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Review Comments

The paper titled “Beyond Triple Negative: molecular markers toward targeted therapy” is interesting. As we head toward more personalized treatments in TNBCs, there is a need to manage the heterogeneity of the disease with finesse, which would require a multi-modal arsenal of biomarker driven targets. More importantly, a uniform and accessible panel of biomarkers is warranted to allow for wider universal adoption. However, there are several minor issues. It would help improve the article if the author could response to the following questions and revise the article manuscript accordingly.

1. Radiation therapy is an important treatment modality for managing breast cancer. RT can also reprogram a fraction of the surviving breast cancer cells into breast cancer-initiating cells, which is supposed to contribute to disease recurrence. Would you please explain how to prevent the occurrence of radiation-induced reprogramming and improve the RT effect of TNBC patients?

Reply 1: Thank you for the question. The aim of the review paper is to address biomarkers in triple negative breast cancer which would help guide systemic therapy. While radiation therapy is an effective modality for locoregional management of breast cancer, its effect on reprograming cancer cells and their current clinical

relevance is unclear and therefore beyond the scope of the review. We understand that there is pre-clinical evidence that ionizing radiation can promote malignant breast cancer phenotypes with expansion of the cancer stem-like population through the Epithelial Mesenchymal Transition (EMT)(1). One would wonder whether the effects of radiation differ according to the biomarker profile of breast cancer, i.e. hormone receptor positive verses HER2 positive verses TNBCs and may have an impact into changing gene expression and subtype of TNBC in favor of mesenchymal subtype however at present time, how much of the radiation effects contribute to disease recurrence remains yet to be solved. Locoregional management of breast cancer with radiation therapy reduces risk of local disease recurrence and appears to be associated with overall survival benefit. There will be a topic on the role of radiation therapy in TNBC, which will be written by one of the other authors of the current special series.

2. What is the progress of PI3K/AKT/mTOR signaling pathway in the treatment of triple-negative breast cancer? Which targeted drugs are included?

Reply 2: Thank you for the question. Please review section of 5 of the review on the role of PI3K/AKT/mTOR as well as the Table included for ongoing clinical trials. We included the section below. It should also be reminded that new therapies in TNBC will be further discussed by one of the authors of the current special series.

The PI3K/AKT/mTOR kinases regulate key pathways essential to cell survival, proliferation and differentiation and are activated through different mechanisms in TNBC (2). *PIK3CA* mutations are associated with luminal cancers and the LAR subtype of TNBCs (3, 4). In basal-like cancers, PI3K/AKT pathway activation is mediated through a different mechanism, i.e., loss of negative regulators of the PI3K

pathway such as *PTEN* and *INPP4B* phosphatase(4-6). PTEN protein expression loss by IHC is significantly associated with large tumor size, high grade, recurrence and TNBC, as well as poorer prognosis (7) while INPP4B loss is associated with higher tumor grade and basal-like breast cancers (5). In mouse models, INPP4B loss led to dose-dependent increase in tumor incidence in *INPP4B* homozygous and heterozygous knockout mice compared to wild-type mice, supporting a role for *INPP4B* as a tumor suppressor in TNBC(6). Another mechanism of pathway activation includes mutations in the catalytic subunit of PI3K (p110α) which occur in about 10% of TNBC cases(4). Contrary to hormone receptor positive breast cancer, *PIK3CA* mutations in TNBCs are associated with improved survival(8).

The LOTUS trial is a phase II clinical trial including patients with treatment native metastatic TNBC who were randomized to paclitaxel plus either ipatasertib, oral ATP-competitive small molecule AKT inhibitor, or placebo. median OS (mOS) was 25.8 months in the ipatasertib plus paclitaxel arm vs 16.9 months in the placebo plus paclitaxel (9). Interestingly, PTEN-low and PIK3CA/AKT1/PTEN altered subgroups had better OS in ipatasertib plus paclitaxel group(9). This, however, did not translate to a meaningful benefit in phase III IPATunity130 that randomized patients with advanced TNBC and alterations in the PIK3CA/AKT1/PTEN pathway to ipatasertib plus paclitaxel verses placebo plus paclitaxel. There was similar overall response rate between the ipatasertib plus paclitaxel verses placebo plus paclitaxel arms (39% vs 35%, respectively). At a median follow-up of 8.3 months, PFS was similar between the experimental and placebo arms (7.4 vs 6.1 months, respectively)(10). This suggests there is likely redundant downstream signaling that bypasses AKT mediated inhibition. This is pending further analysis to explore potential biomarkers of benefit from ipatasertib in this trial. Interestingly, addition of ipatasertib to atezolizumab and chemotherapy (paclitaxel or nab-paclitaxel) in 26 patients with advanced TNBC had

an objective response rate of 73% seen regardless of PD-L1 or PIK3CA/AKT/PTEN pathway alteration status(11), suggesting a promising trend toward combining targeted therapies in TNBC .

