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Mutational Profile in 75 Patients With Anti–Myelin-Associated Glycoprotein Neuropathy

Clinical and Hematologic Therapy Response and Hints on New Therapeutic Targets

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Abstract

Background and Objectives

Neuropathy with antibodies to myelin-associated glycoprotein (MAG) is the most common paraproteinemic IgM neuropathy. Recently, the mutational profile of the *MYD88* and *CXCR4* genes has been included in the diagnostic workup of IgM monoclonal gammopathies. The objective of our study was to assess the prevalence of $MYD88^{L265P}$ and $CXCR4^{S338X}$ gene variants in patients with anti-MAG antibody neuropathy. Secondary aims were to evaluate possible correlations between the mutational profile and neuropathy severity, antibody titers, and treatment response.

Methods

Seventy-five patients (47 men, mean age at molecular analysis 70.8 ± 10.2 years; mean disease duration 5.1 ± 4.9 years) with anti-MAG antibody neuropathy were recruited. Among them, 38 (50.7%) had IgM monoclonal gammopathy of undetermined significance, 29 (38.7%) Waldenstrom macroglobulinemia (WM), and 8 (10.6%) chronic lymphocytic leukemia/marginal zone lymphoma/hairy cell leukemia variant. Molecular analysis was performed on DNA from the bone marrow mononuclear cells in 55 of 75 patients and from peripheral mononuclear cells in 18 of 75 patients. Forty-five patients were treated with rituximab, 6 with ibrutinib, 2 with obinutuzumab-chlorambucil, and 3 with venetoclax-based therapy. All the patients were assessed with the Inflammatory Neuropathy Cause and Treatment (INCAT) Disability Scale, INCAT Sensory Sum Score, and MRC Sum Score at baseline and follow-up. We considered as responders, patients who improved by at least 1 point in 2 clinical scales.

Results

Fifty patients (66.7%) carried the $MYD88^{L265P}$ variant, with a higher frequency in WM and naive patients (77.2% vs 33.3%, $p = 0.0012$). No patients harbored the $CXCR4^{S338X}$ variant. There were no significant differences in hematologic data (IgM levels, M protein, and anti-MAG antibody titers), neuropathy severity, or response to rituximab in *MYD88*-altered and *MYD88* wild-type patients. Nine of 11 (81.8%) patients treated with novel targeted drug, according to the *MYD88* status, responded to treatments.

Discussion

$MYD88^{L265P}$ variant has a high prevalence (66.7%) in anti-MAG antibody neuropathy representing a potential effective mutational target for Bruton tyrosine kinase inhibitors. $MYD88^{L265P}$ variant, however, does not seem to be a prognostic factor of neuropathy severity or response to rituximab. In patients not responding or becoming refractory to rituximab, a tailored therapy with new effective target therapies should be considered.

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Glossary

AS-PCR = allele-specific PCR; **BTK** = Bruton tyrosine kinase; **CLL** = chronic lymphocytic leukemia; **INCAT** = Inflammatory Neuropathy Cause and Treatment; **ISS** = INCAT Sensory Sum Score; **MAG** = myelin-associated glycoprotein; **MGUS** = monoclonal gammopathy of undetermined significance; **MZL** = marginal zone lymphoma; **WM** = Waldenstrom macroglobulinemia.

Anti–myelin-associated glycoprotein (MAG) antibody neuropathy is a chronic sensorimotor demyelinating polyneuropathy, associated with either an IgM monoclonal gammopathy of undetermined significance (MGUS) or lymphoproliferative disorder (Waldenstrom macroglobulinemia [WM], marginal zone lymphoma [MZL], and chronic lymphocytic leukemia [CLL]).[1,2] Despite being slowly progressive, the neuropathy may severely influence patients' functionality and quality of life.[3] Among possible therapies, rituximab, an anti-CD20 chimeric monoclonal antibody, remains the most used treatment efficacious in almost half of the patients and capable of improving disability scales and the response to questionnaires in the global impression of the disease.[4-8] Recently, the discovery of the mutational profile of the *MYD88* and *CXCR4* genes has radically changed the diagnostic and prognostic evaluation of IgM monoclonal gammopathies.

