Malarial protein var2csa improves circulating tumor cell detection from triple-negative breast cancer patients

Abstract Submitter: Deniz Dilay Karayel, Germany*

Co-Authors: Caroline Jacob, Hanna Wietz, Hamna Mahmood, Mette Agerbaek, Ali Salanti, Franziska Meier-Stiegen, Tanja Fehm, Hans Neubauer, André Franken

*University Hospital and Medical Faculty of the Heinrich-Heine University, Department of Obstetrics and Gynecology, Düsseldorf

Abstract

Background: The most aggressive subtype of breast cancer, triple-negative breast cancer (TNBC), is associated with poor prognosis and high recurrence rates. Effective and personalized treatment strategies are crucial for improving patient outcomes. Circulating tumor cells (CTCs) are valuable predictive biomarkers of liquid biopsy, but their scarcity brings about challenges. CTC counts of TNBC patients are shown to be even lower compared to other subtypes which may partly be due to epithelial-mesenchymal transition (EMT). EMT leads to the downregulation of EpCAM, the epithelial cell surface marker used for CTC detection in systems such as the FDA-approved CellSearch. Notably, the malarial surface protein VAR2CSA was shown to bind specifically and independently of the E/M phenotype to oncofetal chondroitin sulfate, a glycosaminoglycan, expressed by embryonic and tumor cells but absent in healthy cells.

Objective: Our goal is to improve CTC detection in metastatic TNBC patients by integrating the recombinant VAR2CSA (rVAR2) protein and to capture TNBC CTCs independently of their E/M phenotype.

Methods: Fluorescently labeled breast cancer cell lines representing all subtypes were spiked into healthy donor blood and enriched with magnetic particles coupled to EpCAM antibodies and rVAR2. CellSearch was used to compare EpCAM-based method to EpCAM- and rVAR2-based method. For patient samples, CTCs were classified as cytokeratin+, DAPI+, CD45-.

Results: Breast cancer cells that were spiked into blood and incubated with rVAR2 could be captured with biotin binder beads for all subtypes. TNBC cell lines showed 80% recovery rates, exceeding HER2+ and luminal cell lines. CellSearch analysis of TNBC cells spiked in blood demonstrated 70% recovery when EpCAM antibodies were combined with rVAR2, compared to 25% with EpCAM antibodies only ($p<0.01$). Similarly, TNBC patient samples exhibited higher CTC counts with EpCAM antibodies and rVAR2, compared to EpCAM antibodies only ($n=13$, $p<0.05$), whereas there was no significant difference for the luminal breast cancer patient samples ($n=10$).

Conclusion: Integrating rVAR2 into the CellSearch system significantly enhances the detection of CTCs in TNBC patients, independent of their epithelial or mesenchymal phenotype. This strategy strengthens the role of CTCs as a predictive biomarker, broadening their potential for guiding personalized therapeutic approaches in TNBC.

Do you have any conflicts of interest?

Yes, I have a conflict of interest.

AS and MA are shareholders in VARCT Diagnostics having the commercial rights to use rVAR2 for cancer diagnostics. The other authors declare no conflict of interest.