The Cmax for Ranibizumab is expected to be well below the upper limit of the free VEGF level. For NanoLuc method, eight out of ten lots have recovery of within ±25% at each QC level. For ECL method, eight out of ten lots have recovery of within ±25% at each QC level. For NanoLuc method, eight out of ten lots have recovery of within ±25% at each QC level. For ECL method, eight out of ten lots have recovery of within ±25% at each QC level.

Electrochemiluminescence Assay for the Measurement of Ranibizumab (VEGF) treatment is an effective therapy shown to reverse the changes in vision and visual symptoms of AMD. Ranibizumab is an approved treatment for macular degeneration. The pharmacokinetics of Ranibizumab requires a high sensitive assay to be developed. We investigated the assay method using the unique novel technology; NanoLuc® from Promega Inc. NanoLuc is a small and ultrabright luciferase with stable luminescence signal that can be used as a reporter in ELISAs.

**INTRODUCTION**

Novocarcinogen or age-related macular degeneration (AMD) is the leading cause of blindness in the elderly population. Aromatase inhibitors in the retina leads to loss of vision. Anti-vascular endothelial growth factor (VEGF) treatment is an effective therapy shown to reverse the changes in vision and visual symptoms of AMD. Ranibizumab is an anti-VEGF antibody that inhibits the VEGF receptor in the retina. Ranibizumab is an affinity matured Fab fragment based on the bevacizumab antibody, specifically designed for higher affinity to VEGF and shorter half-life.

**SYSTEMIC TOXICITY**

Systemic exposure to Ranibizumab after IV injection is very low due to elimination on meeting systemic circulation from the vitreous. Characterization of the pharmacokinetics of Ranibizumab requires a high sensitive assay method to demonstrate that the free VEGF signal in plasma from patients treated with Ranibizumab is within the normal range. Therefore, a method to measure the level of free VEGF in plasma is needed. We investigated the assay method using the unique novel technology; NanoLuc® from Promega Inc. NanoLuc is a small and ultrabright luciferase with stable luminescence signal that can be used as a reporter in ELISAs.

**METHODS**

The method is based on the ELISA kit developed by Somru BioSciences for the measurement of free VEGF in human plasma. Briefly, immobilized VEGF and rabbit anti-Ranibizumab antibody are added to plasma samples. During overnight incubation, these two molecules bind to Ranibizumab and form an immune complex. This immune complex is then captured by streptavidin-coated plate and detected using labelled anti-Rabbit antibody (NanoLuc® labeled). The plates were then analyzed by electrochemiluminescence (ECL) or luminescence (NanoLuc® technology) signal. The recovery accuracy and precision values (See Table 1 and 2) of ECL and NanoLuc® method was similar. The inter assay accuracy and precision were within ±2% for all QC levels except ±5% at LLOQ. The recovery of QC in both ECL and NanoLuc® method was presented in Figure 2 A-D. Matrix effect reactivity of the method was evaluated by fortifying each 10 µL plasma sample at levels of below, at the low and high QC samples of Ranibizumab at 1000 pg/mL and 10,000 pg/mL. Table 3 and Figure 3. For ECL method, eight out of ten lots have recovery of within ±25% at each QC level. For NanoLuc method, eight out of ten lots have recovery of within ±25% at each QC level. The mean recovery of both methods at all QC levels was within 90%-110%.

**Interference of VEGF**

Interference of VEGF in the assay was evaluated by the addition of free VEGF into the plasma prior to addition of Ranibizumab. A dose range from 500–2000 pg/mL free VEGF showed no inhibition of the free VEGF signal at 1000 pg/mL (Table 4). No linearity was assessed as the assays was expected to be well below the upper limit of the concentration (20,000 pg/mL) for expected clinical trials samples.

**RESULTS**

The precision and accuracy of the assay was determined by running a standard curve from 625 pg/mL up to 30,000 pg/mL Ranibizumab with an accuracy of ±100%. Quality control at levels, LLOQ of 625 pg/mL, and the ULOQ at 20,000 pg/mL, 15% CV and ±10% bias were evaluated over multiple days with four parallel runs to generate overall accuracy and precision values (Data shown in Table 1 and 2). For ECL and NanoLuc® method, the accuracy and precision were within ±2% for all QC levels except ±5% at LLOQ. The recovery of QC in both ECL and NanoLuc® method was presented in Figure 2 A-D. Matrix effect reactivity of the method was evaluated by fortifying each 10 µL plasma sample at levels of below, at the low and high QC samples of Ranibizumab at 1000 pg/mL and 10,000 pg/mL. Table 3 and Figure 3. For ECL method, eight out of ten lots have recovery of within ±25% at each QC level. For NanoLuc method, eight out of ten lots have recovery of within ±25% at each QC level. The mean recovery of both methods at all QC levels was within 90%-110%.

**CONCLUSION & FUTURE WORK**

The results from both platforms were comparable. The results from qualification experiments indicate that the assay is "validatable" and meets FDA Bioanalytical Guidance for pharmacokinetic assays and industry best practice standards. The assay sensitivity is reported to be 25 pg/mL. We intend to directly capture the rabbit anti-Ranibizumab antibody to further optimize the method and achieve less than 25 pg/mL sensitivity. Further optimization will be targeting to minimize crossreact and plate washing effects for the NanoLuc method.

**NOVEL ASPECT**

NanoLuc® is a novel technology that can be used as a reporter in ELISA and other immunoassays. NanoLuc® is a small and ultrabright luciferase with stable luminescence signal that can be used as a reporter in ELISAs. The results from both platforms were comparable. The results from qualification experiments indicate that the assay is "validatable" and meets FDA Bioanalytical Guidance for pharmacokinetic assays and industry best practice standards. The assay sensitivity is reported to be 25 pg/mL. We intend to directly capture the rabbit anti-Ranibizumab antibody to further optimize the method and achieve less than 25 pg/mL sensitivity. Further optimization will be targeting to minimize crossreact and plate washing effects for the NanoLuc method.