Specifically, $MYD88^{L265P}$ has been found to be the most common variant reported in WM and IgM-MGUS.[9] Since $MYD88^{L265P}$ interacts with nuclear factor kB signaling, it plays a crucial role in the response to ibrutinib, the first in-class inhibitor of Bruton tyrosine kinase (BTK), which acts by inhibiting the downstream signaling after the interaction between altered MYD88 protein and BTK.[10] In addition, somatic variants in the C-terminal domain of CXCR4 have been reported in WM and shown to be associated with a more aggressive disease. More important, *MYD88/CXCR4* status has been shown to be predictive of the response to ibrutinib in WM.[9]

In a prospective study, WM patients with *MYD88*-altered and *CXCR4* wild-type have been shown to have better and longer response to ibrutinib.[9] Among the 63 studied patients, 9—3 of whom with anti-MAG antibodies—had received ibrutinib for progressive IgM paraproteinemic neuropathy. All 9 patients had a response, with subjective improvement of peripheral neuropathy in 5 patients and stability in 4 patients during the treatment course.

In a subsequent study, 4 of 31 patients with WM had been treated with ibrutinib for the neuropathy: 2 remained stable and 2 had subjective improvement starting from week 9 of treatment, with subsequent complete recovery in 1 patient.[11]

Preliminary data on 20 patients with anti-MAG antibody neuropathy have shown that 60% of the patients carry the $MYD88^{L265P}$ suggesting the use of BTK inhibitors in anti-MAG polyneuropathy.[12] Accordingly, we first reported on 3 patients with WM and anti-MAG antibody neuropathy, who had a subjective, objective, and hematologic response to ibrutinib, 2 after the loss of response to rituximab.[13]

Since the response to ibrutinib strictly depends on the IgM paraprotein alteration profile, the aim of our prospective study was to assess the mutational profile of the *MYD88* and *CXCR4* genes in patients with anti-MAG antibody neuropathy, irrespective of the underlying hematologic conditions.

The results might help identify the presence of a potential mutational target for new therapies (ibrutinib, second generation BTK inhibitors or other target treatments). Moreover, we aimed at assessing possible correlations between the mutational profile of *MYD88* and *CXCR4* genes and neuropathy severity, antibody titers, and treatment response.

Methods

Clinical Evaluation

This is an observational prospective study, involving the Departments of Neurosciences of the University of Padova and University of Pisa. Inclusion criteria were clinical and neurophysiologic diagnosis of anti-MAG antibody neuropathy associated with histologically confirmed IgM monoclonal gammopathy (IgM MGUS or lymphoproliferative diseases); anti-MAG antibody titer >7,000 Bühlmann titre units (BTU).[14,15] All the patients had neurophysiologic evidence of distal acquired demyelinating symmetric neuropathy with low conduction velocities and markedly prolonged distal latencies and no conduction blocks. Patients with atypical forms of anti-MAG antibody neuropathy[16] were not included.

The primary endpoint was to identify the rate of $MYD88^{L265P}$ variant in patients with anti-MAG antibody neuropathy. Secondary aims were to evaluate the presence of $CXCR4^{S338X}$ variant, and the correlation between the mutational profile with neuropathy severity, antibody titers, and treatment response.

All the patients were assessed with the Inflammatory Neuropathy Cause and Treatment (INCAT) Disability Scale,[17] INCAT Sensory Sum Score (ISS),[18] and Medical Research Council (MRC) Sum Score (in 6 muscles, deltoid, biceps, wrist extensor, iliopsoas, quadriceps femoris, tibialis anterior, bilaterally) at baseline and after treatment. We considered as responders, patients who improved by at least 1 point in 2 clinical scales.

Table 1 Characteristics of All the Patients and According to MYD88^{L265P} Variant

Variables	All patients (n = 75)	MYD88^{L265P} (n = 50)	MYD88 wt (n = 25)	*p* Values
Male/female	47/28	32/18	15/10	0.8026
Median age (y)	70.8 ± 10.2	70.5 ± 10.5	71.3 ± 9.8	0.8026
Disease duration (y)	5.1 ± 4.9	4.0 ± 3.6	7.0 ± 6.7	0.2107
Previous rituximab	18 (24.0%)	6 (12.0%)	12 (48.0%)	0.0002
Diseases				0.0107
IgM-MGUS	38 (50.7%)	21 (42.0%)	17 (68.0%)	
WM	29 (38.7%)	26 (52.0%)	3 (12.0%)	
CLL	4 (5.3%)	2 (4.0%)	2 (8.0%)	
MZL	3 (4.0%)	1 (2.0)	2 (8.0%)	
HCLv	1 (1.3%)	0 (0.0%)	1 (4.0%)	
INCAT, median (IQR)	2.5 (1–4)	2 (1–4)	3 (1.25–4)	0.3899
ISS, median (IQR)	4 (2–6)	3 (1–6)	4.5 (2.25–5)	0.5259
MRC, median (IQR)	60 (58–60)	60 (58–60)	60 (58–60)	0.6307
M-protein (g/L)	5.2 ± 4.7	5.6 ± 4.1	4.4 ± 3.6	0.0541
IgM (g/L)	6.6 ± 5.1	7.2 ± 4.8	5.4 ± 4.7	0.0546
Anti-MAG titer (BTU/L)[a]	84.1 ± 79.2	69.4 ± 42.8	112.9 ± 109.0	0.3175

