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Progesterone receptor ligands for the treatment of endometriosis: the mechanisms behind therapeutic success and failure

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TABLE OF CONTENTS

BACKGROUND: Despite intense research, it remains intriguing why hormonal therapies in general and progestins in particular sometimes fail in endometriosis.

OBJECTIVE AND RATIONALE: We review here the action mechanisms of progesterone receptor ligands in endometriosis, identify critical differences between the effects of progestins on normal endometrium and endometriosis and envisage pathways to escape drug resistance and improve the therapeutic response of endometriotic lesions to such treatments.

SEARCH METHODS: We performed a systematic Pubmed search covering articles published since 1958 about the use of progestins, estro-progestins and selective progesterone receptor modulators, to treat endometriosis and its related symptoms. Two reviewers screened the titles and abstracts to select articles for full-text assessment.

OUTCOMES: Progesterone receptor signalling leads to down-regulation of estrogen receptors and restrains local estradiol production through interference with aromatase and 17 beta-hydroxysteroid dehydrogenase type 1. Progestins inhibit cell proliferation, inflammation, neovascularisation and neurogenesis in endometriosis. However, progesterone receptor expression is reduced and disrupted in endometriotic lesions, with predominance of the less active isoform (PRA) over the full-length, active isoform (PRB), due to epigenetic abnormalities affecting the *PGR* gene transcription. Oxidative stress is another mechanism involved in progesterone resistance in endometriosis. Among the molecular targets of progesterone in the normal endometrium that resist progestin action in endometriotic cells are the nuclear transcription factor FOXO1, matrix metalloproteinases, the transmembrane gap junction protein connexin 43 and paracrine regulators of estradiol metabolism. Compared to other phenotypes, deep endometriosis appears to be more resistant to size regression upon medical treatments. Individual genetic characteristics can affect the bioavailability and pharmacodynamics of hormonal drugs used to treat endometriosis and, hence, explain part of the variability in the therapeutic response.

WIDER IMPLICATIONS: Medical treatment of endometriosis needs urgent innovation, which should start by deeper understanding of the disease core features and diverse phenotypes and idiosyncrasies, while moving from pure hormonal treatments to drug combinations or novel molecules capable of restoring the various homeostatic mechanisms disrupted by endometriotic lesions.

Key words: progestins / endometriosis / endometrium / therapeutic failure / innovation / hormonal treatments / selective progesterone receptor modulators / new drugs

Introduction

Endometriosis-like symptoms have been alluded to in ancient medical records dating from about 4000 years ago (Nezhat *et al.*, 2012). Chinese medicinal herbs have been prescribed to alleviate disabling pelvic pain and severe systemic symptoms related to the menstrual period since ancient times, when the pharmacological rationale for the medical treatment of dysmenorrhea was still unknown (Nezhat *et al.*, 2012; Wieser *et al.*, 2007). The first pathological description of endometriotic and adenomyotic lesions was made by Karl von Rokitansky in 1860 (Hudelist *et al.*, 2009; Rokitansky, 1860), but only in the beginning of the 20th century, the morphological and clinical pictures of such diseases were better defined (Cullen, 1908; Cullen, 1920). In the twenties of the last century, John Sampson coined the name 'endometriosis' and proposed the 'retrograde menstruation theory' to explain its pathogenesis (Sampson, 1927). At that time, medical therapies for this condition were virtually non-existent and the severe pelvic symptoms were treated, even in young patients, by aggressive surgical procedures such as hysterectomy and oophorectomy (Brosens and Benagiano, 2011).

Interestingly, some of the first recommendations for endometriosis prophylaxis were early marriage and frequent childbearing (Meigs, 1953). In fact, the attempt at using hormonal therapies for symptomatic management of endometriosis, already performed occasionally, gained an impulse when the idea of inducing a state of 'pseudo pregnancy' came to the light (Andrews *et al.*, 1959; Kistner, 1958). By observing the positive effect of pregnancy on endometriosis evolution, Kistner postulated that the process of decidualisation might cause necrosis and the consequent elimination of superficial ectopic implants (Kistner, 1958). Thereby, the logical reasoning for using progesterone for the clinical control of endometriosis was established.

Progestins encompass the oldest and the newest medical treatments of endometriosis. By binding to progesterone receptors (PRs), these synthetic compounds can induce anti-estrogenic, pro-apoptotic, anti-inflammatory and anti-neurogenic effects, resulting in pain relief and interruption of pathogenic mechanisms within the endometriotic lesions (Gezer and Oral, 2015). The possible routes of progestin administration include oral and vaginal preparations, intramuscular and subcutaneous injections, patches, subdermal implants and intrauterine systems. Derivates of C-21 progesterone (medroxyprogesterone acetate (MPA) and dydrogesterone) and C-19 nortestosterone (levonorgestrel, norethisterone, lynestrenol, desogestrel and dienogest) are representants of the class (Gezer and Oral, 2015). Combined estrogen–progestins were introduced as contraceptives in the 1960s. They have been widely used in patients with endometriosis since then, with good rates of patient adherence and satisfaction (Andrews, 1976; Vercellini *et al.*, 2018b).

Preliminary studies published in 1971 indicated the use of danazol as a therapeutic possibility for endometriosis, mainly due to its alleged anti-gonadotropic properties. At that time, no direct action mechanism of danazol had been demonstrated in endometriotic implants, but some progestational-like changes induced by this substance were observed in the endometrium of estrogen-primed animals (Greenblatt *et al.*, 1971). Later, despite being effective against endometriosis symptoms, its inconvenient androgenic side effects curbed the clinical applicability of danazol (Brosens and Benagiano, 2011). Subsequent initiatives of danazol administration by vaginal or intrauterine routes resulted in much improved tolerance while keeping the therapeutic effects (Cobellis *et al.*, 2004; Igarashi *et al.*, 1998; Razzi *et al.*, 2007a), but the drug never regained its former popularity.

Progestins, either alone or conjugated with estrogens, continue to be successfully indicated to treat endometriosis (Vercellini *et al.*,

2016b). However, some patients have only partial improvement or do not respond to this therapy at all (Barra et al., 2019). In addition, progestins and combined hormonal contraceptives do not eliminate endometriotic lesions, but induce their quiescence at best (Liang et al., 2018). Estrogen-suppressive therapies, such as gonadotropin-releasing hormone (GnRH) agonists and antagonists or aromatase inhibitors, are also far from being a panacea and are still expensive and have bothersome side effects (Brown et al., 2010). Despite intense research, it remains intriguing why hormonal therapies sometimes fail in endometriosis. Thus, the aim of this review is to evaluate the action mechanisms of PR ligands in endometriosis, to identify critical differences between the effects of progestins on normal endometrium and endometriosis and to envisage pathways to overcome the therapeutic failures.

Methods

In this narrative review, we performed a historical bibliographic search for references to the medical treatment of endometriosis and a systematic Pubmed search covering articles in any language published since 1958 with the following terms: ('Endometriosis'[Mesh] OR 'Endometrium'[Mesh]) AND ('Progestins'[Mesh] OR 'Medroxyprogesterone Acetate'[Mesh] OR 'Norethindrone'[Mesh] OR 'Desogestrel'[Mesh] OR 'dienogest' [Supplementary Concept] OR 'Dydrogesterone'[Mesh] OR 'Levonorgestrel'[Mesh] OR 'Mifepristone'[Mesh] OR 'ulipristal acetate' [Supplementary Concept] OR vilaprisan [Supplementary Concept]). Two reviewers screened the titles and abstracts to select articles for full-text assessment. We also searched clinicaltrials.gov and EudraCT for study protocols on hormonal treatments of endometriosis with the status of 'not yet recruiting', 'recruiting', 'active, not recruiting' or recently (until December 2017) 'completed' or 'terminated'.

Progesterone Receptors in Human Endometrium and Endometriosis

The progestational effects of natural progesterone and synthetic progestins are mediated by three categories of specific receptors: the classical nuclear PRs that include subtypes A and B (Hill et al., 2012), a mitochondrial isoform (PR-M) also derived from the same PGR gene (Price and Dai, 2015), and the cell membrane receptors that comprise progesterone receptor membrane components (PGRMC) and membrane receptors (mRPs) belonging to the progestin and adipoQ (PAQR) protein family (Gerdes et al., 1998; Kowalik et al., 2013). In addition, progesterone and progestins may have cross effects on glucocorticoid, mineralocorticoid and androgen receptors (Stanczyk et al., 2013).

Human endometrium expresses both PR isoforms during all phases of the menstrual cycle, but the total amount of receptors and the ratio between PRA and PRB change in response to variations in circulating ovarian steroids, with an overall predominance of PRA over PRB (Bedaiwy et al., 2015; Mote et al., 1999). The receptors are more abundant in the glandular epithelium than in the endometrial stroma and the maximal expression of PRA and PRB is seen at mid-cycle, followed by a gradual decrease that reaches a nadir at the late secretory phase (Fig. 1). In the secretory phase, stromal PR localises specifically in cells with morphological features of decidualisation (Mote et al., 1999), a critical mechanism of endometrial differentiation that ultimately concurs to allow embryo implantation (Gellersen and Brosens, 2014; Wu et al., 2018). Furthermore, stromal PR activation elicits paracrine mechanisms that limit epithelial proliferation (Kim et al., 2013; Wu et al., 2018). Splice variants of the PGR transcript have been found in human endometrium (Yamanaka et al., 2002) and a putative truncated isoform (PRC) was detected in term gestation decidua (Goldman and Shalev, 2007), but their functional significance remains elusive.

