EXPERT OPINION

The prolactin receptor as a therapeutic target in human diseases: browsing new potential indications

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Introduction: Prolactin (PRL) signaling has emerged as a relevant target in breast and prostate cancers. This has encouraged various laboratories to develop compounds targeting the PRL receptor (PRLR). As the latter is widely distributed, it is timely to address whether other conditions could also benefit from such inhibitors.
Areas covered: The authors briefly overview the two classes of PRLR blockers, which involve: i) PRL-core based analogs that have been validated as competitive antagonists in various preclinical models, and ii) anti-PRLR neutralizing antibodies that are currently in clinical Phase I for advanced breast and prostate cancers. The main purpose of this review is to discuss the multiple organs/diseases that may be considered as potential targets/indications for such inhibitors. This is done in light of reports suggesting that PRLR expression/signaling is increased in disease, and/or that systemic or locally elevated PRL levels correlate with (or promote) organ pathogenesis.
Expert opinion: The two immediate challenges in the field are i) to provide the scientific community with potent anti-prolactin receptor antibodies to map prolactin receptor expression in target organs, and ii) to take advantage of the availability of functionally validated PRLR blockers to establish the relevance of these potential indications in humans.

Keywords: antagonists, antibodies, atherosclerosis, autoimmunity, cancer, hair, hyperplasia, inflammation, pain, Stat5, targeted therapy, tumorigenesis

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1. Introduction

In the era of personalized medicine, the identification of novel targets is essential in order to propose to each patient a treatment that takes into account the molecular characteristics of his particular disease. Based on the documented role of the prolactin (PRL) system in breast tumorigenesis (see below), the PRL receptor (PRLR) has emerged as one such potential new target. In mammals (including humans), the PRLR is widely distributed [1], which raises two questions. The first one relates to the safety of drugs that may act in many different tissues, if not all. In this respect, reference can be made to genetically-modified mice lacking the PRLR [2]. Although these mice display several phenotypes [1], most of them appear to be relatively mild with no reported impact on lifetime or morbidity. In fact, numerous studies indicate that female fertility and mammary gland development are the sole functions for which PRL is mandatory. In humans, until recently, no inactivating mutations of PRL or its receptor had been reported to evaluate the clinical phenotypes linked to altered/abrogated PRLR signaling. In 2013, Newey *et al.* reported the first loss-of-function mutation of the PRLR in familial hyperprolactinemia (i.e., excess of

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Article highlights.

- According to the wide distribution of its receptor (the prolactin receptor [PRLR]), prolactin regulates many organs and pathophysiological processes.
- The PRLR has emerged as a relevant therapeutic target in breast and prostate cancers.
- Various prolactin analogs and anti-PRLR antibodies have been validated as potent PRLR signaling blockers in preclinical models.
- This review article discusses the potential relevance of fifteen potential indications for such inhibitors.
- Patient stratification based on PRLR expression is currently challenging using commercial antibodies.

This box summarizes key points contained in the article.

circulating PRL) [3]. The existence of oligomenorrhea and infertility in some (but not all) sisters confirmed that the major impact of PRLR signaling deficiency involved reproductive functions. Hence, one should expect that drug-mediated down-regulation of systemic PRLR-signaling in patients should exert no major interference with any physiological function other than female fertility. The second question that arises from the wide PRLR distribution relates to the potential usefulness of anti-PRLR drugs to treat diseases affecting tissues that are not necessarily considered as primary PRL targets in a physiological context (i.e., other than the breast). As described below, several scientific reports suggest that PRLR expression and/or signaling is increased in many disease states, and/or that systemic or local PRL levels correlate with organ pathogenesis. As anti-PRLR compounds have reached late preclinical or early clinical development stages, it is timely to discuss the multiple organs/diseases that may be considered as potential targets/indications for such future drugs.

2. Mechanism of action of the PRLR

The PRLR belongs to the large family of class I hematopoietic cytokine receptors discovered 25 years ago [1]. These are single pass transmembrane receptors devoid of intrinsic enzymatic activity. Signaling is mediated by associated kinases including Janus kinases (mainly [Jak2]), signal transducers and activators of transcription ([Stat], mainly Stat5), the mitogen-activated protein kinase pathway (mainly ERK1/2), Src and phosphatidylinositol 3 phosphate kinase/Akt [1,4,5].

The molecular mechanism of PRLR activation has been reviewed in recent publications [6,7]. More recent studies have shown that this receptor is (at least in part) predimerized at the cell membrane in the inactivated state [8], which is contrary to the old dogma that PRL-induced receptor dimerization was the trigger of intracellular signaling. In fact, the interaction of one PRL molecule with the preformed PRLR homodimer is assumed to induce conformational changes resulting in the initiation of intracellular signaling (**Figure 1A**). The molecular mechanism of activation that has been recently elucidated for the closely-related growth hormone (GH) receptor (Ref. [9] and references therein) may not fully apply to the PRLR [7].

Of note, although PRL is typically a pituitary-secreted hormone that acts on its target tissues via the endocrine route, local PRL production has been documented in many tissues, including in pathological states where its production seems to be increased in many instances [10]. Whether the effects mediated by endocrine versus autocrine PRL are strictly identical, or in contrast, partly differ and/or involve different molecular mechanisms, remains elusive.

Finally, one should remember that, in humans, the PRLR can be activated by three types of ligands: PRL, GH and placental lactogen. Although they all exhibit binding affinity in the nanomolar range, one should note that GH circulating levels are 10-fold lower than PRL levels, and that placental lactogen is almost exclusively produced during pregnancy [11]. The placental GH variant (GH-V), another member of this gene family, does not activate the PRLR.

3. Different classes of PRL inhibitors

3.1 Dopamine agonists

The pituitary is the main source of circulating PRL, and dopamine is the main physiological negative regulator of pituitary PRL production. This inhibitory effect is mediated by the D2 subclass of dopamine receptors [12]. Drugs mimicking dopamine action have been used in hyperprolactinemic patients for 3 decades (see Section 4.1). Bromocriptine was the pioneering compound. Nowadays, dopamine agonists include a family of D2 receptor ligands (cabergoline, pergolide, quinagolide) that have proven to efficiently down-regulate PRL production by the pituitary, and, hence, reduce circulating PRL levels [13]. However, various adverse effects have been documented (e.g., nausea, headaches etc.) [13]. In addition, as stated above, PRL production has been reported in various non-pituitary sites [10]. The current consensus is that dopamine (and hence dopamine agonists) is unable to down-regulate PRL production in extrapituitary sites. Although this statement should be taken with caution due to the paucity of data in this field, it is one of the arguments that have supported the development of alternative drugs targeting PRLR activation rather than PRL production.

