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A Clinical Biomarker Assay to Quantitate MIP-1α in Human Serum

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Abstract

Macrophage inflammatory protein 1-alpha (MIP-1alpha, MIP-1α, CCL3) is a cytokine that is involved in the acute inflammatory state in recruitment and activation of polymorphonuclear leukocytes through binding to the receptors CCR1, CCR4 and CCR5. MIP-1α is expressed by macrophages, dendritic cells and lymphocytes. Crucial in immune response against infection and inflammation, MIP-1α induces the synthesis and release of IL-6, IL-10 and TNFα. As a proinflammatory chemokines, MIP-1α is primarily associated with cell adhesion and migration of monocyte to inflammatory tissues. In addition, MIP-1 α inhibits the proliferation of hematopoietic stem cells *in vitro* and *in vivo*. Expression levels of MIP-1 α have provided important information in the progression of diseases such as multiple myeloma, lung cancer, multiple sclerosis, HIV infection, allergic asthma and sepsis.

Results: We validated an electro chemiluminescence assay for measurement of MIP-1α in human serum with a lower limit of quantitation of 2.97pg/ml. The assay demonstrated optimal performance characteristics, including precision, accuracy, and sensitivity and dilution linearity. Stability of the analyte and biological variability in serum were also assessed.

Conclusion: The validated MIP-1α assay enables us to analyze circulating MIP-1α present in serum as a Pharmacodynamic marker in the development of pharmaceutical products.

Introduction

Macrophage Inflammatory Protein-1 alpha (MIP-1α; also known as CCL3) is a member of the CC chemokines family which is primarily associated with cell adhesion and migration [1]. Chemokines are small (8-11kD) chemo tactic proteins that that are secreted by various cells under the influence of cytokines, growth factors and cancer cells [2,3]. Upon release, chemokines create a chemical gradient in a local microenvironment and attracts cells such as Neutrophils, macrophages and cancer cells. MIP-1α binds to Chemokines Receptors such as CCR1 (CC Chemokines Receptor 1), CCR5 (CC Chemokines Receptor 5) and CCR9 (CC Chemokines Receptor 9), in these cells which facilitates cell migration [4]. MIP-1α also functions as a hematopoietic stem cell/ progenitor cells regulator in the bone marrow [5]. Both *in vitro* and *in vivo*, MIP-1α could reversibly inhibit colony proliferation and proliferation of hematopoietic stem cells. MIP-1α maintains a quiescent status of stem cells by blocking cell cycle entry [6-8]. As such, the role of MIP-1α in disease progression is well-studied in multiple myeloma [9].

In these patients, MIP-1α is produced by myeloma cells, and its role in migration of cells, tumor growth and bone lesion destruction. The function of MIP-1α is known in osteoclast formation and differentiation. Furthermore, in multiple myeloma patients, it has been observed that MIP-1α levels are elevated and is correlated with the progression of the disease. In addition, MIP-1α levels correlates positively with bone resorption markers and is known to play a role in lytic bone lesions in multiple myeloma. We initiated this study, which includes intra- and inter-assay precision, dilution linearity, spike recovery, stability and inter- and intra-subject biological variability studies, as part of a validation package of the V-PLEX Human MIP-1α assay in serum on the MesoScale Discovery platform. This assay has been fully validated and will be used to analyze MIP-1α in serum collected in clinical samples, as a Pharmacodynamic marker in clinical studies. Since the resurgence of immunotherapy recently, there has been a high interest in MIP-1α [10-14]. This study will provide some insight

into the utility of MIP-1α immunoassays in serum.

Methods

Reagents

Recombinant human CCL3/MIP-1α Protein, (carrier-free; Catalog number 270-LD-010/CF; Lot number CG1216071) was purchased from R & D Systems (Minneapolis, MN). The recombinant protein was E. coli-derived and consists of Ala27-Ala92 of the MIP-1αprotein (which is 8-11kD in size). A stock of 100µg/ml concentration was made by adding 0.1ml of PBS to the vial. Concentration of recombinant protein stock was certified by Amino Acid Analysis Service Laboratory, Inc. (Damascus, OR) to be 99µg/ml. To do a 99-fold dilution, 50ul of the 99µg/ml stock was added to 4.9ml of PBS to create the 1µg/ml stock. From this stock solution, 6500pg/ml top standards stock solution 180µlaliquots were made in Diluent 43 (164.1µl of stock of concentration 99µg/ml was added to 25ml of Diluent 43) and stored at -80˚C.