PGRMC-1 transcript (Kao et al., 2002) and protein (Chen et al., 2009) are abundant in human proliferative endometrium with down-regulation in the mid-secretory phase. Conversely, a detailed study in non-human primate showed that PGRMC-2 expression increases in the mid-secretory phase, especially in the glandular epithelium of the functionalis zone (Keator et al., 2012). Once activated by the extracellular ligand, PGRMC-1 recruits the intracellular serpine mRNA-binding protein 1 and the complex ligand-receptor-binding protein activates protein kinase G to reduce Ca^{2+} levels in the cytosol (Kowalik et al., 2013). PGRMC-1 mediates progesterone effects on cholesterol metabolism, steroidogenesis and apoptosis in ovarian cells in vitro, and these effects have been hypothesised to contribute to progesterone actions on endometrial glands (Keator et al., 2012; Kowalik et al., 2013).

When it comes to endometriosis (Table I), studies that have evaluated superficial peritoneal lesions or ovarian endometriomas indicate that PR expression looses the menstrual cyclic pattern (Beliard et al., 2004), PRB levels are low (Bedaiwy et al., 2015; Beliard et al., 2004; Eaton et al., 2013; Hayashi et al., 2012; Wu et al., 2006), sometimes even undetectable (Attia et al., 2000), and PRA is by far the predominant PR isoform expressed within lesions (Attia et al., 2000; Bedaiwy et al., 2015; Bono et al., 2014). The PRB deficiency is much more evident in the lesions than in the eutopic endometrium of women with endometriosis (Attia et al., 2000; Bedaiwy et al., 2015; Wu et al., 2006). Deep rectal lesions express PR isoforms both at glands and stroma (Vinci et al., 2016; Zanatta et al., 2015), but, when compared to normal endometrium, PRB immunoreactivity is suppressed in DIE and even more in OMA (Liu et al., 2018) (Table I). The predominance of PRA over PRB in endometriosis is explained by the hypermethylation of PGR at the promoter region that starts the full gene transcription and generates PRB, while the downstream promoter region associated with PRA transcription remains unmethylated (Rocha-Junior et al., 2019; Wu et al., 2006). The mechanism responsible for PRB promoter methylation in endometriosis is probably the chronic inflammatory environment with increased release of proinflammatory cytokines (Wu et al., 2008). Other epigenetic mechanisms such as aberrantly expressed micro-RNAs can limit PGR transcription in endometriosis (McKinnon et al., 2018).

Studies in a human myometrial cell line suggest that the balance between PRA and PRB isoforms controls the myometrial responsiveness to proinflammatory stimuli, and that an increased PRA:PRB ratio shifts the pregnant uterus from a quiescent to an active state at late gestation (Amini et al., 2016; Chen et al., 2017; Patel et al., 2018). In myometrial cells, progesterone represses interleukin (IL)-1β-induced proinflammatory genes through co-repressor molecules recruited by the PR DNA-binding domain in cooperation with the amino-terminal
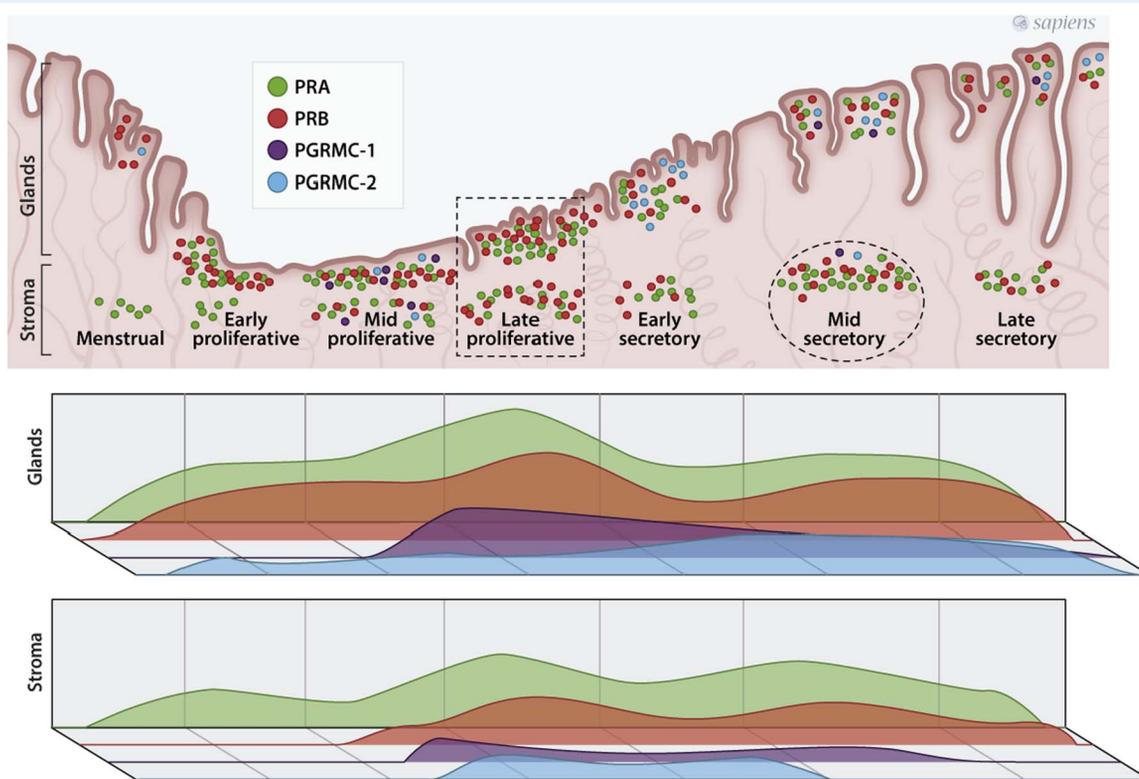
Figure 1 **Schematic representation of nuclear progesterone receptors A (PRA) and B (PRB) and membrane receptors PGRMC-1 and PGRMC-2 during the menstrual cycle in human endometrium.** The dot density indicates the abundance of the represented molecule at each cycle phase and tissue compartment (glands or stroma), according to immunolocalisation (Bedaiwy *et al.*, 2015; Keator *et al.*, 2012; Mote *et al.*, 1999), western blotting (Bedaiwy *et al.*, 2015), real-time PCR and *in situ* hybridisation (Keator *et al.*, 2012). Note, the maximal expression of PRA and PRB in the late proliferative phase (dotted rectangle) and the transient increase in stromal PRA expression at mid-secretory phase (dotted circle).

region that is unique to PRB (Chen *et al.*, 2017). An increased abundance of PRA also represses PRB-mediated transcriptional activity (Amini *et al.*, 2016; Patel *et al.*, 2018). By analogy, one may hypothesise that the relative increase of PRA over PRB in endometriosis contributes to tissue inflammation. However, the evidence available thus far suggests that both PR isoforms mediate the anti-inflammatory actions of progesterone in the endometrium (Patel *et al.*, 2015). More importantly, in immortalised human endometriotic epithelial cell lines expressing either PRA or PRB, dienogest inhibited the transcription of multiple pro-inflammatory genes regardless of the receptor isoform expressed by the cell (Ichioka *et al.*, 2015). Thus, it appears that in endometriosis the PR imbalance toward PRA does not provoke the local inflammatory response.

In summary, endometriosis is characterised by down-regulation of PR in general and PRB in particular through epigenetic inhibition (promoter hypermethylation) of upstream *PGR* transcription by local inflammatory mediators. Endometriotic lesions also lack the menstrual cycle variation of PR distribution that characterises the normal endometrium. As we shall discuss later, PRB down-regulation is an important but not the only mechanism to explain the refractoriness of some endometriotic lesions to progestin therapy.

Therapeutic Effects of Progesterone and Progestins in Human Endometrium and Endometriosis

Progestins are synthetic compounds that mimic the effects of progesterone (Gezer and Oral, 2015). The features that all progestins share are the ability to bind PR and induce the secretory transformation of estrogen-primed uterine endometrium, the so-called progestogenic effect. Steroids with progestational activity have differences in their chemical structures that affect their profile and potency of action on hypothalamic–pituitary axis, reproductive and breast tissues and metabolic processes (Gezer and Oral, 2015). The final progestogenic activity of any substance depends also on the route, the timing and the dose administered (de Lignieres *et al.*, 1995; Rommler *et al.*, 1985). Progestins reduce the frequency and increase the amplitude of pulsatile GnRH release, resulting in a reduction in follicle-stimulating hormone (FSH) and luteinising hormone (LH) secretion. As a result, continuous use of progestins leads to the suppression of ovarian steroidogenesis

Table I Summary of studies that have evaluated progesterone receptor expression and localisation in endometriotic lesions.

