## Results

### Semi-automated dissociation of human tumor tissue

The recently developed Tumor Dissociation Kit in combination with the MACS®_Dissocia_1®_kit_ allowed for the semi-automated preparation of single-cell suspensions from human tumor tissue. The resulting cell suspensions contained significant amounts of single cells with minimal background (BKG) even in cases of single tumors with low concentrations of CSCs (Fig. 1A). This kit facilitates the efficient dissociation of CSCs from various tumor tissue types, which can involve several CSC surface markers, including CD24 and CD44, and cell viability with regard to standard methods. In addition, the kit allows for the dissociation of multiple tumor entities such as melanoma, showed contradictory results with standard methods used. When analyzing putative CSC markers, it was shown that the AC133 epitope is lost upon CSC differentiation, although CD133 expression persists². The optimized tumor dissociation procedure preserves CSC surface markers and provides single cells at high purity and viability, with the integrity of cell surface epitopes being preserved (Fig. 2A). In the shown example (fig. 2B), the AC133 epitope is lost upon CSC differentiation, indicating their true stem cell properties.

### Isolation of breast cancer stem cells

Dissociation yields high amounts of single cells at viability rates of around 90%. The integrity of cell surface epitopes is preserved. Furthermore, we generated conjugates for primary tumor tissue for the quantification and isolation of CSCs from primary tissue, e.g., human melanoma tissue. Human mammary carcinoma stem cells are defined by the expression of CD44 and the absence of CD24. Analysis of further surface markers showed that CSCs have been shown to secrete extracellular matrix proteins that contribute to their self-renewal capacity (Fig. 3A). Using this approach, we established a standardized protocol for CSC enumeration and isolation. This platform is based on an automated, user-independent acquisition process for the determination of cell viability and the isolation of CSC fractions. A strong permanent magnet is placed in the magnetic field of a MACS Separator, in a short 15-min incubation step. Labeling of breast CSCs is achieved by magnetic labeling of CD24+ and CD45+ cells. The flow-through contains the non-labeled CD24+/CD45+ fraction, which is used for further analysis. The isolated CD44+ cells are used for the isolation of breast CSCs. In particular, we demonstrated that the AC133 epitope, which is expressed on CD44+ cells, is preserved. Furthermore, we generated conjugates for primary tumor tissue for the quantification and isolation of CSCs from primary tissue, e.g., human mammary tissue.

### Conclusion

We have developed a standardized platform for CSC enumeration and isolation. This platform is based on an automated, user-independent acquisition process for the determination of cell viability and the isolation of CSC fractions. A strong permanent magnet is placed in the magnetic field of a MACS Separator, in a short 15-min incubation step. Labeling of breast CSCs is achieved by magnetic labeling of CD24+ and CD45+ cells. The flow-through contains the non-labeled CD24+/CD45+ fraction, which is used for further analysis. The isolated CD44+ cells are used for the isolation of breast CSCs. In particular, we demonstrated that the AC133 epitope, which is expressed on CD44+ cells, is preserved. Furthermore, we generated conjugates for primary tumor tissue for the quantification and isolation of CSCs from primary tissue, e.g., human mammary tissue.

### Outlook

By using reliable methods for the analysis and isolation of CSCs, we can further improve our understanding of tumor behavior and provide new insights into the mechanisms of tumorigenesis, which can help in the development of more effective therapeutic strategies.