Cytogenetic profiles in multiple myeloma and monoclonal gammopathy of undetermined significance: a study in highly purified aberrant plasma cells

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Online Supplementary Design and Methods

Analysis of IGH gene rearrangements and CDR3 sequencing

Highly purified bone marrow aberrant plasma cells (aPC) were lysed using the REDExtract-N-Amp Blood PCR Kit (Sigma, St. Louis, MO, USA) following the recommendations of the manufacturer. Briefly, purified aPC were incubated with 20 μL of extraction solution (for 5 min at room temperature); then 120 μL of neutralization solution were added. The malignant rearrangements were identified according to the BIOMED-2 protocol[1] with slight modifications. For each polymerase chain reaction (PCR), 4.8 μL of each individual aPC lysate were used in a final volume of 20 μL containing 10 μL of PCR ReadyMix and 7.8 pmol of each primer. Two different multiplex PCR were tested for the *IGH* gene regions (*VDJ-FR2* and *DJ*). Reverse primers were labeled with 6-carboxyfluorescein (6-FAM). PCR were performed in a Verity™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) using the following conditions: incubation at 95°C for 3 min (pre-activation) followed by 40 cycles of sequential incubation at 95°C for 45 s (denaturation), 60°C for 45 s (annealing), and 72°C for 90 s (extension), with a final extension step for 10 min at 72°C. Clonal *IGH* gene rearrangements were identified by fragment analysis in an ABI PRISM 3130 Avant sequencer, using GENEMAPPER 3.1 software (Applied Biosystems). A clonal population was defined by the presence of either a single peak or a predominant peak over a polyclonal background. A signal was considered to be positive when the fluorescence was at least 250 absorbance units or it was two times higher than the polyclonal background, whenever the latter was present. In all cases, at least two tubes were performed for each PCR to avoid 'pseudoclonality'.

Clonal PCR products were then purified with ExoSap (USB Corp., Cleveland, OH, USA) and directly sequenced in both directions in an ABI 3130 DNA sequence analyzer using the Big-Dye 3.1 Terminator cycle sequencing chemistry (Applied Biosystems). Germline *IGHV*, *IGHD* and *IGHJ* segments from complete *IGH VDJ* gene rearrangements were identified through direct comparison with the IMGT database[2,3] (*http://imgt.cines.fr*) using DNAPLOT (MRC Center for Protein Engineering, Cambridge, UK). *IGHD* and *IGHJ* germline segments from incomplete *IGH DJ* gene rearrangements were identified using BLAST search in the *IGH D-J* germline locus sequence (*http://blast.ncbi.nlm.nih.gov/Blast.cgi*).

References

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Online Supplementary Table S1. Size of different cytogenetically-defined clones of aPC as blindly determined by two independent expert observers (13/13 MGUS and 61/62 MM cases with an *IGH* translocation are shown).