Abbreviations: BTU = Bühlmann titre units; CLL = chronic lymphocytic leukemia; HCLv = hairy cell leukemia variant; IgM MGUS = monoclonal gammopathy of undetermined significances; IQR = interquartile range; M-protein = monoclonal protein; MZL = marginal zone lymphoma; WM = Waldenstrom macroglobulinemia; wt = wild-type.
[a] The included (IgM and anti-MAG) data of previously rituximab-treated patients referred to those obtained before the new treatment.

Standard Protocol Approvals, Registrations, and Patient Consents

The study did not need ethical committee approval being the genetic assessment and treatments performed part of standard of care in the Hematological unit. Signed informed consent was obtained from all the patients.

Anti-MAG Antibody Testing

Anti-MAG antibodies were tested per standard care using a commercially available enzyme linked immunosorbent assay (Bühlmann Laboratories, Schönenbuch, Switzerland) and expressed as BTU/L.

Molecular Analysis

Molecular analysis was performed in all the 75 enrolled patients. In 57 patients on DNA extracted by an automated system (Maxwell 16, Promega Italia, Milano, Italy) from bone marrow mononuclear cells after density gradient separation by Ficoll Hypaque (Sigma-Aldrich). *MYD88^{L265P}* variants were searched by allele-specific PCR (AS-PCR), as previously described[19] with a reported sensitivity of 0.1%. For detecting the most common *CXCR4* variant, i.e., S338X, a highly sensitive AS-PCR assay was developed.[20,e3] Sanger sequencing of *CXCR4* gene (less sensitive) was still be required for both nonsense and frameshift mutations of the C-terminal domain in S338X-negative samples. In 18 patients, molecular analysis was performed from circulating mononuclear cells after density gradient separation by centrifugation. DNA was extracted from isolated cells by an EZ1Qiagen automated system. *MYD88^{L265P}* variants were searched by real time PCR "qBiomarker Somatic Mutation PCR Assay" (Qiagen, 7,900 Applied Biosistem) in combination with amplification refractory mutation system PCR.[21]

