Surface Plasmon Resonance-Based Immunogenicity Assays; Moving Towards a New Paradigm in Detection

Dell, Paul, FDA/CDER/OBP; Mills, Fred, FDA/CDER/OBP; Tolnay, Mate, FDA/CDER/OBP

Abstract

Immunogenicity assays are designed to measure antibodies generated against therapeutic proteins and monoclonal antibodies (mAbs). The current standards for immunogenicity assays are based primarily on plate-based techniques such as ELISA. Surface plasmon resonance (SPR) based assays represent a significant leap forward in the state of the art. Their major advantages include complete binding and dissociation curves rather than single values, which carry useful information about binding kinetics and behavior.

We set out to design and optimize an SPR assay to detect anti-drug antibodies (ADAs) generated against Rituximab (Rituxan®, Genentech), aiming to improve assay design. Our approach was twofold. On one hand we worked to improve the assay tolerance to drug interference by developing a viable method to deplete drug from serum samples using several magnetic-bead and cell-based approaches. On the other we designed and optimized an SPR assay on the Biacore® platform to detect low levels of both high and low affinity ADAs in minimally diluted serum. To be used as positive controls, we developed a panel of polyclonal ADAs in rabbits to adequately simulate patient immune responses at various stages of treatment.

Through several depletions studies we determined that mAb conjugated magnetic beads were optimal for removing significant amounts of drug from serum, reaching ~95% depletion of high-load samples. The assay was shown to be functional and efficient—a single sensor surface can be regenerated up to 120 times, permitting the analysis of about 200 samples per chip. Positive controls were detectable down to nanomolar concentrations in the presence of serum proteins at 1:4 dilution.

The viability of this assay indicates the utility of SPR for clinical monitoring of ADA production, and offers a more powerful and accurate tool for this purpose than was previously available. The assay also has great potential for further development, including the extraction of additional information from complex curves for the purpose of better characterizing antibody-dependent response to mAb therapeutics. The aim of this project is to improve the clinical utility of ADA assays and to provide a more complete picture of the immune response to mAbs and other protein therapeutics during clinical trials.

Introduction

Surface Plasmon Resonance (SPR) is a physical phenomenon that occurs at the thin gold surface on an SPR chip. In an SPR assay, the binding target of the analyte—in our case an antibody—is covalently attached to the surface with dextran linker (fig. 1). When analyte—in our case, ADA—is flowed past the surface, the binding that occurs between it and the surface can be measured directly as a change in local refractive index. This generates binding and dissociation curves as well as binding maximum data (fig. 1).

SPR assays are typically more sensitive than plate-based assays and detect a much wider range of antibodies. This is primarily due to the lack of wash-steps that would typically remove most low-affinity ADA populations. The label-free nature of SPR also removes confounding effects from crosslinking labels, simplifying analysis and cutting down on false-negatives significantly. SPR assays also enable inline isotyping through the addition of anti-Isotype antibodies after the association phase.

Materials and Methods

Assay Optimization: The SPR assay was designed through iterative optimization of several key parameters. All analysis was carried out using CM5 chips (Cytiva) and a Biacore T200 instrument (Cytiva).

We first generated a set of positive controls in rabbits by immunizing them with Rituximab Fab with standard adjuvant and collecting serum at several time-points. These timepoint sera were characterized and purified via either salting out or by affinity purification. The pooled and purified polyclonal controls were designed to mimic different clinical immunogenicity reactions—i.e. some were low-affinity with low on-rate/high off-rate populations, while others were made up of primarily rituximab-specific high-affinity antibodies.

The surface was optimized through extended trials of different molecules and capture levels to give the best signal to noise ratio possible. Following surface optimization, the regeneration solution was optimized through a similar iterative process, and finally an ideal concentration was determined through titration (Fig. 2).

Assay Qualification: A human negative control serum panel was obtained from a commercial supplier, evenly balanced by sex, race, and age. This panel was used to establish cut-points for positivity analysis.

Sensitivity analysis was performed in select serum using serial dilutions of various positive controls. Drug-tolerance of the assay was assessed using a serial dilution of Rituximab spiked into several concentrations of positive controls in serum.

Depletion: The depletion arm of the investigation was carried out using streptavidin-coated magnetic beads (New England Biolabs) and biotinylated anti-Rituximab mAb (BioRad). CD20 peptides and cell-based depletion using Raji/Daudi cell lines were also attempted. Results were assessed via Rituximab ELISA (Eagle Biosciences).

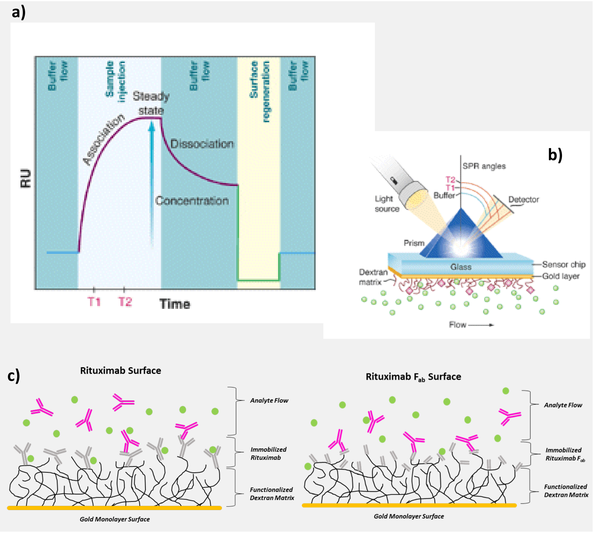


Figure 1. a) typical association and dissociation curves, b) fundamental SPR detector scheme, and c) Rituximab surface compared to Rituximab Fab surface diagram

Results and Discussion

Depletion: Of the depletion methods trialed, the only significantly effective technique was the anti-Rituximab magnetic bead separation, which yielded depletion levels around 83-99% in high-load samples.

