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IGVH segment utilization in immunoglobulin gene rearrangement differentiates patients with anti-MAG neuropathy from others IgM-gammopathies

Jean-Sebastien Allain[1,2], Florian Thonier[3], Morgane Pihan[4], Marie-Laure Boulland[3], Sophie de Guibert[5], Vincent Launay[6], Anne-Violaine Doncker[7], Michel Ganard[2,5], Amyra Aliouat[8], Céline Pangault[2,3,9], Roch Houot[2,5,9], Marie De Tayrac[2,8], Thierry Lamy[2,5,9], Mikael Roussel[3,9], Thierry Fest[2,3,9], Olivier Decaux[1,2,9] and Cedric Pastoret[2,3,9]

Affiliations

[1]Service de Médecine Interne, CHU de Rennes, F-35033 Rennes, France
[2]Université de Rennes 1, Rennes
[3]Laboratoire d'Hématologie, Pole de Biologie, CHU de Rennes, F-35033 Rennes, France
[4]Service de Neurologie, CHU de Rennes, F-35033 Rennes, France
[5]Service d'Hématologie Clinique, CHU de Rennes, F-35033 Rennes, France
[6]Service d'Hématologie, CH Saint Brieuc, France
[7]Hopital Privé Sévigné, Cesson-Sévigné, France
[8]Laboratoire de Bioinformatique Médicale, Pole de Biologie, CHU Rennes, France
[9]INSERM, UMR1236, Equipe labellisée Ligue contre le Cancer, Rennes, France

Correponding author: Cedric Pastoret, Laboratoire d'Hématologie, Pôle de Biologie, CHU de Rennes, 2 rue Henri Le Guilloux, 35 033 Rennes Cedex 9, France. cedric.pastoret@chu-rennes.fr Phone: +33 299 289 142; Fax: +33 299 284 152

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Demyelinating neuropathy associated with antibodies against myelin-associated-glycoprotein (anti-MAG) is a rare acquired immune-mediated neuropathy[1,2]. It predominantly consists of sensory deficiency in the lower limbs. Frequently, invalidating tremor, sensory ataxia, and painful paresthesia are present. Anti-MAG neuropathy is associated with IgM monoclonal protein produced by an underlying B lymphoproliferative disorder, Waldenström macroglobulinemia (WM) or IgM-monoclonal gammopathy of undetermined significance (IgM-MGUS) for the most frequent. Management of anti-MAG neuropathy remains a challenge and no predictive factor has been described to identify patients with IgM monoclonal gammopathy at risk to develop anti-MAG neuropathy. Description of the mutational profile of *MYD88, CXCR4,* and *TP53* genes have radically changed the diagnosis and prognostic evaluation of IgM monoclonal gammopathies[3,4,5]. Acting as a trigger for NFκB signaling, *MYD88*^{L265P} is the most prevalent mutation in WM and IgM-MGUS[6,7]. Somatic mutations in the C-terminal domain of *CXCR4* are frequently described in WM and associated with a more aggressive disease[4]. Moreover, the *MYD88/CXCR4* status is predictive of the response to BCR-inhibitor treatment in WM[8]. The frequency of these mutations is actually unknown in anti-MAG neuropathy and could be relevant for the management of patients. Moreover, analysis of immunoglobulin heavy chain variable *(IGVH)* sequence of clonal tumor B-cells provides information on cell origin and antigen dependence[9]. *IGVH* gene encodes the complementary-determining region 3 (CDR3) that most closely interacts with the antigen. Thus, analysis of the *IGVH* sequence from clonal B cells in patients with monoclonal gammopathy could identify a subset of patients with a biased *IGVH* segment utilization, prone to develop a demyelinating neuropathy. The aim of this study was to analyze the mutational profile of *MYD88*, *CXCR4*, *TP53* gene and *IGVH* sequence in anti-MAG neuropathy with high throughput sequencing (HTS).