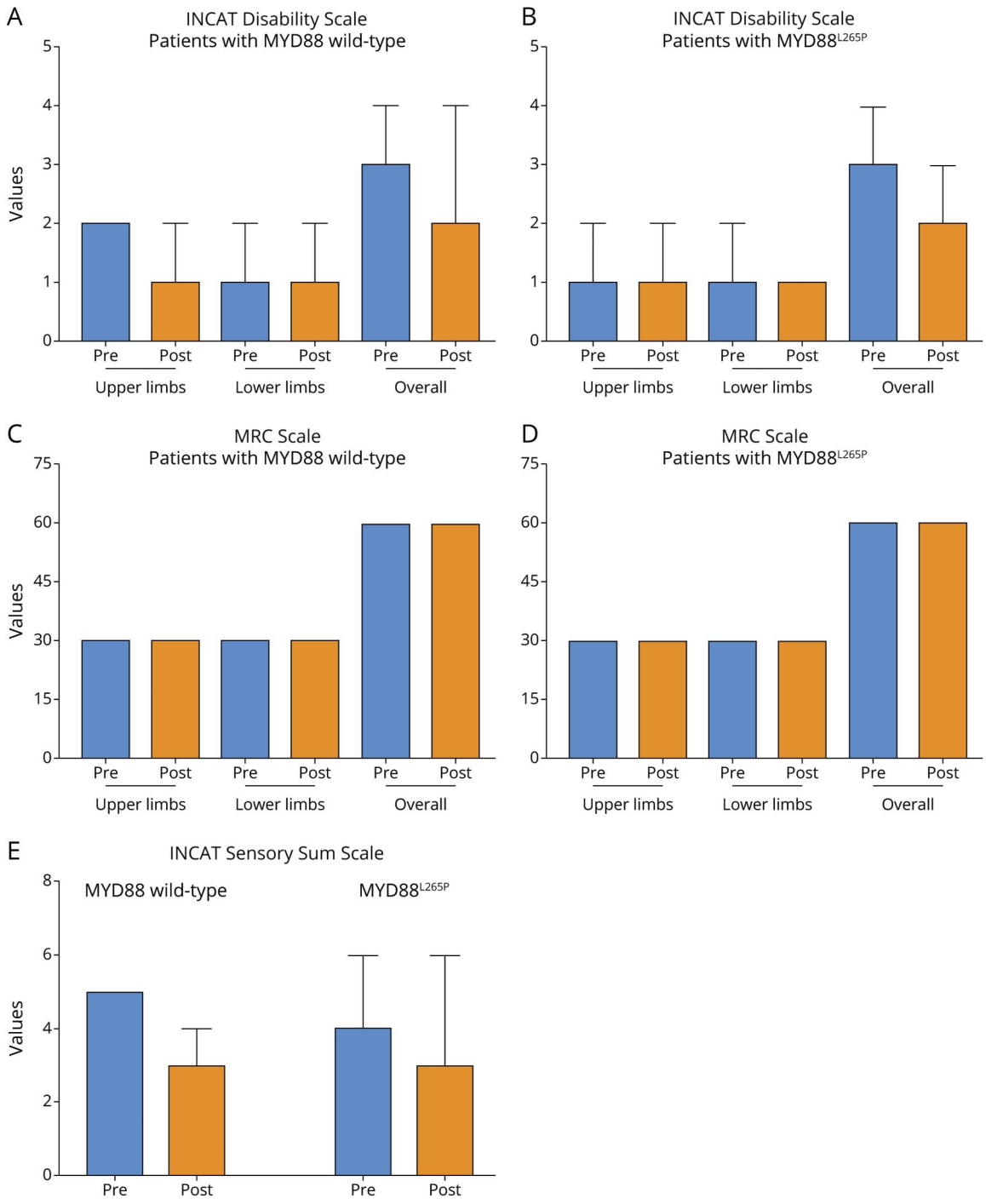
Treatments

Forty-five patients were treated with rituximab (375 mg/m^2 IV weekly for 4 consecutive weeks), 6 with ibrutinib (420 mg daily, orally), 2 with obinutuzumab-chlorambucil (obinutuzumab was given IV at 100 mg on day +1, 900 mg on day +2, then at 1,000 mg on day 8 and 15 of cycle 1 and day 1 of cycles 2–6; chlorambucil orally at 0.5 mg/kg at day 1 and 15 of cycles 1–6), and 3 patients were treated with venetoclax-based therapy (venetoclax 400 mg/d orally after a lead-in weekly ramp-up phase) including 1 with rituximab after the ramp-up phase, at 375 mg/m^2 for the second month and then monthly at 500 mg/m^2 for months 3–7, 1 with obinutuzumab (at the above reported doses) and 1 single agent.

Data Availability

Anonymized data not published within this article will be made available on reasonable request from any qualified investigator.

Figure 1 Assessment of INCAT, ISS, and MRC Scales Before and After Treatment With Rituximab



In the upper panels, the INCAT Disability Scale is applied in patients with MYD88 wild-type (A) and MYD88-altered (B). The Dunn multiple comparison test was applied to upper limbs, lower limbs, or both limbs (each comparison had a $p > 0.05$). In the middle panel, the MRC muscle scale is applied in patients with MYD88 wild-type (C) and MYD88-altered (D). The Dunn multiple comparison test was applied to upper limbs, lower limbs, or both limbs (each comparison had $p > 0.05$). In the lower panels (E), the INCAT Sensory Scale (ISS) is applied in patients with MYD88 wild-type and MYD88-altered. The Dunn multiple comparison test was applied to upper limbs, lower limbs, or both limbs (each comparison had $p > 0.05$). All data are reported as median and interquartile range. INCAT = Inflammatory Neuropathy Cause and Treatment.

Statistical Analysis

Continuous variables were compared with the Mann-Whitney test, while categorial variables with the Fisher exact test or χ^2 test. Comparison of neurologic scales was compared with Kruskal-Wallis tests.

