1.

VEGF and IL-6 as markers of severity and response to treatment in Waldenstrom Macroglobulinemia and IgM-monoclonal gammopathy of undetermined significance: a monocentric experience.

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Background: Angiogenesis plays an important role in the development and the maintenance of hematolymphoid malignancies. Serum levels of angiogenic cytokines, such as vascular endothelial growth factor (VEGF) and interleukin 6 (IL-6), are known to be increased in patients with Waldenstrom Macroglobulinemia (WM) and IgM monoclonal gammopathy of undetermined significance (MGUS). Even if the role of IL-6 and VEGF as markers of disease status and severity has been recognized, only a limited number of studies have addressed the role of cytokines in WM and there is no proof about any correlation between their serum levels in WM and IgM-MGUS patients. Nevertheless, their dosage at various stages of the disease aims to understand if they have a comparable pattern, and therefore they could be used in clinical practice for disease monitoring.

Aims: This study aims to find a correlation of serum levels of VEGF and IL-6 with the burden of disease in our patients with IgM-MGUS and WM and to evaluate a possible relationship between the two angiogenic cytokines, in an attempt to identify potential markers of severity.

Methods: We performed a retrospective monocentric study of 18 patients (ten with WM and eight with IgM-MGUS) with a median age of 73 years (range: 52-90), whose 66% were male. Patients with WM were at different stages of disease (four were naive, four in remission, two relapsed/refractory). A total of 18 controls with a similar age and gender distribution were also tested. Controls were selected among patients with neuropathies in the absence of any hematological condition. We used the 1:1 ratio for the selection of controls: for every male or female WM patient, one control of the same gender and of similar age was included. In order to find a relationship of VEGF and IL-6 with a laboratory parameter, IgM levels dosage for each patient have been measured at the same time-point of the measurement of the cytokines. In all patients and controls, we evaluated serum levels of VEGF and IL-6 using Immunoassay panels (i.e., anti-IL-6 and anti-VEGF ELISA kit).

Results: All patients showed elevated values of angiogenic cytokines when compared with controls. **(P value < 0.05).** Both VEGF and IL-6 serum levels (median values: 623,5 pg/mL and 4,5 pg/mL, respectively; ranges: 113,4-1981 pg/mL and 0,3-21,9 pg/mL, respectively) showed a positive correlation with IgM serum levels (median value: 2055 mg/dL, range: 300-6123 pg/mL) **(Fig. 1a and 1b)**. Furthermore, the relationship between IL-6 and VEGF was statistically significant and could be explained by a linear model **(P value = 0,007) (Fig. 1c)**. No statistically significant differences were observed between the different phases of WM.

Conclusions: Evidence from our sample shows that serum levels of angiogenic cytokines as VEGF and IL-6 have a comparable pattern with IgM levels. Therefore, it seems reasonable to suggest that they could be used in clinical practice as markers of severity of disease and response to treatment. Moreover, since the cytokines profiles are comparable, IL-6 can be used interchangeably with VEGF, allowing the monitoring of the disease with easily availability and unexpensive techniques.

Figure 1a) IgM values and VEGF levels correlation in IgM-MGUS and WM patients; **1b)** IgM values and IL-6 levels correlation in IgM-MGUS and WM patients; **1c)** IL-6 and VEGF levels correlation in WM and IgM-MGUS patients.

Figure 1a)

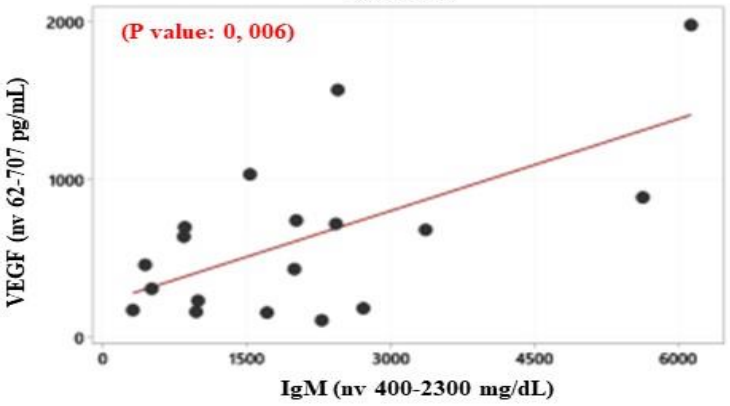


Figure 1b)

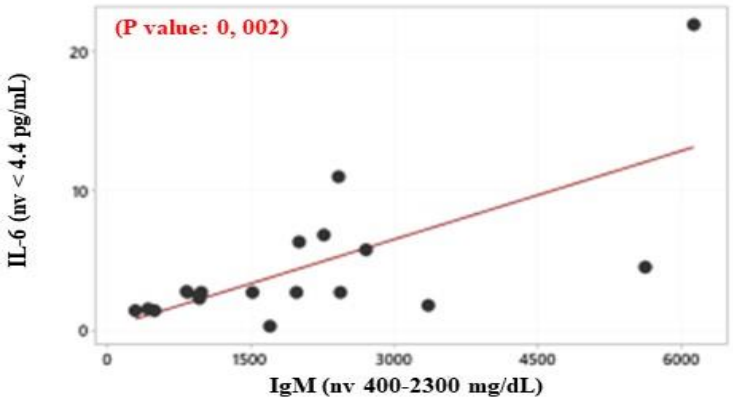
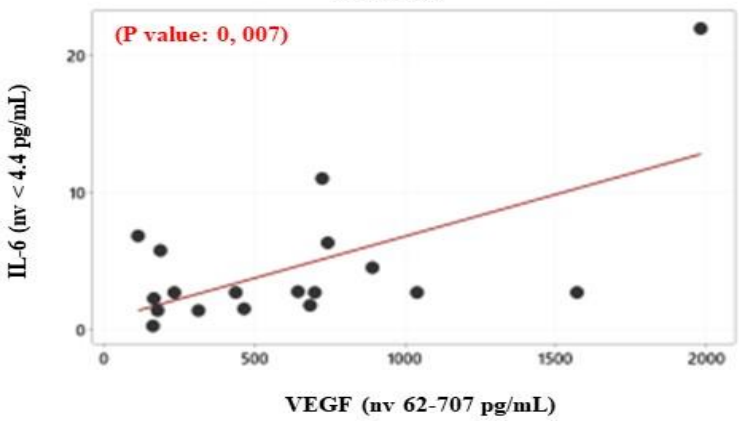


Figure 1c)



2.

The subsequent mortality of patients with Waldenstrom macroglobulinemia who are progression-free after initiation of first-line immunochemotherapy may be similar to that of the general population

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In patients with symptomatic Waldenström macroglobulinemia (sWM), the prognosis is assessed with the International Prognostic Scoring System for WM or its revised version, *MYD88* and *CXCR4* mutational status, among others abnormalities, may also influence the outcome in this setting. Later during the clinical course, we recently showed that the difference in subsequent outcomes between patients with early progression (within the first 24 months) and patients who were progression-free at 24 months was mainly related to the prolonged subsequent progression-free survival (PFS) of the latter patients (Hematol Oncol. 2022 Apr 6. doi: 10.1002/hon.2996). Therefore, we sought to check the standardized mortality ratio (SMR) of those patients who achieved a prolonged PFS after first line therapy.

The filo database for symptomatic WM merges the data of 11 available local databases, according to the French legislation on observational studies of routine clinical practice and the methodology specified by the French National Data Protection Commission. Although these databases had been set-up at different date (between 1990 and 2012), they all enrolled consecutive patients. All patients gave their informed consent. We used the updated version of the survexp.fr package in R, release 4.0.2 (The R foundation for Statistical Computing, Vienna, Austria). Appropriate

follow-up (at least until 2020) was available for 325 consecutive patients from 9 centers. In a first step, we focused our study on the 214 patients who received immunochemotherapy frontline between 2002 and 2018. Median age was 69.8 years (Interquartile range: 62-77), M/F=1.87, median survival after first treatment initiation: 162 months (95% confidence interval [95CI]: 162-NR), 19 deaths were recorded, median PFS was 52 months (95CI: 42-65). International prognostic index (IPSSWM) was low, intermediate and high in 15, 42 and 43% of patients respectively. The revised IPSSWM was very-low, low, intermediate, high and very high in 22, 24, 20, 18 and 16% of patients respectively. *Myd88* was mutated in 92% of the 84 evaluated patients. The SMR calculated for mortality after the following time-point: 12, 24, 36, 48 and 60 months of patients progression-free at these time points was constantly not significantly different from 1 (Table 1). In a second step, the same analyses were repeated in patients who received chemotherapy front-line and we were unable to identify long-term progression-free patients with a subsequent outcome similar to that of the general population. The analyses could not be performed in patients who received ibrutinib frontline because of their limited number.

We conclude that sWM patients with prolonged response to first-line immunochemotherapy delivered before 2018 may have a subsequent mortality that does not differ significantly from that observed in the general population.

Table 1: Subsequent mortality of patients treated with immunochemotherapy frontline and event-free at several time points

Study population	Number of patients	Observed	Expected	SMR [95%CI]	p value (Poisson)
Patients progression-free at 12 months	171	12	14.02	0.85 (0.48-1.50)	0.59
Patients progression-free at 24 months	136	7	8.66	0.8 (0.38-1.69)	0.57
Patients progression-free at 36 months	89	2	5.03	0.39 (0.10-1.59)	0.19
Patients progression-free at 48 months	72	2	3.33	0.60 (0.15-2.4)	0.47
Patients progression-free at 60 months	47	1	1.72	0.58 (0.08-4.12)	0.59

Abbreviation: SMR: standardized mortality ratio; CI: confidence interval.

3.

Minimal residual disease status improved the response evaluation in patients with Waldenström's macroglobulinemia

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Abstract:

Minimal residual disease (MRD) has been recognized as an important prognostic factor for survival in hematological malignancies. However, the prognostic value of MRD in Waldenström's macroglobulinemia (WM) remains largely unexplored. We analyzed 108 newly diagnosed WM patients receiving systematic therapy and assessed MRD by multiparameter flow cytometry (MFC) in the bone marrow every two courses and then every 3 months after the induction therapy for 2 years. At best response, 34 patients (31.5%) were undetectable MRD (uMRD). Hemoglobin>115 g/L (P=0.03), serum albumin>35 g/L (P=0.01), β_2-MG≤3 mg/L (P=0.03), and low-risk International Scoring System for Waldenström's Macroglobulinemia (ISSWM) stage (P<0.01) were associated with a higher rate of uMRD. Improvements in IgM (P<0.01) and hemoglobin (P=0.03) were more evident in uMRD patients compared with MRD-positive patients. The 3-year progression-free survival (PFS) was better for uMRD patients compared with those MRD-positive patients (96.2% vs. 52.8%; P<0.01). Patients who achieved partial response (PR) and uMRD had a 3-year PFS of 100%, which was significantly higher than that in MRD-positive patients with PR (62.6%, P=0.03). Multivariate analysis showed that MRD positivity was an independent factor for PFS (HR 4.25, P<0.01). Moreover, the combination of the 6th International Workshop on WM assessment (IWWM-6 Criteria) and MRD had a higher 3-year AUC compared with the IWWM-6 criteria only (0.71 vs. 0.67). In conclusion, MRD based on MFC was an independent prognostic factor for survival in WM, which could improve the precision of response evaluation.

4.

FINAL RESULTS OF THE PHASE I/II HOVON124/ECWM-R2 STUDY INCLUDING 2-YEAR RITUXIMAB MAINTENANCE AFTER INDUCTION WITH IXAZOMIB, RITUXIMAB AND DEXAMETHASONE IN RELAPSED WALDENSTRÖM'S MACROGLOBULINEMIA

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ABSTRACT

Background In the phase I/II HOVON124/ECWM-R2 trial, induction treatment with the combination of ixazomib, subcutaneous (s.c.) rituximab and dexamethasone (IRd) showed promising efficacy with manageable toxicity in patients with relapsed Waldenström's Macroglobulinemia (WM).

Aims To report the final analysis of the trial after two years of rituximab maintenance with a median follow-up (FU) of 45.6 months (range, 12.4-72.2).

Methods In total, 59 patients were enrolled (median age, 69 years; range, 46-91 years) of which 48 patients completed at least six cycles of IRd induction; 41 patients (median age 66 years, 66% male) with at least a minimal response (MR) continued to 2 years of rituximab maintenance (1400 mg s.c., q 3 months) starting 3 months after the last induction cycle. The primary endpoint of the study was overall response rate (ORR, ≥ MR)) after 8 induction cycles and was 71% (Kersten/Amaador et al, JCO, 2022). Secondary endpoints included progression-free survival (PFS), overall survival (OS) and ORR after 2 years of rituximab maintenance and improvement of response after maintenance. *MYD88* mutation was determined in peripheral blood (PB) by droplet digital PCR (ddPCR).

Results In total, 22 (54%) out of 41 patients completed 2 years of rituximab maintenance. The median number of cycles was 8 (range, 1-8). *MYD88* and *CXCR4* mutations determined by next generation sequencing (NGS) on BM biopsy/aspirate, were present in 51 of 55 (93%) and 14 of 52 (27%) patients. Nineteen patients did not complete maintenance treatment due to progression (n =16), excessive toxicity (n =1), non-compliance (n =1), and other unknown reason (n =1). The best ORR after maintenance was 85% (2% complete response [CR], 24% very good partial response [VGPR], 39% partial response [PR], and 20% MR). Improvement of response after maintenance occurred in 9 (22%) patients; VGPR to CR in one (2%), PR to VGPR in 6 (15%), and MR to PR in 2 (5%). A further decrease in IgM levels was seen after 2 years of maintenance (IgM 3.62 g/dl at baseline; 1.3 g/dl after induction and 0.42 g/dl after maintenance, p-value<0.001; Figure 1A), accompanied by a further increase in Hb levels (Hb 10.5 to 14.3 g/dl, p-value<0.001; Figure 1B). The median progression-free survival (PFS) was 23.6 months (95% CI, 13.4 to 43.2; Figure 1C), and median overall survival (OS) was not reached; at 45 months, 85% of patients were alive (95% CI, 0.72 to 0.92; Figure 1D). After a median FU of 45.6 months, 29 patients had received subsequent therapy and the median

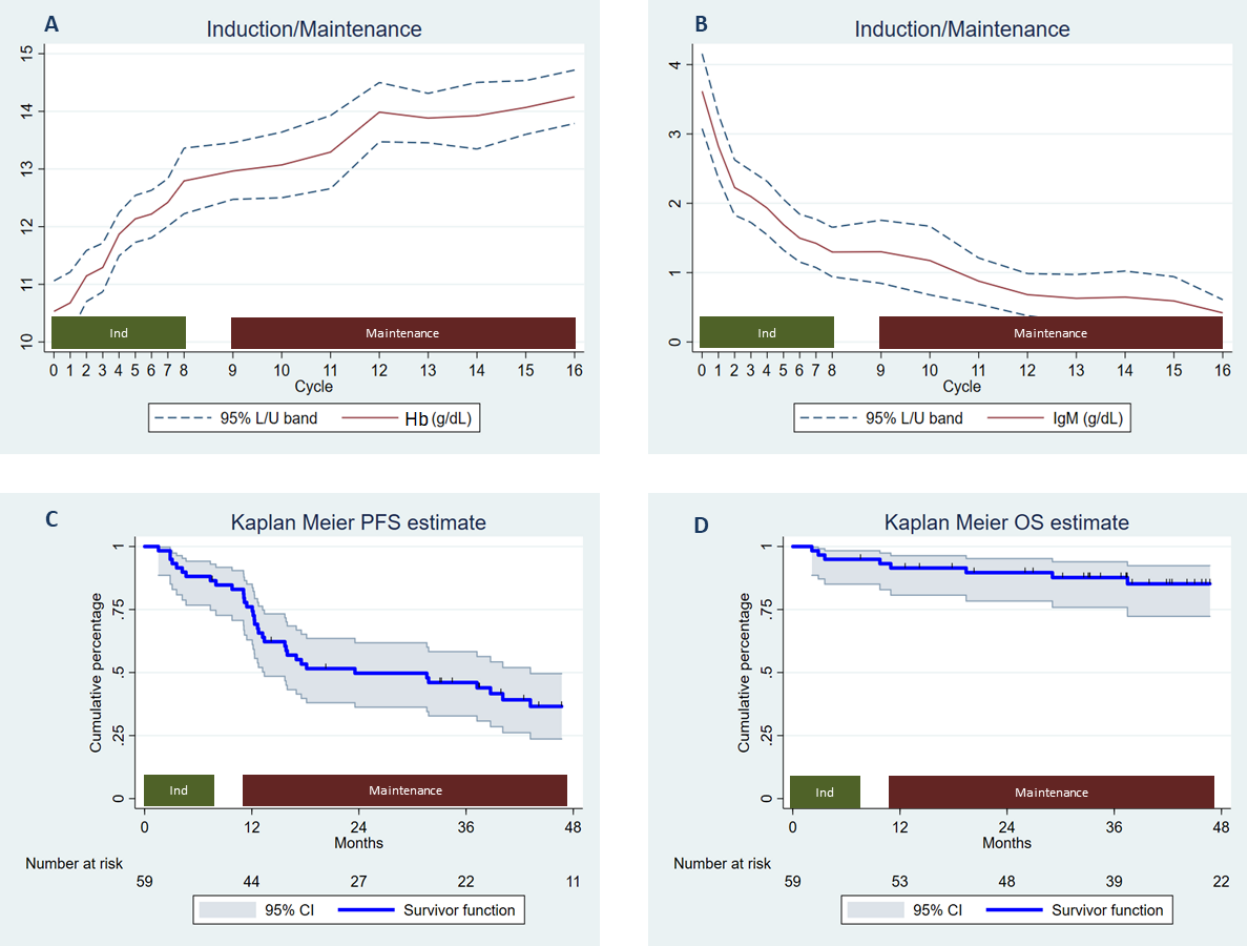
time to progression after subsequent therapy was 53.7 months. During maintenance, rituximab was administered i.v. instead of s.c. in 7 (17%) patients. None of the patients treated with s.c. rituximab developed systemic hypersensitivity reactions but 2 patients had an injection site reaction (1 grade 1, 1 grade 2). Grade 3/4 toxicity was seen in 10% and 5% of patients, respectively (grade 3/4 neutropenia (n=1 and n=2), grade 3 chronic kidney disease (n=1), and grade 3 elevations of transaminases (n=2)). In the 41 patients who started maintenance, 8 SAEs were reported in 6 patients, of which 4 occurred during maintenance (mostly infections) and 4 (mostly secondary malignancies, considered unrelated to study drug) during FU. Three patients died during maintenance therapy due to progressive disease, intracranial bleeding, and acute myeloid leukemia, respectively. *MYD88* ^{L265P} determined by ddPCR in PB was measured in a selected group of patients and present in 26 of 27 (96%) patients. The median variant allele frequency (VAF) decreased significantly after maintenance (P<0.007). At baseline the *MYD88*^{L265P} VAF determined by ddPCR in PB correlated strongly with the immunohistochemically estimated BM involvement (r=0.48; P<.007).

