Cytogenetic aberrations in multiple myeloma are associated with shifts in serum immunoglobulin isotypes distribution and levels

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SUPPLEMENTARY DATA

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Supplementary Methods

Serum Immunoglobulin measurements

The concentrations of serum intact immunoglobulins and free light chains (FLCs) were measured by a latex enhanced immunoassay on a Behring 2 nephelometer, except for IgG which was measured by electrophoresis. The reference ranges were used according to the manufacturer's instructions as follows: 3.3–19.4 mg/L for kappa light chain, 5.7–26.3 mg/L for lambda light chain and 0.3–1.6 for kappa/lambda ratio. The FLC test was considered positive when the criteria of both an abnormal FLC ratio and an elevation of the involved light chain above the respective upper range were met. Immunofixation of serum was performed using the Paragon Electrophoresis System kit by Beckman Coulter. In case of an ambiguous result, the immunofixation was repeated after prior denaturation with 2-mercaptoethanol and kits by Dako, Binding Site or Technoclon were applied additionally.

Supplementary Tables

Supplementary Table S1. Patient characteristics from GMMG-MM5 and GMMG-HD4 clinical trials.

	GMMG-MM5 (Total =523)	**GMMG-HD4 (Total=325)**
Male	312 (60%)	191 (59%)
Age (years)	58±8 (32–70)	55±7 (27–65)
ISS[a] (Stage 1/2/3)	197 (38%)/180 (34%)/146 (28%)	125 (41%)/105 (34%)/76 (25%)
Myeloma with involved heavy/light chain		
IgGκ	230 (44%)	154 (48%)
IgGλ	88 (17%)	37 (11%)
IgAκ	64 (12%)	36 (11%)
IgAλ	42 (8%)	38 (12%)
LCO-κ	56 (11%)	28 (9%)
LCO-λ	34 (6%)	27 (8%)
Others[b]	9 (2%)	4 (1%)

Age is presented as mean± standard deviation (range). LCO: light chain only disease; ISS: international staging system. [a] GMMG-HD4 had 19 missing values.
[b] included additional 9 patients (1 IgMλ, 1 IgDκ and 7 IgDλ) in GMMG-MM5 and 5 patients (1 IgMκ, 1 IgDκ, 2 IgDλ and 1 misssing) in GMMG-HD4.

Supplementary Table S2. Proportion of positivity for cytogenetic abnormalities (CAs) in IgG isotypes.

CA	Proportion		P
	IgGκ	**IgGλ**	**P**
GMMG-MM5	**(N=230)**	**(N=88)**	
t(4;14)	0.06 (13/215)	0.05 (4/79)	1.0
t(11;14)	0.15 (31/210)	0.31 (24/78)	**3.7×10⁻³**
t(14;16)	0.02 (2/96)	0.19 (5/27)	**5.6×10⁻³**
Hyperdiploidy	0.70 (145/208)	0.55 (42/77)	**0.02**

GMMG-HD4	**(N=154)**	**(N=37)**	
t(4;14)	0.10 (14/142)	0.10 (3/31)	1.0
t(11;14)	0.18 (25/142)	0.25 (8/32)	0.48
t(14;16)	0.01 (1/135)	0.03 (1/31)	0.34
Hyperdiploidy	0.68 (94/138)	0.55 (17/31)	0.23

Proportion: each entry represents proportion of CA positive cases. In parentheses are shown the number of CA positive cases and the number of cases tested for this CA.
N: total number of cases with this MM isotype.
P: P value from a Chi-square test for comparison between isotype machted Igs (e.g. IgGκ vs IgGλ). Fisher's exact test was used when the expected count was less than 5.
Bold types: significant differences (P≤0.05) are shown in bold.

Supplementary Table S3. Concentrations of serum M-protein in IgG MM and IgA MM cases based on cytogenetic abnormalities (CAs), showing median values together with ranges.

