

# The Window Is Wide Open: Evaluating the Rationale for Window of Opportunity Studies in Breast Cancer with a Focus on Immune Therapies

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## Abstract

Drug development in oncology is inefficient with high rates of attrition, making the process of bringing a new therapy to market highly costly. As agents with novel mechanisms of action become standard of care, it is imperative that clinical trials be strategically designed to optimize success. Appropriate biomarker selection is key to identify which subgroup of patients should be included in later stage testing and ultimately treated with these expensive targeted agents. Window of opportunity (WOO) studies, in which treatment-naïve patients are briefly exposed to therapy to observe tumor biology, are emerging as a strategy to identify biomarkers. This strategy allows evaluation of changes in a known target following exposure to therapy *in vivo*. With modern high throughput technologies that allow for more complex hypothesis testing, we are able to more efficiently apply preclinical knowledge of the main drug target. Additionally, molecular analysis testing can be conducted on treatment-naïve patients who are less likely to harbor resistance mutations. WOO trials also allow for evaluation of treatments combined with, or instead of, standard of care therapy that are important to understand the pharmacodynamics of new treatments and identify biomarkers.

Immune targeted therapies are rapidly changing the landscape of cancer treatment in many solid tumors, including melanoma, lung, kidney, and head and neck cancers. As they make their way into studies in breast cancer—the most common malignancy among women worldwide—strategic trial designs are imperative. In this paper, we review important considerations surrounding WOO trials with a focus on breast cancer, and examine these considerations in the context of immune therapy.

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## Introduction

Oncology drug development is a rapidly progressive field. When compared with other therapeutic indications, oncology drugs are more often first-in-class, biotech, or orphan medications.<sup>1,2</sup> In a 2014 review, approval rates for 5820 drugs for various indications were analyzed, including 1803 oncologic medications. It was found that oncology medications had the lowest likelihood of approval at only 7%.<sup>3</sup> This, in part, is due to the fact that a common setting to investigate novel therapies is in an unselected, heterogeneous end-stage cancer population.<sup>4,5</sup> Attempting to conduct translational research to identify predictive biomarkers is challenging and inefficient when analyzing heavily pre-treated tumors with high degrees of heterogeneity. In order to increase the success rate of drugs in later stage clinical trials, accurate, and efficient methods of understanding pharmacodynamics and identifying predictive biomarkers is paramount, and this is even more important as we consider development and integration of targeted therapies.

Window of opportunity (WOO) studies are emerging as a solution to address these issues. In this study design, treatment-naïve patients are exposed to 1 or more doses of a new therapy in order to observe changes in tumor biology. It is distinct from a neoadjuvant treatment trial in which the primary aim is to improve disease outcomes and evaluate surrogate endpoints, such as pathologic response. In a WOO trial, the investigational agent or intervention is given prior to the definitive treatment strategy, whether it be local or systemic. This strategy offers several advantages, particularly in the age of precision medicine. First, it allows evaluation of changes in a known target following exposure to targeted therapy *in vivo* as opposed to traditional pre-clinical mouse models. With modern high throughput technologies that allow for more complex hypothesis testing, we can more efficiently apply preclinical knowledge of the main drug target. Secondly, molecular analysis testing can be done on treatment-naïve patients that are less likely to harbor resistance mutations, or molecular alterations that occur as a result of having been previously treated. Thirdly, it allows for evaluation of treatments combined with, or instead of, standard of care therapy that would not be possible in traditional clinical trials, but is important to understand the pharmacodynamics of new treatments and identify biomarkers.

These advantages together allow for higher success rates in later trials by appropriately selecting patients that are likely to benefit.

In a 2014 review of oncology WOO trials done by Marous et al, 56 trials were identified that fit inclusion criteria. Of these, breast cancer was evaluated in 33 (59%) making it the most common tumor site utilizing this approach.<sup>6</sup> The earliest WOO study in breast cancer was published in 1993, where tamoxifen given prior to surgery was shown to statistically decrease Ki-67 expression level in breast tumors. Since that time, there have been many WOO studies in breast cancer evaluating endocrine therapies, targeted therapies, and other, non-breast cancer-specific medications such as metformin and statins.<sup>7</sup> Looking forward, the need to use the WOO platform is increasing with the rapid introduction of novel therapeutic strategies with high potential for success. A particular interest is the evaluation of immune therapies either individually or in combination with other therapies. Immune therapies have been found to be effective in a number of tumor sites, however the evidence for breast cancer, based on traditional metastatic trials has not shown a clear signal. In order to observe similar success in breast cancer, strategic study designs are imperative to identifying the patient population that is most likely to benefit. Here, we review important considerations surrounding this unique trial design with a focus on breast cancer, and examine these considerations in the context of immune therapy.

