Application Note
Dr. Jones and colleagues
University of Cambridge, UK

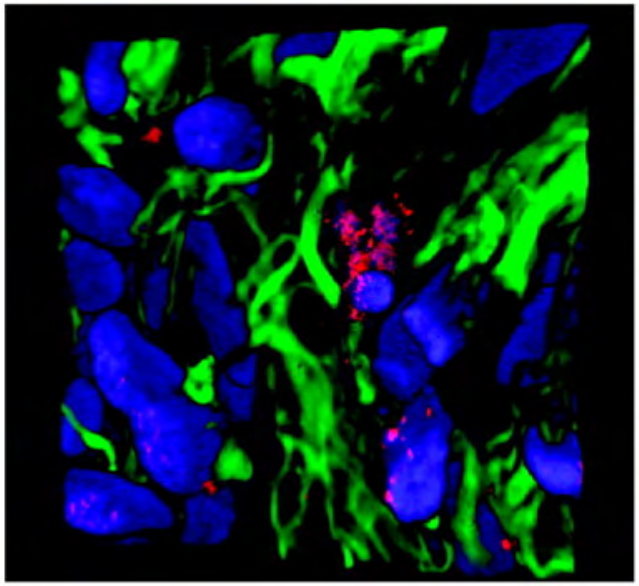
Studies of the prodrug AQ4N in patients with advanced solid tumors

Most solid tumors have an abnormal vasculature that results in poor delivery of O_2 to localized regions. These hypoxic tumor cells are resistant to radiotherapy and cytotoxic compounds and therefore new agents need to be developed that specifically target these regions. One potential candidate is the novel bioreductive prodrug, AQ4N, which is currently in clinical trials. Evidence has shown that AQ4N undergoes bioreduction in hypoxic regions to form the active drug AQ4, a potent cytotoxic topoisomerase II inhibitor.

As part of this phase I clinical study, researchers investigated whether the activated AQ4 selectively accumulates in hypoxic regions. Thirty two patients with various forms of cancer (glioblastoma, bladder, head and neck, breast and cervical) received a 200 mg/ m^2 dose of AQ4N. The concentrations of AQ4 and Glut-1 (an endogenous marker of tumor hypoxia) were then measured and the results showed a positive correlation between the AQ4 tumor concentrations and tumor hypoxia. This was further examined by exploiting the intrinsic ability of AQ4 to fluoresce. Researchers analyzed the tissue distribution of AQ4 in relation to tumor hypoxia using confocal microscopy.

Samples were imaged on a confocal microscope using a 633 nm laser to excite AQ4 fluorescence. The projected z-sections were then rendered using Volocity Visualization software. The results showed that AQ4 fluorescence colocalized with regions containing the highest levels of Glut-1. They also found that AQ4 was detected in areas that lacked cellular architecture, a feature consistent with necrosis. 3D rendering showed that, peripheral to these necrotic regions, AQ4 was found at high levels in cells exhibiting morphologic features of apoptosis, including nuclear fragmentation and cytoplasmic bulging (figure).

The study showed proof of concept for the preferential activation of AQ4N in hypoxic compartments of several important solid tumors, leading to potentially therapeutic levels of AQ4 in regions of the tumor microenvironment that are typically refractory to conventional chemotherapy and radiotherapy. This is the first time that the activation of a bioreductive agent in clinical tumor samples has been shown. It will hopefully lead the way for future clinical trials and ultimately, will enhance therapies for cancer patients.



The figure shows a reconstructed 3D z-stack image of a sample taken from a glioblastoma (GBM) tumor, demonstrating AQ4 abundance in cells undergoing nuclear fragmentation. The image shows an overlay of glial fibrillary acidic protein (GFAP) tumor marker (green), DAPI stained tumor nuclei (blue) and AQ4-dependent fluorescence (red).

Adapted with permission from the American Association for Cancer Research: Albertella M R *et al*. (2008). Hypoxia-Selective Targeting by the Bioreductive Prodrug AQ4N in Patients with Solid Tumors: Results of a Phase I Study. *Clin. Cancer Res.* 14: 1096-1104 (Figure 5).