

Repurposing Tamoxifen to Treat Molecularly Stratified Metastatic Triple Negative Breast Cancer

Currently available targeted therapies are ineffective in metastatic triple negative breast cancer (TNBC). Chemotherapy, although effective at the outset, fails to block the progression and metastasis of TNBC. As a result, newer approaches that battle TNBC from a different vantage point have been developed, namely immunotherapy. However, while immunotherapy alone or in combination with chemotherapy is effective in some patients with metastatic TNBC, a large percentage of those patients are unresponsive. Furthermore, adverse side-effects from immunotherapy are of serious concern. Hence, there remains an urgent need to develop new therapeutic strategies.

There are two estrogen receptors (ERs) in breast cancer: ER-alpha (ER α) and ER-beta (ER β). Historically, based on the presence or absence of ER α breast cancer has been broadly classified as ER-positive and ER-negative. Although the second receptor, ER β , was discovered more than twenty years ago, it is still not considered for classification of breast cancers to ER-positive and ER-negative categories. Because of the absence of targetable canonical ER α , hormone therapy with drugs such as **Tamoxifen (Tam)** has been thought to be not useful against TNBC. The current proposal challenges this dogma. Although TNBC does not have ER α , a majority of them have ER β . Another important player in breast cancer is the major tumor suppressor called p53. In more than 80% of TNBC, p53 is dysfunctional (mutant p53) as a tumor suppressor. Furthermore, mutant p53, in addition to losing its tumor suppressor capabilities, gains tumor promoting or 'oncogenic' functions. Such mutants are called "gain-of-function p53 mutants".

ER β has been reported to have both pro- and anti-tumor functions. Our pre-clinical studies showed that ER β directly binds to both normal and mutant p53. Furthermore, we showed that when normal p53 is present, ER β increases TNBC cell proliferation. On the other hand, when mutant p53 is present, ER β blocks TNBC cell proliferation and causes cell death. Importantly, our retrospective analysis of a large breast cancer clinical trial database METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) showed that TNBC that have both mutant p53 and high levels of ER β have better breast cancer-specific survival. We have reported these findings in the *Journal of the National Cancer Institute (JNCI)*, 2019, 111(11):1202-1215).

Surprisingly, we observed that Tam increases the ability of ER β to bind and prevent mutant p53 from driving TNBC. **Doxorubicin (Doxo) (Adriamycin)** is a chemotherapeutic agent widely used to treat TNBC. Doxo is known to damage cancer cell DNA to kill the cells. At the same time, Tam helps ER β to disable oncogenic mutant p53. Thus, Doxo-Tam combination becomes a double whammy to TNBC. Although Doxo is effective initially against TNBC, it has the potential for severe side effects (including cardiotoxicity) and has a cumulative effect on the patient thereby limiting its efficient therapeutic usage. Importantly, our experiments showed that when Doxo is combined with Tam, cell killing ability of Doxo increased by three-fold. Based on these novel findings, *we are proposing repurposing Tam to fight stage IV metastatic TNBC that have both mutant p53 and ER β proteins.* The combination therapy should be effective in patients with doses of Doxo much lower than what is currently used in the management of TNBC, thereby reducing major side effects of Doxo. Tam is one of the most tolerated drugs patients can take for long periods. Furthermore, Tam has been recently reported to suppress TNBC brain metastasis by blocking the tumor's immune evasion. Importantly, a stage IV TNBC metastatic patient, after noticing our research published in *JNCI*, persuaded her oncologist to put her on Tam therapy. Intriguingly, multiple brain metastatic lesions in this patient have been under impressive remission for the past two years (*Clinical Case Report* manuscript submitted for publication). Immune checkpoint inhibitors (ICIs), such as anti-PD-1 and anti-PD-L1 drugs are now FDA-approved for use with certain chemotherapeutics in metastatic TNBC, but its efficacy is still limited. We posit that ICI efficacy will be enhanced when combined with this new drug regimen (Tam+Doxo) due to its impact on the immune system.

The proposed studies in mouse models of TNBC systems that closely resemble human metastatic TNBC will provide important insights into future clinical strategies potentially combining immunotherapy with this novel therapeutic regimen for metastatic TNBC. As majority of TNBCs have both mutant p53 and ER β , this therapeutic strategy has the potential to impact a huge number of TNBC patients with metastasis. Furthermore, Tam, Doxo, and ICIs such as anti-PD-1 and anti-PD-L1 are FDA-approved enabling repurposing of Tam to move faster in the clinical trial pipeline. *The proposed pre-clinical studies will inform future precision medicine trials in metastatic TNBC toward a novel combination therapy that is fundamentally better in terms of time to reach the patients and efficacy.*