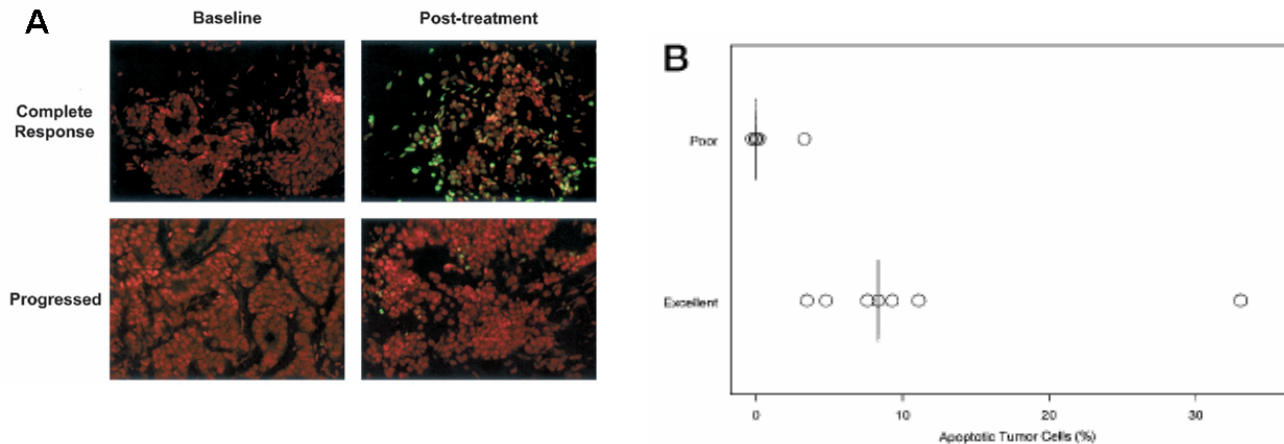


## Case Study #11 - Development of a Clinical Diagnostic that Predicts Efficacy of Chemotherapy in Breast Cancer



It is currently impossible to determine whether or not cancer therapy is effective before radiographic changes are evident, which typically takes weeks to occur. Physical examinations are relatively imprecise, lacks sensitivity and specificity, and meaningful findings tend to occur near the second cycle of therapy, well into the treatment course. Thus, effective therapeutic intervention is often delayed or never obtained, resulting in unnecessary, prolonged exposure to agents that possess significant toxicity and affect quality of life. Scientists at ApoCell developed an automated, laser scanning quantitative assay that measures the percentage of breast cancer cells in tumor biopsies undergoing DNA fragmentation, which is characteristic of apoptosis. Eighteen-gauge core breast cancer biopsies were obtained before and after neoadjuvant therapy with docetaxel plus doxorubicin or paclitaxel as part of two prospective clinical trials. The results significantly predicted patient-specific clinical response to therapy. This study was featured on the Cover of Clinical Cancer Research in 2003 (Davis, D.W., et. al.).



**A**, Qualitative analysis of DNA fragmentation: Apoptosis-associated DNA fragmentation was detected by fluorescent TUNEL (green), and total cell nuclei were detected by counterstaining with propidium iodide (red). Representative fluorescence microscopic (X200 objective) images obtained from baseline and after treatment (48 h). Tumors are presented for 1 of the patients who demonstrated a pathological CR and 1 of the patients who progressed on therapy. **B**, Changes in apoptosis (48 h) correlate with response. Tumors were grouped into two categories, those that demonstrated excellent responses and those that demonstrated a poor response. Data points corresponding to the percentage of change in individual tumor cell apoptosis at 48 h, are indicated by the open symbols. Group medians (excellent = 8.32%, poor = -0.09%) are indicated by the vertical lines. By these criteria rates of apoptosis correlated directly with response for 48 h ( $P=0.0023$ ) but not at 24 h ( $P=0.82$ ) using the Wilcoxon rank sum test.

The apoptosis data reported in this case study are consistent with results obtained by others using different methods, suggesting that levels of therapy-induced apoptosis correlate with response. However, kinetics can be a critical factor for detecting apoptotic cells. Our analysis of apoptosis in biopsies obtained 24 h after initiation of therapy did not correlate with clinical response. Furthermore, ER, PR, and Her2 measured by IHC at 24 h or 48 h did not show any significant correlation with clinical outcome. Thus, the need for a sensitive, accurate and reproducible test that can predict response to therapy is warranted. ApoCell is in the process of validating the clinical utility of this assay using a proprietary DNA fragmentation probe (apotag) in a larger patient cohort. This would provide clinicians with a more rational means for maximizing therapeutic benefit on a case by case basis.

### Reference

1. Clin Cancer Res. 2003 Mar;9(3):955-60