Development of a targeted mass spectrometry serum assay to quantify M-protein in the presence of therapeutic monoclonal antibodies

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Supporting Information

Figure S-1. Interference of therapeutic monoclonal antibodies with the M-protein in serum protein electrophoresis and immunofixation electrophoresis

Table S-1. M-protein proteotypic tryptic peptides for five multiple myeloma patients from The Multiple Myeloma Research Foundation CoMMpass Study (dbGAP accession phs000748.v6.p4)

Table S-2. List of parent masses, charge states, fragment masses and fragment types (b and y ions) that have given signal between the integration boundaries

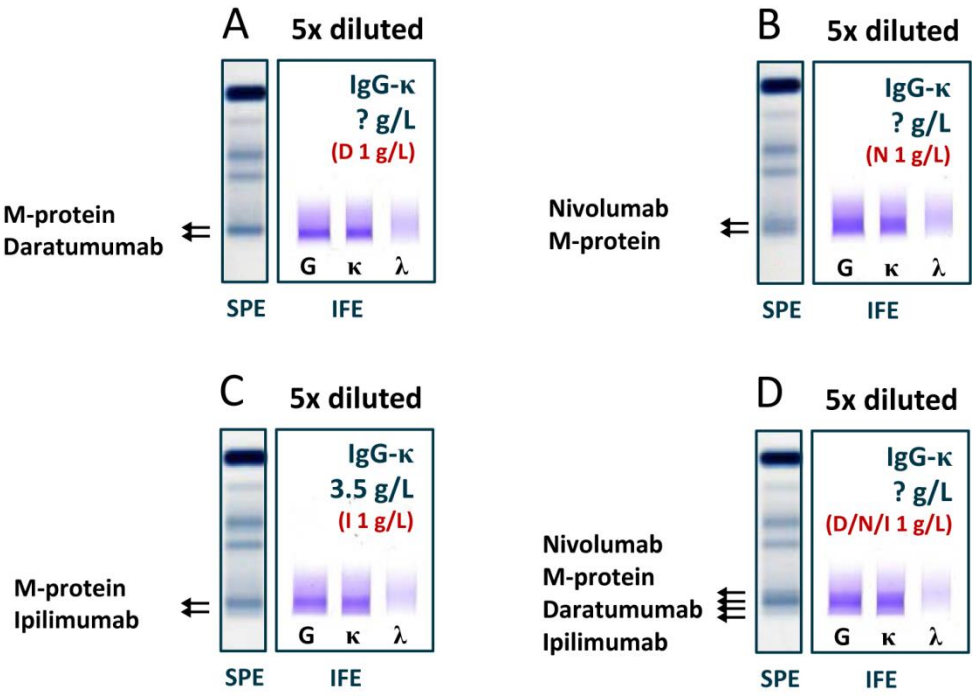

Figure S-1.

Interference of therapeutic monoclonal antibodies with the M-protein in serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE). Therapeutic monoclonal antibodies (mAbs) are added to the 5 times diluted multiple myeloma (MM) patient serum in a concentration of 1 g/L (D, daratumumab; N, nivolumab; I, ipilimumab in A, B, C, respectively). They are detected with SPE and IFE and may be misinterpreted as an M-protein and interfere with quantification. As the bands are hardly (ipilimumab) or not at all resolved (daratumumab, nivolumab) the arrows indicate the position of M-protein and therapeutic mAbs on the SPE/IFE. The SPE and IFE patterns become complicated and M-protein detection compromised if a combination of therapeutic monoclonal antibodies (D) is administered to a multiple myeloma patient. Question marks indicate cases in which it is not possible to determine M-protein concentration due to the co-migration with therapeutic mAbs. SPE and IFE scans without added therapeutic monoclonal antibodies are shown in Figure 1. in the main text.