In this study approved by our institutional ethics committee, we analyzed and compared the genomic profile of 26 anti-MAG neuropathy patients with 46 cases of IgM monoclonal gammopathies without neurologic symptoms or anti-MAG antibodies detection (24 WM and 22 IgM-MGUS). All patients underwent bone marrow (BM) investigations in our lab. Genomic DNA was extracted from BM mononuclear cells using Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA). Target enrichment was performed using the Access Array System (Fluidigm San Francisco, CA, USA) from 50 ng of DNA. Then, purified libraries were sequenced with MiSeq (Illumina, San Diego, CA, USA). Somatic mutations were defined as frameshift, stop gain or missense variants not reported as a polymorphism, with a variant allele frequency (VAF) higher than 2%. In parallel, *MYD88*^{L265P}

and *CXCR4*^{S338X} mutations were screened by alleles specific – polymerase chain reactions (AS-PCR), with a sensitivity of 0.1%[10,11]. *IGH* gene was sequenced by HTS using a two-step PCR protocol adapted from Biomed-2 recommendations from 100 ng of genomic DNA[12]. Libraries were sequenced on Illumina MiSeq platform and analysis were performed with Vidjil software[13]. A clonotype was defined as a dominant sequence showing a frequency of at least 1% of total reads well separated from the polyclonal background reads. Patients' characteristics are reported in Table 1.

In the anti-MAG group, all the patients (N=26) presented an IgM monoclonal gammopathy, high anti-MAG antibodies titers, and clinical and electrophysiological evidence of demyelinating neuropathy[2]. Anti-MAG titers ranged from 3,620 to >70,000 BTU. For 24 out of 26 patients (92.3%), anti-MAG was >7,000 BTU, including 9 strongly positive (>70,000 BTU) patients (34.6%). The underlying lymphoproliferative disorders were 15 IgM-MGUS, 9 WM, one splenic marginal zone lymphoma (SMZL), and one monoclonal B-cell lymphocytosis (MBL) with a Matutes score at 5. *MYD88*^{L265P} was detected in 19 subjects (73.1%) (Figure 1A). Among them, 9 are identified by AS-PCR only and 10 by both HTS and AS-PCR. *MYD88*^{L265P} was found in 10 IgM-MGUS (66.7%), in 8 WM (88.8%) and in the SMZL. Three patients (11.5%) were mutated for *CXCR4* in the anti-MAG group and one harbored a TP53 mutation (3.8%) (Figure 1A and supplemental table S1).

In control WM group, we detected *MYD88*^{L265P} mutations for 23 patients (95.8%). Truncating mutations of *CXCR4* genes were detected for 12 patients (50.0%): 7 patients with HTS and 5 AS-PCR positive cases. *TP53* gene was mutated in two WM patients (8.3%). In control IgM-MGUS group, we evidenced a *MYD88*^{L265P} mutation for 13 patients (59.1%) including 5 detected by AS-PCR only. Three patients harbored a *CXCR4* variant (13.6%) and no *TP53* mutation was detected in this group (Figure 1A and supplemental table S1).

Our study demonstrates that the *MYD88*^{L265P} mutation is highly prevalent in a cohort of anti-MAG patients. The *MYD88* mutational rate in anti-MAG neuropathy is closely related to the underlying B cell disorder. Indeed, the prevalence observed is comparable to our internal control groups and previous larger cohorts of WM or IgM-MGUS without anti-MAG[3,6,7]. With HTS, no *CXCR4* mutation was detected for anti-MAG patients while 13.6% of IgM-MGUS and 29.1% of WM were mutated. *CXCR4*^{S338X} represents 70% of *CXCR4* mutations detected by HTS and a third of variants consist of frameshift mutations. Combining HTS with AS-PCR for the most common variant *CXCR4*^{S338X}, we showed that 10% of anti-MAG patients exhibited a mutation in *CXCR4* independently of the associated B-cell malignancy while up to 50% of patients were mutated in our WM group. This difference is

partly explained by a relative lower BM infiltrate for anti-MAG patients with WM compared with our control WM group (means of 22.6 vs. 44.6% respectively, ns). Consequently, the *CXCR4* mutational rate in anti-MAG group might be slightly underestimated by the lack of detection of frameshift variants by AS-PCR for lower infiltrated samples.