Assay Optimization: Optimal assay parameters were determined before final qualification began. The ideal running buffer was shown to be a 1x HEPES buffer with 5 mM EDTA. Similarly, the best sample dilution buffer was shown to contain 20 mM EDTA for a 1:4 serum dilution.

The most critical element of the optimization was determining the ideal surface: the best performance was given by a rituximab Fab surface at a medium density of 600-1200 RU. Optimal regeneration was achieve using a short exposure to basic conditions in the presence of mixed surfactants. Other parameters were easily optimized, such as association/dissociation times, flow rate, etc.

Assay Qualification: The negative control panels (Fig. 2) showed a general clustering around 30 RU of background binding signal, with a traditional cut point of 50 RU. Sensitivity analysis demonstrated an ability to detect ADA concentrations down to ~50 ng/mL, with a lower limit of detection around 45 ng/mL for affinity purified positive control. Drug tolerance results are forthcoming.

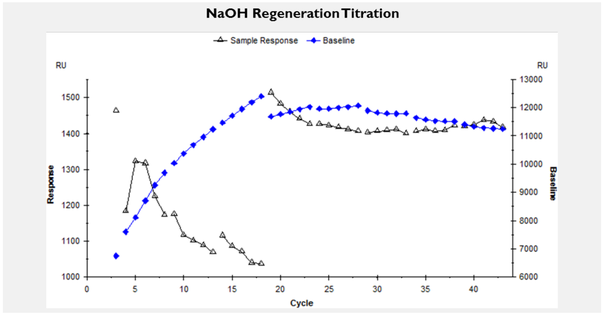


Figure 2. Regeneration optimization, NaOH concentration series

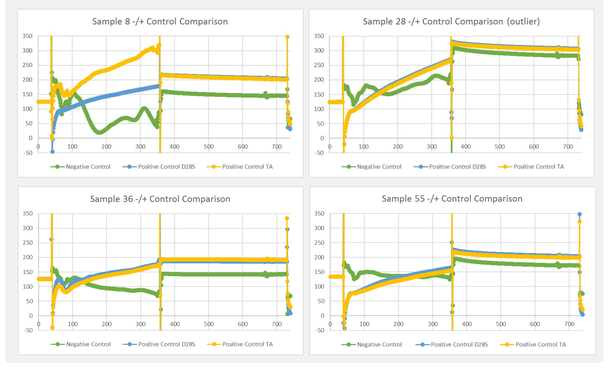
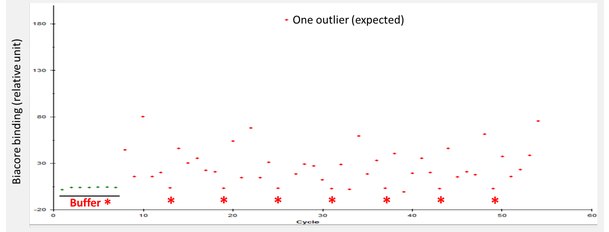


Figure 3. Positive control in serum curves (blue and yellow, medium and high affinity respectively) plotted with the corresponding negative human serum control sample curve (green)

Figure 4. Negative control panel with regularly interspersed buffer controls marked with *) to measure stable assay performance



Conclusion

Based on the preliminary performance of the assay in qualification studies, SPR immunogenicity assays can offer improvement over plate-based traditional assay design. They demonstrate excellent sensitivity, enable flexible isotyping within the workflow, and require very small volumes of patient serum compared to plate-based assays.

SPR immunogenicity assays also demonstrate a clear ability to differentiate positive and negative samples based on analysis of the shape/behavior of generated curves. Even when serum behavior is non-standard, the ability to detect ADAs is maintained, as demonstrated in Figure 3. It is straightforward to differentiate positive curves from negative curves visually, but also mathematically. Further work on the quantification of positive vs. negative patient samples is ongoing as we collect large positive control datasets to enable a comprehensive analysis.

Further improvements in SPR assay design, along with increased use of the SPR platform in all stages of clinical development, can lead to SPR-based assays with superior performance over traditional plate-based assays in immunogenicity screening and confirmation. Additional innovations based on surfaces engineered to produce little to no background binding could enable even greater use of SPR for samples with complex matrix constituents—including serum, lymph, whole blood, and cell lysates. Even in the absence of such improvement, SPR offers clear improvements over previous assay formats and greatly simplifies the overall workflow involved in immunogenicity assessments.

Future work on SPR immunogenicity assays using real patient samples in a clinical setting will strengthen the use-case for this technology. We expect further work in this area to focus on the validation of such assays using a similar format to that described above.

References

1. Wilson, W. Science 295, 2103 (2002)