GYROS PROTEIN Technologies

Gyrolab® Assays

Keytruda® (pembrolizumab) ADA Assay

INTRODUCTION

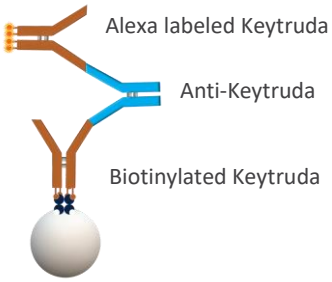
Keytruda® (pembrolizumab) is a cancer immunotherapy biopharmaceutical that belongs to the group of PD-1 (programmed cell death protein 1) inhibitors. Keytruda is a humanized antibody of IgG4 isotype that blocks a protective mechanism of cancer cells, allowing the immune system to destroy them.

Anti-drug antibodies (ADA) can be generated by patients when biopharmaceuticals are administered. The presence of ADA can inhibit biopharmaceuticals binding the target, impact the PK profile, and affect safety and efficacy. Detection and characterization of ADA is an integral part of biopharmaceutical drug development.

We have developed a homogenous bridging Gyrolab ADA assay to determine levels of anti-Keytruda antibodies in human serum samples. An MRD of 10 gives a specific and drug tolerant assay. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a homogenous bridging assay following acid dissociation, 0.5 M Glycin-HCl pH 2.6, with biotinylated Keytruda as a capture molecule and Keytruda labeled with Alexa Fluor® 647 as a detection molecule. The positive control used for this assay was a human anti-idiotypic mAb from Bio-Rad Laboratories.



ASSAY PERFORMANCE

Assay Sensitivity

The assay sensitivity was estimated from the screening cut point as 7.68 ng/mL of positive control antibody, see Figure 1. Due to the qualitative nature of ADA assays the assay sensitivity is not an absolute concentration of anti-drug antibody, and may be different in a clinical setting. Use of a surrogate positive control is, however, best industry practice and accepted by regulatory agencies.

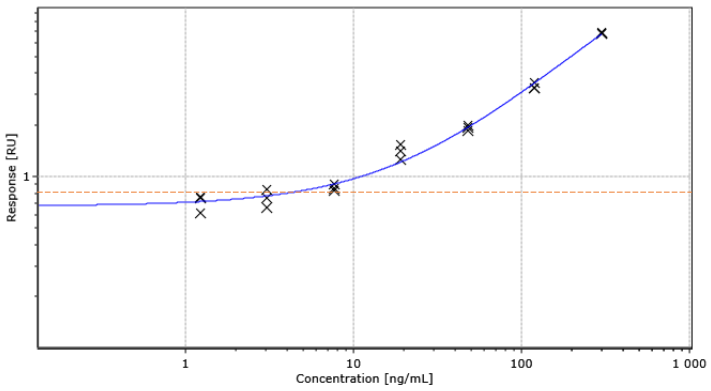


Figure 1 Positive control titration in Rexxip ADA with 10% serum. Screening cutpoint at 0.822 RU. Concentrations in neat serum.

Assay screening cut point

A floating cut point with 5% false-positive rate was estimated from 35 healthy mixed gender individuals, analyzed over three runs. This would not constitute a validated cut point assessment but was estimated from the principles outlined in Shankar et al. (Recommendations for the Validation of Immunoassays Used for Detection of Host Antibodies Against Biotechnology Products. Journal of Pharmaceutical and Biomedical Analysis 2008;48;1267-1281). The responses for individuals are shown in Figure 2. The normalization factor was set at 1.117 from the responses of this population.