Case ID	Type of PCD	% of aPC by observer 1/% of aPC by observer 2					
		Non-altered† clone	Altered clone I	Altered clone II	Altered clone III	Other patterns‡	Clonal profile§
13691	MGUS	26/20	74/80	-	-	-	II (1)
13703	MGUS	15/20	63/70	-	-	22/10	II (1)
14223	MGUS	-	62/75	17/3	-	21/22	I (1)
14263	MGUS	17/22	74/73	-	-	9/5	II (1)
14956	MGUS	10/24	72/48	-	-	18/28	II (1)
15174	MGUS	-	65/69	16/20	-	19/11	II (1)
22722	MGUS	-	26/45	21/30	24/16	29/9	II (1)
15022	MGUS	-	49/49	16/10	-	35/41	II (2)
14176	MGUS	32/35	21/31	21/27	-	26/7	II (1)
13875	MGUS	30/38	70/62	-	-	-	II (1)
13898	MGUS	73/70	27/29	-	-	0/1	II (1)
14713	MGUS	12/18	85/82	-	-	3/0	II (1)
23811	MGUS	20/25	80/75	-	-	-	I (4)
13733A‖	MM	-	84/70	16/20		0/10	I (3)
13733B		-	92/89	-	-	8/11	I (3)
13849	MM	-	56/66	36/28	-	8/6	II (1)
14169	MM	-	88/96	-	-	12/4	II (1)
14206	MM	-	72/60	12/23	-	16/17	I (1)
14738A	MM	-	40/40	24/30	8/20	28/10	II (1)
14738B		-	10/52	43/17	18/13	29/18	II (1)
15252	MM	-	52/57	16/24	-	32/19	II (1)
15279	MM	-	37/35	20/19	15/20	28/26	I (1)
15496	MM	-	94/93	-	-	6/7	II (1)
15858	MM	26/18	53/70	-	-	21/12	II (1)
15916	MM	-	61/52	19/12	-	20/36	I (1)
16529	MM	-	92/88	-	-	8/12	II (1)
16568	MM	29/40	48/54	-	-	23/6	II (1)
16587	MM	-	68/62	21/23	-	11/15	I (1)
17309	MM	14/19	61/66	-	-	25/15	I (1)
17595	MM	17/20	46/54	15/23	-	22/3	I (1)
18215	MM	-	58/41	0/31	-	42/28	I (1)
18324	MM	-	84/90	-	-	16/10	II (1)
19627	MM	-	66/55	11/35	-	23/10	I (3)
20022	MM	-	70/83	20/10	-	10/7	II (1)
20427	MM	15/16	42/49	42/30	-	1/5	II (1)
23411	MM	-	90/78	-	-	10/22	I (1)
23588	MM	-	79/91	-	-	21/9	II (1)
23898	MM	-	98/94	-	-	2/6	II (1)
24508	MM	-	85/70	11/20	-	4/10	II (1)
24715	MM	-	90/75	-	-	10/25	I (5)
13818	MM	-	37/32	26/33	-	37/35	I (3)
14050	MM	-	93/85	-	-	7/15	I (1)
14548	MM	9/18	74/76	-	-	17/6	I (1)
14625	MM	15/20	46/63	18/7	-	21/10	II (1)
14733	MM	9/17	51/50	11/21	-	29/12	I (1)
14739	MM	-	23/52	30/15	19/11	28/22	I (1)
15268	MM	-	47/48	31/32	-	22/20	I (3)
15604	MM	47/50	47/45	-	-	6/5	II (1)
17026	MM	-	54/61	-	-	46/39	I (1)
20934A	MM	-	100/100	-	-	-	I (2)
20934B		-	100/100	-	-	-	I (2)

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20934C		24/25	64/60	-	-	12/15	I (2)
20934D		-	100/100	-	-	-	I (2)
21227A	MM	-	78/80	-	-	22/20	I (6)
21227B		-	43/55	19/24	-	38/21	I (6)
22240	MM	-	39/62	33/11	-	28/27	I (1)
22824	MM	-	63/70	24/20	-	13/10	I (2)
23430	MM	-	54/70	-	-	46/30	I (1)
23549	MM	20/21	63/65	17/14	-	-	I (5)
15481	MM	-	58/72	21/20	-	21/8	I (1)
16971	MM	-	51/48	12/25	8/17	29/10	I (1)
23544	MM	-	90/79	-	-	10/21	I (1)
19528A	MM	-	49/58	23/25	-	28/17	I (4)
19528B		23/22	57/63	-	-	20/15	I (4)
14048	MM	14/20	86/80	-	-	-	II (1)
14053	MM	-	79/80	-	-	21/20	I (1)
14269	MM	-	69/58	11/26	20/16	-	I (1)
14302A	MM	23/30	58/50	19/20	-	-	II (3)
14302B		-	60/66	40/34	-	-	II (3)
14319	MM	28/33	72/61	-	-	0/6	I (1)
15394	MM	-	69/75	19/15	-	12/10	II (1)
15626	MM	-	88/90	-	-	12/10	I (1)
19492	MM	-	54/60	46/40	-	-	II (1)
19614	MM	23/22	37/41	40/37	-	-	II (2)
19723	MM	12/19	88/81	-	-	-	I (1)
20157	MM	87/80	-	-	-	13/20	II (1)
20964	MM	-	79/79	21/21	-	-	I (4)
22741	MM	-	64/44	19/36	-	17/20	I (1)
22850	MM	-	79/82	-	-	21/18	I (2)
23899	MM	17/21	65/61	18/18	-	-	I (4)
24379A	MM	-	78/74	20/26	-	2/0	I (4)
24379B		46/44	40/37	14/17	-	0/2	I (4)
24610	MM	14/20	51/37	29/33	-	6/10	I (4)