Results

We enrolled 75 consecutive patients, 47 men and 28 women, with a mean age at the time of molecular analysis of 70.8 ± 10.2 years, and mean disease duration of anti-MAG antibody

neuropathy at the time of molecular analysis of 5.1 ± 4.9 years (Table 1). Of them, 38 (50.7%) had IgM-MGUS, 29 (38.7%) WM, 4 (5.3%) CLL, 3 (4.0%) MZL, and 1 (1.3%) hairy cell leukemia-variant. All the 75 patients were assessed for *MYD88*^{L265P} variant, while 47 of 75 patients were assessed for *CXCR4*^{S338X} variant, by AS-PCR as mentioned above. Although none of the tested patients harbored the *CXCR4* variant, 50 of 75 patients (66.7%, 32 men, mean age 70.5 ± 10.5 years, mean disease duration 4.0 ± 3.6 years) carried the MYD88^{L265P} variant. Conversely, 25 of 67 patients (33.3%, 15 men, mean age 71.3 ± 9.8 years, mean disease duration 7.0 ± 6.7 years) were *MYD88* wild-type. The 2 groups were homogeneous regarding sex, age, and duration of follow-up (Table 1). Considering the underlying hematologic disease, the alteration was present in 26 of 29 (89.7%) patients with WM, 21 of 38 (55.3%) IgM MGUS, and 3 of 8 (37.5%) CLL/MZL. According to the literature,[9] *MYD88*^{L265P} variant was significantly more common in patients with WM ($p = 0.0107$) (Table 1). In addition, there was no significant difference between *MYD88*-altered and *MYD88*-wild-type patients regarding anti-MAG antibody titer and neuropathy severity (Table 1). In particular, the median INCAT Disability Scale was 2 in *MYD88*-altered patients and 3 in *MYD88* wild-type patients; the median ISS was 3 in *MYD88*-altered and 4.5 in *MYD88* wild-type; the median MRC was 60 in both groups (Table 1). We found a trend for slightly higher IgM and M-protein levels in patients MYD88^{L265P} compared with wild-type patients (both $p = 0.054$, Table 1).

At the time of molecular analysis, 18 patients (6 *MYD88*^{L265P}-altered patients and 12 wild-type) had already been treated with rituximab, while 57 (44 *MYD88*^{L265P}-altered and 13 wild-type) were therapy-naive. *MYD88*^{L265P} variant was significantly more common in therapy-naive patients ($p = 0.0002$). Of the 75 enrolled patients, 44 of the 57 (77%) therapy-naive patient carried the MYD88^{L265P} variant, vs 6 of the 18 (33.3%) previously treated patients ($p = 0.0012$). In the latter group, the mean delay between treatments and molecular analyses was 5.6 ± 4.1 years (5.7 ± 4.9 years in *MYD88* wild-type, 5.2 ± 1.9 years in *MYD88*^{L265P}-altered) (eTable 1 in eAppendix 1, links.lww.com/NXI/A855).

Of the 57 therapy-naive patients, 21 *MYD88*^{L265P}-altered and 6 wild-type were treated with rituximab after molecular analysis. Overall, among the 45 patients treated with rituximab, 40 (23 *MYD88*-altered and 17 wild-type) had a follow-up of at least 12 months for evaluating the treatment response. Twenty-six of 40 (65%) were responders to treatment, including 14 of 23 (61%) *MYD88*-altered and 12 of 17 (70.6%) wild-type ($p = 0.7385$). Detailed analysis of the adopted clinical scales is reported in Figure 1. Among the 18 patients genetically tested after treatment with rituximab, 14 (18.7% of all patients) needed additional cycles because of relapse: 5 of 50 (10.0%) *MYDD88*-altered and 9 of 25 (36.0%) wild-type ($p = 0.0109$).

Six patients (all with WM, *MYD88*^{L265P}-altered and *CXCR4* wild-type, previously treated with rituximab with lack or loss of benefit) were treated with ibrutinib 420 mg/d orally, with early and persistent clinical benefit as shown by improvement in clinical scales (Table 2). We now have a longer follow-up of the first 3 treated and described patients[13] showing a maintenance of the response up to 36 months.

Three additional patients have been treated with ibrutinib. One patient (4, Table 2) was a 79-year-old man with anti-MAG antibody neuropathy and WM, who was treated with rituximab in September 2020 with only transient (3 months) benefit. For worsening of the gait stability and several falls, the patient was started on ibrutinib in June 2021. At neurologic evaluation 6 months later, the patient reported reduced hypoesthesia and improved motility at feet, and absence of falls. Unfortunately, the patient, who had been suffering for several years from severe depression, died 2 months later of unrelated causes.