In vivo, dopamine is released in the median eminence by the terminals of hypothalamic dopaminergic neurons and is carried to the anterior pituitary by the long portal vessels. The PRLR is expressed in dopaminergic neurons, and PRL has been shown to positively regulate dopamine production [14]. In other words, PRL exerts a negative feedback on its own production by maintaining the dopamine tone. This regulatory loop is obviously of concern regarding the potential opposing effect of drugs aimed at inhibiting PRLR signaling in peripheral tissues.

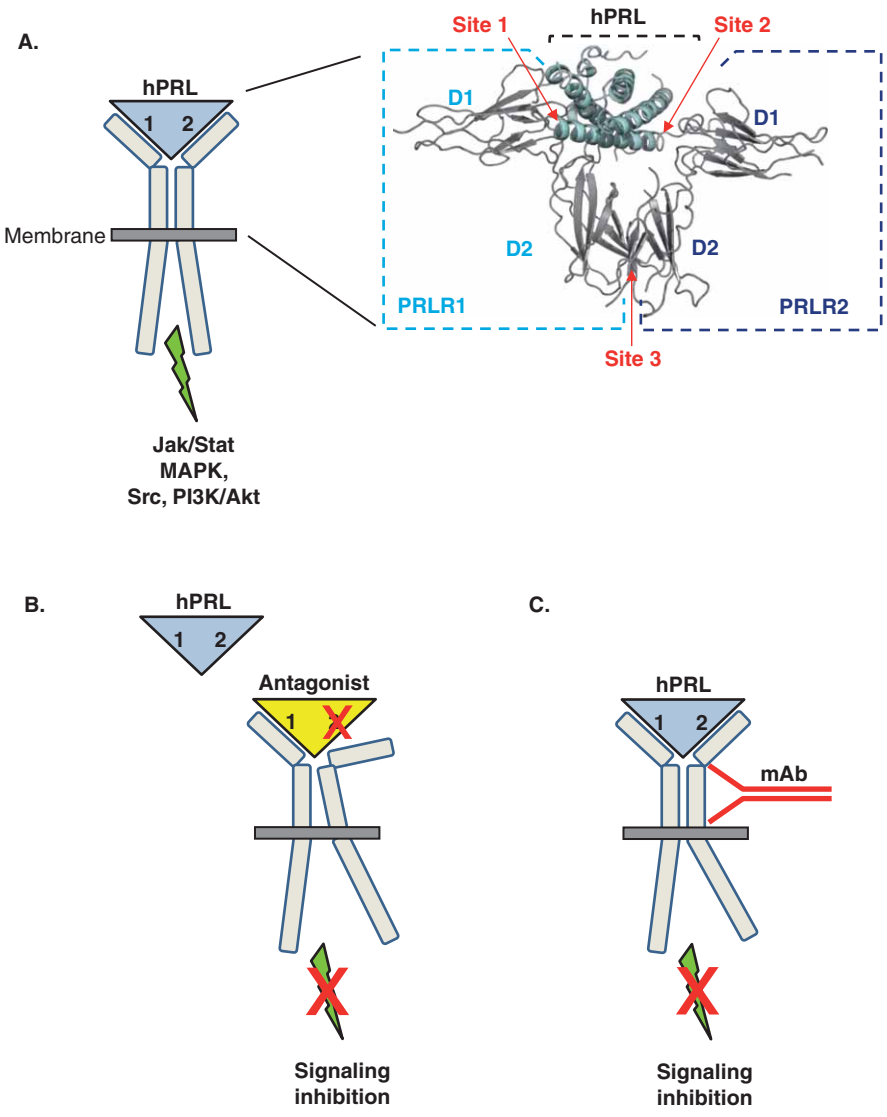

Figure 1. Activation and inhibition of the prolactin receptor (PRLR). (**A**) The activation of intracellular signaling by prolactin (PRL) requires the formation of a ternary complex involving one PRL moiety bound to a PRLR homodimer (referred to as PRLR1 and PRLR2). The crystal structure of this ternary complex involving the PRLR extracellular domain is shown on the right (Protein databank accession code 3NPZ). The latter is made of two domains (D1 and D2) each containing 7 beta strands. PRL is a 4-helix bundle protein that binds to the PRLR homodimer via one high-affinity binding site (site 1) and one low-affinity binding site (site 2). Site 3 involves contacts between the two receptor extracellular domains. (**B**) All PRLR antagonists contain one steric mutation (usually Gly129 to Arg) that abolishes proper interaction of binding site 2 with the PRLR. Such antagonists compete with PRL for binding to the receptor (via site 1), but are unable to activate the PRLR. (**C**) The PRLR neutralizing mAb LFA102 binds to the PRLR in a non-competitive manner. Although it was shown to inhibit downstream signaling, the actual molecular mechanism is unknown.

3.2 PRLR antagonists

In 1996, our laboratory was the first to report the antagonistic properties of an engineered human PRL (hPRL) variant [15]. This pioneering compound called G129R-hPRL involved the steric Gly129-to-Arg mutation within the low-affinity binding site 2 of hPRL to the PRLR homodimer (**Figure 1B**). As a result, G129R-hPRL leads to the formation of a signaling incompetent ligand/receptor complex. A few years later, we developed Del1-9-G129R-hPRL by deleting the 9 N-terminal amino acids of G129R-hPRL. This deletion was performed to abolish the residual agonistic activity of G129R-hPRL that was detected using sensitive *in vitro* cell-based assays [16]. As indicated below, Del1-9-G129R-hPRL has been used by many groups in many experimental systems which all confirmed its pure antagonistic properties. Other variants have been developed by others on the hPRL core.

They include i) the combination of G129R-hPRL with other functional proteins, for example, endostatin or interleukin-2, or ii) S179D-PRL, a molecular mimic of phosphorylated PRL that was initially described as a competitive antagonist before being recognized as a selective PRLR agonist that signals to antagonistic ends. We invite the reader to look at dedicated reviews for detailed composition and properties of these compounds [6,17,18].

The double weakness of PRL core-based antagonists involves their lower binding affinity compared to hPRL (~ 10 fold) and their short half-life *in vivo* (< 1 h) due to rapid renal clearance (their 23 kDa size is below the renal glomerular cut-off). By inserting mutations within the competent binding site 1, a high affinity version of G129R-hPRL was recently developed and validated in cell-based assays [19]. Others coupled G129R-hPRL to albumin binding peptides, which was shown to improve pharmacokinetics *in vivo*; this however was at the expense of its potency (decreased by 5-fold *in vitro*) [20]. We recently developed a long half-life prototype of Del1-9-G129R-hPRL by polyethylene glycol (PEG) coupling. Although this modification decreased the affinity by 5 – 10-fold, mouse experiments showed that PEGylated Del1-9-G129R-hPRL remained active up to 48 h after a single injection, and was detectable in serum up to 72 h (unpublished data). Of note, PEGylation and mutations increasing binding affinity are strategies that were successfully combined to develop the GH receptor antagonist (Pegvisomant) used to treat acromegalic patients since the early 2000s [21].

