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Isolation and detection of CTCs in HCC pat

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- Blood from Cellsave Preservative Tubes was transferred into 50 ml tubes and made up to the 15 ml mark with PBS

1 (without Ca2+ and Mg2+)

2 - this was transferred to the filter of the OncoQuick tube.

3 - Centrifuge this OncoQuick tube for 20 minutes at 1600g and +4 °C. The acceleration as well as the deceleration of the centrifuge were set to level five (medium) as specified by greiner bio one. The pipette and a 50 ml tube were moistened with the FACS buffer to minimize the binding of tumor cells to the walls of these materials. This step was also called "pre-wetting".

4 - The supernatant from the centrifuged Oncoquick tube was carefully pipetted off completely and transferred with the moistened pipette to the 50 ml tube that had also been prepared. The walls of the Oncoquick tube were washed again with 5 ml FACS buffer by gentle swiveling during pipetting in order to transfer any adherent tumor cells. The same 5 ml FACS buffer was then added to each 50 ml tube.

5 - The first washing of the resulting sample was performed by refilling to the 50 ml mark with FACS buffer and swivelling the tube five times. The tubes were then centrifuged for 10 min at 200 g and +4 °C. The acceleration and deceleration were set to the highest level (nine).

6 - Pipette 45 ml of the solution washed in step 5, leaving the cells at the bottom of the tube undisturbed.

7 - For the second wash, the same tube was refilled to the 50 ml mark with FACS buffer and swivelled five times. The tube was then centrifuged again for 10 min at 200 g and +4 °C.

8 - The air bubbles formed on the surface of the sample were then removed using a Pasteur pipette and the tube decanted.

9 - Finally, the remaining sample was carefully mixed using the pipette and all remaining liquid (including the remaining cells at the bottom of the tube) was transferred to a FACS tube.

10 - After decanting the sample, ideally mainly CTCs have remained at the bottom of the tube. The leucocytes still within the sample could be separated from the CTCs in flow cytometry.

11 - Immediately after tumor cell isolation the liquid in the FACS Tubes was stained with the mixture of the fluorescence labeled antibodies.

12 - 30 min incubation of the antibodies at +4 °C in the dark

13 - a wash cycle was performed to eliminate unbound antibodies

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14 - 1 ml FACS buffer was added to each sample

15 – it was centrifuged for 5 min at 1500 g and at room temperature.

16 - Finally, the samples were filled up with 500 µl FACS buffer each.

17 -To detect the fluorescent antibodies and thus the tumor cells, the samples were measured with the BD FACSAria Fusion Flow Cytometer of the Flow Core Unit (FCU) in the Georg-Speyer-Haus at the University Hospital Frankfurt am Main, Germany, and then analyzed with the FACS Diva Software. **0 µl**

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