Targeting the progesterone receptor in breast cancer: mind the short form!

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SUMMARY:
The pre-surgical window of opportunity trial (WOT), MIPRA, provides evidence that neoadjuvant treatment with the progesterone receptor (PR) antagonist Mifepristone (RU486) may benefit patients with ER+ breast cancer characterized by a high ratio of PR-A versus PR-B isoform (>1.5) suggesting that PR may be targeted in a subset of patients.

MAIN TEXT:
In this issue of *Clinical Cancer Research*, Elía and colleagues make elegant use of a WOT to test the clinical relevance of their preclinical findings that PR can be a therapeutic target in a subset of ER+ BC postmenopausal patients (1).

While the role of ER signaling as a driver of ER+ BC has long been recognized and successfully exploited therapeutically, the role of other nuclear receptors, progesterone (PR), androgen (AR), and glucocorticoid receptor (GR) frequently co-expressed in this disease, has been less clear. Because endocrine resistance is an important clinical problem there has been substantial interest in finding alternative strategies to current ER-focused approaches. In particular PR signaling has long been implicated in breast carcinogenesis (2) yet it is currently only used prognostic marker in the clinics.

Elía and colleagues argued that a reason for the limited success of PR targeting strategies in preclinical and clinical studies lies with the complexities of PR signaling and these need to be take into account in patient selection. The PR gene encodes at least two major proteins, isoform B (PRB) of higher molecular weight and isoform A (PRA), which lacks the first 164 amino acids (3). This N-terminal part contains the PR-B specific activating function 3 (AF3) (3,4) shown to mediate interactions with p300 (5) and likely accounts for distinct transcriptional activities of the two forms in various in vitro models (4).
The functional importance of the 164 aa difference in vivo was demonstrated by elegant genetic studies in mice which revealed that the PR-A form is important in mediating physiological functions of progesterone in the uterus whereas the long PR-B form is most important in the mammary gland (6). The long form mediates progesterone-induced Wnt4 and Rankl expression required for stem cell activation and cell proliferation central roles of PR signaling in pubertal and adult mammary gland development (7) whereas PRA is important and sufficient for alveologenesis during pregnancy (8).
In the normal human breast epithelium, the two forms are expressed in equimolar amounts, the ratio changes in tumors most frequently in favor of the PR-A form (9–11). Elía and collaborators argued that the ratio of PR-A and PR-B in the tumor cells determines the response to anti-progestins and tested this hypothesis in preclinical models using mifepristone because of its availability. They characterized the PR-A/B ratio in 220 tumor samples by Western blot and showed an enrichment of the A form, in line with previous studies (9–11). Ex vivo exposure of fresh tissue slices to mifepristone showed that all 19 samples with PR-A/B protein ratio > 1.2 responded to treatment with decreased Ki67 index (12) when the response was varied in tumors with more PR-B than PR-A form.

Now, Elía et al. have taken this hypothesis to the bedside using a WOT which nicely illustrates the challenges and opportunities of translational research:
ER+ breast cancers typically grow slowly; in clinical practice, it is difficult to obtain enough tissue for the different assays required in this study and the presurgical biopsies may not contain sufficient tumor cells. As there are no isoform-specific antibodies for the PR, Western blots were required for quantifying the two PR forms. Because of varying quantity and quality of tissue samples, Elía and colleagues had to recruit more patients than initially planned, interviewing a total of 140 patients to obtain the 20 patients that fulfilled all the inclusion criteria. These practical issues restrict experimental possibilities, the study is small and lacks the control arm.

Yet, because of the smart design and the consistency of the different datasets, the present results together with the results of another WOT examining the effects of ulapristate in premenopausal BC (13) support the important notion that PR antagonists can be exploited clinically.

The first strength of this study is the use of a change in Ki67 index as endpoint. Ki67 index has been used extensively in high quality clinical trials and reflects longterm clinical outcome (14). Within only 14 days of Mifepristone treatment, 70% of the tumors showed 30% reduction of the Ki67 index. This reduction might be even higher if the window was extended. A potentially stronger beneficial effect is suggested by the transcriptomic analysis of a subset of 8 patients from whom paired RNA seq data was obtained. Four of these were responders and four were non-responders. Yet, downmodulation of cell proliferation pathways was significant across these samples corroborating the finding that PR signaling impinges on cell proliferation and suggesting that additional patients may benefit if a more sensitive readout is used.

Excitingly, the RNA seq data provide new insights into the role of PR signaling activities. Gene set enrichment analysis shows that mifepristone treatment upregulates tissue remodelling, apoptosis, early and late estrogen-related genes, as well as immune bioprocesses. Immunostainings on a larger set of samples validate these findings and quantification of tumor infiltrating lymphocytes (TILs) shows an increase in 81% of the tumors in line with the immunomodulatory effects.
As the cherry on the cake, Elía et al. succeed in obtaining nuclear and cytosolic extracts by LC-MS/MS from 10 matched samples. The findings align well with the RNA seq data and lend further support to the conclusion that mifepristone treatment reduces cell proliferation and impinges on immune pathways and the extracellular matrix.

Recent guidelines from ASCO (15) recommend the use of molecular screening tools, such as Oncotype DX, MammaPrint, Breast Cancer Index (BCI), and EndoPredict to better assign treatment for ER+ breast cancer patients. The use of RNA-seq data in WOT trials may ultimately help to link the molecular underpinnings of the disease with the clinical outcomes.
The authors have made their data publicly available, following recommendations of open science guidelines (16) and enable other researchers to reuse the data, evaluate their findings and further explore them. Functional data from patient tumors are invaluable when most ER+ BC research relies on a limited number of cell line models. We recently revealed a

surprising interpatient heterogeneity in response to physiologic hormone stimulation with a small set of ER+ BC PDXs (17) highlighting the need to acquire more functional clinical datasets ofr a better understanding of drug responses.

The findings raise many new questions. In particular, how do tumors with smaller ratios of PR-A/PR-B respond to mifepristone and other SPRMs? How stable is the ratio during tumor progression and therapy? How carefully do the tumors need to be selected? Which other parameters need to be considered? What factors determine the ratio of the two isoforms?

Will SPRMs benefit patients as monotherapy and/or in combination with other therapies? Should they be administered concomitantly or alternating with SERMs? It is conceivable that the two forms differentially interact with ER; how does this affect combine endocrine therapy?

Do the striking effects of mifepristone on matrix remodelling result in decreased breast density? Is there a role for SPRMs in breast cancer prevention in women with high radiographic breast density who are known to be particularly at risk?

Can the effects of mifepristone on TIL recruitment and the innate immune response be therapeutically exploited?

More smart WOTs, ideally multi-centered, together with preclinical studies are likely to provide answers in the near future

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