3.3 PRLR neutralizing antibodies

We are aware of two companies that have developed neutralizing monoclonal antibodies (mAb) directed against the human PRLR. The first one (LFA102) was recently reported by Novartis in two academic publications [4,22], whereas information of the mAb developed by Bayer (Mat3) can be obtained only from their patents (Ref [23] and citations therein). Basically, both mAbs were shown to efficiently down-regulate PRL-induced activity *in vitro* and *in vivo*. Of note, the preclinical validation of LFA102 in cancer models involved the inhibition of endogenous mammary tumors (i.e., non-human tumors) in long-term treated rodent models, whereas evidence for PRLR inhibition in human systems has been restricted to short-term inhibition of PRL-induced Stat5 signaling in breast cancer cells cultured *in vitro* or xenografted into immunocompromised mice [22]. As expected, LFA102 was shown to increase circulating PRL levels in rats, suggesting interference with negative feedback mechanisms [22]. In contrast to competitive antagonists (Section 3.2), LFA102 does not compete with PRL binding to the receptor (**Figure 1C**). Although its actual mechanism of action remains uncharacterized, this suggests that LFA102 efficacy may rely more on the mAb/PRLR molar ratio in target tissues than on PRL levels. Whether this provides an advantage over competitive antagonists should be addressed in dedicated experiments. Clearly, one advantage of antibodies over PRL-core antagonists is their long half-life due to their bigger size (~ 150 kDa), which reduces renal clearance.

3.4 Small molecule inhibitors

Although some academic groups have initiated the search for small molecule inhibitors [24], we are not aware that any of them has been published or has achieved a proof-of-concept of *in vivo* efficacy. Although the binding affinity of small molecules is often in the micromolar rather than the nanomolar range, one of the advantages of such compounds over biomolecules is the easier production at much lower cost.

4. Potential indications for PRLR inhibitors

As stated above, the main goal of this review article is to discuss various pathological contexts for which there is a medical need that may potentially benefit from these new PRLR signaling inhibitors. Obviously, the prerequisite to be eligible as a candidate target tissue for such compounds is to express the PRLR. As this will be discussed in Section 5, the poor quality of commercial antibodies has been a recurrent obstacle for accurate (reliable) determination of the PRLR expression status in health and disease. Therefore, potential indications that are supported only by increased PRLR levels in pathological states should be considered with caution.

4.1 Dopamine-resistant hyperprolactinemia

Hyperprolactinemia is defined as circulating PRL levels above the normal range (~ 8 – 23 ng/ml in women). The most common cause of pathological hyperprolactinemia involves prolactinomas, that is, pituitary tumors affecting PRL-secreting cells (lactotropes); another important cause is antipsychotic medication. The outcomes of hyperprolactinemia are suppression of reproductive function, osteopenia (due to suppressed sex steroids), and, in some instance growth of the pituitary tumor in the case of an aggressive disease. Dopamine agonists are the first line therapy of prolactinomas. However, 10 – 15% of patients do not fully respond, or display dopamine resistance, or are intolerant to the drug. Alternative strategies exist to treat the pituitary tumor (surgery, radiotherapy) but are not always applicable. In non-normalized patients, female fertility can be restored by hormone-replacement therapy or ovulation induction therapy, and osteopenia can be treated by hormone therapy or bisphosphonates. However, these medications circumvent the dysfunctions induced by hyperprolactinemia in a limited set of tissues while maintaining PRL activity above physiological levels in target tissues. As increased cancer risk has been associated with high-normal PRL levels (breast cancer; see Ref. [25]) and hyperprolactinemia (see Ref. [26] and sections below), reducing PRL activity in patients with non-normalized PRL levels may be valuable. Given the high prevalence of prolactinomas in the general population, this represents a non-negligible number of patients.

By analogy to acromegalic patients who display somatostatin resistance (~ 40%) and are therefore candidates for GH antagonist therapy [21], dopamine-resistance may be considered as an indication for anti-PRLR drugs. However, in contrast to acromegaly, hyperprolactinemia is not a life-threatening and debilitating disease therefore the use of expensive drugs is economically questionable. Furthermore, assuming that large compounds such as anti-PRLR antibodies or PEGylated antagonists reach the central nervous system (which has not yet been demonstrated), they may have adverse effects on the pituitary tumor by interfering with i) the negative PRL feedback loop at the hypothalamic level, leading to further elevation of circulating PRL levels as observed in preclinical models [22]; and ii) the anti-proliferative and pro-apoptotic effects of PRL on pituitary cells (for review, Ref. [27]). Against this hypothesis, prolactinomas were not detected in familial hyperprolactinemia due to mono-allelic loss-of-function PRLR mutation [3], suggesting that partial inhibition of PRLR signaling, as expected to occur using anti-PRLR drugs, may not necessarily result in pituitary growth. Regardless, similarly to acromegalic patients treated with the GH antagonist Pegvisomant, the evolution of adenoma volume should be a concern.

In conclusion, although treating dopamine-resistant prolactinomas with drugs targeting PRLR signaling is scientifically sound, medical (pituitary volume) and economical (cost/benefit) issues should be carefully evaluated.

4.2 Female reproductive tissues

4.2.1 Cancer

Before discussing the indications *per se*, it is worthwhile to mention that the design of experimental settings to assess the anti-tumor potency of anti-PRLR compounds is challenging. One of the gold standards for drug validation in oncology involves xenografts of human immortalized cancer cell lines or of patient-derived tumors into immunocompromised mice. Although attractive, these models face several limitations when applied to the PRL field. First, the human PRLR is insensitive to most non-human PRLs, including mouse PRL [28], therefore such heterologous *in vivo* models fail to provide the tumor with the physiological level of endocrine PRL stimulation. Second, extrapituitary PRL production that has been described in many human tissues [10] appears to be more rarely detected in immortalized human cell lines than initially expected [29]. As a result, human xenografts may have to grow in an environment virtually lacking both endocrine and autocrine PRL stimulus, which may prevent anti-PRLR drugs from having marked effects. Finally, with the notable exception of the rat Nb2 cell line, we are not aware of immortalized cancer cell lines that are dependent on PRL signaling for growth. This may be due to the fact that these cell lines have been cultured for decades in media containing bovine PRL (present in fetal calf serum) as the source of lactogen; as mentioned, bovine PRL is a poor agonist of the human PRLR, which may have contributed to select cells that are not dependent on PRL for survival/growth. These limitations have been well discussed in the paper reporting on LFA102 mAb, whose inhibitory properties towards human breast cancer cells *in vivo* were assessed only by demonstrating its capacity to prevent PRL-induced activation of Stat5 phosphorylation in short-term experiments [22]. The recent development of PRL-humanized mice (knock-in strategy) by the group of Gregerson [30] should solve this inter-species incompatibility.