CA	IgG MM		IgA MM	
	M-protein (g/l)	**Number of cases**	**M-protein (g/l)**	**Number of cases**
GMMG-MM5				
All cases	36.0 (1.5, 125.6)	318	32.3 (1.1, 81.8)	106
t(4;14)	50.5 (5.7, 125.6)	17	45.3 (7.8, 81.8)	22
t(11;14)	40.2 (3.5, 82.1)	55	28.3 (10.9, 60.1)	13
t(14;16)	34.5 (3.1, 102.9)	7	3.7 (2.4, 4.9)	2
any IgH	42.6 (3.5, 125.6)	67	42.4 (2.4, 81.8)	28
Hyperdiploidy	34.0 (1.5, 95.6)	84	19.0 (1.5, 79.2)	18
GMMG-HD4				
All cases	42.0 (0.6, 106.1)	191	35.8 (0, 73.0)	74
t(4;14)	54.1 (20.9, 90.5)	17	53.9 (0, 67.9)	18
t(11;14)	45.1 (3.1, 86.8)	33	44.9 (29.2, 54.5)	8
t(14;16)	37.0 (24.5, 49.5)	2	54.6 (54.6, 54.6)	1
any IgH	46.1 (3.1, 90.5)	39	44.9 (0, 73.0)	21
Hyperdiploidy	41.7 (3.5, 91.1)	93	39.1 (13.1, 72.7)	24

Supplementary Table S4. Concentration of serum uninvolved immunoglobulins and free light chains (FLCs) depending upon cytogenetic abnormalities (CAs) in IgG MM, IgA MM and LCO MM cases.

CA	Laboratory parameter	Reference range[*]	Median (m₁, m₂)	Cases (n₁, n₂)	BETA	P
IgG MM (N=318)						
t(4;14)	IgA (g/l)	0.7–3.8	0.4, 0.3	17, 275	0.14	0.17
	IgM (g/l)	0.4–2.8	0.2, 0.2	17, 275	0.07	0.47
	FLCκ (mg/l)	3.3–19.4	31.0, 89.5	17, 274	0.13	0.51
	FLCλ (mg/l)	5.7–26.3	8.6, 8.2	17, 274	0.10	0.52
t(11;14)	IgA (g/l)	0.7–3.8	**0.25, 0.33**	54, 232	-0.14	**0.03**
	IgM (g/l)	0.4–2.8	**0.16, 0.20**	54, 232	-0.13	**0.02**
	FLCκ (mg/l)	3.3–19.4	37.9, 99.3	54, 231	0.01	0.95
	FLCλ (mg/l)	5.7–26.3	9.9, 8.0	54, 231	-0.03	0.77
t(14;16)	IgA (g/l)	0.7–3.8	0.27, 0.34	7, 116	0.11	0.57
	IgM (g/l)	0.4–2.8	0.08, 0.20	7, 116	-0.14	0.38
	FLCκ (mg/l)	3.3–19.4	13.0, 124.5	7, 116	-0.13	0.74
	FLCλ (mg/l)	5.7–26.3	218.0, 6.9	7, 116	0.37	0.16
gain 1q21	IgA (g/l)	0.7–3.8	0.28, 0.33	99, 188	0.04	0.45
	IgM (g/l)	0.4–2.8	0.2, 0.2	99, 188	0.00	0.99
	FLCκ (mg/l)	3.3–19.4	23, 118	100, 186	-0.12	0.27
	FLCλ (mg/l)	5.7–26.3	**9.3, 7.4**	100, 186	0.20	**0.01**
Hyperdiploidy	IgA (g/l)	0.7–3.8	0.33, 0.26	187, 96	0.05	0.40
	IgM (g/l)	0.4–2.8	0.2, 0.2	187, 96	0.01	0.77
	FLCκ (mg/l)	3.3–19.4	107.5, 52.2	186, 96	-0.01	0.93
	FLCλ (mg/l)	5.7–26.3	7.4, 9.3	186, 96	-0.09	0.27
del (13q)	IgA (g/l)	0.7–3.8	0.28, 0.33	123, 170	-0.03	0.57
	IgM (g/l)	0.4–2.8	0.21, 0.18	123, 170	0.03	0.50
	FLCκ (mg/l)	3.3–19.4	43.9, 102.1	122, 170	-0.02	0.83
	FLCλ (mg/l)	5.7–26.3	8.5, 7.4	122, 170	-0.06	0.43
del (17p)	IgA (g/l)	0.7–3.8	0.4, 0.3	30, 264	-0.01	0.92
	IgM (g/l)	0.4–2.8	0.2, 0.2	30, 264	-0.02	0.79
	FLCκ (mg/l)	3.3–19.4	276.5, 62.6	30, 263	0.07	0.64
	FLCλ (mg/l)	5.7–26.3	5.6, 8.5	30, 263	-0.19	0.12
IgA MM (N=106)						
t(4;14)	IgG (g/l)	7.0–16.0	3.0, 3.6	22, 75	-0.10	0.10
	IgM (g/l)	0.4–2.8	0.17, 0.18	21, 75	-0.05	0.58
	FLCκ (mg/l)	3.3–19.4	31.4, 26.0	22, 75	-0.05	0.83
	FLCλ (mg/l)	5.7–26.3	8.0, 9.0	22, 74	-0.15	0.46
t(11;14)	IgG (g/l)	7.0–16.0	3.1, 3.5	13, 82	-0.08	0.26
	IgM (g/l)	0.4–2.8	0.1, 0.2	13, 81	-0.13	0.24
	FLCκ (mg/l)	3.3–19.4	24.6, 30.8	13, 82	-0.40	0.16
	FLCλ (mg/l)	5.7–26.3	6.4, 8.7	13, 81	-0.13	0.62
t(14;16)	IgG (g/l)	7.0–16.0	3.5, 3.3	2, 43	-0.09	0.65
	IgM (g/l)	0.4–2.8	0.1, 0.2	2, 42	-0.28	0.12
	FLCκ (mg/l)	3.3–19.4	16.5, 31.4	2, 43	-0.59	0.37
	FLCλ (mg/l)	5.7–26.3	3005.8, 8.4	2, 43	0.58	0.30
gain 1q21	IgG (g/l)	7.0–16.0	**3.0, 4.1**	54, 41	-0.15	**3.7×10⁻³**
	IgM (g/l)	0.4–2.8	**0.17, 0.20**	53, 41	-0.19	**0.01**
	FLCκ (mg/l)	3.3–19.4	21.1, 31.7	54, 41	-0.13	0.51