MYD88 mutations is an early event in the pathogenesis of monoclonal gammopathy, promoting the emergence of a B-cell clone responsible for IgM paraprotein production[3,11]. *CXCR4* has been shown to occur later in the lymphomagenesis, explaining the progression from IgM-MGUS to WM[11]. In our study, the similar prevalence of *MYD88* mutations between anti-MAG and control and the low prevalence of *CXCR4* mutations in anti-MAG patients suggest that these mutations do not influence the occurrence of anti-MAG neuropathy during this oncogenic process. However, since $MYD88^{L265P}/CXCR4^{WT}$ genomic profile has been shown to predict a better efficiency of treatment with BCR-inhibitor in WM, the high frequency of this genomic profile in our anti-MAG cohort suggests the potential interest of BCR inhibitors for the treatment of anti-MAG patients[8].

In the second part of this study, we compared by HTS the *IGH* locus sequences from anti-MAG patients with our control WM and IgM-MGUS patients (Figures 1B, 1C, and supplemental table S1). In the anti-MAG group, 29 clonotypes were identified for 16 patients: 7 patients with WM (77.7%), 7 with IgM-MGUS (46.7%), and the 2 patients with SMZL and MBL (Figure 1A). In comparison, we detected 44 different clonotypes from 19 patients in the WM control group (79.2%) and 17 clonotypes from 11 patients in the IgM-MGUS control group (50.0%). The frequency of clonotypes detection suggests that our anti-MAG group is comparable with our control groups. As recently reported for WM, we evidenced a high frequency of *VH3* segment usage, particularly *VH3-23* and *VH3-11* in our three groups, suggesting a pathogenic link between anti-MAG, IgM-MGUS, and WM (Figure 1B)[9]. Interestingly, *VH4-34* segment was more frequently used in dominant clonotypes from anti-MAG patients independently of the underlying B-cell proliferation. Indeed, 6 anti-MAG patients (3 MW, 2 IgM-MGUS, and 1 SMZL) (23.1%) exhibited at least one *VH4-34* clonotype whereas only one patient among the 46 control IgM gammopathies presented one *VH4-34* clonotype (2.2%) (p=0.008) (Figure 1B). *VH4-34* segment was significantly more used in anti-MAG clonotypes (24.1%) compared with the control WM group (2.3%) (p=0.002) and with the control IgM-MGUS group clonotypes (0.0%) (p=0.019) (Figure 1C). VH4-34 is a human heavy chain family that has germline-encoded polyreactivity towards multiple self-antigens[14]. *VH4-34* segment has been identified as a diagnostic marker of variant hairy cell leukemia. Its over-representation has been found in other demyelinating processes

such as multiple sclerosis and other autoimmune diseases[14,15]. Therefore, our results suggest the potential interest of detecting *VH4-34* segment utilization in *IGH* loci of patient with IgM monoclonal gammopathy for an earlier identification of patients with higher risk of developing an anti-MAG neuropathy. For these patients, a treatment might be initiated before the installation of irreversible demyelinating lesions. However, a prospective study evaluating the occurrence of anti-MAG neuropathy in a large cohort of IgM gammopathies according to their *IGH* sequence might be useful to exclude a potential bias based on the small size of our sample.

In conclusion, our results demonstrate that $MYD88^{L265P}/CXCR4^{WT}$ is the most frequent genomic profile in anti-MAG patients supporting the initiation of clinical trials with BCR inhibitors in anti-MAG patients. *VH4-34* segment utilization in immunoglobulin gene recombination could predict a higher risk of developing an anti-MAG neuropathy in patients with an IgM monoclonal gammopathy. Earlier identification of high-risk patients and genomic adapted therapy could improve the management of anti-MAG neuropathy, however further studies are needed to address the use of these markers in daily practice.

References

1. D'Sa S, Kersten MJ, Castillo JJ, et al. Investigation and management of IgM and Waldenström-associated peripheral neuropathies: recommendations from the IWWM-8 consensus panel. Br J Haematol. 2017;176(5):728–742.