Reference	Sample	Phenotype	Findings
Bedaiwy et al., 2015	C, E, L	SUP, OMA	PRA (Western blot): C < E > OMA > SUP PRB (Western blot): C = E > OMA > SUP
Beliard et al., 2004	E, L	SUP	PRA + PRB (immunohistochemistry): C = E > L Down-regulation in the secretory phase in C and E, but not in L
Bono et al., 2014	L	OMA	PRA + PRB (immunohistochemistry): detected in 10/10 samples PRB (immunohistochemistry): detected in 6/10 samples
Eaton et al., 2013	C, L (stromal cells)	OMA	PRA (western blot): C > L PRB (western blot): C > L
Hayashi et al., 2012	C, L	OMA	PRA (immunohistochemistry): C = L PRB (immunohistochemistry): C > L
Wu et al., 2006	C, E, L	SUP, OMA	PRB mRNA (real-time PCR): C > E > L Hypermethylation or PGR at the PRB promoter region in L
Attia et al., 2000	E, L	'extra-ovarian'	PRA (western blot): E > L PRB (western blot): only E
Beranič and Rižner, 2012	L (Z-12 cell line)	SUP	Expressed PRB mRNA (real time PCR) and protein (western blot)
Vinci et al., 2016	L	DIE	PRA + PRB (immunohistochemistry): detected in 34/112 samples
Zanatta et al., 2015	L	DIE	PRA + PRB (immunohistochemistry): detected in 17/18 samples PRB (immunohistochemistry): detected in 17/18 samples
Liu et al., 2018	C, L	OMA, DIE	PRB (Immunohistochemistry): C>DIE>OMA

C: control endometrium from healthy women; E: eutopic endometrium from women with endometriosis; L: endometriotic lesion; SUP: superficial peritoneal endometriosis; OMA: ovarian endometrioma; DIE: deep infiltrating endometriosis; PRA: progesterone receptor A; PRB: progesterone receptor B; PGR: progesterone receptor gene

with anovulation and low serum levels of ovarian steroids (Horne and Critchley, 2011) (Fig. 2).

The long-standing hypoestrogenic and hypergestagenic state supports the use of progestins for treating endometriosis. In fact, the condition induced by these compounds causes decidual transformation of the eutopic endometrium and the same effect is observed to some extent in ectopic lesions (Vercellini et al., 2016b). The use and effectiveness of progestins for treating endometriosis is not just related to their growth inhibiting actions, but also to their induction of anovulation, inhibition of blood vessel growth and anti-inflammatory properties, creating a steady hypoestrogenic and progestogenic milieu for disease quiescence (Vercellini et al., 2008).

Effect on hypothalamic–pituitary–ovary axis

Hormonal therapies for endometriosis inhibit gonadotropin release, reduce ovarian steroid secretion and can induce amenorrhea, thereby preventing repeated endometrial reflux (Fig. 2). Combined oral contraceptives (COCs) inhibit the pituitary production and secretion of both FSH and LH (Mishell et al., 1977; Vandenberg et al., 1974). Inhibition of pituitary gonadotropins by progestins takes place at the hypothalamus, blocking the normal production of GnRH (Kastin et al., 1972), and also at the pituitary level, as shown by lower pituitary secretion of FSH and LH in response to the administration of GnRH (Robyn et al., 1974).

It has been claimed that androgenic effects contribute to the anti-gonadotropic effects of progestins (Bullock and Bardin, 1977; Neumann, 1978). However, some 17-hydroxyprogesterone derivatives, such as MPA or cyproterone acetate, which have either synandrogenic or anti-androgenic action, also inhibit gonadotropin secretion (Bullock and Bardin, 1977; Couzinet et al., 1986). In addition,

the 19-norprogesterone derivative without androgenic activity, nomegestrol acetate (NOMAC), has a similar anti-gonadotropic activity compared to the 19-nortestosterone derivative with androgenic activity, norethisterone acetate (NETA) (Couzinet et al., 1996).

Danazol has anti-gonadotropic activities, and daily oral administration of 400 mg of danazol inhibits both ovulation and LH surge (Barbieri and Ryan, 1981; Lauersen and Wilson, 1977; Wood et al., 1975). However, ovulation is not inhibited by daily vaginal administration due to the lower serum concentration of danazol reached by this route (Mizutani et al., 1995; Razzi et al., 2007a). Regarding intrauterine progestin administration, i.e., levonorgestrel (LNG)-releasing intrauterine system (LNG-IUS), plasma FSH concentrations were found below the normal upper limit of luteal phase concentrations, plasma LH secretion was normally pulsatile and preovulatory LH peaks were seen. Therefore, it seems that pituitary function remained essentially unchanged (Nilsson et al., 1980). Higher percentages of ovulatory cycles were found after 6 years of use, and no case of complete suppression of ovulation was found (Xiao et al., 1995).

Effects on estrogen receptors and estrogen synthesis

Perhaps the most remarkable therapeutic mechanisms of progestins in endometriosis derive from the fact that PR signalling leads to down-regulation of estrogen receptors (ERs). This effect has been clearly demonstrated not only in the eutopic endometrium but also in the glands and stroma of endometriotic lesions following in vivo treatment with different progestational formulations (Brichant et al., 2018; Engemise et al., 2011; Gomes et al., 2009).
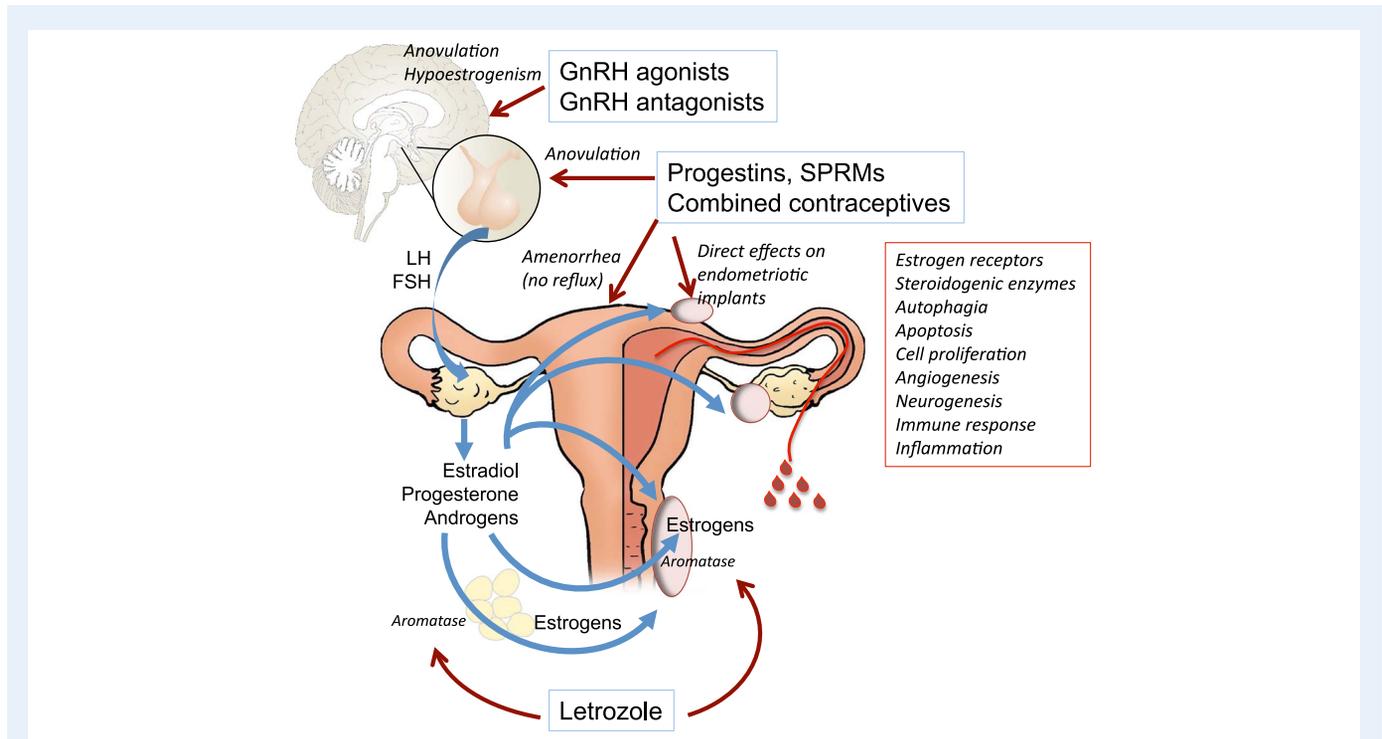
Figure 2 Therapeutic mechanisms of hormonal treatments for endometriosis.   Progestins, selective progesterone receptor modulators (SPRMs) and combined contraceptives block ovulation through central inhibition of gonadotropin release, induce amenorrhea which prevents repeated menstrual reflux and have many direct effects on endometriotic implants. Gonadotropin-releasing hormone (GnRH) agonists and antagonists block ovulation and induce hypoestrogenism, while aromatase inhibitors such as letrozole reduce androgen aromatisation into estrogens, both in the adipose tissue and within endometriotic lesions.

Another front of anti-estrogen action of progestins is the restraint of local estradiol production through interference with certain enzymes. As shown by experimental evidence, dienogest inhibits aromataseexpression and, consequently, local estrogen production in immortalised human endometrial epithelial cells (Shimizu *et al.*, 2011). Similarly, a decreased aromatase transcription was reported after MPA and dydrogesterone administration to nude mice implanted with human endometrial fragments (Fechner *et al.*, 2007). Furthermore, in the Z-12 epithelial cell line of peritoneal endometriosis, different types of progestin, like MPA, dydrogesterone and dienogest, were able to inhibit the expression of 17 beta-hydroxysteroid dehydrogenase (17β-HSD) type 1, the enzyme that catalyses the reduction of estrone to estradiol (Beranič and Rižner, 2012; Mori *et al.*, 2015). Similarly, they upregulate the expression of the oxidative 17β-HSD type 2, which inactivates estradiol (Beranič and Rižner, 2012).

However, some progestins derived from 19-nor-testosterone such as norethisterone and gestodene are metabolised into non-phenolic A-ring-reduced compounds that display estrogenic activity mediated by ERα (Larrea *et al.*, 2001).