V-PLEX human MIP-1α Kits (which include pre-coated Human MIP-1α plates, SULFO-TAG anti-human MIP-1α antibody, Diluent 3 and Diluent 43), were purchased from Meso Scale Discovery (MSD) (Rockville, MD; Catalog number K151NQD-4, lot number Z0046151). Both capture and detection antibodies used in the kit were mouse monoclonal antibodies. HBR1 (Heterophilic Blocking Reagent 1) was purchased from Scantibodies Laboratory, Inc. (Santee, CA; Part number: 3KC533). Serum samples of healthy subjects and cancer patients were purchased from Bioreclamation, Inc. (Westbury, NY). Six time-points were collected from each cancer patient, with 3-4 days between adjacent collection time points. Prior to each analysis, the MIP-1α top standard stock of 6500pg/ml Three sets of Quality Control (QC)samples (high, medium and low) were prepared using human serum of healthy subjects purchased from Bioreclamation, Inc. (Westbury,NY). Endogenous levels of MIP-1αin the QCs were 435pg/ml (BRH1228689), 69.2pg/ml (BRH1228511) and 19.8pg/ml (BRH1228535) respectively for high, medium and low QCs. The QC samples were aliquoted as 150ul per aliquot and stored at -80˚C.

MIP-1α ECL assay procedure and sample analysis

All samples (serum, standards, QC and blank) were analyzed in duplicate on a MSD V-PLEX 96-well plate Electro Chemi Luminescent (ECL) platform following the same protocol. Diluent 43 assay buffer was used as blank. All samples were tested with a 2-fold dilution. Fifty µl of diluted sample were added into each well of a V-PLEX plate. The plate was incubated for 2 hours at room temperature with vigorous shaking (1000 rpm). The plate was then washed three times on a Biotech ELx405 microplate washer (Winooski, VT) with 150µl per well of MSD Wash Buffer (1X PBS + 0.05% Tween-20; Rockville, MD). Antibody solution was prepared by adding 60µl of detection antibody from MesoScale Discovery to 3ml of Diluent 3. After washing, 25µl of detection antibody solution made in Diluent 3 was added to the plate. The plate was incubated for 1 hour at room temperature with vigorous shaking (1000 rpm). Upon completion of the capture antibody incubation, the plate was washed three times with 150µl per well of MSD Wash Buffer. Prior to reading the plate, 150µl of 1 × Read Buffer T (1:4 dilution of 4 × Read Buffer T in distilled water) was added to each well. The plate was read on the MSD Sector Imager 6000.

Software and Statistical Analysis

The Watson LIMS analysis software (Thermo Fisher Scientific; Waltham, MA) was used for data acquisition and data analysis. Signals from standards with known levels of MIP-1α were used to calculate the concentration of MIP-1α in unknown samples. The software utilized a 5-parameter logistic model (using the 5-PL auto estimate function in Watson) and included a 1/Y2 weighting function to determine the mean, Standard Deviation (SD), and % CV (Coefficient of Variation).

Results and Discussion

To support early stage clinical studies, we validated an immunoassay that is able to measure MIP-1α in human serum using the V-PLEX ECL kit from MSD (Rockville, MD). We performed a fit-for-purpose validation of the MIP-1α ECL assay to assess whether circulating MIP-1α can be a Pharmacodynamic marker in drug development. In this validation package, we tested the precision, sensitivity, dilution linearity and accuracy of the assay, as well as stability of the MIP-1α analyte in various conditions. Finally, we surveyed the inter- and intra-subject variability of endogenous concentration of MIP-1α in serum collected from normal healthy subjects (for inter-subject variability) and cancer patients (both inter- and intra-subject variability). To this end, we determined that we have a validated assay which passes our predetermined criteria for analysis of MIP-1α concentration in patients involved in our clinical trials.