	FLCλ (mg/l)	5.7–26.3	9.1, 8.5	53, 41	-0.17	0.34
Hyperdiploidy	IgG (g/l)	7.0–16.0	**4.2, 3.0**	50, 44	0.13	**0.01**
	IgM (g/l)	0.4–2.8	0.20, 0.17	49, 44	0.08	0.28
	FLCκ (mg/l)	3.3–19.4	32.8, 13.2	50, 44	0.19	0.30
	FLCλ (mg/l)	5.7–26.3	7.2, 8.8	49, 44	0.26	0.10
del (13q)	IgG (g/l)	7.0–16.0	**3.0, 4.2**	53, 44	-0.11	**0.03**
	IgM (g/l)	0.4–2.8	0.17, 0.20	52, 44	-0.09	0.24
	FLCκ (mg/l)	3.3–19.4	30.9, 21.2	53, 44	-0.07	0.74
	FLCλ (mg/l)	5.7–26.3	**7.8, 9.0**	52, 44	-0.34	**0.04**
del (17p)	IgG (g/l)	7.0–16.0	2.6, 3.5	13, 84	-0.02	0.80
	IgM (g/l)	0.4–2.8	0.1, 0.2	12, 84	-0.13	0.23
	FLCκ (mg/l)	3.3–19.4	10.3, 28.4	13, 84	0.03	0.93
	FLCλ (mg/l)	5.7–26.3	24.7, 8.3	13, 83	-0.08	0.74

