

## Targeting Prolactin Receptor (PRLR) Signaling in PRLR-Positive Breast and Prostate Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

### INTRODUCTION

In this issue, Agarwal et al. report negative results from a phase I trial of LFA102, a humanized monoclonal antibody (mAb) that binds to and inhibits prolactin receptor (PRLR) signaling, in patients with PRLR-positive metastatic breast cancer or metastatic castration-resistant prostate cancer (mCRPC) [1]. Although no anticancer activity was noted in the study, questions remain regarding the role of PRLR signaling in these and other cancers and whether further efforts to develop PRLR blockers are warranted. It should be noted that hormonal manipulation of the hypophyseal-pituitary axis has benefited patients through the use of gonadotropin-releasing hormone agonists in prostate and breast cancer and somatostatin analogs in neuroendocrine tumors [2]. Therefore, it may be possible to exploit the biology of these and other hypophyseal-pituitary hormones for the benefit of other cancer patients.

The authors are to be commended for publishing results of this carefully conducted study in the Clinical Trial Results section of *The Oncologist* (Panel 1). Although “negative” in terms of antitumor activity, the study provides useful pharmacokinetic and pharmacodynamic information. A rise in serum prolactin levels followed drug administration, possibly an indication of the effective blockade of the receptor by LFA102. However, a temporal delay was noted between pharmacokinetic parameters and pharmacodynamic response, possibly because of the time required for LFA102 to spread to peripheral tissues and inhibit peripheral PRLR, resulting in an elevation in serum prolactin levels as a compensatory feedback mechanism. The lack of response could have many explanations: a failure to maintain inhibition of the pathway throughout the treatment interval, an ineffective degree of inhibition of the receptor, or a failure to translate receptor inhibition into biologically important effects on tumor growth. The study in question did not biopsy tumor during the trial, so we have no information on these issues. Nor was there a selection strategy for tumors highly expressing the receptor, an approach that might have led to a more positive outcome.

Returning to the rationale for further work on inhibitors of the prolactin receptor, there is an obvious need to augment current hormonal therapies of breast and prostate cancers [3].

Antiandrogens and antiestrogens play key roles in the therapy of hormone-sensitive breast and prostate cancer, respectively, but metastatic tumors inevitably develop resistance to current endocrine-based therapies. Expression of prolactin in both breast and prostate tissue has been well-documented, and tumor production of prolactin has been implicated in the growth of breast and prostate cancer via autocrine or paracrine pathways [4, 5]. Wen et al. noted that G129R, an antagonist peptide of prolactin (PRL), blocked the tumoral PRL/PRLR axis, resulting in inhibition of tumor growth in orthotopic models of human ovarian cancer [6]. Prolonged treatment with G129R caused redundant autolysosomes in cancer spheroids, resulting in type II programmed cell death, or inducible autophagy [6]. Additionally, carboxypeptidase-D and nitric oxide levels are upregulated by PRL and testosterone in vitro, which favors survival of prostate cancer cells. However, inhibition of PRLR and the androgen receptor (AR) prevented carboxypeptidase-D and nitric oxide production in this setting, which implies that combined blockade of PRLR and AR may have a role in the treatment of prostate cancer [7]. It has been noted that human epidermal growth factor 2 (HER2)-positive breast cancers are more likely to proliferate and metastasize in the presence of autocrine PRL, which suggests that PRLR and HER2 interact to promote tumorigenesis and breast cancer progression. Therefore, investigators studied the combination of the monoclonal antibody trastuzumab and G129R in two PRL-expressing human breast cancer cell lines (T-47D and BT-474) with varying levels of HER2 and PRLR expression. The combination of trastuzumab and G129R additively inhibited cellular proliferation in vitro and in vivo as measured by inhibition of the growth of both cell line xenografts in athymic nude mice [8]. Therefore, targeting prolactin signaling appeared to be a logical strategy for further study in breast and prostate cancer.

### THE BIOLOGY OF PROLACTIN AND THE PRLR

Prolactin is primarily produced by lactotrophs in the anterior pituitary gland of vertebrates [9]; however, it has also been found in many other extrapituitary sites, including reproductive, immune, neural, and integumentary tissues and other locations such as lacrimal glands, adipose tissue, blood

