amplification (57). Schimke (58,59) and co-workers conducted most of the early, detailed research on the mechanisms of gene amplification, in the context of studies explaining how cancer cells in patients treated with methotrexate became resistant to that drug.

4. *Circulating tumor cells as a prognostic biomarker for colon cancer – the Cohen study*

Circulating tumor cells can be used as a biomarker. In a study of colon cancer, Cohen et al. (60) stratified patients according to baseline levels of tumor cells circulating in the bloodstream. Prior to initiating chemotherapy, blood was withdrawn, tumor cells present in the bloodstream were analyzed, and patients were divided into two subgroups. The two subgroups were:

- **First subgroup.** Three or more tumor cells/7.5 mL whole blood; and
- **Second subgroup.** Less than three tumor cells/7.5 mL whole blood.

Tumor cells were detected by an immunological method sensitive to cytokeratin. All of the patients were subsequently treated with one of the drugs bevacizumab, irinotecan, or exaliplatin. There was no placebo group.

The median PFS was 4.5 months (high circulating tumor cells) and 7.9 months (low circulating tumor cells). The median overall survival was 9.4 months (high circulating tumor cells) and 18.5 months (low circulating tumor cells). The results demonstrated that lower baseline circulating tumor cells, as compared with higher baseline circulating tumor cells, are correlated with greater PFS ($P = .0002$) and also correlated with greater overall survival ($P < .0001$). The authors concluded that the biomarker of circulating tumor cells is a strong predictor for PFS and overall survival.

The circulating tumor cell biomarker test has the following uses. First, it can serve as a stratification factor for clinical trials. Second, it can inform the physician if more aggressive chemotherapy is needed (high circulating tumor cells), or if a less toxic chemotherapy is acceptable (low circulating tumor cells). Third, it can identify patients who can safely have prolonged treatment breaks in chemotherapy versus those who need to resume chemotherapy more quickly.

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