Conclusion Rituximab maintenance after IRd induction is feasible and well tolerated. The ORR improved in 22% of patients, confirming the efficacy of this chemo-free regimen in combination with rituximab maintenance in relapsed/refractory WM. Additionally, ddPCR on PB for MYD88 mutational screening is feasible and highly sensitive even in relapsed WM.

***The course of MYD88 VAF related to changes in response in bone marrow and peripheral blood is currently being analyzed and this data will be present at the time of the workshop.**

Keywords: Waldenström's macroglobulinemia; minimal residual disease; multiparameter flow cytometry; progression-free survival; prognosis.

Figure 1 (A) Hb during induction and maintenance **(B)** IgM during induction and maintenance **(C)** Progression free survival **(D)** Overall survival



5.

Oligosecretory Waldenström Macroglobulinemia patients exhibit excellent treatment response and outcomes

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Introduction:

WM is defined as lymphoplasmacytic lymphoma (LPL) with bone marrow involvement and an IgM monoclonal paraprotein but does not require the quantification of IgM. Most WM patients present with elevated IgM. However, there is no clear definition and further study of these patients with low IgM levels.

According to the current efficacy evaluation criteria, serum IgM quantitation reduces ≥90% from baseline could be classified as very good partial response (VGPR), and ≥50% as partial response (PR). When the patients have a low IgM level, within twice of the upper limit of normal value, it is not possible to make an accurate assessment of PR or VGPR. In multiple myeloma (MM), newly diagnosed MM patients are classified into measurable and unmeasurable diseases according to the secretory status of monoclonal immunoglobulin. In previous studies, the concept of oligosecretory MM is defined due to the sensitivity of electrophoresis assays. Similarly, in WM study, when the IgM level is low, it was also impossible to accurately evaluate the efficacy of WM patients, we thus define patients with initial IgM status within twice the upper limit of normal as "oligosecretory WM patients" and twice higher than the upper limit of normal as "measurable WM patients".

Methods:

The database of the Chinese Registration Network for Waldenstrom Macroglobulinemia (CRNWM) included 1274 newly diagnosed WM patients between July 2003 and September 2020 in thirty-three hematologic centers in China. We combined marrow biopsy, extramedullary disease, and clinical manifestations for oligosecretory WM patients to make a comprehensive judgment. When the tumor cells of bone marrow biopsy (BMB) declined by more than 50% accompanied by the reduction of spleen volume and lymph node size and improvement in clinical symptoms, we uniformly designated it as PR/VGPR. And complete remission (CR) was the same as before.

Results:

Among the 1274 WM patients, eighty patients (6.3%) were classified as oligosecretory WM according to our definition. The clinical characteristics of oligosecretory WM and measurable WM were described in Table 1. Compared with measurable WM group, oligosecretory WM patients had a higher proportion of thrombocytopenia (41.2% vs 27.4%, P=0.008) and a lower proportion of hypoalbuminemia (32.9% vs 64.6%, P<0.001) as well as elevated serum β2-microglobulin (57.1% vs 73.8%, P=0.002).

We identified four dimensions to evaluate the patient's tumor burden: flow cytometry (FCM) of bone marrow, bone marrow biopsy (BMB), splenomegaly, and lymphadenopathy (Table 2). Overall, no significant difference was observed in median malignant cells of bone marrow by FCM (9.85% vs 8.96; P=0.0114), the percentage of abnormal cells by BMB of different ratio ranges, and patients with splenomegaly (38.4% vs 35.2%; P=0.583) and lymphadenopathy (44.8% vs 39.1%; P=0.390) (Table 2, Figure 1). However, oligosecretory WM patients had a significantly higher percentage of patients with greater than 50% abnormal cells compared to the measurable WM patients (22.7% vs 12.4%; P=0.048), suggesting some patients with low IgM levels still had high tumor infiltration of bone marrow (Figure 1).

The proportion of patients with no indication of treatment was significantly higher in oligosecretory WM group than in measurable WM group (6.1% vs 1.0%; P=0.024). And all other treatment regimens were not significantly different between the two groups. The proportion of patients who achieved CR was significantly higher in oligosecretory WM group than in measurable WM group (14.7% vs 5.4%, P=0.0043).

According to our comprehensive efficacy evaluation, oligosecretory WM patients with different treatment responses experienced significantly different values for PFS (P=0.0085). Until the follow-up deadline, five patients achieved CR and no one developed disease progression. Importantly, oligosecretory WM patients had a significantly better survival outcome. Patients with measurable disease and oligosecretory WM had a 3-year PFS rate of 59.6% and 78.8% (P=0.0013) and a 3-year OS rate of 83.4% and 87.3% (P=0.89) respectively (Figure 2).

Conclusions:

Herein, we characterize a cohort of WM patients with low IgM levels. The oligosecretory WM was a special type of WM, and the patients exhibited excellent treatment response and outcomes.

Table 1 Baseline characteristics of newly diagnosed WM with measurable disease and oligosecretory WM

Characteristic	Oligosecretory WM (N=80)	Measurable WM (N=1194)	P
Age			0.831
Median (range) — year	65 (31-88)	64 (27-90)	0.836
＜65 years—no. (%)	43 (53.7)	656 (54.9)	
≥65 years—no. (%)	37 (46.3)	538 (45.1)	
Gender			0.685
Female—no. (%)	57 (71.3)	874 (73.2)	
Male—no. (%)	23 (28.7)	320 (26.8)	
B symptoms			0.891
Absent—no. (%)	55 (76.4)	848 (77.1)	
Present—no. (%)	17 (23.6)	252 (22.9)	
Lymphadenopathy ≥1.5 cm — no. (%)	26 (44.8)	296 (39.1)	0.390
Splenomegaly ≥15 cm — no. (%)	28 (38.4)	321 (35.2)	0.583
$MYD88^{L265P}$ mutation—no. (%)			0.065
No	39 (83.0)	427 (70.3)	
Yes	8 (17.0)	180 (29.7)	
Hemoglobin level			
Median (IQR) — g/L	84 (67-105)	84 (69-103)	0.998
>115—no. (%)	11 (13.8)	178 (15.0)	0.764

≤115—no. (%)	69 (86.2)	1010 (85.0)	
Platelet count			
Median (IQR) — 10^9/L	137 (61-237)	164 (95-250)	0.045
>100—no. (%)	47 (58.8)	836 (72.6)	0.008
≤100—no. (%)	33 (41.2)	316 (27.4)	
ALC Median (IQR) — 10^9/L	1.69 (0.94-2.9)	1.63(1.13-2.54)	0.766
Serum β2-microglobulin			
Median (IQR) — mg/L	3.25 (2.43-3.54)	4.1 (2.91-5.80)	0.874
≤3 mg/L—no. (%)	30 (42.9)	261 (26.2)	0.002
>3 mg/L—no. (%)	40 (57.1)	736 (73.8)	
LDH			
Median (IQR) — U/L	175 (142.3-236.5)	146.4 (112-197.4)	0.04
<250 U/L—no. (%)	63 (82.9)	945 (87.3)	0.275
≥250 U/L—no. (%)	13 (17.1)	138 (12.7)	
Serum albumin			
Median (IQR) — g/L	37.7(32.1-42.0)	32.0 (27.5-36.7)	0.000
≥35 g/L—no. (%)	53 (67.1)	411 (35.4)	0.000
<35 g/L—no. (%)	26 (32.9)	749 (64.6)	
κ/λ ratio Median (IQR)	1.42 (0.8-6.88)	3.43(1.13-2.54)	0.766

LDH, lactic dehydrogenase; ALC, absolute lymphocyte count

Table 2 Tumor load of newly diagnosed WM with measurable disease and oligosecretory WM

Parameters	Oligosecretory WM	Measurable WM	P
Percentage of bone marrow abnormal cells of FCM—Median (range) %	9.85 (3.57-44.5)	8.96 (2.5-26.0)	0.114
Greater than 50% abnormal cells of BMB—no. (%)	14 (53.8)	111 (54.4)	1.0
Splenomegaly ≥13 cm — no. (%)	28 (38.4)	321 (35.2)	0.583
Lymphadenopathy ≥1.5 cm — no. (%)	26 (44.8)	296 (39.1)	0.390

FCM, flow cytometry; BMB, bone marrow biopsy

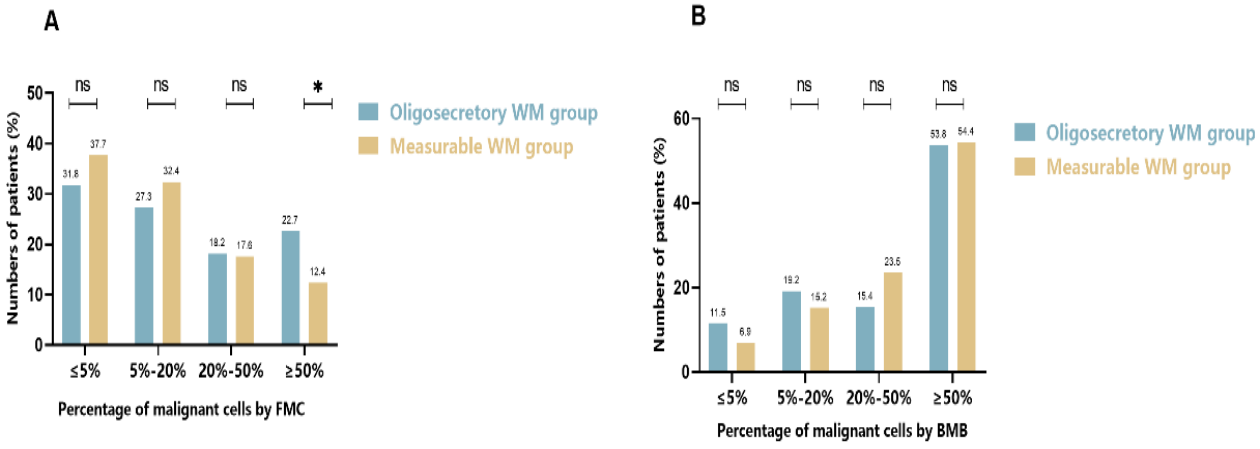

Figure 1 The percentage of malignant cells by FCM of bone marrow and BMB.

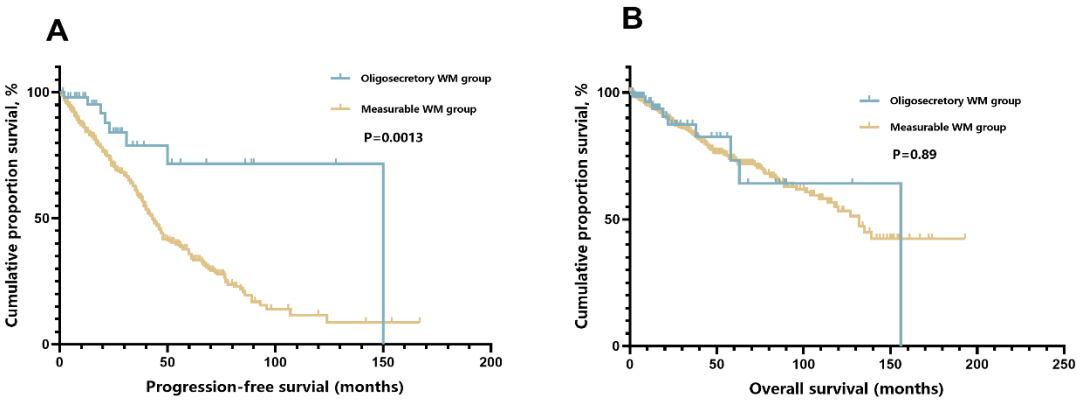

Figure 2 Kaplan-Meier curves for PFS and OS of oligosecretory WM and measurable WM patients; (A) The PFS of two groups, (B) The OS of two groups.

6.

T-regulatory cell-mediated immunosuppression plays a pivotal in supporting Waldenström Macroglobulinemia pathogenesis

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Recent studies have improved our understanding of the molecular aberrations supporting Waldenström Macroglobulinemia (WM) biology and disease progression; however, whether immunosuppressive mechanisms could also contribute to WM pathogenesis has not been described.

With the present studies we aimed: to characterize the transcriptome profiling of WM-Tregs; to dissect the functional WM-Treg phenotype: and to investigate the WM cell-to-Tregs interactions, at single-cell level.
We performed transcriptome profiling of primary WM patients'-derived CD4+/CD25+/FOXP3+ regulatory T cells (Tregs); and identified a peculiar mRNA signature that differentiates WM- from healthy donor (HD)-derived Tregs, characterized by a significant enrichment for NF-κB-mediated TNF-α signaling, MAPK-, PI3K/AKT-related genes. This molecular signature was paralleled by different Treg functional phenotype, depicted by a significantly higher Treg-induction, -expansion and -proliferation triggered by WM cells as compared to their normal cellular counterpart. Of note, a significantly higher effect was induced by $CXCR4^{C1013G}$-mutated, as compared to CXCR4-wild type WM cells. By assessing a Treg suppression assay, using WM patient-derived Tregs, a significantly higher suppressive activity was demonstrated, as compared to HD-derived Tregs. By interrogating the transcriptome profiling of WM- versus HD-derived Tregs, we found EBI3, FGL2, GZMB and PRF1 to be significantly up-regulated in primary WM patient-derived Tregs, as compared to their normal cellular counterpart. In parallel, WM-Tregs presented with a significant up-regulation of TIM3 and enrichment for CTLA4, a known player in Treg-mediated suppression.

To characterize the specific signaling events responsible for the induction of Treg differentiation, we next evaluated the B-T-cell interactions at single cell level, adopting a B-to-T cross-talk model. Briefly, WMCL1 or WMCL1-$CXCR4^{C1013G}$ clusters were identified as potential *sender* cells, hence responsible for the release of the immunosuppressive signal. Treg cells were considered as *receiver* and the Treg transcriptional program was identified by comparing Treg normalized counts against the transcriptionally closest T CD4+ cell cluster. This cross-talk model allowed to identify CD40/CD40-ligand axis as the potential regulator of the WM cell/Treg cross-talk. To confirm the biological relevance of these findings, functional validation assays were performed by using DRI-C21045, a potent inhibitor of the CD40/CD40-ligand interaction. Treg induction was tested in the presence of WM cells (BCWM.1; MWCL.1), exposed to DRI-C21045; and found a significant decrease in Treg induction, and Treg proliferation upon CD40/CD40-ligand inhibition. We next investigated whether CD40/CD40-ligand inhibition could also modulate the Treg phenotype within the context of CXCR4-mutated WM cells. Our findings demonstrated that halting CD40/CD40-ligand interaction could also target Treg inhibition and Treg growth within the context of CXCR4-mutated WM. Finally, we investigated the modulation of pro-survival signaling pathways, and found how

CD40/CD40-ligand blockade led to inhibition of p-AKT and p-ERK in Tregs exposed to ether CXCR4/wild-type- or CXCR4-mutated WM cells.

Taken together, our findings demonstrate, for the first time, the existence of a Treg-mediated immunosuppressive phenotype in WM; and suggest how halting CD40L/CD40 axis may represent a strategy to inhibit the Treg-mediated immunosuppressive scenario in WM.

7.

Single-cell transcriptome profiling reveals tumor cell heterogeneity and immunosuppressive microenvironment in Waldenström Macroglobulinemia

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Purpose: In this study, we delineated the multicellular ecosystem in bone marrow (BM) of Waldenström macroglobulinemia (WM), and elucidate the immune cell dysfunction, which would provide novel insights into the disease development and progression. **Experimental Design:** BM samples from 6 healthy donors and 14 patients were subjected to scRNA-seq using 3' or 5' mRNA & TCR & BCR library and further analysis. **Results:** ScRNA-seq analysis provides a comprehensive single-cell transcriptomic atlas to characterize cellular ecosystems in WM BM. We firstly delineated a novel model for the ecosystem of WM, wherein tumor cells and immune cells co-evolve kinetically, and clarified an aberrant immune suppressive milieu. Besides malignant B cells, malignant plasma cells, and plasmacytoid lymphocytes, two novel sub-populations co-expressing T-cell marker genes, $CD19^{+}CD3^{+}$ and $CD138^{+}CD3^{+}$ cells were identified with distinct transcriptomic profiles. $CD19^{+}CD3^{+}$ malignant cells present an early stage of B cell differentiation compared with $CD19^{+}CD3^{-}$ canonical B cells. Colony formation assay further identified that $CD19^{+}CD3^{+}$ malignant cells acted as potential WM precursors. Moreover, we observed tumor-derived perturbations of T cells in WM milieu. A precursor exhausted CD8-T cells and functional deletion of natural killer cells were identified, which was involved in the development of an immunosuppressive

microenvironment. Our study indicated that CD47 would be a potential therapeutic target to reverse immune cell dysfunction in WM.

Conclusions: Our study identified the biological heterogeneity of malignant cells and the altered functional states of immune cells in WM. This integrative analysis provides novel insights into the pathogenesis of WM and enhances the rational development of precision therapies to benefit patients with the greatest need.

Acknowledgments

We thank Dr. Steven Treon for his insightful and encouraging comments about our work. This work was supported by the National Natural Science Foundation of China (82170194 and 81920108006), CAMS Innovation Fund for Medical Sciences (CIFMS 2021-I2M-1-040).

8.

EFFICACY OF RITUXIMAB IN NEUROPHYSIOLOGICALLY SELECTED PATIENTS AFFECTED BY IGM-RELATED, ANTI-MAG DEMYELINATING POLYNEUROPATHY: INTERDISCIPLINARY CHARACTERIZATION END EVALUATION OF LONG TERM RESPONSE

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Introduction. IgM-related polyneuropathy (PNP) is an underdiagnosed condition associated to Waldenström Macroglobulinemia (WM) and IgM monoclonal gammopathy of undetermined significance (MGUS). It is a chronic, disabling PNP, characterized by anti-myelin-associated glycoprotein (anti-MAG) IgM antibodies in the serum. Previous studies with rituximab (RTX), though promising, were hampered by not well-defined diagnostic criteria and limited follow-up (FU).

Methods. Between 2017 and 2019 patients (pts) with monoclonal gammopathy (MG) and suspected PNP were prospectively evaluated by a multidisciplinary team. All pts underwent

marrow biopsy, flow cytometry, and *MYD88*^{L265P} mutational screening by digital PCR: WM or IgM MGUS cases matching diagnostic criteria for demyelinating PNP were selected. Neurological evaluation by clinical and disability scales and standardized neurophysiological study (ENG) was performed before treatment (t0), at 12 (t1) and 24 (t2) months. To assess PNP severity and disability, we employed Inflammatory Neuropathy Cause and Treatment disability scale (INCAT-ds) and modified Inflammatory Sensory Scale (mISS) for sensory deficits.

