Development of Non-Cell Based Potency Assays for Bispecific Antibodies

W1530-02-09

Wesley Kendle, Joanna Robbie, Anais Sotomayor Albino, Karina Mejia Cardenas, Lakshmi Pillai-Kastoori, Jessica Weaver, Jeff Patrick

BioAgilytix Labs, Durham, North Carolina 27713

CONTACT INFORMATION: wesley.kendle@bioagilytix.com , jeff.patrick@bioagilytix.com

PURPOSE

In recent years, the development of Bispecific Antibodies (BsAb) has become an increasingly explored approach in cancer immunotherapy. Unlike traditional chemotherapeutics which indiscriminately kill all rapidly dividing cells, BsAb immunotherapy is a targeted approach to cancer treatment. Since 2014, nine BsAbs have been approved by the FDA, including 2 approvals in 2023 for the treatment of B-cell lymphoma.

Potency assays are essential in the development of biologics such as BsAbs. During the development of BsAbs, potency assays measuring the ability of the drug to bind to its intended targets are a critical part of the manufacturing process. However, the development of such assays for BsAbs is complicated by the need to effectively assess interactions between the BsAb and both target antigens. In this study, two potency assays were developed for a Phase I BsAb, henceforth referred to as Antibody X. Antibody X was designed to bridge effector T-cells and target tumor cells to promote T-cell activation and the subsequent lysis of tumor cells. Antibody X contains antigen binding sites targeting a cell surface protein X (CSPX) on T-cells, and a cell surface protein Y (CSPY) on certain tumor cells (Figure 1). CSPX plays a vital role in T-cell activation while CSPY is a tumor-associated antigen expressed in various solid tumor types. To align with the mechanism of action (MOA) of Antibody X, two potency assays were developed to measure the binding of the BsAb to each target antigen independently. Following assay development, validation was completed for both the CSPX- and CSPY-based potency assays and facilitated continuation of product development.

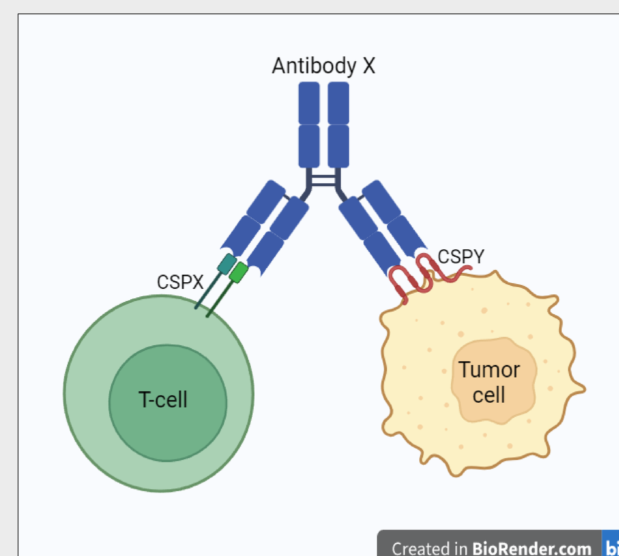

Figure 1. Antibody X bridges T-cells and tumor cells through interactions with CSPX and CSPY.

OBJECTIVES

The objective was to develop a potency assessment for Antibody X through the development of two independent potency assays, one designed for each of the two antigen binding sites of Antibody X. We aimed to establish a process in which potency assays can be developed for early phase BsAb studies, overcoming current obstacles to traditional BsAb potency assays.

METHODS

Capture protein, either CSPX or CSPY, was immobilized on standard Meso Scale Discovery (MSD) multi-array plates. Serial dilutions of Antibody X (analyte) and a negative control were added to the plates. Ruthenylated secondary antibody was bound to Antibody X and used to generate an electrochemiluminescence (ECL) response directly proportional to the amount of analyte detected in each well. The ECL units were measured on an MSD plate reader. Mean, R^2, %CV, and Relative Potency (REP) were calculated using a 4-parameter nonlinear logistic (4PL) curve using Gen5 Secure, version 3.02 (Agilent Technologies). During the development of these potency assays, capture protein concentration and ratio of capture protein to primary antibody were explored and optimized. To achieve optimal 4PL curves with sufficient data points in the upper and lower asymptotes for each potency assay, the Antibody X dose concentration ranges were optimized independently.

RESULTS

CSPX-based potency assay

- 10,000ng/mL was determined to be optimal for the highest concentration of the reference standards, and 1.6-fold serial dilution was used to generate an 11-point reference curve.
- Relative potency at 50%, 100% and 150% nominal drug concentrations (NDCs) for the test samples was measured. Representative 4PL curves with test samples at 50%, 100% and 150% NDC are shown (Figure 2).
- R^2 value of the mean relative potencies for test sample linearity assessment during assay validation was 0.9998 (Figure 3).

