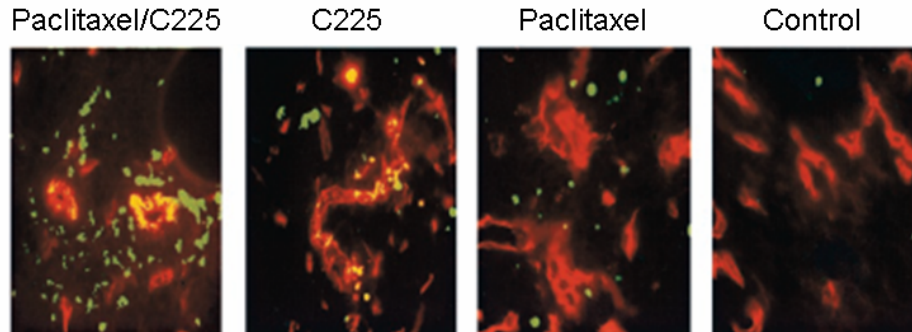


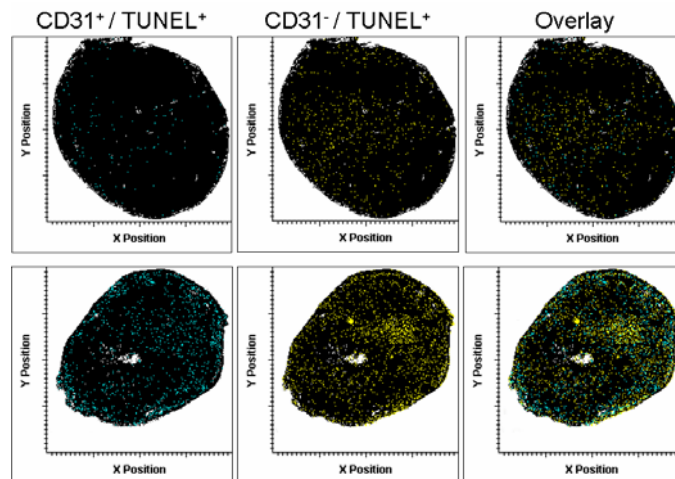
Case Study #2 - Quantification of Cell Specific Apoptosis to Determine Optimization of Combination Therapy

Molecular targeted therapies offer several therapeutic benefits compared to conventional therapies. Preclinical studies indicate that biological agents such as antagonists of EGFR or HER-2/neu receptor modulate host responses and enhance the efficacy of chemotherapy. In fact, various combinatorial approaches are now being tested in clinical trials. Scientists at ApoCell developed an innovative, reproducible detection method for identifying apoptosis in endothelial cells and tumor cells. Detecting cell specific apoptosis has been critical for assessing the effects of combination therapies. Results from many preclinical studies using this biomarker assay have been pivotal for optimizing and advancing combinatorial therapy into the clinical setting.



Effects of Paclitaxel + C225 on apoptosis in orthotopic bladder tumors. Representative immunofluorescent images show endothelial cells (CD31, red) and DNA fragmentation identified by TUNEL (green). These images are superimposed to show co-localization of apoptosis in endothelial cells (yellow) and tumor cells (green). Manual high-powered quantification of apoptosis in selected fields revealed significant differences between control and treatment regimens. Pretreatment control 0.4 ± 0.9 (0.0–2.0); Control (PBS) 0.7 ± 1.5 (0.0–3.3); Paclitaxel 11.8 ± 3.8 (8.8–16.3); C225 24.0 ± 9.1 (14.3–35.3); Paclitaxel/C225 49.5 ± 11.3 (37.5–63.2).

Visualization of cell specific apoptosis allows for manual quantification. However, it is difficult for the human eye to simultaneously distinguish multiple fluorochromes and the results are limited to the number of microscopic fields analyzed by the investigator. Scientists at ApoCell developed an automated approach for quantifying cell specific apoptosis in tissues sections.



Effects of DC101 on apoptosis in orthotopic bladder tumors. Tissues were scanned by LSC to generate maps of the intra-tumoral distribution of apoptotic endothelial cells (CD31+/TUNEL+, blue pixels) and apoptotic tumor cells (CD31-/TUNEL+, yellow pixels) within whole tumor cross-sections (black pixels represent total cell nuclei). Superimposing CD31+/TUNEL+ cells and CD31-/TUNEL+ cells reveals that DC101-induced apoptosis is heterogeneous, with clusters of dead CD31-positive cells in the tumor periphery. Note the increase in blue and yellow pixels in the DC101-treated tumors (bottom panel) compared with the controls (top panel). Tissue maps reveal localization of apoptotic cells and quantification (flow based) can be performed on specific regions or the whole tumor cross section.

ApoCell's quantitative biomarker assays for determining synergistic effects of targeted therapies have shown significant correlation with clinical outcome on FDA approved therapies such as Avastin + Erlotinib therapy (see Case Study # 8).

References

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