LCO MM (N=90)						
t(4;14)	IgA (g/l)	0.7–3.8	0.3, 0.4	5, 71	-0.15	0.45
	IgG (g/l)	7.0–16.0	4.3, 4.8	5, 71	-0.18	0.07
	IgM (g/l)	0.4–2.8	0.12, 0.20	5, 70	-0.23	0.16
	FLCκ (mg/l)	3.3–19.4	2810, 486	5, 71	0.26	0.48
	FLCλ (mg/l)	5.7–26.3	11.2, 13.3	5, 71	0.08	0.80
t(11;14)	IgA (g/l)	0.7–3.8	0.4, 0.3	29, 42	-0.08	0.37
	IgG (g/l)	7.0–16.0	4.8, 4.6	29, 42	0.02	0.63
	IgM (g/l)	0.4–2.8	0.18, 0.19	28, 42	-0.08	0.31
	FLCκ (mg/l)	3.3–19.4	**437, 719**	29, 42	-0.40	**0.02**
	FLCλ (mg/l)	5.7–26.3	16.3, 11.4	29, 42	-0.11	0.45
t(14;16)	IgA (g/l)	0.7–3.8	2.0, 0.3	2, 23	-0.08	0.79
	IgG (g/l)	7.0–16.0	2.4, 4.9	2, 23	0.21	0.39
	IgM (g/l)	0.4–2.8	0.14, 0.21	2, 23	0.07	0.81
	FLCκ (mg/l)	3.3–19.4	6503, 633	2, 23	0.87	0.38
	FLCλ (mg/l)	5.7–26.3	2213.7, 11.2	2, 23	0.73	0.25
gain 1q21	IgA (g/l)	0.7–3.8	**0.2, 0.5**	25, 50	-0.29	**0.01**
	IgG (g/l)	7.0–16.0	**4.1, 5.0**	25, 50	-0.12	**0.03**
	IgM (g/l)	0.4–2.8	**0.1, 0.2**	25, 49	-0.21	**0.02**
	FLCκ (mg/l)	3.3–19.4	7, 733	25, 50	-0.22	0.25
	FLCλ (mg/l)	5.7–26.3	250, 11	25, 50	-0.18	0.25
Hyperdiploidy	IgA (g/l)	0.7–3.8	**0.3, 0.4**	23, 49	0.21	**0.04**
	IgG (g/l)	7.0–16.0	4.9, 4.3	23, 49	0.06	0.23
	IgM (g/l)	0.4–2.8	0.20, 0.18	23, 48	0.09	0.30
	FLCκ (mg/l)	3.3–19.4	**506, 534**	23, 49	0.36	**0.05**
	FLCλ (mg/l)	5.7–26.3	13.5, 11.2	23, 49	0.23	0.14
del (13q)	IgA (g/l)	0.7–3.8	0.4, 0.4	48, 27	-0.05	0.62
	IgG (g/l)	7.0–16.0	4.2, 6.1	48, 27	-0.09	0.11
	IgM (g/l)	0.4–2.8	0.17, 0.25	47, 27	-0.13	0.14
	FLCκ (mg/l)	3.3–19.4	496, 633	48, 27	0.22	0.27
	FLCλ (mg/l)	5.7–26.3	12.4, 11.5	48, 27	-0.07	0.65
del (17p)	IgA (g/l)	0.7–3.8	0.3, 0.4	7, 70	0.02	0.91
	IgG (g/l)	7.0–16.0	4.0, 4.9	7, 70	0.02	0.81
	IgM (g/l)	0.4–2.8	0.1, 0.2	6, 70	-0.12	0.43
	FLCκ (mg/l)	3.3–19.4	**2130, 464**	7, 70	0.78	**0.01**
	FLCλ (mg/l)	5.7–26.3	8.0, 13.4	7, 70	-0.11	0.64

m1, m2: median concentration of serum immunoglobulins and FLC in CA positive and CA negative cases, respectively.

n1, n2: number of CA positive and CA negative cases, respectively.

BETA, P: estimate of coefficient and p value for CA in a multiple linear model involving logarithmic transformed laboratory parameter as dependent variable and international staging system (ISS), sex, light chain type, bone marrow cell count and secondary CAs as independent variables.

Bold types: median concentrations shown in bold for P≤0.05.

[*] Reference ranges were as reported at the http://www.laborlexikon.de/Vision.htm or by the International Myeloma Foundation.

LCO: light chain only