Development of an Anti-Idiotype Pharmacokinetic PY314 Assay to Increase Specificity and Sample Throughput

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Abstract

PY314 is an afucosylated humanized monoclonal antibody (mAb) that is undergoing evaluation in a Phase 1 clinical trial (NCT 04691375) for the treatment of patients with advanced solid tumors. PY314 binds to triggering receptor expressed on myeloid cells 2 (TREM2) which is present on tumor associated macrophages (TAMs) and functions as a negative regulator of inflammatory responses. PY314 is designed to deplete TREM2 positive TAMs and repolarize the immune cell populations in the tumor microenvironment (TME) to promote anti-tumor immunity.

To support the phase 1 clinical trial, a ligand-binding assay (LBA) was developed. The assay uses human TREM2 coated onto a plate to capture the PY314 which is then detected using a sulfo-tagged mouse anti-human IgG1. The assay was successfully validated to measure PY314 levels and support clinical sample testing.

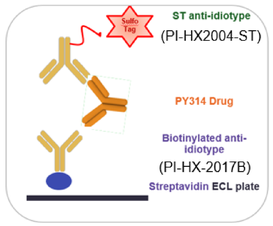
Here, we present a new assay to measure PY314 using an anti-idiotype mouse monoclonal antibody (mAb) pair to increase assay sensitivity, specificity, sample throughput, and eliminate the dependence on vendor supplied recombinant human TREM2 in the LBA format. The anti-idiotype antibody based PY314 pharmacokinetic (PK) assay is a typical sandwich immunoassay developed on the Meso Scale Discovery (MSD) platform. The biotinylated anti-idiotype (PI-HX-2017B) is first coated on a streptavidin plate to capture PY314. The standards, QCs (quality controls), and serum samples are then resuspended in an assay buffer containing heterophilic blocking reagent 1 (HBR1) which greatly improves assay specificity through heterophilic interference and prevention of non-analyte mediated bridging. A second anti-idiotype antibody (PI-HX-2004-ST) which is sulfo-tagged is then used for detection.

Assay Parameters Summary

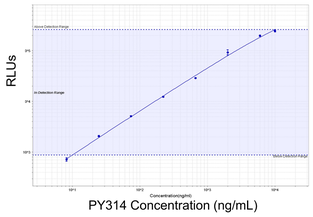
Anti-idiotype MSD ECL Assay

Minimum Required Dilution	1:20
Method Description	MSD-ECL using Streptavidin plate
Matrix	Human Serum
Coating	(PI-HX-2017B) Biotinylated capture anti-idiotype 1 h @ room temperature (RT)
Blocking	None required
Sample Incubation	1 h @ RT in high salt buffer (HSB) 0.35 M NaCL with HBR1 added at 10 µg/mL final concentration
Detection	(PI-HX-2004-ST) Sulfo-Tag anti-idiotype 1 h @ RT
Total assay run time	3 h
Lower Limit of Quantitation (LLOQ) (in-assay)	1-2 ng/mL
Upper Limit of Quantitation (ULOQ) (in-assay)	300 ng/mL

PY314 PK Assay Principle



PY314 PK Standard Curve



Anti-idiotype Antibody Screening

Sample	PY314 10 ng/mL	SC120926 (Head and Neck Cancer)	H1121002 Breast Cancer	C8121018 NSCLC Cancer	SC1211382 Ovarian Cancer	A1121306 Ovarian Cancer	SC1210056 Breast Cancer	A1120779 Breast Cancer	011211547 Breast Cancer	Normal 389762	Normal 389763
Coating/Detection	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs	RLUs
PI-HX-2013B/PI-HX-2002ST	13626	45	36	93	627	37	6898	35	32	101	33
PI-HX-2013B/PI-HX-2005ST	8612	33	31	39	388	29	4004	28	27	44	31
PI-HX-2017B/PI-HX-2004ST	4759	37	49	71	517	40	5835	33	44	72	40
PI-HX-2017B/PI-HX-2008ST	3834	47	50	71	442	37	3610	30	42	78	51
PI-HX-2018B/PI-HX-2005ST	2020	28	26	32	958	31	4951	25	26	38	26
PI-HX-2018B/PI-HX-2010ST	1054	56	38	83	1458	39	7434	161	30	170	73
PI-HX-2019B/PI-HX-2008ST	2681	36	49	68	338	38	3232	34	36	62	42
PI-HX-2020B/PI-HX-2005ST	417	194	2829	211	433	132	2073	170	136	1216	72

