Omobolaji Akala, MD, PhD

CRC IRB Proposal

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The prognostic role of CD47 in Triple negative Breast Cancer

1). Study Purpose and Rationale

Breast cancer is the most common malignancy in women worldwide and is one of the leading causes of cancer-related mortality. More than 1.2 million cases are diagnosed each year, affecting 10–12% of the female population and accounting for almost 500,000 deaths per year worldwide. Breast cancer mortality rates have been declining in the USA and several other countries since the early 1990s, for the most part due to the introduction of adjuvant systemic therapies. However, the large majority of primary breast cancer patients who receive adjuvant systemic therapy do not benefit from this intervention. Molecular biology has greatly enhanced our understanding of the heterogeneity of the disease, but only a few molecular tumor features (hormone receptor and HER2 status) are used in the clinic to guide the choice of a systemic treatment strategy. For estrogen receptor (ER)-positive disease, allocation to about 5 years of adjuvant endocrine therapy reduces the annual breast cancer death rate by approximately 30%. The addition of trastuzumab to adjuvant chemotherapy has improved outcome of HER2-positive breast cancer patients considerably. For ER-negative, progesterone receptor (PgR)-negative and HER2-negative breast cancer, defined as 'triple negative breast cancer', only conventional chemotherapy, which lacks an established therapeutic target, is available as an effective treatment option. Chemotherapy has certainly improved over the years, but with chemotherapy alone the residual risk remains substantially higher, between 30% and 40%. Improving outcomes in triple-negative disease will require better understanding of the biology and drug targets in this subtype. Triple negative breast cancer account for approximately 10–20% of the whole breast cancer cases and are associated with relatively early clinical relapses within 3 years, with frequent progression to distant metastasis, particularly, visceral metastasis and poor prognosis. One important goal is therefore the identification of prognostic factors and markers to reliably select high and low risk subsets of patients with triple-negative disease for different treatment approaches and also for development of targeted approaches.

Epithelial tumors contain a heterogeneous population of cells, and this cellular heterogeneity can be characterized by differences in the histopathology and functional properties (e.g.,anchorage-independent growth, proliferative capacities, and apoptotic responses to therapies). Some suggest that such intratumoral heterogeneity arises from genetic and epigenetic differences of tumor cells through selective pressure during tumor evolution. Recently emerging evidence supports the existence of a cellular hierarchy within epithelial tumors. At the top of this hierarchy is a tumor-initiating cell (T-IC) or cancer stem cell (CSC) population that can self-renew and differentiate to progeny cells, thus resulting in the observed cellular and functional heterogeneity of epithelial tumors. Candidate T-ICs have been isolated in a variety of solid tumors, including breast, brain, colorectal, head and neck, pancreatic, prostate, and melanoma, based primarily on the expression of CD44, CD133, ALDH, and ABCB5. Particularly looking in breast cancer overall CD44 and CD133 expression has been linked to a negative prognosis with regards to overall survival and relapse free survival. However neutralizing antibodies against these markers have not been effective in pre-clinical studies for targeted therapy. In the case of CD47, another cancer stem cell marker found in leukemias, a neutralizing antibody has been proven to be effective for targeted therapy in pre-clinical xenograft experiments and from anecdotal data is expressed in epithelial tumors.

Analysis of mouse myeloid leukemias show an increased expression of CD47 based on flow cytometry and mRNA levels compared to normal bone marrow, and on analysis of human leukemic stem cells it was shown that CD47 was more highly expressed when compared to normal HSCs. The Weissman group went on to explore the clinical significance of this observation based on the concept that leukemic progenitors are co-opting a strategy utilized by normal HSCs to evade macrophage mediated phagocytosis. In their studies they found that CD47 expression levels across subgroups of AML patients correlates with the presence of FLT3-ITD mutations and is associated with worse overall survival. Using CD47 expression as an independent variable to stratify 285 AML patients with diverse cytogenetic and molecular characteristics into low and high CD47 expression groups they found that the CD47 high group had a significant decrease in overall survival. Relying on xenotransplantation engraftment models in immunocompromised mice they went on to show that blocking antibodies against Human CD47 prevents engraftment of AML leukemic stem cells and clears already engrafted disease in these mice. In order to ascertain toxicity to normal tissues they repeated treatment with blocking antibodies against CD47 in a mouse model of leukemia. Again engraftment of transplantable leukemia was prevented and disease burden was cleared. There was no depletion of normal HSCs in these experiments. The mechanism of depletion and prevent of engraftment was shown to be a consequence of macrophage phagocytosis, after abrogation of the CD47-SIRP-alpha mediated negative regulation of phagocytosis by administration of blocking antibodies no longer allowed escape from immunosurveillance.