Results are expressed as percentages of aPC from all aPC analyzed as determined by two independent expert observers (separated by /). Each clone encompassed >15% of counted aPC by at least one of the two expert observers. PCD, clonal plasma cell disorder; aPC, aberrant plasma cell; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma. [†]none of the tested cytogenetic alterations identified. [‡]sum of minor clones, each encompassing ≤15% of counted aPC. [§]numbers refer to Table 2 of the manuscript. [¶]Two aPC populations with different cytogenetic evolution patterns sorted by flow cytometry.

Online Supplementary Table S2. Cytogenetic patterns based on the presence or absence of del(13q14), del(17p13), +17p13, t(14q32) and deletions/gains of *IGH*, *FGFR3*, *CCND1* and *MAF* in the absence of t(14q32) in MM or MGUS patients classified according to their overall DNA ploidy.

Cytogenetic pattern	MM	MGUS
Hypodiploid		
I [t(14q32)+, del(13q14)+]		
del(13q14) & t(11;14) → pattern change[1]	1	0
III [t(14q32)-, del(17p13)+]		
del(13q14) & del(17p13) → del(14q32)[2]	1	0
None[3] → del(13q14) → del(17p13)	1	0
Diploid		
I [t(14q32)+, del(13q14)+]		
del(13q14) & t(4;14)	2	0
del(13q14) & t(4;14) → pattern change	2	0
del(13q14) & t(11;14)	2	0
del(13q14) & t(11;14) → pattern change	1	1
del(13q14) & t(11;14) → pattern change & +16q23	2	0
del(13q14) & t(11;14) → pattern change & del(16q23)	1	0
none → del(13q14) & t(11;14) → pattern change	1	0
del(13q14) & t(14;16)	1	0
del(13q14) & t(14;16) → pattern change	1	0
del(13q14) & t(14;16) → translocation second chromosome	1	0
del(13q14) & other t(14q32)	2	0
del(13q14) & other t(14q32) → pattern change	2	0
del(13q14) & del(17p13) & other t(14q32)	1	0
t(4;14)→ del(13q14) → pattern change & +16q23	1	0
t(4;14) → del(13q14) → translocation second chromosome	1	0
none → t(11;14) → del(13q14) → pattern change	1	0
A[4]: t(11;14) → del(13q14), B: t(11;14)	1	0
del(13q14) & t(4;14) & t(14;16) (biclonal)	1	0
none → A: del(13q14) → other t(14q32) → del(14q32), B: del(13q14) → other t(14q32)	1	0
none → del(13q14) & del(16q23) → other t(14q32)	0	1
del(13q14) & +11q13 → other t(14q32) → del(16q23)	1	0
none → del(13q14) & t(4;14) → del(17p13)	1	0
II [t(14q32)+, del(13q14)-]		
none → t(4;14)	1	0
t(4;14) & +11q13 → pattern change	1	0
t(11;14)	4	0
none → t(11;14)	0	3
t(11;14) → translocation second chromosome	1	0
none → t(11;14) → translocation second chromosome	1	0
t(11;14) → pattern change	4	2
none → t(11;14) → pattern change	2	1
t(11;14) & +16q23 → pattern change	1	0
none → t(14;16) → pattern change	0	1
none → other t(14q32)	1	1
other t(14q32) & +11q13 → pattern change & del(11q13)	1	0
III [t(14q32)-, del(17p13)+]		
none → del(14q32) → del(17p13)	1	0
del(13q14) & del(17p13) & del(14q32)	1	0
none → del(13q14) & del(16q23) → del(17p13) & del(14q32)	1	0
IV [t(14q32)-, del(13q14)+, del(17p13)-]		
del(13q14)	3	1
none → del(13q14)	6	1
del(13q14) & del(14q32)	1	2