		CoMMpass Study Patients				
		1	**2**	**3**	**4**	**5**
M-protein peptide sequence	**LC**	DIQLTQSPS**S**LSASVGDR ASQ**SVGTN**L**N**WYQQ**T**PG**R** **V**LI**FG**ASTLQ**A**GVPSR	NNQRPSGV**A**DR SEDEADYYCAAWDDSLSG**R**	EIVLTQSP**V**TLSLSPGER AT**V**SCR AS**E**S**IR** **T**YLAWYQQKPGQAPR LLIYD**TF**NR	LL**L**YDAS**K**	IQ**L**TQSPSSLSASVGDR ASQGISNYLAW**Y**QQKPGK **L**LIYAASSLQSGVPSR
	HC			LSC**T**ASGFTFDD**F**AMHWVR GLEWVSLI**V**G**N**G**DT**T**R** YA**G**SVK **S**SLYLQMNSL**K** TEDTALYYC**V**K D**LGQCGIITCYD**NWFD**R** WGQGT**R**	QVQLVESGGGLV**R** LSC**S**ASGFT**L**S**H**YYMSWIR GLEWVSYIS**PT**G**E**TIYY**S**D**F**V**E**GR NSL**F**LQMNSLR **V**EDTA**L**YYCAR **AAYNWNFGSR** GT**F**VTVSSASTK	QV**P**LVQSGAEVK PG**D**SVK VSGY**S**LTELSMHWVR GLEWMGG**VH**PE**N**GET**V**YAQK VT**L**TEDTSTDTAYMELS**G**LR **F**EDTAVYYCATPR
Germline peptide sequence	**LC**	DIQLTQSPSFLSASVGDR ASQGISSYLAWYQQK* LLIYAASTLQSGVPSR	NNQRPSGVPDR SEDEADYYCAAWDDSLSG*	EIVLTQSPATLSLSPGER ATLSCR ASQSVS* SYLAWYQQKPGQAPR LLIYDASNR	LLIYDASN*	IQMTQSPSSLSASVGDR ASQGISNYLAWFQQKPGK SLIYAASSLQSGVPSR
	HC			LSCAASGFTFDDYAMHWVR GLEWVSLISGDGGSTY* *YADSVK NSLYLQMNSLR TEDTALYYCAK DNWFDS* *WGQGTLVTVSSASTK	QVQLVESGGGLVK LSCAASGFTFSDYYMSWIR GLEWVSYISSSGSTIYYADSVK* NSLYLQMNSLR AEDTAVYYCAR DAFDVWGQ* GTMVTVSSASTK	QVQLVQSGAEVK PGASVK VSGYTLTELSMHWVR GLEWMGGFDPEDGETIYAQK VTMTEDTSTDTAYMELSSLR SEDTAVYYCATPR

Table S-1.

M-protein potential proteotypic tryptic peptides for five multiple myeloma patients from The MMRF CoMMpass Study (dbGAP accession phs000748.v6.p4). Patients 1 and 2 have light chain multiple myeloma, therefore there are no peptides for the heavy chains. Mutations from the germline are in bold and underlined. LC, light chain; HC, heavy chain.

*M-protein and germline tryptic peptides are different in lengths if mutation occurs at a lysine (K) or arginine (R) residue

Protein	Peptide	m/z	z	HCD Collision Energy (%)	Fragment mass [fragment type]
Daratumumab	GLEWVSAISGSGGGTYYADSVK	735.4	3	30	171.1 [b2]; 246.2 [y2]; 300.2 [b3]; 333.2 [y3] 448.2 [y4]; 486.2 [b4]; 519.3 [y5]; 585.3 [b5] 672.3 [b6]; 682.3 [y6]; 743.4 [b7]; 845.4 [y7] 856.5 [b8]; 943.5 [b9]; 946.5 [y8]; 1000.5 [b10] 1003.5 [y9]; 1060.5 [y10]; 1087.5 [b11]; 1204.5 [y12] 1261.6 [y13]; 1348.6 [y14]; 1359.7 [b15]; 1461.7 [y15]
Daratumumab	GLEWVSAISGSGGGTYYADSVK*	738.0	3	30	171.1 [b2]; 254.2 [y2]; 300.2 [b3]; 341.2 [y3] 456.3 [y4]; 486.2 [b4]; 527.3 [y5]; 585.3 [b5] 672.3 [b6]; 690.4 [y6]; 743.4 [b7]; 853.4 [y7] 856.5 [b8]; 943.5 [b9]; 954.5 [y8]; 1000.5 [b10] 1011.5 [y9]; 1068.5 [y10]; 1087.5 [b11]; 1212.6 [y12] 1269.6 [y13]; 1356.6 [y14]; 1359.7 [b15]; 1469.7 [y15]
Ipilimumab	TGWLGPFDYWGQGTLVTVSSASTK	853.4	3	34	159.1 [b2]; 248.2 [y2]; 335.2 [y3]; 345.2 [b3] 406.2 [y4]; 458.2 [b4]; 493.3 [y5]; 515.3 [b5] 580.3 [y6]; 612.3 [b6]; 679.4 [y7]; 759.4 [b7] 780.4 [y8]; 879.5 [y9]; 992.6 [y10]; 1037.5 [b9] 1093.6 [y11]; 1150.6 [y12]; 1223.6 [b10]; 1278.7 [y13] 1280.6 [b11]; 1335.7 [y14]; 1408.6 [b12]; 1465.7 [b13]
Ipilimumab	TGWLGPFDYWGQGTLVTVSSASTK*	856.1	3	34	159.1 [b2]; 256.2 [y2]; 343.2 [y3]; 345.2 [b3] 414.2 [y4]; 458.2 [b4]; 501.3 [y5]; 515.3 [b5] 588.3 [y6]; 612.3 [b6]; 687.4 [y7]; 759.4 [b7] 788.4 [y8]; 887.5 [y9]; 1000.6 [y10]; 1037.5 [b9] 1101.6 [y11]; 1158.6 [y12]; 1223.6 [b10]; 1280.6 [b11] 1286.7 [y13]; 1343.7 [y14]; 1408.6 [b12]; 1465.7 [b13]
M-protein	GLEWVSYISSGGGSTYYADSVK	1163.5	2	21	246.2 [y2]; 300.2 [b3]; 333.2 [y3]; 448.2 [y4] 486.2 [b4]; 519.3 [y5]; 585.3 [b5]; 672.3 [b6] 682.3 [y6]; 835.4 [b7]; 845.4 [y7]; 946.5 [y8] 948.5 [b8]; 1033.5 [y9]; 1035.5 [b9]; 1090.5 [y10] 1122.5 [b10]; 1147.5 [y11]; 1179.6 [b11]; 1204.5 [y12]

