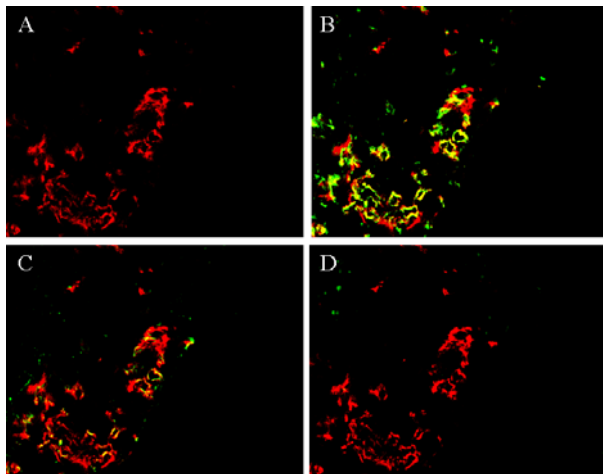
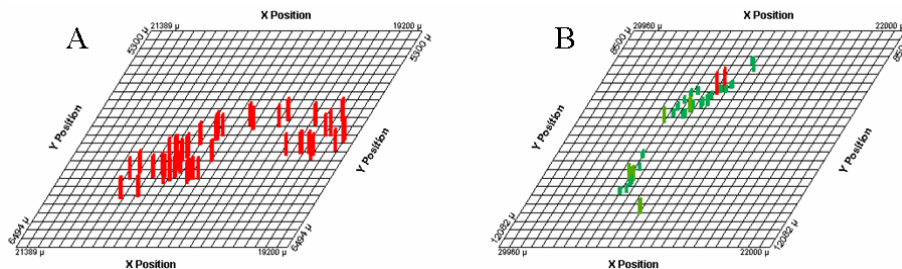


Case Study #8 - Multiplex Assay for Simultaneous Quantification of RTK Target Inhibition and Apoptosis in Endothelial Cells and Tumor Cells

Molecular targeted therapies against multiple receptor tyrosine kinases (RTKs) offer an attractive therapeutic advantage compared to single targeted agents. One of the first multi-targeted tyrosine kinase inhibitors to enter clinical trials was SU6668. Preclinical studies with SU6668, a nonspecific TK inhibitor of VEGFR-2, PDGFR, and bFGF, revealed that inhibition of these RTKs has the potential to induce apoptosis in tumor-associated endothelial cells and tumor cells, significantly reducing tumor burden. However, clinical studies with SU6668 revealed lack of anti-tumor activity observed in patients. Therefore, it was critically important to determine whether SU6668 inhibited phosphorylation of the intended RTKs in human tumors, especially to determine the validity of the RTKs as potentially beneficial anti-angiogenic targets. Scientists at ApoCell developed a multiplex assay for the detection and quantification of RTKs and apoptosis. The results revealed that SU6668 had little biological activity in human tumors.



Effects of SU6668 on multiple RTK targets. Representative patient biopsy obtained after 28 days of SU6668 treatment was processed and immunofluorescently stained (simultaneously) for endothelial cells [CD31, (red)], phosphorylated PDGF- β (green), phosphorylated VEGFR-2 (green), and DNA fragmentation (terminal-deoxynucleotidyl-transferase dUTP nick-end labeling, TUNEL) (green). Images shown are laser generated scanned images superimposed and pseudo-colored for visualization of (A) CD31+ cells, (B) CD31+/phosphorylated-PDGFR- β +, (C) CD31+/phosphorylated-VEGFR-2+, and (D) CD31+/TUNEL+. Laser scanning quantitative analysis was performed using different wavelengths to determine expression levels for each biomarker.



Laser scanning quantitative analysis of the effects of SU6668 on phosphorylated-VEGFR-2. Patient biopsies obtained before (A) and 28 days after (B) SU6668 treatment were immunofluorescently stained (see Figure above) and scanned by LSC to determine the levels of phosphorylated-VEGFR-2 in endothelial cells. The 3-D tissue maps display the location of endothelial cells positive for phosphorylated-VEGFR-2 (bars) within each tissue cross section and the intensity of phosphorylated-VEGFR-2 (red, high phosphorylation; green/blue, low phosphorylation) before and after SU6668 treatment. In this particular patient, SU6668 treatment slightly decreased phosphorylation of VEGFR-2.

Quantification of RTK activity has become an important pharmacodynamic endpoint for monitoring the effects of tyrosine kinase inhibitors. Because SU6668 lacked clinical activity, it was not clear whether the lack of significant clinical activity was due to a poor choice of drug targets or ineffective target inhibition. The latter is more likely as 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. Biotechniques. 2003 May;34(5):1048-50, 1052, 1054 passim