4.2.1.1 Breast cancer

Hundreds of papers have addressed the role of PRL signaling in breast cancer. To make a long story short, arguments have accumulated since four decades to support the pro-tumor role of PRL on the breast based on its ability to stimulate breast cancer cell proliferation, survival, motility and chemoresistance of immortalized breast cancer cell lines [5,6,31]. This is in agreement with the increased risk of estrogen receptor-positive breast cancer associated with PRL levels in the high-normal range [25] and the observation that systemic or mammary PRL over-expression in mouse models leads to mammary tumorigenesis (for a review, see Ref. [32]). However, an alternative hypothesis has emerged more recently based on clinical observations suggesting that the activation of Stat5, the major signaling pathway downstream of the PRLR, is of good prognosis in breast cancer patients [33]. At the cellular level, two independent groups have shown that this effect was potentially linked to the intrinsic (physiological) capacity of the PRL/Stat5 pathway to promote mammary cell differentiation, which may contribute to maintain a certain level of differentiation of breast cancer cells, to promote their homotypic adhesion and prevent epithelial-to-mesenchymal transition [34,35]. It should be mentioned, however, that PRL also activates other signaling pathways in breast cancer cells, some of which (e.g., Src) have been shown to promote tumor cell growth depending on extracellular matrix stiffness [36]. To reconcile such versatile roles of PRL signaling in breast cancer cells, one tentative model suggests that PRL may participate in tumor initiation, whereas in established breast cancer, it may contribute to reduce aggressiveness and dissemination [37].

If true, abrogating PRLR signaling in patients diagnosed with breast cancer may be counter-productive, at least without careful patient stratification. Clearly, additional studies are required to elucidate the complexity of PRL effects in breast cancer. Of note, older studies showed no improvement in long term-survival or disease-free interval in breast cancer patients treated with bromocriptine (for a review, see Ref. [5]). These disappointing results were subsequently used to support the involvement of autocrine/paracrine more than endocrine PRL in disease progression. The availability of potent PRLR-targeting drugs (antagonists, neutralizing antibodies) now allows addressing this hypothesis. The ongoing Phase I study aimed at evaluating LFA102 anti-PRLR antibody (Novartis) in patients with PRLR-positive metastatic breast cancer (trial #NCT01338831) will be the determinant

for delineating the actual outcome of PRLR signaling in this type of human breast cancer.

4.2.1.2 Ovarian and endometrial cancers

PRL levels are elevated in patients with endometrial cancer or ovarian cancer, with no differences between early- or late-stage diseases [38]. Accordingly, PRL was among a four circulating protein signature that was shown to discriminate between disease-free and epithelial ovarian cancer patients [39]. Expression of both PRL and its receptor were increased in endometrial and ovarian cancer samples, arguing for the involvement of an autocrine loop in addition to endocrine over-stimulation [38]. Of note, high PRL/PRLR expression was also reported to be associated with cell survival in cervical cancer [40]. *In vitro* studies using immortalized endometrial and ovarian cancer cell lines have indicated that PRL promotes cell proliferation, survival and invasion [38,41,42]. Furthermore, PRL was shown to transform immortalized normal human ovarian epithelial cells through activation of the Ras pathway [38]. Finally, a recent study demonstrated that blockade of PRLR signaling in epithelial ovarian cancer using G129R-hPRL antagonist led to autophagy-mediated programmed cell death [43].

In summary, there is emerging evidence that PRL signaling may play a role in ovarian and endometrial tumorigenesis, although the causality was not formally demonstrated. This potential indication warrants further investigation to establish its relevance.

4.2.2 Benign disorders

4.2.2.1 Benign breast diseases

The etiology of benign breast diseases (BBDs) is currently poorly understood. Several endocrine factors (mainly estrogens and progesterone) have been suspected to be involved with little impact on therapeutic management of the patients, which remains empirical. PRL is part of the list of hormones that may be linked to BBD etiology [44]. However, although BBDs are frequently associated with high PRL levels, causative evidence is lacking. Some years ago, we identified the first gain-of-function PRLR genetic variant (PRLR-I146L) in a rare BBD named multiple breast fibroadenoma. Based on the higher prevalence of PRLR-I146L in the patient versus the control cohorts, a potential association was suggested, although a causative link could not be demonstrated here as well [45]. Of note, we showed that Del1-9-G129R-hPRL was able to down-regulate the basal activity exhibited by PRLR-I146L in reconstituted cell-based assays. This indicates that, in addition to competing with PRL for PRLR activation (**Figure 1B**), Del1-9-G129R-hPRL can, in some instances, act as a reverse agonist.

In conclusion, although it appears relevant to consider PRLR signaling as a candidate in BBD etiology, there is currently limited-suggestive evidence for a causative role.

4.2.2.2 Endometriosis/adenomyosis

Endometriosis is characterized by the presence of ectopic endometrium either in the pelvic cavity (endometriosis externa) or within the uterus (endometriosis interna, adenomyosis). Therapeutic management of this chronic disease is symptomatic and not curative, and includes estro-progestins, progestins, GnRH agonists and surgery. Many of them have important side effects (venous thromboembolism, bone loss, hot flushes, body weight gain, depression) and are often not able to provide pain relief. There is thus a strong medical need.

Association of elevated PRL levels with endometriosis has been suggested [46]. Although some authors showed that the PRLR mRNA was detected in normal endometrium but rarely in endometriotic lesions [47], Bayer's group reported totally opposite data supporting upregulation of PRLR (and PRL) expression in human endometriotic lesions [23]. Using a mouse model of PRL-induced adenomyosis, the same group reported the protective effect of their neutralizing PRLR mAb Mat3 [23].

Although the data are still limited, and mainly involve animal models that may not perfectly reflect the human disease, endometriosis is probably an interesting indication to consider further for anti-PRLR drugs, especially because there is a real medical need.

4.2.3 Contraception

Oral combined contraceptives (estrogens + progestins) are not recommended for some categories of women (overweight, smokers, women suffering from lupus, etc.). Furthermore, oral contraceptives increase venous thromboembolism risk [48]. Progestin-alone contraceptives can cause irregular bleeding patterns, spotting and amenorrhea. There is thus a real medical need to develop new contraception without vascular risk.

Both hyperprolactinemia (see Section 4.1) and hypoprolactinemia interfere with female fertility. Mice deficient for the PRLR are infertile [2], and mice with reduced PRLR signaling due to Del1-9-G129R-hPRL expression are fertile but display irregular cycles [27]. In humans, as stated above, loss-of-function PRLR mutations correlate with reproduction failure in some patients [3]. Together these observations support the notion that PRLR inhibitors able to completely abolish, and not only to interfere with, female fertility could be used as contraceptives. The possible advantage of such compounds includes absence of above-mentioned side effects, no restriction of the targeted population, and non-proliferative action on the breast epithelium as opposed to estrogens and progestins.

