Detection of occult HER2 mRNA-positive tumor cells in the peripheral blood of patients with operable breast cancer: evaluation of their prognostic relevance

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Unfortunately, one of the paragraphs contained some mistakes. Please find the corrected paragraph below.

Nested reverse transcription-polymerase chain reaction (RT-PCR)

A previously described nested RT-PCR assay for the detection of HER2 mRNA [21] with minor modifications was used [22]. Reverse transcription of RNA was carried out with the Thermoscript RT-PCR system (Invitrogen,

Paisley, UK). cDNA was synthesized according to the manufacturer's instructions. Two different PCR reactions, with the respective negative controls, were performed with each sample in order to amplify fragments of HER2 and β -actin. The sequences of primers used for HER2 (synthesized by Gencet, Paris, France) were as follows: 5'TC CTCCTCGCCCTCTTGC3' (sense Her-2-A); 5'GCGGGT CTCCATTGTCTA3' (antisense Her-2-B); 5'AGCCGCGA GCACCCAAGT3' (sense Her-2-C); 5'TTGGTGGGCAGG TAGGTGAGTT3' (antisense Her-2-D). To amplify cDNA, 2 μ l were subjected to first PCR in 20 μ l buffer [10 mM of HCL, buffer (pH 8.3), 50 mM KCL and 2.5 mM $MgCl_2$] containing 1 mM deoxynucleotide triphosphate, 3 μ M of primers (Her-2-A and Her-2-B) and 2.5 units platinum Taq DNA polymerase (Invitrogen). For the second round of amplification (nested RT-PCR) a 2 μ l aliquot of first PCR product was added to the same PCR buffer with 1 mM deoxynucleotide triphosphate, 3 μ M of primers (Her-2-C and Her-2-D) and 2.5 units platinum Taq DNA polymerase. The nested RT-PCR was performed using a modified touchdown program as previously described [22]. All PCR products were electrophoresed in agarose 2% gel, stained with ethidium bromide and photographed under UV conditions. In order to determine the sensitivity of the assay, MCF-7, T47D and SKBR3 cells were mixed with normal PBMCs in a cell ratio ranging from 1:10 to 1:10⁶, and total RNA was extracted from these cell dilutions and tested for HER2 mRNA by nested RT-PCR.

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