The 14th WRIB

# Pharmacodyanamic Analysis of Crizanlizumab by Surface Plasmon Resonance: A Case for Implementing Unique Analytical Platforms in Clinical Trials

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## BACKGROUND

- Crizanlizumab is the first and only FDA-approved monoclonal antibody (marketed as Adakveo®) that reduces the frequency of vaso-occlusive crises (VOCs) or pain crises in adults and pediatric patients, aged 16 years and older, with sickle cell disease.
- This antibody binds to P-selectin on endothelial cells and platelets thereby inhibiting its interaction with P-selectin glycoprotein ligand-1 (PSGL-1) expressed on blood cells. The P-selectin-PSGL-1 interaction promotes sticking of blood cells leading to sickle cell related pain crisis.
- Surface Plasmon Resonance (SPR) has been used routinely in drug discovery due to its ability to measure molecular interactions in a label free high throughput manner. However, this powerful approach has not been applied in pivotal clinical studies.
- We describe here the development and validation of an SPR based pharmacodynamics (PD) assay utilized to demonstrate the inhibition of P-selectin-PSGL-1 interaction by crizanlizumab and the key challenges encountered with introduction of this technology into a clinical trial setting.

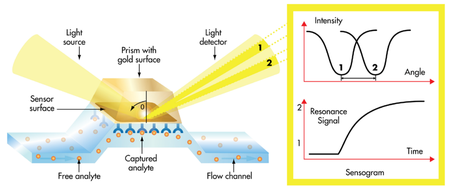



**Figure 1. Schematic configuration of a label-free detection using SPR.**
(Adapted from www.bruker.com)

## METHODS

- The SPR assay to quantify inhibitory (PD) activity of crizanlizumab was designed to mimic in vivo biological interactions, wherein, a biotinylated glycosulfopeptide-6 (GSP6) representing the PSGL-1 binding domain was immobilized on a streptavidin coated sensor chip.
- Serum samples from patients treated with crizanlizumab were injected over the GSP6 sensor chip in the presence of excess P-selectin and inhibitory activity of the drug (function of serum concentration) was measured as change in response units from the baseline sample.

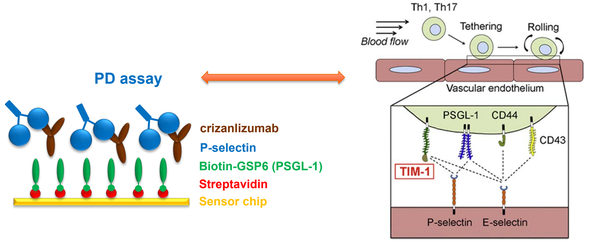



**Figure 2. In vivo biology derived PD assay design principle.**
(Right figure is from Immunity 2014, Volume 4 issue 4, page 542-553)

## ASSAY DEVELOPMENT

- Optimize GSP6 immobilization density to improve the longevity of the sensor chip's surface
  - Minimize avidity and P-selectin binding signal loss
  - Include surface conditioning cycles prior to clinical sample testing

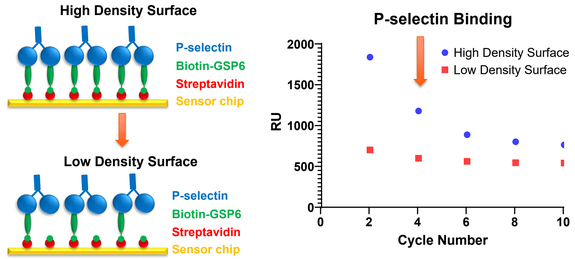
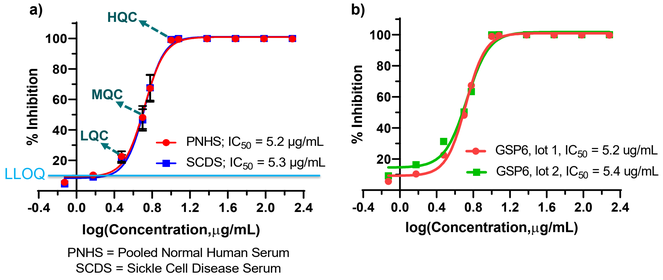



**Figure 3. Lowering the immobilization density of GSP6 has shown to minimize the signal loss from P-selectin binding and increase longevity of biosensor surface.**

