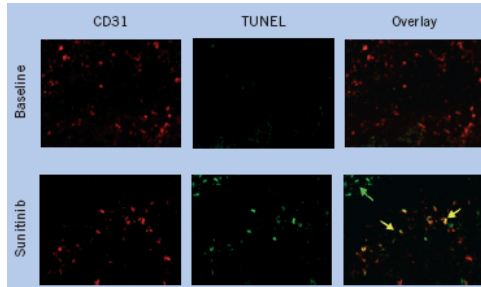


Case Study #7 - Novel Assay Development for Monitoring the Effects of Angiogenesis Inhibitors

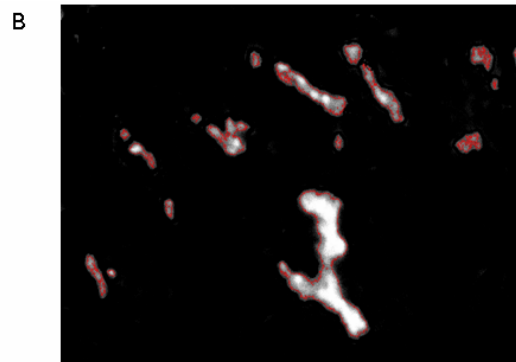
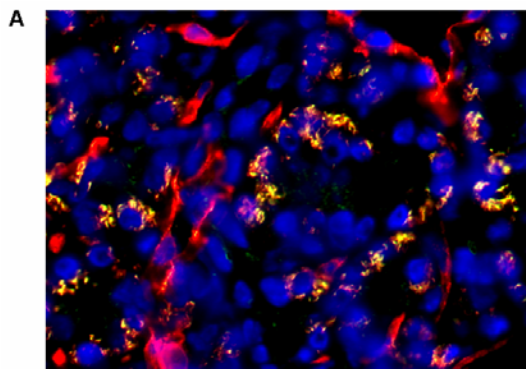


Angiogenesis inhibitors (AIs) offer several therapeutic benefits compared to conventional therapies, and appear to be more effective against solid tumors and less toxic than chemotherapy. However, as a result of the early clinical trials of AIs, investigators are beginning to appreciate the complexity of targeting angiogenesis and realize that developing clinically useful AIs is more challenging than originally thought. Scientists at ApoCell have developed innovative detection technologies that directly assess the biological activity of AIs using biopsies or indirectly through surrogate marker assays. Results from some of these assays have been validated on FDA approved drugs and show significant correlation with clinical outcome. Other innovative assays are undergoing validation studies and may offer strategic and/or regulatory advantages and overall economical benefit during clinical drug development.

Scientists at ApoCell developed a novel assay to visualize apoptosis in endothelial cells (red), DNA fragmented cells (green), and total cell nuclei (not shown) *in situ*. Featured on the cover of Biotechniques in 2000 (Davis, D.W.), this technique has been featured in more than 100 publications and is routinely used to identify the effects of AIs in xenografts and clinical specimens.



ApoCell's scientists have developed an automated, quantitative process to enumerate apoptotic endothelial and tumor cells in tissue sections. This biomarker and assay have demonstrated significant correlation with clinical outcome of FDA approved drugs. Laser generated images (left Panel) show apoptosis in tumor and endothelial cells from a patient who benefited from Sunitinib therapy. In a Phase II study, tumors from patients with clinical benefit displayed an overall 10- and 6-fold ($P < 0.05$) increase from baseline in endothelial and tumor cell apoptosis, respectively. In contrast, tumors from patients with progressive disease exhibited little or no change from baseline in endothelial and tumor cell apoptosis. Automated quantification of apoptosis is a proprietary clinical utility of ApoCell.



Scientists at ApoCell have optimized tissue antigen retrieval methods to allow multiplex, quantitative analysis of proteins with low expression levels, particularly phosphorylated epitopes. **A**, Immunofluorescent image showing endothelial cells (red), phosphorylated (p)-VEGFR-2 (green) [ApoCell Ab ACCL-14], and total cell nuclei (blue). **B**, Laser generated image showing quantification of tumor microvessels detected by immunofluorescent anti-CD31 staining. This breakthrough detection technology allowed the quantification of protein expression levels within the tumor vessels, e.g., pVEGFR-2.

ApoCell's biomarker assay for measuring levels of phosphorylated-VEGFR-2 and PDGFR in the endothelium has shown significant correlation with clinical outcome on FDA approved therapies such as Sunitinib (see Case Study #3) and Avastin + Erlotinib combination therapy (see Case Study # 8).

Reference

1. Cancer Res., 59: 5412-5416, 1999
2. Clin Cancer Res. 2004 Jan 1;10(1 Pt 1):33-42
3. Abstract #57, EORTC-NCI-AACR - Prague, Czech Republic 2006