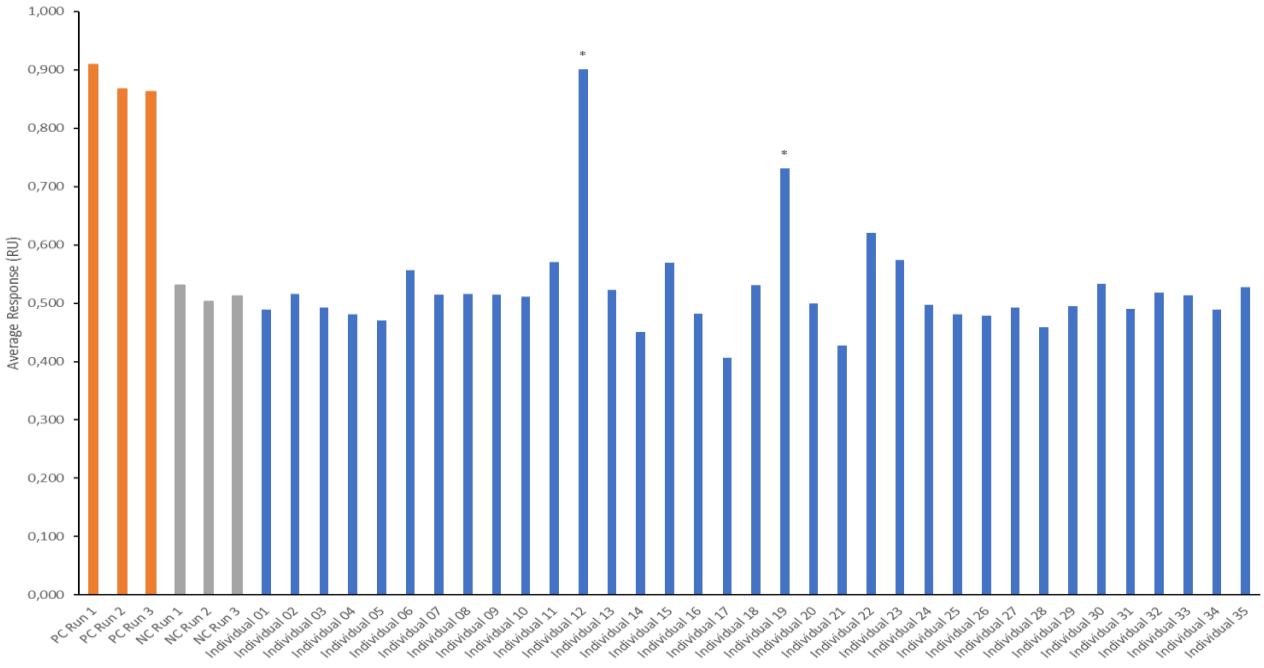


Figure 2 Average responses from Positive Controls (PC) at 20 ng/mL, Negative Controls (NC) and individual human serum samples (Individual 01 – 35) in Rexxip ADA containing 10% human serum. Concentrations in neat serum. Outliers identified by Grubbs Test marked with *.

Screening assay precision

The assay precision across three runs for the positive control at 20 ng/mL and for the negative control of pooled mixed gender human serum is shown below in Table 1.

Table 1 Precision assessment of the screening assay over three runs

Parameter	Negative Control (Pooled human serum)	Positive Control (20 ng/mL)
Average response	0.516 RU	0.879 RU
Intra-assay precision	≤14.3%	≤11.8%
Inter-assay precision	3.4%	3.1%

Confirmatory assay

Confirmatory analysis was performed with Keytruda in Rexxip® ADA where mixed gender individuals were analyzed in three runs following a 1-in-10 MRD using Rexxip® ADA with 500 µg/mL Keytruda and without Keytruda to determine the level of inhibition equivalent to a 1% false positive rate. The confirmatory cut point was determined from this set of individuals as 38% inhibition in the presence of Rexxip ADA with 500 µg/mL Keytruda.

Drug tolerance

Drug tolerance was assessed by titrating concentrations of Keytruda in the presence of 100 ng/mL ADA positive control in pooled human serum. At 100 ng/mL of positive control drug tolerance was estimated at 260 µg/mL of Keytruda as this is where the curve intersects the screening cut point, see Figure 3. Full inhibition of 100 ng/mL of ADA positive control was achieved at 640 µg/mL of Keytruda, equivalent to average response of assay blank at 0.721 RU; inhibition results are shown in Table 2. The anticipated trough level of Keytruda in human serum is ≤ 50 µg/mL.