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del(14q32) → del(13q14)	2	1
none → del(13q14) → del(14q32)	1	0
del(13q14) → del(14q32) → del(14q32) second chromosome	1	0
none → del(13q14) & del(14q32)	2	0
none → A: none, B: del(13q14) → del(14q32)	0	1
none → del(13q14) & del(14q32) → +17p13	1	0
del(13q14) → +17p13 → further del(13q14) & ++17p13	1	0
V [t(14q32)-, del(13q14)-, del(17p13)-]		
none	6	14
none → del(14q32)	2	0
none → +14q32	1	0
del(14q32)	0	2
+17p13	1	0
none → +17p13	0	1
none → +17p13 → ++17p13	1	0
Hyperdiploid		
I [t(14q32)+, del(13q14)+]		
del(13q14) & t(4;14) → tetraploidization	1	0
del(13q14) & t(4;14) → del(16q23)	1	0
del(13q14) & t(4;14) & tetraploidization → pattern change	1	0
del(13q14) & other t(14q32)	2	0
del(13q14) & other t(14q32) & tetraploidization & del(16q23)	1	0
none → del(13q14) & del(17p13) & t(4;14) & del(11q13) → pattern change	1	0
del(13q14) & del(17p13) & t(4;14) → pattern change	1	0
none → +11q13 → del(13q14) → other t(14q32)	1	0
del(13q14) & t(11;14) → del(17p13)	1	0
A: del(13q14) & t(4;14), B: del(13q14) & t(4;14) → +17p13 → pattern change	1	0
II [t(14q32)+, del(13q14)-]		
t(11;14) → pattern change	1	0
other t(14q32)	1	0
none → other t(14q32)	1	1
other t(14q32) & del(16q23)	0	1
other t(14q32) & del(4p16) & +11q13 → pattern change	1	0
+17p13 & t(4;14) → +16q23	0	1
none → +17p13 & other t(14q32) → pattern change & +16q23 & +11q13	1	0
none → +17p13 & +11q13 → other t(14q32) & del(4p16)	1	0
III [t(14q32)-, del(17p13)+]		
del(17p13)	2	0
none → del(17p13)	2	0
del(14q32) → del(17p13)	1	0
none → del(13q14) & del(17p13)	1	0
none → del(17p13) → del(13q14)	1	0
IV [t(14q32)-, del(13q14)+, del(17p13)-]		
del(13q14)	5	2
none → del(13q14)	3	0
del(13q14) & del(14q32)	1	2
none → del(13q14) & +14q32	0	1
none → del(13q14) → +4p16	1	0
del(13q14) → del(14q32) → del(16q23) & del(4p16)	1	0
del(13q14) & +17p13	2	0
none → del(13q14) & +17p13	2	0
none → del(14q32) → del(13q14) & +17p13	1	0
del(13q14) & +17p13 → +14q32 & ++17p13	1	0
+17p13 → del(13q14)	1	0

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V [t(14q32)-, del(13q14)-, del(17p13)-]		
none	17	13
none → del(14q32)	0	2
none → +14q32	1	0
none → del(14q32) → pattern change	1	0
del(14q32) & +11q13 → del(14q32)	1	0
+17p13	0	1
none → +17p13	5	3
+17p13 → ++17p13	1	0
none → +17p13 & +16q23 & +11q13 & del(14q32) → ++17p13	1	0

