Development of Total and Free PK Assays for Detection of PI-114-AB1 and PI-114-AB2 Antibodies in the Serum of Cynomolgus Monkey

Kara Mojica, Linda Liang, Xi Yang, Shilpa Mankikar, Sergio Lacayo, Sayantan Mitra, Nadine Jahchan, Kevin P. Baker, and Xiaoyan Du

Pionyr Immunotherapeutics, 2 Tower Place, Suite 800, South San Francisco, CA 94080

Abstract #

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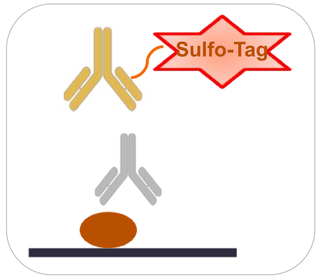
Abstract

Pionyr Immunotherapeutics (Pionyr) has generated anti-human monoclonal antibodies (mAb), termed PI-114-AB1 and PI-114-AB2 that target a receptor expressed on tumor-associated macrophages (TAMs) in the tumor microenvironment (TME) to promote anti-tumor immunity. A surrogate anti-mouse antibody has shown strong anti-tumor responses using syngeneic mouse tumor models by inducing immune actuation and activating intra-tumor immunity. To assess drug exposure and safety of the humanized drug candidates, Pionyr has developed total and free pharmacokinetic (PK) assays to quantify PI-114-AB1 and PI-114-AB2 antibody concentrations in an exploratory single dose non-human primate (NHP) PK and tolerability study. The single dose study consisted of four animals (1 male and 1 female per group) dosed at 10 mg/kg. The single dose study demonstrated that PI-114-AB1 and PI-114-AB2 antibodies have an acceptable PK and are well tolerated.

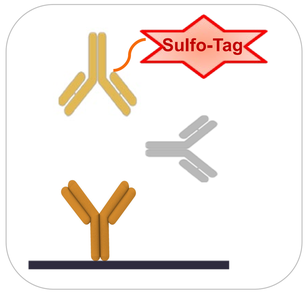
Method Principle

Ligand Binding (LBA) PK Assay Format



- Sulfo-tag labeled anti-hIgG CH2 antibody
- Drug
- Recombinant Human Protein
- MSD ECL Plate

Total PK Assay Format



- Sulfo-tag labeled anti-hIgG CH2 antibody
- Drug
- Biotin labeled anti-human kappa antibody
- MSD Streptavidin Plate

The ligand binding PK assay and total PK assay are designed to measure "free" and "total" antibody, respectively.

Assay Procedure

Ligand Binding PK Assay Format

Coat Standard MSD Plate with 40 µL of recombinant human protein @ 1 µg/mL.

Seal plate. Incubate with shaking at RT for 60 min.

Wash plate (3x). Block plates with 5% BSA, 150 µL/well for 60 min at RT.

Total PK Assay Format

Coat Streptavidin MSD Plate with 40 µL of biotinylated anti-human kappa antibody @ 1 µg/mL.

Seal plate. Incubate with shaking at RT for 60 min.

Wash plate (3x).

Dilute STDs, QCs and Samples
Add 40 µL of prepared STD, QCs and samples to coated plate with high salt buffer.

Seal plate. Incubate with shaking at RT for 60 min.

Wash plate (3x).

Add Detection Antibody
Add 40 µL of anti-human IgG CH2 @ 0.5 µg/mL.

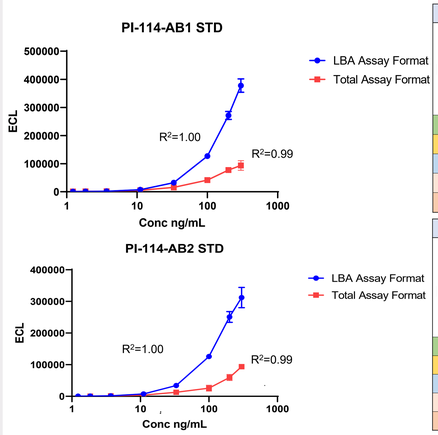
Seal plate. Incubate with shaking at RT for 60 min.

Wash plate (3x).

Add 150 µL of 1x Read Buffer to MSD plate and read plate on MSD Sector Imager.

