Reviewer's report

Title: High incidence of microsatellite instability and loss of heterozygosity in three loci in breast cancer patients receiving chemotherapy: a prospective study

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Reviewer: Yih-Horng Shiao

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Authors reported that chemotherapy for less than 3 months induced microsatellite instability/loss of heterozygosity (MSI/LOH) in peripheral blood DNA in >70% patients and these genetic alterations associated with immunohistochemical stainings of mismatch repair (MMR) proteins in breast cancer tissues. Although few reports have described this new paradigm of frequent chemotherapy-mediated MSI/LOH (Fonseca et al., Breast Cancer Res 7:R28, 2005; Pinto et al., J Pharm Pharmacol 63:931, 2010), such observations were made by gel electrophoresis only without further validation by DNA sequencing. It is known that PCR tends to generate stutter microsatellite products due to polymerase slippage or misalignment of complementary DNA strands. Denaturing gel/capillary electrophoresis is commonly used to resolve microsatellite repeats and to prevent artifacts from partially denatured PCR products (Slovak et al., Breast Cancer Res Treat 119: 391, 2010). Since conditions of denaturing gel/capillary electrophoresis vary from lab to lab, it is important to validate the electrophoresis assay with DNA sequencing first before large-scale screening of MSI/LOH. In the current study, “normal genotypes” in Figure 1 appear to have more than 2 alleles, indicating that the electrophoresis conditions have not been optimized and may bias the MSI/LOH detection. It is not clear what the biological relevance of MSI/LOH in peripheral blood is to the low levels of MMR proteins in archived breast cancer tissues. The time point, before or after chemotherapy, for the collection of those archived tissues was not given also. These technical uncertainties refrain authors from making meaningful conclusion.

Major compulsory revisions

1. Need to validate the electrophoresis conditions for detecting true MSI/LOH, not artifacts, by DNA sequencing.

2. It is necessary to include an internal molecular weight control to determine the correct PCR products or de novo alleles.

3. MSI/LOH should be also examined in archived breast cancer tissues to correlate with immunohistochemical signal of MMR proteins.

Minor essential revisions

1. The primer concentration of “4 pmol/L”, described in DNA extraction and LOH
and MSI analysis, is incorrect. The 4 pmol/L means 4 pM. The primer of 0.05-0.5 uM final concentration is commonly used.

2. Please define the abbreviations, “F”, “CAR”, “TAR”, “TTCR”, “AACR”, and “GCCR” in Table 2. In this same table, the “No. of repeats” should be “unit of repeat”.

3. Label subject ID to each panel in Figure 1. Define the color code.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

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

**Declaration of competing interests:**

I declare that I have no competing interests.