormones are ineffective (156, 158). Moreover, the fact that RU486 is not antiproliferative in PR-negative breast cancer cell lines also argues for a receptor-mediated mechanism of action (156).

The antiproliferative actions of RU486 in these models of breast cancer would support a logical treatment strategy if it were not for one disturbing fact: progestin agonists, including R5020, also inhibit their growth. This effect of R5020 is seen even at low doses in the T47Dco cells (110) but requires higher doses in the clone 11 cells, in which, interestingly, low doses actually protect the cells from the antiproliferative effects of RU486 (156). Furthermore, in the estrogen-responsive clone 11 cells, R5020 inhibits only the estradiol-stimulated growth fraction where it is cytostatic, while RU486 reduces cell numbers below the estrogen-untreated baselines, suggesting that the latter has a more profound cytotoxic effect (160, 161). Thus the antiproliferative mechanisms for progestin agonists like R5020 and antagonists like RU486 may be fundamentally different. R5020 appears to have dual proliferative/antiproliferative effects depending on the dose that is tested, and the antiproliferative doses produce growth stasis. By contrast, RU486 is more purely antiproliferative even at low doses, and it can produce growth regression. Indeed, ultrastructural studies show effects of RU486 on cell and chromatin condensation and pyknosis consistent with the induction of cell death by apoptosis (161). This cytotoxic effect is prevented by low doses of R5020. To explain these results, Bardon et al. (161) propose that there are three different mechanisms by which progestins inhibit growth of breast tumor cells: 1) "PR-mediated cytotoxic" mechanisms that apply to antagonists like RU486. These are observed only in PR-positive cells using "physiological" hormone doses and are preventable by receptor occupancy with an agonist. This cytotoxicity is characterized by ultrastructural evidence of cell death. 2) "PR-mediated cytostatic" effects are produced by physiological doses of antagonists or agonists and are characterized by inhibition of the growth-stimulatory actions of unrelated (nonprogestin) growth factors including estradiol. 3) "Nonspecific cytotoxic" effects are not receptor-mediated and are seen with most steroid hormones at high doses. The molecular explanations that underlie these three mechanisms are unknown, but they serve as an important departure for further research. For example, the prolonged DNA occupancy time of RU486-bound PR compared to R5020-bound PR may account for cytotoxic vs. cytostatic effects (97). As described above, variables like the gene or cell being tested (99) and regulation by either the A or B receptor (71) may dictate an agonist or antagonist response. Alternatively, it has been suggested that R5020 is inhibitory because it is "antiestrogenic" (35) while RU486 is inhibitory through a direct antiproliferative effect involving PR (156). While these explanations may begin to address the paradox that allows both R5020 and RU486 to be growth inhibitory in the appropriate physiological setting, it is clear that a considerable amount of research remains to be done. Preliminary analyses of cell cycle parameters show no definitive differences between agonists and antagonists. Both appear to inhibit growth by significantly decreasing the proportion of cells in the S-phase of the cell cycle; cells accumulate in G0/G1 possibly due to an increase in the G1 transit time (158, 162).

While the majority of studies using cell culture models ascribe growth-inhibitory properties to progestins and to antiprogestins through direct effects involving PR, contradictory results have also been reported. Given the fact that, at physiological levels, progesterone is believed to be mitogenic in the normal breast (see above), it is not entirely surprising that Hissom and Moore (33) consistently report proliferative effects of R5020 at all doses in T47D cells. It is surprising, however, that RU486 also stimulates growth in these cells (163). The explanation for this discrepancy is unknown. However, it is now clear that T47D cells are exceptionally unstable; during prolonged time in culture, subpopulations can develop that are phenotypically different from the parental stocks, and some of these subpopulations may respond to hormones in a manner that differs markedly from the expected response. For example, some sublines or subpopulations of T47D cells respond to high doses of tamoxifen by growth stimulation (48, 164). In the case of tamoxifen, these aberrant responses may be mediated by the presence of mutant or variant ER (164). By analogy, it is possible that the T47D cells of Moore and his colleagues have arisen from a subpopulation harboring a mutant PR. This scenario would be very interesting, but the PR of these cells have not been analyzed in detail.

