ORIGINAL ARTICLE

The effects of prolactin receptor blockade in a murine endometriosis interna model

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Abstract

Endometriosis in an estrogen-dependent disease that is characterized by the presence of endometrial tissue outside the uterine cavity leading to pain and infertility in many affected women. Highly efficient treatment options which create a hypo-estrogenic environment can cause side effects such as hot flushes and bone mass loss that are not favorable for premenopausal women. Previous work has demonstrated that increased local or systemic prolactin seems to be involved in the pathogenesis of endometriosis. Here we examined two prolactin receptor (PRLR) blocking antibodies in a murine endometriosis interna model which relies on the induction of systemic hyperprolactinemia in female SHN mice. The severity of the disease is determined by the degree of endometrial invasion into the myometrium. In this model, endometriosis was inhibited by clinical gold standards such as progestins and anti-estrogenic approaches. PRLR blockade completely inhibited endometriosis in this mouse model to the same extent as the anti-estrogen faslodex or the GnRH antagonist cetrorelix. In contrast to cetrorelix and faslodex, the PRLR antibodies did not decrease relative uterine weights and were thus devoid of anti-estrogenic effects. We therefore hypothesize that PRLR antibodies may present a novel and highly efficient treatment option for endometriosis with a good safety and tolerability profile. Clinical studies are on the way to test this hypothesis.

KEYWORDS
endometriosis, murine endometriosis interna model, prolactin, prolactin receptor antibody

1 | INTRODUCTION

Endometriosis is an estrogen-dependent, gynecological disease that affects up to 15% of premenopausal women and is characterized by the presence of endometrial tissue outside the uterine cavity.[1] Endometriosis is associated with pain symptoms such as dysmenorrhea as well as with infertility [2, for review]. There is growing evidence that endometriosis interna (presence of ectopic endometrium in the myometrium) and endometriosis externa (presence of ectopic endometrium in the pelvic cavity) represent two phenotypes of the same disease.[3] Treatment of endometriosis relies on laparoscopy as well as hormonal interventions using combined oral contraceptives, progesterone, GnRH agonists, and antagonists. In addition, nonsteroidal anti-inflammatory drugs are used for the treatment of

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inflammatory pain [4, for review]. As the hormonal and antihormonal approaches often cause symptoms such as hot flashes, vaginal dryness, and loss of bone mass density, there are continuous efforts to identify novel treatment options devoid of these side effects.[4]

There are several hints in the literature that the hormone and proinflammatory cytokine prolactin might be involved in the pathogenesis of endometriosis. Elevated systemic prolactin levels or occult hyperprolactinemia as well as changed nocturnal peaks of prolactin secretion have been described in infertile women suffering from endometriosis [5, for review]. A case report describing the galactorrhea-endometriosis syndrome[6] pointed toward a link between systemic hyperprolactinemia and endometriosis. Recently, it was demonstrated that prolactin-lowering drugs such as dopamine 2 receptor (D2R) agonists effectively reduced lesion burden in preclinical experiments in mice[7] as well as in clinical studies in hyperprolactinemic women suffering from endometriosis.[8]

Prolactin mediates its effects by the prolactin receptor (PRLR) that belongs to the class 1 cytokine receptor superfamily. The PRLR has three different isoforms, the short, the long, and the intermediate form that differ by the length of their cytoplasmic tails.[9] Prolactin binding leads to dimerization of two PRLR molecules and predominant activation of the Janus Kinase/Signal transducer and activator of transcription (JAK/STAT) pathway stimulating the transcription of prolactin target genes.[9]

Prompted by our findings that prolactin as well as its receptor are upregulated in human endometriotic lesions when compared to eutopic endometrium, we generated the hypothesis that not only systemic hyperprolactinemia but also enhanced local PRLR–mediated signaling in endometriotic lesions might contribute to the pathophysiology of endometriosis.[10] In humans, prolactin secretion from pituitary and extra-pituitary sites is controlled by different promotors[11] and D2R agonists are only able to interfere with pituitary prolactin secretion.[12] To achieve complete blockade of PRLR-mediated signaling activated by prolactin from pituitary as well as extra-pituitary origin PRLR antagonists are required. We previously identified and characterized the PRLR antibodies 005-C04[13] and Mat3 which is closely related to its precursor antibody 005-C04.[14,15] Both antibodies act as PRLR antagonists in vitro and in vivo.[10,13–15]

Here, we analyze the effects of these two PRLR antibodies in a murine endometriosis interna model in comparison to the D2R agonist bromocriptine and several (anti)hormonal approaches to support further clinical development of the antibody Mat3 for the treatment of women suffering from endometriosis.

