A number of studies of colorectal cancer, for example, have used the PCR for measuring the number of tumor cells present in the bulk of unpurified PBMCs. Iinuma et al. (68) used PCR for quantifying tumor cells, where the target genes were carcinoembronic antigen and cytokeratin 20. In a careful methodological study, these authors found a statistical difference in CTC counts, when comparing normal control subjects with cancer patients, and found some overlap in count numbers between these two sets of subjects. Iinuma et al. (69), report a detection limit of one tumor cell in 3 million PBMCs.

6. Cytokeratin as a Soluble Protein Biomarker for Colon Cancer —The Koelink Study

This study concerned the tumor antigen, cytokeratin. The type of cytokeratin that was measured was CK18-Asp396, a degradation product of cytokeratin-18.

Koelink et al. (70) demonstrated that soluble cytokeratin is elevated in the blood plasma of patients with colon cancer and that it is correlated with outcome. Outcome was according to the endpoint of disease-free survival. In this study, patients were divided into two groups, namely, those with cytokeratin greater than the median plasma concentration (for the group of patients), and those with cytokeratin lower than the median concentration (for the group of patients). Scott et al. (71) reported similar findings on cytokeratin's use as a prognostic biomarker for colon cancer.

7. Tumor-Infiltrating T Cells as a Prognostic Biomarker for Colon Cancer—The Galon Study

The number and activation state of immune cells found to infiltrate a tumor can be prognostic of outcome, for example, prognostic of metastasis of the tumor (72). Figure 19.4, from a biopsy of a patient with colorectal cancer, illustrates the infiltration of tumor cells (blue in original article) with lymphocytes (T cells) (brown in original article) (73,74). The study found that the density of T cells near tumor cells was a better predictor of survival than traditional staging based on tumor size. The researchers divided their biopsy samples into two groups, depending on whether the concentrations of T cells were high or low. Patients whose tumors had an abundant

[68]Iinuma H, Okinaga K, Egami H, et al. Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. Int. J. Oncol. 2006;28:297–306.

[69]Iinuma H, Okinaga K, Egami H, et al. Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. Int. J. Oncol. 2006;28:297–306.

[70]Koelink PJ, Lamers CB, Hommes DW, Verspaget HW. Circulating cell death products predict clinical outcome of colorectal cancer patients. BMC Cancer 2009;9:88.

[71]Scott LC, Evans TR, Cassidy J, et al. Cytokeratin 18 in plasma of patients with gastrointestinal adenocarcinoma as a biomarker of tumour response. Br. J. Cancer 2009;101:410–7.

[72]Deschoolmeester V, Baay M, Specenier P, Lardon F, Vermorken JB. A review of the most promising biomarkers in colorectal cancer: one step closer to targeted therapy. Oncologist 2010;15:699–731.

[73]Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.

[74]Couzin J. T Cells a boon for colon cancer prognosis. Science 2006;313:1868–9.