They went on to examine other malignancies for the expression of CD47 and found that in B- cell non-Hodgkin lymphomas there was also an increased expression CD47 compared to normal peripheral blood and germinal center B cells. They examined a large subset of primary patient samples from multiple subtypes of B cell NHL including large B cell lymphoma (DLBCL), B cell chronic lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL), follicular lymphoma (FL), marginal zone lymphoma (MZL), and pre-B acute lymphoblastic leukemia (pre-B ALL) and found differing levels of CD47 expression within the various subtypes. In three subtypes, diffuse large B cell lymphomas, B cell chronic lymphocytic leukemia***,*** and mantle cell lymphomas they found that higher expression of CD47 was associated with an increased risk of death and adverse molecular features, once again providing prognostic utility. In xenotransplantation experiments performed with immunocompromised mice, incubation of human NHL cells derived from a DLBCL and FL patient's with blocking anti-CD47 antibodies prevented tumor engraftment. They then went on to show that combination of blocking anti-CD47 antibodies and anti- CD20 antibody, rituximab was capable of eliminating already established lymphoma in primary human NHL xenotransplant mouse models.

The purpose of this study will be to determine if CD47 protein expression can serve as a prognostic marker for relapse-free survival in patients diagnosed with triple negative breast cancer

2). Study Design, Statistical Procedures, and Study Procedures

The study will be a retrospective cohort study with the goal of analyzing 350 cases of triple negative breast cancer in patients at NYP/Columbia Medical Center who have undergone current standard of care, which is local therapy (surgery and or radiation) and adjuvant chemotherapy. Patients will have an immunohistochemical diagnosis of triple negative status based on established standard pathologic guidelines for staining for ER, PR, and HER2. A pathological database chart review will allow for identification of these patients and archival paraffin embedded samples will be obtained. Clinical information such as age, tumor size,

histological grade, TNM stage and axillary lymph node status will also be obtained from medical records and pathology reports.

Immunohistochemical staining will be performed for CD47 protein expression. Samples will be scored as CD47 high if there is > 75% positive out total of a 500 counted cells under × 400 magnifications in 5 fields freely selected under a light microscope, and scored CD47 low if there is < 30% positive out total of a 500 counted cells under × 400 magnifications in 5 fields freely selected under a light microscope. All sections will be scored by two different investigators unaware of individual patient clinical outcome.

In order to power the study it was assumed that approximately 60% of patients would have high CD47 protein expression level of 75% based on expression levels seen in leukemia samples and bladder cancer samples. Using a two sided chi-square test for 80% power, testing at P=0.05, it was determined that I would need 107 patients in each group. Therefore analysis of 350 patients for the entire study should be sufficient.

Relapse-free survival (RFS) will be defined as the length of time from the date of definitive surgery to the last follow-up or first event. Relapse free survival will be estimated by using the Kaplan–Meier method, and the differences in survival curve between these two groups of the patients will be assessed by the log–rank test.

3). Study Drugs or devices: None, not applicable

4). Study Questionnaires: None, not applicable

5). Study Subjects

Exclusion: Patients who did not receive adjuvant chemotherapy, who did not have axillary lymph node dissections, and who received any targeted biologic therapies.

6). Recruitment of Subjects: Not applicable

7). Confidentiality of Study data: Individual patient information will be de-identified for confidentiality purposes.

8). Potential Conflict of Interest: There are no potential conflicts of interest

9). Potential Risks: As a retrospective cohort study, the only risk possible is the loss of confidentiality. The steps put in place to safeguard patient identity should negate this risk

10). Potential Benefits: This study does not offer direct benefits to patients whose tissue samples are analysed

11). Alternatives: Not applicable