Assay Performance

PK Calibration Curve Range: 300 ng/mL – 1.85 ng/mL



PI-114-AB1 Controls						
	LBA Assay Format			Total Assay Format		
Nominal Conc. ng/mL	Detected Mean Conc. (ng/mL)	Calc. Mean Conc. CV	% Recovery Mean	Detected Mean Conc. (ng/mL)	Calc. Mean Conc. CV	% Recovery Mean
240	233	5	**94**	280	10	**116**
100	109	5	**106**	120	3	**120**
50	46	5	**96**	56	0	**111**
10	9	5	**89**	10	5	**100**
5	4	3	**77**	5	6	**91**

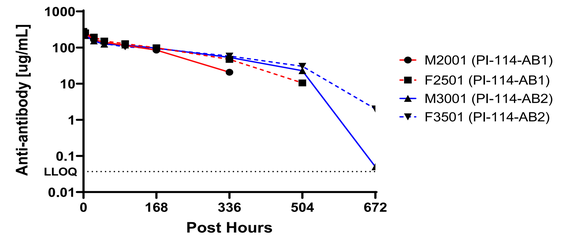
PI-114-AB2 Controls						
	LBA Assay Format			Total Assay Format		
Nominal Conc. ng/mL	Detected Mean Conc. (ng/mL)	Calc. Mean Conc. CV	% Recovery Mean	Detected Mean Conc. (ng/mL)	Calc. Mean Conc. CV	% Recovery Mean
240	217	4.1	**88**	295	0	**123**
100	98	0.8	**97**	88	21	**88**
50	47	2.2	**94**	58	9	**116**
10	10	1.8	**99**	10	16	**104**
5	4	2.4	**85**	6	3	**129**

NHP Study Design

Group No.	Test Material	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals	
					Males	Females
1	Control Article	0	2	0	1	1
2	PI-114-AB1	10	2	5	1	1
3	PI-114-AB2	10	2	5	1	1

LBA PK Result

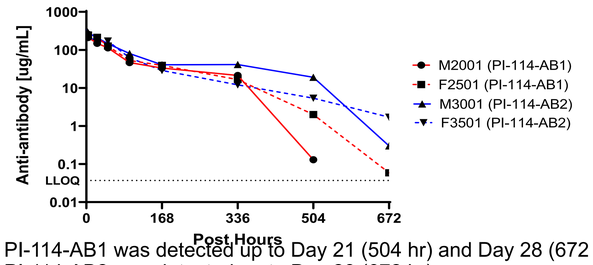
Concentration-Time Profiles of PI-114-AB1 and PI-114-AB2 in the NHP Prepilot Study



- PI-114-AB1 showed no detectable drug levels post Day 21 (504 hr)
- PI-114-AB2 was detected up to Day 28 (672 hr)

Total PK Result

Concentration-Time Profiles of PI-114-AB1 and PI-114-AB2 in the NHP Prepilot Study



- PI-114-AB1 was detected up to Day 21 (504 hr) and Day 28 (672 hr)
- PI-114-AB2 was detected up to Day 28 (672 hr)

- Both total and free assay formats measured similar drug concentrations for each antibody.
- Additional drug levels were quantified for PI-114-AB1 in F2501 on Day 28 and M2001 on Day 21 using the total PK assay format, but the actual levels are both below 0.2 µg/mL.
- Animals dosed with PI-114-AB2 showed slightly better exposure than dosed with PI-114-AB1. Changes in exposure is likely due to target mediated drug disposition (TMDD) or anti-drug antibodies (ADA).

Summary

- **The PK assays were successfully developed to measure drug concentrations in the exploratory NHP study.**
- **The PK assay range was 1.85 ng/mL to 300 ng/mL (in assay) with a minimum required dilution (MRD) of 20.**
- **The LLOQ was 1.85 ng/mL and the ULOQ was 240 ng/mL. The quality controls (QCs) ranged from 5 ng/mL to 240 ng/mL.**
- **The assay demonstrated acceptable accuracy and precision.**
- **The PK results for PI-114-AB1 and PI-114-AB2 are comparable using both the total and free assay.**
- **PI-114-AB1 and PI-114-AB2 antibodies have an acceptable tolerability and good exposure following the single dose study.**