Effects on tissue morphology, growth, vascularisation and regeneration

Endometrial tissues undergo physiological continuous changes resulting from endogenous ovarian hormonal secretion. The use of exogenous steroids with estrogenic, androgenic and progestogenic activity induces a spectrum of histologic changes in endometrial glandular and stromal architecture, blood vessels and cytology (Dinh *et al.*, 2015). These changes are observed not only in eutopic endometrium but also in endometriosis. The direct effects of progestins include the attenuation of the inflammatory state and the creation of a pseudo-pregnancy condition with increased apoptosis and atrophy of the endometriotic implants (Liang *et al.*, 2018; Mabrouk *et al.*, 2018). At the beginning of treatment, progestins induce secretory differentiation, then, following the down-regulation of the ERs after several cycles, the endometrium appears atrophic with simple tubular glands, weak secretory vacuolation and low cellular density at the stroma. There are no consistent histologic features that allow differentiation between the endometrial and vascular effects of different progestins. However, depot MPA and progestin implants early on induce decidualisation and then atrophy of both glands and stroma (Dinh *et al.*, 2015).

During treatment with dydrogesterone in endometriosis, the ectopic tissue undergoes different changes, including decidualisation, undifferentiation or involution (Cornillie *et al.*, 1987). Histological evaluation after a short term treatment of endometriosis with gestrinone or danazol showed a degree of cellular inactivation and degeneration of the endometriotic implants (Brosens *et al.*, 1987). In endometriomas, dienogest induces a low frequency of proliferative cells and a high rate of apoptotic cells (Miyashita *et al.*, 2014). A recent study evaluated the histopathological effect of short term dienogest treatment in endometriotic tissue *in vivo*, showing a high frequency of decidualisation

and a tendency to a reduced inflammation. No differences were shown in terms of necrosis, glandular atrophy and angiogenesis between medically treated and untreated lesions (Mabrouk *et al.*, 2018).

In vitro, dienogest induces a dose-dependent inhibition of human endometrial stromal cell (hESC) proliferation together with morphological and functional changes, including the production of prolactin, a typical marker of decidualisation (Okada *et al.*, 2001). Furthermore, dienogest treatment of endometriotic cells suppresses protein kinase B (AKT) and extracellular signal-regulated kinase (ERK)1/2 activity, thereby inhibiting mammalian target of rapamycin (mTOR), inducing autophagy, and promoting apoptosis (Choi *et al.*, 2015). Also, NETA showed a direct anti-proliferative effect on endometriotic stromal cells *in vitro* without apparent cytotoxicity, as assessed by lactate dehydrogenase release, while inducing apoptosis through increased caspase 3/7 activity (Minami *et al.*, 2013). The role of progestins in inhibiting cell proliferation, inflammation and neovascularisation in endometriosis has been demonstrated by the lack of lesion expansion, the maintenance of ERα and PR and the attenuation in Ki67 (cell proliferation marker), CD31 (neovascularisation marker), pro-inflammatory cytokine expression and macrophage infiltration, in experimental endometriosis treated with progesterone (Li *et al.*, 2016). Furthermore, progestins reduce nerve fibre density and nerve growth factor signalling in peritoneal endometriotic lesions (Tokushige *et al.*, 2009) and in cultured endometriotic stromal cells (Makabe *et al.*, 2017).

Effects on local immune response

Endometriosis is associated with changes in both cell-mediated and humoral immunity (Riccio *et al.*, 2018). The peritoneal fluid of women with endometriosis contains a number of immune cells secreting several cytokines and growth factors, which play a role in the development and maintenance of endometriotic implants.

Progesterone, dienogest and danazol attenuate the expression of IL-8 by reducing tumour necrosis factor (TNF)-induced nuclear factor-kappa B (NF-κB) activation in endometriotic stromal cells (Horie *et al.*, 2005; Lazzeri *et al.*, 2010). Notably, dienogest reverses some alterations of the immune system in endometriosis, as it increases the activity of natural killer (NK) cells in the peritoneal fluid and in the spleen, decreases the number of peritoneal fluid cells, decreases IL-1β production by peritoneal macrophages (Katsuki *et al.*, 1998) and inhibits IL-1β-induced CCL20 release by an endometriotic epithelial cell line (Mita *et al.*, 2017). Also, danazol may interfere with the immune system involvement in endometriosis (Hill *et al.*, 1987). The drug inhibits spontaneous peripheral blood lymphocyte-mediated cytotoxicity toward endometrial stromal cells (Vigano *et al.*, 1994) and suppresses spontaneous and activated NK cell cytotoxicity *in vitro* (Vigano *et al.*, 1992). Danazol treatment counteracts peripheral blood monocyte-mediated enhancement of endometrial cell proliferation. There are also direct effects of danazol on endometrial cells, suggesting that this drug affects both monocyte-derived growth-stimulating factors and endometrial cell response to these factors (Braun *et al.*, 1994). Moreover, danazol suppresses the production of IL-1β by human monocytes *in vitro* (Mori *et al.*, 1990) and, coherently with this mechanism, danazol administration to women with endometriosis results in a decrease in serum IL-6 and IL-1α levels (Koumantakis *et al.*, 1994).

In short, the therapeutic effects of progestins, COCs and other hormonal drugs used in endometriosis include inhibition of estrogen synthesis and action through down-regulation of steroidogenic enzymes and ER, inhibition of endometriotic cell survival and proliferation, limitation of local angiogenesis and neurogenesis and, linking all these mechanisms, attenuation of the immune-inflammatory response (Fig. 2). Nonetheless, experimental studies comparing directly endometrial and endometriotic tissues show a net difference in their response to such drugs.

Differential effects of progesterone and progestins between normal endometrium, eutopic endometrium of females with endometriosis and endometriotic lesions

In animal models

In spite of the limitations of murine models of endometriosis to emulate the human disease, they help to understand the morphological and functional changes induced by hormonal treatments in endometriotic implants. In nude mice transplanted subcutaneously with human endometrial fragments, treatment with depot MPA induced low or atrophic surface epithelium, decidual reaction and either narrow or dilated glands. In the same mouse model, danazol treatment induced dilated glands and a medium to the high epithelium, although with variable responses within the treatment group. Interestingly, this study suggested that an intact stroma is necessary for the glandular epithelium of the endometriotic tissue to respond to hormonal treatments (Bergqvist *et al.*, 1985). In another study with human endometrial grafting into the peritoneal cavity of nude mice, MPA administration in a dose that caused 50% reduction of uterine weight was unable to cause regression of endometriotic lesions induced with endometrial fragments of normal volunteers or women with endometriosis (Palmer *et al.*, 2016). Using the same experimental model, the subcutaneous administration of slow-release progesterone over 2 weeks reduced the number and size of endometriotic lesions in nude mice injected with normal human endometrium, but the same treatment was ineffective when the animals had been implanted with endometrial fragments from women with endometriosis (Bruner-Tran *et al.*, 2006).

For obvious reasons, the effects of hormonal treatments on heterotopic endometrial xenografts cannot be compared with the effects of the same treatments on the eutopic endometrium of the human donors or of the mouse host. However, in the model of autologous uterine tissue transplantation, the effects of hormonal treatments may vary considerably between the eutopic endometrium and the endometriotic lesions. For example, in rats autografted with uterine tissue in the inguinal region, treatment with MPA resulted in significant atrophy of the ectopic implants but not of the eutopic uterine horn (Pereira *et al.*, 2015). In rats autotransplanted with uterine sections into the parietal peritoneum and mesenteric vessels, the antiprogestin onapristone induced severe atrophic changes in luminal and glandular epithelium of ectopic implants and, conversely, induced epithelial cell proliferation and hypertrophy in the eutopic endometrium (Stoeckemann *et al.*, 1995). However, bazedoxifene administration reduced gland size and number in mouse endometrium as well as in autologous endometriotic lesions (Kulak Jr *et al.*, 2011).

The ability of endometriosis to respond to hormonal treatments is better understood as a dynamic rather than a static phenomenon,

experimental evidence suggests. The mouse model of peritoneal transplantation of uterine cell suspension from syngeneic donors revealed that progesterone treatment, if initiated 4 days before transplantation, effectively inhibits the establishment and growth of endometriotic lesions through anti-proliferative, anti-angiogenic and anti-inflammatory mechanisms. In contrast, when progesterone treatment starts later, i.e. 4 days after transplantation, the local expression of PR and its target genes is markedly reduced and the treatment effects are not seen anymore (Li *et al.*, 2016).

In cultured human cells and tissues
Primary cultures of hESCs and epithelial cells provide an insightful model to understand the differential effects of hormonal treatments between normal endometrium, eutopic endometrium in the presence of endometriosis and endometriotic lesions. With this approach, our group found out that both stromal and epithelial cells from endometriotic lesions produce aberrantly high amounts of reactive oxygen species (ROS) such as hydrogen peroxide and, unlike normal endometrial cells, endometriotic cells fail to reduce their hydrogen peroxide production in response to danazol (Ngô *et al.*, 2009). Another study observed that an eosinophil-attracting chemokine, CCL11, was produced by epithelial cells isolated from endometriosis when stimulated with estradiol, MPA and pro-inflammatory cytokines, but not in the absence of inflammatory stimulus, whereas normal endometrial cells did not release CCL11 *in vitro* regardless of hormonal and/or inflammatory stimuli (Hornung *et al.*, 2000).