2 | MATERIALS AND METHODS

2.1 | Murine endometriosis interna experiments

To compare the in vivo effects of the PRLR antibodies 005-C04 and Mat3 we used an endometriosis interna (= adenomyosis) model in SHN mice.[16] We applied this model previously to study the effects of danazol (androgenic progestin), cetrorelix (GnRH antagonist), and faslodex (estrogen receptor antagonist).[17] It turned out that these treatment approaches that are efficacious in the treatment of human endometriosis were also able to reduce endometriosis interna in mice.[17] SHN mice develop endometriosis interna spontaneously with increasing age whereby they pass between 4 and 9 weeks of age a critical phase in which the foundation for later disease development is built.[18] Increasing prolactin levels in young-adult SHN mice by treatment with either dopamine antagonists or pituitary grafting (into the uterine cavity or under the kidney capsule) accelerates development and increases the incidence of endometriosis interna in these animals at a younger age [for review see 18].

Here we describe two experiments using this endometriosis interna model following the previously described procedures.[17] In brief, the model relies on the induction of endometriosis interna in female SHN mice by systemic hyperprolactinemia. Female mice receive a male donor pituitary under their kidney capsule at the age of 8 weeks.[16,17] Two weeks after pituitary transplantation, treatment with the antibodies and comparator compounds was started and performed for 8 weeks (experiment 1) and 7 weeks (experiment 2) before the animals were sacrificed.

The timelines were established in pre-experiments that were performed based on literature data to identify conditions that reliably offer a sufficient window of measurement when testing different compounds, that is, a high disease score in pituitary grafted, untreated animals with as much homogeneity of the disease score as possible compared to unoperated, untreated animals that develop the disease slower and spontaneously.

2.1.1 | Experiment 1

This experiment analyzed the effects of the antibody 005-C04 (30 mg/kg, once weekly i.p., $n = 9$) in comparison to cetrorelix (100 μg per mouse s.c., $n = 10$), danazol (25 mg/kg, s.c., $n = 10$), and faslodex (5 mg/kg, s.c., $n = 10$) that were administered on 6 days per week. Additional experimental groups encompassed unoperated, untreated control animals ($n = 10$, depicted as control in Figure 1) and pituitary grafted, untreated animals ($n = 10$; depicted as graft in Figure 1). The results from this experiment obtained with the comparator compounds were published previously, whereas the antibody data were not disclosed.[17] To facilitate for the reader direct comparison of the antibody data (that are published here for the first time) with the previously published data from the comparator compounds, the data obtained with the comparator compounds are reproduced in Figure 1 (permission from the publisher was obtained).

2.1.2 | Experiment 2

This experiment analyzed the effects of the PRLR antibodies 005-C04 and Mat3 (both in the murine IgG2a format) in endometriosis
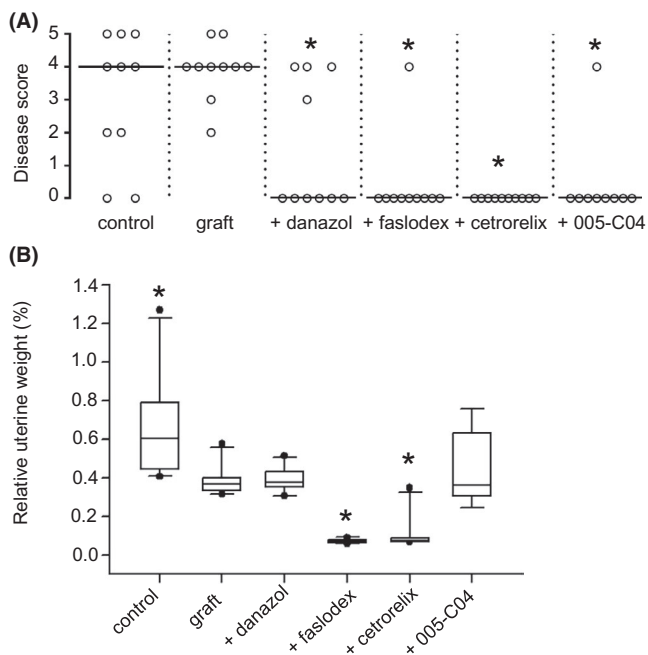
FIGURE 1 Disease scores (A) and relative uterine weight (B) from experiment 1. SHN mice remained unoperated (control) or received pituitary grafts followed by subsequent treatment with danazol, faslodex, cetrorelix, or PRLR antibody 005-C04. Median disease scores are indicated as horizontal bars, a score of 0 indicates healthy animals. Asterisks indicate scores significantly different from the score of the pituitary-grafted, untreated group (*p < .05, Dunn's method) (A). Antibody 005-C04 at a dose of 30 mg/kg inhibited endometriosis interna to comparable extent as the anti-estrogen faslodex or the GnRH antagonist cetrorelix (A). Relative uterine weights are depicted as box plots. The middle hash of the box indicates the median; 25–75th percentiles are presented by end caps of the box, and the whiskers indicate the 10th and 90th percentiles. *p < .05 (by Dunnett test vs. the pituitary-grafted, untreated group) (B)