Results. Overall, 97 pts were evaluated: 28 were confirmed as IgM-related PNP and were included in this study; 21 were male, median age was 72. 25/28 had symptomatic PNP while 3 were still asymptomatic but showed typical progressive ENG pattern. 24/28 pts had WM, 4 MGUS; involved light chain was K in 25/28. Anti-MAG antibodies were present in 23 pts, *MYD88*^{L265P} was mutated in 24/27 (89%). Median IgM level was 833 mg/dl (range 121-4325). 4 pts had been pretreated with RTX. Overall, 23 pts received 4 weekly RTX 375 mg/m2, while 5 WM pts were treated for concurrent hematologic indications, receiving 6x RTX, cyclophosphamide (CTX), dexamethasone (RCD). Median FU after therapy was 29.5 months (15-40). RTX had a manageable safety profile, with only 9 G2 infusion related reactions. Median IgM level after therapy was 398 (83-1152) at t1 and 376 (76-1732) at t2. 8/28 pts received subsequent treatment, 5 with additional RTX for PNP relapse. At last FU, 5 pts were in very good partial response, 9 in partial response, 10 in minimal response, 3 had stable disease and 1 progressive disease.

Selecting only anti MAG positive patients treated with RTX single agent (n=23), both clinical and ENG parameters improved significantly from t0 to T1 and T2, particularly in upper limbs. There was a significant reduction from for mISS and for INCAT-ds, with $p<0.001$ at the inferential Friedman's test. Ulnar nerve terminal latency index and distal motor latency significantly changed from T0 to T1 and in the overall analysis ($p=0.001$ and $p=0.002$), and ulnar nerve sensory nerve action potential (SNAP) amplitude significantly increased at T2 from T1 ($p<0.001$). The analysis of the ROC curves showed that a 41.8% increase in SNAP amplitude of ulnar nerve at T2 from T0 was a fair predictor of mISS reduction $\geq$2 points (AUC 0.85; $p=0.005$).

Conclusions. This study showed for the first time long-term patient-reported and objective efficacy of RTX in a well-defined population of patients with IgM MG and anti-MAG neuropathy. Early multidisciplinary evaluation might improve outcome, and some neurophysiological parameters might be useful for monitoring RTX efficacy.

CLINICAL SCALES (EVALUATION OF CHANGE OVER TIME)

	Baseline	Time 1	Time 2	$\Delta_{T1 - Baseline}$	$\Delta_{T2 - Baseline}$	p value	
						Overall	*Comparisons*
MRC scale	58.3 ± 2.09 58.5 (52.0 - 60.0)	58.7 ± 1.89 59.0 (52.0 - 60.0)	58.8 ± 1.91 60.0 (52.0 - 60.0)	0.4 ± 0.96 0.0 (0.0 - 4.0)	0.5 ± 1.29 0.0 (0.0 - 6.0)	0.010	T0vsT1 0.058 T1vsT2 0.257 T0vsT2 0.078
mISS scale	9.6 ± 4.99 9.0 (0.0 - 21.0)	8.8 ± 5.00 9.0 (0.0 - 17.0)	8.5 ± 5.23 7.5 (0.0 - 21.0)	- 0.8 ± 1.52 0.0 (-4.0 - 2.0)	-1.1 ± 1.65 0.0 (-4.0 - 2.0)	0.003	T0vsT1 0.024 T1vsT2 0.319 T0vsT2 0.012
INCAT Disability Score	2.0 ± 1.43 2.0 (0.0 - 6.0)	1.6 ± 1.32 1.0 (0.0 - 5.0)	1.5 ± 1.45 1.0 (0.0 - 6.0)	- 0.4 ± 0.69 0.0 (-2.0 - 0.0)	- 0.5 ± 0.64 0.0 (-2.0 - 0.0)	< 0.001	T0vsT1 0.013 T1vsT2 0.589 T0vsT2 0.006

ULNAR - MEAN DX/SX (LOCF)

DML	4.7 ± 1.97 4.5 (2.3 - 9.9)	4.3 ± 1.90 3.9 (2.1 - 10.8)	4.3 ± 2.16 3.5 (1.9 - 12.5)	-0.4 ± 0.91 -0.2 (-3.3 - 0.9)	-0.4 ± 0.99 -0.5 (-2.8 - 2.5)	0.028	T0vsT1 0.048 T1vsT2 0.44 T0vsT2 0.06
CMAP amplitude	5.8 ± 2.54 5.8 (1.6 - 10.8)	6.1 ± 2.42 6.3 (1.6 - 10.8)	6.4 ± 2.56 6.6 (1.8 - 11.3)	0.3 ± 0.85 0.0 (-1.0 - 2.0)	0.6 ± 0.92 0.8 (-1.3 - 2.0)	0.007	T0vsT1 0.19 T1vsT2 0.101 T0vsT2 0.015
SAP amplitude	5.1 ± 6.09 2.8 (0.0 - 19.2)	5.6 ± 5.93 3.6 (0.0 - 19.2)	7.5 ± 7.61 4.5 (0.0 - 27.5)	0.4 ± 1.82 0.0 (-3.3 - 6.5)	2.2 ± 4.40 0.7 (-3.0 - 15.7)	0.017	T0vsT1 0.34 T1vsT2 0.006 T0vsT2 0.033

Figure 1: Clinical evaluation (top panel) and ENG responses (bottom panel) at baseline, t1 and t2 timepoints; ENG parameters were calculated for each patient as mean of right and left nerve conduction values.

9.

IDENTIFICATION OF MULTIPLE IMMUNE ESCAPE MECHANISMS INVOLVED IN WALDENSTRÖM MACROGLOBULINEMIA : A THERAPEUTIC PERSPECTIVE

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Keywords : B cell Lymphomas ; Waldenström Macroglobulinemia ; MYD88 ; Immunomodulation

B cell lymphomas are subdivided into aggressive and indolent forms. Waldenström's macroglobulinemia (WM) is an indolent LymphoPlasmacytic Lymphoma (LPL) characterized with bone marrow infiltration and the presence of a monoclonal IgM peak in the blood. To note, L265P mutation of *MYD88* is commonly found in this lymphoma (90% for WM).

In our team, we developed a mouse model that expresses the $Myd88^{L252P}$ mutation (the ortholog of the human L265P mutation). This expression is restricted to B cell compartment thanks to Cre-lox technology. Mice developed a B cell lymphoma with some features of WM : (i) Development of an indolent lymphoma (between 8 and 12 months) ; (ii) development of a LPL (in the spleen) (iii) Progressive apparition of a blood monoclonal IgM peak (Ouk *et al.* 2021).

To understand the mechanisms involved in LPL development in our model, we are focusing our work on Immune Escape Mechanisms (IEMs). Nowadays, IEMs are of growing interest and seems to be implicated in the development of multiple cancers et lymphomas. A previously published review from our team carefully highlighted the implication of IEMs in lymphomas development through engineered mouse models (Lemasson *et al.* 2021).

In the present work, we are showing many IEMs and their consequences:

- Tumor B cells exhibit an immunosuppressive phenotype with high expression of PD-L1 associated to a decrease of MHC class II surface expression.

- Concomitantly, Tumor infiltrated T cells (TILs) show an exhausted phenotype with an overexpression of PD-1, CTLA-4, TIM3 and LAG3.
- Regulatory T cells proportion increases in LPL lymphomas.
- Tumor Associated Macrophages (TAMs) population decreases drastically in LPL, due to the loss of CD80 and MHC II expression.

Taken together, these results show an evident implication of multiple IEMs in the development of lymphoma in our mouse model of WM. It is now interesting to research these phenomena in human disease.

One of the most famous molecule use for B cell lymphomas treatment is the well-known Btk inhibitor Ibrutinib. However, despite the promising results given by Ibrutinib, many patients remain refractory or develop resistances. In consequence, novel strategies to treat WM seem to be necessary.

We are also using our mouse model as a powerful pre-clinical tool. To begin mice were treated with Ibrutinib. In addition to its ability to induce B cells depletion, we observed an original effect of Ibrutinib which seems to be also able to reduce T cells exhaustion. In fact, a reduction of PD-1, CTLA-4, TIM-3 and LAG-3 surface expression was observed on CD4 and CD8 T cells following a 42 days Ibrutinib treatment.

To go further, we want to add different targeted therapies (anti-PD-1, anti-TIM-3, …) to Ibrutinib. We hope to obtain a synergic effect which act directly on tumor B cells but also indirectly by reactivating T cells against the tumor.

In essence, the presence of IEMs and especially T cells exhaustion in our mouse model of WM could open the perspective of novel therapies based on anti-tumor immune system reactivation.

10.

Early progression was an inferior predictor of survival in patients with Waldenström's macroglobulinemia

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Running heads: MRD predicts outcomes in WM

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Abstract: Early progression of disease within 24 months (POD24) of diagnosis is associated with inferior overall survival (OS) in follicular lymphoma, but its prognostic role in Waldenström's macroglobulinemia (WM) is still unclear. Here, we performed a retrospective analysis of 373 patients pooled from the database of the Chinese Registration Network for Waldenström's Macroglobulinemia (CRNWM) to determine the outcomes of early progressors. Patients were randomly partitioned into the training group (n=226) and validation group (n=147) at a ratio of 3:2. POD24 occurred in 26.5% of evaluable patients in the training group, with 3-year OS rates of 41.7% (vs 90.1% for those without POD24, P<0.001). This prognostic impact was confirmed in the validation group, in which early progression was observed in 38 out of 147 (25.9%) evaluable patients, with 3-year overall survival rates of 60.7% in the group with POD24 and 99.0% in the reference group (P<0.001). Moreover, POD24 still maintained its predictive ability of inferior OS in patients treated with rituximab based-therapy, protease inhibitor based-therapy, or traditional

chemotherapy alone. Patients with a very high-risk Revised-International Prognostic Scoring System for Waldenström's Macroglobulinemia stage were more likely to have early disease progression (*P*=0.020). In conclusion, POD24 was associated with poorer survival and may represent a useful endpoint in future prospective clinical trials.
Keywords: Waldenström's macroglobulinemia; Early progression of disease within 24 months; Overall survival.

11.

Bortezomib efficacy and tolerability in frontline and relapsed Waldenström Macroglobulinaemia

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Background:

Waldenström macroglobulinemia (WM) is an incurable low grade lymphoma which typically follows a prolonged disease course following a remitting and relapsing trajectory. Treatment options include… rituximab-containing combinations, bortezomib-containing regimens and Bruton tyrosine kinase inhibitors (BTKi). Treatment selection is based on patient performance status, disease characteristics, drug tolerability and local availability.

Aims:

We assessed the efficacy and tolerability of bortezomib-containing regimens in patients with WM.

Methods:

Adult patients who received a subcutaneous bortezomib-containing regimen for WM between 2010-2021 across six centres in the United Kingdom were retrospectively reviewed. Data was acquired from the national WMUK Rory Morrison Registry. Research ethics was obtained.

Results:

Thirty-five patients (24 male, 11 female) were identified: 33 had one bortezomib-containing regimen and two had >1 bortezomib-containing regimen, totalling 38 regimens administered. At bortezomib administration, the median age was 60 years (37-87), performance status was 1 (0-2), *MYD88*^{L265P} was present in 72% (13/18) and *CXCR4* mutated in 33% (4/12). 31% (11/35) were treated with a bortezomib-containing regimen at frontline and 69% (24/35) at relapse. Overall, the median prior lines of treatment was

2 (0-5), and time to bortezomib-containing treatment was 36 months (0-422) from WM diagnosis. 14% (5/35) were refractory/intolerant to BTKi.

At bortezomib initiation, the median M-protein was 30g/l (5-60) with bone marrow LPL infiltration 70% (5-100) [characterised in n=12: 28% lymphoid (0-90), 10% plasma cell (0-90) infiltrate]. A median of 5 cycles (1-11) were delivered; 62% (13/21) received 1.6mg/m^2 and 38% (8/21) received 1.3mg/m^2 bortezomib dose.

Grade (G) 1-2 neuropathy occurred in 25% (8/32) but did not result in treatment cessation in any case. 22% (7/32) required a dose reduction, predominantly due to G1-2 neuropathy (n=4). Gastrointestinal disturbance occurred in 6% (2/32), one patient required admission with G4 diarrhoea. Major response rate ($\geq$PR) was 79% (5 CR, 3 VGPR, 14 PR, n=28). Major response rate was 69% (9/13) in those receiving a dose of 1.6mg/m^2 and 78% (7/9) in those receiving 1.3mg/m^2. Three of 5 patients who had prior BTKi achieved PR, 1 MR, 1 SD. Median bone marrow infiltration after treatment was 10% (0-85).

Median follow up was 38 months (1-131) from bortezomib administration. 2-year overall survival (OS) was and progression-free survival (PFS) was 90% (95% CI 73-96) and 75% (95% CI 55-87), respectively; median OS not reached and median PFS was 39 months. Median time to best response was 81 days from end of treatment. Two patients died during treatment due to infection (COVID; respiratory sepsis), not attributable to disease relapse. No patients developed secondary MDS; 1/34 developed high-grade transformation.

Conclusion:

Bortezomib-containing regimens are highly active with a 2-year OS 90% and PFS 78%, effective even in those multiply relapsed with heavy marrow infiltration and BTKi failure. Gastrointestinal and neurotoxicity are manageable with dose reductions. No patients required treatment discontinuations in this real-world cohort, suggesting a manageable safety profile.

12.

Changes to platelet quality and function in Waldenström Macroglobulinaemia

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Word Count: 320

Background:

Platelet glycoprotein (GP) receptors are essential for initiating platelet adhesion, activation and aggregation. Reduced levels can occur when thrombopoiesis is disturbed, or coincident with bleeding in patients with thrombocytopenia, trauma or haematological malignancies. Both GPVI and GPIbα are metalloproteolytically shed from activated or aged platelets. Waldenström Macroglobulinaemia (WM) is a B-cell lymphoma with common symptoms including bleeding/bruising and thrombocytopenia. WM is treated with Bruton's tyrosine kinase inhibitors (BTKis) and chemotherapies, which can exacerbate bleeding. Platelet function and quality are under-investigated in WM.

Objectives:

To evaluate platelet quality and function, and clotting potential in WM.

Patients/Methods:

Relative platelet receptor levels, reticulated platelets and platelet activation were measured by whole blood or PRP flow cytometry in 16 WM patients compared with 66 healthy donors (HD). Blood was enumerated by automated analyser; metalloproteolytically-shed soluble GPVI was measured by ELISA; and whole blood clotting potential was evaluated using ROTEM.

Results:

WM blood displayed significantly reduced (**$p<0.01$) but enlarged platelets (**$p<0.01$) bearing less GPVI (**$p<0.01$), GPIbα (*$p<0.05$) and reticulation (*$p<0.05$), and increased tetraspanin CD9 (***$p<0.001$) and P-selectin (*$p<0.05$) compared with HDs. Plasma soluble GPVI was within HD ranges. Samples from WM patients displayed normal ROTEM parameters in intrinsic and extrinsic clotting assays but markedly reduced thrombus size (****$p<0.0001$) compared to HD values in FIBTEM. When recalcified (NATEM), WM patient blood formed thrombi 58% faster (****$p<0.0001$), that were enlarged 22% (****$p<0.0001$) over HDs.

Conclusions:

WM patient platelets bore reduced levels of key receptors governing platelet function. Reductions may be due to changes in platelet maturation/production as GPVI shedding was not evident and reticulated platelets were reduced. Global clotting capacity was normal, however the relative contributions of platelets and plasma components were abnormal, consistent with deranged haemostatic regulatory capacity in WM. Future work will assess the utility of these measurements in stratifying patients for bleeding risk.

13.

Non-invasive screening of *MYD88*^{L265P} mutation by droplet digital PCR in IgM-gammopathies: results of the multicentric "BIOWM" trial of the Fondazione Italiana Linfomi (FIL).

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Introduction. *MYD88*^{L265P} mutation has become increasingly important in the clinical management of IgM-gammopathies. Although liquid biopsy has been tested in Waldenström Macroglobulinemia (WM) as a non-invasive mutational approach, alternative to the bone marrow (BM) aspiration, no prospective comparison between tissues was performed in large patients' series, so far. The primary endpoint of the multicenter, observational FIL_BIOWM (NCT03521596) trial, sponsored by the Fondazione Italiana Linfomi (FIL) and the International WM Foundation/Leukemia and Lymphoma Society, was to compare the levels of detectable *MYD88*^{L265P} by droplet digital PCR (ddPCR) in plasma vs BM, to demonstrate a negligible difference between these two specimens. Moreover, *MYD88*^{L265P} screening was performed in parallel by next generation sequencing (NGS) when BM-CD19+ selected cells were available.

Methods. From 2018 to 2020 the trial enrolled 300 patients with primary diagnosis of WM or IgM monoclonal gammopathy of undetermined significance (IgM-MGUS). Paired BM, peripheral blood (PB) and plasma samples for cell-free DNA (cfDNA) analysis, were collected in 272 cases (91%) in the centralized laboratories of Torino, Pavia and Salamanca: overall, 210 WM and 62 IgM-MGUS patients. *MYD88*^{L265P} detection was performed by ddPCR [Drandi, Haematologica 2018]. NGS targeted resequencing was performed on Illumina Hiseq 2500 with a median coverage of 2369x. Agreement was estimated by k-statistic and Passing-Bablok regression.

Results. *MYD88*^{L265P} rate in WM patients was 95% in BM, 82% in PB and 91% in cfDNA, while in IgM-MGUS it was 76%, 56%, and 69%, respectively. Median *MYD88*^{L265P} quantification in cfDNA was superimposable to BM, both in WM (1.4E-02 vs 1.80E-02) and in IgM-MGUS (1.5E-03 vs 2.6E-03), while PB values were always about 1 log lower (1.2E-03 and 8.1E-04, respectively). Of note, IgM-MGUS patients showed significantly lower levels of mutation than WM, in all specimens. The qualitative concordance was excellent between cfDNA and BM both in WM (agreement 95.2%, K-statistic: 0.614) and IgM-MGUS (agreement 92%, K-statistic: 0.800) but only fair between PB and BM (88%, 0.384 and 83%, 0.636 respectively). The Passing-Bablok regression showed a negligible difference in the quantitative dosage of cfDNA compared to BM (intercept=0.0422, slope =0.868), also including 68 patients with CD19+ cells showing a highest quantitative mutation level (median 4.7E-01) (Figure 1).

