

Validation of pharmacodynamic assays to evaluate the clinical efficacy of an antisense compound (AEG 35156) targeted to the X-linked inhibitor of apoptosis protein XIAP.

Cummings J, Ward TH, LaCasse E, Lefebvre C, St-Jean M, Durkin J, Ranson M, Dive C. *Br J Cancer*. 2005 Feb 14;92(3):532-8.

The inhibitor of apoptosis protein, XIAP, is frequently overexpressed in chemoresistant human tumours. An antisense oligonucleotide (AEG 35156/GEM 640) that targets XIAP has recently entered phase I trials in the UK. Method validation data are presented on three pharmacodynamic assays that will be utilised during this trial. Quantitative RT-PCR was based on a Taqman assay and was confirmed to be specific for XIAP. Assay linearity extended over four orders of magnitude. MDA-MB-231/U6-E1 cells and clone X-G4 stably expressing an RNAi vector against XIAP were chosen as high and low XIAP expression quality controls (QCs). Within-day and between-day coefficients of variation (CVs) in precision for cycle threshold (CT) and delta CT values (employing GAPDH and beta 2 microglobulin as housekeepers) were always less than 10%. A Western blotting technique was validated using a GST-XIAP fusion protein as a standard and HeLa cells and SF268 (human glioblastoma) cells as high and low XIAP expression QCs. Specificity of the final choice of antibody for XIAP was evaluated by analysing a panel of cell lines including clone X-G4. The assay was linear over a 29-fold range of protein concentration and between-day precision was 29% for the low QC and 23% for the high QC when normalised to GAPDH. XIAP protein was also shown to be stable at -80 degrees C for at least 60 days. M30-Apoptosense plasma Elisa detects a caspase-cleaved fragment of cytokeratin 18 (CK18), believed to be a surrogate marker for tumour cell apoptosis. Generation of an independent QC was achieved through the treatment of X-G4 cells with staurosporine and collection of media. Measurements on assay precision and kit-to-kit QC were always less than 10%. The M30 antigen (CK18-Asp396) was stable for 3 months at -80 degrees C, while at 37 degrees C it had a half-life of 80-100 h in healthy volunteer plasma. Results from the phase I trial are eagerly awaited.