Sensitivity

The sensitivity of the assay in human serum is expressed by the Lower Limit of Quantification (LLOQ), which is the lowest concentration of MIP-1α in human serum that the MIP-1α ECL assay can reliably quantitate. The purpose of this assay is an exploratory biomarker assay in early stage clinical studies, LLOQ was determined as the lowest standard concentration that can be measured with an inter-assay precision within 25% CV. Among the 8-point standards in the MIP-1 assay across 10 runs, the lowest standard (2.97 pg/ml) had an inter-assay precision of 21% CV (Table 1). Thus, the assay sensitivity was 2.97pg/ml.

Standard sample	Expected MIP-1α(pg/mL)	Observed MIP-1α			
		n (runs)	Mean (pg/mL)	Stdev (pg/mL)	% CV
S1	6500	10	6370	152	2.4
S2	2170	10	2320	108	4.7
S3	722	10	737	16.1	2.2
S4	241	10	228	10.3	4.5
S5	80.0	10	77.1	2.60	3.4
S6	26.7	10	27.8	1.45	5.2
S7	8.9	10	10.0	0.77	7.7
S8	3.0	10	2.6	0.55	21

Table 1: MIP-1αStandard Curve.

Upper Limit of Quantification (ULOQ)

We determined the assay Upper Limit of Quantification (ULOQ) using a method very similar to the method for LLOQ determination. ULOQ of the assay is the top standard curve point that is measured with an inter-assay CV within 20%. The measured concentration of the highest standard was 6500pg/ml. Across 10 runs; this standard demonstrated an inter-assay precision of 2.4% CV (Table 1). Thus, the ULOQ for MIP-1αmeasurement in human serum was 6500pg/ml.

Precision

An 8-point standard curve was prepared for each run. ECL signals of standards were measured in duplicate and a back-calculated concentration (observed concentration) for each individual standard data point was obtained using a 5-parameter logistic model fitted curve (Table 1). Replicate measurements with >20 % CV were excluded from standard curve and precision analysis. The acceptance criteria for a run-in assay validation are the following: at least 75% of standard points have ±20 % CV (or ±25 % CV for LLOQ) between duplicated analysis. Intra-assay precision was determined by the mean of six individual wells for three QC samples (high QC, mid QC and low QC), standard samples and blank wells, were all run in duplicate. The intra-assay precision ranged from 3.83 to 5.51% CV (Table 2).

	Intra-Assay[a]			Inter-Assay[b]		
	High QC	Medium QC	Low QC	High QC	Medium QC	Low QC
n	6	6	6	10	10	10
Mean (pg/mL)	429	64.9	16.4	453	69.4	19.6
Stdev (pg/mL)	20.5	2.5	0.9	30.1	8.2	2.7
% CV	4.8	3.8	5.5	6.7	12	14
a: n=6 samples, each sample was run in duplicate; b: n=10 samples, each sample was run in duplicate.						

Table 2: MIP-1αIntra- and inter-assay Precision.

Inter-assay precision was determined in ten independent runs consisting of an 8-point standards and blank sample (consisting of only the assay buffer) analyzed in duplicate, and two sets of each of the three QC samples (high QC, mid QC and low QC). The inter-assay precision for the QC samples was calculated using the first set of the QC samples in each run during the assay two values on the plate for each validation (n=10) ranged from 6.65% to 14% CV (Table 2). Therefore, inter-assay precision over 10 independent runs met pre-specified acceptance criteria <20% CV. Thus, the assay has acceptable precision in quantitation of MIP-1α in human serum.

