**Reviewer's report**

**Title:** MTDH Mediates Trastuzumab Resistance in HER2 Positive Breast Cancer by Decreasing PTEN Expression through an NF-kappa B dependent Pathway.

**Version:** 3  
**Date:** 14 August 2014  
**Reviewer:** Carmen Blanco-Aparicio

**Reviewer's report:**

Du et al describe that there is a correlation between high levels of MTDH in breast cancer patients and positive nodal status, advanced pathological stage and high Ki67 index, and in a small population of patients treated with trastuzumab there is a trend toward higher clinical benefit rate and a longer PFS in patients with low MTDH expression and those patients show higher levels of PTEN. To validate the hypothesis that MTDH upregulation could be associated with PTEN downregulation and trastuzumab resistance in HER2 positive breast cancers. The authors have generated a resistant HER2 cell line to trastuzumab, SKBR3-R cell line. This resistant cell line in comparison with the parental cell line showed higher levels of MTDH, lower levels of PTEN and activation of AKT. The authors showed by knocking down or overexpressing MTDH in resistant and parental cell lines respectively that MTDH regulates the expression of PTEN via NF#B. Moreover, restoration of PTEN levels downregulates AKT activation and inhibits cellular proliferation and restores partial response to trastuzumab. The same effect was observed in vivo when resistant and parental cell lines where injected in nude mide. Tumors from the resistant cell line in which MTDH has being knockdown grew slower and respond to trastuzumab treatment what correlates with higher levels of PTEN. By contrary tumors from the parental cell line in which MTDH has been overexpressed grew faster and do not respond to trastuzumab and this correlates with lower levels of PTEN. All these results pointed out to do further studies with human samples to translate the hypothesis of the role of MTDH levels to the clinic.

Before the paper should be accepted minor essential and major compulsory revision.

**Minor essential revision.**

1. Results, paragraph “Forced expression in SKBR-3/r cells restored trastuzumab sensitivity.” When they say “ Furthermore, SKBR-3 cells with GFP-PTEN, should say SKBR-3-R

2. In legend of Figure 2A should be indicated the concentration of Trastuzumab used.

3. In legend of Figure 3B should be indicated the duration of the trastuzumab treatment and the concentration used.

4. In figure 3B, 3C and 3D should be indicated that knockdown is down in SKBR-3/R cells and overexpression in SKBR-3.
5. In figure S1 should be indicated that knockdown is down in SKBR-3/R cells and overexpression in SKBR-3.
6. In figure S2C, correct X axis.

Major compulsory revision
1. In figure 4C: The knockdown of p65 or MTDH has a similar effect in the relative levels of PTEN luc activity and the knockdown of both proteins has an additive effect, do the authors have check the effect of an NF#B inhibitors in this resistant cell line in the levels of PTEN and if this treatment restore the response to trastuzumab.

2. In figure 5A: the authors show the expression of endogenous PTEN and PTEN-GFP, they claim that they see an increase in PTEN levels, but the size of PTEN and PTEN-GFP is different, the band that they show in the picture correspond to PTEN or PTEN-GFP? It could be better if the show in the same picture both the endogenous and PTEN-GFP.

3. Also in figure 5A there is not a complete downregulation of AKTP in those cells lines that overexpress PTEN, do the authors could explain that. If they treat SKBR3/R cells with a PI3K inhibitor do they see a complete inhibition of AKTP?

4. In figure 5C: overexpression of PTEN-GFP clearly disminish the proliferation capacity of the cells but eventhough statistically significant when they treat this cells with trastuzumab they don’t see a strong response. Do the authors have any explanation for this result?

5. As the authors claim that MTDH upregulation in resistant cell lines regulates the levels of PTEN inducing an activation of AKT, do this resistant cell line respond to PI3K inhibition and combination with trastuzumab restore its sensitivity. As this combination treatments are already in the clinics and it would be important to see that those resistant tumors with high levels of MTDH could also respond to the combination treatment with PI3K inhibitors and trastuzumab.

6. In figure 6C: IHC for MTDH should be included to show that knockdown and overexpression of MTDH has been keep a long the treatment.


Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.