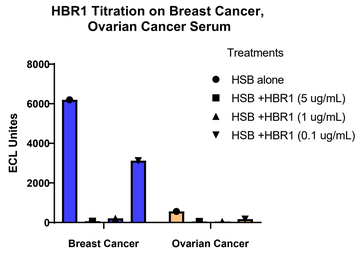
- Screening was performed using a panel of serum from 8 cancer individuals, 2 normal sera, 1 pooled sera and a fixed concentration of PY314 (10 ng/mL)
- PI-HX-2017B/PI-HX-2004-ST, and PI-HX-2013B/PI-HX-2005-ST were among the top pairs with PI-HX-2017B/PI-HX-2004ST being the finalist
- 2 Individual cancer sera showed consistent elevated signals (SC1211382- Ovarian cancer, SC1210056- Breast Cancer) regardless of anti-idiotype pair screened

Blocker Evaluation and HBR1 Titration

HBR1 is the best blocker

Blocker Treatments	HSB (0.35M NaCL)	HSB (0.35 M) +HRB1 (500 µg/mL)	HSB (0.35 M) + mIgG(100 µg/mL)	HSB (0.35M) + Diluent 2 (MSD) 1:1 mix	HSB (0.35 M) +HRB1 (500 µg/mL)	HSB (0.35 M) + MIgG(100 µg/mL)	HSB (0.35M) + Diluent 2 (MSD) 1:1 mix
2017B/2004ST Pair	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 2	Treatment 3	Treatment 4
Sample	RLUs	RLUs	RLUs	RLUs	%Reduction signal over Treatment 1 HSB		
PY314 10 ng/mL	8946	7567	8340	5101	15.4	6.8	43.0
SC120926 (Head & Neck)	61	58	106	67	4.9	-73.8	-9.8
H1121002 Br. Cancer	80	72	125	81	10.0	-56.3	-1.3
SC1211382 Ovarian	348	73	163	58	79.0	53.2	83.3
SC121005014 Br. Cancer	8166	125	792	67	98.5	90.3	99.2
1112098 (Head and Neck Cancer)	76	67	125	99	11.8	-64.5	-30.3
C8121018 NSCLC	113	99	168	119	12.4	-48.7	-5.3
NPS (389760-67)	94	71	131	104	24.5	-39.4	-10.6

HBR1 Titration



- To reduce non-specific background in human sera specimens, anti-idiotype pairs were further evaluated with blockers including mouse IgG, HBR1, HSB, MSD diluent 2 (proprietary MSD blocker)
- Treatment with HSB + HBR1 led to a decrease in nonspecific signal while maintaining specific signal to PY314
- HBR1 was further titrated, from 5 µg/mL to 0.1 µg/mL in cancer serum samples; recommendation is to use HBR1 at a final concentration of 10 µg/mL

Selectivity with Cancer Serum

Cancer Serum Sample	300 ng/mL spike (in-assay)		1 ng/mL spike (in-assay)	
	Calc. Conc. Mean	Recovery (%)	Calc. Conc. Mean	Recovery (%)
1	310.7	103.6	0.9	87.0
2	186.4	62.1	1.1	109.9
3	265.5	88.5	1.2	115.1
4	278.6	92.9	0.8	75.4
5	265.2	88.4	1.2	115.7
6	297.7	99.2	1.0	101.7
7	379.9	126.6	1.0	97.2
8	353.1	117.7	2.9	294.2
9	267.1	89.0	0.8	79.1
10	256.7	85.6	1.0	98.4
Overall Pass Rate (%)		80.0		90.0

- Selectivity was evaluated using 10 individual cancer serum samples spiked at the LLOQ (1 ng/mL) and the ULOQ (300 ng/mL)
- Pass rate was 80% and 90% at the ULOQ/LLOQ respectively
- Selectivity with normal serum also passed (data not shown)