The fifth patient (5, Table 2) was a 76-year-old man previously (2014–2015) treated with benefit with rituximab and bendamustine for anti-MAG antibody neuropathy in WM. From 2019, the gait instability worsened forcing the patient to use unilateral support for walking; he also developed inability to botton and perform precision tasks (INCAT lower limbs 2, upper limbs 3). An additional rituximab cycle (September 2020) had no benefit. The patient was started (June 2021) on ibrutinib with no amelioration, but clinical stability was achieved.

The sixth patient (6, Table 2) was a 46-year-old man who complained of distal sensory loss (toes and fingers) with neurophysiologic evidence of diffuse symmetric demyelinating polyneuropathy. Three years earlier, he was accidently found to carry an IgM/k (0.59 g/L) monoclonal gammopathy. Bone marrow biopsy showed the presence of *MYD88*-altered and *CXCR4* wild-type WM. Anti-MAG antibody was positive (140.000 BTU). For worsening of symptoms at lower limbs (with the onset of ataxic gait) and occurrence of symptoms at the hands (INCAT 1 + 1), the patient was treated with rituximab with subjective worsening, despite neurologically he was unchanged. Ten months later, however, a further worsening of sensory symptoms and onset of stepping gait occurred, so the patient was started on ibrutinib with arrest of deterioration. Neurologic evaluation 12 months after the beginning of ibrutinib showed a mild improvement (INCAT 0 + 1).

In all the 6 patients, treatment with ibrutinib was well tolerated. Interesting enough, M-protein and IgM levels showed a constant decrease in all the 6 patients, while anti-MAG antibody titers showed a fluctuating pattern, despite clinical improvement (Table 2).

Two therapy-naive patients with CLL received obinutuzumab-chlorambucil. Patient 7 was MYD88 wild-type, whereas patient 8 was *MYD88*^{L265P}-altered; both were *CXCR4* wild-type. Both patients had improvement in clinical scales and hematologic data

Table 2 Neurologic Scales and Biochemical Parameters in Patients Treated With Ibrutinib, Obinutuzumab, or Venetoclax

		INCAT Disability Score (U.E. + L.E.)	ISS	MRC Sum Score	M-protein (g/L)	IgM (g/L)	Anti-MAG titer (BTU/L)
Ibrutinib							
Patient 1	Baseline	2 + 2	8	53	6.50	7.2	52.9
	6 mo	2 + 1	5	55	5.40	4.3	67.9
	12 mo	2 + 1	5	55	4.30	4.0	60.4
	24 mo	2 + 1	4	55	3.25	3.5	>70.0
	36 mo	2 + 1	4	55	3.4	3.6	>70.0
Patient 2	Baseline	4 + 4	9	51	7.20	13.7	>70.0
	6 mo	3 + 3	6	53	3.30	6.6	>70.0
	12 mo	3 + 3	6	54	2.8	6.4	68.0
	24 mo	3 + 3	6	55	1.73	6.1	>70.0
Patient 3	Baseline	0 + 1	5	60	10.60	15.9	51.5
	6 mo	0 + 1	3	60	5.60	8.8	>70.0
	12 mo	0 + 0	3	60	3.70	7.7	>70.0
	24 mo	0 + 0	3	60	2.80	7.2	>70.0
Patient 4	Baseline	1 + 1	5	59	1.90	1.88	49.1
	6 mo	0 + 0	4	60	0.94	1.27	47.3
Patient 5	Baseline	3 + 2	6	54	24.8	31.8	>70.0
	6 mo	3 + 2	6	54	12.4	19.7	>70.0
	12 mo	3 + 2	6	55	8.00	13.4	>70.0
Patient 6	Baseline	1 + 1	4	58	6.01	10.6	140.0
	6 mo	1 + 1	4	58	5.73	9.78	>70.0
	12 mo	0 + 1	2	58	5.61	9.54	48.6
Obinutuzumab							
Patient 7	Baseline	1 + 4	6	54	15.80	14.8	>70.0
	6 mo	1 + 3	3	56	8.40	6.7	68.3
Patient 8	Baseline	1 + 1	3	60	1.10	1.05	>70.0
	6 mo	0 + 0	0	60	0.00	0.60	5.4
	12 mo	0 + 0	0	60	0.00	0.62	4.7
	24 mo	0 + 0	0	60	0.00	0.63	4.8
	36 mo	0 + 0	0	60	0.00	0.60	3.9
	48 mo	0 + 0	0	60	0.00	0.59	4.3
Venetoclax							
Patient 9	Baseline	3 + 0	3	60	7.36	10.8	>70.0
	4 mo	1 + 0	1	60	4.40	6.6	10.3
Patient 10	Baseline	3 + 3	5	60	0.85	1.16	18.5
	12 mo	1 + 1	1	60	0.00	0.46	8.74
	24 mo	0 + 1	1	60	0.00	0.44	3.71