Using PRLR signaling inhibitors as contraception is thus scientifically relevant, but the elevated cost of such drugs to be used chronically probably renders this indication unrealistic. This indication may be reconsidered once low-cost small molecules are available.

4.3 Male diseases

4.3.1 Prostate cancer

Epidemiological evidence indicates that circulating PRL levels are not linked to prostate cancer risk [49]. Moreover, prostate cancer risk seems to be decreased in hyperprolactinemic patients, but this may be a consequence of concomitant hypoandrogenism in these patients as a result of PRL interference with gonadotropin-releasing hormone pulsatility [26]. Although these observations do not argue for a role of endocrine PRL in prostate tumorigenesis, there is a large body of evidence showing that local expression of PRL and/or Stat5 activation is increased in human prostate cancer (see Ref. [50] and references therein for statements below). This observation correlates with disease progression and predicts early recurrence. Experimental studies have demonstrated the ability of PRLR/Stat5 signaling to promote proliferation and progression of established prostate cancer, for example, using immortalized cell lines xenografted into mice. In addition, there is genetic evidence for amplification of PRLR and Stat5 loci in some prostate cancer specimens. Finally, PRLR over-expression has been documented in ductal prostate cancer compared to acinar adenocarcinoma (the most frequent form of prostate cancer), suggesting that PRLR signaling may be particularly relevant to the etiology/progression of this subtype of prostate cancer.

Of interest, cell-based and tissue explant studies have highlighted the functional synergy between PRL/Stat5 and androgen receptor (AR) pathways [51], which was later demonstrated to be mediated by physical interaction between Stat5 and AR [52]. The synergy was reciprocal, as activated Stat5 was shown to favor AR activity independently of androgen stimulation, whereas ligand-bound AR enhanced Stat5 activity regardless of Stat5 phosphorylation [52]. One of the results of this synergy was the increased survival of prostate cancer cells in culture shown to involve increased nitric oxide production via upregulation of carboxypeptidase-D [53]. Of note, these effects were abolished only when cells were co-treated using a combination of antiandrogens (flutamide) and Del1–9-G129R-hPRL [54].

The current challenge of prostate cancer therapy involves the advanced stages of the disease, that is, when cancer escapes androgen ablation therapy. As AR expression persists in such a context, the crosstalk between AR and Stat5 signaling might be critical for prostate tumor recurrence and progression. Supporting this possibility, we recently showed that increased PRLR/Stat5 signaling in the mouse prostate led to marked amplification of basal/stem cells [55,56], which are androgen-independent and have the capacity to generate tumors in transplantation models when transformed by oncogenes relevant to prostate cancer etiology [57]. Of note, chronic hyperprolactinemia was shown to induce mild hyperplasia of dorsolateral prostate in castrated mice, suggesting that PRL can exert growth-promoting effects in the absence of androgens [58].

Taken together, these data suggest that the combination of antiandrogen and anti-PRLR signaling targeted therapies may be relevant for the treatment of advanced prostate cancer. The Phase I study of LFA102 mAb (Novartis) in patients with PRLR-positive castration-resistant prostate cancer should help determine the strength of this indication.

4.3.2 Benign prostate hyperplasia

In rats, marked enlargement of the lateral prostate was observed when chronic hyperprolactinemia was pharmacologically induced using dopamine antagonists that lead to elevated systemic PRL levels [59]. In mice, systemic over-expression of a PRL transgene (called Mt-PRL mice) led to benign prostate hyperplasia (BPH), including stromal hyperplasia and focal areas of epithelial dysplasia (prostate intraepithelial neoplasia) [60]. These effects were shown to be independent of elevated androgen levels [58]. Prostate-specific expression of a PRL transgene in mice (so-called Pb-PRL mice) resulted in very similar phenotypes, including dramatic prostate hypertrophy of the three lobes [55,61], stromal inflammation [62] and urinary retention [63]. We showed that prostate hypertrophy and associated histological defects were closely linked to increased Stat5 signaling [55,56]. Thus, there is now a large body of evidence supporting the role of PRL/Stat5 as a mitogen for prostate epithelial cells able to initiate BPH in rodents and promote progression of established prostate cancer (see Section 4.3.1).

Such a proliferative effect of PRL on human prostate epithelial cells was also reported using primary cultures derived from BPH specimens [64]. However, expression of PRLR was not found to be increased in BPH compared to normal prostate samples [65] but it must be stressed that the latter study was performed using an anti-PRLR mAb that was later shown to be poorly specific [66]. At the epidemiological level, small case-control studies failed to correlate circulating PRL levels with BPH [67]. Additionally, a more recent prospective, case–control study involving 20 hyperprolactinemic young men and 20 healthy controls concluded that PRL excess had no effect on prostate volume [68]. Again, this may be due to the fact that hyperprolactinemia is accompanied by low testosterone and dihydrotestosterone levels, a major risk factor for prostate growth.

In conclusion, although animal models converge to support increased PRL/Stat5 signaling as a potential mechanism for BPH induction, this pathway is very poorly characterized in the human disease. These are ongoing studies in our laboratory that should help to delineate the potential relevance of this pathway in human BPH etiology. Given the huge population targeted (half of men aged 50 years present with BPH), positive results should position BPH as an interesting indication for PRLR inhibitors.

4.3.3 Prostatitis

There is increasing evidence for the association of chronic prostate inflammation (prostatitis) with prostate tumorigenesis in men. It has also been proposed that BPH is an

inflammatory disease, based on the observation that BPH nodules are often associated with chronic inflammatory infiltrates mainly composed of chronically activated T cells and macrophages. Although involvement of PRL in human prostatitis has yet to be established, mild-to-moderate chronic inflammation involving lymphocyte and macrophage infiltrates has been reported in prostates of Pb-PRL transgenic mice [61,62]. The mechanisms underlying this prostate phenotype are unknown. PRL has been suggested to mediate estrogen-induced prostate inflammation in rodents. Interestingly, no inflammation was observed in estrogen-deficient aromatase knockout mice, despite their elevated levels of circulating PRL [69]. This suggests that elevated PRL alone may not be sufficient to exert pro-inflammatory effects. Accordingly, the anti-inflammatory and anti-estrogenic actions documented for androgens in a rat model of non-bacterially-induced prostate inflammation were proposed to be partially mediated through decreased PRL-induced responses [70].

In conclusion, although animal studies support a role of PRL in prostate inflammation, there is clearly a lack of data for human prostatitis. Additional studies are required to link the status of PRL/PRLR expression and Stat5 signaling with human prostate inflammation.