interna in dose response (30, 10, 3, and 1 mg/kg of each antibody were administered s.c. once weekly). For comparison and control, an unspecific IgG2a anti-FITC antibody (30 mg/kg s.c. once per week, depicted as FITC in Figures 2 and 3) and the D2R agonist bromocriptine (Sigma B2134; 10 mg/kg s.c. on 5 consecutive days per week) were used. The experiment was performed as published previously[17] with the exception that the antibodies were administered s.c. and not i.p. There were the following treatment groups: no pituitary grafting and no treatment (n = 10, depicted as control in Figures 2 and 3), pituitary grafting and no treatment (n = 9, depicted as graft in Figures 2 and 3), pituitary grafting and treatment with unspecific control antibody (n = 10), pituitary grafting and treatment with 005-C04 at doses of 1 mg/kg (n = 10), 3 mg/kg (n = 10), 10 mg/kg (n = 10), 30 mg/kg (n = 10), pituitary grafting and treatment with Mat3 at doses of 1 mg/kg (n = 10), 3 mg/kg (n = 10), 10 mg/kg (n =10), and 30 mg/kg (n = 9), pituitary grafting and treatment with bromocriptine at a dose of 10 mg/kg (n = 9).

We switched from i.p. administration of the PRLR antibody in experiment 1 to s.c. administration for both antibodies in experiment 2, once we had established that the pharmacokinetics of the PRLR antibodies in mice were comparable after i.p. and s.c. administration and to mimic the envisaged later s.c. antibody administration in humans.

2.2 | Animals

SHN mice were from the Japanese RIKEN BRC Institute, and the breeding colony was maintained at Taconic (Denmark). Mice were kept on a 14-h light/10-h dark cycle and provided with food and water ad libitum. All animal procedures were carried out according to German animal welfare law with the permission of the District Government of Berlin.

2.3 | Histological analysis

Uteri from both experiments were processed for histopathological analysis. We modified a previously described scoring system[19] which is based on the depth of endometrial invasion into the myometrial layers into a six-ordered-level scoring system to reflect the degree of endometriosis interna in the animals[17]: 0, no endometriosis interna; 1, the concentricity of the inner circular myometrial layer is lost; 2, endometrial stroma and glands invade the inner circular layer of the myometrium; 3, endometrial stroma and glands are located between the inner circular and outer longitudinal myometrial layer; 4, endometrial stroma and glands infiltrate the outer myometrial layer; 5, endometrial stroma and glands pass the outer myometrial layer and have direct contact with the peritoneum. Whereas a score of 0 indicates a healthy animal, increasing scores reflect increasing disease severity. For each disease score, exemplary histological pictures are depicted in supplemental Figure 1.