One important mechanism activated by progesterone that opposes estrogen and limits endometrial growth is the induction of phosphatase and tensin homolog deleted from chromosome 10 (PTEN), a tumour suppressor that promotes autophagy and apoptosis in normal endometrial stromal cells by inhibiting AKT and mTOR activation (Choi *et al.*, 2017). The normal endometrium increases PTEN expression and decreases AKT phosphorylation upon progesterone exposure *in vivo* or *in vitro*. However, stromal cells derived from endometriotic cyst do not increase PTEN expression when exposed to progesterone *in vitro*, and eutopic endometrium from women with endometriosis lacks PTEN increase in the secretory phase of the menstrual cycle (Choi *et al.*, 2017). Consequently, the AKT/mTOR pathway remains active during the whole cycle in the eutopic endometrium and is not inhibited by progesterone treatment in endometriotic lesions, allowing abnormal survival of endometriotic stromal cells.

As summarised in Fig. 3, a number of morphological and functional changes that follow progestin or progesterone stimulation of normal endometrial cells are not seen when the same stimulus is applied to endometriotic cells or eutopic endometrial cells from women with endometriosis (Aoyagi *et al.*, 2017; Bruner-Tran *et al.*, 2002; Cheng *et al.*, 2007; Novembri *et al.*, 2011; Sultana *et al.*, 2017; Tsuno *et al.*, 2009; Yu *et al.*, 2014). Among the molecular targets in normal endometrium that resist progestin or progesterone action in endometriotic cells are the nuclear transcription factor FOXO1, whose deficiency impairs stromal cell decidualisation (Yin *et al.*, 2012), matrix metalloproteinases (Bruner-Tran *et al.*, 2002), the transmembrane gap junction protein connexin 43 (Yu *et al.*, 2014) and paracrine regulators of estradiol metabolism (Cheng *et al.*, 2007). These *in vitro* experiments provide useful hypotheses to explain why hormonal treatments may not affect endometriotic lesions as expected, resulting in therapeutic failure.
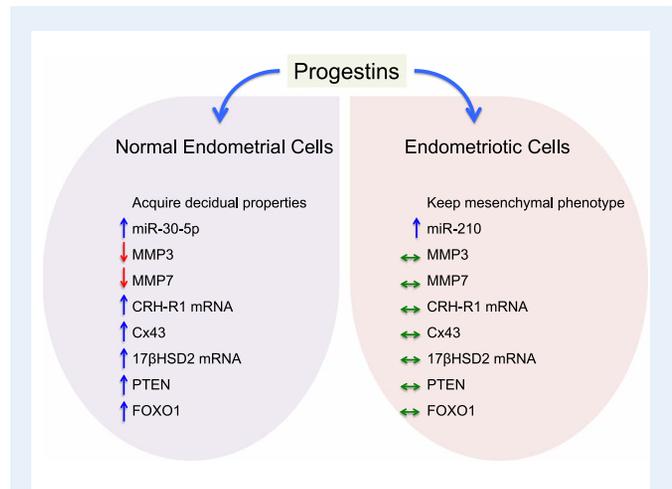

Figure 3 **Effects of progestin treatment *in vitro* on normal endometrial cells compared to endometriotic cells cultured in parallel and under the same conditions.** The arrows indicate that progestin treatment stimulates (↑), inhibits (↓) or has no effect (⇔) on the target gene, RNA or protein.

In human studies in vivo
Clinical studies evaluating human endometriotic lesions before and after medical treatments are rare, as current practices recommend avoiding multiple surgeries. Nevertheless, two independent clinical trials obtained samples of both eutopic endometrium and endometriotic lesions before and 6 months after the insertion of LNG-IUS in symptomatic women with endometriosis (Engemise *et al.*, 2011; Gomes *et al.*, 2009). Although none had a control group without endometriosis or a placebo group, these studies consistently showed that 6 months of LNG-IUS use resulted in decreased expression of estrogen and progesterone receptors, decreased cell proliferation and increased expression of an apoptosis marker in both eutopic endometrium and endometriosis. These results suggest that levonorgestrel treatment delivered into the uterus is able to induce progestin-like effects in pelvic endometriosis that are surprisingly similar to the effects induced in the eutopic endometrium that is in close contact with the drug releasing system.

Why Do Progestins Sometimes Fail in Endometriosis?

Characteristics of the type of hormone

All progestins used to treat endometriosis have been labelled for contraception and/or menopausal hormone therapy in doses and regimens sufficient to induce endometrial decidualisation and prevent endometrial hyperplasia. Direct comparisons between different types of progestins is rare in endometriosis clinical studies, with the scarce evidence suggesting that when given by the same route and the same regimen, the effectiveness may be similar. This is the case, for example, of an observational study comparing NETA versus dienogest used sequentially by the same patients, each drug for 6 months, resulting in the same rates of symptom control, health-related quality of life

and overall satisfaction with treatment (Vercellini *et al.*, 2016a). Nevertheless, it is largely unknown whether they are all equally capable of acting on endometriosis lesions to induce apoptosis or to inhibit cell proliferation, adhesion, invasiveness, angiogenesis and inflammation. *In vitro* studies may give some clues to this question, by comparing face-to-face the effects of different progestins on primary cell or tissue cultures derived from endometriosis.

In endometrial explants of women with endometriosis, chlormadinone acetate consistently inhibited cyclooxygenase-2 synthesis and prostaglandin F2α release, contrasting to a weaker effect of dienogest and drospirenone (Roth *et al.*, 2019). In isolated endometrial stromal cells challenged with the proinflammatory cytokine TNF, incubation with high concentrations (10^{-5} M) of MPA failed to inhibit the *in vitro* secretion of the chemokine CCL2, while equimolar doses of dienogest or NETA were sufficient to block CCL2 release by these cells (Grandi *et al.*, 2016). The same study, however, showed that MPA was capable of inhibiting IL-6 secretion by endometrial stromal cells despite a paradoxical increase of IL-6 mRNA transcription (Grandi *et al.*, 2016). Still in hESCs, dienogest inhibited the in-vitro transcription of the growth factor midkine, whereas NETA and MPA did not (Nirgianakis *et al.*, 2016).

Another reason for therapeutic failure may be the concomitant administration of an estrogen with the progestin. Face-to-face comparisons between hormonal treatments with and without estrogen have been done in randomised controlled trials (RCTs) or patient preference trials and have not shown meaningful differences between groups in terms of pain relief (Cheewadhanaraks *et al.*, 2012; Leone Roberti Maggiore *et al.*, 2014; Morotti *et al.*, 2014; Razzi *et al.*, 2007b; Vercellini *et al.*, 2016b; Vercellini *et al.*, 2002; Vercellini *et al.*, 2005). However, in general, the trial participants receiving progestin treatments are more satisfied than those treated with combined hormonal therapies (Leone Roberti Maggiore *et al.*, 2014; Morotti *et al.*, 2014; Scala *et al.*, 2018; Vercellini *et al.*, 2002; Vercellini *et al.*, 2005). In fact, for most women who do not respond to COC, changing to NETA may be a successful strategy to treat endometriosis pain and improve general well-being (Vercellini *et al.*, 2018c).

Casper (2017) provides an empirical framework to explain why combined hormonal contraceptives may be less effective than isolated progestins to alleviate endometriosis symptoms and probably ineffective to inhibit endometriosis progression. Briefly, endometriotic lesions have endogenous estradiol production, reduced estradiol inactivation and progesterone insensitivity, while combined hormonal contraceptives have supraphysiological doses of estrogen, creating an imbalance in favour of estrogen action in the endometriotic implants (Casper, 2017). On the other hand, estrogen priming may be necessary to induce PR in endometriotic lesions (Bono *et al.*, 2014).

It may be reasonable to avoid estrogen administration and even to inhibit estrogen synthesis to achieve maximal suppression of endometriotic implants. The later strategy has long been pursued with pituitary gonadotropin blockade by GnRH analogues (extensively reviewed by Brown *et al.* (2010)) and, more recently, with the use of aromatase inhibitors such as anastrozole and letrozole (Amsterdam *et al.*, 2005; Ferrero *et al.*, 2009). In mice, letrozole therapy is superior to MPA to induce endometriosis regression and also to inhibit stem cell migration to the endometriotic lesions (Ersoy *et al.*, 2017). However, aromatase inhibitors promote pituitary gonadotropin release with consequent ovarian stimulation; therefore, they must be combined with a progestin or other method of gonadotropin inhibition to treat endometriosis in premenopausal women (Ailawadi *et al.*, 2004). In two non-randomised, open-label trials, the use of letrozole (2.5 mg/day) combined with NETA (2.5 mg/day) over 6 months was more effective than NETA alone to reduce the intensity of chronic pelvic pain and deep dyspareunia associated with rectovaginal endometriosis (Ferrero *et al.*, 2009) as well as the size of ovarian endometriomas (Ferrero *et al.*, 2014). Nonetheless, both treatments were effective, none had lasting effects after discontinuation and the addition of letrozole to NETA did not increase patient satisfaction at the end of treatment (Ferrero *et al.*, 2009; Ferrero *et al.*, 2014). This evidence places letrozole as a second-line therapy for selected patients who fail to respond to progestins (Table II).

