Heterogeneity of Mesenchymal Markers Expression—Molecular Profiles of Cancer Cells Disseminated by Lymphatic and Hematogenous Routes in Breast Cancer

Aleksandra Markiewicz [1,2], Magdalena Książkiewicz [1], Barbara Seroczyńska [3], Jarosław Skokowski [3,4], Jolanta Szade [5], Marzena Wełnicka-Jaśkiewicz [6] and Anna J. Zaczek [1,*]

Correction on page 1497

The correction concerns the following section, which should be shown in the main text, between Sections 4.1 and 4.2 on page 1497:

CTCs Isolation/Enrichment

Blood samples (5 mL), were diluted with 1 × PBS buffer (pH 7.2) to obtain 9 mL solution and layered on a density gradient comprising of two layers: a denser lower layer (Polymorphprep, Axies-Shield, Oslo, Norway) and an upper layer (Nycodenz, Axies-Shield, Oslo, Norway). Samples were centrifuged at 450 g for 20 min at 20 ℃. A fraction containing tumor cells was collected, washed with 10 mL of cold PBS, spun down, suspended in 1 mL of DB buffer (1 × PBS, 2 mM EDTA, 1% FBS) and subjected to negative immunoselection with anti-CD45-covered magnetic particles (CD45 Dynabeads, Invitrogen, Warsaw, Poland; 250 µL of magnetic particles per 5 mL of initial blood volume). Samples were incubated in a Hoola Mixer (Invitrogen), allowing sample rotation and shaking, for 30 min at 4 ℃. Samples were then diluted with DB buffer and incubated on a magnet (DynaMag 15, Invitrogen) for 10 min. The collected solutions were incubated a second time in a magnet to assure depletion of magnetic particles. The CD45-depleted samples were then diluted with a DB buffer to 50 mL and centrifuged at 600 g for 15 min at 4 ℃. From the pellets RNA was isolated with 1 mL Trizol reagent (Invitrogen) according to the manufacturer's instructions.