2. Joint Task Force of the EFNS and the PNS. European Federation of Neurological Societies/Peripheral Nerve Society Guideline* on management of paraproteinemic demyelinating neuropathies. Report of a Joint Task Force of the European Federation of Neurological Societies and the Peripheral Nerve Society – first revision. J Peripher Nerv Syst. 2010;15(3):185–195.

3. Varettoni M, Zibellini S, Defrancesco I, et al. Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. Haematologica. 2017;102(12):2077–2085.

4. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenström macroglobulinemia. Blood. 2014;123(18):2791–2796.

5. Poulain S, Roumier C, Bertrand E, et al. *TP53* Mutation and Its Prognostic Significance in Waldenstrom's Macroglobulinemia. Clin Cancer Res. 2017;23(20):6325–6335.

6. Treon SP, Xu L, Yang G, et al. MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia. N Engl J Med. 2012;367(9):826–833.

7. Landgren O, Staudt L. MYD88 L265P somatic mutation in IgM MGUS. N Engl J Med. 2012;367(23):2255-2256-2257.

8. Treon SP, Tripsas CK, Meid K, et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. N Engl J Med. 2015;372(15):1430–1440.

9. Gachard N, Parrens M, Soubeyran I, et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenström macroglobulinemia/lymphoplasmacytic lymphomas. Leukemia. 2013;27(1):183–189.

10. Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenstrom's macroglobulinemia and related lymphoid neoplasms. Blood. 2013;121(13):2522–2528.

11. Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenström Macroglobulinaemia. Br J Haematol. 2016;172(5):735–744.

12. van Dongen JJM, Langerak AW, Brüggemann M, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17(12):2257–2317.

13. Giraud M, Salson M, Duez M, et al. Fast multiclonal clusterization of V (D) J recombinations from high-throughput sequencing. BMC Genomics. 2014;15(1):409.

14. Rawlings DJ, Metzler G, Wray-Dutra M, Jackson SW. Altered B cell signalling in autoimmunity. Nat Rev Immunol. 2017;17(7):421–436.

15. Baranzini SE, Jeong MC, Butunoi C, Murray RS, Bernard CC, Oksenberg JR. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. J Immunol. 1999;163(9):5133–5144.