Another phase II trial, the PAKT trial, investigated capivasertib, an oral AKT inhibitor, with paclitaxel versus paclitaxel alone as first-line treatment of metastatic TNBC. With capivasertib, PFS improved (5.9 vs 4.2 months, respectively) and in patients with *PIK3CA/AKT1/PTEN* alterations, this benefit was prominent (PFS, 9.3 vs 3.7 months, respectively). An improvement in median OS was seen in the entire population (19.1 vs 12.6 months; HR, 0.61; *P*=.04). A better understanding of the redundancy in the pathway and the main downstream drivers is required to drive precision medicine. Clinical trials are currently ongoing to evaluate PI3K/AKT/mTOR inhibitors in treating TNBC, Table 2.

3. Triple-negative breast cancer liver metastasis is associated with poor prognosis and low patient survival. What are the genomic/transcriptome characteristics of TNBC liver metastasis? What is its effect on the recurrence of potential therapeutic targets?

Reply 3: Thank you for a very interesting question. To summarize, the question is referring to whether there are different characteristics of TNBC related organ metastases including liver metastases. The molecular heterogeneity within any given tumor, between tumors in different organs and with time and treatment is certainly key to understanding the biology of any cancer and developing targeted therapies. This will certainly play an important role in the future with the advent of comprehensive genome profiling/Next Generation Sequencing on tissues and in blood through liquid biopsy as well as assessment of tumor expression profiles, epigenetic changes, miRNA regulation, proteomic changes and microbiome but at present time other than occasional unfunded NGS testing, other techniques are not used clinically and therefore beyond the scope of the review. There is indeed a differential

expression profile of breast cancer metastases to the liver with downregulation of extracellular matrix/stromal genes but specific targeting of these changes for patients with liver metastases from TNBC is not being investigated in clinical trials to our knowledge(12). There may be additional gene signatures for organ specific metastases but again this would be beyond the scope of the review as it would likely require a whole review paper in its own accord. Whether these gene signatures are prognostic or predictive remains to be determined.

4. What is the current status of nanotherapy options for TNBC patients? How to identify promising molecular targets? What are the challenges related to the development of targeted nanotherapeutics?

Reply 4: The only clinical use for nanoparticles in oncology is within a formulation of nab-paclitaxel/abraxane where albumin-bound to a nanoparticle is used instead of cremphore to dissolve paclitaxel. Other than this, we found limited publications on breast cancer and nanotherapy and it seems largely preclinical(13). Few are specific to TNBC(14). We look forward to seeing what this field would offer.

5. How to identify subtypes of breast cancer that may respond to neoadjuvant chemotherapy? How to analyze the range of chemotherapy regimens and response rates used?

Reply 5: This will be the scope of the topic “Adjuvant vs. neoadjuvant chemotherapy in triple negative breast cancer”, also part of the current special series.

6. There have been many studies on triple-negative breast cancer. What is the difference between this study and previous studies? What is the innovation? These

need to be described in the introduction.

Reply 6: Thanks for the question. We appreciate it and find this to be key for a paper review. This has been updated and highlighted in the introduction below.

Triple negative breast cancer (TNBC) is clinically defined by the lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and low expression of human epidermal growth factor receptor (HER2). As these cancers are defined by what they are not rather than what they are, they naturally represent a heterogenous group of cancers that are still largely managed as a single entity disease.

TNBCs represent 15-20% of breast cancers, are more common in younger women and those of African American descent (15, 16), as well as, in *BRCA* mutation carriers(17). Women with TNBC tend to present with large tumors that are usually higher grade and involve the lymph nodes(18). TNBCs have been characterized by an aggressive natural history with higher rates of relapse within the first five years, in addition to higher rates of distant recurrences, worse disease-free survival (DFS) and overall survival (OS) compared to other breast cancer subtypes(18). Despite molecular advances in characterizing TNBCs and the availability of few targeted therapies in the advanced setting, the overall survival of women with metastatic TNBC remains low(18).

