Quantification of sBCMA in Human Plasma using a High-Throughput Mass Spectrometry Workflow for Exploratory, CAP/CLIA or Regulated Studies

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Background

- **B cell maturation antigen (BCMA) in Multiple Myeloma patients**
  - BCMA contributes to multiple myeloma pathophysiology and is a precision medicine target
  - Several drugs and immunotherapies*/** target BCMA
  - The soluble form (sBCMA) is a potential biomarker for disease prognosis and monitoring
- **ELISA assays for sBCMA measurement in patient serum/plasma**
  - The BCMA ligands (BAFF, APRIL) and/or therapeutic drug in patient serum/plasma are known to interfere with sBCMA measurement, thereby impacting assay reliability
- **Our goal was to develop a MS-based assay to circumvent the limitations of ELISA and to provide reliable measurement of the biomarker sBCMA**

** CAR-T cells, antibody-drug-conjugates*
*** Under development: Next gen CAR-T cells, bispecific T-cell engagers, bispecific molecules, and bi-/tri-specific antibodies…*

Workflow - Hybrid LBA-LC/MS

Samples (62.5 µL plasma / well) → **Immunoaffinity Enrichment** (Anti-sBCMA; Kingfisher, Thermo) → **Digestion to Peptides** (Trypsin) → **MRM Analysis** (QTRAP 6500, SCIEX) → **Peak Integration** (MultiQuant. SCIEX)

- **Sample reception to data delivery = 3 days**
- **Rapid TAT, suitable for CAP/CLIA clinical laboratory testing**

LC-MS Method

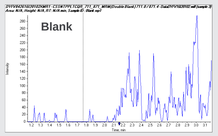
- **Mobile phase A: 97/3 Water/DMSO + 0.2% Formic acid**
- **Mobile phase B: 97/3 Acetonitrile/DMSO + 0.2% Formic acid**
- **Column: ACQUITY UPLC BEH C18 Column, 130Å, 1.7 µm, 1.0 mm X 50 mm**
- **Total runtime: 10 min**
- **Quantifier transition: CSSNTPPLTCQR 711.0 → 871.4**
- **Qualifier transition: CSSNTPPLTCQR 711.0→ 972.5**

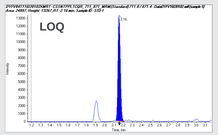
Precision and Accuracy

- **Experimental design for 3 independent P&A runs:**

Workflow	Calibration Curve	QC Samples
1. Calibration and QC standards prepared 1X using spiked recombinant human (rh) sBMCA protein	— 8 STDs (1-1000ng/mL) — In surrogate matrix (blank rat plasma)	— 4 levels (LOQ/Endo/Mid/High) — LOQ in surrogate matrix (1ng/mL in blank rat plasma) — Endo/Mid/High in sample matrix (pooled human plasma)
2. Immunoaffinity enrichment 3X over different days by 2 operators	— Single replicate	— Duplicates*
3. MRM analysis	— Single replicate	— Single replicate

- **Data show good P&A over the quantitative range**



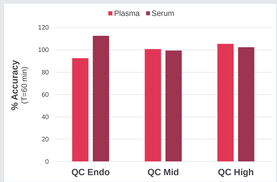


	Calibration Curve								Quality Controls			
	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8	QC-LOQ	QC-Endo	QC-Mid	QC-High
Nominal	1.000	2.00	10.00	80.0	250.0	500.0	800.0	1000.0	1.00	3.02**	48.0	753.0
Exp 1	1.02	1.96	9.34	65.0	248.8	575.1	843.7	1056.6	0.79	3.02	38.4	786.0
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	0.71	3.13	38.6	831.5
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	2.89	N/AP	N/AP
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	2.90	N/AP	N/AP
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	3.09	N/AP	N/AP
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	3.08	N/AP	N/AP
Exp 2	1.04	1.79	10.92	61.4	266.9	555.2	847.5	856.0	0.95	2.64	39.7	817.3
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	0.99	2.61	35.5	736.2
Exp 3	1.00	2.01	9.84	68.4	273.7	561.8	793.9	910.4	0.96	3.28	41.0	721.6
	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	N/AP	1.11	2.78	41.1	766.0
Mean	1.02	1.92	10.03	64.90	263.14	564.01	828.36	941.02	0.92	2.94	39.07	776.40
% CV	2.2%	5.9%	8.1%	5.3%	4.9%	1.8%	3.6%	11.0%	15.8%	7.4%	5.3%	5.6%

** For Exp1 only, 6 replicates of pooled human plasma were extracted and analyzed to establish the nominal 'QC-Endo' value.*
*** Nominal value as determined in Exp1.*

Blood Kinetic

- **Whole blood collected onsite and spiked with rh sBMCA at 2 levels**
- **Plasma and serum were generated at 2 timepoints**
  - T= 0 min
  - T= 60 min post-collection at 4 ºC
- **sBCMA was stable in whole blood at 60 min post-collection**