Table 2 Drug tolerance assessment in the presence of 100 ng/mL positive control

Keytruda Conc. (µg/mL)	100 ng/mL Positive Control	
	Average response (RU)	% Inhibition (Blank subtracted)
0	2.46	N/A
10	2.41	2.7
20	1.93	30.5
40	1.67	45.4
80	1.26	69.2
160	0.987	84.6
320	0.774	96.9
640	0.670	102.9
1 280	0.618	105.9

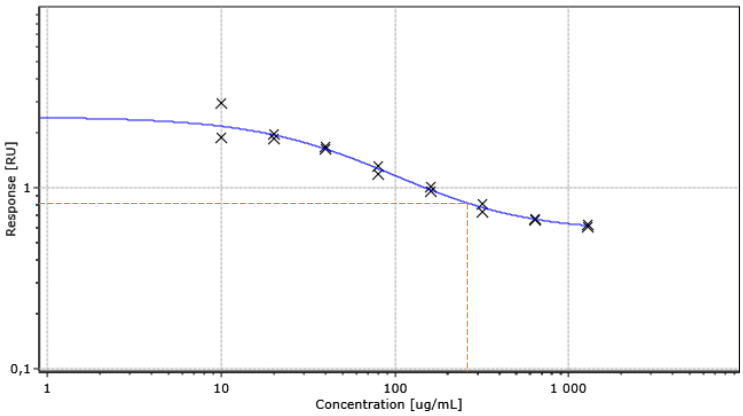


Figure 3 Titration of Keytruda in Rexxip ADA with 10% serum and 100 ng/mL of positive control. Concentrations in neat serum.

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Mixing CD 96. The assay was set up using a 1-step Gyrolab method with two wash solutions (Mixing96-1W-003-A) and a 5% PMT setting. The assay buffer was Rexxip ADA with 10% human serum, the confirmatory assay buffer was Rexxip ADA with 10% human serum and 500 µg/mL of Keytruda. The acid dissociation step was performed using 0.5 M Glycin-HCl pH 2.6. A bridging assay format was used with equal concentrations of capture and detection reagents in 1 M Tris-HCl pH 8.

The capture reagent was the humanized IgG4 monoclonal antibody pembrolizumab (Keytruda) from Merck Sharp & Dohme. 100 µg of Keytruda (1 mg/mL in PBS) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 1:12 challenge ratio of biotin. Capture reagent was used in mastermix at a concentration of 2 µg/mL in Rexxip ADA.

The detection reagent was the humanized IgG4 monoclonal antibody pembrolizumab (Keytruda) from Merck Sharp & Dohme. 100 µg of Keytruda (1 mg/ml in PBS) was labeled with one vial of Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide). The detection reagent was used in mastermix at a concentration of 2 µg/mL in Rexxip ADA.

The positive control used was a human anti-pembrolizumab monoclonal antibody, clone AbD30689_hIgG1, sourced from Bio-Rad (HCA298), diluted in Rexxip ADA with 10% human serum.

Summary table

Capture	Biotin labeled pembrolizumab (Keytruda, Merck Sharp & Dohme), 12:1 molar challenge ratio
Detection	Alexa Fluor 647 labeled pembrolizumab (Keytruda, Merck Sharp & Dohme)
Mastermix	2 µg/mL detection reagent and 2 µg/mL capture reagent in 1 M Tris-HCL pH 8
Acidic Buffer	0.5 M Glycin-HCl pH 2.6
Analyte	Human anti-pembrolizumab monoclonal antibody (HCA298; Bio-Rad) in Rexxip ADA with 10% human serum
CD-type	Gyrolab Mixing CD 96
Method	Mixing96-1W-003-A
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
PMT-setting	5%
Negative Control inter assay precision	3.4%
Suggested Positive Control concentration	PC: 20 ng/mL
Confirmatory drug concentration	500 µg/mL
Assay sensitivity	7.68 ng/mL*
Drug tolerance	260 µg/mL*
Screening Cut point	1.117 x Mean NC RU*
Confirmatory Cut point	38%*

* These values are dependent on the pool and individuals used in the assay validation.

Recommendations

When developing this assay for a specific drug development purpose, it is important to perform in-house screening of matrices and determine population screening cutpoint correction factor. Parameters, such as sensitivity and positive control concentrations should be validated in-house. Data given in this document should only be considered as guidance. Additional details on recommendations for optimizing an ADA method on the Gyrolab system can be found via the Gyrolab User Zone.

For additional support contact your local Field Application Support