2.4 | PRLR antibodies

The antibody 005-C04 and its derivative Mat3 have been characterized previously.[10,13–15] Both behave as noncompetitive, selective PRLR antagonists in vitro and in vivo. As antibody 005-C04 displayed no cross-reactivity to the rhesus monkey PRLR and toxicity studies were required in this species for subsequent clinical development, Mat3 was derived from 005-C04 by a two-staged mutagenesis approach. In a first step, variants of the Fab part of antibody 005-C04 carrying position-specific mutations in the complementarity-determining regions (CDRs) were generated by site-directed mutagenesis using so-called NNK-trinucleotide cassettes (whereby N represents a 25% mix each of adenine, thymine, cytosine, and guanine, and K represents a 50% mix each of thymine and guanine nucleotides). The variants were assessed for improved affinity by an ELISA-based high-throughput screen. In a second step, the mutations of the most beneficial substitutions were recombined
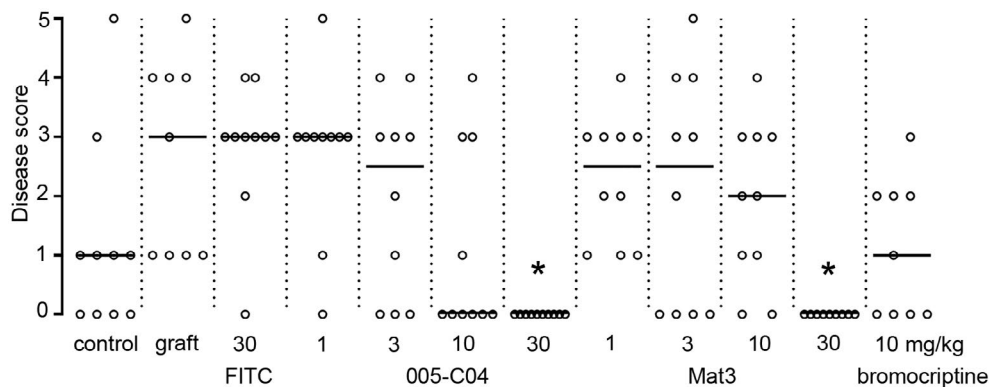
FIGURE 2 Disease scores (experiment 2). SHN mice remained unoperated (control) or received pituitary grafts and remained untreated or were treated with IgG2a anti-FITC control antibody (30 mg/kg), with 005-C04 or Mat3 at doses of 1–30 mg/kg, or with 10 mg/kg bromocriptine. Median disease scores are indicated as horizontal bars, a score of 0 indicates healthy animals. Asterisks indicate scores significantly different from the score of the pituitary-grafted, untreated group (*$p < .05$, Dunn's method). Pituitary iso-grafting induced endometriosis interna in all animals. In contrast to the unspecific control antibody, 005-C04 and Mat3 inhibited endometriosis interna in a dose-dependent manner and to full extent at a dose of 30 mg/kg
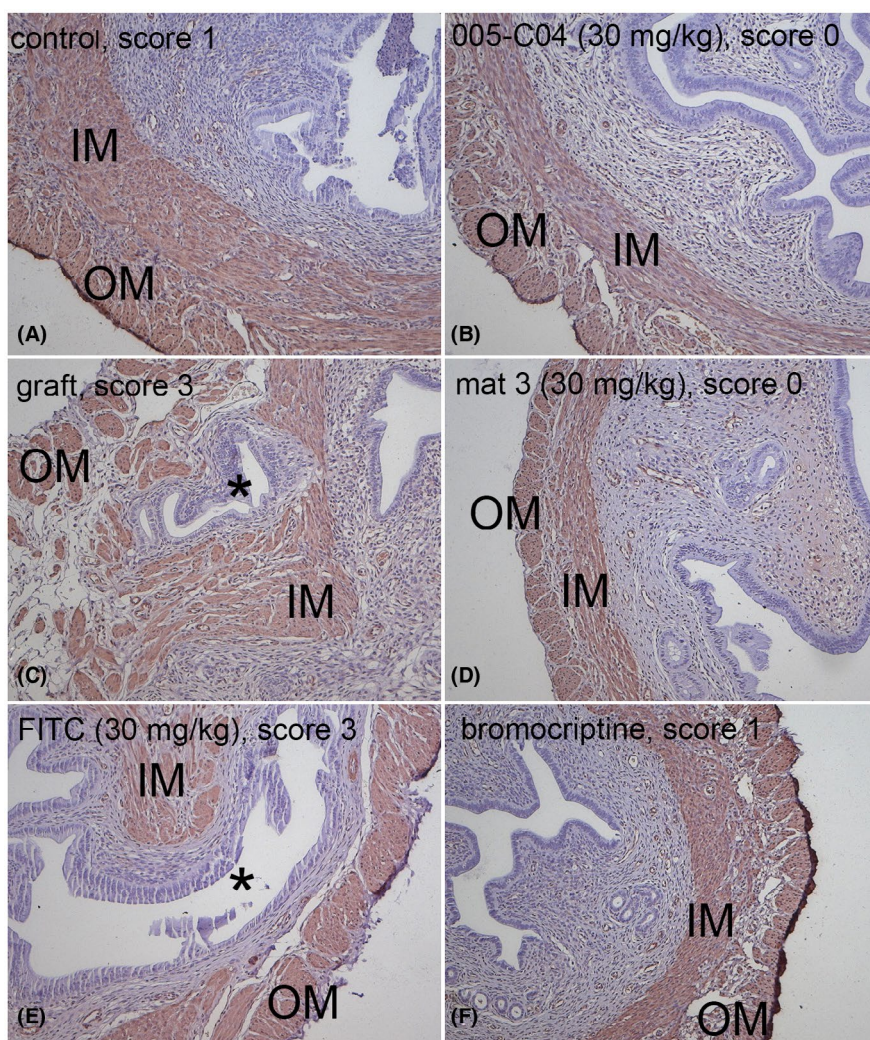
FIGURE 3 Uterine sections (experiment 2). Representative images (taken at the same magnification) reflecting the median disease score of each experimental group are depicted for uteri from unoperated, untreated animals (A) or from animals with pituitary isografts (B–F) receiving either no treatment (C) or treated with unspecific IgG2a anti-FITC control antibody at a dose of 30 mg/kg (E), with 005-C04 (B) or Mat3 at a dose of 30 mg/kg (D), or with 10 mg/kg bromocriptine (F). Asterisks indicate endometriotic foci invading between the inner (IM) and/or outer muscular (OM) layer of the uterus. Disease severity increased in pituitary-grafted animals that were either untreated (C) or treated with unspecific control antibody (E) compared to nongrafted control mice (A). While the unspecific control antibody was devoid of any treatment effect (E), 005-C04 (B) and Mat3 (D) at 30 mg/kg inhibited endometriosis interna. Treatment with bromocriptine (F) suppressed the median disease score from 3 to 1 which was statistically not significant

