Peer Review File

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Comment 1:

There are genomic changes in the ESR1 and PIK3CA genes. How do these mutations provide information for new combinations to improve future clinical efficacy?

Response:

Thank you for the comment. The value of assessing ESR1 and PIK3CA mutations has been predominantly studies in HR positive BC patients treated with CDK4/6 inhibitor in combination with fulvestrant. In RCT settings, the exploratory analysis of PALOMA-3 trial revealed that baseline mutations in ESR1 and PIK3CA genes, may attenuate the response to treatment with CDK4/6 inhibitors. However, in another study employing paired baseline and end of study sequencing of ctDNA, from the PALOMA-3 study, newer driver mutations in the PIK3CA and ESR1 genes emerged during treatment in both the arms. This suggested that mutations in these 2 loci might have therapeutic implications for treatment with other endocrine drugs like aromatase inhibitors. The clinical significance of these mutations with respect to stage of disease and line of treatment was assessed by a study by Takeshita et al (Takeshita, T., Yamamoto, Y., Yamamoto-Ibusuki, M. et al. Clinical significance of plasma cell-free DNA mutations in PIK3CA, AKT1, and ESR1 gene according to treatment lines in ER-positive breast cancer. Mol Cancer 17, 67 (2018). https://doi.org/10.1186/s12943-018-0808-y). They analysed the ctDNA of early and metastatic BC patients and identified that the prevalence of mutations in PIK3CA and ESR1 genes were high in late treatment settings. This suggested the role of endocrine therapy in driving the mutations leading to resistance to endocrine therapy. The same study also revealed shorter time to treatment failure in PIK3CA mutated patients treated at early lines of treatment while there was no statistically significant difference in patients with ESR1 mutations. Hence the current evidence suggests a role for mutations in PIK3CA and ESR1 genes to impact treatment outcomes with endocrine therapy. This should be considered while designing future trials to evaluate the efficacy of drugs with similar molecular targets. A part of this explanation is included in the revised manuscript.

Comment 2:

Clonal evolution leads to frequent driver gene mutations in patients with advanced treatment. Do early and late progressions have different resistance mechanisms? What is the difference between the treatment of early and late patients?

Response:

Thank you for the comment. We acknowledge the role of driver mutations in influencing the treatment outcome. Based on the current level of evidence, there seems to be not much difference with respect to the mechanism of resistance in early and late progressions. But the baseline resistance in certain genetic hotspots seems to influence the time to progression in patients on therapy. As mentioned above, the exploratory study from the PALOMA 3 trial provided the role of ESR1 and PIK3CA mutations during therapy. The study revealed that the emergence of driver mutation was low in early progression than in comparison to late progression. This could be due to the fact that in early progression, resistance in distinct genetic loci were already present at baseline. The current treatment strategy for early and late progression depends on whether the clinical setting is early breast cancer or advanced/metastatic breast cancer. Sequential treatment strategies are provided in NCNN guidelines and are beyond the scope of this review.

Comment 3:

How many endocrine resistance mechanisms of ER+/HER2-advanced breast cancer to estrogen receptor modulators play a role in endocrine therapy?

Response:

Thank you for the comment. Since we had focussed on biomarkers in RCTs, we had included only PIK3CA and ESR1 mutation in the current review. Apart from these, translocation in ESR1, YAP1 genes and up regulation of miRNAs (miR-155, miR-221/222, miR-21, miR-125b) also seems to influence resistance to endocrine therapies in cell line studies and observational studies.

Comment 4:

How effective is the AKT inhibitor MK-2206 in advanced breast cancer patients with PIK3CA or AKT mutations and/or PTEN deletion/PTEN mutations?

Response:

Thank you for the comment. The phase 2 study in advanced breast cancer patients treated with MK-2206 did not reveal good treatment outcome in patients selected for PIK3CA or AKT mutations and/or PTEN deletion/PTEN mutations. The same as been mentioned in the revised manuscript as follows,

‘’ Similarly, MK-2206, which is an allosteric AKT inhibitor, has been evaluated in a recent phase-2 study. The study revealed limited clinical activity for MK-2206 in patients with PIK3CA/AKT1 mutation (ORR:5.6%; 1/18 partial response) and PTEN loss/mutation (ORR:0%).’’