Table 1. Baseline characteristics of anti-MAG, WM and IgM-MGUS

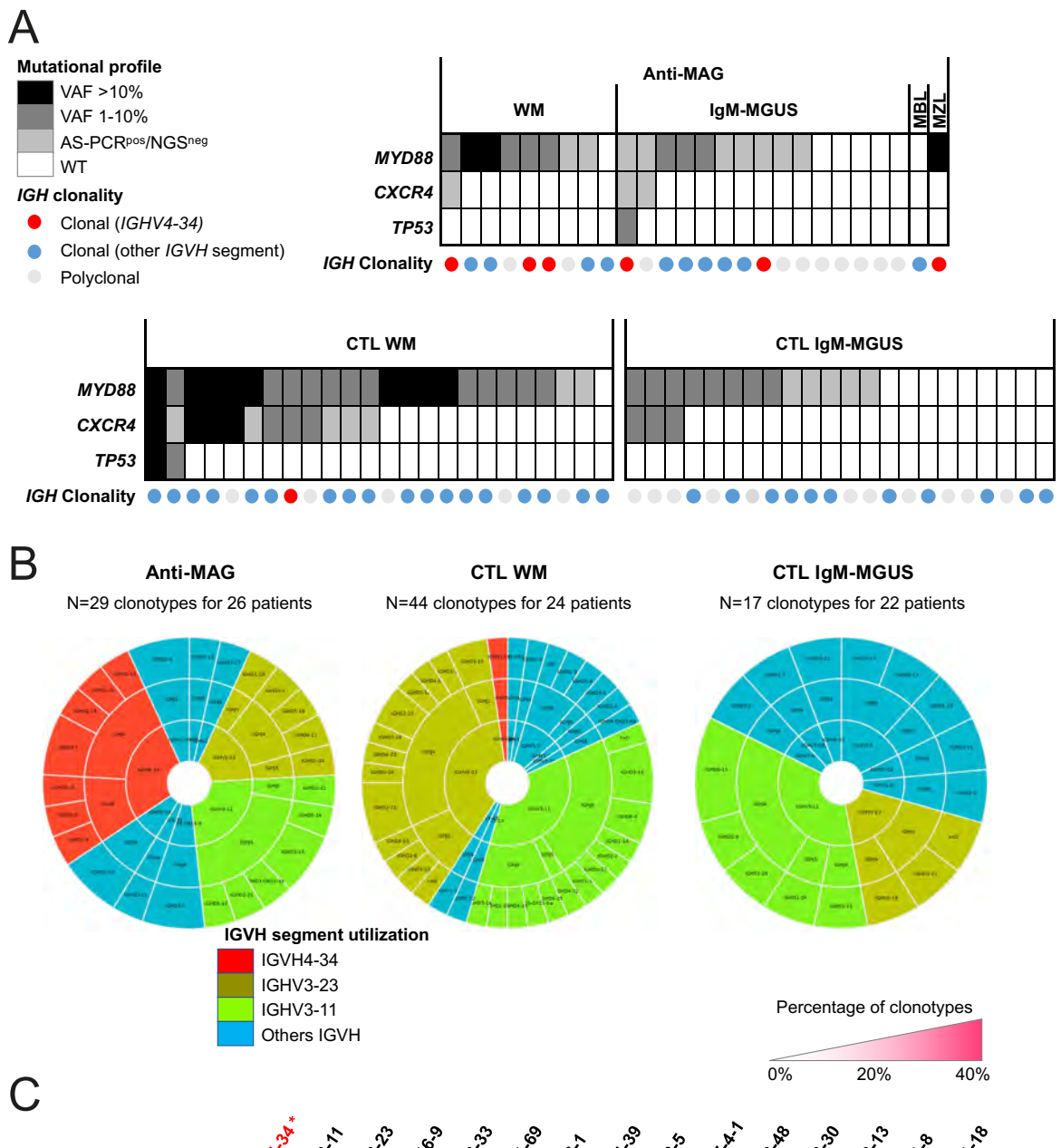
Characteristics	Anti-MAG group (n=26)	WM control group (n=24)	IgM-MGUS control group (n=22)
Male sex - n (%)	18 (69.2%)	14 (58.3%)	14 (63.6%)
Median age (IQR) – year	61 (58-71)	75 (60 - 78)	68 (63-79)
Median anti-MAG titers (IQR) – BTU	43666 (21206->70 000)	-	-
Symptoms - n (%)			
Paresthesia / Dysesthesia	21 (80.8%)	-	-
Sensory deficit	20 (76.9%)	-	-
Motor deficit	6 (23.1%)	-	-
Ataxia	11 (42.3%)	-	-
Pain	7 (26.9%)	-	-
Median monoclonal protein (IQR) - g/L	6.4 (2.9-18.7)	12.3 (2.8-25.8)	2.6 (1.0-7.9)
Light chain isotype† - n (%)			
Kappa	21 (80.8 %)	20 (83.3%)	18 (81.8%)
Lambda	6 (23.1%)	4 (17.4%)	4 (18.2%)
Median hemoglobin level (IQR) - g/dL	14.0 (13.1-14.6)	11.5 (9.5-13.3)	13.4 (11.0-14.5)
Median platelet count (IQR) – 10^9/L	232 (209-283)	188 (116-272)	239 (209-292)
Median neutrophil count (IQR) - 10^9/L	3.5 (2.6-4.5)	3.9 (2.6-5.0)	4.0 (3.1-4.9)
Median lymphocyte count (IQR) - 10^9/L	1.7 (1.5-2.2)	1.6 (1.2-2.9)	1.6 (1.0-2.0)
Median bone marrow infiltrate (IQR) -%	1 (<1-12)	37.5 (13.8-80)	1 (<1-2)
Somatic mutations – n (%)			
MYD88^{L265P}	19 (73.1%)	23 (95.8%)	13 (59%)
CXCR4	3 (11.5%)	12 (50.0%)	3 (13.6%)
TP53	1 (3.8%)	2 (8.3%)	0 (0.0%)
Detection of clonality with HTS			
Positive patients– n (%)	17 (65.4%)	19 (79.2%)	11 (50%)
IGVH segment utilization			
VH4-34 – n (% of patients)	6 (23.1%)	1 (4.2%)	0 (0.0%)
VH4-34 – n (% of clonotypes)	7 (24.1%)	1 (2.3%)	0 (0.0%)