Characteristics of therapeutic regimen and route of administration

A retrospective cross-sectional study of deep endometriotic lesions compared women who were using different medical treatments pre-operatively (COC, oral progestins or GnRH agonists) to a group without treatment, and found a large intra-lesion, intra-patient and intra-treatment variance in the expression levels of ER, which was more evident than any difference between treatments or between treated and non-treated lesions (Brichant *et al.*, 2018). This irregular effect of systemic progestins contrasts with the consistent inhibition of ER expression in endometriotic lesions after LNG-IUS insertion (Engemise *et al.*, 2011; Gomes *et al.*, 2009). A more consistent drug delivery to pelvic lesions would explain why LNG-IUS was found to be more effective than oral danazol (even in standard dose of 600 mg/day) to treat endometriosis-related pain (Taneja *et al.*, 2017). However, an open-label RCT suggested that subdermal etonogestrel implant and LNG-IUS may be equally effective to treat endometriosis-associated pelvic pain and dysmenorrhea (Carvalho *et al.*, 2018). Independently from pain reduction, the degree of satisfaction with treatments to prevent relapse after endometriosis surgery appears to be higher among LNG-IUS users compared to women on COC (Morelli *et al.*, 2013).

A systematic review concluded that continuous administration of COC after conservative surgery for endometriosis results in a faster reduction in frequency and severity of dysmenorrhea compared with the cyclic regimen (Seracchioli *et al.*, 2009). A subsequent patient preference trial of a COC containing ethinyl estradiol and dienogest showed a net superiority of the continuous over the cyclic regimen to reduce pain scores at 3 months and also to decrease the prevalence of dysmenorrhea, dyspareunia, non-menstrual pelvic pain and dyschezia both at 3 months and 6 months of follow-up (Caruso *et al.*, 2016). Not surprisingly, the continuous regimen achieved faster and better improvement in the patients' quality of life (Caruso *et al.*, 2016).

Characteristics of the disease phenotype

Endometriotic implants are very heterogeneous in clinical features and evolution, being currently classified into three distinct phenotypes: superficial peritoneal endometriosis, ovarian endometrioma and deep-infiltrating endometriosis (Johnson *et al.*, 2017; Nisolle and Donnez, 1997). The overwhelming majority of clinical studies on medical therapies for endometriosis either mixes various disease phenotypes or chooses one main phenotype, making it difficult to evaluate whether a

Table II Potential approaches for patients who have failed initial therapy for endometriosis and the currently available evidence.

Cause of progestin failure	Potential approaches	Evidence
Hormone type	To change the type of progestin	Observational study: NETA and dienogest may have similar effects (Vercellini *et al.*, 2016a). In-vitro studies: different progestins may have different mechanisms of action (Grandi *et al.*, 2016; Nirgianakis *et al.*, 2016; Roth *et al.*, 2019).
	To avoid estrogen association	RCTs did not show meaningful differences between isolated progestins and progestin-estrogen associations (Cheewadhanaraks *et al.*, 2012; Razzi *et al.*, 2007b; Vercellini *et al.*, 2002; Vercellini *et al.*, 2005). Prospective self-control study: for patients who do not respond to COC, changing to NETA may be beneficial (Vercellini *et al.*, 2018c).
	To inhibit estrogen synthesis	Non-randomised open-label trials: for patients who fail to respond to progestins, letrozole can be tried as a second line therapy (Ferrero *et al.*, 2009; Ferrero *et al.*, 2014).
Therapeutic regimen	To change therapeutic regimen	Systematic review: continuous administration may be better than cyclic regimens (Seracchioli *et al.*, 2009).
Route of administration	To change the route of administration	RCT: systemic and local progestins may be similar (Carvalho *et al.*, 2018).
Progesterone resistance	To associate NSAIDs to progestin therapy	No high-quality evidence for NSAIDs effectiveness on endometriosis symptoms, but the association with hormonal treatments might diminish the inflammatory response that boosts progesterone resistance (Brown *et al.*, 2017).
	To associate antioxidants	Multi-centre open-label non comparative clinical trial: antioxidant preparations containing N-acetyl-cysteine may mitigate symptoms (Lete *et al.*, 2018).

NETA: norethisterone acetate; RCTs: randomised controlled trials; COC: combined oral contraceptive; NSAIDs: non-steroidal anti-inflammatory drugs

therapy is equally effective to treat superficial peritoneal disease, ovarian endometrioma or the several manifestations of deep endometriosis. Yet, knowing which hormonal treatment is more appropriate to treat each endometriosis phenotype would lead to a lesion-based therapeutic decision (Vercellini *et al.*, 2018a) and possibly maximise patient adherence and treatment results.

Morphological endpoints

Structurally and functionally, endometriotic lesions are not all the same (Tosti *et al.*, 2015). Nerve fibres expressing protein gene product 9.5 are much more abundant in deep-infiltrating than in superficial peritoneal endometriosis (Wang *et al.*, 2009a) and, among the deep lesions, the intestinal forms (either at sigmoid, appendix or rectum) are more densely innervated with myelinated, unmyelinated, sensory and autonomic nerve fibres than other forms of deep endometriosis such as nodules infiltrating the uterosacral ligament (Wang *et al.*, 2009b). Nerve fibre density in superficial peritoneal endometriosis is reduced to approximately half in women treated with progestins or combined pills compared to women not receiving hormonal treatment (Tokushige *et al.*, 2009) but, in deep rectovaginal lesions, which are more innervated, the effect of hormone therapy on nerve fibre density seems to be even more pronounced (Tarjanne *et al.*, 2015).

Vascularisation also differs among the various types of lesions. For instance, blood vessel density evaluated by von Willebrand factor staining is twice as higher in rectal endometriosis than in bladder or ovarian endometriotic implants (Machado *et al.*, 2008). Progestin therapy is associated with a lower number of microvessels and a larger vascular area, suggesting inhibited angiogenesis and increased

vasodilation in both superficial and deep (uterosacral, vaginal and vesical) endometriotic lesions. Again, the effect of progestins varied according to the lesion type and the reduction of blood vessels associated with the therapy was more intense in superficial than in deep endometriosis (Jondet *et al.*, 2006). As for ovarian endometrioma, a controlled clinical trial showed that dienogest treatment for 6 months before surgery, despite inducing decidualisation, is unable to inhibit angiogenesis within the endometriotic cysts (Mabrouk *et al.*, 2018).

Another endpoint of hormonal treatment that markedly differs according to the endometriosis phenotype is the effect on lesion size. Ovarian endometriomas tend to have a substantial reduction in volume after 6 months or more of progesting therapy, be it norethisterone (Taniguchi *et al.*, 2017), drospirenone (Harada *et al.*, 2017; Taniguchi *et al.*, 2015) or dienogest (Lee *et al.*, 2018; Park *et al.*, 2016). Conversely, deep endometriosis appears to be more resistant to size regression upon medical treatments. A cohort study that followed 83 women with deep rectovaginal endometriosis treated over 12 months with NETA or desogestrel observed a mean reduction of 20 to 30% in the volume of the endometriotic nodules, as estimated by ultrasonography with bowel preparation (Ferrero *et al.*, 2013). Our group noted a 50% reduction of rectovaginal plaque volume after 12 months of dienogest therapy (Razzi *et al.*, 2007a), whereas another cohort study saw no volumetric change in deep lesions of intestine or posterior fornix with the same treatment (Leonardo-Pinto *et al.*, 2017), although symptoms improved leading to a better quality of life. Indeed, intestinal endometriosis can be so resistant to medical therapy
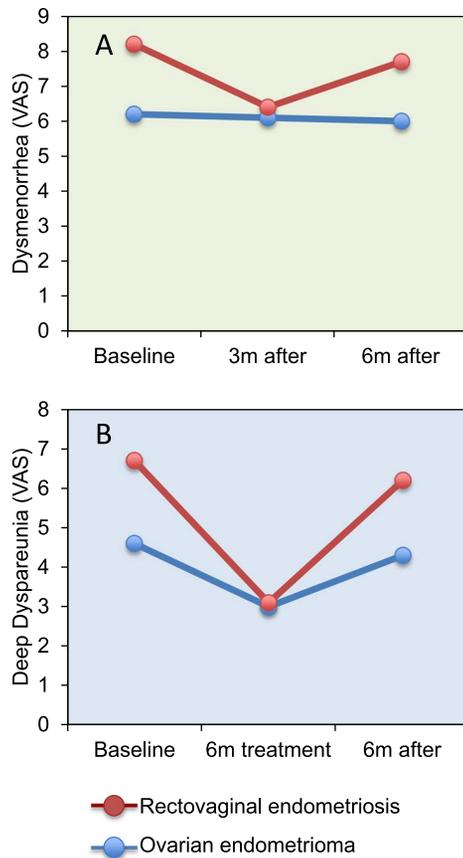
Figure 4 Endometriosis phenotype may affect the therapeutic response to progestins. The graphs summarise dysmenorrhea (**A**) and deep dyspareunia (**B**) scores obtained by visual analogue scale (VAS) in women with rectovaginal endometriosis (Ferrero et al., 2009) and in women with ovarian endometrioma (Ferrero et al., 2014) treated with norethisterone acetate for 6 months. Data were extracted from published tables of two separate studies and plotted together for better visualisation.

that in some cases it keeps growing and invading the organ wall despite continuous medication (Millochau et al., 2016).

Clinical endpoints

In a patient preference, parallel cohort trial comparing oral progestin (NETA) therapy versus second-line laparoscopic surgery for women with persistent or recurrent endometriosis after a first surgery, the outcomes varied according to the treatment chosen but also depending on the type of endometriosis. Among women with rectovaginal disease, the rate of satisfaction after 12 months was around 50% in both treatment groups, whereas among the participants with other types of endometriosis (mostly ovarian endometriomas), the satisfaction rate was much higher with progestin than with surgical treatment (Vercellini et al., 2012). The effects of 6 months progestin (NETA) therapy on pain relief were assessed in a prospective study of women with rectovaginal endometriosis (Ferrero et al., 2009) and subsequently by the same authors in another study including only women with ovarian endometrioma (Ferrero et al., 2014). When the results of the two

studies are viewed together (Fig. 4), it becomes clear that patients on progestin therapy ended with the same level of pain scores in the rectovaginal endometriosis cohort as in the ovarian endometrioma cohort, but in the former the therapeutic effect was more evident because the patients had higher pain intensity at baseline.