and screened again.[15] In addition, each substitution of the most affine recombined variants which negatively impacted the molecules' thermal stability were reverted to the original amino acid of the parental antibody 005-C04 to ensure developability of those molecules. The thermal stability was assessed by Differential Scanning Calorimetry (DSC) as described previously.[20]

Table 1 summarizes the species specificity of both antibodies, 005-C04 and Mat3, in the human IgG1 format as derived from previously published cellular antiproliferation assays.[10,13,15] Both antibodies show comparable potency at the murine PRLR. Mat3 is more potent on the human PRLR compared to 005-C04 and shows the required cross-reactivity to the rhesus PRLR (Table 1).

For the preclinical in vivo experiments, the antibodies 005-C04, Mat3, as well as an unspecific control antibody (anti-FITC) were applied in the murine IgG2a format. The switch of the constant fragment (Fc) of these antibodies from a human to a murine variant (i.e., mIgG2a) was done in order to reduce the risk that mice develop "anti-drug" antibodies (ADAs) during the course of the experiment which might negatively impact the pharmacodynamics of the applied molecules. In experiment 1, the stock solution for antibody 005-C04 had a concentration of 3.75 mg/ml and mice were injected i.p. on the treatment days with 200 μl/25 g body weight to obtain a dose of 30 mg/kg. In experiment 2, stock solutions of different antibody concentrations were prepared (i.e. 9, 3, 0.9, and 0.3 mg/ml) and stored frozen at −30°C. At the morning of the treatment days, one aliquot of each stock solution was thawed, and 100 μl/30 g bodyweight were injected s.c. (experiment 2) into mice to achieve the respective doses of 30, 10, 3, and 1 mg/kg.