*Results are expressed as number of cases. Abbreviations are explained in Online Supplementary Table S1. [1]Defined as a deletion or a gain of 14q32 (*IGH*) or of the respective partner region (*FGFR3, CCND1 *or* MAF*) in cases already carrying a translocation or numeric alteration of the* IGH *gene in a previous clone. [2]del(14q32) in the absence of t(14q32) (including deletion potentially associated with t(14q32) but absence of t(4;14), t(11;14) and t(14;16)). [3]>15% of aPC without any of the cytogenetic alterations tested ('diploid'). [4]Two aPC populations with different cytogenetic evolution patterns sorted by flow cytometry. +: cytogenetic alteration present. -: cytogenetic alteration absent. →: sequential acquisition. &: simultaneous acquisition.*

Online Supplementary Table S3. Distribution of patients with an ancestral aPC clone (>15% aPC) without any cytogenetic alteration tested in different cytogenetic subgroups of patients with MM or MGUS.

Karyotypic pattern	MM (n=148)	MGUS (n=60)
I [t(14q32)+, del(13q14)+]	6/39 (15%)	1/2 (50%)
II [t(14q32)+, del(13q14)-]	8/23 (35%)	7/11 (64%)
III [t(14q32)-, del(17p13)+]	7/12 (58%)	0/0 (0%)
IV [t(14q32)-, del(13q14)+, del(17p13)-]	17/36 (47%)	3/11 (27%)
V [t(14q32)-, del(13q14)-, del(17p13)-]	35/38 (92%)	33/36 (92%)
Total	73/148 (49%)*	44/60 (73%)*

*Results are expressed as number of patients and percentages are given between brackets. Abbreviations are explained in Online Supplementary Table S1. *P=0.002.*

Online Supplementary Table S4. Cytogenetic patterns in three exemplary MM cases studied at different disease stages during follow-up.

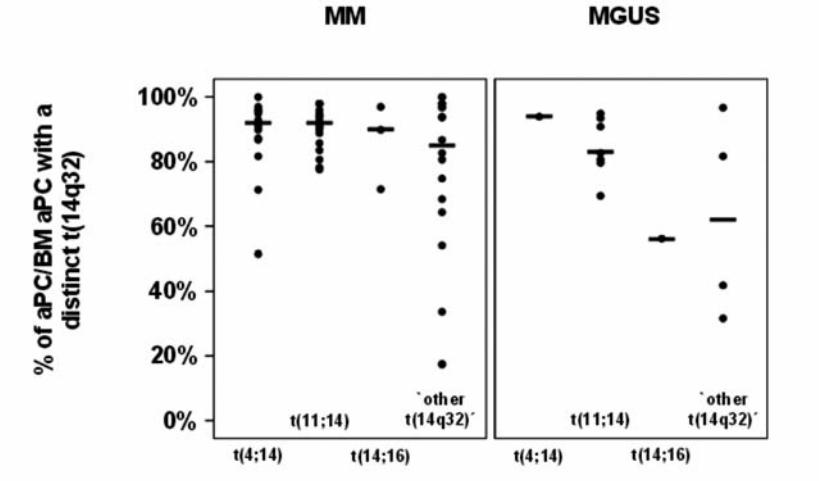
Case ID	Moment of study	Clone I [%]	Clone II [%]	Clone III [%]
23549	diagnosis (10/02/2010)	non-altered[1] (20%)	del(13q14) & t(4;14) (63%)	del(17p13) (17%)
	progression (29/10/2010)	non-altered (51%)	del(13q14) & t(4;14) (49%)	disappeared
20595	diagnosis (14/11/2008)	non-altered (20%)	del(13q14) (80%)	-
	relapse (9/06/2010)	non-altered (18%)	del(17p13) (21%)	del(13q14) (61%)
14548	diagnosis (17/01/2006)	non-altered (18%)	del(13q14) & t(4;14) (82%)	-
	MRD (12/07/2011)	non-altered (60%)	del(13q14) & t(4;14) (40%)	-

Results are expressed as percentage of aPC in different cytogenetically-defined clones from all counted aPC. MRD, minimal residual disease. [1]none of the tested cytogenetic alterations was identified.