MAG : myelin associated glycoprotein ; WM : Waldenström macroglobulinemia ; IgM-MGUS: monoclonal gammopathy of undetermined significance; IQR : interquartile range ; BTU : Buhlmann titer units; HTS : high throughput sequencing

† One patient in the anti-MAG group has biclonal IgM Kappa and Lambda gammopathy.

‡ Bone marrow infiltrate was quantified by flow cytometry.

Figure 1: Genomic profile of anti-MAG neuropathy. (A) Comparison of *MYD88, CXCR4,* and *TP53* gene mutational status in anti-MAG goup, WM and IgM-MGUS control groups. All patients have been tested both with HTS and AS-PCR for $MYD88^{L265P}$ and $CXCR4^{S338X}$. Below, results of clonality assessment with *IGH* gene rearrangement sequencing revealed an over-representation of *VH4-34* segments (red circles) in the anti-MAG group (p=0.008). (B) Sunburst representation of VDJ segments utilization in the dominant clonotypes identified in the anti-MAG, the WM and the IgM-MGUS group (C) Heatmap showing the *IGVH* segment utilization frequency (% of dominant clonotypes) in anti-MAG samples, compared to WM and IgM-MGUS control groups. * indicates a significant *VH4-34* over-representation in anti-MAG clonotypes vs WM (p=0.002) and IgM-MGUS clonotypes (p=0.019).


A
Mutational profile
VAF >10%
VAF 1-10%
AS-PCRpos/NGSneg
WT
IGH clonality
Clonal (IGHV4-34)
Clonal (other IGVH segment)
Polyclonal
Anti-MAG
WM
IgM-MGUS
MBL
MZL
MYD88
CXCR4
TP53
IGH Clonality
CTL WM
CTL IgM-MGUS
MYD88
CXCR4
TP53
IGH Clonality
B
Anti-MAG
N=29 clonotypes for 26 patients
CTL WM
N=44 clonotypes for 24 patients
CTL IgM-MGUS
N=17 clonotypes for 22 patients
IGVH segment utilization
IGVH4-34
IGHV3-23
IGHV3-11
Others IGVH
Percentage of clonotypes
0%
20%
40%
C
IGVH4-34 *
IGVH3-11
IGVH3-23
IGVH16-9
IGVH3-33
IGVH1-69
IGVH6-1
IGVH4-39
IGVH2-5
IGVH7-4-1
IGVH3-48
IGVH3-30
IGVH3-13
IGVH1-8
IGVH1-18
Anti-MAG
CTL WM
CTL IgM-MGUS

Supplemental Table S1: Somatic mutations and *IGH* clonotypes description for each patient included in the study

