Choosing an Appropriate Neutralizing Antibody Assay Format: A Case Study in Parallel Method Development of Cell-Based and Competitive Ligand Binding Neutralizing Antibody Assays

Andrew J. Brown[a], *Kristina Newton*[a], *Mark Dysinger*[b], *and Xun Wang*[a]

a. QPS, LLC; b. Alexion Pharmaceuticals

BACKGROUND

The 2019 FDA Immunogenicity Guidance, and Wu, et. al., recommend using a cell-based assay format for neutralizing antibody (NAb) assays when a therapeutic has a cell-based mechanism of action (MoA). This case study focuses on ALXN2220, a novel recombinant human IgG monoclonal antibody (Mab) therapeutic which targets mis-folded amyloid transthyretin (ATTR) in the extra-cellular space of the heart and facilitates its removal via antibody mediated phagocytosis (Michalon, et. al,, Nat. Commun., 2021). Since the Fc domain in ALXN2220 facilitates phagocytosis through binding the Fc receptor on macrophages, this cell-based mechanism aligns with effector function Mabs per Wu, et. al., suggesting a cell-based NAb assay be developed. However, due to the lack of a clear cell-based endpoint and challenges in developing cell-based NAb assays with sufficient drug tolerance, we concurrently developed and compared a cell-based assay and a competitive ligand binding assay (CLBA) to determine the most appropriate format.

Evaluating both cell-based and competitive ligand-binding neutralizing antibody assay formats allows for a data-driven decision that aligns with the therapeutic's mechanism of action and clinical needs. This approach ultimately enhances the robustness and applicability of the assay and contributes to more accurate and effective therapeutic monitoring.

CELL-BASED ASSAY FORMAT

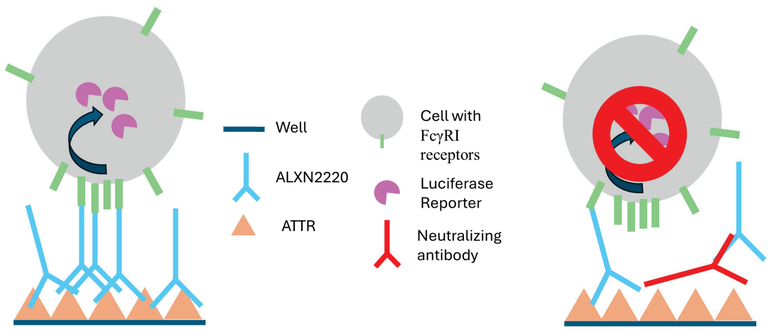

Figure 1. The cell-based assay with solid phase extraction and acid dissociation (SPEAD) extracts neutralizing antibodies from samples, which are then incubated with ALXN2220. A FcγRI ADCP Bioassay Kit (Promega GA1341) is then used, which is a bioluminescent cell-based assay measuring the presence of Fc domains (contained in ALXN2220) through the expression of luciferase. The ALXN2220 forms a bridge between the cells and ATTR coated onto the plate producing luciferase, which is inhibited by neutralizing antibodies.

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COMPETITIVE LIGAND BINDING ASSAY FORMAT

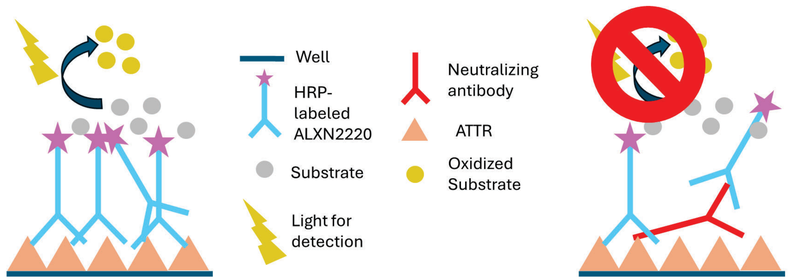

Figure 2. The CLBA with solid phase extraction and acid dissociation (SPEAD) extracts neutralizing antibodies from serum samples, which are then incubated with HRP-labeled ALXN2220. This mixture is then added onto ATTR coated plates. The HRP-labeled ALXN2220 binds to the ATTR and produces signal with a chemiluminescent substrate, which is inhibited by neutralizing antibodies.

METHODOLOGY

Table 1. Summary of a few key assay parameters specifically optimized while developing the assays, and the reason each condition was optimized.