Less interesting trivial explanations for discrepancies among laboratories studying growth regulation by progestins in cell culture were discussed above (see also Ref. 1).
B. Animal models of mammary cancer

The antiproliferative properties of progesterone antagonists are well documented in animal models of hormone-dependent mammary cancer. These include rats bearing DMBA-induced or nitrosomethylurea (NMU)-induced tumors, and mice bearing the transplantable MXT tumor line. Growth of these tumors is inhibited by ovariectomy and maintained by physiological doses of estrogens (165–170). While treatment of rats with progestins at the time of DMBA administration accelerates tumor formation (21-23), prophylactic treatment of rats with RU486 at the time of DMBA administration delays the initial appearance of tumors from an average of 39 days, to an average of 81 days (157). The reversal by progestosterone of the inhibition of tumor induction produced by tamoxifen can in turn be blocked by RU486 (30). These implicate a PR-mediated mechanism. Treatment of established tumors with RU486 for 3 weeks prevents their further enlargement, but tumor remission is not observed (165). In contrast to the stasis seen with RU486, ovariectomy leads to a decrease in tumor size (169), which in MXT tumors is accompanied by necrosis and cytolysis of the tumor cells (168). While ovariectomy-induced tumor regression is known to be accompanied by extensive loss of PR (29), loss of tumor PR was also seen with RU486 treatment (165). Since in the latter, the PR assay was not performed under exchange conditions or by immunological methods, the validity of this decrease requires reexamination in light of other studies that show persistently high levels of PR in RU486-treated human breast cancer cells (97). Since adrenal weights are unchanged by RU486, participation of the antiglucocorticoid effects in the antitumor activity is considered to be unlikely (169). This is supported by studies in human breast cancer cell lines, where the inhibitory effects of RU486 cannot be rescued by dexamethasone (156). Antigonadotrophic effects have also been excluded as a mechanism (169).

In established DMBA tumors inhibition of growth by tamoxifen resembles the inhibition seen with RU486. The two hormones have equal growth-inhibitory effects when each is used alone (165). When the two drugs are combined, however, the inhibitory effects are additive, and tumor remission similar to that induced by ovariectomy is observed (166). This effect of combined treatment with an antiprogestin and antiestrogen is extremely exciting and has considerable therapeutic promise. The mechanisms underlying these effects remain unclear, but several proposals have surfaced. First, tamoxifen can have agonist actions among which is the induction of PR (153). A tumor with increased, or restored, PR may have greater or more sustained sensitivity to RU486. This hypothesis could be tested by the use of an antiestrogen having no agonist activity. Second, among the physiological effects seen in RU486-treated intact female rats are increased plasma levels of LH, PRL, estradiol, and progesterone, as well as the persistence of numerous and actively secretory corpora lutea associated with hypertrophic pituitaries (165, 166, 168, 169). It has therefore been proposed that the efficacy of simultaneous tamoxifen results from its ability to counteract the proliferative effects of the high estrogen levels.

Several newer antiprogestins, ORG31710 and ORG31806 (166), and ZK98299 and ZK112993, have equal or greater antiproliferative actions than RU486 (166, 168). In the hormone-dependent MXT-transplantable tumor model, treatment with ZK98299 or RU486 starting 1 day after transplantation led to an almost complete inhibition of tumor growth. Their effect on established tumors was equivalent to that of ovariectomy (168, 169). In this model, the potent antiproliferative actions of the antiprogestins completely counteracted the growth-stimulatory actions of estradiol, or of approximately equimolar doses of MPA, but at higher MPA doses, the agonist actions of the progestin prevailed (170). It appears that antiprogestins inhibit growth by direct antagonism of progesterone action at the tumor, probably mediated by PR. This conclusion is bolstered by the fact that the hormone-independent MXT tumor is antiprogestin-resistant (168).