Comment 5:

How to explore the activity of immune checkpoint modulators in breast cancer treatment in advanced and neoadjuvant settings?

Response:

Thank you for the comment. Kindly note that currently 8 different clinical studies with PD-1 and PD-L1 inhibitors have been completed in breast cancer patients in different settings. Among the studies, only atezolizumab in combination with nab-paclitaxel have been assessed in phase 3 study in TNBC patients. Except the Keynote 014 phase 2 study and Javelin phase 1 study, all the other studies have evaluated the effectiveness of PD-1/PD-L1 inhibitors in TNBC patients either in advanced or metastatic settings. The Keynote 014 study evaluated the effectiveness of pembrolizumab in advanced HER2 positive patients who had progressed on trastuzumab treatment. The study reported good tumor response and survival outcomes in PD-L1 expression positive patients. Whereas the Javelin study with avelumab reported a ORR of 22.1% in TNBC patients, 2.8% in ER positive patients and 0% in HER2 positive patients. None of the studies had evaluated the role of ICIs in neoadjuvant settings. Since the purpose of this review is to predominantly assess biomarkers in RCTs, we had not included the above studies in the review.

Comment 6:

The latest advances in genome sequencing indicate that the genetic diversity between patients and between different subclonal cell populations within the same patient may evolve at the location of metastatic tumors and during treatment. Translate this knowledge into better clinical care?

Response:

Thank you for the comment. We acknowledge the role of genome sequencing in predicting therapeutic response. But the difference in genetic mutation landscape within sub clonal cell populations needs further studies for providing conclusive results. In RCTs, mutation landscape is either assessed in stored tumor samples or in ctDNA both of which may not provide the complete mutation landscape. This could be the reason for outliers in exploratory studies and in observational studies evaluating the role of mutations on the therapeutic outcomes. In future, patients may have to be assessed for mutation periodically from ctDNA for improving the clinical care. But assessment of mutation landscape in metastatic sites and continuous monitoring of tumor samples may not be practically possible. A part of this explanation is included in the revised manuscript.

Comment 7:

How to use biomarkers alone or in combination with other immunotherapy, chemotherapy, radiotherapy and small molecule inhibitors?

Response:

Thank you for the comment. Kindly note that assessment of biomarkers alone may not improve patient outcomes. With the current level of evidence, biomarkers could predict treatment outcome in different patients with breast cancer and could be used for stratifying patients in future clinical trials. Biomarkers could also assist in devising combination therapy. PD-1/PD-L1 expression could assist in selecting

patients for treatment with PD-1/PD-L1 inhibitors. For chemotherapy and radiotherapy, no conclusive biomarkers have been established yet. Hence, they were not mentioned in the review.

Comment 8:

Although we have made significant progress in understanding the molecular basis of hormone receptor-positive breast cancer, endocrine drug-resistant diseases are still the main cause of breast cancer mortality. How to deepen our understanding of changes in breast cancer under treatment pressure to reveal the mechanism of resistance and apply precision medicine in biomarker-driven clinical trials?

Response:

Thank you for the comment. The majority of patients with endocrine resistant disease present with mutation is ESR1 and PIK3CA genes. As mentioned as response to query 3, the other modes of resistance have not been conclusively proved in RCT settings. The understanding of endocrine resistance attributed to ESR1 and PIK3CA mutations has led to the novel combination therapy with PI3K inhibitors. In this context, exploration of biomarkers and expanding the available targeted therapy drugs could lead to enhanced application of precision medicine. Future clinical trials have to stratify patients based on biomarkers to improve patient outcomes. Further, all the biomarker studies from pivotal clinical trials are exploratory or post-hoc analysis which may not have the statistical power to predict the biomarker related endpoints. This also needs to be accounted for in future trials.