Continued

Table 2 Neurologic Scales and Biochemical Parameters in Patients Treated With Ibrutinib, Obinutuzumab, or Venetoclax *(continued)*

		INCAT Disability Score (U.E. + L.E.)	ISS	MRC Sum Score	M-protein (g/L)	IgM (g/L)	Anti-MAG titer (BTU/L)
Patient 11	Baseline	2 + 1	4	59	2.37	4.72	>70.0
	6 mo	2 + 1	3	59	0.00	3.97	>70.0
	9 mo	1 + 1	1	59	0.00	3.07	>70.0

Abbreviation: INCAT = Inflammatory Neuropathy Cause and Treatment; ISS = INCAT Sensory Sum Score.

(Table 2). Unfortunately, patient 7 developed grade 4 neutropenia, and patient 8 died of pneumonia 2 months after the end of treatment. At the last follow-up after 4 years from therapy, patient 7 persisted improved (INCAT 0); also neurophysiologic evaluation had ameliorated compared with baseline.[22] Three patients were treated with venetoclax alone or in combination with anti-CD20 monoclonal antibody (Table 2).

Patients 9 was an elderly man with untreated CLL and concurrent anti-MAG antibody neuropathy with severe impairment at upper limbs (distal tremor, sensory loss, and incapability of writing and buttoning without help; INCAT upper limbs 3), while gait was quite preserved. The patient had *MYD88*-altered and *CXCR4* wild-type. He was treated with venetoclax-obinutuzumab. Despite a fast improvement, being able of buttoning up independently already after the first month, he developed a severe SARS-CoV-2 pneumonia during the fourth month of treatment and died (month +7).

Patient 10 was a 62-year-old woman affected by anti-MAG neuropathy and CLL, both *MYD88* and *CXCR4* wild-type, previously treated with rituximab-cyclophosphamide with only partial benefit and subsequent relapse was treated with venetoclax-rituximab, with dramatic clinical and hematologic response (Table 2). Treatment was well tolerated. Early assessment has been already reported.[23] According to the treatment schedule, venetoclax was stopped after 2 years. After 24 months, the patient continues to show a clinical response with further improvement at upper limbs (no longer tremor, the patient is now capable of buttoning up, she regained the capability of knitting and using the computer keyboard).

Patient 11, a 74-year-old man, with a long history of anti-MAG antibody neuropathy and MGUS IgM, had been treated with 4 cycles of rituximab (2 in 2007, 1 in 2008, and the latest in 2010), followed by plasma-exchange, that was discontinued for side effects. When we first evaluated the patient, he had an ataxic unstable gait, left steppage. Bone marrow biopsy revealed a WM without *MYD88* and *CXCR4* alterations, so he was started treatment with venetoclax. After 6 months of follow-up, neuropathy stabilized, and at month 9, he improved in gait stability, Sensory Sum Score, and upper limbs functionality (Table 2).

In conclusion, 65% of patients were responders to rituximab treatment, regardless of *MYD88* alteration. Nine of 11 (81.8%) patients treated with novel targeted drug responded to treatments, being able to improve at least 1 point in 2 different neurologic scales. Caution is however warranted for potential adverse effects in aged patients, especially risks of infection that may be lethal. In the first report by Rakocevic et al.,[24] obinutuzumab was ineffective but safe in 2 patients with anti-MAG antibody neuropathy, whereas in our experience, obinutuzumab combined with either chlorambucil or venetoclax was effective in all the 3 patients, but burdened with serious side effects. For unknown reasons, obinutuzumab-based treatment seems to be highly toxic in patients with anti-MAG neuropathy as compared with other real-world evidence studies in patients with CLL.[25,26]

Discussion

Anti-MAG antibody neuropathy is a chronic, potentially disabling demyelinating polyneuropathy for which adequate immunotherapy is eagerly needed.[27] According to clinical trials and case reports, rituximab is effective in nearly 50% of patients, but no clear predicting factors of therapy response have so far been identified.[8] A recent systematic review on rituximab in chronic immune-mediated neuropathies showed that rituximab was effective in 48% of anti-MAG antibody neuropathy.[28] The review included 23 studies, of which 2 were randomized controlled trials, and 6 prospective and 15 retrospective studies. Neurophysiologic improvement was evident in 40% of patients with anti-MAG neuropathy.