2.5 | Statistical analysis

The endometriosis disease score was measured on an ordinal scale. The pituitary-grafted untreated experimental group was considered as reference against which all other experimental groups were compared at the 5% significance level using Dunn's method for all group comparisons to a designated control group which controls for the familywise error rate.[21]

Relative uterine weights are depicted as box plots. The experimental groups were compared to control animals with pituitary graft using a significance level of 5% and assuming log-normally distributed data. Dunnett's test[22] was applied keeping the familywise error rate under control. Since the performed experiments were exploratory in nature, no across variable α-adjustments were applied.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.

org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY[23] and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22.[24] According to Concise Guide to PHARMACOLOGY 2021/22, the PRLR belongs to the family of catalytic receptors.[25]

3 | RESULTS

In experiment 1, we analyzed the effects of the PRLR antibody 005-C04 as well as of several hormonal and antihormonal approaches used for endometriosis treatment in a murine model and compared these effects to those seen in pituitary-grafted untreated animals. All experimental data were published previously except the antibody data.[17] The progestin danazol, the estrogen receptor antagonist faslodex and the GnRH antagonist cetrorelix (leading to estradiol depletion) inhibited endometriosis interna when compared to pituitary grafted animals which exhibited a median disease score of 4 (Figure 1A). The PRLR antibody 005-C04 at a dose of 30 mg/kg suppressed endometriosis interna (median disease score 0) to comparable extent as faslodex and cetrorelix (Figure 1A).

Pituitary-grafting is known to increase progesterone levels[16] and progesterone by inhibiting the proliferative activity of estradiol in the endometrium can slightly decrease relative uterine weights.[17] Therefore, it was expected that normal control animals showed the highest uterine weights and the highest variability when compared to pituitary-grafted untreated animals as they were in random cycle (Figure 1B). Faslodex and cetrorelix, both resembling anti-estrogenic approaches, strongly reduced relative uterine weights (Figure 1B). The progestin danazol and the PRLR antibody 005-C04 had no effect whereby a higher variability was seen with the PRLR antibody treatment (Figure 1B).

Since antibody 005-C04 was not suited for further development, we generated antibody Mat3 exhibiting the desired activity at the rhesus PRLR (Table 1) from antibody 005-C04 by site-directed mutagenesis. In experiment 2, we analyzed the effects of both PRLR antibodies 005-C04 and Mat3 in the murine IgG2a format in dose response whereby an unspecific control antibody and the D2R agonist bromocriptine served as negative and positive controls, respectively. The results are depicted in Figure 2 (median disease score) and Figure 3 (representative histological pictures for the median disease score of the respective treatment group). As already evident from former experiments,[16–18] pituitary grafting accelerates disease development compared to unoperated SHN mice which spontaneously develop the disease (Figure 2). Therefore, a higher disease incidence and a higher disease severity is observed in pituitary grafted compared to age-matched unoperated control animals when sacrificed 8 weeks after treatment start and 10 weeks after pituitary grafting. The median disease score increased from 1 in unoperated animals to 3 in animals receiving a pituitary graft (Figure 2). The inner circular muscular layer was losing its concentricity in unoperated animals (Figure 3A), whereas at a disease score of 3 endometrial tissue was found between the inner circular and outer longitudinal layer

TABLE 1 Species specificity of antibody 005-C04[10,13] and antibody Mat3[15] in the human IgG1 format as derived from in vitro cellular antiproliferation assays

Antibody	IC$_{50}$ mPRLR [nM]	IC$_{50}$ hPRLR [nM]	IC$_{50}$ rhPRLR [nM]
005-C04	4.9	3.6–8.6	—
Mat3	3	0.7	4.6

Abbreviations: h, human; m, murine; rh, rhesus monkey.

in pituitary-grafted untreated animals (Figure 3C). The unspecific control antibody at a dose of 30 mg/kg had no effect on endometriosis interna (median disease score 3, Figure 3E). 005-C04 as well as Mat3 inhibited endometriosis interna in a dose-dependent manner and to comparable extent (Figure 2). When both PRLR antibodies were applied at a dose of 30 mg/kg, all uteri appeared to be healthy (median disease score 0, Figure 2) as also shown in Figure 3B (005-C04) and in Figure 3D (Mat3). All other employed antibody doses (i.e., 1–10 mg/kg) as well as bromocriptine (median disease score 1, Figure 3F) had effects that where statistically not significantly different from the disease scores obtained in pituitary-grafted, untreated animals (Figure 2).