Online Supplementary Table S5. Sequences of the CDR3 region and clone characteristics assessed by iFISH analyses in selected cases of MGUS (n=2) and MM (n=7).

Case ID/PCD	Sensitivity limit [%]	CDR3 sequence	Cytogenetic alteration[1]	Cytogenetic pattern[2]
85888/MGUS	≤0.1	GTATTACGATCTTTTGACTGGTTAT TATCTAAATTCCTAAATCGATGAC AGCCTTTGACTACTGGGGCCAGG	+15 & +17 (80%); del(17p13) (79%); del(13q14) (22%);	none → del(17p13) & +17 → del(13q14)
85312/MGUS	≤0.1	TGTGCGAGAGGTCCCCATTACAGT GACTTGACTGGATACAGTTGGTAC CTTGATTCCTGG	del(13q14) (37%)	none → del(13q14)
87672/MM	not done	ATTACTGTGTGAAAGATCGATTGT CGGATTTTTGGAGCGGTGTCCCCTTT CTTGACTGGGGCCAG	non-altered	non-altered
87673/MM	≤0.1	TGTGCGAAAGTCGTAGGGGGGTC TCATCTATTGTAGTAGTACCAGCT GCTATTGGTTTGACGGTTTTTGCTT TTGATTCCTGG	non-altered	non-altered
85864/MM	≤0.1	AGGATATTGTAATAGAACCAGCTG CTAAGCCGAGCTACAACAGTACA TGTACTACTTTGACTACT	del(13q14) (86%); t(11;14) (77%; different translocation patterns in 36%, 29%, 14%, 13% and 8%)	del(13q14) & t(11;14)→ pattern change
81175/MM	≤0.06	TGTGTATTATTGTGGGAGAGACAA GTATGTGGTAGCGACTACTGCGG TCGCGCA	other t(14q32) (77%); +4 (71%); +15 (70%); +11 (67%); ++17 (54%); ++17p13 (46%); +17p13 (17%); +17 (16%)	none → other t(14q32) & +17→ ++17
93759/MM	<0.23	GTAGAGATGACTACACCGCCGAA AAATACCCGACAATGACTACTGG GGCCAGGGAACCCTGGTCACCGT CTCCTCA	del(13q14) (74%); t(4;14) (64%); del(17p13) (59%)	none → del(13q14) & t(4;14) & del(17p13)
96955/MM	<1.2	TGTTTATTACTGTGCGAAGGACAG GAACAGTGGCTGGGACAAGTGGT TCGCCCCCTGGGGCAAGGGAACCC	del(13q14) (84%); del(14q32) (82%)	none → del(13q14) & del(14q32)
96240/MM	<1.2	TGACACGGCCTTGTATTACTGTGT AAAAGATCGGGGCAGCTCGTCCT TGCTCCCGACGTTAGACTTCTGGG GCCA	t(14q32) (93%; different translocation patterns in 74%, 12%, 7% and 7%); del(17p13) (40%)	other t(14q32) → del(17p13) → pattern change

The CDR3 sequences are shown in bold and underlined nucleotides highlight the position of sense ASO primers. One thousand aPC with a purity >99% were analyzed in every case. Abbreviations are explained in Online Supplementary Table S1. [1]Percentages of aPC with distinct cytogenetic alterations (from all aPC in the bone marrow), [2]Cytogenetic patterns as defined in Online Supplementary Table S2. → : sequential acquisition. &: simultaneous acquisition.



Online Supplementary Figure S1. Percentage of aPC with different types of t(14q32) from all bone marrow (BM) aPC in MM *versus* MGUS (circles indicate individual patients and bars correspond to the median values of altered cells). There were no statistically significant differences, either between MM and MGUS or between different types of t(14q32) among MM and MGUS, respectively (the patient with coexisting t(4;14) together with t(14;16) has been excluded from this analysis).