Group	Case	Underlying hemopathy	BM infiltrate (% by FCM)	*MYD88* variant (VAF%)	*CXCR4* variant (VAF%)	*TP53* variant (VAF%)	Number of clonotypes	*IGH* clonotypes description and frequency (% of total reads)	
Anti-MAG	#1	WM	4	L265P (7%)	S338X *	WT	3	IGHV2-5/IGHJ4 (48.4%)	**IGHV4-34/IGHJ4 (15.5%)**
	#2	WM	70	L265P (19%)	WT	WT	2	IGHV3-23/IGHJ4 (81.2%)	IGHV3-11/IGHJ4 (1.6%)
	#3	WM	45	L265P (41%)	WT	WT	1	IGVH3-23/IGJH5 (50.8%)	
	#4	WM	25	L265P (7%)	WT	WT	1	IGHV3-23/IGHJ4 (70.2%)	
	#5	WM	15	L265P (5%)	WT	WT	0		
	#6	WM	12	L265P (4%)	WT	WT	2	IGHV3-11/IGHJ5 (5.6%)	**IGHV4-34/IGHJ4 (1.3%)**
	#7	WM	10	L265P *	WT	WT	2	IGHV1-69/IGHJ4 (6.9%)	**IGHV4-34/IGHJ6 (5.9%)**
	#8	WM	10	L265P *	WT	WT	0		
	#9	WM	12	WT	WT	WT	2	IGVH3-11/IGJH4 (25.3%)	IGVH3-33/IGJH4 (14.2%)
	#10	IgM-MGUS	0	L265P *	S338X *	R248Q (3%)	1	IGVH3-23/IGJH4 (1.0%)	
	#11	IgM-MGUS	0	L265P *	S338X *	WT	2	IGHV3-23/IGHJ3 (7.4%)	**IGHV4-34/IGHJ6 (3.2%)**
	#12	IgM-MGUS	5	L265P (3%)	WT	WT	0		
	#13	IgM-MGUS	0	L265P (3%)	WT	WT	1	IGVH3-11/IGJH4 (8.9%)	
	#14	IgM-MGUS	0	L265P (1%)	WT	WT	1	IGHV4-39/IGHJ4 (43.9%)	
	#15	IgM-MGUS	5	L265P *	WT	WT	2	IGHV6-1/IGHJ6 (16.3%)	IGHV3-11/IGHJ4 (8.7%)
	#16	IgM-MGUS	0	L265P *	WT	WT	1	IGHV3-11/IGHJ4 (32.6%)	
	#17	IgM-MGUS	0	L265P *	WT	WT	1	IGVH3-11/IGJH4 (1.2%)	
	#18	IgM-MGUS	0	L265P *	WT	WT	1	**IGHV4-34/IGHJ6 (22.0%)**	
	#19	IgM-MGUS	0	L265P *	WT	WT	0		
	#20	IgM-MGUS	0	WT	WT	WT	0		
	#21	IgM-MGUS	0	WT	WT	WT	0		
	#22	IgM-MGUS	0	WT	WT	WT	0		
	#23	IgM-MGUS	0	WT	WT	WT	0		
	#24	IgM-MGUS	0	WT	WT	WT	0		
	#25	MZL	55	L265P (20%)	WT	WT	2	IGHV3OR16-9/IGHJ4 (10,5%)	IGHV3-23/IGHJ4 (3,5%)
	#26	MBL	2	WT	WT	WT	2	**IGHV4-34/IGHJ4 (30.1%)**	**IGHV4-34/IGHJ4 (5.3%)**
CTL WM	#27	-	45	L265P (25%)	S338X (20%)	L206fs (23%)	1	IGHV3-11/IGHJ4 (77.5%)	
	#28	-	nd	L265P (3%)	S338X *	R259M (3%)	3	IGHV3-11/IGHJ6 (8.2%)	IGHV3-23/IGHJ4 (6.6%)