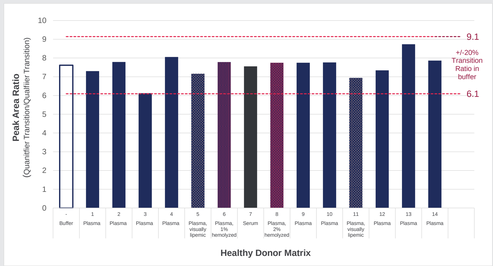
Matrix Effect

- **Testing of 8 different matrix samples at 2 spiked levels (QC-Mid, QC-High)**
  - Plasma, serum from various healthy donors, including hemolyzed and lipemic samples
- **All matrices and spiking levels met acceptance criteria (Accuracy ≤ 25%)**
- **No matrix effect was observed**

	QC-MID (~49ng/mL)					QC-High (~754 ng/mL)				
	Peak Area	IS Area	Area Ratio	Calculated Concentration	Accuracy	Peak Area	IS Area	Area Ratio	Calculated Concentration	Accuracy
Plasma 1	1647585	1226440	1.3434	51.56	106.1	25126935	1131537	22.2060	846.41	112.3
	1296731	1057072	1.2267	47.12	96.9	24491826	1137347	21.5342	820.81	108.9
Plasma 2	1485550	1122980	1.3229	50.78	100.9	22919618	1107480	20.6953	788.85	104.4
	1340668	1073304	1.2491	47.97	95.3	21284691	989205	21.5170	820.16	108.6
Plasma 3	1082422	1008265	1.0735	41.28	83.7	21538329	1063128	20.2594	772.25	102.4
	1334518	1056292	1.2634	48.51	98.4	18722978	864264	21.6635	825.74	109.5
Plasma 4	1359330	1080613	1.2579	48.30	97.3	21580100	984290	21.9245	835.69	110.7
	1302755	1088489	1.1968	45.98	92.6	21301157	1037828	20.5247	782.35	103.7
Plasma, visually lipemic	1407870	1137534	1.2377	47.53	98.0	23997971	1151113	20.8476	794.66	105.5
	1587021	1270674	1.2490	47.96	98.9	19098620	849105	22.4927	857.33	113.8
Plasma, 1% hemolyzed	1444555	1080087	1.3374	51.33	103.5	28759639	1197543	24.0155	915.35	121.3
	1414841	1048571	1.3493	51.79	104.4	22960049	1059360	21.6735	826.12	109.5
Serum	1443378	1137230	1.2692	48.73	97.9	18201159	872382	20.8638	795.27	105.4
	1509600	1197442	1.2607	48.41	97.3	21794493	991467	21.9821	837.88	111.0
Plasma, 2% hemolyzed	1324703	1090514	1.2148	46.66	91.7	23779038	1124704	21.1425	805.89	106.6
	1309861	1051293	1.2460	47.85	94.1	22496059	1047133	21.4835	818.88	108.3
Average	1393212	1107925	1.2561	48.24	97.3	22378291	1037993	21.5516	821.48	108.9
% CV	16.9%	10.5%	5.3%	N/AP	N/AP	11.7%	10.3%	4.2%	N/AP	N/AP

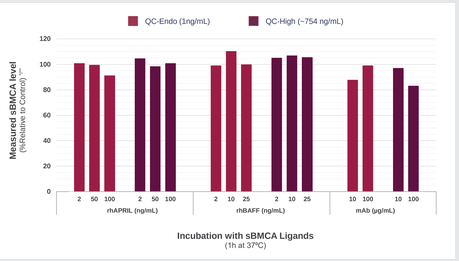
Specificity

- **Testing of plasma/serum samples from 14 individual healthy donors**
- **Due to the presence of endogenous analyte in the sample matrix, assay specificity was assessed by comparing the ratios of the quantifier and qualifier peptide transitions in sample matrix vs buffer**
- **Assay specificity was confirmed in all 14 donors tested**



Total sBCMA Testing

- **Total sBMCA measurement by ELISA is susceptible to interference due to binding of natural ligands (BAFF, APRIL) and/or therapeutic drugs**
- **Measurement of sBCMA in QC samples +/-bioactive BCMA ligands (BAFF, APRIL, or anti-sBCMA mAb) using hybrid LBA-LC/MS showed no interference**



** Control = no BCMA ligand, no incubation*
*** sBCMA Peak Area Ratio (Treated)/ Peak Area (Control) X 100%*

Conclusions

- **We developed a hybrid LBA-LC/MS assay for total sBCMA measurement in human plasma/serum with a 3-day TAT**
- **Performance data for the hybrid LBA-LC/MS assay shows:**
  - Good precision and accuracy
  - Good assay specificity
  - Absence of matrix effects.
  - No interference* from binding ligands (BAFF, APRIL, anti-sBCMA mAb)
- **ELISA-associated limitations due to ligand interference are avoided**
- **Hybrid LBA-LC/MS provides reliable sBCMA measurement in plasma/serum and is suitable for Exploratory, CAP/CLIA or Regulated Studies**

** Each distinct BCMA-binding drug is subject to ad-hoc assay verification prior to deployment for clinical sample analysis*