Finally, 129 BM-CD19+ selected cells were analyzed by NGS and compared with ddPCR (68 BM-CD19+ and 61 BM unselected cells), with a level of agreement between the two techniques of 97% (K-statistic: 0.801). 125/129 samples were concordant (116 mutated (MUT) for both techniques, 9 wild-type (WT)) while 4 showed discordant results: 1 ddPCR-WT/NGS-MUT (that revealed the non-canonical *MYD88*^{M232T}) and 3 ddPCR-MUT/NGS-WT, all characterized by low mutational levels (median 3.8E-03).

Conclusion. The primary objective of the FIL_BIOWM study was met: *MYD88*^{L265P} mutation rates and quantitative values detected in plasma by ddPCR were superimposable to BM. PB showed up to 10% false negative results, as well as lower median quantitative levels, suggesting that is a suboptimal tissue for mutational screening. Overall, these data are in favor of implementing *MYD88*^{L265P} ddPCR assay in plasmatic cfDNA for non-invasive and prompt mutational diagnostics in clinical practice for WM and IgM-MGUS patients.

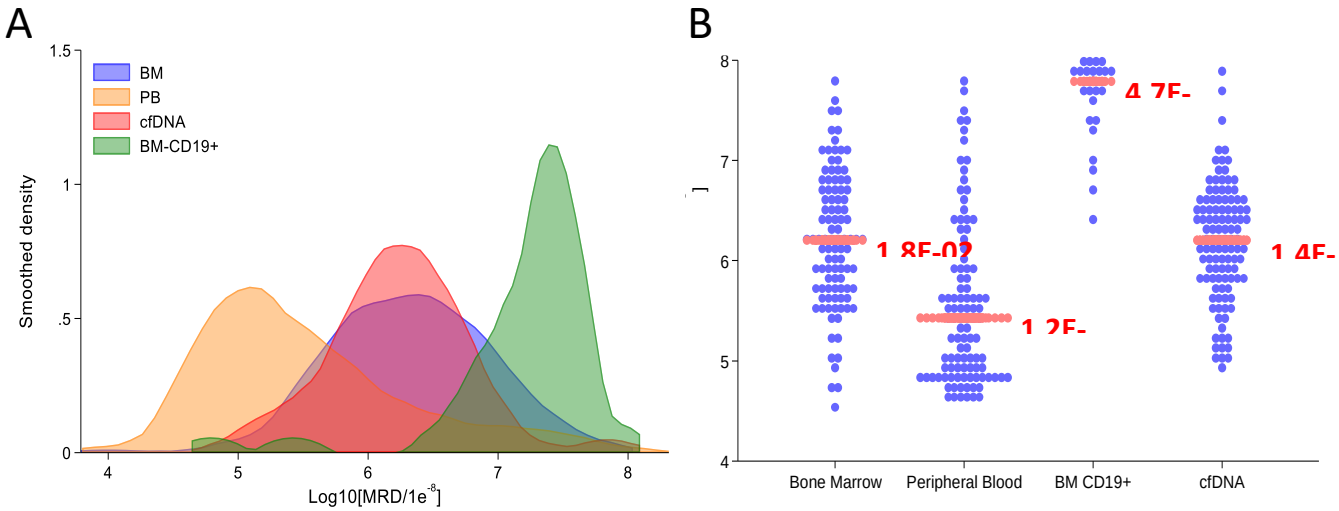

Figure 1. Comparison of *MYD88*^{L265P} quantitative values among different specimens in WM patients. (A) Density plot and (B) quantitative values of *MYD88*^{L265P} in bone marrow (BM), peripheral blood (PB), BM-CD19+ selected cells and plasma cfDNA.

14.

Treatment and survival outcomes in patients with Waldenström macroglobulinemia complicated by AL amyloidosis.

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Background: Light chain (AL) amyloidosis is an uncommon complication of Waldenström macroglobulinemia (WM). WM-associated AL (WM-AL) amyloidosis has unique morphological, genetic, and clinical features (Sidana Leukemia 2020). Currently, there is a paucity of data to guide the treatment of WM-AL amyloidosis. We sought to describe the treatment and survival outcomes of a large cohort of patients with WM-AL amyloidosis from the Boston University Amyloidosis Center.

Methods: We identified consecutive patients with WM-AL amyloidosis evaluated between 2006 and 2022. All patients met consensus criteria for WM and had positive Congo red staining of a biopsy specimen with typing confirming AL amyloidosis. Hematologic and organ responses to treatment were assessed using consensus definitions from the International Society of Amyloidosis. Event-free survival (EFS) was defined as the time between frontline treatment and subsequent line of therapy or death, whichever occurred first. Overall survival (OS) was defined as the time between AL amyloidosis diagnosis and death from any cause or last follow-up.

Results: We identified 49 patients with WM-AL amyloidosis. AL amyloidosis was diagnosed after the WM diagnosis in 80% of patients. Median serum IgM level was 1418 mg/dL (range, 284–5498); lymphoplasmacytic bone marrow (BM) involvement was 20% (range, 10–60%); 61% had lambda isotype; and 15% had a serum creatinine >2 mg/dL. Organ involvement included: renal (51%), cardiac (35%), peripheral nervous system (33%), autonomic nervous system (20%), gastrointestinal (16%), pulmonary (16%), and hepatic (6%). MYD88 and CXCR4 mutations were present in 81% and 33% of tested patients, respectively; no patient had t(11;14).

A total of 44 patients (90%) received at least one treatment. The response and survival treatment outcomes for each frontline regimen are summarized in **Table 1**. The overall, complete, and very good partial hematologic response rates to frontline therapy were 77%, 26%, and 26%, respectively. Cardiac, renal, and hepatic organ response rates were 67%, 52%, and 67%, respectively. The median EFS was 4.9 years (95% CI 2.3–NR), and the 5- and 10-year EFS estimates were 48% and 37%, respectively. The median OS was 7.3 years (95% CI 5.4–NR), and the 5- and 10-year OS estimates were 70% and 38%, respectively. On multivariate analysis, a serum creatinine >2.0 mg/dL was independently associated with shorter EFS (0.7 versus 7.2 years; HR 4.20; 95% CI 1.51–11.7; p=0.006) and OS (2.5 versus 10 years; HR 3.91; 95% CI 1.29–11.8; p=0.02). Depth of categorical hematologic response impacted both EFS (p<0.001) and OS (p=0.002). Age >65 years, male sex, lambda light chain isotype, hemoglobin ≤11.5 g/dL, platelets ≤100 K/uL, beta2-microglobulin >3 mg/L, serum IgM >4000 mg/dL, BM >10%, proteinuria >5000 mg/24h, alkaline phosphatase >150 IU/L, brain natriuretic peptide >81 pg/mL, troponin-I >0.1 ng/mL, and WT MYD88 were not significantly associated with EFS or OS. Patients who received maintenance rituximab (n=7/42; 17%) had a higher 5-year EFS (100% vs. 41%; p=0.01) and OS (100% vs. 67%; p=0.03).

Conclusion: A baseline serum creatinine >2.0 mg/dL adversely impacts EFS and OS in patients with WM-AL amyloidosis. We describe treatment outcomes in a large cohort comprised only of patients with WM-AL amyloidosis.

Table 1. Treatment outcomes to the frontline regimen used for AL amyloidosis in patients with Waldenström macroglobulinemia.

Regimen	N	FLC Response, n (%)		Organ Response, n (%)		Survival, median (years)	
		ORR	CR/VGPR	Cardiac	Renal	EFS	OS
Benda-R	15	12/15 (80)	8/15 (53)	3/4 (75)	5/6 (83)	5.4, 5-yr: 65%	7.3, 5-yr: 86%
BDR	9	6/9 (67)	6/9 (56)	1/2 (50)	2/6 (33)	4.4, 5-yr: 48%	7.3, 5-yr: 57%
HDM/SCT	9	9/9 (100)	8/9 (89)	2/2 (100)	5/6 (83)	NR, 5-yr: 88%	NR, 5-yr: 86%
CPR	5	3/5 (60)	1/5 (20)	--	0/2 (0)	1.7	12.0
CyBorD±R	2	2/2 (100)	0/2 (0)	--	0/2 (0)	0.7	2.5
Melphalan	2	1/2 (50)	0/2 (0)	--	0/1 (0)	2.8	7.7
Rituximab	1	0/1 (0)	0/1 (0)	--	--	1.3	1.6
Flu-R	1	0/1 (0)	0/1 (0)	0/1 (0)	--	0.9	1.2

All patients treated with HDM/SCT received pre-transplant induction therapy (BDR: n=8; Benda-R: n=1).

Benda-R, bendamustine and rituximab; BDR, bortezomib, dexamethasone, and rituximab; HDM/SCT, high-dose melphalan and stem cell transplantation; CPR, cyclophosphamide, prednisone, and rituximab; CyBorD±R, cyclophosphamide, bortezomib, dexamethasone, and rituximab; Flu-R, fludarabine and rituximab; FLC, free light chain; ORR, overall response rate; CR, complete response; VGPR, very good partial response; EFS, event free survival; NR, not reached.

15.

Patient Reported Outcomes Measures (PROMs) in 155 patients with Waldenstroms Macroglobulinaemia: A real-world data analysis from the WMUK Rory Morrison Registry project

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Background

Beyond treatments' safety and efficacy, quality of life (QoL) is one of the major concerns for WM patients. Several PROMs instruments are used to assess patient experience (1). PROMs are as valuable as therapy and clinical outcomes and should be considered in clinical decision making (2). The Rory Morrison Registry (RMR) uses 4 questionnaires; the EORTC QLQ-C30, BIPQ, HADS and EQ-5D-5L*.

Aims

To gauge the applicability of 4 standard questionnaires* in UK patients with WM by embedding their use in the RMR.

Methods

The 4 questionnaires (58 questions) are distributed digitally on quarterly basis to patients registered for PROMs in the RMR. Responses from Quarter-4 2021 were analysed. Differences between groups were analysed by Mann-Whitney U test or Kruskal-Wallis test, as appropriate, with GraphPad Prism v9.0.

Results

As of November 2021, clinical data for 1188 patients with WM were entered by medical teams on the RMR. 64/1188 patients completed digital PROM questionnaires and 91 patients self-entered PROMs data via online-questionnaires. M:F ratio was 1.3:1 (n=155). Median age was 69yrs (60/155 Age <66 years and 95/155 >=66 years), median age at diagnosis was 60yrs (33-77), and median time from diagnosis was 7yrs (23/65 Years of Disease: 0-5yrs and 42/65 Years of Disease: 6+). 26/52 received 0-1 and 26/52 ≥2 prior therapy lines. 35/52 evaluable patients were not on any treatment, 13/17 patients were taking a BTKi and 4/17 on Chemotherapy-based regimen.

40/58 questions across 4 questionnaires elicited a median response which was not notable; most likely because these questions were not specific to WM. Subgroup analysis was conducted on the

18/58 questions which elicited a notable median (Table 1.). HADS Anxiety score was significantly higher in Age <66 years (P=0.0149) and Females (P= 0.0077) where 0–7 represents a "normal" score (Table 2.). HADS Depression Score were higher in Prior Lines of Treatment: 0-1, Years of Disease: 0-5yrs, those not on treatment and those on chemotherapy vs BTKi. Patients reported feeling less in control in Prior Lines of Rx: 0-1, Years of Disease: 0-5yrs, and if currently on Chemotherapy-based regimen vs BTKi. Prior Lines of Rx: 0-1 also felt WM affected life to a greater extent. Understanding of WM was reported to be lower in Prior Lines of Rx: 0-1. Usual activity was felt to be more affected in those not on treatment and in those taking chemotherapy vs BTKi. There was no observable difference in responses for those with a WM-Associated condition i.e. BNS/Cryoglobulinemia/Peripheral Neuropathy/Schnitzler vs those without.

Conclusion

Digital self-entry of PROMs has been successfully set up, providing valuable insights into the QOL of patients living with WM in the UK. However, 70% of the questions in the 4 standard questionnaires failed to elicit a notable median. This suggests that a more relevant questionnaire designed specifically to assess PROMs in WM is required. Such a tool could collect data with greater applicability and utility to clinical practice, potentially improving quality of care and treatment decision making.

References:

1. Wiering B, de Boer D, Delnoij D. Patient involvement in the development of patient-reported outcome measures: a scoping review. Health Expect. 2017 Feb;20(1):11-23. doi: 10.1111/hex.12442. Epub 2016 Feb 18. PMID: 26889874; PMCID: PMC5217930.
2. Nobels-Janssen E, van der Wees PJ, Verhagen WIM, Westert GP, Bartels RHMA, Boogaarts JD. Patient-reported outcome measures in subarachnoid hemorrhage: A systematic review. Neurology. 2019 Jun 4;92(23):1096-1112. doi: 10.1212/WNL.0000000000007618. Epub 2019 May 10. PMID: 31076533; PMCID: PMC6556093.

Table 1. Median responses for 18/58 questions that elicited a notable result amongst entire sample population (n=155)

	Question	Median (Range)	Scale
1.	**Your own health state today**	75 (15-100)	0-100: 0 = The worst health you can imagine, 100 = The best health you can imagine
2.	**Mobility**	1 (1-4)	1-5: 1 = I have no problems in walking about, 5 = I am unable to walk about
3.	**Usual activity**	1 (1-5)	1-5: 1 = I have no problems doing my usual activities, 5 = I am unable to do my usual activities
4.	**Pain / discomfort**	2 (1-5)	1-5: 1 = I have no pain or discomfort, 2 = I have extreme pain or discomfort
5.	**HADS Anxiety score**	5 (0-19)	0-21: 0-7 = Normal, 8-10 = Borderline abnormal (borderline case), 11-21 = Abnormal (case)
6.	**HADS Depression Score**	4 (0-15)	0-21: 0-7 = Normal, 8-10 = Borderline abnormal (borderline case), 11-21 = Abnormal (case)
7.	**How would you rate your overall quality of life during the past week**	5 (1-7)	1-7: 1 = Very Poor, 7 = Excellent
8.	**How would you rate your overall health during the past week**	5 (2-7)	1-7: 1 = Very Poor, 7 = Excellent
9.	**Have you had trouble sleeping**	2 (1-4)	1-4: 1 = Not at all, 4 = Very much
10.	**Has your physical condition or medical treatment interfered with your social activities**	2 (1-4)	1-4: 1 = Not at all, 4 = Very much
11.	**Were you tired**	2 (1-4)	1-4: 1 = Not at all, 4 = Very much
12.	**Did you need to rest**	2 (1-4)	1-4: 1 = Not at all, 4 = Very much
13.	**Have you felt weak**	2 (1-4)	1-4: 1 = Not at all, 4 = Very much
14.	**How much control do you feel you have over your illness?**	4 (0-10)	0-10: 0 = Absolutely no control, 10 = Extreme amount of control
15.	**How well do you feel you understand your illness?**	8 (0-10)	0-10: 0 = Don't understand at all, 10 = Understand very clearly
16.	**How concerned are you about your illness?**	6 (0-10)	0-10: 0 = Not at all concerned, 10 = Extremely concerned
17.	**How much do you experience symptoms from your illness?**	3 (0-10)	0-10: 0 = No symptoms at all, 10 = Many severe symptoms
18.	**How much does your illness affect your life?**	5 (0-10)	0-10: 0 = No affect at all, 10 = Severely affects my life

*

- The Brief Illness Perception Questionnaire (BIPQ): Q1-4
- Hospital Anxiety and Depression Scale (HADS): Q5-6
- EuroQol-5D-5L: Q7-13
- European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire QLQ-C30 (EORTC QLQ-C30): Q14-18
- Table 2. Median responses and P-Values if statistical significance for subgroup to the key 18/58 questions

Table 2. Median responses and P-Values if statistical significance for subgroup to the key 18/58 questions

		Question (see Table 1)																
Group	**n=**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Age <66	60	73	1	1	2	6	5	5	5	2	2	2	2	2	4	8	6.5	3	6
Age >=66	95	79.5	1	1	2	4	4	5	6	2	2	2	2	1	4	8	6	3	5
P-Value if Significant						0.0149													
Male	87	80	1	1	2	4	4	5	5	2	2	2	2	2	5	8	6	3	6
Female	68	73.5	1	1	2	5	5	5	5	2	2	2	2	2	4	8	6	3	5
P-Value if Significant						0.0077													
Prior Lines of Treatment: 0-1	26	66.5	1.5	2	2	5	5	5	5.5	2	2	2	2	2	4	7	7	4	7
Prior Lines of Treatment: 2+	26	75	1	1	2	4	2.5	5.5	5	2	2	1	2	2	6	8	6	4	5
On Treatment	17	76	1	1	1	5.5	2	6	6	2	2	1	2	1	6	8	6	4	5
Off Treatment	35	73	1	2	2	4	5	5	5	2	2	2	2	2	4	8	6	4	7
BTKi	13	77.5	1	1	1	4.5	1	6	6	2	2	1	2	1	6	8	6	3	3
Chemo	4	53.5	1	2	1	6	5	5.5	5	2.5	2	1.5	1	2	2	9	9	6	6.5
Years of Disease: 0-5yrs	23	73	1	2	2	6	5	5	6	2	2	2	2	2	4	8	7	3	6
Years of Disease: 6+	42	74	1	1	2	4	3	5	5	2	2	2	2	2	5	8	6	4	6
With WM-Associated Condition	8	71	1	1	2	6.5	3.5	5	6	2	2	2	2	2	6	8	7.5	5	7
Without WM-Associated Condition	147	75	1	1	2	4.5	4	5	5	2	2	2	2	2	4	8	6	3	5

16.

Immune response in Waldenström Macroglobulinaemia patients after BTK inhibition

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Background & Aims

The bone marrow tumour microenvironment (TME) in Waldenström Macroglobulinaemia (WM) has been shown to have a role in WM disease progression and therapy resistance, however the effect of BTK inhibitors (BTKi) on the TME has been under explored. BTK is an essential element of the B-cell receptor (BCR) signalling cascade and plays an important role in pathways that regulate tumour microenvironment interactions. The aim of this study was to evaluate the immune microenvironmental changes in the bone marrow, following BTKi treatment in WM and correlate with molecular profiling.

Materials & Methods

A targeted gene expression assay was performed using the Oncomine™ Immune Response Research assay, using an Ion S5™ sequencing system to interrogate 400 genes involved in the tumour microenvironment. RNA was extracted from FFPE bone marrow trephine biopsies for 31 samples from 15 patients plus 1 control. Each patient has a sample prior to BTKi treatment and 12 months after starting BTKi treatment. Correlation with existing *MYD88* mutation status was performed and differential analysis between the two time points was performed using the Affymetrix™ Transcriptome Analysis Console software.

Results

14/31 samples passed the quality control threshold for this analysis. Of the 14, there were 9 $MYD88^{L265P}$ positive samples and 5 $MYD88^{L265P}$ negative samples. All patients had a minor response or better at the 12 month time point.