4 | DISCUSSION

Here, we analyzed two PRLR antibodies, Mat3 and its precursor antibody 005-C04 in a murine endometriosis interna model which has been previously validated by using hormonal and anti-hormonal principles that are also employed in clinical practice for the treatment of endometriosis.[17] We observed that blockade of PRLR-mediated signaling inhibited endometriosis interna in mice to the same extent as anti-estrogenic approaches. Based on these data, we hypothesize that PRLR blockade might be also suitable to treat human endometriosis.

SHN mice develop endometriosis interna spontaneously with increasing age and pass a critical period before 10 weeks of age where the foundation for later disease development is built.[18,26] Pituitary grafting in this critical age phase accelerates disease development and leads to higher disease incidence and more homogenous disease severity already at a younger age.[18] The question whether the employed experimental setting was purely preventive or purely therapeutic is difficult to solve. As treatment started 2 weeks after pituitary grafting in 10 weeks old animals which had already passed the critical point and would develop the disease (although it was not histologically evident at treatment start) it would be a therapeutic setting compared to an experimental design with a treatment start at 4 weeks of age in either unoperated or simultaneously pituitary grafted animals.[26] If a therapeutic setting would be defined by the presence of fully developed disease at treatment start (as in the clinical situation) the presented experiments were performed in a preventive setting. Regarding the translatability of the results to the human situation it might be of more relevance that the animal model in its present setting has been validated by testing approaches that are successful in the clinic and that PRLR antibodies reduced endometriosis interna in this model to the same extent as anti-estrogenic drugs. The SHN model like all animal models for endometriosis as well as the human disease is estrogen dependent.[18] Ovariectomized SHN mice—with or without pituitary graft—do not develop the disease.[18] The fact that disease development in unoperated SHN mice is also prolactin-dependent[18] is supported by the observation that PRLR blockade reduced the disease score to 0 in almost all pituitary grafted animals and this score was

lower than the median disease score seen in unoperated animals (Figures 1 and 2).

Whereas the SHN mouse model relies on systemic hyperprolactinemia due to pituitary grafting, human endometriosis might be the consequence of either systemic hyperprolactinemia or enhanced local PRLR-mediated signaling. Several authors have shown that systemic hyperprolactinemia can accompany human endometriosis.[5,6,8] We could demonstrate previously that prolactin and its receptor are upregulated in human endometriotic lesions compared to normal endometrium providing evidence for enhanced local PRLR signaling in human endometriosis.[10] Whereas D2R agonists such as bromocriptine or quinagolide can only interfere with systemic hyperprolactinemia due to enhanced pituitary prolactin secretion, they are not able to block extra-pituitary prolactin production due to different promotor usage for pituitary and extra-pituitary PRL synthesis.[11,12] In contrast, the PRLR blocking antibodies Mat3 and 005-C04 completely block PRLR signaling due to enhanced pituitary or extra-pituitary prolactin production and are supposed to be beneficial for the treatment of endometriosis with or without systemic hyperprolactinemia. Therefore, a superior treatment effect in endometriosis is expected for PRLR blocking antibodies compared to D2R agonists.

There are several case-reports describing women suffering from prolactin-producing leiomyomas leading to systemic hyperprolactinemia which was treatment-refractory to D2R agonists.[27,28] Myomectomy but not D2R agonist treatment led to normalization of prolactin levels[27,28] thus proving the inability of D2R agonists to inhibit extra-pituitary prolactin synthesis. It remains to be established whether systemic hyperprolactinemia seen in a subgroup of endometriosis patients is always due to increased pituitary prolactin production or in some cases also due to local prolactin production in the endometriotic lesions. D2R agonists in contrast to PRLR antibodies would not be effective in the latter scenario as already demonstrated in prolactin-producing leiomyoma patients.[27,28]

For feasibility reasons and to reduce the injection burden for the animals, bromocriptine was only administered on 5 days and not 7 days per week. Therefore, insufficient dosing could explain its reduced efficacy in experiment 2 when compared with the effects of the PRLR antibodies at a dose of 30 mg/kg. Bromocriptine showed a positive treatment trend in the murine model that did not reach statistical significance which could be also due to the fact, that the treatment window in experiment 2 (median disease score 3 in pituitary-grafted animals) was slightly smaller than in experiment 1 (median disease score 4 in pituitary-grafted animals).