	Assay Parameter	Reason
CLBA	Coating buffer	Optimize sensitivity and dynamic range
	Readout/detection reagent	Optimize sensitivity and dynamic range
	Sample pre-treatment	Improve drug tolerance and reduce matrix effects
	SPEAD acids and bases	Ensure sufficient PC recovery
Cell-based	Coating buffer	Optimize sensitivity and dynamic range
	Cell and reagent concentrations	Optimize sensitivity and dynamic range
	Sample pre-treatment	Improve drug tolerance and reduce matrix effects
	SPEAD acids and bases	Ensure sufficient PC recovery and cell viability
	Incubation times	Ensure feasible timing without impacting assay

RESULTS—Assay Sensitivity

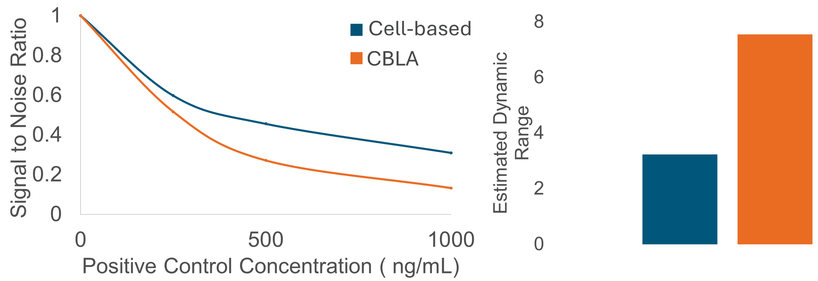

Figure 3. Positive control antibody response across physiologically relevant concentrations in both assay formats (left). An estimate of the dynamic range for both assay formats calculated as the inverse of the high positive control SNR (right).

RESULTS—Robustness and Drug Tolerance

Table 2. Negative Control (NC) and Positive Control (PC) performance in both assay formats. Displayed values are the average raw signal (Relative Light Units, RLU) for the NC and average Signal to Noise Ratio (SNR) for the PC levels across several runs. %CV is calculated as: [standard deviation] / [average] * 100%.

Assay Type	Assay Parameter	NC (RLU)	250 ng/mL PC (SNR)	500 ng/mL PC (SNR)	1,000 ng/mL PC (SNR)
CBLA	Average	200,643	0.517	0.271	0.133
	%CV	21.8	17.5	24.2	23.7
Cell-based	Average	210,707	0.598	0.456	0.310
	%CV	32.7	26.3	37.5	41.3

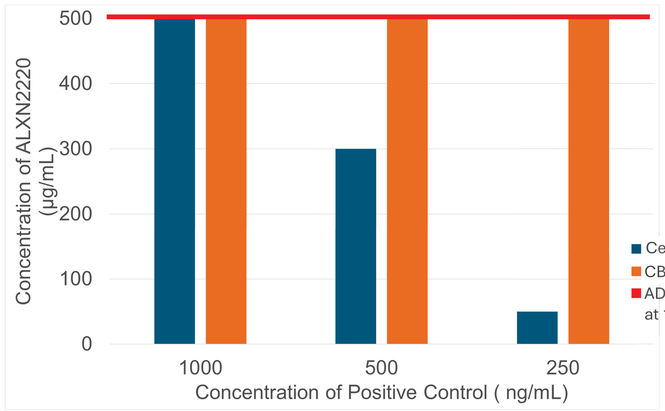

Figure 4. Comparison of Expected drug tolerance for both assay formats. Displayed as the highest concentration of ALXN2220 that can be tolerated for a positive control concentration and still expected to screen positive.

CONCLUSIONS

- Functional CLBA and cell-based assay formats were developed with sufficient sensitivity (<250 ng/mL of positive control).
- The CLBA demonstrates a more robust dynamic range, and critically, a more clinically relevant expected drug tolerance (250 ng/mL of PC can tolerate ≥ 500 µg/mL of ALXN2220)—the trough concentrations of ALXN2220 in clinical trials are expected to be as high as 500 µg/mL.
- The CLBA was chosen to continue into assay validation even though ALXN2220's MoA would imply that a cell-based assay would be more appropriate.

REFERENCES

1. FDA. Guidance for Industry: Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. Jan 2019
2. Wu B, et. al. Strategies to Determine Assay Format for the Assessment of Neutralizing Antibody Responses to Biotherapeutics. AAPS J. 2016 Nov;18(6):1335-1350.
3. Michalon A, et. al. A human antibody selective for transthyretin amyloid removes cardiac amyloid through phagocytic immune cells. Nat Commun. 2021 May 25;12(1):3142.