4.4 Other tumors

4.4.1 Gastrointestinal tumors

PRLRs are present in the gastrointestinal (GI) tract of various species. In rabbits, the most intense immunoreactivity was associated with the esophageal epithelium, chief (zymogenic) cells of the fundic mucosa, pancreatic islets of Langerhans and surface epithelial cells of the duodenum and jejunum [71]. In humans, PRLR expression was observed throughout the cancerous progression of the colonic and gastric mucosa from adenomas to colonic liver metastasis and GI cancer cell lines at various stages of growth and differentiation [72].

The association of GI cancers with PRL levels comes from a recent epidemiological study [26] that investigated the cancer risk in a cohort of 969 hyperprolactinemic patients (in the vast majority normalized at the time of follow up) versus 9618 control subjects. This is the largest study ever reported for hyperprolactinemia, which may explain why diseases proposed to be associated with high circulating PRL levels were missed in earlier studies involving smaller cohorts. In the Berinder *et al.* study, there was an overall increased cancer risk in the hyperprolactinemic population (HR 1.31; 95% CI: 1.02 – 1.68), that was mainly attributed to an increased risk of upper GI cancer (HR 3.69; 95% CI: 1.70 – 8.03). This risk applied to both genders. Even before hyperprolactinemia diagnosis, there were more hyperprolactinemic patients than expected with upper GI cancer, with preponderance for head and neck cancers. This finding is intriguing and the mechanism unknown [26].

Others have reported that patients with advanced tongue cancer had higher PRL levels compared with controls and that PRL in a multivariate analysis was an independent prognostic risk factor of survival [73]. These authors also showed that tongue cancer cells produce PRL, possibly acting as a major local growth promoter by means of autocrine and paracrine mechanisms as the PRLR was also detected in several cases.

Finally, high PRL levels have been proposed to be an early marker of colon cancer recurrence, but the causality was not demonstrated in these studies [74]. In a recent study, higher levels of PRLR expression was observed in colon cancer samples and cell lines compared to normal colonic epithelial cells. PRL stimulation was found to induce various components of Notch signaling, which is known to regulate colorectal cancer stem cell populations [75].

Taken together, these results suggest that PRL may be an active player in GI cancer and may constitute a new interesting indication to investigate further for PRLR signaling inhibitors.

4.4.2 Hematologic tumors and leukemia

Acute myeloid leukemia (AML) is a neoplasia characterized by the rapid expansion of immature myeloid blasts in the bone marrow. Current treatments of AML include chemotherapy and allogeneic hematopoietic cell transplantation. There are significant side effects, and, despite remission rates of > 50%, most patients relapse and die within 2 years. There is thus a need for alternative/complementary treatments.

PRL is involved in the regulation of the immune and haematopoietic systems, and there is a large body of experimental evidence supporting that PRL regulates leukemia and hematopoietic tumors [76]. For example, Clevenger *et al.* suggested that PRL plays a role in the proliferation/survival of blood cells [77]. Moreover, an autocrine/paracrine mechanism has been suggested based on the fact that AML cells produce PRL [78]. These older observations corroborate the recent epidemiologic study mentioned above [26], which identified hematopoietic cancer in females as the second most frequent cancer that could be linked to hyperprolactinemia (HR 3.51; 95% CI: 1.06 – 11.6).

In summary, immune and hematopoietic tumors appear to be relevant indications for PRLR signaling inhibitors.

4.5 Non-tumor indications

4.5.1 Hair loss

Treatment of hair loss is still an unmet need. Drugs such as finasteride (an androgen pathway inhibitor) are used for the treatment of androgenetic hair loss ('androgenic alopecia'), whereas glucocorticoids are used for alopecia areata. These treatments have side-effects (finasteride: libido loss and impotence in men; glucocorticoids: diabetes, weight gain, osteoporosis), and the problem of treating hair loss is far from being solved.

Several studies involving mouse or human models have shown that PRL regulates hair cycle (for review, see Ref. [79]). Thanks to the use of Del1-9-G129R-hPRL

antagonist, the involvement of autocrine/paracrine PRL could be demonstrated in human explant cultures [80]. Shaving adult rodents induces the shift from telogen (resting phase) to anagen (i.e., growing phase). In such models, PRL was shown to exert an inhibitory role on hair regrowth, which was recently proposed to involve the maintenance of hair follicle stem cells in quiescence [81]. Accordingly, the Bayer neutralizing PRLR antibody was shown to stimulate hair regrowth after shaving hyper- and normo-prolactinemic male and female mice [23].

In humans, the situation is obviously more complex than in rodents as the response of human scalp hair follicles appears to be highly gender- and/or location-dependent [82]. Although it may be deduced from one report that PRLR signaling inhibitors should be useful for treating male androgenic alopecia [83], such interpretation could actually be misleading as that study involved occipital male scalp hair follicles, which are not lost during male pattern balding and are androgen-insensitive. Finally, although hyperprolactinemia has been tentatively proposed to be associated with human hair loss, a recent epidemiological study concluded with the absence of causative evidence [84].

Given the huge inter-species differences in this field, simplistic animal-to-human extrapolation cannot support the potential benefit of PRLR signaling inhibitors to treat human hair loss disorders.

4.5.2 Pain

Clinical pain management is a considerable challenge in health care. The identification of the superfamily of transient receptor potential (TRP) cation channels, particularly TRPV1 and TRPA1, has shed light on the molecular basis of pain signaling during inflammatory conditions. TRPV1 and TRPA1 are considered as potential targets in the treatment of inflammatory pain due to their ability to be activated by nociceptive signals and sensitized by pro-inflammatory mediators, including in the context of visceral inflammation and pain in the GI and urinary tracts [85]. Apart from TRPV1, the pharmacology of this channel family is still insufficiently known [86].

It is well documented that PRL is elevated in serum during many pain conditions, including postoperative pain [87,88], migraine [89] and cancer pain [90]. The group of A. Akopian discovered that PRL was also locally elevated during certain pain conditions [88,91]. Importantly, such extrapituitary PRL was elevated more in females than males [88,91,92]. Of interest, peripheral and spinal blockade of PRL action using Del1-9-G129R-hPRL produced analgesia for all modalities for females, but not males [92]. We are not aware of other examples for sex-specific pain treatment.

PRL acts on several identified nociceptors ('pain' receptors), that is, TRPV1, TRPA1 and TRPM8 [91]. These calcium channels are located (among others) on peripheral nerve terminals. When activated (e.g., by inflammation), they induce nerve terminal depolarization triggering neuronal excitation, which results in pain sensation. PRL has no effect by itself on neuronal excitability, but it potentiates these channels resulting in a lower pain detection threshold, hence an increased pain sensation for a given stimulus. Of interest, Del1-9-G129R-hPRL prevented/reversed PRL effects involving TRPV1 [93]. Furthermore, inflammation at the site of injury led locally to huge PRL release (autocrine/paracrine effect), and our Del1-9-G129R-hPRL almost totally reversed PRL-induced sensitization of capsaicin (TRPV1 activator) responses in rat sensory neurons in both genders [92].