In DMBA-induced tumors, ZK98299 was more potent than an equal concentration of RU486 (168). It produced tumor regression analogous to that of ovariectomy, rather than the tumor stasis observed with RU486. A similar trend was observed with NMU-induced rat mammary tumors (168–170). However, lack of comparative metabolic and pharmacokinetic data on the two antiprogestins in rats and mice makes these quantitative differences uninterpretable at present. Also of interest is the finding that strong antitumor activity was noted at 20% of the doses needed to obtain abortifacient actions in these rodent systems. This is important because by use of lower doses of antiprogestins, their antiglucocorticoid effects may be mitigated. After treatment with the antiprogestins, the morphology of the hormone-dependent MXT and DMBA tumors showed signs of differentiation of the mitotically active polygonal epithelial tumor cells toward the nonproliferating glandular secretory pattern with formation of acini and evidence of secretory activity (168). Based on this it is suggested that the antiproliferative efficacy of the antiprogestins is related to their ability to induce terminal differentiation. Recall that they block tumor cells in G0/G1 (156, 162). Note that an antiproliferative mechanism based on induction of terminal differentiation is fundamentally different from a mechanism involving tumor cell death. Tumor cell degeneration and cytolysis were features of ovariectomy-
induced regression (168). The mechanisms underlying the antitumor effects of antiprogestational agents require further study, especially in human tissues and cells.

C. Human clinical trials

The enormous promise of progestin antagonists in the treatment of breast cancer remains largely unexplored in clinical practice. Only two small clinical trials using RU486 have been reported, both from European laboratories. The first involved a series from France (171) of 22 oophorectomized or postmenopausal patients in whom chemotherapy, radiotherapy, or tamoxifen and other hormonal therapy had already been used. RU486 at 200 mg/day led to partial regression or stabilization of lesions in 12 of 22 (53%) women after 4 to 6 weeks of treatment. The response rate at 3 months had dropped to 18%. It is important to note that for ethical reasons, this untried therapy was used only in patients with advanced breast cancers in whom other treatment modalities had already failed. PR levels were not measured in all patients, but of the responders, four of four were PR-positive, while of the nonresponders zero of four were PR-positive. In general, RU486 was well-tolerated in long-term treatment with few symptoms of adrenal dysfunction, but plasma cortisol levels were elevated. Of interest was the fact that a strong analgesic effect was observed in most of the patients with bone metastases.

The second trial, from the Netherlands (166, 170), involved 11 postmenopausal patients with metastatic breast cancer who were treated with 200–400 mg RU486 for 3–34 weeks as second-line therapy after first-line treatment with tamoxifen, irrespective of the response to tamoxifen. Six of 11 patients had a short-term (3 to 8 months) stabilization of disease, and one had an objective response lasting 5 months after RU486 treatment. Again, response was associated with presence of PR in the tumors. In this study, which involved prolonged use of RU486, two patients had undesirable side-effects associated with the antiluglucocorticoid actions of the drug. Three days of treatment with dexamethasone reversed these symptoms after RU486 was stopped. As in the animal studies, plasma estradiol levels increased despite the fact that these women were postmenopausal, and it is suggested that the simultaneous administration of tamoxifen might be beneficial because of its ability to block tumor ER. Alternatively, symptoms of adrenal hypersecretion and elevated plasma estradiol levels might be reduced with concurrent aminoallthulimide or aromatase inhibitors. By modification of the dose and time of RU486 administration, its antiluglucocorticoid effects might be further minimized (172), although maintenance of high sustained blood levels of the drug is likely to be important.

VI. Progestin Resistance

The emergence of hormone-resistant cells eventually reduces the effectiveness of all therapies in advanced breast cancer, and progestin agonists or antagonists are unlikely to be exceptions. This is essentially an unexplored field. Unlike the case for other members of the steroid receptor family, no examples of natural PR mutants have yet been reported. It is possible that unlike mutations in androgen receptors, systemic mutations in PR are incompatible with life. It is likely, however, that acquired mutations can develop in tumors as one mechanism for the development of resistance, and that a systematic search would demonstrate them. In a similar manner, resistance to tamoxifen appears to be due in part to the development of mutant ER (164). To address other possible mechanisms of progestin resistance Murphy et al. (173) generated a subline of T47D cells that are resistant to the growth-inhibitory effects of progestins. This was done by sequential selection in medium containing 1 μM MPA. The cells remained PR-positive, but receptor levels were halved. Transforming growth factor-α and EGF receptor mRNA levels were both increased. The investigators suggest that increased growth factor expression and action and decreased PR levels may be involved in the development of progestin resistance. Finally, it is likely that extensive heterogeneity in PR content exists within cell subpopulations of tumors that are PR-positive based on analyses of solid tumors (174) and of human breast cancer cell lines (48). Factors or treatments that lead to the selection and expansion of PR-poor or PR-negative populations would, in the long run, produce progestin resistance.

