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A Cell-Based Assay to Assess the Binding Activity of the Monoclonal Antibody Component of an Antibody Drug Conjugate

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Introduction

Antibody Drug Conjugates (ADCs) are cancer therapeutic agents designed to direct a cytotoxic drug to cells expressing a cell-surface antigen recognized by an antibody. The antibody and drug are linked through chemistries that enable the release of the cytotoxic drug upon internalization and digestion of the ADC by the cell. The efficiency of any ADC can be evaluated by Cell-based Potency and Cytotoxicity Assays.

A competitive cell-based potency assay was developed for determination of relative binding potency of a newly-constructed ADC, consisting of a monoclonal antibody against CD19 antigen (overexpressed on the surface of cancer-modified B lymphocytes) and a Pyrrolobenzodiazepine (PBD) as a cytotoxic DNA damage agent.

The assay utilized CD19 expressing Ramos cells (RA-1, ATCC® CRL1596™). A SULFO-TAG® anti-CD19 antibody was used as a competitor to unlabeled ADC. Conjugated and unconjugated anti-CD19 antibodies were recognized to a similar extent by the antigen. Luminescence was measured by a Mesoscale Discovery (MSD) Sector Imager plate reader and was proportional to the competition by ADC of the binding of SULFO-TAG® antibody to the CD19 antigen

Method

A competitive cell-based binding immunoassay with electrochemiluminescent (ECL) detection was developed to determine the relative binding potency of antibody drug conjugate and antibody intermediate relative to their respective fully-characterized reference standards. The assay utilizes CD19 expressing Ramos cells (RA-1). MSD plates are coated for at least one hour with Concanavalin A. Reference standard, QC and test samples are prepared in dilution buffer containing SULFO-TAG labeled ADC (anti-CD19), which is used as a competitor for the unlabeled ADC. RA-1 cells are pre-incubated with diluted reference standard, QC and test samples at room temperature (RT) for at least one hour. Plates are then washed three times, and the contents transferred to the Concanavalin A coated plates.

These were then incubated for at least two hours at RT. The plates were washed and read buffer is added to the wells. Signals are detected following the application of a voltage to the plate electrodes within the MSD Sector Imager 6000 (Meso Scale Discovery), causing the bound SULFO-TAG to emit light which is detected by the Sector Imager.

Method Qualification

During the method qualification, System Suitability, Accuracy, Precision, Specificity, Linearity and Range were examined.

System Suitability: System Suitability passed in all qualification assays: %CV ≤25%, R2≥0.99, A, B, D values 75%-125% of Reference Standard.

Accuracy: Accuracy was tested by preparation of the ADC at five levels across a range corresponding to 200%, 150%, 100%, 75% and 50% (each percent denoted as Test Material and 100% as Assay Control) of theoretical working concentration. Accuracy was assessed as percent recovery, or measured Relative Potency divided by the Theoretical

Theoretical Potency(%)	Measured Potency (%)					
	ADC			Antibody Intermediate		
	Relative Potency	%CV	Percent Recovery	Relative Potency	%CV	Percent Recovery
50	56	7	111	49	5	99
75	78	5	104	74	6	99
100	102	8	102	100	3	100
150	161	16	107	155	5	103
200	187	5	93	195	1	98

Table 1. Standard solutions prepared at five levels across a range corresponding to the theoretical working concentration.

Potency, multiplied by 100. The ADC test solutions were diluted to the five target concentration levels and compared with the ADC or Antibody Intermediate prepared at 100%. Accuracy was calculated assuming all preparations were at the theoretical concentrations. Each of the five concentration levels was tested three times. Recoveries for individual runs were between 94% and 114% of theoretical concentrations, and the CV between the recovery data of each tested level was between one and 16 percent.

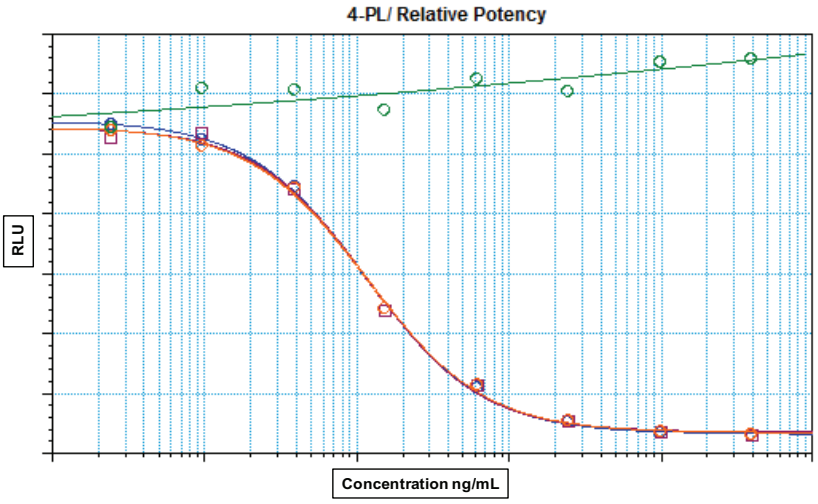

Figure 1. Specificity of the method assayed by use of a non-specific anti-CD25 antibody drug conjugate.