### Panel 1. Clinical Trial Results

Clinical Trial Results, or CTR, is a section of *The Oncologist* dedicated to the idea that no clinical trial should go unpublished. One of the ethical obligations engendered by the enrollment of patients in a clinical trial is that their contribution to the greater good will be shared. Yet rates of publication in oncology consistently fall short of this mark. In a survey of ASCO abstracts, we recently observed that only 75% of randomized trials and only 54% of nonrandomized trials were published within 4–6 years. The CTR section provides a menu-driven, simple format to quickly upload the results of a clinical trial, and it will draw the Kaplan-Meier and waterfall plots and generate tables. CTR then provides space for a short discussion of what went right or wrong. Whether a positive or negative trial, large or small, CTR is a cutting-edge venue for reporting clinical trials.

endothelial cells, and kidney [10]. Prolactin is encoded by the *PRL* gene found on chromosome 6 in humans [11]. The mature 23-kDa protein is composed of 199 amino acids; however, isoforms of prolactin have been identified in the pituitary gland and plasma as a consequence of posttranslational modification of the 23-kDa protein [12]. There are several different isoforms of prolactin with regard to size. The shorter isoforms, such as the 23-kDa protein, are predominant and have greater biological activity than longer isoforms ( $\geq 48$  kDa) [13]. Extrapituitary sites of secretion of a 16-kDa isoform, which has antiangiogenic and antitumor properties, have been identified in the myocardium, retina, chondrocytes, and mammary gland [14]. The 23-kDa prolactin isoform binds to the monomeric, transmembrane PRLR, initiating intracellular signaling through several distinct pathways: Jak/Stat, mitogen-activated protein kinases, Src, and phosphatidylinositol 3 phosphate kinase/Akt [15]. The wide distribution of PRLR on human tissues facilitates its role in various physiological processes, including cellular growth and differentiation, angiogenesis, hematopoiesis, reproduction, and lactation [16].

#### THE ROLE OF PROLACTIN AND PRLR IN ONCOGENESIS

The role of prolactin and PRLR in tumor growth remains controversial. In addition to prolactin produced by lactotrophs in the anterior pituitary, prolactin produced locally could promote tumorigenesis in an autocrine fashion in breast cancer [15]. However, cellular, molecular, and epidemiological studies investigating the role of prolactin in breast cancer have yielded conflicting results [17–20]. This observation could be because prolactin has a diverse role in the pathogenesis of breast cancer. For example, preclinical data suggest that prolactin may contribute to the development of breast cancer; in established cases, however, it may act instead to reduce the aggressiveness and spread of disease [21]. Interestingly, the long isoform of PRLR has been implicated in supporting the development of breast cancer metastases. In one study of two orthotopic and highly metastatic breast cancer cell lines (mouse 4T1 and human BT-474), long PRLR knockdown in three-dimensional culture resulted in apoptosis of 95% of tumor-initiating/cancer stem cells [22].

Autocrine and/or paracrine prolactin may also contribute to the pathogenesis of prostate cancer; for example, prostatic hyperplasia is seen in mice that overexpress prolactin in the prostate, and these changes may progress to intraepithelial neoplasia and even to adenocarcinoma of the prostate [23, 24]. In epidemiologic studies, the presence of prolactin and phosphorylated signal transducer and activator of transcription 5 (STAT5) in human prostate cancers is linked with high-grade tumors and more aggressive disease [25];

therefore, targeting autocrine and/or paracrine prolactin-mediated activation of STAT5 may represent a novel therapeutic target for patients with prostate cancer. For example, the efficacy of a Jak2 inhibitor, AZD1480, in preventing the growth of mCRPC via its effects on Jak2-dependent STAT5a/b signaling has been studied. Nine of 12 clinical prostate cancers responded to AZD1480 by profound cellular apoptosis, which was associated with reduced levels of nuclear STAT5a/b, suggesting that further clinical development of AZD1480 was warranted [26]. AZD1480 was also the subject of a phase I study in solid tumors conducted by Plimack et al.; however, development was halted given unusual dose-limiting toxicities, such as dizziness, anxiety, and ataxia, and limited clinical activity [27].

Prolactin analogs and anti-PRLR antibodies have already been developed as clinical candidates (Table 1), including LFA102 [28], as per the study by Agarwal et al. These compounds will potentially be developed to treat a variety of conditions apart from cancer, such as dopamine-resistant hyperprolactinemia, endometriosis, prostatitis, and autoimmune and cardiovascular diseases [28]. Both LFA102 and another neutralizing mAb, Mat3, effectively downregulate PRL-induced signaling both in vitro and in vivo, and two clinical trials of these compounds have been conducted to date (NCT01338831 and NCT01610050).