Although the amount of evidence in the field is still limited, this discovery opens the doors to the potential relevance of using PRLR inhibitors for sex-gender specific treatment of a variety of acute or chronic pain conditions, including postoperative pain, cancer pain and migraine.

4.5.3 Autoimmune diseases

PRL acts on innate and adaptive immunity, consistent with the wide distribution of its receptor in cells of the immune system. Many autoimmune diseases have been associated with PRL [94]. Importantly, transcriptomic analyses revealed that PRL over-expression in the mouse prostate (Pb-PRL mice) affected the expression of a series of genes linked to autoimmunity, including genes identified as autoimmune disease markers (complement C3 up, its inhibitor CCRY down, two markers of *myasthenia gravis*) [95]. The fact that such a regulation was observed in the prostate, that is, a non-immune tissue (although inflammatory cells are present in Pb-PRL prostate tumors – see above) that is not known to be a target of autoimmune diseases, probably underlines the general property of PRL signaling to regulate autoimmune-related genes.

Systemic lupus erythematosus (SLE) is probably the hallmark of PRL-associated autoimmune diseases [96]. The association relies on hyperprolactinemia reported in a high proportion of patients, and on the higher prevalence of SNPs, for example, G1149T polymorphism in the distal promoter of the PRL gene (which is assumed to direct extrapituitary PRL expression) leading to higher transcriptional activity [97]. This association, however, was not confirmed in another study [98]. Thanks to our human PRLR-specific bioassays, we could demonstrate that elevated serum bioactive PRL levels are associated with SLE activity, as well as with specific organ involvement [99].

Psoriasis is a skin autoimmune disease that connects immune and skin regulations, both of which involve (local) PRL [79]. Recent studies suggest that PRL levels, either systemic or local, may be correlated to psoriasis. This field has been ill-explored until now; the recent review by Paus *et al.* nicely summarizes the data currently available [100].

In summary, autoimmune diseases are presumably a field of interest for PRLR inhibitors. However, connections with diseases such as SLE have been discussed for a while in the literature, with no translation into the clinics. In contrast, psoriasis probably deserves to be explored further.

4.5.4 *Cardiovascular diseases*

The relationship between the occurrence of cardiovascular diseases and endocrine disorders has long been acknowledged. However, as summarized below, the role of PRL in this process remains elusive. Extensive discussion of this issue and relevant references can be found in a recently published review article [101].

Atherothrombosis, that is, narrowing of the arteries as a result of the deposition of cholesterol and other lipid substances within the arterial wall, is the major cause of cardiovascular diseases. Recently, PRLR expression has been documented in human atherosclerotic plaques, more precisely in T cells and macrophages located near the lipid core and shoulder regions of the plaques. Whether PRL stimulation of these macrophages results in a pro- or anti-inflammatory response is unknown. As local PRL expression was not detected in atherosclerotic plaques, the involvement of endocrine probably more than local PRL as a modulator of plaque formation and potentially also of the events occurring after rupture of the plaque could be suggested.

A number of patients with prolactinomas display distinctive clinical features that may be associated with the metabolic syndrome, although one should note that this association has not been recognized in all clinical studies. Of note, several metabolic parameters improve significantly after treatment with dopamine agonists, further suggesting a role for endocrine PRL. However, as such treatments also reverse the hypogonadotropic state of the patients, these observations do not definitely identify PRL as an independent cardiovascular risk factor. In this matter, we recently showed that systemic expression of Del1-9-G129R-hPRL in the atherosclerosis-susceptible LDL receptor knockout mouse model improved the plasma atherogenic index (decrease in plasma cholesterol levels upon feeding a Western-type diet), but did not alter the susceptibility for initial development of atherosclerotic lesions [102]. Local administration of PRLR inhibitors in such preclinical models may help further elucidate the actual involvement of PRLR signaling in atherosclerosis.

In conclusion, although PRL may have the potential to contribute to cardiovascular diseases through modulation of local cellular processes taking place within atherosclerotic plaques and/or of conventional cardiovascular metabolic risk factors, it is likely premature to consider cardiovascular diseases as an indication for new anti-PRLR drugs.

4.5.5 *Lymphangioleiomyomatosis*

Lymphangioleiomyomatosis (LAM) is a chronic cystic lung disease. It is characterized by uncontrolled proliferation of smooth muscle-like LAM cells due to bi-allelic inactivating mutations of the tuberous sclerosis complex gene resulting in over-activation of the mTORC1 pathway [103]. In sporadic cases, LAM can be accompanied by renal angiomyolipomas and retroperitoneal lymphadenopathy. Clinical trials have shown that the mTORC1 inhibitor Sirolimus could stabilize pulmonary function in moderately severe LAM, but that this beneficial effect was lost after drug discontinuation.

The involvement of endocrine components in disease progression has been suspected based on the fact that this pathology affects almost exclusively women. Estrogen and other steroid hormones have been proposed as top candidates (see Ref. [103] and references therein). A single recent study [104] suggested that PRL signaling could also promote LAM disease based on: i) the association of elevated PRL levels with disease progression in patients; ii) elevated PRL and PRLR expression (mRNA and immunoreactivity) in LAM lesions compared with vascular smooth muscle cells in the same region of the tissue; and iii) increased PRLR expression, activation of PRLR signaling pathways and PRL-induced proliferation of cells lacking tuberous sclerosis complex 2 gene.

Although currently available data are restricted to a single study, this indication should be considered further based on the medical need for the treatment of this pathology.

5. Expert opinion

PRL is a versatile hormone. Due to the wide distribution of its receptor, it acts on a wide variety of tissues. However, with the exception of female reproductive functions, it often acts as a physiological modulator rather than a master regulator. In other words, as nicely reflected by the numerous phenotypic studies of PRLR knockout mice, the absence of PRLR signaling does not seem to have any dramatic consequences other than on female reproductive functions. In pathology, and more specifically in the cancer context, elevated PRL levels and/or increased expression of PRLR and/or PRL in the tumor have been documented (more often at the mRNA level than protein level – see below), and this has been used to support the notion of increased PRLR signaling via endocrine and/or autocrine/paracrine routes. Whether this is a cause or a consequence of the pathological process is unknown. Cell and animal studies have shown the capacity of enforced PRLR signaling to induce transformation in some tissues (e.g., mammary gland, ovary). In humans, although epidemiological evidence suggest that high PRL levels are correlated with increased cancer risk [25,26], this does not formally demonstrate a causative link. Regardless, as PRLR signaling involves pathways commonly activated in cancer (Jak/Stat, MAPK, PI3K/Akt, Src), the participation of increased PRLR signaling in disease progression is likely, which is the point regarding the therapeutic relevance of targeting this receptor.

