Author's response to reviews

Title: Extra-Virgin Olive Oil (EVOO)-derived Secoiridoids and lignans: Two new families of anti-HER2 (erbB-2) phytochemicals

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Dear Editor-in-Chief,

Please find attached the manuscript entitled: “Extra-Virgin Olive Oil (EVOO)-derived secoiridoids and lignans: Two new families of anti-HER2 (erbB-2) phytochemicals”. Since we understand that the Editorial Board of the BMC CANCER must ensure the rapid publication of manuscripts of exceptional importance, with a broad scientific audience, and that their consideration is based upon a comparative pool and therefore highly selective, we truly appreciate this opportunity to consider our manuscript for evaluation in your prestigious journal.

We here designed a systematic approach to investigate the effects of Extra Virgin Olive Oil (EVOO)-derived phenolic compounds on the expression and oncogenic activity of the HER2 tyrosine kinase receptor in human breast cancer-derived cell lines. First, semi-preparative high-performance liquid chromatography (HPLC) was used to isolate phenolics from commercial EVOO. Second, analytical HPLC was performed to check for the purity of the isolated compounds and to confirm their identity. Third, the effects of micromolar amounts of EVOO phenolics on breast cancer cell viability, proliferation and apoptosis were assessed using MTT, crystal violet staining, and Cell Death ELISA assays, respectively. Fourth, the effects of EVOO phenolics on both the activation status and the expression levels of HER2 oncoprotein HER2-specific ELISAs. All the major EVOO polyphenols (i.e. secoiridoids and lignans), but not EVOO single phenols and phenolic acids, were found to induce strong tumoricidal effects within a micromolar range by selectively triggering apoptotic cell death in HER2-overexpressing breast cancer in vitro models. Mechanistically, EVOO polyphenols drastically reduced the tyrosine kinase activity of HER2 (up to 95% when using the lignan 1-(+)-acetoxypinoresinol). Short-term exposure to EVOO polyphenols blocked HER2 signaling by rapidly reducing the activation status of the 1248 tyrosine residue (Y1248), the main autophosphorylation site of HER2. Long-term exposure to EVOO polyphenols further suppressed the content in HER2 protein (up to 87% when using the secoiridoid ligstroside aglycone) regardless the molecular mechanism contributing to HER2 overexpression (i.e. naturally by gene amplification in SKBR3 cells and ectopically driven by a viral promoter in MCF-7 cells transduced with the human HER2 cDNA).

Considering that an EVOO-rich Mediterranean diet can supply ~10–20 mg of polyphenols per day, the plasma concentration of these compounds will exceed $10^{-6}$-$10^{-5}$ M and,
therefore, the tumoricidal and anti-HER2 effects at micromolar concentrations of EVOO lignans and secoiridoids are within the concentration range expected after nutritional intake from EVOO-rich Mediterranean diets. Therefore, our current results provide new insights on the ultimate mechanisms by which EVOO (i.e. the juice of the olive obtained solely by pressing and consumed without any further refining process), may significantly decrease the number of HER2-positive breast carcinomas in populations consuming a predominantly EVOO-based Mediterranean-style diet. Moreover, the fact that humans have safely been ingesting significant amounts of secoiridoids and lignans as long as they have been consuming olives and OO, strongly suggest that the stereochemistry of these phytochemicals might provide an excellent and safe platform for the design of new HER2-targeted anti-breast cancer drugs.

We here took a complex experimental approach including chemical isolation, characterization and purification of EVOO phenolics, retroviral generation of experimental breast cancer in vitro models mimicking HER2 oncogene-driven breast cancer disease, and cellular/molecular characterization of the anti-HER2 effects of EVOO phenolics. Because of the biological and clinical relevance of this original approach we therefore urge you to evaluate this manuscript for publication in BMC CANCER.

All authors of this manuscript have directly participated in the planning, execution, and analysis of the study. All authors are aware of and agree to the content of the manuscript, and all authors have approved the final version submitted and their being listed as an author on the manuscript. The contents of this manuscript have not been copyrighted or published previously. There are no directly related manuscripts or abstracts, published or unpublished, by one or more authors of this manuscript. The contents of this manuscript are not now under consideration for publication elsewhere. Neither the submitted manuscript nor any similar manuscript, in whole or in part, will be copyrighted, submitted, or published elsewhere while the Journal is under consideration.

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Thank you in advance for evaluating this manuscript for publication in your influential journal. Please do not hesitate to contact me at your earliest convenience.

We look forward to seeing our work published in BMC CANCER.

Yours sincerely,

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