$MYD88^{L265P}$ variant has been shown to be the most frequent alteration in patients with WM and IgM MGUS.[19] In this study, we confirm, in the largest population so far assessed, the high prevalence of the $MYD88^{L265P}$ variant in patients with anti-MAG antibody neuropathy, especially in therapy-naive patients (77%). These data are interesting because the $MYD88^{L265P}$ variant represents a potential effective mutational target for ibrutinib that has been demonstrated to be effective in patients with anti-MAG neuropathy also after failure or loss of efficacy of previous therapies.[13] $MYD88^{L265P}$ variant, however, does not seem to be a prognostic factor of neuropathy severity or response to rituximab. In our study, as

expected, we did not find a significant difference in clinical scales or in hematologic parameters, or in neurophysiologic findings (data not shown) between *MYD88*-altered and wild-type patients indeed. However, assessment of *MYD88* alteration might be useful for hematologic diagnosis and for selecting the optimal target therapy besides rituximab.

In *MYD88* wild-type patients, likely nonresponders to ibrutinib, venetoclax, an oral inhibitor of BCL2, alone or in combination with rituximab has been shown to be highly active in ibrutinib-resistant hematologic malignancies and has so far been used with benefit in a single patient with anti-MAG antibody neuropathy.[23]

Limitations of our study are the different genetic assessment in a subgroup (24%) of patients, in which *MYD88* alteration was assessed in the peripheral blood rather than from bone marrow cells. A comparative study showed a high, but not complete concordance between the peripheral blood and bone marrow tests.[29] Possible future strategies to avoid bone marrow assessment might be the use of droplet digital PCR rather than AS-PCR on peripheral blood or plasma cell-free DNA.[21,29]

Moreover, the rarest *CXCR4* variants have not been searched for in all the patients. However, data coming from the literature report that only a small percentage of patients with anti-MAG antibody neuropathy carry the *CXCR4* variation.[30] Although neurophysiologic evaluation was performed in all the patients at the diagnosis and in most also after therapy (almost 50% of patients),[13,23,31] still neurophysiologic data were not included among the criteria of response, considering that neurophysiologic assessment was often performed in different centers with different laboratory reference values. Moreover, often axonal loss at lower limbs is present before starting therapy, so neurophysiologic improvement may be difficult to be demonstrated if not in the more spared regions (e.g., upper limbs).[4]

In conclusion, the mutational analysis of IgM-paraprotein associated with anti-MAG antibody neuropathy has revealed that $MYD88^{L265P}$ is the most frequent variant, opening new avenues to potential efficacious therapy, especially in patients who are not responsive or become refractory or might be unfit to rituximab, due to comorbidities. Currently, rituximab should be the first-line therapy in patients with anti-MAG antibody neuropathy, with the awareness that efficacy may occur up to 6 months from the administration.[7]

Genetic assessment should therefore be performed in patients with anti-MAG antibody neuropathy to help identify possible mutational targets for potential new therapies (ibrutinib, second-generation BTK inhibitors or other target treatments) for the most common and often disabling paraproteinemic neuropathy. In our center, genetic assessment is routinely performed on bone marrow cells because this compartment showed a higher sensibility than peripheral blood assessment. However, ongoing studies are evaluating the new role of non–invasive serum cell-free DNA in IgM monoclonal gammopathies.[29]

The lower rate of $MYD88^{L265P}$ alteration in rituximab-relapsed patients poses some questions because we do not know if *MYD88* wild-type patients might have a higher relapsed rate than MYD88-altered, despite a similar response rate. Furthermore, it is not known whether rituximab might eradicate MYD88-altered cells favoring the occurrence of a *MYD88* wild-type B-cell clone. Therefore, prospective sequential studies are warranted.

In conclusion, rituximab is still the standard of care for the treatment of anti-MAG antibody neuropathy because of the presence of prospective clinical trials and the longer follow-up.

In patients who relapse after rituximab, if not done at diagnosis, we suggest to evaluate *MYD88* alteration and to tailor the next treatment based on the genetic results, considering ibrutinib for $MYD88^{L265P}$-altered patients and venetoclax for *MYD88* wild-type cases. In addition, rituximab-responsive patients may benefit also from retreatment in case of a late relapse.[32]

Caution should be used in elderly patients with obinutuzumab-based therapy especially for potentially life-threatening infections. The availability of new more selective BTK inhibitors, such zanubrutinib, with a safer profile and activity also in *MYD88* wild-type patients, further support the use of target therapies in patients with anti-MAG neuropathy.[33,34]

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