Parallelism

Parallelism was assessed by dilution linearity of human serum samples without spiking recombinant MIP-1α. Dilution linearity of the assay was assessed by serially diluting six endogenous serum samples in seven serial dilutions, by 2-fold, up to 128-fold (Table 3). Five separate serum samples were used without spiking recombinant MIP-1α endogenous. The concentrations of each diluted sample were interpolated and then corrected for the dilution factor. The recovery of these diluted samples was determined. The Minimal Required Dilution (MRD) was established to be 2-fold dilution during validation. Dilution linearity is demonstrated if multiple dilutions show a difference within 20% from the MRD. The linear range of serum dilution is 1:2 to 1:4.

Subject name	Concentration (pg/ml)	Dil Factor	Corrected conc. (pg/ml)	% diff from 2-fold
BRH1213689(normal serum)	701	1	701	-16.9
	422	2	844	0
	211	4	844	0
	94.2	8	754	-10.7
	45.3	16	725	-14.1
	22	32	704	-16.6
	8.22	64	526	-37.7
	BLOQ	128	BLOQ	BLOQ
BRH1213688 (normal serum)	55.5	1	55.5	-27.2
	38.1	2	76.2	0
	16.8	4	67.2	-11.8
	6.66	8	53.3	-30.1
	BLOQ	16	BLOQ	BLOQ
	BLOQ	32	BLOQ	BLOQ
	BLOQ	64	BLOQ	BLOQ
	BLOQ	128	BLOQ	BLOQ
BRH1228509(normal serum)	89	1	89	0.907
	44.1	2	88.2	0
	18	4	72	-18.4
	5.83	8	46.6	-47.1
	BLOQ	16	BLOQ	BLOQ
	BLOQ	32	BLOQ	BLOQ
	BLOQ	64	BLOQ	BLOQ
	BLOQ	128	BLOQ	BLOQ
BRH1228511(normal serum)	141	1	141	-7.24
	76	2	152	0
	31.9	4	128	-16.1
	13.7	8	110	-27.9
	5.15	16	82.4	-45.8
	BLOQ	32	BLOQ	BLOQ
	BLOQ	64	BLOQ	BLOQ
	BLOQ	128	BLOQ	BLOQ
BRH1228543(normal serum)	983	1	983	-17.5
	596	2	1190	-0.168
	277	4	1110	-6.88
	121	8	968	-18.8
	60.1	16	962	-19.3
	26.2	32	838	-29.7
	8.65	64	554	-53.6
	BLOQ	128	BLOQ	BLOQ

BRH1228548(normal serum)	31.4	1	31	-45.9
	29	2	58	0
	14	4	56	-3.4
	BLOQ	8	BLOQ	BLOQ
	BLOQ	16	BLOQ	BLOQ
	BLOQ	32	BLOQ	BLOQ
	BLOQ	64	BLOQ	BLOQ
	BLOQ	128	BLOQ	BLOQ

Table 3: MIP-1α Parallelism.

BLOQ (Below Limit of Quantification)

Accuracy

We assessed the assay accuracy by measuring the recovery of spiked recombinant MIP-1α in human serum samples. Recoveries of spiked MIP-1α were assessed by spiking high (400pg/ml) and low (100pg/ml) levels of recombinant human MIP-1α protein into six human serum samples of normal healthy individuals, and into assay buffer. Each spike recovery experiment was assessed using three samples: spiked serum was prepared by adding the MIP-1α spike solution into serum; non-spiked serum was prepared by adding the assay buffer into serum and spike-in buffer was prepared by adding the spike solution into the assay buffer. These three samples had the same volume and were analyzed in the same run. Table 4 shows the data of spikes in the assay buffer (observed MIP-1α in spike), spiked (MIP-1α in spiked serum) and non-spiked (endogenous MIP-1α in serum) serum. Spike recovery was calculated using the formula:

% Recovery = ([Spiked Serum - Non-Spiked Serum] ÷ Spike in Buffer) × 100. The recoveries of the high and low spikes were at 88.5–117% in these six human serum samples (except for one sample with 136% recovery), demonstrating acceptable assay accuracy.