## ASSAY VALIDATION

- Standard inhibition curve of crizanlizumab
  - 11 concentrations titrated with a fixed concentration of P-selectin
  - Negative Control (buffer only) and Positive Control (P-selectin only) are included to calculate %binding of P-selectin and %inhibition of crizanlizumab (100% -%binding = %inhibition)
  - $IC_{50}$ value is generated and used as the bench mark to bridge critical reagent changes, such as different lots of P-selectin and GSP6
  - Crizanlizumab shows comparable $IC_{50}$ values in different matrix
  - Three levels of assay quality controls (QCs) are determined: HQC, MQC and LQC




PNHS = Pooled Normal Human Serum
SCDS = Sickle Cell Disease Serum

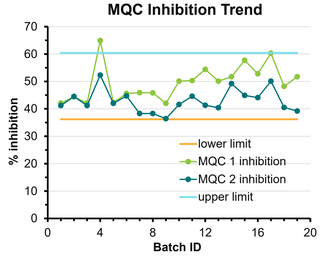
**Figure 4. a) Crizanlizumab inhibition curve in PNHS and SCDS**
**b) Crizanlizumab inhibition curve in PNHS with two different lots of GSP6**

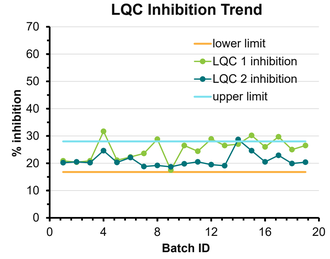
## ANALYTICAL VALIDATION RESULTS SUMMARY

| Parameter | Result | Acceptance Criteria | Outcome (P/F) |
|---|---|---|---|
| **Inhibition Curve (Linearity of dilution)** | Concentration range: 0.75 – 192 µg/mL<br>%CV = 19.2%<br>$IC_{50}$ = 5.2 µg/mL | %CV: ≤ 20% (≤ 25% at LLOQ) | Pass |
| **Total Precision** | Highest %CV = 21.9% (at LLOQ) | %CV: ≤ 20% (≤ 25% at LLOQ) | Pass |
| **Accuracy** | Highest % Recovery = 20.5%<br>Highest %CV = 10.9% | % RPD within ±25%<br>%CV: ≤ 20% (≤ 25% at LLOQ) | Pass |
| **Reportable Inhibition Range** | ULOQ = 100.0%<br>LLOQ = 10.4% | N/A | N/A |
| **Interference** | PNHS $IC_{50}$ = 5.2 µg/mL<br>SCDS $IC_{50}$ = 5.3 µg/mL | $IC_{50}$ from SCD ± 20% from PNHS $IC_{50}$ | Pass |

## ASSAY PERFORMANCE

- One set of QCs is tested at the beginning of the assay run and another set is tested at the end of the assay run.
- Clinical sample results are only considered valid when QC meets pre-defined acceptance criteria.
- The trend of QC results is monitored for each clinical trial to ensure the proper PD assay performance.
- HQC inhibition near or at 100% with minimum variation and therefore not shown here.

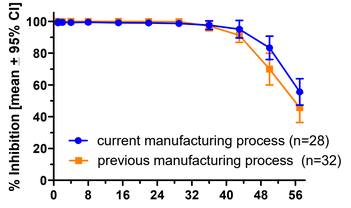


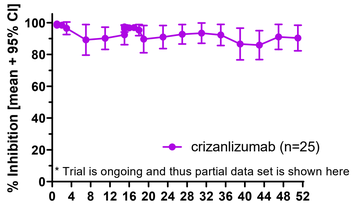

## PD RESULTS FROM CLINICAL TRIALS

Crizanlizumab showed inhibition of P-selectin binding in clinical studies of sickle cell disease patients, and, the inhibitory properties of crizanlizumab were comparable amongst different manufacturing methods.

**Single dose of crizanlizumab with different manufacturing processes in Healthy Volunteers**




**Multiple dose of crizanlizumab in Sickle Cell Disease Patients***




## CONCLUSIONS

- A systematic approach was taken to qualify the instrumentation and novel reagents and perform advanced assay validation commensurate with regulatory guidelines for use of a ligand binding method for a co-primary endpoint of a clinical trial supportive of drug registration.
- The validated SPR assay has been applied to over 3000 serum samples from clinical trials of crizanlizumab to support a co-primary endpoint and aid in determining the impact of crizanlizumab on P-selectin binding, thus, enabling use of the SPR assay across ongoing sickle cell disease trials.

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