Comparative analysis of all $MYD88^{L265P}$ positive pre (5) and post (4) BTKi samples showed differences within the type 2 interferon signalling pathway, checkpoint pathway

and T cell receptor pathway with the most significant upregulation in NK cell marker, *NCR1* and downregulation of *CXCL13* post BTKi (figure 1).

The analysis of *MYD88*^{L265P} negative pre (2) and post (3) BTKi samples did not show the same pattern in terms of gene expression, but instead showed a downregulation of *B3GAT1*, *IL6* and *TLR7*.

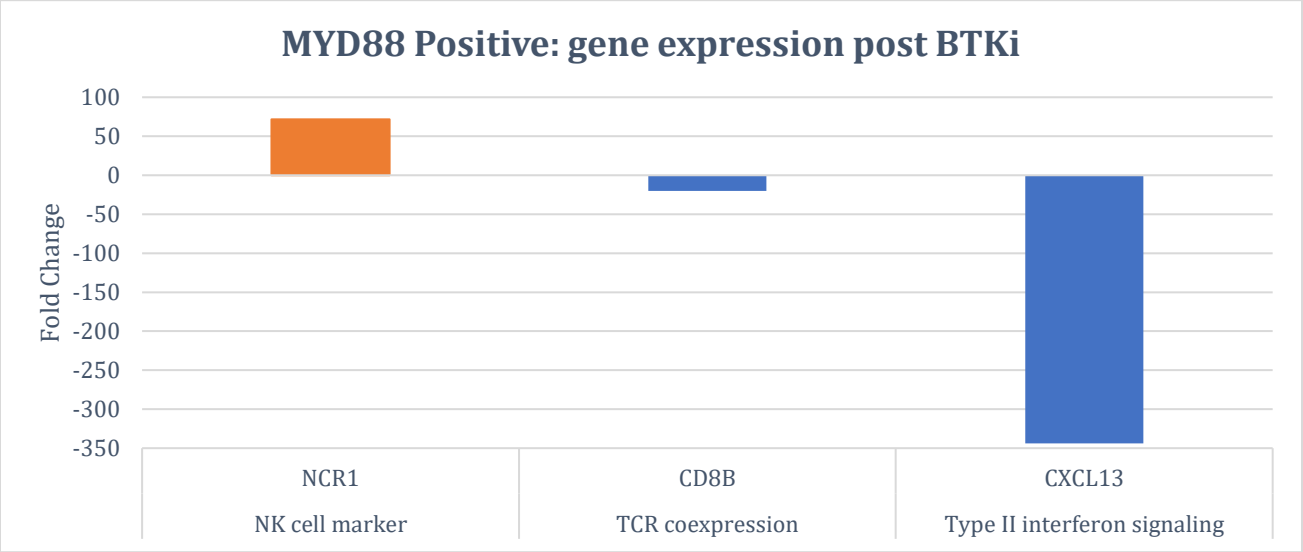

Figure 1 – comparative analysis of gene expression by MYD88 mutation status, pre vs post BTKi, and treatment type showing the fold change in gene expression between pre and post BTKi treatment for MYD88^{L265P} *positive patients only, all with a significant p-value of 0.03. BTKi treatment types include acalabrutinib (4 patients), zanubrutinib (1 patient) & ibrutinib (1 patient).*

Differential expression of *NCR1*, which encodes for a cytotoxicity-activating receptor on NK cells, has not previously been associated with Waldenström macroglobulinaemia but is consistently upregulated post BTKi in our cohort. *NCR1* has been shown to be downregulated in patients with B-CLL, but not in patients with SLL, causing an impaired cytotoxic function from NK cells in these patients (1). Ibrutinib has also been reported to suppress NK-cell cytotoxicity after one month in patients with Mantle Cell Lymphoma (2). Downregulation of *CXCL13*, previously shown to be produced by LPL cells, was predominantly seen in our cohort in patients treated with acalabrutinib. This is consistent with published data where *CXCL13* has shown a significant decrease in levels after ibrutinib inhibition in WM (3).

Conclusion

This pilot study has shown regions of the WM immune microenvironment with significant changes in gene expression after BTKi therapy, namely in pathways involving NK cells and type 2 interferon signalling. A larger cohort with paired samples pre and post BTKi treatment is warranted.

1. Parry HM, Stevens T, Oldreive C, Zadran B, McSkeane T, Rudzki Z, et al. NK cell function is markedly impaired in patients with chronic lymphocytic leukaemia but is preserved in patients with small lymphocytic lymphoma. Oncotarget. 2016 Oct 18;7(42):68513–26.
2. Flinsenberg TWH, Tromedjo CC, Hu N, Liu Y, Guo Y, Thia KYT, et al. Differential effects of BTK inhibitors ibrutinib and zanubrutinib on NK-cell effector function in patients with mantle cell lymphoma. Haematologica. 2020;105(2):e76–9.
3. Vos JM, Tsakmaklis N, Patterson CJ, Meid K, Castillo JJ, Brodsky P, et al. CXCL13 levels are elevated in patients with Waldenström macroglobulinemia, and are predictive of major response to ibrutinib. Haematologica. 2017 Nov;102(11):e452–5.

17.

Genomic Pathways Differentiating IgM-MGUS from Waldenstrom Macroglobulinemia: The Integrated microRNA and Gene Expression Landscape

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Introduction

Waldenstrom Macroglobulinemia (WM) is a rare non-Hodgkin lymphoma that is preceded by an IgM monoclonal gammopathy of undetermined significance (IgM-MGUS). The factors underlying this malignant transformation remain poorly understood, but the stepwise accumulation of genomic abnormalities has been identified as a potential mechanism. Non-coding aspects of the genome are increasingly recognized as drivers of disease pathogenesis but remain under-explored in this disease spectrum. In this study, we aimed to identify the microRNAs (miR) and genomic pathways which may facilitate the IgM-MGUS to WM progression.

Methodology

Sorted CD19+ and CD138+ B cells from prospectively collected bone marrow samples of patients with IgM-MGUS (n=7) and WM (n=25) underwent total RNA extraction and sequencing. Profiling of miRNAs was conducted, and selection of targets of interest was performed through identifying differentially expressed miRNA between IgM-MGUS and WM with a log2 fold change (FC)>0.5 or <-0.5 and false discovery rate (FDR) <0.05. Next, mRNA sequencing was conducted on the same patient samples, and differential expression was performed the same way as the miRNA data to identify mRNA candidates. Differentially expressed miRNA/mRNA pairs between WM and IgM-MGUS with correlated biological expression, i.e. either upregulated (up) miRNA experimentally predicted to regulate downregulated (down) mRNA or vice versa, were selected. The miRNA/mRNA pairs identified here were evaluated using Ingenuity Pathway Analysis (IPA) to discover dysregulated genomic pathways between IgM-MGUS and WM. Only pathways with

an absolute activation Z-score of ≥2 were selected, and dysregulated miRNA and associated genes involved in altered pathways were identified.

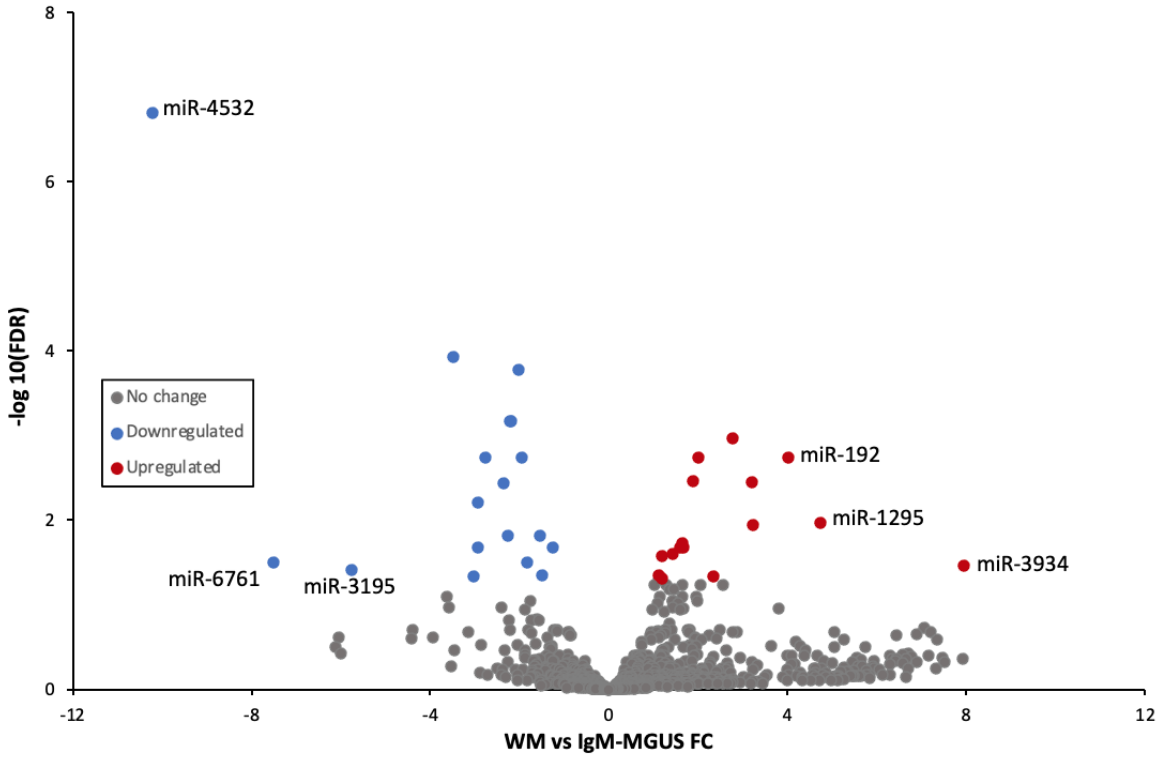
Results

We found 34 miRNAs (16 up/18 down) to be significantly differentially expressed between IgM-MGUS and WM (**Figure**). Among these, miR-4532, miR-192, miR-3195, miR-6761, miR-1295, and miR-3934 had the highest FC between these two conditions. After correlation of miRNA/mRNA pairs, the most differentially regulated pathways with recognized oncological relations were identified, among which, the ID-1, IL-8, and STAT3 pathways were found to be downregulated in WM compared to IgM-MGUS, while the PTEN pathway was found to be upregulated. More broadly, the G protein-coupled receptor (GPCR), and the tumor microenvironment (TM) pathways were both found to be downregulated in WM compared to IgM-MGUS. Some of the main miRNAs driving multiple pathways with key associated genes included: miR-20 (GPCR, TM, IL8, ID1) through regulation of cyclin D1 (*CCND1*), matrix metalloproteinase-2 (*MMP2*), and leukemia inhibitory factor (*LIF*), miR-1260 (ID1, GPCR, STAT3, TM) through regulation of B-cell lymphoma 2 (*BCL-2*), chemokine receptor 6 (*CCR6*), and ETS proto-oncogene 1 (*ETS1*), and miR-1295 (IL8, TM, GPCR) through regulation of vascular cell adhesion molecule 1 (*VCAM1*), matrix metallopeptidase 19 (*MMP19*), and hydroxycarboxylic acid receptor 2 (*HCAR2*).

Conclusion

Our study is one of the first to identify the altered non-coded transcriptomics in WM and IgM-MGUS. We found several differentially expressed miRNAs with a known oncological role, such as miR-4532, miR-20, and miR-1260, highlighting the role of miRNAs as potentially important epigenetic regulators in these two diseases. The genomic pathways found to be potentially regulated by miRNA may underlie the transition between the premalignant to malignant disease of IgM-MGUS to WM.

Figure 1: Volcano plot representing differentially expressed miRNA between WM and IgM-MGUS



18.

Waldenström Macroglobulinemia and the Clinical Implications of Acquired von Willebrand Syndrome

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Introduction

Waldenström macroglobulinemia (WM) is a lymphoplasmacytic lymphoma that can rarely lead to the development of acquired von Willebrand syndrome (AVWS), a bleeding disorder generally associated with another systemic process. There is limited literature on clinical outcomes of WM-associated AVWS (WM-AVWS). Our study aimed to assess the prevalence of this condition at our institution and determine the associated clinical manifestations and outcomes of WM-AVWS.

Methodology

Consecutive patients with a diagnosis of WM evaluated at Mayo Clinic, MN, FL, and AZ who underwent von Willebrand factor (VWF) testing from 01/2002 to 01/2022 were included. Laboratory confirmation of AVWS was obtained in the Special Coagulation Laboratory at Mayo Clinic, and those with a confirmed diagnosis of WM and AVWS were included in our study. Bleeding symptoms, WM- and AVWS-related treatment, and associated laboratory data were abstracted.

Results

Of 2210 patients with a diagnosis of WM, 73 (3%) received VWF testing, and 11 (0.5% of all patients) were diagnosed with WM-AVWS. The median follow-up for these 11 patients was 34.0 months (95% CI, 30.1 – 163.6). Baseline testing at time of initial AVWS diagnosis [median (range, normal)] revealed: platelet count 170 (49-281, 135-317), VWF-activity (latex immunoassay) 25% (12-31, 55-200), ristocetin co-factor activity 21% (12-31, 55-200), VWF-antigen 32 (13-44, 55-200), and Factor VIII activity 40% (16-64, 55-200). Multimer analysis available in 6 patients revealed a normal distribution pattern.

The most common underlying reason for VWF testing was bleeding symptoms (n=9 [82%]). Other reasons for testing included pre-surgical (n=1) and routine screening (n=1). Eight (73%) patients were diagnosed with AVWS prior to frontline treatment initiation for WM. Of these 8 patients, 5 (45%) patients had a concurrent diagnosis of AVWS and WM, where WM was diagnosed a median of 35 days (range, 4-83) after the diagnosis of AVWS **(Table 1)**. All patients received WM-directed treatment.

Recurrent epistaxis was the most observed bleeding symptom (n=6 [55%]) **(Table 1)**. Seven (64%) patients had a major bleeding event leading to hospitalization; due to spontaneous hematoma (n=2), GI bleeding (n=2), epistaxis (n=2), and subarachnoid hemorrhage (n=1). Pharmacologic hemostatic treatments were administered to 6 (55%) patients: Factor VIII/VWF complex (n=4 [36%]), desmopressin (n=4 [36%]), intravenous immunoglobulin (IVIG) (n=3 [27%]) and prednisone (n=1 [9%]). These therapies led to temporary improvement of bleeding symptoms (<3 months) in all 6 patients. Of the 9 patients who had bleeding symptoms, 6 (67%) had improvement in their symptoms after WM-directed therapy. Three patients who were refractory to WM-directed therapy had no improvement or worsening in bleeding symptoms while on WM therapy.

Conclusion

We found that <1% of patients had an identified diagnosis of AVWS secondary to WM, highlighting the rarity of this diagnosis and its potential under-recognition. The high morbidity associated, leading to events such as CNS bleeding and spontaneous hematomas, signals that one needs to evaluate patients with WM who are presenting with symptomatic bleeding for AVWS. Additionally, given a fraction of patients had worsening bleeding events after therapy initiation, agents with lower bleeding risk should be considered in patients with this diagnosis.

Table 1: Summary of Individual Outcomes in Patients with Acquired von Willebrand Syndrome (AVWS) and Waldenstrom Macroglobulinemia (WM)

ID	Presenting Symptoms of AVWS	Reason for AVWS Testing	AVWS-Directed Therapy	Response to AVWS-Directed Therapy	Diagnosis of WM and AVWS	WM-Directed Therapy After AVWS Diagnosis	Response of AVWS to WM-Therapy	Best WM Response
1	Spontaneous intraabdominal hematoma	Bleeding	Desmopressin, Prednisone	Improvement	Prior to WM diagnosis	Zanbrutinib	Resolution	MR
2	Recurrent GI bleeding	Bleeding	IVIG, desmopressin, Factor VIII/VWF	Improvement	Prior to WM-directed frontline therapy	BR	Resolution	VGPR
3	Spontaneous wrist hematoma	Bleeding	IVIG, Factor VIII/VWF	Improvement	Prior to WM diagnosis	BDR	Resolution	MR
4	Subarachnoid hemorrhage, retinal hemorrhage, epistaxis	Bleeding	None		Progressive WM, after frontline therapy	2nd line: DRC	Resolution	PR
5	Retinal hemorrhage, epistaxis	Bleeding	Desmopressin, Factor VIII/VWF	Improvement	Prior to WM diagnosis	Plasma exchange followed by BR	Resolution	PR
6	Epistaxis	Bleeding	None		Prior to WM diagnosis	BDR	Resolution	MR
7	Epistaxis	Bleeding	Desmopressin	Improvement <3 months	Prior to WM-directed frontline therapy	Rituximab	Continued bleeding symptoms	PD
8	Epistaxis	Bleeding	None		Prior to WM diagnosis	Ibrutinib	Worsening epistaxis after 2 weeks of therapy	PD
9	GI Bleeding, Epistaxis	Bleeding	None		During WM-directed frontline therapy	BR	Worsening cutaneous bleeding after cycle 1	SD
10	None	Pre-surgical screening	IVIG, Factor VIII/VWF	Improvement	Prior to WM-directed frontline therapy	DRC	None	PR
11	None	Routine Screening	None		Progressive WM, after frontline therapy	2nd line: Rituximab	None	PR

BDR, Bortezomib-rituximab-dexamethasone; BR, bendamustine-rituximab; DDAVP, desmopressin; DRC, Dexamethasone-rituximab-cyclophosphamide; MR, minimal response; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

19.

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Effectiveness And Safety Of Ibrutinib In Waldenström's Macroglobulinemia (WM) In Belgian Routine Clinical Practice With A 3-Year Follow-Up

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Background: Ibrutinib is a first-in-class, once-daily oral inhibitor of Bruton's tyrosine kinase approved as monotherapy or in combination with rituximab for the treatment of adults with WM. The Belgian ibrutinib Real-world Data (BiRD) study was designed to investigate the effectiveness and safety of ibrutinib treatment in routine clinical practice.

Methods: BiRD is a retrospective and prospective, multicenter, non-interventional, observational, cohort study of adults with chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), or WM. Eligible patients initiated reimbursed ibrutinib treatment upon or after commercial availability in Belgium; or participated in the Medical Need Program for CLL, MCL, or WM; or received free-of-charge ibrutinib for WM as a single patient request and switched to reimbursed ibrutinib with commercial availability. Primary outcome measures were progression-free survival (PFS) and overall response rate (ORR); secondary outcome measures included overall survival (OS), time-to-next-treatment (TTNT), and safety. Data were collected both prospectively and retrospectively; retrospectively collected adverse events (AEs) included only those considered related to ibrutinib, whereas prospectively collected AEs included all treatment-emergent AEs, therefore AEs are reported separately for each group. Total population effectiveness results were adjusted with left truncation. Here we present results from the third interim analysis for patients with WM, with a median follow-up of 26.9 months.

