**Abstract (#3)**

HIF1α is an important regulator of the response to hypoxic stress. A number of cancer therapeutics that are aimed at targeting hypoxia have been shown in preclinical models to have a significant effect on tumor growth, including topotecan and indenoisoquinoline NSC 743400. However, the exact mechanism of action for these therapeutics is unknown. The goal of this study was to quantitate the expression levels of HIF1α in Biopsy specimens from xenografts treated with topotecan and indenoisoquinoline NSC 743400 and to use this data to help guide future clinical trials.

**Materials and Methods**

- The assay is a two-site chemiluminescent enzyme linked immunosorbent assay. The assay was performed according to the manufacturer’s instructions (R&D Systems, Abingdon, United Kingdom) using a 2-site chemiluminescent enzyme-linked immunosorbent assay (ECL) detection system. The assay is performed in 96 well plates and employs a capture monoclonal antibody and a detection monoclonal antibody (R&D Systems, Abingdon, United Kingdom) to detect the target antigen. The plates are coated overnight with the capture antibody and incubated for 2 hours with the detection antibody. The plates are then washed and incubated with the specimen to be tested. After incubation, the plates are washed again and incubated with the detection antibody conjugated to horseradish peroxidase. The plates are then washed and incubated with the chemiluminescent substrate. The chemiluminescent signal is measured using a plate reader.

**2-Hydroxylglutarate (2-HG) as a PHD Inhibitor by Competitively Inhibiting HIF1α in Tumors (2-HG), a Substrate of PHD**

- Assay Variability & Accuracy by Spike Recovery

- Expression in Immunoassay (PC3 Cells)

**Conclusions**

- HIF1α expression in Biopsy specimens was determined using a competitive immunoassay. The assay was shown to be useful in recovering HIF1α levels. Different lysis buffers were compared (CEB vs. CEB + 2-HG) with similar results. A high degree of variability was observed within and between mice.

**Design Experiment**

- Animal: ALB-2 line; inbred BALB/c nude mice. Six-week-old males. 
- Treatment: Vehicle or Topotecan (1 mg/kg) or topotecan (1 mg/kg) + indenoisoquinoline NSC 743400 (10 mg/kg) or topotecan (1 mg/kg) + indenoisoquinoline NSC 743400 (10 mg/kg) + topotecan (1 mg/kg).
- Tumor collection: 1st post-dose 5
- Tumor size was evaluated, divided in 2 groups, and was used to diagnose buffer. Different methods of homogenization were compared using 2 different beads (a 3.0 mm, 1 mm, 0.8 mm, 0.5 mm).
- Different buffer were compared (CEB vs. CEB + 2-HG). CEB. Credibility of the assay was evaluated.