					1236.6 [b12]; 1291.6 [y13]; 1293.6 [b13]; 1378.6 [y14] 1380.6 [b14]; 1481.7 [b15]; 1491.7 [y15]
M-protein	GLEWVSYISSGGGSTYYADSVK*	1167.6	2	21	254.2 [y2]; 300.2 [b3]; 341.2 [y3]; 456.3 [y4] 486.2 [b4]; 527.3 [y5]; 585.3 [b5]; 672.3 [b6] 690.4 [y6]; 835.4 [b7]; 853.4 [y7]; 948.5 [b8] 954.5 [y8]; 1035.5 [b9]; 1041.5 [y9]; 1098.5 [y10] 1122.5 [b10]; 1155.5 [y11]; 1179.6 [b11]; 1212.6 [y12] 1236.6 [b12]; 1293.6 [b13]; 1299.6 [y13]; 1380.6 [b14] 1386.6 [y14]; 1481.7 [b15]; 1499.7 [y15]
M-protein	NSLSLQMNNLR	645.3	2	23	175.1 [y1]; 202.1 [b2]; 288.2 [y2]; 315.2 [b3] 402.2 [b4]; 402.2 [y3]; 515.3 [b5]; 516.3 [y4] 643.3 [b6]; 647.3 [y5]; 774.4 [b7]; 775.4 [y6] 888.4 [b8]; 888.5 [y7]; 975.5 [y8]; 1002.5 [b9] 1088.6 [y9]; 1115.6 [b10]; 1175.6 [y10]
M-protein	NSLSLQMNNLR*	650.3	2	23	185.1 [y1]; 202.1 [b2]; 298.2 [y2]; 315.2 [b3] 402.2 [b4]; 412.3 [y3]; 515.3 [b5]; 526.3 [y4] 643.3 [b6]; 657.3 [y5]; 774.4 [b7]; 785.4 [y6] 888.4 [b8]; 898.5 [y7]; 985.5 [y8]; 1002.5 [b9] 1098.6 [y9]; 1115.6 [b10]; 1185.6 [y10]
Nivolumab	ASGITFSNSGMHWVR	825.4	2	28	159.1 [b2]; 175.1 [y1]; 216.1 [b3]; 274.2 [y2] 329.2 [b4]; 430.2 [b5]; 460.3 [y3]; 577.3 [b6] 597.3 [y4]; 664.3 [b7]; 728.4 [y5]; 778.4 [b8] 785.4 [y6]; 865.4 [b9]; 872.4 [y7]; 922.4 [b10] 986.5 [y8]; 1053.5 [b11]; 1073.5 [y9]; 1190.5 [b12] 1220.6 [y10]; 1321.6 [y11]; 1376.6 [b13]; 1434.7 [y12] 1475.7 [b14]
Nivolumab	ASGITFSNSGMHWVR*	830.4	2	28	159.1 [b2]; 185.1 [y1]; 216.1 [b3]; 284.2 [y2] 329.2 [b4]; 430.2 [b5]; 470.3 [y3]; 577.3 [b6] 607.3 [y4]; 664.3 [b7]; 738.4 [y5]; 778.4 [b8] 795.4 [y6]; 865.4 [b9]; 882.4 [y7]; 922.4 [b10]

					996.5 [y8]; 1053.5 [b11]; 1083.5 [y9]; 1190.5 [b12] 1230.6 [y10]; 1331.6 [y11]; 1376.6 [b13]; 1444.7 [y12] 1475.7 [b14]

Table S-2.

List of parent masses, charge states, fragment masses and fragment types (b and y ions) that have given signal between the integration boundaries.

* stable isotope labeled amino acid