#### DISCUSSION AND CONCLUSION

Despite promising preclinical data, no clinical activity was observed in the phase I study by Agarwal et al., in which 73 patients were treated, including 53 at the highest dose level. Overall, LFA102 was safe and well-tolerated; the maximum tolerated dose was not reached, as there were no dose-limiting toxicities. Pharmacokinetic and pharmacodynamic data analyses confirmed that serum LFA102 maximum concentration and last area under the curve were approximately linearly dose-dependent. The recommended dose for phase II evaluation was 60 mg/kg LFA102; at that dose, the estimated half-life was 9 days, slightly lower than the typical half-life of mAbs, which is approximately 25 days. Therefore, the authors postulate that LFA102 administration may have been too infrequent, given the fact that LFA102 was administered only once every 4 weeks. Although in vitro studies showed that the drug had a high binding affinity for PRLR, the degree of inhibition of this target in human tissues was not assessed. Serum prolactin levels were used as a surrogate marker for PRLR inhibition, but this may not have been an optimal alternative biomarker of antitumor activity, as either the tumor or the pituitary gland could be responsible for increases in serum prolactin levels.

**Table 1.** Potential clinical indications for PRLR inhibitors [28]

Potential clinical indication	PRLR antagonist	Evidence for use		Comments
		Preclinical data	Clinical data	
Breast cancer	LFA102 (PRLR neutralizing antibody)	Strong	Conflicting	Potential role as combination therapy in ER-positive and/or HER2-positive disease
Prostate cancer	LFA102 (PRLR neutralizing antibody), Del1-9-G129R-hPRL (PRLR antagonist)	Strong	Strong but limited	Therapies to block PRLR indicated given autocrine prolactin signaling mechanisms
Ovarian/endometrial cancer	G129R-hPRL antagonist (PRLR antagonist)	Limited (existing data appears promising)	Limited	Need for further research; high clinical need
Gastrointestinal tumors	NA	Limited	Epidemiologic	Most commonly observed malignancy in patients with hyperprolactinemia; need for further research
Leukemias and other hematologic malignancies	NA	Strong	Epidemiologic studies only	Potentially strong indication; further research needed
Benign breast disease	Del1-9-G129R-hPRL (PRLR antagonist)	Weak	Weak	No association identified
Benign prostatic hyperplasia	Anti-PRLR mAb (PRLR antagonist)	Strong	None	Prevalent condition; high clinical need
Prostatitis	NA	Strong	None	High clinical need; further research warranted
Endometriosis/adenomyosis	Mat3 (PRLR neutralizing antibody)	Weak	Weak	High medical need; further research warranted
Contraception	NA	Strong	Experimental data: LOF, prolactin mutation	Consider further clinical development when cheaper agents are available
Cardiovascular disease	NA	Ongoing	Limited, conflicting	No clear association identified
Autoimmune disease	NA	Limited	Controversial	Clinical development for patients with psoriasis should be considered
Pain	Del1-9-G129R-hPRL (PRLR antagonist)	Experimental: ongoing	Limited	High medical need; further research warranted
Hair loss	Del1-9-G129R-hPRL (PRLR antagonist)	Experimental: strong	Limited	High clinical need; clinical outcomes disappointing to date
Dopamine-resistant hyperprolactinemia	NA	Strong	Strong	Consider further clinical development when cheaper agents are available; agents not fully effective in 10%–15% of patients with hyperprolactinemia

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor 2; LOF, loss of function; mAb, monoclonal antibody; NA, not applicable; PRLR, prolactin receptor.

The potential agonistic and antagonistic effects of prolactin signaling and receptor blockade in breast cancer as discussed above illustrate the need for further studies to better inform the therapeutic potential of the prolactin axis. Moreover, as the prolactin signaling pathway intersects with other key pathways involved in breast cancer pathogenesis such as HER2 and estrogen, combination trials may be warranted [29]. Combination therapy with antiandrogens may also be beneficial in castration-resistant prostate cancer. Another challenge in clinical development is that detection of PRLR expression has not been validated using currently available commercial antibodies, making it difficult to accurately identify a subgroup of patients who may benefit from PRLR

blockers [28]. Additionally, single nucleotide polymorphisms of the PRLR have been reported, some of which may affect receptor function and response to receptor blockade [29]. Despite the lack of clinical efficacy observed in this phase I study, future trials evaluating PRLR blockers alone and in combination with other agents may still be warranted in patients with breast and prostate cancer. Future research should aim to clarify the importance and complexities of PRLR signaling and develop accurate assays for identifying patient subgroups that may benefit from PRLR blockade.

#### DISCLOSURES

The authors indicated no financial relationships.

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**EDITOR'S NOTE:** See the related article, "Phase I Study of the Prolactin Receptor Antagonist LFA102 in Metastatic Breast and Castration-Resistant Prostate Cancer," by Neeraj Agarwal et al. on page 535 of this issue.