Characteristics of individuals: the pharmacogenomics

Individual genetic characteristics can affect the bioavailability of hormonal drugs used to treat endometriosis and, hence, explain part of the variability in the therapeutic response. A semiquantitative study of deep endometriosis biopsies from women undergoing hormonal treatments (progestins, COC or GnRH agonist) revealed a large heterogeneity of ERα and PR immunostaining between patients and even between endometriotic glands from the same lesion, particularly in women treated with a progestin (Brichant et al., 2018). N-Acetyltransferases (NAT) control drug kinetics by acetylating active substances and converting them into inactive metabolites. Different combinations of *NAT2* alleles are associated with different acetylation activity and speed. In a sample of Russian women with adenomyosis or endometriosis, a greater rate of effective hormonal treatment was associated with genotypes that contained more than one mutant allele, which predisposes to slower inactivation of drugs (Iskhakova et al., 2006).

The cytochrome P450 3A (CYP3A) system is one of the main enzymatic pathways involved in the metabolism of progestins and anti-progestins (Jang and Benet, 1997; Moreno et al., 2012). *CYP3A*, *CYP2C19*, and other gene polymorphisms involved in hormone metabolism have been investigated in women with endometriosis (Bozdag et al., 2010; Moreno et al., 2012; Sapkota et al., 2017), but the association of such polymorphisms with the individual response to hormonal therapy remains largely unknown. In other hormone-dependent conditions, however, evidence shows that single-nucleotide polymorphisms of genes involved in steroid hormone synthesis, metabolism or transport have important influence on the therapeutic response to steroid hormones in conditions as diverse as renal transplantation (Rekers et al., 2017), nephrotic syndrome (Chiou et al., 2012), epilepsy (Grover et al., 2010), prostate cancer (Binder et al., 2016) and breast cancer (Wang et al., 2010).

The *PGR* gene variant PROGINS is characterised by a 306-bp insertion in intron G that renders the receptor less responsive to ligands and predisposes the carrier to progesterone insensitivity and thereby to increased risk of spontaneous abortion (Schweikert et al., 2004), endometrial cancer (Junqueira et al., 2007) and endometriosis (De Carvalho et al., 2007). An *in vitro* study with eutopic endometrial cells from women with endometriosis showed that cells harbouring the PROGINS variant when incubated with progesterone responded with more proliferation, more viability and less apoptosis than control cells expressing the wild-type PR (D'Amora et al., 2009).

Progesterone resistance in endometriosis: an insurmountable barrier?

Progesterone resistance is defined as subnormal cellular response to the effects of natural progesterone, but the concept can be extended
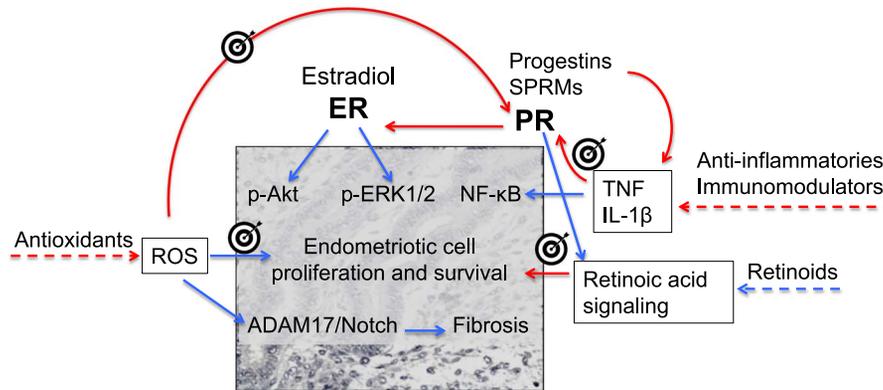
Figure 5 Hypothetical targets and strategies to overcome progesterone resistance in endometriosis based on currently available drugs. Progesterone receptor (PR) expression is inhibited by gene hypermethylation induced by proinflammatory cytokines and reactive oxygen species (ROS), which could be inhibited by anti-inflammatory drugs/immunomodulators and antioxidants, respectively. The same drugs and others, such as retinoids, may activate mechanisms downstream PR signalling that prevent endometriosis proliferation and fibrosis. Blue arrows: stimulation; red arrows: inhibition. ER: estrogen receptor; p-Akt: phosphorylated serine/threonine protein kinase B; p-ERK1/2: phosphorylated extracellular signal-regulated kinase 1/2; NF-κB: nuclear factor-kappa B; ADAM17: A disintegrin and metalloproteases metallopeptidase domain 17; TNF: tumour necrosis factor; IL: interleukin.

to encompass a poor response to therapeutic progestins. The putative mechanisms and consequences of progesterone resistance in endometriosis have been comprehensively updated in recent reviews (McKinnon *et al.*, 2018; Patel *et al.*, 2017; Yilmaz and Bulun, 2019). Briefly, the central feature of the low response of endometriosis to progestins is the insufficiency of PRB transcription, translation and biological activity. This defect may be congenital, as exemplified by the PROGINS variant (D'Amora *et al.*, 2009), or acquired, through the epigenetic influence of an inflammatory milieu (Patel *et al.*, 2017). Hence, we may consider alternatives to revert the epigenetic mechanisms that inhibit PRB synthesis by adding an anti-inflammatory substance or an immunomodulator as adjuvant to progestin therapy.

It is surprising that the effectiveness of non-steroidal anti-inflammatory drugs to treat endometriosis symptoms remains uncertain as no high-quality evidence is available to date (Brown *et al.*, 2017). The association of anti-inflammatories with hormonal treatments might potentially decrease the levels of proinflammatory cytokines such as TNF and IL-1β and consequently reduce PRB methylation (Fig. 5). Immunomodulators can also be useful to the same scope. For example, Ibrutinib (an FDA-approved inhibitor of the B cell receptor signalling mediator Btk) is able to skew activated B lymphocytes towards regulatory B cells and lower serum TNF levels in endometriotic mice (Riccio *et al.*, 2019).

Oxidative stress is another mechanism involved in progesterone resistance in endometriosis, as endometriotic lesions and their surrounding peritoneal fluid are rich in ROS (Santulli *et al.*, 2015), which stimulate the A disintegrin and metalloproteases metallopeptidase domain 17 (ADAM17)/Notch signalling pathway (Gonzalez-Foruria *et al.*, 2017), and Notch signal induces *PGR* hypermethylation (Su *et al.*, 2016). Antioxidants like *N*-acetyl-cysteine inhibit ROS production and cell proliferation in endometriotic lesions (Ngô *et al.*, 2009) and mitigate symptoms (Giorgi *et al.*, 2016; Lete *et al.*, 2018), but their potential to reduce *PGR* methylation and overcome progesterone resistance remains to be investigated.

Moreover, strategies to bypass PR signal and directly activate its downstream targets might be a more complex, albeit rational approach (Fig. 5). These would include antioxidants because ROS are capable of promoting endometriosis growth and fibrosis by mechanisms independent of sex hormones (Gonzalez-Foruria *et al.*, 2017). A complementary approach to antioxidants could be the use of retinoids to compensate for the deficiency of endometriotic lesions in a mechanism of apoptosis induced by PR and mediated by retinoic acid signal (Pavone *et al.*, 2016; Pavone *et al.*, 2010).

In summary, clinical studies support at least four explanations for a suboptimal response to progestin therapy in endometriosis (Table II): the type of progestin used may not be ideal for that patient, the presence of estrogen in the formulation may be harmful, the pill-free interval of a cyclic regimen may weaken the drug efficacy and the progesterone insensitivity of endometriotic tissues may demand the association of a non-hormonal drug to overcome this resistance.

Selective Progesterone Receptor Modulators to Treat Endometriosis: Rationale and State of the Art

Selective progesterone receptor modulators (SPRMs) comprise a relatively recent class of synthetic molecules capable of interacting with PR. The interest in these new drugs is based on their affinity to PR, especially considering their antagonist effects (Bouchard *et al.*, 2011; Clemenza *et al.*, 2018; Fu *et al.*, 2017). SPRMs have a wide spectrum of action and are related to a number of therapeutic as well as adverse effects. By binding PR, SPRMs can perform an isolated agonist or antagonist function, but a mixed activity is also possible (Wagenfeld *et al.*, 2016). For instance, onapristone acts only as an antagonist while mifepristone and asosprinil have both antagonist and agonist effects and

ulipristal acetate (UPA), in its turn, has a predominant PR antagonist action in the uterus (Benagiano *et al.*, 2014).

The effect produced by the interaction of SPRMs and PR depends on the conjunction of several factors, including the amount of PRA and PRB in each tissue and the changes in receptor conformation promoted by the different ligands. The final action also relies on a complex machinery of co-regulators and co-repressors, whose proportion will determine a greater or lesser transcriptional activity in target genes (Bouchard *et al.*, 2011; Chabbert-Buffet *et al.*, 2012). Apparently, the intrinsic mechanisms are distinct among the different SPRMs. In fact, a study showed that UPA and mifepristone lead to diverse gene expression patterns in endometrium (Kannan *et al.*, 2018).

