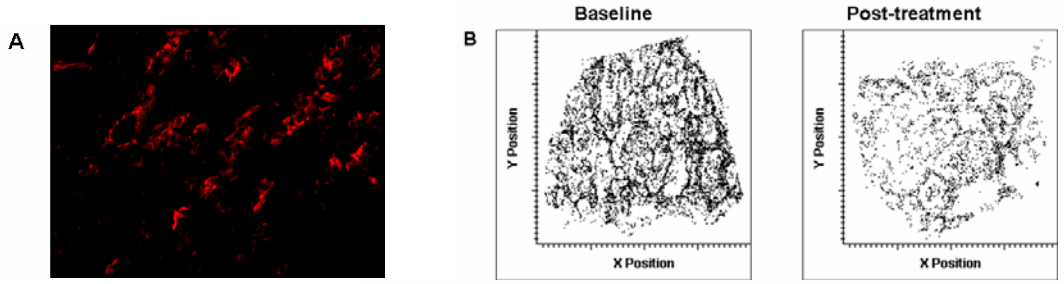
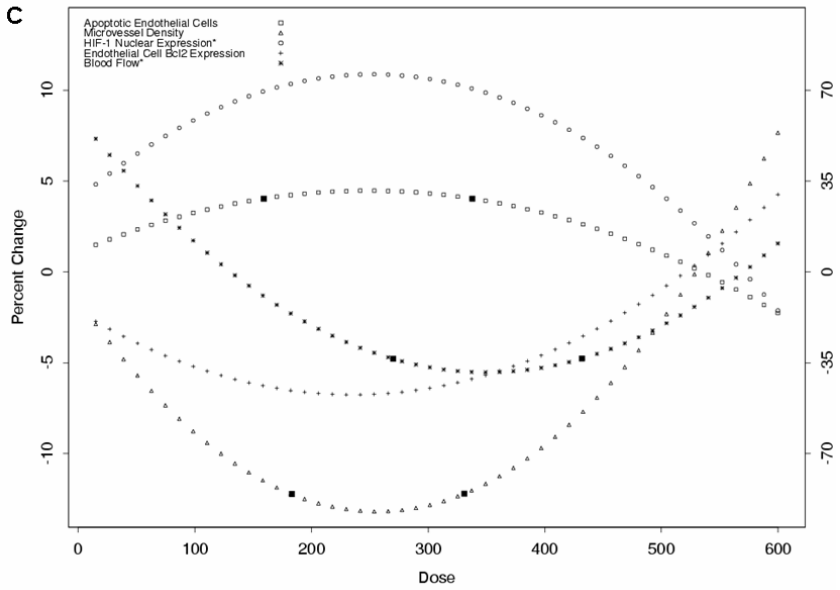
Case Study #9 - Phase I Study to Identify Optimal Biological Dose

Angiogenesis is a target for the treatment of cancer and other diseases, and its complex biology suggests that establishing the appropriate dose and schedule for anti-angiogenic drugs will require novel approaches. In a Phase I dose-finding study of endostatin, ApoCell's scientists developed biomarker assays to quantify apoptosis in tumor cells (TC) and tumor-associated endothelial cells (EC), microvessel density (MVD), BCL-2 and nuclear localization of HIF-1α levels in tumor biopsy sections. The results showed significant increases in EC apoptosis and decreases in tumor MVD and blood flow. In contrast, levels of TC death were uniformly low and did not correlate with endostatin dose.





A, Laser generated image of anti-CD31 immunofluorescence in a tumor biopsy. **B,** Each biopsy was scanned and microvessels were enumerated. Tissue maps show MVD before and after endostatin therapy. The number of microvessels decreased in this patient's tumor and demonstrated stabilization of disease at a dose of 180 mg/m2. **C,** LSC was used to quantify apoptosis, microvessel density, HIF-1α, and BCL-2 in EC in each tumor biopsy. Blood Flow was measured by PET imaging. The change in each biomarker after endostatin therapy was calculated for each patient (total of 17) at a dose of 15, 30, 60, 120, 180, 300, and 600 mg/m2. The data were fit to a quadratic polynomial model to test significance. The results revealed a significant increase in EC apoptosis and decrease in MVD, with maximal effects of endostatin at a dose of 249 mg/m2 (95% confidence interval, 159–338) and 257 mg/m2 (95% confidence interval,183–331), respectively. Maximal nuclear HIF-1α and minimal EC Bcl-2 levels were observed at 250 mg/m2, although the changes did not reach statistical significance.

The sensitivity of these biomarker assays made it possible to detect subtle but significant changes in biomarker expression patterns. For example, levels of EC apoptosis were so low that manual quantification made it difficult to identify significant changes in apoptosis. ApoCell's automated assay allowed quantification of a larger population of cells (hundreds of thousands), and revealed that EC apoptosis was 0.2% before therapy and increased to 1.1% after endostatin therapy. This actually represented a 5-fold increase in apoptosis, which is comparable to the fold increases observed in TC lines exposed to cytotoxic agents *in vitro*. These data suggest that endostatin had optimal biological activity at around 250 mg/m2 in this cohort of patients. Endostatin's failure to induce high levels of TC apoptosis may explain its lack of significant clinical activity in this Phase I trial.

References

1. Clin Cancer Res. 2004 Jan 1;10(1 Pt 1):33-42
2. J Clin Oncol. 2002 Sep 15;20(18):3804-14

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