TNBCs have been characterized at the genetic and epigenetic levels (4, 19-21), yet therapeutic targets have been lagging. Chemotherapy has been the backbone line of treatment for TNBCs. Recent advances in treatment include immune checkpoint inhibitors (ICIs), such as Atezolizumab with nab-paclitaxel or Pembrolizumab in combination with chemotherapy for PD-L1 positive TNBCs in the metastatic setting

(22-24), and PARP inhibitors for previously treated *BRCA* mutation carriers with metastatic TNBCs (25-27). Antibody drug conjugate (ADC), sacituzumab govitecan, has recently been FDA approved in patients with metastatic TNBC who received at least two prior therapies(28, 29). Current challenges include translating the heterogeneity within TNBC to individualized treatment plans for the patient, identifying and utilizing biomarkers that predict survival and/or treatment response and identifying optimal tools to help guide precision medicine. This is in addition to a need to better understand mechanisms of chemoresistance in TNBC. The landscape of biomarker driven targeted therapy in TNBC is rapidly changing, and there are several ongoing clinical trials with potential to personalize the standard care of treatment for this heterogenous disease. Here, we present a review of the recent literature and our current knowledge of the molecular characteristics of this unique subset of breast cancer. Furthermore, we highlight clinically relevant biomarkers that have been described for TNBC, and we focus on emerging potential therapeutic targets.

7. What is the efficacy and safety of antibody-drug conjugates in breast cancer?

Reply 7: Thank you for the question. This has been updated and highlighted in the section below.

Antibody drug conjugates (ADC) are multiagent drugs aimed at tumor targeted delivery of therapeutic small molecules and have shown promising results in TNBC. ADCs include three agents: an antibody directed to a tumor antigen, a cytotoxic molecule, and a linker in between (28). Sacituzumab govitecan is an anti-trophoblast cell-surface antigen (Trop-2) antibody conjugated to a DNA damaging agent, SN-38, via a pH-sensitive cleavable linker. Elevated expression of Trop-2 in breast cancer is correlated with poor prognosis (28). In a single-arm phase I/II study, 108 patients with

metastatic TNBC treated with at least two prior therapies received sacituzumab govitecan with objective response rate (ORR) of 33.3%, median PFS of 5.5 months and median OS of 13.0 months regardless of Trop-2 expression in tumors (28). In a phase III trial, the study compared sacituzumab govitecan with single-agent chemotherapy in 468 patients with relapsed/refractory TNBC. Median PFS was significantly longer with sacituzumab govitecan versus control group (5.6 vs. 1.7 months, respectively). Median OS was 12.1 months with sacituzumab govitecan compared to 6.7 months with chemotherapy and objective response rates were 35% and 5%, respectively (29). Side effect profile is similar to other chemotherapy drugs and include but are not limited to neutropenia, anemia, GI symptoms such as nausea, diarrhea as well alopecia and fatigue. Common grade 3 or 4 toxicities included neutropenia, diarrhea, anemia. This led to accelerated approval by FDA for adult patients with metastatic TNBC who had received at least two prior therapies. Another ADC, Ladiratuzumab vedotin, is a humanized antibody targeting the zinc transporter LIV-1 conjugated with a microtubule-disrupting agent, monomethyl auristatin E (MMAE) by a proteolytically cleavable linker. LIV-1 is a multi-span transmembrane protein with putative zinc transporter and metalloproteinase activity expressed in 68% of metastatic TNBC tumors (30). Interim results of the phase I study showed favorable antitumor activity and tolerability of ladiratuzumab vedotin with key adverse events including GI symptoms, neutropenia and peripheral neuropathy (31). Trastuzumab deruxtecan, a humanized antibody against HER2 conjugated with a topoisomerase I inhibitor, extecan derivative (DXd) by a cleavable peptide linker, has shown activity in low HER2 (IHC 1+ or 2+/ISH negative) expressing metastatic breast cancer (32) and is currently under further investigation. Ongoing clinical trials with ADCs in metastatic TNBC are outlined in Table 7.

8. How effective is the new combination therapy with PDGFRβ aptamer and anti-PD-L1 mAb in TNBC?

Reply 8: Thank you for an interesting question. While it is interesting, it is in preclinical models. We are referring to human and murine in vitro studies. This will also be discussed in the topic “New therapies in triple negative breast cancer”.