The occurrence of cross-reaction with other steroid receptors is not rare. In effect, mifepristone, the first SPRM discovered, in 1981, arose from anti-glucocorticoid studies (Chen *et al.*, 2012). This drug is currently used in some countries for medical abortions in combination with prostaglandins such as misoprostol. Because of its several properties, other clinical applications are being studied, to cite Cushing's disease, psychosis, contraception, Alzheimer's disease, uterine fibroids, endometrial cancer and, lately, endometriosis (Chabbert-Buffet *et al.*, 2012; Rozenberg *et al.*, 2017).

The interest of developing a SPRM available for use in endometriosis lays on the selective inhibition of endometrial proliferation, without causing estrogen deprivation and its undesirable side effects. Other expected effects would be pain relief, obtained by the suppression of prostaglandin and cytokine synthesis in endometrial cells, and the action on the PRs present in the spiral arterioles, generating reversible suppression of endometrial bleeding (Merviel *et al.*, 2013).

With this background, some basic and clinical studies have tested the putative actions of SPRMs on endometriosis. Studies with animal models demonstrated regression of endometriotic lesions with subcutaneous implants of mifepristone (Mei *et al.*, 2010). A Cochrane systematic review including 10 RCTs with 960 women assessed the effectiveness and the safety of SPRMs for pain relief in endometriosis (Fu *et al.*, 2017). Mifepristone was more effective than placebo against dysmenorrhea (moderate-quality evidence) and dyspareunia (low-quality evidence), at least when used at 5- or 10-mg doses. A trial excluded from the Cochrane meta-analysis suggested that post-operative treatment with mifepristone for 6 months is able to reduce endometrial thickness and alleviate pelvic pain in patients with endometriosis, being as effective as gestrinone. Spontaneous pregnancy rates after drug discontinuation were also similar between the mifepristone and the gestrinone groups (Zhang, 2016). Asoprisnil was tested in industry-sponsored phase II trials in the early 2000s and was able to induce amenorrhea, but no further drug development to treat endometriosis has been reported (Fu *et al.*, 2017; Guo and Groothuis, 2018).

Ulipristal acetate

UPA is currently approved for emergency contraception and as a presurgical therapy for symptomatic women with uterine fibroids. For the last indication, its use for 3 months reduces uterine bleeding and fibroid size (Donnez *et al.*, 2014). UPA inhibits human endometrial cell proliferation *in vivo* (Whitaker *et al.*, 2017), and suppresses the growth and development of endometriotic implants in mice (Liang *et al.*, 2018). In the latter study, there was a higher accumulation of antral follicles post-UPA use, probably due to a delay in follicular rupture induced by the drug. However, the regrowth of endometriosis lesions after treatment interruption was slower with UPA than with dydrogesterone or dienogest (Liang *et al.*, 2018).

One of the consequences observed with the exposure to SPRMs is the so-called progesterone receptor modulator-associated endometrial changes (PAECs), characterised by extensive cyst formation, vascular and glandular changes. The morphologic features of PAEC may vary with different SPRMs. Exposure to UPA for a period exceeding 6 months leads to PAEC, but such alterations do not seem to contain cytological atypia and are reversible after the drug discontinuation (Whitaker *et al.*, 2017). There is a paucity of information about the histomorphological effects of SPRMs in endometriosis (Kannan *et al.*, 2018). A case report of a patient that underwent surgical intervention for uterine fibroids after receiving three 90-day courses of UPA showed the presence of PAEC in an endometriotic lesion incidentally found in the surgical specimen. This finding demonstrates histomorphological evidence of a pharmacological effect of UPA in endometriosis tissue (Bateman *et al.*, 2017). Further reassurance comes from the finding that exposure to UPA for over 24 weeks did not lead to suppression of heart and neural crest derivatives expressed 2 protein (HAND2), a transcription factor whose inhibition might predispose to the development of endometrial hyperplasia and adenocarcinoma (Kannan *et al.*, 2018). In spite of a predictable benignity of PAEC, further evaluation is needed to guarantee a safe longstanding use of UPA (De Milliano *et al.*, 2017).

In 2018, the European Medicines Agency (EMA) provided an alert about a serious risk of liver injury caused by UPA and recommended some measures to minimise the negative outcomes. The medication is contraindicated in patients with liver disease; liver tests are required before, during and after the drug administration; repeated therapeutic courses can be offered only to women not eligible for surgery; and patients have to be widely oriented about the risks. Further studies have to be developed in order to clarify the mechanisms of UPA hepatotoxicity and to certify that such prophylactic measures are in fact effective (European Medicines Agency, 2018).

Vilaprisan

Vilaprisan is a new SPRM that differs from other drugs of the same class by a distinct metabolic clearance, with the potential benefit of not presenting liver toxicity. A 2B phase trial evaluated the safety and efficacy of the drug in patients with uterine fibroids during 12 weeks of treatment and a 24-week post-treatment follow-up. Improvement in uterine bleeding, occurrence of amenorrhea and reduction of fibroid volume were observed and a daily oral dose of 2 mg was considered safe for further investigation (Bradley *et al.*, 2019). However, vilaprisan has an antagonistic effect on PR that is five times that of UPA (Schultze–Mosgau *et al.*, 2018), which makes this new SPRM unlikely to become a better option to treat endometriosis.

In summary, although some studies performed with SPRMs in endometriosis showed a potential clinical use for symptom relief (Wagenfeld *et al.*, 2016), there is no drug representative of this class currently approved for clinical use in endometriosis therapy. While mifepristone may have some benefit, mainly by inducing amenorrhea, the available evidence does not allow any conclusion about the therapeutic value and clinical safety of other SPRMs to be used in the long-term treatment of endometriosis (Fu *et al.*, 2017).

Table III New progesterone receptor modulating drugs for endometriosis treatment with study protocols registered in ClinicalTrials.gov

Class	Substance	Route	Trial number	Completed
Progesterone	Progesterone	Subcutaneous	NCT02793908	No
Progestin	Etonogestrel (Nexplanon)	Subcutaneous implant	NCT02669238	No
SPRM?	Danazol	Vaginal ring	NCT00117481	Yes
PR antagonist	PF-02413873	Oral	NCT00800618	Yes
Analgesic + progestin	Low dose naltrexone + norethindrone acetate	Oral	NCT03970330	No

Strategies to Overcome the Therapeutic Shortcomings: Innovative Drug Design, Better Phenotyping, Personalised Medicine

A number of PR-related treatments having endometriosis as inclusion criterion and its symptoms as primary outcomes are under investigation in registered clinical trials (Table III). It is clear that current clinical trials privilege single compounds over combined therapies, which could have synergic effects by hitting hormonal, inflammatory, oxidative stress and pro-fibrotic targets. Novel substances with the potential to gather all these mechanisms in a single product remain unavailable, but not unattainable. The recognition that endometriosis requires a multisided medical therapy contemplating its multifaceted pathophysiology may be the first step towards a real breakthrough in the field.

Although the process of decidualisation involves many PR targets (Gellersen and Brosens, 2014), the transcription factor named promyelocytic leukemia zinc finger (PLZF) emerged as a critical mediator with a pivotal role in inducing the transcriptional reprogramming of endometrial stromal cells that ultimately leads to the decidual phenotype (Kommagani et al., 2016; Szwarc et al., 2018). This transcription factor exemplifies a promising, yet underused strategy to induce endometriosis regression through drugs acting beyond PR and therefore unaffected by progesterone resistance.

Future searches for new medical treatments of endometriosis should include better phenotyping of the included patients, considering the many specificities of each type of lesion and the possible variation in therapeutic results obtained with the same treatment administered to patients with different disease phenotypes (Tosti et al., 2015). Moving beyond the macroscopic disease picture, future therapeutic developments may lead us to consider molecular phenotypes to predict the effectiveness of a drug for every single patient. This personalised medicine approach makes sense in endometriosis, since endometrial stromal cells respond diversely to hormonal treatments according to their constitutive expression of the primary molecular targets of such drugs (Hou et al., 2017).

Conclusions

Progestins have been used to treat endometriosis for over 60 years with large success rates, but still some patients do not respond to this therapy as expected. Progestins depend on the expression of PR to exert their actions in the target cells, but PR expression is often blunted and disrupted in endometriotic foci, which cannot be resolved by simple dose increment or by small modifications in the molecular design of the drug. Combined hormonal contraceptives have the same limitations of isolated progestins, plus a possible counterproductive effect of the estrogenic compound.

Understanding the mechanisms of therapeutic success and failure is essential to guide clinical decisions and inform future research in this field. The development of new molecules for medical treatment of endometriosis should aim at strategies to overcome the resistance mechanisms and aim at new targets. However, current clinical trials consist of old drugs with new delivery (danazol vaginal ring), new drugs with old mechanisms (oral GnRH antagonists) or new mechanisms with old concepts (steroidogenic enzyme inhibitors) (Barra et al., 2019). Innovation is urgently needed and should start by deeper understanding of the disease core features, diverse phenotypes and idiosyncrasies, while moving from pure hormonal treatments to drug combinations or novel molecules capable of restoring the various homeostatic mechanisms disrupted by endometriotic lesions.

Authors' roles

F.M.R., F.P. and F.B. conceived the review. F.M.R., L.M.C. and S.V. performed the bibliographic search and drafted the manuscript. F.P., F.B. and C.C. revised the manuscript for important intellectual content. All authors approved the final version.

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Conflict of interest

The authors report no conflict of interest.

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