Meanwhile, clinical proof of concept was obtained for the use of the D2R agonist quinagolide in women suffering from endometriosis associated with hyperprolactinemia. When these women were treated for 18–20 weeks with orally administered quinagolide, endometriotic lesions either disappeared or showed reduced size at the preplanned second look laparoscopy.[8] These clinical findings were further supported by experiments demonstrating that quinagolide reduced lesion volume in an autologous rat endometriosis model (rats transplanted with own uterine fragments on the peritoneum)[29] and a heterologous mouse model

(nude mice transplanted with human endometrial fragments on the peritoneum).[7] Considering the translatability of the D2R agonist effects from the preclinical to the clinical situation and the large effect size of PRLR blockade in the murine endometriosis interna model, we assume that PRLR blockade may provide a new treatment opportunity for endometriosis.

Antibody Mat3 was derived from antibody 005-C04 as non-human primate toxicity studies were required for further clinical development, and 005-C04 in contrast to Mat3 was devoid of any activity on the rhesus monkey PRLR. Here, we show that both PRLR antibodies exhibit comparable in vivo activity on the murine PRLR. In line with previous findings, where a dose of 30 mg/kg 005-C04 inhibited lactation and fertility in mice,[13] the same dose of 30 mg/kg for both antibodies completely suppressed endometriosis interna in mice. Compared to D2R agonists and approaches inducing a hypo-estrogenic environment, PRLR antibodies are expected to show a superior tolerability profile. Major side effects of bromocriptine include nausea, vomiting, edema, hypotension, dizziness, hair loss, headache, and hallucinations,[30] whereas GnRH antagonists and anti-estrogens can induce postmenopausal symptoms such as hot flushes, vaginal dryness, and bone mass loss in young women.[4] Here we could demonstrate that PRLR antibodies are devoid of anti-estrogenic properties since they did not reduce relative uterine weights compared to pituitary-grafted untreated animals as it was the case for the anti-estrogenic compounds cetrorelix and faslodex. The lack of any anti-estrogenic effect of PRLR antibodies is further substantiated by the observation that administration of PRLR antibodies to female mice or monkeys did not interfere with their estrous cycle (Otto, unpublished data), whereas mice and monkeys treated with anti-estrogens lack estrous cycles. Mat3 proved to be safe and well tolerated in a multiple dose phase I study conducted in postmenopausal women and there were no differences in treatment emergent adverse events between placebo and Mat3-treated women.[14]

Taken together, in contrast to D2R agonists, PRLR blocking antibodies offer for the first time the opportunity to completely block PRLR-mediated signaling due to systemic hyperprolactinemia or due to enhanced local prolactin production. PRLR antibodies showed similar efficacy as compounds inducing a hypo-estrogenic environment in a validated murine endometriosis interna model relying on systemic hyperprolactinemia. A phase II clinical study with Mat3 (antibody HMI-115) has been initiated by Hope Medicine Inc. to test the hypothesis whether PRLR blockade will offer a novel treatment option with improved tolerability for women suffering from endometriosis.

AUTHOR CONTRIBUTIONS
The authorship responsibility form has been signed by all authors and has been uploaded as a separate document. The authors were involved in the following tasks: Christiane Otto (conception and design of the study, data acquisition, data interpretation, writing, and final approval of the manuscript); Hannes-Friedrich Ulbrich (statistical analysis of data, data interpretation, writing, and final approval of the manuscript); Christoph Freiberg (conception and design of the study, data acquisition, data interpretation, writing, and final approval of the manuscript).

ETHICS APPROVAL STATEMENT
All animal procedures were carried out according to German animal welfare law with the permission of the District Government of Berlin.

PERMISSION TO REPRODUCE MATERIAL
Experiment 1 analyzed the effects of comparator compounds and the antibody 005-C04 head- to-head in a murine endometriosis interna model. The results obtained with the comparator compounds in experiment 1 were previously published (Exp Ther Med 2012;3:410–414). The antibody data obtained in experiment 1 are published here for the first time. To facilitate for the reader the direct comparison of the antibody data with the previously published comparator compounds, the data obtained with the comparator compounds are reproduced in Figure 1 (permission from the publisher was obtained).

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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