Response

The enumeration of circulating tumor cells (CTCs) holds great promise for clinical decision making. The CellSearch method has proven prognostic value in advanced breast, colorectal, and prostate cancers (1). However, this fact does not imply that there is no room for improvement. As mentioned by Hayes and Cristofanilli, CTCs cannot be detected in a substantial number of patients with metastatic breast cancer. Lack of expression of a marker essential for CTC detection may account for this problem. Epithelial cell adhesion molecule (EpCAM) expression on CTCs is crucial for several CTC assays. However, as acknowledged by Hayes and Cristofanilli and by Connelly and colleagues, EpCAM is not a perfect marker. Clinical breast cancer is a highly heterogeneous disease, also in terms of EpCAM expression, as shown in a single invasive breast tumor in Figure 1 (a color version is available online in Supplementary Figure 1).

To circumvent the problem of EpCAM heterogeneity in clinical breast cancers, we used homogeneous cell lines (2). It was by this approach that we were able to demonstrate that cell lines of the intrinsic normal-like breast cancer subtype were not captured by EpCAM-dependent approaches. Accordingly, in their series of 59 human breast tissue samples, Van Laere and coworkers found decreased EpCAM expression in those samples from breast tumors with the normal-like subtype. To further substantiate their findings, we performed a similar analysis on 969 publicly available Affymetrix U133A chip data from five different clinical breast cancer studies (3–7). We used *z*-score normalization for data from each study to evaluate differences in EpCAM expression between the different breast cancer subtypes. In analogy with the results of Van Laere and coworkers, we found that the gene encoding EpCAM, *TACSTD1*, was expressed statistically significantly lower in the 68 tumors of the normal-like subtype (mean rank = −0.84, 95% confidence interval [CI] = −1.22 to −0.46) than in the other subtypes (mean rank = 0.06, 95% CI = 0.00 to 0.12; two-sided Mann–Whitney test, $P < .001$).

It is also apparent from these findings and Figure 1 that EpCAM is not completely absent in clinical breast cancer samples with a normal-like phenotype. The heterogeneous expression of EpCAM in breast tumors also indicates that some disseminated breast tumor cells might lack EpCAM expression. Such cells will be missed by EpCAM-dependent approaches. However, whether detection of these EpCAM-negative CTCs holds clinical relevance remains to be determined.

Future research into whether markers other than those currently used could increase the sensitivity of CTC assays is important. In the search for such markers, cell lines, such as those we have used, and clinical breast cancer samples are indispensable. If antibodies enabling the detection of EpCAM-negative CTCs are identified, they can be added to currently available techniques such as CellSearch. Of course such techniques would have to be thoroughly validated before clinical use, exactly as we have stated previously (2). By developing assays that are able to

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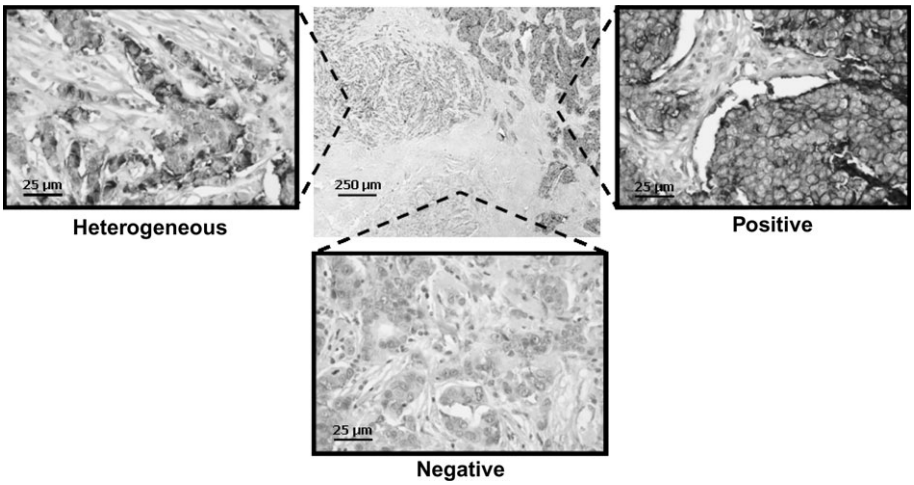

Figure 1. Example of heterogeneous epithelial cell adhesion molecule (EpCAM) staining within one invasive breast tumor with a normal-like phenotype. Representative formalin-fixed paraffin-embedded tissue section of a normal-like human breast tumor stained for expression of EpCAM protein with a mouse anti-human EpCAM antibody (clone VU1D9; Cell Signaling, Danvers, MA; 1:250 dilution, stained overnight after an antigen retrieval step in citrate buffer at pH 6.0; DAKO, Glostrup, Denmark). Anti-EpCAM (**dark stain**) was visualized with the peroxidase-conjugated Envision method from DAKO. The specificity of immunostaining was controlled by using normal mouse IgG and by omitting the primary antibody. Magnifications: middle overview = ×40 and bar length = 250 µm; boxed detail sections = ×400 and bar length = 25 µm. (For color version, see Supplementary Figure 1, available online.)

visualize both EpCAM-negative and EpCAM-positive CTCs and applying these assays in well-designed clinical studies, the clinical relevance of CTC detection could be further augmented.

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References

1. Sleijfer S, Gratama JW, Sieuwerts AM, Kraan J, Martens JW, Foekens JA. Circulating tumour cell detection on its way to routine diagnostic implementation? *Eur J Cancer* 2007;43(18):2645–2650.
2. Sieuwerts AM, Kraan J, Bolt J, et al. Anti-epithelial cell adhesion molecule antibodies and the detection of circulating normal-like breast tumor cells. *J Natl Cancer Inst*. 2009;101(1):61–66.
3. Hess KR, Anderson K, Symmans WF, et al. Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. *J Clin Oncol*. 2006;24(26):4236–4444.
4. Loi S, Haibe-Kains B, Desmedt C, et al. Definition of clinically distinct molecular subtypes in estrogen receptor-positive breast carcinomas through genomic grade. *J Clin Oncol*. 2007;25(10):1239–1246.
5. Miller LD, Smeds J, George J, et al. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc Natl Acad Sci U S A* 2005;102(38):13550–13555.
6. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature*. 2005;436(7050):518–524.
7. Pawitan Y, Bjohle J, Amler L, et al. Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. *Breast Cancer Res*. 2005;7(6):R953–R964.

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