Author's response to reviews

Title: A novel deleterious PTEN mutation in a patient with early-onset bilateral breast cancer

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Author's response to reviews: see over
Response to the comments by Dr. Michel Longy

Dear Dr. Longy,

thank you for your stimulating observations; you will find below the answers to each point.

- The tumour from which PTEN and AKT immunostaining was performed is not specified. It would be interesting to repeat the analysis on the three breast tumours presented by this patient and compare the results. 

PTEN and AKT immunostaining was performed on the first invasive and on the in situ tumors (the third tumor was not available), with consistent results; we have changed figure 2D, where both the tumors are now shown.

- According to the material available, the same remark could also concern the somatic mutation screen of the PTEN, AKT and PIK3CA genes. 

Unfortunately, tumor DNA was available for the in situ breast cancer only.

I disagree with the authors when they say that PTEN acts as monoallelic defect in breast cancers occurring in Cowden patients. Literature data are consistent with the Knudson model in this situation (cf Banneau et al. Breast Cancer Res. 2010;12(4):R63). PTEN IHC remains positive in this patient because the missense mutation does not activate the NMD and maintain a protein expression.

The absence of LOH at the PTEN locus is not sufficient to rule out an inactivation of the wild type allele in the tumour cells. Such an inactivation could be the result of another somatic PTEN mutation or a loss of expression by promoter hypermethylation. These hypotheses must be either explored (eventually by screening for allelic exclusion at the RNA level) or discussed.

The adherence of PTEN to the Knudson model is, indeed, a controversial issue in the literature. We agree that for breast cancer arising in Cowden patients the results of the studies by your group suggest the complete loss of PTEN and no data exist, to the best of our knowledge, demonstrating the opposite, which suggests that, at least in this situation, the two-hit mechanism is followed. We also agree that, in the case here described, positive PTEN immunostaining does not exclude the loss of functional protein, since the mutant protein is expected to be expressed anyway; we have performed complete PTEN sequencing without finding any additional mutation, but we couldn’t exclude epigenetic defects because DNA was not sufficient for additional tests and RNA was not available. We have discussed these issues in the conclusions section.

Minor points:

- PHTS not PTHS
- oral papillomatous papules
Both have been corrected in the text

-what is QUART? (quadrantectomy?)

QUART means Quadrantectomy plus Axillary dissection plus RadioTherapy according to Veronesi, but we understand that it is not a universally used term, therefore we have changed it in the text.

-Is the IDC ER/PR negative HER2 positive an apocrine carcinoma with AR positive?
Unfortunately we are not able to answer this question, since pathologic diagnosis was performed in another institution and we could not obtain tissue from this tumor.

- SIFT score is 0.01
  We have repeated the in silico analysis and consistently found the value of 0, as shown in the screen-capture below. Maybe you mean that, being a probability, a 0 value should not be allowed, but we chose to report faithfully the prediction provided by the in silico tool.

- What is the origin of the XTC UC1 cell line?
  XTC.UC1 is an immortalized cell line, derived from a metastasis of thyroid oncocytic follicular carcinoma (Zielke et al, Thyroid 8: 75-83, 1998), which our group has demonstrated to be PTEN null (unpublished data, now reported in supplementary material).

Response to the comments by Dr. Robert Pilarski

Dear Dr. Pilarski,
Thank you for your gratifying comments; you will find below our answer to each point.

- “Clinical History” section: since the initial breast cancer was ER/PR positive, HER2/neu negative, while the relapse was ER/PR negative, HER2/neu positive, the authors should discuss the possibility that this represents a third primary breast cancer, rather than a relapse.
  We completely agree and have changed the text accordingly.

- “Conclusions” section: the first paragraph would should include a short discussion of the available data on breast cancer risk in women with PTEN mutations, particular the limited data on bilateral risk.
  We have included such a discussion.

- “Background” section, line 16: “PTEN Hamartoma Tumor Syndrome” should be abbreviated “PHTS”, not “PTHS”.
- “Functional assessment of the mutation” section: first line, “length” is spelled incorrectly.
  Both have been corrected in the text