#### Timing of Biomarker Evaluation

In determining the appropriate window to take study biopsy specimens, consideration must be made to the pharmacokinetics of the treatment. Ideally, measurements would be taken when the agent under investigation has reached steady state that is generally 5 half-lives. Thus, depending on the half-life of the agent, there may be increasing risk of how delays in treatment could affect patient outcomes. There are also concerns surrounding how time influences the marker of interest. In a retrospective analysis by Chen et al, 276 patients with matched core needle biopsies and untreated surgical specimens were analyzed for consistency in ER, PR, HER2 and Ki-67 measurements.<sup>8</sup> There was agreement in ER, PR, and HER2 evaluation between the core biopsy specimen and surgical resection specimen; however, the Ki-67 expression was significantly higher in the surgical specimen compared to the core, with levels of 29.1% and 26.2%, respectively ( $P < .001$ ).

WOO trials that investigate immunotherapy face similar challenges when considering the optimal time for biomarker evaluation. There are many factors that can affect levels of immune cell infiltrate and immune cell subtype apart from immune-targeted therapies. It is known that cell lysis from chemotherapy can cause release of tumor antigens stimulating the immune system to recognize and destroy malignant cells. Additionally, many chemotherapy agents require steroids as part of the pre-medication regimen which can further impact the immune milieu.

#### Selection of Endpoints

It is important in WOO studies that the primary endpoint has clinically relevant implications. Ideally, an endpoint would be chosen that has proven correlation to survival outcomes. In many of the WOO trials conducted to date in breast cancer, Ki-67 expression level is chosen based on evidence demonstrating its association with long-term outcomes.<sup>9,11</sup> In the IMPACT (Immediate Preoperative Anastrozole, Tamoxifen or combined with Tamoxifen) study, molecular changes were evaluated following brief exposure to randomized endocrine therapy.<sup>12</sup> They were able to show that Ki67 suppression was different between the anastrozole and tamoxifen arms, and the suppression levels were predictive of the recurrence free outcomes of the ATAC (Arimidex, Tamoxifen Alone or in Combination) trial.<sup>13</sup> The continued use of this outcome was demonstrated in a 2016 review of WOO in breast cancer, where 26 of 33 trials identified included Ki67 as an outcome measure.<sup>7</sup> There are, however, many limitations to using this marker as a primary endpoint. Firstly, there are high levels of inter-observer and inter-center variability in scoring.<sup>14</sup> Beyond that, there is evidence to suggest that in triple negative and HER2-positive breast cancer, there may be an increase in Ki67 from baseline, even in the absence of treatment prior to surgery.<sup>15</sup> Additionally, it is not specific to any particular or targeted treatment modality, and thus does not take advantage of molecular information of targeted therapies.

One of the key utilities for WOO trials is assessing changes in known targets of specific treatments. In a study looking at bevacizumab, an antibody to vascular endothelial growth factor (VEGF) in breast cancer, phosphorylated VEGF was evaluated.<sup>16</sup> It was shown in this study to decrease following treatment with bevacizumab however, in a single arm trial, with small numbers it was not possible to draw any predictive or prognostic conclusions. Similarly, trials evaluating EGFR-inhibitors have attempted to assess changes in levels of phosphorylated EGFR, but results have been mixed, likely partly due to cross talk with hormone receptors.<sup>7</sup>

In the case of immune therapies with a defined target, such as the anti-PD-1 antibodies, there has been mixed evidence to suggest whether measurement of PD-L1 is an effective biomarker for predictive or prognostic purposes.<sup>17</sup> Most studies evaluating PD-L1 status show trends towards increasing response rates with increasing expression,<sup>18,20</sup> while other studies fail to show a correlation.<sup>21</sup> There are several other limitations to using PD-L1 expression as a biomarker. There remains a need to determine what the clinically meaningful cutoff is, as values have been variable in clinical trials.<sup>17</sup> Multiple PD-L1 immunohistochemistry (IHC) antibodies have been used including 28-8, 5H1, MIH1, and 405.9A11, and comparative performance characteristics are not well known. Additionally, there are 2 distinct mechanisms by which PD-L1 is biologically active; through dynamic interferon-gamma (IFN $\gamma$ ) expression (inflammation-driven) or via constitutive oncogene activation.<sup>22</sup> Inflammation occurs focally at

sites of IFN $\gamma$ -mediated immune attack, oncogene-driven expression is diffuse.<sup>23</sup> Measurement in a window of opportunity study where the inflammation-driven expression would be the biomarker of interest, would be subject to inaccuracies.

There is growing evidence to support that tumor infiltrating lymphocytes (TILs) have prognostic implications in breast cancer.<sup>24,26</sup> There is also evidence that TILs predict higher pathologic complete response rates.<sup>27,28</sup> There are still however, questions that must be answered before TILs can be considered a standard endpoint. The current standardized approach for measurement is based on the Salgado criteria for immunohistochemistry using the haematoxylin and eosin (H&E) staining technique.<sup>29</sup> While this is commonly cited in the literature, it fails to distinguish pro- versus anti-inflammatory lymphocytes, which may be more accurately distinguished using techniques such as multispectral IHC.<sup>30</sup> Another potential method of analyzing tumor antigen-specific T-cell activation is by T-cell receptor (TCR) sequencing, which has been shown to correlate with H&E staining of TILs.<sup>31</sup> When T cells are developing in the thymus, the complementary determining region 3 of the T-cell receptor gene undergoes modifications to generate a large population of T-cell clones, each with its own unique TCR that gives the cell its antigen specificity. Sequencing the receptor thus gives deeper information on clonal diversity.