Subject name	MIP-1α in Spiked Plasma (pg/mL)	MIP-1α in Non-Spiked Plasma (pg/mL)	Observed MIP-1α in spike in buffer (pg/mL)	Recovery of spiked MIP-1α (pg/mL)	% Recovery of spiked MIP-1α
BRH1228509	552	30.9	444	521	117
	171	30.9	103	140	136
BRH1228510	489	9.4	444	480	108
	108	9.4	103	99	95.7
BRH1228511	574	60.3	444	514	116
	169	60.3	103	109	106
BRH1228520	473	20	444	453	102
	130	20	103	110	107
BRH1228535	411	18	444	393	88.5
	126	18	103	108	105
BRH1228541	524	29.6	444	494	111
	130	29.6	103	100	97

Table 4: MIP-1α Spike Recovery.

Stability

MIP-1α stability in human serum was assessed in three aspects: freeze-thaw cycles and short-term storage stability at room temperature and 4°C. We assessed the effects of freeze-thaw cycles on serum MIP-1α stability using human serum samples of three apparently healthy individuals. Samples were stored at -80°C for at least 24 hours, then thawed unassisted at room temperature. Samples were then re-frozen for at least an overnight period at -80αC before thawing again. Aliquots of the frozen serum went through one to three freeze-thaw cycles prior to the MIP-1α measurement. MIP-1αlevels in the original serum samples (thaw cycle 1) and serum samples that had gone through additional freeze-thaw cycles from all three individuals were analyzed in the same run. The concentration dif-

ference was compared to the original serum sample (thaw cycle 1). Table 5 shows that compared with the serum sample that has not gone through additional freeze-thaw cycles, MIP-1α levels in serum displayed less than 6.9% difference after one to three additional freeze-thaw cycles in three individuals. Thus, we conclude that MIP-1α in serum is stable up to three freeze-thaw cycles.

Subject name	Condition	MIP-1α (pg/mL)	% Difference from the fresh frozen plasma
BRH1228689	fresh frozen	407	0
	1 additional F/T	415	2
	2 additional F/T	418	2.7
	3 additional F/T	426	4.7
BRH1228611	fresh frozen	73	0
	1 additional F/T	73.9	1.2
	2 additional F/T	73.5	0.7
	3 additional F/T	74.2	1.6
BRH1228635	fresh frozen	18.9	0
	1 additional F/T	18.9	0
	2 additional F/T	20.2	6.9
	3 additional F/T	19.9	5.3

Table 5: MIP-1α Freeze Thaw Stability.

We also examined serum MIP-1α stability for short-term storage using three human serum samples. The human serum samples were stored at the following conditions (a) 4 hours at room temperature, (b) 4 hours at 4°C (c) 24 hours at room temperature, (d) 24 hours at 4°C. All samples were assayed on a single plate run and stability was evaluated by calculating the percentage change based on the mean concentration from frozen controls. As shown in Table 6, MIP-1α levels in human serum samples displayed less than 12% difference from the control and are stable after storage at all the above described conditions. MIP-1α levels are stable after nine months of storage at -80°C (Table 7).

Run	MIP-1**α** (pg/mL)		
	High QC	Medium QC	Low QC
Non-treated	407	73	18.9
4hr @ RT	432	75.3	19.8
% Difference	6.1	3.2	4.8
4hr @ 4C	456	71.5	20.4
% Difference	12	-2.1	7.9
24hr @ RT	407	70.7	18.7
% Difference	0	-3.2	-1.1
24hr @ 4C	417	69.2	18
% Difference	2.5	-5.2	-4.8
9 months long-term stability	443	70.9	19.4
% Difference	1.8	2.5	-2

Table 6: MIP-1α Short-Term Stability.

Run	MIP-1**α** (pg/mL)		
	High QC	Medium QC	Low QC
T= 0 months	407	73	18.9
T= 9 months	477	74.6	17.9
% Difference	17.2	2.2	-5.3
Run	MIP-1**α** (pg/mL)		
	BRH1254593	BRH1254594	BRH1254595
T= 0 months	9	7.5	6.3
T= 6 months	11.2	8.8	7.4
% Difference	24.2	16.8	18.5

Table 7: MIP-1α Long-term Stability.