This review article browses a list of potential indications that extends far beyond cancer and includes a series of benign disorders. Some of them have been documented for years, whereas others have recently emerged and are supported by a much weaker array of evidence (**Table 1**). In many instances, evidence comes from animal models showing that increased PRLR signaling induces/cooperates to disease development/progression. These studies face the limits of animal-to-human extrapolation. In the absence of strong genetic arguments for

Table 1. Potential indications for PRLR blockers: summary.

Potential indication	Experimental/clinical evidence	Comment	Strength of the indication
Dopamine-resistant hyperprolactinemia	Obvious	10 – 15% of hyperprolactinemic patients are not normalized. PRLR inhibitors may have adverse effect on pituitary volume	Weak (current high-cost compounds unjustified). To be reconsidered once cheap drugs are available
Female reproductive tissues			
Breast cancer	Experimental: strong Humans: controversial (risk factor vs good prognosis)	High medical need, especially for triple negative and metastatic breast cancer. PRL signaling may be protective once the tumor is established	Declining indication? Needs careful patient stratification?
Endometrial/ovarian cancer	Experimental: limited Humans: limited	Only few studies available but rather convincing	Deserves further investigation to be confirmed
Benign breast diseases	Experimental: weak Humans: weak	No causative link demonstrated	Weak
Endometriosis/ adenomyosis	Experimental: limited Humans: limited and controversial	High medical need	Deserves further investigation to be confirmed
Contraception	Experimental: strong Humans: LOF PRLR mutation	Medical need to develop contraceptives without (with less) adverse effects	Weak (current high-cost compounds unjustified). To be reconsidered once cheap drugs are available
Male diseases			
Prostate cancer	Experimental: strong Humans: strong (but mainly from a single research group)	High medical need (advanced stages). Anti-PRLR strategies appropriate as cell-autonomous mechanisms (autocrine PRL) seem to be involved	Potentially strong indication
Benign prostate hyperplasia	Experimental: strong Humans: none	Very highly prevalent disease (50% of men after 50 year) and high medical need	Deserves further investigation to state whether animal studies are relevant to the human context
Prostatitis	Experimental: strong Humans: none	High medical need	Deserves further investigation to state whether animal studies are relevant to the human context
Other tumors			
Gastrointestinal tumors	Experimental: weak Humans: epidemiological	Most frequent malignancy in hyperprolactinemic patients (both genders). Very limited number of experimental investigations	Potentially strong indication, deserves further investigation (mechanisms involved)
Hematologic tumors and leukemia	Experimental: strong (old data) Humans: epidemiological	Second most frequent malignancy in hyperprolactinemic females. Potentially mediated by cell autonomous mechanisms (autocrine PRL)	Potentially strong indication, deserves further investigation
Non tumor			
Hair loss	Experimental: strong Humans: site- and gender-specific	High medical need	Mouse-to-human extrapolation likely misleading
Pain	Experimental: emerging (females) Humans: limited	High medical need. Very limited pharmacology of TRP channels, so alternative targets needed	Deserves further investigation to state whether animal studies are relevant to the human context
Autoimmune diseases	Experimental: limited Humans: numerous (controversial)	High medical need. Anti-PRLR strategies appropriate as cell-autonomous mechanisms (autocrine PRL) seem to be involved	An 'old' indication. Among others, psoriasis may deserve further investigation
Cardiovascular diseases	Experimental: emerging Humans: limited, controversial	High medical need. No involvement of autocrine PRL (making dopamine agonists suitable)	Weak (no clear evidence for a causal link)
Lymphangioleio-myomatosis	Experimental: one study Humans: speculative	High medical need	Deserves further investigation to state on its relevance

PRL: Prolactin; PRLR: Prolactin receptor.

most of these indications (e.g., highly potent gain-of-function PRL or PRLR mutations), involvement of PRLR signaling in the human context almost exclusively relies on descriptive studies (e.g., expression level of the actors). The current challenge in the field is to identify which of these indications are really relevant to human pathology. One of the key issues to validate pathological states that may be relevant for PRLR signaling inhibitors is adequate disease classification and subsequent patient stratification. Accordingly, the relevance of the PRLR as a predictive biomarker able to identify potential responders to PRLR targeted therapy will have to be established. Currently, very little is known regarding the actual levels of PRLR protein expression in tissues/diseases and the poor quality of commercial antibodies is a recurrent obstacle to progress in this matter. For example, with respect to prostate cancer, one study comparing PRLR expression in healthy versus pathological prostates concluded that PRLR levels were increased in pre-neoplastic stages (dysplasia) [65]. However, this study was performed using so-called mAb B6 (commercialized as B6.2) that was later proposed to recognize a PRLR-associated protein [66]. Another study [105] used an antibody that was not validated in the field (called SPM2123) to support PRLR over-expression in ductal versus acinar adenocarcinoma. Although the latter results were confirmed by transcriptional analysis of microdissected tumors, the reliability of the IHC data remains a concern. Therefore, one of the issues that will have to be solved in the future is the development of potent (specific, high affinity) anti-PRLR antibodies able to map PRLR expression in the targeted organs. Hopefully, the companies that are currently developing therapeutic anti-PRLR mAbs have already considered the need to develop companion tools for patient stratification.

Finally, the type of PRLR inhibitors that should be identified as the most potent for clinical use is also an issue. Pioneering studies involved knowledge-based development of PRL-based antagonists that are assumed to maintain the receptor binding specificity of the natural agonist. However, the compounds that were developed for proof-of-concept studies usually have reduced affinity and short half-life, and therefore require further improvements. It should be noticed that this was the track followed for the development of Pegvisomant, the antagonist of the closely-related GH receptor. Although antibodies undoubtedly have better pharmacokinetics than PRL-core antagonists, off-target effects may be an issue, especially in view of the structural similarity between cytokine receptors [106]. The generation of these biomolecules (antagonists, mAbs) is complex and costly. In this respect, generating small molecules able to inhibit PRLR signaling should be welcome. We are not aware of small molecule inhibitors able to block cytokine receptor activation, which may reflect the difficulty of such a strategy regarding affinity, specificity and/or any other mandatory parameters to develop potent drugs. Specific inhibitors of downstream effectors (Jak2, Stat5) are in development, but off-target effects are of concern given the wide distribution of these molecules in many different cell types/tissues [107].

In summary, our Expert opinion is that one should take advantage of the availability of functionally validated PRL blockers to establish the relevance of the numerous potential indications in humans. Although scientifically and clinically sound, this perspective may be hampered by the high cost of clinical trials.

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Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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