Observing RNA level changes of genes that are known to interact with the therapy of interest has been proposed as a strategy of assessing response to immune therapy is. One method of detection of changes in RNA is with microRNAs (miRNAs) which are small non-coding post-translational regulators. The signal transducers and activators of transcription (STAT) pathway is under control of such miRNAs. STAT1 is a transcriptional regulator that recruits effector cells and prevents tumor progression, whereas STAT3 downregulates this response. There are data from trials using IFN $\alpha$ 2b in melanoma to show that the ratio of STAT1/STAT3 is predictive of response and overall survival.<sup>32</sup> In breast cancer, there is evidence to suggest importance of the STAT pathway in endocrine resistance,<sup>33</sup> and that STAT1 expression is upregulated by HER2,<sup>34</sup> however, its utility as a biomarker is not known. In a recent WOO study in HER2-positive breast cancer, Varadan et al demonstrated that an immune signature evaluated after a single dose of trastuzumab predicted response in HER2-positive breast cancer, suggesting with further validation these signatures may enable early evaluation of treatment response.<sup>35</sup>

### Potential Risks

A challenge of WOO studies is that they are not designed to provide direct clinical benefit for individual patients. Instead, the goal is to identify predictive biomarkers to appropriately design and enrich study population in subsequent studies. It is therefore imperative that there is no harmful outcome to the patients involved as a result of their participation. There is potential for adverse events, although small, associated with receiving single doses of

treatment, and with the additional biopsy required. In their review of 56 oncology WOO trials, Marous et al identified 2 deaths (0.05%) related to study drugs. In 180 patients (4%) treatment had to be interrupted due to adverse events. Across all studies, 7% could not undergo surgery per protocol, but only 1% was due to an adverse event. Arnaout et al evaluated the feasibility of window of opportunity trials in breast cancer. The authors found that 18 of 20 patients experienced mild-to-moderate (grade 1-2) adverse effects. All patients were able to proceed along the planned timeline to surgery.<sup>36</sup> Beyond that, 100% of patients approached were willing to participate in the study, demonstrating patients are willing to accept the minimal risks associated with participating in a WOO trial.

While generally well tolerated, there are a diverse range of unique toxicities that are associated with immunotherapy, ranging from cytokine therapies inducing capillary leakage to checkpoint inhibitors that induce auto-immune related adverse events.<sup>37-39</sup> In order to ensure WOO trials are safe, the side effects of the therapies must be adequately managed and ideally prevented. With increasing education and awareness of what to anticipate, the drugs are becoming increasingly safe for use.<sup>38</sup> We still await, however, prospective trials for management of immune related adverse events to guide practice. With these considerations, it is important that WOO trials with immune therapy be conducted by physicians at centers that have experience using such agents, to avoid delays in definitive therapy.

### A Proposed WOO Design for Immune Therapy in Breast Cancer

In order to address the issues discussed above, we propose a generalized window of opportunity study design to evaluate immunotherapy in breast cancer (Figure 1). Patients with early-stage breast cancer with sufficient tumor for analysis (T >1.5cm) are randomized to 1 of 3 arms. A single dose of therapy is given prior to treatment according to standard of care. Treatment arms include chemotherapy, combination therapy, and immune therapy alone allowing evaluation of how cytotoxic therapy affects the biomarker analysis in the presence and absence of immune therapies, and their associated supportive therapies. Depending on the subtype of breast cancer being evaluated, therapies targeting the estrogen receptor, and/or HER2-receptor, could be included in separate arms. The primary goal is to use pre- and posttreatment core biopsy specimens for biomarker analysis. The biomarker studies include a comprehensive look at the tumor microenvironment to evaluate known candidate biomarkers, and explore potential biomarkers within the same study. The pre- and posttreatment biopsies are separated by 1 cycle duration to allow for treatment effect to be observed. Following the second biopsy, the patient may go on to have further neoadjuvant therapy, or directly to surgery depending on the opinion of the oncologic team regarding overall risk of disease recurrence.

## Conclusions

WOO trials present an exciting opportunity to learn about mechanisms of action of novel therapies, molecular activity, and efficacy. They also provide an opportunity for biomarker evaluation to appropriately enrich study population for later stage clinical trials. Careful multidisciplinary considerations, however, must be made in designing these studies. A knowledge of tumor biology as well as biology of the novel agents is fundamental to a successful design. Clinically meaningful surrogate endpoints should be sought, with attention paid to safety of the agent and avoiding any disruption or delay of standard therapy.

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