	#29	-	70	L265P (39%)	S338X (38%)	WT	3	IGHV3-7/IGHJ6 (7.9%)	IGHV3-23/IGHJ4 (7.2%)
	#30	-	80	L265P (23%)	T318fs (29%)	WT	1	IGHV3-23/IGHJ4 (88.8%)	
	#31	-	95	L265P (21%)	S338fs (21%)	WT	0		
	#32	-	95	L265P (14%)	S338X *	WT	2	IGHV3-11/IGHJ4 (70.2%)	IGHV3-23/IGHJ5 (4.3%)
	#33	-	10	L265P (6%)	p.S338X (5%)	WT	2	IGHV3-13/IGHJ6 (6.7%)	IGHV3-7/IGHJ5 (5.6%)
	#34	-	95	L265P (6%)	p.S338X (6%)	WT	3	IGHV3-11/IGHJ4 (29.1%)	**IGHV4-34/IGHJ5 (4.4%)**
	#35	-	80	L265P (4%)	p.S338X (4%)	WT	0		
	#36	-	15	L265P (9%)	S338X *	WT	3	IGVH3-23/IGJH4 (18.7%)	IGVH1-18/IGJH4 (2.3%)
	#37	-	40	L265P (7%)	S338X *	WT	2	IGHV3-30/IGHJ4 (16.0%)	IGHV3-48/IGHJ4 (4.9%)
	#38	-	5	L265P (5%)	S338X *	WT	0		
	#39	-	80	L265P (43%)	WT	WT	1	IGHV3-23/IGHJ4 (76.5%)	
	#40	-	60	L265P (27%)	WT	WT	3	IGVH3-7/IGJH6 (74.1%)	IGVH3-11/IGJH6 (4.5%)
	#41	-	25	L265P (14%)	WT	WT	2	IGVH3-23/IGJH5 (19.2%)	IGVH3-23/IGJH5 (5.3%)
	#42	-	20	L265P (14%)	WT	WT	2	IGHV3-23/IGHJ4 (6.0%)	IGHV3-23/IGHJ4 (4.3%)
	#43	-	10	L265P (9%)	WT	WT	1	IGHV1-8/IGHJ6 (6.3%)	
	#44	-	35	L265P (8%)	WT	WT	0		
	#45	-	nd	L265P (8%)	WT	WT	1	IGHV3-23/IGHJ2 (4.5%)	
	#46	-	30	L265P (7%)	WT	WT	3	IGVH3-11/IGJH6 (3.1%)	IGVH3-11/IGJH5 (1.7%)
	#47	-	20	L265P (6%)	WT	WT	2	IGHV6-1/IGHJ4 (2.3%)	IGHV3-11/IGHJ4 (2.0%)
	#48	-	10	L265P *	WT	WT	0		
	#49	-	50	L265P *	WT	WT	1	IGHV3-11/IGHJ4 (29.3%)	
	#50	-	10	WT	WT	WT	3	IGHV3-11/IGHJ6 (80.8%)	IGHV3-11/IGHJ6 (5.3%)
	#51	-	0	L265P (6%)	S338X (2%)	WT	0		
	#52	-	0	L265P (6%)	S338X (14%)	WT	0		
	#53	-	1	L265P (4%)	R334X (3%)	WT	0		
	#54	-	0	L265P (10%)	WT	WT	1	IGVH3-11/IGJH6 (2.2%)	
	#55	-	0	L265P (4%)	WT	WT	0		
CTL IgM-MGUS	#56	-	2	L265P (3%)	WT	WT	2	IGVH3-23/IGJH6 (6.2%)	IGVH2-5/IGJH6 (4.8%)
	#57	-	0	L265P (2%)	WT	WT	0		
	#58	-	1	L265P (2%)	WT	WT	1	IGVH5-51/IGJH6 (20.4%)	
	#59	-	nd	L265P *	WT	WT	0		
	#60	-	0	L265P *	WT	WT	0		
	#61	-	0	L265P *	WT	WT	1	IGVH3-11/IGJH5 (2.7%)	
	#62	-	nd	L265P *	WT	WT	2	IGVH3-23/IGJH4 (19.0%)	IGVH3-1/IGJH4 (1.8%)

#63	-	0	L265P *	WT	WT	1	IGVH2-5/IGJH5 (31.1%)	
#64	-	0	WT	WT	WT	2	IGVH16-9/IGJH4 (3.5%)	IGVH1-8/IGJH4 (1.7%)
#65	-	1	WT	WT	WT	0		
#66	-	nd	WT	WT	WT	2	IGVH3-11/IGJH4 (14.2%)	IGVH3-11/IGJH4 (2.3%)
#67	-	0	WT	WT	WT	0		
#68	-	0	WT	WT	WT	0		
#69	-	1	WT	WT	WT	1	IGVH4-39/IGJH4 (9.6%)	
#70	-	0	WT	WT	WT	0		
#71	-	0	WT	WT	WT	1	IGVH7-4/IGJH4 (20.2%)	
#72	-	0	WT	WT	WT	2	IGVH2-5/IGJH6 (6.3%)	IGVH3-23-IGJH6 (4.2%)

BM: bone marrow, FCM: flow cytometry, MBL: monoclonal B-cells lymphocytosis, MZL: marginal zone lymphoma, MGUS: monoclonal gammopathy of undetermined significance, WM: waldenstrom macroglobulinemia, WT: wild type, nd: not done

* indicates variant only detected with allele specific PCR