Matrix Effects in Lipemic and Hemolyzed Samples

240 ng/mL QC in pooled lipemic serum

Sample	Conc. Mean (ng/mL)	Conc. CV (%)	Recovery (%)
NPS	229.4	5.4	95.6
2.5 % lipemic	209.5	8.8	87.3
5 % lipemic	195.2	3.2	81.3
10 % lipemic	198.1	9.1	82.5
25 % lipemic	188.6	5.7	78.6

2.4 ng/mL QC in pooled lipemic serum

Sample	Calc. Conc. Mean	Conc. CV (%)	Recovery (%)
NPS	2.3	10.2	96.8
2.5 % lipemic	2.0	1.7	82.1
5 % lipemic	2.0	6.7	83.4
10 % lipemic	2.1	2.5	89.4
25 % lipemic	1.7	15.3	71.6

240 ng/mL QC in pooled hemolyzed serum

Sample	Conc. Mean (ng/mL)	Conc. CV (%)	% Recovery
NPS	241.9	3.8	100.8
2.5 % hemolysis	225.0	0.9	93.8
5 % hemolysis	249.5	6.7	104.0
10 % hemolysis	197.7	9.8	82.4
25 % hemolysis	175.6	4.6	73.1

2.4 ng/mL QC in pooled hemolyzed serum

Sample	Calc. Conc. Mean	Conc. CV (%)	% Recovery
NPS	2.2	1.0	90.8
2.5 % hemolysis	2.2	1.1	92.1
5 % hemolysis	2.4	3.3	98.3
10 % hemolysis	2.0	0.0	83.2
25 % hemolysis	2.1	1.9	86.2

- Lipemic and hemolyzed serum samples were pooled from individual donors
- PY314 QC's were prepared with pooled hemolyzed or lipemic serum at 240, 24, and 2.4 ng/mL levels
- QC recovery was within 80%-120% limit with up to 10% lipemic or hemolyzed serum
- PY314 QC's at 24 ng/mL all passed, data not shown

Dilution Linearity

2501 _dilution linearity test starting conc: 80.6 µg/mL

Dilution	Theoretical PY314 Conc (ng/mL)	Mean RLUs	Conc. Mean (ng/mL)	Calc. Conc. CV	Detection Range	Final (ug/mL)	Recovery (%)
50	1612	340801	1114.5	45.6	ALQ	55.7	69.1
150	537.3	274619	472.5	10.7	ALQ	70.9	87.9
450	**179.1**	**179365**	**189.7**	**7.9**	**In Detection Range**	**85.4**	105.9
1350	**59.7**	**69195**	**50.3**	**1.9**	**In Detection Range**	**67.8**	84.2
4050	**19.9**	**26415**	**17.0**	**5.2**	**In Detection Range**	**68.7**	85.3
12150	**6.6**	**9107**	**5.5**	**5.3**	**In Detection Range**	**67.0**	83.1
36450	**2.2**	**3159**	**1.8**	**4.5**	**In Detection Range**	**66.5**	82.5
109350	0.7	1155	0.6	0.2	BLQ	66.9	83.0

ALQ= Above Limit of Quantitation BLQ= Below Limit of Quantitation

- Dilutional linearity was evaluated using 4 serum samples from non-GLP Cyno study; samples were brought to MRD then serial dilutions were performed in matrix matched assay buffer
- For sample 2501, the results show that within the assay range (1-300 ng/mL), recovery was acceptable 80-100%
- Similar results were obtained for 3 other samples evaluated (2101, 2002, 2502), data not shown

Summary

- A PY314 anti-idiotype PK assay (PI-HX-2017B capture, PI-HX-2004 ST detection) has been developed and qualified for use with both normal and cancer serum samples after evaluating 22 mouse-anti-idiotype mAb antibodies.
- Blocking reagent HBR1 at a final concentration of 10 µg/mL was selected to reduce nonspecific signal from cancer sera while maintaining specific signal to PY314.
- The assay passed selectivity in diseased-cancer serum, normal serum, and tolerates up to 10% lipemic and hemolyzed samples, the assay also demonstrated acceptable dilution linearity.
- The assay has a run time of 3 hours which is an improvement over the currently validated ligand-based assay which has a run time of 7 hours.
- In addition, the anti-idiotype PK assay minimizes the dependence on externally supplied reagents for capture and detection of PY314 and is more compliant with current industry standards.