CSPY-based potency assay

- 4,000ng/mL was determined to be optimal for the highest concentration of the reference standards, and 1.5-fold serial dilution was used to generate a 9-point reference curve.
- Relative potency at 50%, 100% and 150% nominal drug concentrations (NDCs) for the test samples was measured. Representative 4PL curves with test samples at 50%, 100% and 150% NDC are shown (Figure 4).
- R^2 value of the mean relative potencies for test sample linearity assessment during assay validation was 0.9995 (Figure 5).

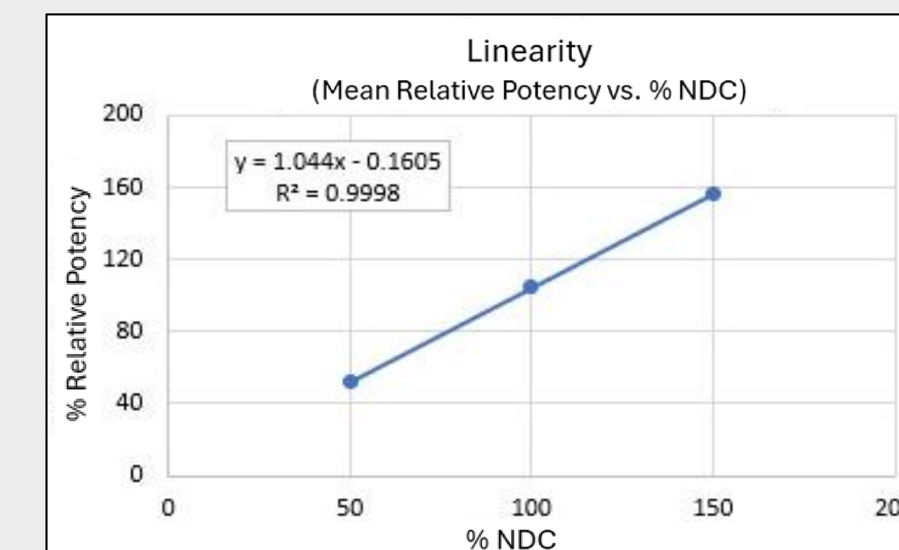

Figure 3. Sample linearity for CSPX potency assay

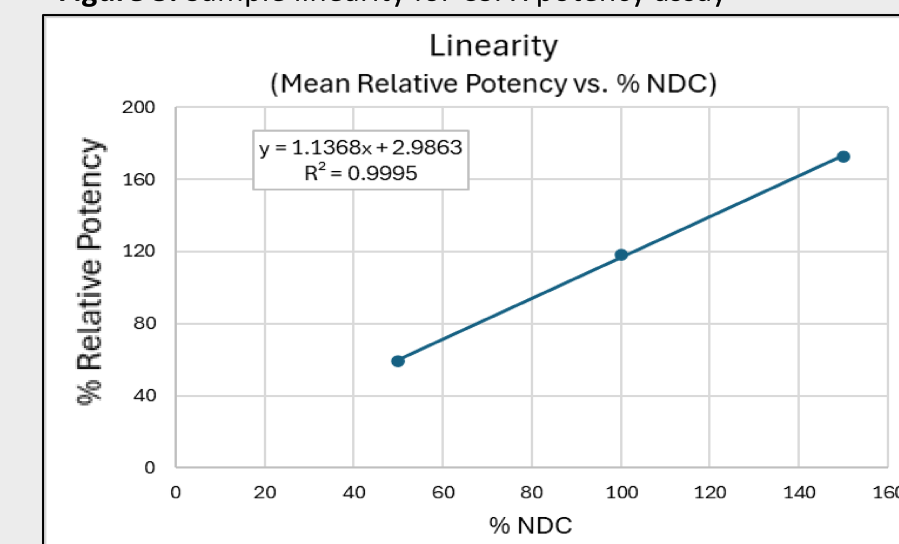

Figure 5. Sample linearity for CSPY potency assay

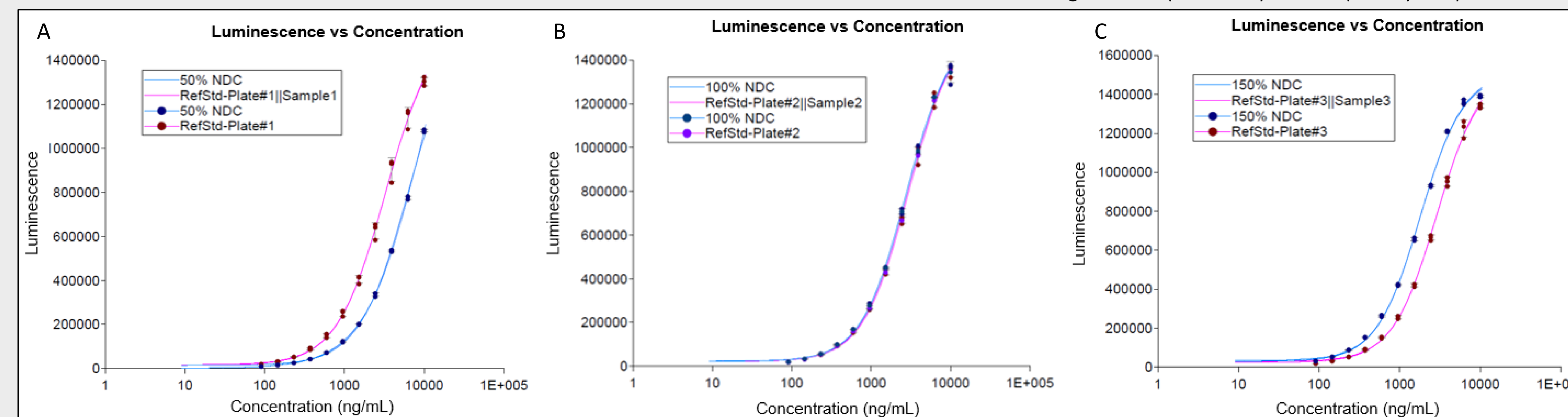

Figure 2. CSPX dose-response curves at (A) 50%, (B) 100% and (C) 150% NDC

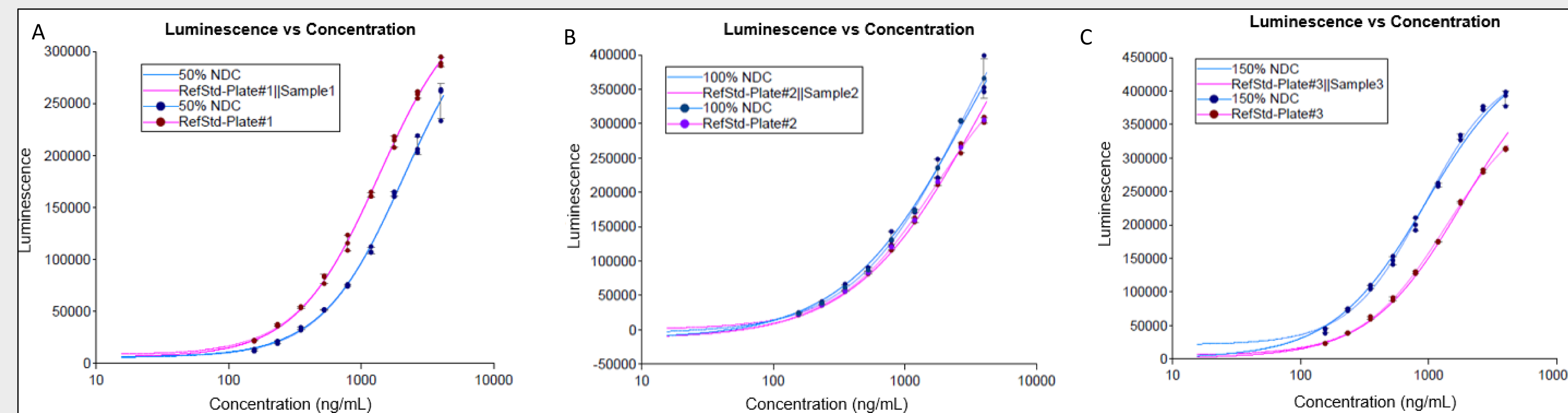

Figure 4. CSPY dose-response curves at (A) 50%, (B) 100% and (C) 150% NDC

CONCLUSIONS

Potency assays are a critical aspect of the drug development process with design of appropriate assay methods being driven by the unique MOA considerations of the therapeutic modality. With many BsAbs, the binding interactions with target molecules represent an essential step in the MOA. The development of potency assays for BsAbs therefore has unique challenges, such as the requirement for multiple specific methods around the binding of the BsAb and each target antigen.

Here, the development of two potency assays, one to measure the BsAb (Antibody X) binding to target 1 (CSPX) and the second to measure binding to target 2 (CSPY) are described. The results shown here, demonstrate the appropriate performance of two independent binding assays for potency determination of a single bispecific therapeutic. This strategy represents a valuable approach to gaining essential insights into antigen binding of BsAbs during the early phases of drug development.

REFERENCES

1. Food and Drug Administration. (2023). Bispecific Antibodies: An Area of Research and Clinical Applications.
2. FDA-2019-D-0621. Bispecific Antibody Development Programs Guidance for Industry. May 2021, Pharmaceutical Quality/CMC.
3. FDA-2023-D-4299. Potency Assurance for Cellular and Gene Therapy Products. December 2023.