Inter-Subject Biological Variability

MIP-1**α** levels in serum samples of 52 cancer patients and 12 normal healthy individuals from BioReclamationIVT were measured and the data are shown in Table 8 and Figure 1. Of the 52 cancer patients, the patient distribution was 20 non-small cell lung cancers, 20 multiple myelomas and 12 melanomas for the serum samples from non-small cell lung cancer patients, the inter-subject variability was 60.2% CV, ranging from 4.51 to 26.9pg/ml, and averaging at 11.3pg/ml. MIP-1**α** levels in serum samples of the multiple myeloma patients were measured. Except one individual whose MIP-1**α** level was unquantifiable (below LLOQ), the inter-subject variability was 307% CV among these twenty individuals, ranging from 3.03 to 404pg/ml, and averaging at 28.8pg/ml. In addition, except three individual whose MIP-1**α** level was unquantifiable (below LLOQ), in the serum of 12 melanoma patients measured, the variability was 82.2% CV, ranging from3.36-12.2pg/ml and averaging at 5.55pg/ml. Finally, for the 12 normal healthy subjects, except for three individual whose MIP-1**α** level was unquantifiable (below LLOQ), inter-subject variability was at 305% CV. The MIP-1**α** concentration of the serum samples ranged from 3.41-598pg/ml and averaged at 56.1pg/ml.

Indication	Subject	MIP-1α (pg/mL)	Mean (pg/mL)	Stdev (pg/mL)	CV%
Non-small cell Lung Cancer	1	8.41	11.3	6.81	60.2
	2	7.79			
	3	11.7			
	4	6.47			
	5	10.7			
	6	9.43			
	7	4.51			
	8	23.5			
	9	14.8			
	10	23.5			
	11	10.4			
	12	20.1			
	13	26.9			
	14	8.2			
	15	5.57			
	16	8.96			
	17	8.14			
	18	6.81			
	19	4.69			
	20	5.53			
Multiple myeloma	1	14.7	28.8	88.6	308
	2	5.25			
	3	19.5			
	4	3.3			
	5	8.78			
	6	6.22			
	7	16.2			
	8	6.51			
	9	4.42			
	10	5.49			
	11	7.55			
	12	32.2			
	13	3.19			
	14	6.03			
	15	10.7			
	16	9.61			
	17	404			
	18	9.11			
	19	3.03			
	20	0			

Melanoma	1	9.58	5.5	4.5	82.2
	2	4.29			
	3	3.77			
	4	8.6			
	5	8.66			
	6	3.36			
	7	11.7			
	8	12.2			
	9	3.57			
	10	0			
	11	0			
	12	0			
Normal	1	598	56.1	171	305
	2	5.27			
	3	7.1			
	4	8.12			
	5	7.98			
	6	6.25			
	7	29.7			
	8	7.19			
	9	3.41			
	10	0			
	11	0			
	12	0			

Table 8: MIP-1α Inter-subject Biological Variability.