Results: In total, 42 patients were included in the study, 39 in the effectiveness population (prospective, n=16; retrospective, n=23) and 41 in the safety population (prospective, n=17; retrospective, n=24). Median age at ibrutinib initiation was 67 years (range, 49-89); 64.1% were male; median time from diagnosis to ibrutinib initiation was 8.6 years (range, 0.1-20.5); and all evaluable patients (n=29) had *MYD88* mutation. Prior therapies included combination therapy (33.3%), monotherapy (15.4%), or both (51.3%); and 7.7%, 38.5%, and 53.8% received 1, 2, or ≥3 prior lines of therapy, respectively. The most recent prior therapies were rituximab (20.5%) and bortezomib-dexamethasone-rituximab, rituximab-bendamustine, rituximab-cyclophosphamide-dexamethasone, and rituximab-cyclophosphamide-vincristine-prednisolone (all 10.3%). The most common reason for initiating ibrutinib treatment was immunoglobulin M–related pathology (63.4%), and most patients received single-agent ibrutinib (97.6%).

With ibrutinib treatment, median PFS was 50.6 months, best ORR was 87.2%, and median time to first response was 2.9 months. Median duration of response, median OS, and median TTNT were not estimable. The median duration of ibrutinib treatment was 22.8 months (range, 2.6-55.7), and dose modifications were reported in 15 patients (36.6%). AEs and serious AEs were reported in 79.2%/94.1% and 20.8%/41.2% of patients in the retrospective/prospective groups, respectively. AEs led to dose reduction, interruption, or withdrawal in 20.8%/5.9%, 12.5%/35.3%, and 8.3%/35.3% of patients in the retrospective/prospective groups, respectively. AEs of interest in the retrospective/prospective groups were: major bleeding (0/11.8%), infection (58.3%/47.1%), hypertension (12.5%/11.8%), atrial fibrillation (0/5.9%), arthralgia/myalgia (20.8%/5.9%), diarrhea (20.8%/11.8%), and rash (12.5%/5.9%). Ibrutinib was discontinued in 14 patients (34.1%), most commonly due to disease progression (n=5).

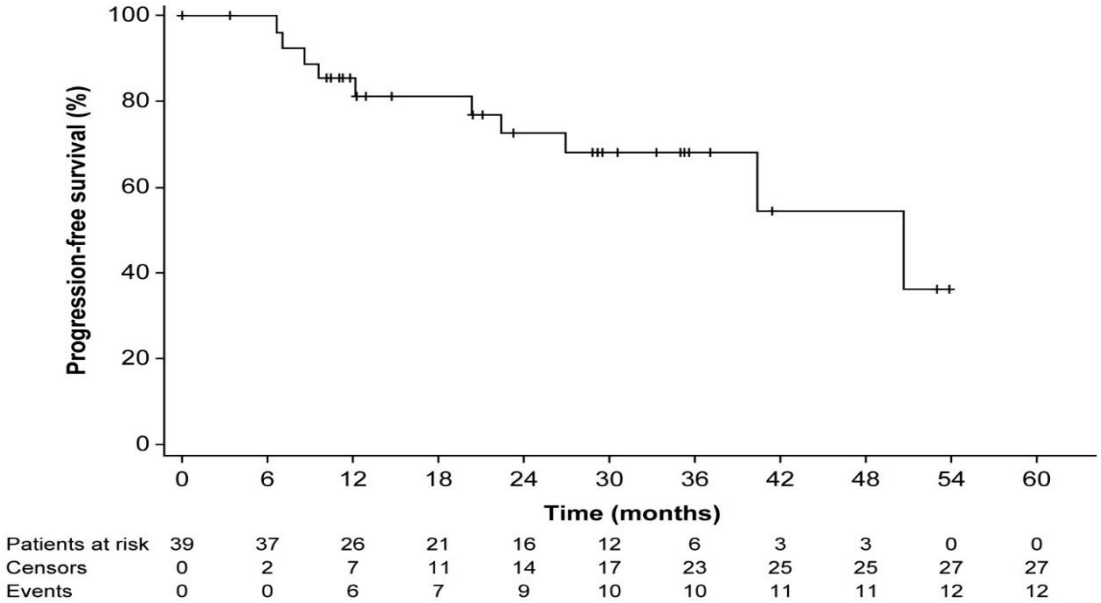
Summary/Conclusion: Ibrutinib is effective for the treatment of patients with WM in a real-world setting. No new safety signals were observed, and AEs were consistent with the known safety profile of ibrutinib.

Table. Effectiveness Results

	Total WM Effectiveness Population (N=39)
Median PFS, months (Q1-Q3)	50.6 (22.4-NE)
Estimated 36-months PFS, %	68.2
Best ORR, % Very good partial response Partial response Minor response	87.2 33.3 43.6 10.3
Median duration of response, months (Q1-Q3)	NE (19.3-NE)
Median OS, months (Q1-Q3)	NE (40.3-NE)
Estimated 36-month OS, %	81.4
Median TTNT, months (Q1-Q3)	NE (22.8-NE)
Time to first response, months (Q1-Q3)	2.9 (2.7-4.1)

NE, not estimable.

Figure. Progression-Free Survival of Patients With WM



20.

Molecular Clusters and Tumor-Immune Drivers of IgM Monoclonal Gammopathies

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The IgM MGUS and Waldenstrom Macroglobulinemia (WM) include a wide range of conditions whose management varies from observation to immunochemotherapy. The current classification relies solely on clinical features and does not explain the heterogeneity that exists within each of these conditions. To shed light on the biology that may account for the clinical differences, we used bone marrow (BM) clonal $CD19^+$ and $CD138^+$ cells and matched BM plasma from 32 patients (7 IgM MGUS, 25 WM) and 5 healthy controls to perform the first multi-omics approach including whole exome sequencing, RNA-seq, proteomics, metabolomics and mass cytometry.

Applying PCA to gene expression profiling, most of WM patients clustered together while a small subset of them grouped separately with MGUS patients, suggesting a biologic dichotomy within WM. The healthy controls formed a distinct group from most WM and MGUS. We then applied a non-negative matrix factorization consensus clustering to the gene expression data and identified three robust clusters. Cluster 1 (C1) included only patients with WM, C2 included patients with both WM and MGUS, C3 included all normal controls as well as a small number of WM and MGUS patients. When mutations commonly identified in WM were analyzed, there was no difference among the three groups (excluding controls) in mutation burden of *MYD88 L265P* and *CXCR4*. Interestingly, aberrant expression of *TNFAIP3* seemed a distinct feature of C1 as deletion of 6q (which encodes for *TNFAIP3*) and *TNFAIP3* mutations were each significantly enriched in C1 compared to C2 and C3 (p=0.04). Individual clusters associated with specific transcriptional signature and clinical features. While C1 displayed downregulation of genes involved in cell cycle and immune response with aggressive behavior, C2 showed upregulation of inflammatory response and senescence genes with indolent behavior. C3 had an intermediate feature with combined proliferative and inflammatory signatures. In accordance with transcriptomics, the proteomic hallmark of C1 was increase of proteins involved in proliferation (eg AKT, MAPK) and downregulation of inflammatory proteins (eg IL4, IL10) while the opposite was observed in C2. Once more, C3 confirmed intermediate features with combined upregulation of proliferation and inflammatory proteins. The metabolism was rewired towards mitochondrial anabolism in C1 and C3 while to glycolysis in C2. Accordingly, C1 and C3 showed undetectable concentration of 3-hydroxybutyric acid as opposed to C2 which had increased levels of malic and lactic acids, as end products of fatty acid oxidation and glycolysis respectively. Next, we explored whether C1 and C2 displayed a distinct immune profile. tSNE analysis showed that $CD4^+$ T cells were more abundant in C2 compared to C1 while the opposite was observed for $CD8^+$ T cells. Among $CD4^+$ T cells, activated follicular helper (T_{FH}; p=0.02) and T regulatory (T_{reg}; p=0.008) cells were predominantly expressed in C2. Conversely, C1 showed a higher expression of senescent T effector memory ($T_{EM;}$ p=0.001) cells. In support of this, SPADE clustering analysis identified three main clusters including T_{FH}, T_{reg} and T_{EM} cells.

In conclusion, we identified three distinct molecular clusters, suggesting a potential biologic classification that may have therapeutic implications.

21.

HISTOLOGICAL TRANSFORMATION DEVELOPING AFTER IBRUTINIB THERAPY IN WALDENSTROM MACROGLOBULINEMIA

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INTRODUCTION

Histological transformation (HT) to diffuse large B-cell lymphoma (DLBCL) is a rare event in Waldenström macroglobulinemia (WM) and associated with a poor prognosis. It can occur at anytime during the course of the disease, even before any treatment for WM or during response to WM therapy. Previous studies on HT in WM were mainly conducted in patients treated with chemoimmunotherapy for WM. Few data are available on HT developing in WM patients treated with novel agents, in particular ibrutinib.

METHODS

We retrospectively searched in our international multicenter database of 279 patients with transformed WM for patients treated with ibrutinib prior to HT.

RESULTS

Seventeen patients (10 men and 7 women) were identified. Median age at WM diagnosis was 63 years (range 36-86). *MYD88*^{L265P} and *CXCR4* mutations were present in 93% (13/14) and 57% (4/7) of patients respectively. At time of HT, patients had received a median of 4 lines of therapy (range 1-9) for WM (including ibrutinib). Only 2 patients received ibrutinib as primary therapy. Of the 15 patients treated prior to ibrutinib therapy, 93% were previously exposed to rituximab, 67% to bendamustine, 60% to proteasome inhibitors, 33% to RCD, 13% to chlorambucil, and 13% to fludarabine-based regimen.

At time of HT, 10 patients (59%) were on active treatment with ibrutinib. Response to ibrutinib was available in 9 patients: CR in 1, VGPR in 2, PR in 5 and PD in 1. The median time from WM diagnosis to HT was 4.2 years (range, 1-17 years). Extranodal involvement was present in 59% of patients. Of note, CNS involvement occurred in 5 patients (3 at HT diagnosis, including 2 on ibrutinib, and 2 at relapse). Serum LDH was elevated in 62% of patients. According to Hans' algorithm, 82% of cases harbored a non-GC phenotype. EBER was negative in the 8 informative cases. Of the 15 patients who received treatment for HT, 7 (47%) were treated with R-CHOP, 3 (20%) with R-ICE, 2 (13%) with R-DHAP and 1 with high-dose methotrexate. The treatment was unknown in 2 patients. Response to first-line treatment for HT was available in 14 patients: CR was achieved in 7 patients (50%), PR in 3 (21%) and PD in 4 (29%). Autologous SCT and alloSCT were performed in 1 patient each. Progression occurred in 86% of patients. Median PFS was 5.5 months. At time of last follow-up, 53% of patients have died. The majority of deaths were attributed to progressive disease (78%) or infections (11%).

CONCLUSIONS

HT in WM can occur in patients treated with ibrutinib, even on active treatment. Clinical presentation and outcome seem similar to previous studies on transformed WM. Further studies are needed to evaluate the incidence of HT after ibrutinib therapy in treatment-naïve and in previously treated patients with WM and to elucidate if HT developed after treatment with novel targeted agents differ from that developed after chemoimmunotherapy.

22.

Increased tumor-associated CD66b+ Myeloid-derived suppressor cells in Waldenstrom macroglobulinemia inhibit T-cell immune function

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Background:
Waldenstrom macroglobulinemia (WM) is a discrete clinicopathologic entity resulting from the accumulation of lymphoplasmacytic lymphoma (LPL) cells in the bone marrow (BM) that secrete a monoclonal immunoglobulin (Ig)M protein. *MYD88* (>90%) and *CXCR4* (30-35%) mutations are the most commonly identified mutations in this disease. While much is known about the genomics of WM, far less is known about the role of the BM microenvironment in this disease. Emerging evidence has attested to the role of myeloid-derived suppressor cells (MDSCs) and their potent immunosuppressive activity, however, there is limited information about these cells in WM and monoclonal gammopathy of undetermined significance (MGUS). This study was therefore designed to explore the phenotype of MDSCs and their interaction with other cells in the BM in WM.

Methods:
We performed flow cytometry in normal BM (NBM; n=11) and WM symptomatic /MGUS (n=18) to analyze the number of MDSCs in the specimens. A detailed phenotypic study was then performed using CyTOF (mass cytometry) on NBM (n=4) and WM (n=8) specimens. We then performed Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE-seq) on WM (n=3) and NBM (n=2) specimens to establish a better understanding of the transcriptome profile on MDSC subsets. Finally, the immune function of the MDSC subset was studied by co-culturing with Activated T-cells.

Results:
In the present study, using flow cytometry, we found that MDSCs are more abundant in WM symptomatic /MGUS compared to controls (p=0.005). We also found that most MDSCs in WM specimens had a granulocytic phenotype (Lin- HLA-DR- CD11b+ CD33+ CD15+). The CyTOF analysis identified 3 subtypes of MDSCs including CD66b+ MDSCs with a granulocytic phenotype (G-MDSCs) and confirmed the expansion of CD66b+ MDSCs in WM symptomatic patients when compared to MGUS/smoldering WM and normal controls. Our previous studies have shown that granulocyte colony-stimulating factor (G-CSF) expression is increased in the

bone marrow of WM patients (Jalali et al., 2018). Therefore, to determine whether G-CSF accounted for the expansion of CD66b+ G-MDSCs, we treated WM-BM MDSCs with G-CSF and found that the CD66b+ G-MDSC population substantially increased (p=0.0017).

Further, CITE-seq analysis showed significantly high expression of NLRP12, IGFBP7, CXCR4, IL2, CD55, SOX4, and FoxP1 genes in CD66b+ MDSCs as compared to other MDSC populations. This gene expression signature suggested upregulation of inflammatory pathways potentially affecting immune function. Therefore, to evaluate the effect of CD66b+ G-MDSCs on the immune function of other cells in the BM, CD66b+ and CD66b- MDSCs were co-cultured with activated autologous T-cells. We found that while all populations of MDSCs suppressed T-cell activation, CD66b+ G-MDSCs had the greatest suppressive effect.

Conclusions:

In summary, this study has shown that CD66b+ G-MDSCs are expanded in WM patients, exhibit a unique inflammatory transcriptome, and substantially inhibit T-cell activation and proliferation. Furthermore, CD66b+ G-MDSCs are expanded due to increased G-CSF in the BM microenvironment in WM. Clinical strategies to inhibit the expansion of CD66b+ G-MDSCs may enhance immunological therapies' efficacy in patients with WM.

23.

Clinical and biological features of WM in the elderly (≥75years): description of a case series from an Italian University Hospital

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Introduction. Waldenström's Macroglobulinemia (WM) is a rare low-grade non-Hodgkin lymphoma (NHL) displaying bone marrow infiltration by neoplastic lymphoplasmacytes, which produce an IgM monoclonal paraprotein. People in the advanced age group are frequently affected by WM and despite growing evidence indicates that stratification by age, namely <75 vs ≥75 years, could segregate diseases with different clinical and biological features, data in this regard are poor.
Aims. Our purpose was to compare elderly (≥ 75 years old) with younger MW patients to verify if peculiar characteristics could be recognized as prognostic factors or as guide for therapeutic decisions.
Methods. Medical records complete of relevant information of 161 WM patients followed at the Hematology Unit of the Padova University Hospital, Italy, between 1990 and 2022 were retrospectively reviewed. One hundred fifty-three cases resulted suitable for the analysis and were stratified according to age at diagnosis (<75 vs ≥75 years, 120 vs 33 patients, respectively). Differences in clinical and biological features, first-line therapy administered and outcome between the two groups were examined. Statistical analysis compared continuous variables in univariate model using U-Mann-Whitney test and Chi-square test for categorical ones. Outcome analyses were focused on overall survival (OS) and treatment free survival (TFS).
Results. Median age at diagnosis was 66 years (range: 38-91 years). Median follow-up was 6 years. Of the 153 patients, 21.6% (33) were ≥75 years old at diagnosis. Comparing the two age cohorts (<75 vs ≥75 years), we found that the elderly group was characterized by more cases with renal disfunction (p=0.05), higher b_2-microglobulin levels (p=0.04) and cytogenetic aberrations (p=0.02). With the limitation due to the small number of patients with an available complete cytogenetics, the occurrence of a complex karyotype was consistently more frequent among the elderly compared to younger patients (40% vs 12.5%, respectively). Moreover, this population showed a trend for decreased lactate dehydrogenase (LDH, p=0.07), increased value of IgM (p=0.06) and higher incidence of other tumors (p=0.06). No differences in the frequency of *MYD88* and *CXCR4* gene mutations were found. Also, degree of anemia, presence of lymphadenopathy/splenomegaly and C reactive protein levels were similar. These latter variables are considered suggestive for the inflammatory form of WM. Moreover, we found no substantial differences in the type of therapy (chemotherapy, immunotherapy, proteasome and BTK inhibitors) and quality of response between the two cohorts. The aged population presented a median OS of 81.9 months vs 321.4 months of patients < 75 years (Log rank, p=0.0008). Elderly compared to younger patients had a worse TFS (81.9 months vs 173.0 months, respectively, Log rank, p=0.001) (Tables 1 and 2 and Figure 1).
When compared to age-matched general population it appears that WM influences OS in the elderly.
Conclusions. In our case series, elderly patients (≥75 years) had worse clinical and biological features. They received same-intensity therapies with similar response rates. The role of unfavorable WM characteristics (especially cytogenetics) versus other comorbidities in determining the survival outcome remains to be addressed in this population.

24.

Identification of robust predictors for ibrutinib response by multi-omic genomics in MYD88 mutated Waldenstrom's Macroglobulinemia.

Richardson K, Hunter ZR, Sarosiek SR, Branagan AR, Flynn CA, Meid K, Leventoff CR, White TP, Little M, Gustine JN, Liu X, Kofides A, Liu S, Canning A, Wolf JL, Kacena K, Patterson CJ, Guerrera ML, Tsakmaklis N, Castillo JJ, Treon SP.

Background: Waldenstrom's Macroglobulinemia (WM) is characterized by highly recurring mutations in MYD88 (95-97%) and CXCR4 (40%). *MYD88* and *CXCR4* mutations also impact ibrutinib activity, including time to major response, depth of response and progression-free survival (PFS). Mutation testing for *CXCR4* is challenging and can result in false negatives in up to 2/3 of WM patients by next generation sequencing (Gustine et al, BJH 2021). Moreover, *CXCR4* mutations do not fully predict response activity to BTK-inhibitors. Attainment of a major response (≥PR) by month 6 is a validated predictor for long-term PFS with ibrutinib (Castillo et al, BJH 2021). As such, we sought to identify biomarker(s) to predict response activity to ibrutinib using a multi-omic approach that could easily be adopted to the clinical setting.

