Reviewer's report

Title: Estrogen receptor alpha and aryl hydrocarbon receptor independent growth inhibitory effects of aminoflavone in breast cancer cells

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Reviewer: Vincenzo Pezzi

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The study by Brinkman et. al examined the effects of Aminoflavone (AF), an activator of AhR signaling, on two triple negative breast cancer (TNBC) cell lines. The authors conclude that AF have growth inhibitory effects also on ER-negative BR cells and that the mechanisms involved are AhR-independent. In particular they suggest that the mechanisms of cytotoxicity activated are complex and likely, cell line- and tumor specific.

The paper is very interesting and most of the experiment are well performed. However, the paper is weaken for two reason:

1. In figure 3B western blot analyses show that AhR expression are not completely abrogated (especially in MDA-MB-468). Considering also that the concentration of AF able to induce the effects on cell proliferation is relatively low, the expression of AhR could be sufficient to mediate AF action. In fact also the results showed in Fig. 3 C and D suggest that the transcription of a AhR-dependent gene as CYP1A1 is present even though at very low levels. For these reason more accurate experiments are necessary before to affirm that MDA-MB-468 and Cal51 growth inhibition induced by AF “occurs in the presence and absence of endogeneous levels of AhR protein and signaling”.

2. The different effects of AF on Cell cycle of two cell lines should be supported also by the measurement of the expression of markers (Cyclins, CDKs, CDKIs...) of cell cycle progression.

Addition of these two major points will contribute to make stronger the date supporting the authors’ conclusions.

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.