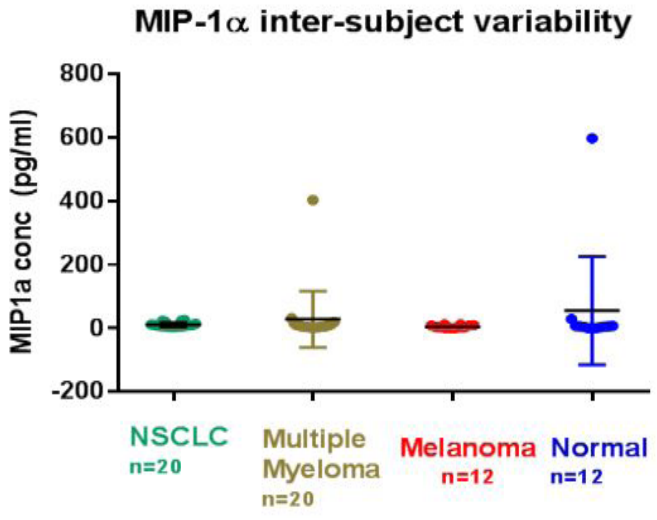

Figure 1: Inter-subject variability of MIP-1α concentration

Intra-Subject Biological Variability

Intra-subject biological variability for serum samples collected from nine different subjects was measured of the nine subjects, there were three breast cancers, two head and neck cancer and four lymphoma patients. For each patient, serum was collected at six different time points. All time points were separated by 3 to 4 days between each time point. These results were shown in Table 9 and Figure 2 of the three breast cancer patients, one had five-time points which were unquantifiable (below LLOQ), and the other two subjects had intra-subject variabilities ranging from 19.8-57.8% CV. The intra-subject average level of MIP-1α in the two patients was 6.98 and 7.02pg/ml. For the head and neck cancer patients, one had 24.7% CV intra-subject variability and the other had 61.3% CV. For the two patients, the average level of MIP-1α across the different days was 5.14 and 7.06pg/ml. Finally, for the lymphoma patients, intra-subject variability ranged from 22.9-34.9% CV. The averages ranged between 5.13-19.7pg/ml.

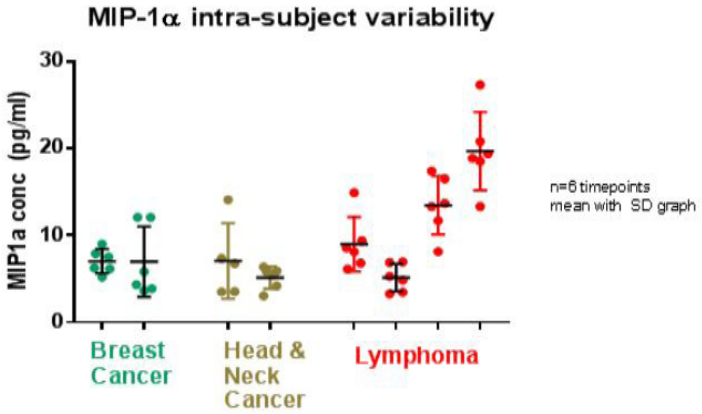

Figure 2: Intra-subject variability of MIP-1α concentration.

Indication	Subject	Time point	MIP-1α(pg/mL)	Mean(pg/mL)	Stdev (pg/mL)	CV%
Breast Cancer	1	1	9.02	7	1.4	19.8
		2	7.54			
		3	6.27			
		4	7.92			
		5	5.21			
		6	6.17			
	2	1	3.64	7	4	57.8
		2	4.35			
		3	3.88			
		4	12.1			
		5	12.1			
		6	5.83			
	3	1	BLOQ	3.5	N/A	N/A
		2	BLOQ			
		3	BLOQ			
		4	3.45			
		5	BLOQ			
		6	BLOQ			

Head and Neck Cancer	1	1	6.77	7.1	4.3	61.3
		2	3.51			
		3	14.1			
		4	BLOQ			
		5	7.36			
		6	3.54			
	2	1	4.19	5.1	1.3	24.7
		2	5.85			
		3	3.04			
		4	6.38			
		5	5.9			
		6	5.45			
Lymphoma	1	1	14.9	9	3.1	34.9
		2	6.82			
		3	9.38			
		4	8.12			
		5	8.56			
		6	6.11			
	2	1	6.95	5.1	1.6	31.1
		2	3.28			
		3	6.89			
		4	3.47			
		5	4.86			
		6	5.3			
	3	1	17.4	13.5	3.4	24.9
		2	8.14			
		3	13.3			
		4	13.7			
		5	16.5			
		6	11.7			
	4	1	19.4	19.7	4.5	22.9
		2	13.3			
		3	18.5			
		4	18.9			
		5	20.8			
		6	27.3			

Table 9: MIP-1α Intra-subject Biological Variability.

Conclusion

We presented the development, optimization and fit-for- purpose validation of an ECL assay for the quantitation of MIP-1α in human serum. This validated assay will allow testing of clinical samples which require MIP-1α as pharmacokinetic and Pharmacodynamic marker, or safety biomarker.

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