Methods: We performed multi-omic sequencing using whole exome, RNA-seq, methylome and ATAC sequencing in treatment-naïve, symptomatic MYD88 mutated WM patients who received ibrutinib on a prospective clinical trial with long-term follow-up (Treon et al, JCO 2018; Castillo et al, Leukemia 2022). ElasticNet regression was performed on differentially expressed transcripts and ATAC regions expressed in the adjusted model using al2fc > |1| and adj p of < 0.1. Models were adjusted for gender, age and B_2M.

Results: PFS was longer among patients attaining a major vs. non-major response at 6 months (median not reached vs 64.5 months; p=0.10). By whole exome sequencing, the only mutation that showed significant association with major response at 6 months was *CXCR4* (p=0.002). While mutated *CXCR4* associated with non-responders, 3/13 (23%) *CXCR4* mutated patients were major responders at 6 months. By RNA-Seq, 13 DE genes were identified that included *WNK2* (p=0.00005), *DUSP22* (p=0.0008), *GPER1* (p=0.0008) as the top hits that were expressed in *CXCR4* wild-type and *CXCR4* mutated major responders. Other DE genes identified by RNA-Seq in major responders included *OSBPL3*, *PRDM15*, *GPLD1*, *GPR18*, *NEB*, *PPP1R3E*, *HYI*, *AC012368.1*, *WDR19*, and *DDR1*. ATAQ-Seq revealed one significantly open genomic region in chromosome 12 which mapped to the 5' region of the *KIF21A* locus, whose transcript was also DE between response groups. ElasticNet regression analysis using RNA-Seq and ATAC-Seq subjected to 500 bootstraps showed many regulators of ERK1/2 signaling including *WNK2*, *DUSP22*, *GPER1*, *TNIK*, and *PRDM15* among top hits (**Fig. 1**). Attainment of a major response at 6 months was strongly associated with expression of *WNK2* (p=0.0043), as well as *DUSP22*, *GPER1*, *TNIK* and *PRDM15* (all p<0.0001). Long-term PFS correlated with attainment of major response at 6 months for these biomarkers. Expression of *WNK2* by immunohistochemistry (IHC) was validated in bone marrow biopsy samples to discern *WNK2* expressors.

Conclusions: By use of comprehensive multi-omic genomics, we identified robust biomarkers to predict ibrutinib major response at 6 months in *MYD88* mutated WM patients that included many regulators of ERK1/2 signaling such as *WNK2*, *GPER1*, *DUSP22*, *TNIK* and *PRDM15*. Expression of these markers correlated with long-term PFS. The feasibility of using IHC to identify biomarker expression was demonstrated, thereby providing a framework for clinical investigation of novel multi-omic identified genes as predictive biomarkers for BTK-inhibitors in WM.

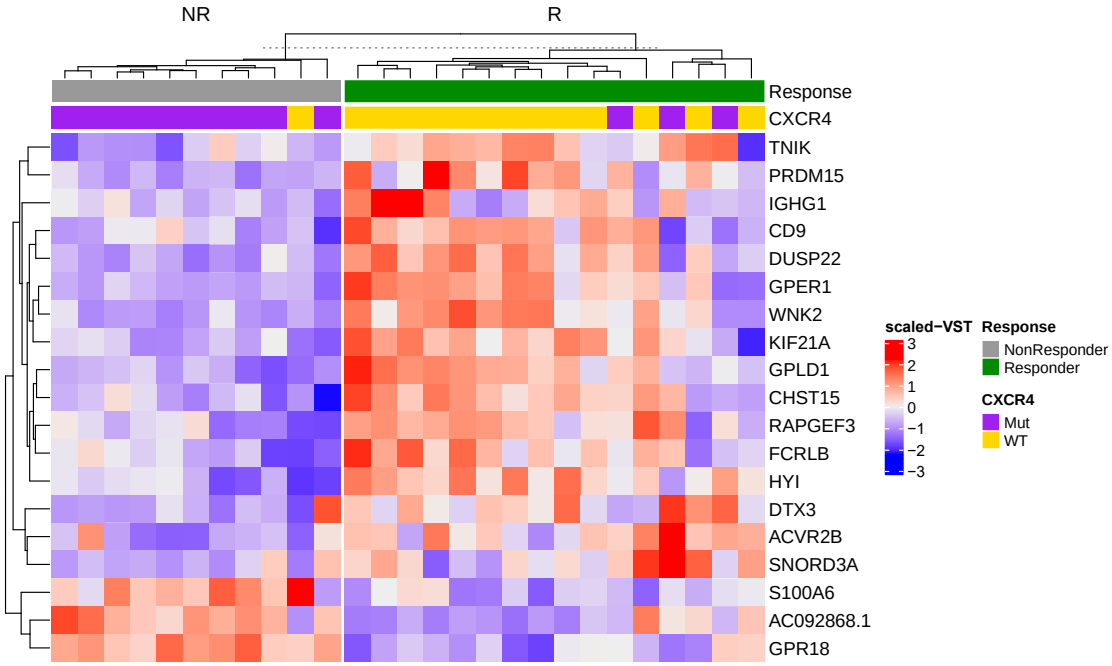

Fig. 1. A heatmap for baseline expressed genes in MYD88 mutated WM patients who received ibrutinib monotherapy on a clinical trial, and who attained a major response (green) or no major response (gray) at 6 months. The heatmap shows the scaled VST gene levels selected by ElasticNet which best predicts differences observed in response between the two groups. Each patient's CXCR4 is shown above the heatmap.

25.

Minimal residual disease (MRD) in Waldenstrom macroglobulinaemia (WM): Depletion of WM-phenotype B-cells is strongly associated with progression following rituximab-based therapy.

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Minimal residual disease (MRD) has been shown to be an independent predictor of patient outcomes in several mature B-cell malignancies, most notably myeloma and CLL. Rituximab-based regimens are widely used as primary therapy in Waldenstrom macroglobulinaemia (WM) and many patients have excellent response durations despite the apparent rarity of complete responses (CR). It is recognised that rituximab-based therapies can result in depletion of monotypic B-cells but a persistence of CD20- plasma cells. This phenomenon can result in delayed serological responses and may also explain the low CR rates. We have therefore developed a sensitive flow cytometry assay for WM B-cells, based on the unique $CD22^{wk}$ CD25+ immunophenotype, to quantify the extent of B-cell depletion with rituximab-based therapies (limit of detection of 0.004%).

Aim: To determine the prognostic significance of residual neoplastic B-cells in WM following rituximab-based therapy in the context of the UK R2W clinical trial.

Method: 60 treatment-naïve symptomatic WM patients were randomised 2:1 to treatment with BCR (Bortezomib; Cyclophosphamide; Rituximab) or FCR (Fludarabine; Cyclophosphamide; Rituximab). Bone marrow (BM) and peripheral blood (PB) samples were collected at baseline, after three cycles of treatment and at 3 months following the end of treatment (EOT).

Results: Neoplastic B-cells with a WM-phenotype were found in the BM of all evaluable patients at baseline. Persistent WM B-cells were demonstrable in BM samples of 24/44 (54.5%) patients (median 0.99%, range 0.004-32.0%) following three cycles and in 24/53 (45.3%) patients (median 0.22%, range 0.004-11.2%) at the EOT.

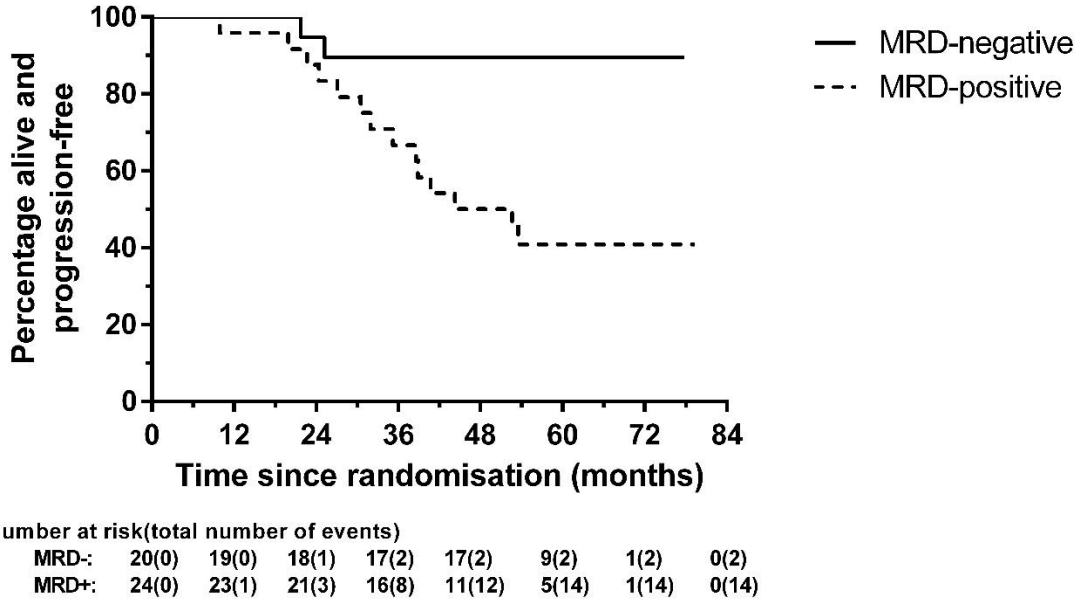
Progression-free survival (PFS) was longer for patients without detectable WM B-cells in the BM, considered MRD-negative, compared to those with detectable WM B-cells, considered MRD-positive. This was the case following three cycles of therapy with 3-year PFS of 89.5% versus 66.7% (HR=0.14, 95%CI: 0.03-0.62, p=0.010) and at the EOT with 3-year PFS of 89.3% versus 58.3% (HR=0.14, 95%CI: 0.04-0.48, p=0.002); see Figures 1A&B. This association with PFS was independent of IgM response and IPSSWM status. Low level PB involvement was noted in 43/54 (79.6%) patients at baseline with median neoplastic B-cells of 1.66% of leucocytes (0.01-36%). Residual neoplastic B-cells could

be demonstrated in the PB in a lower proportion of patients: 5/51 (9.8%) patients following three cycles (median 0.03%, range 0.01-3.14%) and in 3/51 (5.9%) at EOT (median 0.53%, range 0.01-2.67%).

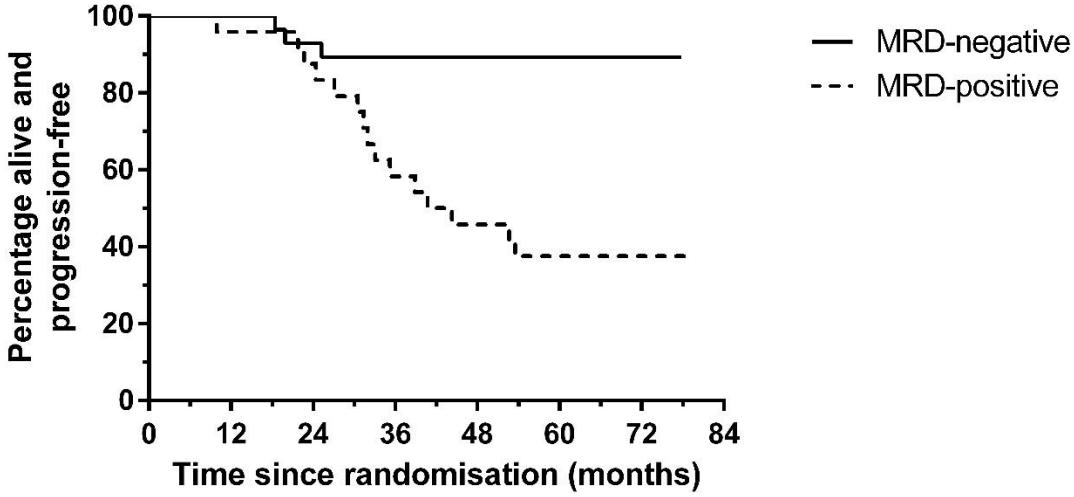
Conclusion: We have shown that sensitive and quantitative flow cytometric assessment of BM B-cell depletion is possible in WM and that this has a significant impact on PFS. Assessment of the peripheral blood appears less sensitive which likely reflects rituximab effect as has been demonstrated in CLL. BM B-cell response appears to be a better predictor of outcome than conventional response assessment and should be utilised when evaluating novel drug combinations in future trials.

Figure 1: Progression-free survival by MRD status after cycle 3 (1A) and at the end of treatment (1B)

(1A)



(1B)



Number at risk(total number of events)

MRD-:	29(0)	28(0)	26(2)	24(3)	23(3)	14(3)	3(3)	0(3)
MRD+:	24(0)	23(1)	21(3)	14(10)	11(13)	6(15)	1(15)	0(15)

26.

Direct-acting antivirals as primary treatment for HCV-associated indolent non-Hodgkin Lymphomas: updated results of the prospective *BArT study* of the *Fondazione Italiana Linfomi*

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Introduction

The most convincing evidence supporting the etiologic role of hepatitis C virus (HCV) in indolent non-Hodgkin's lymphomas (iNHL) is represented by retrospective observations of tumor regression after viral eradication by interferon (IFN)-free direct-acting antivirals (DAAs). However, no prospective studies in this setting have been published so far.

Methods

In 2016 the *Fondazione Italiana Linfomi* started the prospective, multicenter, phase 2, *BArT study* (NCT02836925), evaluating IFN-free DAAs regimens in untreated, HCV-RNA+, non-cirrhotic, iNHL patients without criteria for immediate conventional treatment. Patients with genotypes 1 and 4 received ledipasvir (LDV)/sofosbuvir (SOF) for 12 (naïve) or 24 weeks (IFN experienced), genotype 2 patients SOF + ribavirin (RBV) for 12 weeks and genotype 3 patients LDV/SOF + RBV for 24 weeks. After amendment (July 2017), patients with genotypes 2 or 3 received the novel SOF/velpatasvir (VEL) regimen for 12 weeks. The primary objective was sustained virological response (SVR) while main secondary objectives were overall response rate (ORR) of

lymphoma, progression-free survival (PFS) and toxicity. Herein we update results published in JCO by Merli et al. in 2022.

Results

Forty patients (17 males, 23 females) were enrolled, including 27 marginal zone lymphomas (MZL, 14 MALT, 9 nodal and 4 splenic), 6 lymphoplasmacytic lymphoma (LPL) (4 with Waldenström Macroglobulinemia and 2 with non-IgM LPL), 4 CD5-negative iNHL not otherwise specified, 2 small lymphocytic lymphoma and 1 follicular lymphoma grade 2. Median age was 68 years (45-83). Stage was III/IV in 34 patients (85%). Extranodal sites were involved in 14 (40%) and bone marrow in 23 patients (58%). Genotype was 1 in 17, 2 in 21, 3 in 2 patients. Four patients previously failed an IFN-based regimen. All patients received genotype-appropriate DAAs: 17 LDV/SOF, 8 SOF + RBV, 15 SOF/VEL. The primary endpoint was met as all patients achieved SVR (100%). DAAs were well tolerated, with 17 patients (43%) experiencing 30 grade 1-2 (including 2 RBV-related grade 1 anemia) and 4 grade 3-4 adverse events (grade 4 lipase increase; grade 3 basal cell carcinoma and 2 grade 3 breast cancers, unrelated to study treatments). ORR of lymphoma was 45%, including 8 patients (20%) achieving complete response (CR) and 10 (25%) partial response (PR), while 16 (40%) exhibited stable disease and 6 (15%) progressed (Table 1). Among MZL, the best ORR was recorded in MALT MZL (71%). At a median follow-up of 39 months (95%CI 35-43), two patients died (lymphoma progression and cause unrelated to disease) (4-year OS 94%, 95%CI 76-98%), 3 additional patients progressed (4-year PFS 71%, 95%CI 64-84%) (Figure 1). Notably, no patient with LPL progressed (Figure 2). No significant differences in ORR (48 vs 38%, p=0.74) and PFS (73 vs 77%, p=0.68). were recorded between MZL and non-MZL cases. Four-year duration of response for CR/PR patients was 89% (95%CI 62-97%). Correlative biologic studies (MRD, NGS) are underway and preliminary results will be presented at the meeting.

Conclusions

HCV eradication by DAAs was achieved in 100% in HCV-positive patients with indolent lymphomas not requiring immediate conventional treatment and resulted in non-negligible rate of lymphoma responses. Treatment with DAAs should be considered as the first-line therapy in this setting.

Correlative molecular studies (IGHV repertoire, MRD and mutational data) are ongoing and first results will be presented at the conference.

Table 1 - Lymphoma Responses after direct-acting antivirals (DAAs) in 40 patients with HCV-positive Indolent Lymphomas

Histology	**Response, N (%)**				
	CR	**PR**	**SD**	**PD**	**ORR**
All (N=40)	**8 (20)**	**10 (25)**	**16 (40)**	**6 (15)**	**18 (45)**
MZL (N=27)	**7 (26)**	**6 (22)**	**10 (37)**	**4 (15)**	**13 (48)**
Splenic (N=6)	0	0	4	2	0 (0)
Nodal (N=7)	3	0	3	1	3 (43)

MALT (N=14)	4	6	3	1	10 (71)
Non-MZL (N=13)	**1 (8)**	**4 (30)**	**6 (46)**	**2 (16)**	**5 (38)**
LPL (N=6)	-	1	5	-	1 (17)
CD5- NOS (N=4)	1	1	1	1	2 (50)
SLL (N=2)	-	1	-	1	1 (50)
FL (N=1)	-	1	-	-	1 (100)

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; ORR: overall response rate; MZL: marginal-zone lymphomas; MALT: mucosa-associated lymphoid tissue- marginal-zone lymphomas; CD5-NOS: low-grade CD5-negative B-cell lymphomas not otherwise specified; SLL: small lymphocytic lymphomas; LPL: lymphoplasmacytic lymphomas; FL: follicular lymphomas.