VII. Summary and Future Prospects

The foregoing suggests that progesterone antagonists could have an important place in the routine management of hormone-dependent breast cancers. Our knowledge of the actions of these compounds is rudimentary, however. The following points provide an outline for future directions:

1. If endogenous progesterone has the mitogenic actions in normal breast epithelia that the current data would indicate, then it is likely that physiological progesterone is also a mitogen in breast cancers. Blockade of endogenous progesterone with antiprogestins, especially in premenopausal women, would seem to be an important therapeutic goal. However, nothing is known about the pattern of mitosis in breast cancer cells during the normal menstrual cycle. Obtaining such data is important, and it should be possible to analyze the mitotic patterns of tumors taken from cycling patients. The problem, of course, is the difference in mitotic indices among tumors. Ideally, each tumor should serve as its
own control, with multiple samples analyzed for proliferative activity at different times of the cycle. This approach is fraught with ethical problems. However, it might be possible to obtain a fine needle aspirate of a tumor for initial mitotic analysis, then to acutely treat the patient with RU486 before the tumor is removed 24 h later for reanalysis. Additionally, it is important to know whether RU486 is cytostatic or cytolytic in human breast cancers. Electron microscopy of tumors taken from patients entered into trials may provide answers to this. However, while it is always preferable to ask biological questions using clinical tissues, much of the work on the mechanisms of the mitogenic actions of progestins and progestin antagonists will require well-controlled studies using organ-cultured human breast tumors, human breast cancer cell lines, and human tumors implanted into nude mice.

2. There are sufficient theoretical and preclinical data to justify large-scale clinical trials. Trials must include accurate measurements of ER and PR in tumors, and analysis of pre- and posttreatment levels of endogenous hormones to monitor the status of the pituitary-adrenal-ovarian axis. Progestin antagonists may be useful both for adjuvant endocrine therapy when used either alone or in combination with tamoxifen, and for therapy of locally advanced or metastatic cancers again used either alone or in combination with tamoxifen. The usual issues of drug doses and of metabolism and other pharmacokinetic parameters must be addressed. Schedules in which the antiprogestins and antiestrogens are combined or alternated must be tested. Is an antiestrogen with agonist properties preferable because it induces PR or is a pure antiestrogen preferable because it blocks the actions of elevated circulating estrogens? Is the answer different in premenopausal vs. postmenopausal women?

3. The antiglucocorticoid side effects of RU486 remain a major impediment to its long-term use. However, as the newer Schering antiprogestins show, it appears to be possible to design molecules with maximal antiprogestin activity and minimal antiglucocorticoid activity. Further, even RU486 may be used for long-term treatment if it is combined with drugs that block adrenal steroidogenesis or prevent peripheral aromatization of adrenal steroids to estrogens. Additionally, since the antiglucocorticoid effects of RU486 are apparently tolerable in the short-term (i.e. 2 to 3 months), perhaps it is reasonable to ask whether alternating RU486 with tamoxifen maximizes its antiprogestin effects while it minimizes its antiglucocorticoid ones.

4. Basic tumor biological and molecular research must continue in order for us to understand the precise molecular targets and mechanisms of antagonist action. If different antagonists target different molecular sites, which antagonist would be best for clinical use? What are the genes in breast cells that are regulated by progestins, whose inhibition is associated with lowered cell proliferation? What is the underlying cause of the tissuespecific differences in progestin action at the breast and uterus? What is the molecular explanation for the dual agonist/antagonist effects seen on only some promoters, with only one or the other PR isoform, and in only some cells when using RU486? Can pure progesterone antagonists devoid of antiglucocorticoid activity be synthesized? Are there mutant PR in breast cancers?

Where do we begin? Ensuring that scientists and clinicians have access to antiprogestins, unencumbered by the Byzantine bureaucratic obstacles and the “antagonistic” political climate currently encountered in the United States, is a good place to start.

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