Reply

We thank George Tsavellas and his group for their interest in our paper. This letter refers to the recently published controversial prognostic significance of the reverse transcription-PCR-based CTC detection assays. These results can be explained partly by their limitation in the determination of the cellular source of the reverse transcription-PCR signal. Immunopoeetic cells can definitely express either epithelial proteins or RNA (1, 2). We found in routine gastric and colon biopsy histological sections that CK1-positive cells could be detected in the lamina propria, submucosa. Even CK+ follicles could be found (Fig. 1). The degree of gastritis does not correlate with the number of the CK+ subepithelial cells (Table 1).

A reliable CTC-labeling method should include double or triple fluorescent labeling, using a pancytokeratin or epithelial antibody cocktail and a counter labeling for a common hematopoietic marker, like the CD45, as it is suggested by Tsavellas (3) and Racila (4).

For magnetic separation and consecutive fluorescent labeling, the technique used in our study (Carcinoma Enrichment Kit; Miltenyi Biotec, Bergisch-Gladbach, Germany) is not applicable, because the fixation is done by formaldehyde, which causes a high autofluorescence. For fluorescent labeling, another endothelial surface marker-based technique would be required. The application of another known antiepithelial cell marker, the antiepithelial cell adhesion molecule (known as HEA125 or Ber-EP4, too) antibody, is limited for magnetic isolation, because its expression in mobile CTCs is theoretically not really applicable.

For the automated detection of the isolated, fluorescent-labeled CTCs, the application of flow cytometers is limited, as was shown by George Tsavellas.

Recently, we started the development of a new technique, called SFM for the detection of fluorescent-labeled CTCs and cell clusters on cytopins. In this work, we developed a program for the autofocussed, three-channel scanning of a cytospin specimen with the help of a motorized microscope (X/Z/Y movement, filter changer, Axioplan 2 MOT; Carl Zeiss, Gottingen, Germany) and a digital camera (Axiocam B/W; Carl Zeiss). The slide is electronically recorded in a digital slide format. We have performed a comparison between the recovery rate by SFM, LSC (Compucyte), and flow cytometry (FacsCalibur; BD Biosciences) on an in vitro specimen of HT29 and human blood dilution series (dilutions: 1:1, 1:2, 1:5, 1:10, 1:100, 1:1000, 1:10000, and 1:100000). The samples were stained by CAM5.2- FITC (CK8; BD Biosciences) and CD45-PE-Rhodamin (Coulter). A 4,6-diamidino-2-phenylindole nuclear staining was applied as counter staining. Here we found that a significant correlation can be found between the three methods (Fig. 2). However, in lower concentrations (from 1:10000 to 1:1000000), SFM and LSC (SFM: r = 0.85, LSC: r = 0.90) showed significantly higher recovery of the theoretical dilutions (P < 0.05, Pearson correlation, Statistica; Statsoft, Tulsa, OK), as compared with flow cytometry (r = 0.51). The higher correlation was obtained by the detection of the cell clusters of the microscope based methods.

We agree with the proposal about the evaluation of CTC molecular characteristics. The presence of CTCs in the blood is not equal to metastases. Their number, cluster building ability, and biochemical characteristics significantly influence the patients’ survival and prognosis. Using mRNA expression arrays...
or proteomics is very difficult in this particular case, because the amount of cells and this way the available RNA or proteins is extremely low. Ree et al. (5) described recently a model for gene recovery studies from CTCs from rats. In this first experiment, they used a minimum of 6000 cells, which is far more than the available 10–50 cells from 20 ml of blood.

We thank Dr. Tsavellas for the letter and suggestions that are in close correlation to our experiences and plans in the field of CTC labeling, detection, and examination.

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Table 1  Detection of CK+ cells in routine gastric sections

<table>
<thead>
<tr>
<th>Grade/No.</th>
<th>Lamina propria</th>
<th>Subepithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of + cases</td>
<td>No. of CK+ cells/</td>
</tr>
<tr>
<td></td>
<td></td>
<td>field of view</td>
</tr>
<tr>
<td>Healthy gastric epithelium</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Antral gastritis</td>
<td>Mild 5</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>Middle 5</td>
<td>4–5</td>
</tr>
<tr>
<td></td>
<td>Severe 5</td>
<td>3–4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1–2</td>
</tr>
</tbody>
</table>

We thank Dr. Tsavellas for the letter and suggestions that are in close correlation to our experiences and plans in the field of CTC labeling, detection, and examination.

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References
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