Intra Assay Precision (Repeatability)

Intra assay precision was assessed using Reference Standard (RS), Assay Control (AC), ADC and Ab. Intermediate solutions each at 100%, tested by the same analyst on the same day (repeatability) on three independently prepared plates for each. Intra assay precision for the Antigen Binding Assay was analyzed by determining the percent CV of the relative potency of the Assay Control from three individual plates run on the same day by a single analyst.

Intermediate Assay Precision

The intermediate assay precision was determined for the Antigen Binding Assay for five levels of potency tested experimentally for ADC and Ab. Intermediate. This was done by calculating the percent CV of relative potency values from three individual experiments performed at each level and each tested article (ADC and Ab. Intermediate) to assess 200%, 150%, 75%, and 50% potency levels by two analysts on different days. (Two sets of assays were done by Analyst 1, and one set was done by Analyst 2 for each tested article). The relative potency for the 100% Assay Control was used to assess the percent CV for 100% potency level; six individual runs were used in this determination. The results are illustrated in Table 3.

Analyst 1	%Relative Potency	
	ADC	Ab. Intermediate
Plate 1	105	101
Plate 2	97	106
Plate 3	118	98
Mean	107	102
%CV	10	4

Table 2: Intra Assay Precision for the Antigen Binding Assay was analyzed by determining the percent CV of the Relative Potency of the Assay Control from three individual plates run on the same day by a single analyst.

Specificity

The specificity of the method was assayed by use of non-specific anti-CD25 antibody drug conjugates. As shown on Figure 1, non-specific antibody that showed any specific binding and potency was outside ±50% relative to the Reference Standard.

Linearity & Range

Linearity was evaluated to test the ability of the method to accurately distinguish and quantitate the Relative Potency between and throughout a range of potencies. Five levels of potency for the ADC and Ab. Intermediate; namely, 200%, 150%, 100%, 75%, and 50%, were determined and linearity in the range of 50%-200% was seen. The experimentally acquired mean relative potency of each level relative to their theoretical concentrations was charted using linear regression statistics. Linearity was achieved as shown by the coefficient of determination, R2 = 0.98 for ADC and 1.00 for Ab. Intermediate.

Conclusions

Several assay parameters were evaluated during qualification exercises; including Accuracy, Precision, Specificity, Linearity and Range.

The method has been qualified for assessment of the relative binding potencies of an ADC or its corresponding Antibody Intermediate under cGMP guidelines and regulations.

Data obtained during method qualification and analysis of system suitability criteria tracked through the course of a two-year stability study (Table 4) demonstrated the assay's suitability and robustness for

determining the potency of binding activity, LOD determination; and LOQ determination met the acceptance criteria specified in the validation protocol. The current method is deemed validated and suitable.

Analyst	Relative Potency									
	200%		150%		100%		75%		50%	
	ADC	Ab. Inter.	ADC	Ab. Inter.	ADC	Ab. Inter.	ADC	Ab. Inter.	ADC	Ab. Inter.
Analyst 1	197	196	133	165	104	97	81	73	60	53
					112	99				
Analyst 1	177	192	166	147	109	101	80	80	53	48
					95	98				
Analyst 2	186	198	184	153	88	98	74	70	53	47
					104	98				
					103	107				
Mean	187	195	161	155	102	100	78	74	56	49
%CV	5	1	16	5	8	3	5	6	7	5

Table 3. The Relative Potency for the 100% Assay Control was used to assess the percent CV for 100% potency level; six individual runs were used in this determination.

Time Point	Measured Potency (%)			
	ADC		Ab. Intermediate	
	Relative Potency (Mean of 3 assays), %	%CV	Relative Potency (Mean of 3 assays), %	%CV
T0	102	11	108	18
0.5M	102	9	95	21
1M	103	9	94	18
3M	114	6	104	20
6M	98	15	98	23
9M	92	18	109	5
12M	99	23	99	23
18M	97	12	105	2
24M	101	18	110	8

Table 4. Collected results of two years' worth of stability evaluations of ADC and Antibody Intermediate using Antigen Binding Assay.