Figure 1 – Overall survival (OS) and progression-free survival (PFS) in 40 patients with HCV-positive indolent lymphoma treated with interferon-free direct-acting antivirals (DAAs)

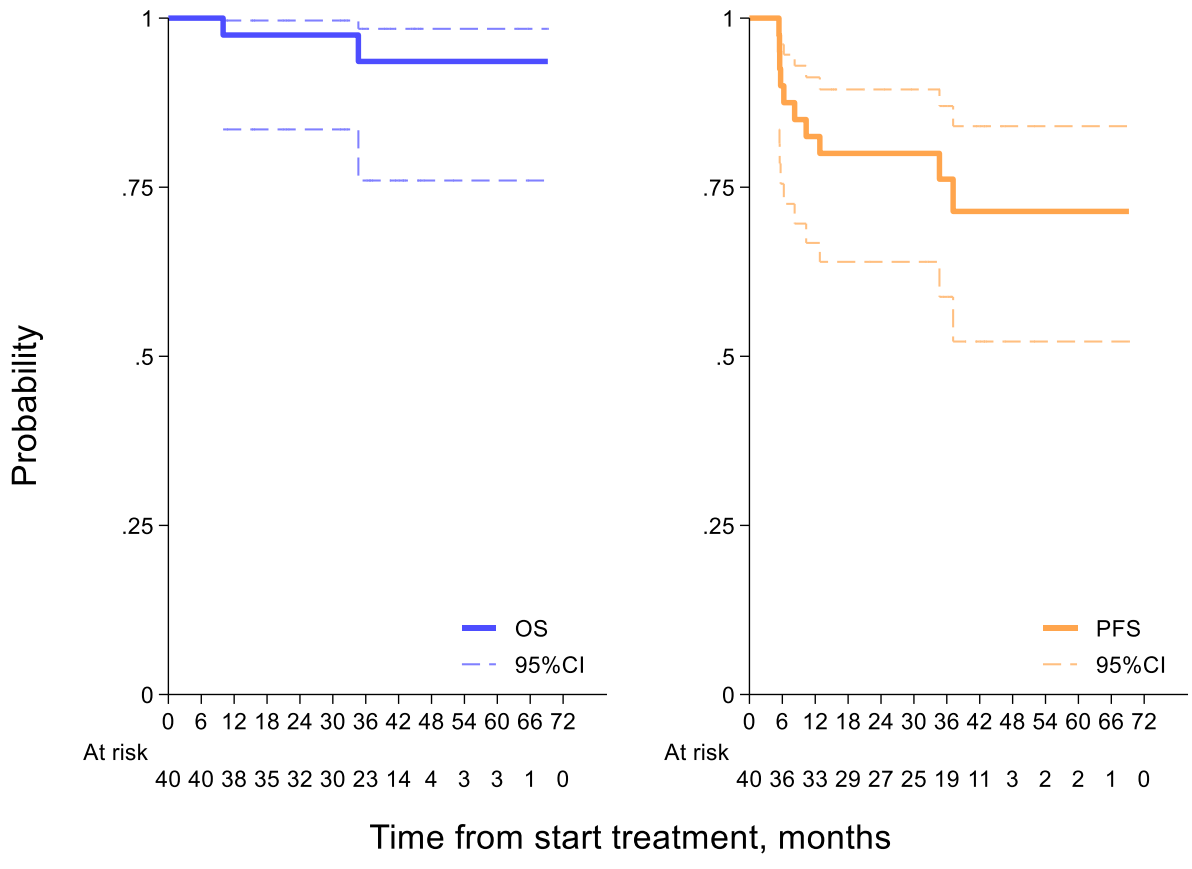
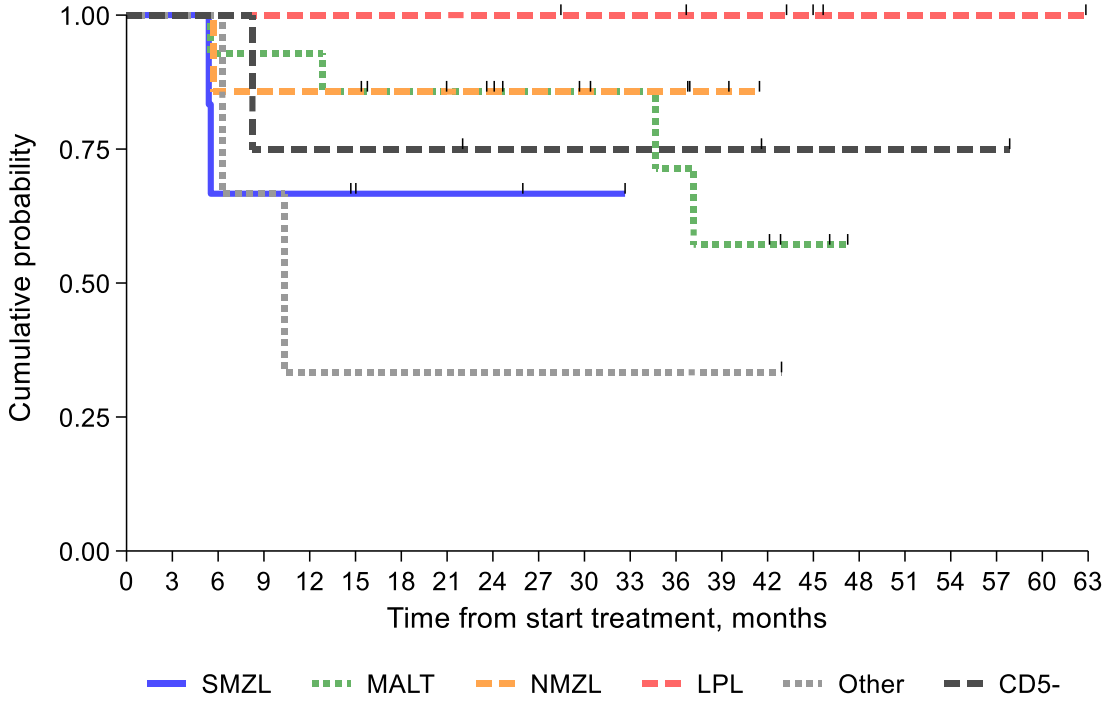


Figure 2 – Progression-free survival (PFS) in 40 enrolled patients according to histologic subtype (SMZL n=4; MALT n=14; NMZL n=9; LPL n=6; CD5- NOS n=4, Other n=3*) (p=0.3).



SMZL: splenic marginal-zone lymphomas; MALT: mucosa-associated lymphoid tissue- marginal-zone lymphomas; NMZL: nodal marginal-zone lymphomas; LPL: lymphoplasmacytic lymphomas; CD5-: CD5-negative B-cell lymphomas not otherwise specified (NOS).
*"Other" includes small lymphocytic lymphomas (n=2) and follicular lymphomas (n=1)

27.

How age, comorbidities and concomitant medications influence ibrutinib management and survival in Waldenstrom macroglobulinemia

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Data on Waldenstrom Macroglobulinemia (WM) receiving ibrutinib outside of clinical trials are sparse, showing a proportion of patients requiring permanent dose reduction (PDR) and toxicity-related definitive treatment discontinuation (Tox-DTD). Furthermore, the impact of concomitant medications is still unexplored. With this aim we evaluated which fitness parameters/concomitant drugs are significant for treatment outcome and management in WM patients receiving ibrutinib in common practice.

In this multicentric retrospective study we analyzed the impact of age (≥75y), CIRS (>6), major comorbidities (at least one organ with a CIRS score ≥3, CIRS3+), ECOG-PS (>1), polypharmacy (>3 concomitant medications), type of concomitant medications, CrCl (<50 ml/min), baseline nephropathy, cardiopathy, hypertension, neuropathy, neutropenia and disease characteristics on tox-DTD; PDR; EFS (event: tox-DTD, PDR, progression/death); PFS and OS. Medical conditions deemed to be WM complications and WM diagnosis itself, were not included in CIRS score calculation.

From Aug 2016 to Apr 2022, 206 patients were analyzed. Patients' characteristics are reported in table1. After a median follow-up of 26.6 months (range 0.5-70.5 months), 139 patients (67.5%) are still on ibrutinib. Temporary interruptions occurred in 60 patients (29.6%) for a median of 18

consecutive days. Overall, 67 patients discontinued ibrutinib due to: PD in 30 (14.6%); toxicity in 23 (11.2%, Tox-DTD); other reasons in 14 (6.8%). Median time to Tox-DTD was 7.5 months. Atrial fibrillation (AF) was the most common reason in 10/23 pts.

Fifty-three patients (25.7%) required dose reduction at least once, followed by PDR in 40 (19.4%) of them. Most common reasons for PDR were AF and diarrhea, both occurring in 7 patients. Median time to PDR was 7.1 mo.

Patients with very-high revised-IPSSWM and CrCl<50 showed higher risk of Tox-DTD, that was confirmed also at multivariate (*p .002* and *p .025*, respectively). While ECOG-PS>1, CIRS>6 and cardiocomorbidity significantly influenced PDR at univariate analysis, only ECOG-PS maintained its independent role (*p <.001*). Number and type of concomitant medications had no impact on ibrutinib management.

Median PFS, EFS and OS for the whole population were not reached (73.3%, 54.1% and 85.3% at 2 years, respectively). Age, ECOG-PS, CrCl>50 and nephropathy negatively predicted all survival outcomes at univariate analysis. CIRS3+, but not CIRS>6 impacted on OS. At Cox regression model, only ECOG-PS>1 confirmed its role on PFS, EFS and OS (*p <.001*), while CrCl<50 was significant for EFS (*p .007*) and OS (*p .003*). PFS was significantly impacted by PDR (p<.0001), also showing a trend in pts temporarily interrupting ibrutinib. Results from univariate analysis are summarized in Table 2.

To our knowledge this is the first series analyzing the role of age, comorbidities and concomitant medications in patients with WM receiving ibrutinib. Poor ECOG-PS and compromised renal function emerged as significant prognostic baseline parameters on both treatment management and survival. High comorbidity burden, but not major comorbidity associated with schedule modifications. Age *per se* did not result as a risk factor for toxicity-related discontinuations/ dose reductions, nevertheless patients with very-high revised-IPSSWM were at higher risk of Tox-DTD. Importantly, neither polypharmacy, nor type of concomitant medication interfere with ibrutinib management.

Table 1. Patients' and disease characteristics at ibrutinib initiation

Characteristic	Value N° (%)
Median Age y (range) <65 y/65-75 y/>75 y	70.4 (45.1-92.1) 45 (21.8)/57(27.7)/104 (50.5)
Sex: Male/Female	138 (67)/68 (33)
ECOG-PS 0-1/>1	 160 (77.6)/ 46 (22.4)
CIRSMedian (range) **CIRS ≤6/CIRS*>6**	3 (0-18) 174(84.5)/32(15.5)
CIRS3+	44 (21.4)
CIRS*>6 and CIRS3+	18 (8.73)
CrCl ml/min <50 ≥50	 45/203 (22.2) 158/203 (77.8)
Pts with Cardio-Comorbidity	46 (22.3)
Pts with Hypertension	95 (46.1)
Pts with neuropathy	39 (18.9)
Pts with nephropathy	28 (13.6)
Median N° concomitant medications (range) **Pts with >3 concomitant medications**	3 (0-13) 88 (42.7)
Pts treated with CYP3A4 inhibitors/inducers	12 (5.8)
Pts treated with anticoagulants and/or antiplatelets Anticoagulants only Antiplatelets only Anticoagulant+antiplatelets Dual antiplatelet therapy	39 (18.9) 13 (6.3) 26 (12.6) 3 (1.5) 0 (0)
Revised-IPSS WM very low low intermediate high very high	 38/167 (22.8) 43/167 (25.7) 58/167 (34.7) 25/167 (15) 3/167 (1.8)
Prior Tx median (range) 1-2 ≥ 3	1 (1-7) 159 (77.2) 47 (22.8)
MYD88 mutated	148/153 (96.7)
CXCR4 mutated	13/60 (21.7)
IgM mg/dl median (range)	2510 (115-7800)
Hb g/dl median (range)	9.8 (6.6-16)

Grade 3-4 neutropenia	21(10.2)

CIRS, cumulative illness rating scale; CrCL, Creatinine Clearance; Tx, therapy; Pts, patients
*medical conditions that deemed to be complications of CLL not included as part of the total CIRS score

Table 2. Univariate analysis of association of patients and disease characteristics with PFS, EFS, OS, tox-DTD, PDR*

	PFS		EFS		OS		tox-DTD		PDR	
**	*HR (95% CI)*	*p*	*HR (95% CI)*	*p*	*HR (95% CI)*	*p*	*HR (95% CI)*	*p*	*HR (95% CI)*	*p*
Age	1.87 (1.29-3.09)	.016	1.79 (1.18-2.70)	.006	2.09 (1.07-4.08)	.031		NS		NS
ECOG-PS	3.05 (1.85-5.04)	**<.001**	4.2 (2.78-6.36)	**<.001**	4.47 (2.34-8.53)	**<.001**		NS	3.92 (2.10-7.34)	**<.001**
CIRS3+		NS	1.6 (1.01-2.52)	.046	2.26 (1.15-4.44)	.018		NS		NS
CIRS>6		NS	1.7 (1.03-2.82)	.039		NS		NS	2.16 (1.05-4.44)	.036
Cardiocomorb		NS	1.66 (1.06-2.62)	.028	2.52 (1.29-4.91)	.007		NS	2.09 (1.07-4.08)	.030
Hypertention		NS	1.52 (1.01-2.28)	0.04	2.35 (1.21-4.56)	.011		NS		NS
CrCl<50	3.86 (2.32-6.44)	<.001	2.56 (1.65-3.98)	**<.001**	3.94 (2.03-7.66)	**<.001**	3.18 (1.33-7.60)	**.009**		NS
Nephropathy	3.09 (1.74-5.48)	<.001	2.4 (1.45-3.97)	<.001	2.55 (1.20-5.43)	.015		NS		NS
Polypharmacy	1.89 (1.15-3.10)	.012		NS	3.06 (1.55-6.05)	.001		NS		NS
Antiplatelets		NS	1.85 (1.04-3.29)	.036	2.42 (1.05-5.58)	.037		NS		NS
CYP3A4		NS		NS	2.91 (1.13-2.49)	.026		NS		NS
r-IPSSWM		NS		NS		NS	4.90 (1.88-12.82)	**.001**		NS

NS: not significant
*p values in bold refer to variables significant also at multivariate analysis
**All factors considered: Age, sex, ECOG-PS, CIRS3+, CIRS>6, cardiocomorbidity, hapertention, CrCl, nephropathy, neuropathy, neutropenia, polypharmacy, anticoagulants, antiplatelets, CYP3A4 inhibitors/inducers, revised IPSSWM, MYD88 mutation, CXCR4 mutation

28.

The prognostic role of depth of response depends on the time of assessment after first-line immunochemotherapy in patients with symptomatic Waldenstrom macrogobulinemia (WM).

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The current criteria for the assessment of response after treatment of patients with symptomatic WM (sWM) have been proposed following the 6th International Workshop on WM with the main aim to promote uniform reporting of clinical trial data but also to retain a predictive role for subsequent outcome. This guideline recommended to take into account the deepeest response observed during follow-up (Owen, BJH 2013, 160 ; 171). On one hand, attainment of complete/very good partial response (VGPR) following immunochemotherapy as first-line or salvage therapy has been shown to be an important determinant to progression-free survival (PFS) (Treon BJH 2011). On the other hand, time-dependent models failed to identify significant prognostic value for survival after first treatment intiaition associated with any level of response (Guidez, Blood Adv 2018 2 ; 3102). In order to explain both results, we assessed the changes in the linear predictor (LP) (and consequently hazard ratio [HR]) associated with partial response (PR) in Cox models of subsequent PFS and survival performed at 72 landmark points (every 2 weeks for the first 3 years after treatment initiation). Dynamic landmarking was performed according to van Houwelingen and Putter (Lifetime Data Anal 2008 14: 447). For this purpose, we updated the records of 134 symptomatic patients who received chemotherapy (cCT 74 patients) or immunochemotherapy (ICT, 60 patients) as front line therapy (median age : 69 Interquartile range: 61-78, M/F=1.85, median PFS and survival after treatment intiation : 31 months ; 95% confidence interval [95CI] : 36-44 and 108 months 95CI: 83-155, respectively). No patient received maintenance therapy. Using sandwich estimators to take into account the clustering of the data, significant favorable effect of PR achievement for subsequent PFS was only observed from the 8th to the 20th landmark points i ;e. between 4 and 10 months only in patients treated with ICT. No significant effet of PR on subsequent survival was observed at any landmark point. More importantly, a regression analysis showed a significant change in the LP with the landmark point rank in patients treated with CT ($p<0.0001$for subsequent PFS and survival) and ICT ($p<0.0001$for subsequent PFS and survival).

Overall, our findings highlight the importance of the timing of response assessment in WM : They confirmed the prognostic value of response after ICT frontline, for subsequent PFS, when assessed 4 to 10 months after treatment intiation (the usual time of completion of ICT). Conversely response status may retain less prognostic value outside of these timepoints. The change in the prognostic importance of response during the evolution likely explain the absence of prognostic value of response coded as a time dependent covariate over the whole period of follow-up after first treatment intiation ($p=0.06$ for PR in the present series). Thus, landmark and time-dependent analyses provide complementary information. Similar analyses should confirm the prognostic value of PR achievement at 6 months in patients wo received ibrutinib frontline or later (Castillo BJH 2021, 192, 542–550).

29.

Clinical and clonal characteristics of Immunoglobulin M-associated type I cryoglobulinaemia

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Background

Type 1 cryoglobulinaemia is defined by monoclonal immunoglobulins which precipitate at temperatures <37°C and redissolve on warming. IgM-monoclonal protein may be associated with cryoglobulinaemia with underlying Waldenström macroglobulinemia (WM), non-Hodgkin lymphoma (NHL) or monoclonal gammopathy of undetermined significance (MGUS). We analysed the incidence, clinical and clonal characteristics of the largest reported series of IgM-associated type I cryoglobulinaemia.

Methods

Data for consecutive adult patients with IgM-associated type I cryoglobulinaemia identified between 2013-2022 from two centres (UCLH, UK and AMC, Netherlands) were retrospectively analysed.

Results

423 samples were screened for cryoglobulinaemia between 2013-2022 from all patients with an IgM-associated disorder at the specialist centre, UCLH. 140 (33%) were positive for all-type cryoglobulinaemia; 94 (22%) patients had type I cyoglobulinaemia. Four additional patients were recruited from UMC, without incidence data. In total, 98 patients (58 male, 40 female) were identified with type I cryoglobulinaemia; the majority with underlying WM (77; 79%), and remaining IgM MGUS (16; 16%) or NHL (4 marginal zone lymphoma, 1 CLL; 5%). There was a kappa light-chain predominance (85%). *MYD88*^{L265P} was present in 44/49 (90%) of WM and 3/8 (38%) MGUS cases. *CXCR4* was mutated in 5/13 (38%) WM cases. IgM-associated disorders were co-existent in 25: cold agglutinin disease/syndrome (CAD/CAS, 15%), Bing-Neel syndrome (7%), anti-MAG antibodies (5%), Schnitzler syndrome (1%). Those with coexistent CAD/CAS had characteristics of a CAD clone, significantly associated with MGUS (vs WM/NHL, p<0.0001) and *MYD88*-wild type (p<0.0001). Patients with anti-MAG antibodies were more likely have MGUS (p=0.0245). Median M-protein at diagnosis of cryoglobulinaemia was 12g/l (0-63), lowest in the MGUS cohort vs WM vs NHL (4g/l vs 16g/l vs 10g/l, p=0.0003). The majority were diagnosed with cryoglobulinaemia >3 months after the underlying IgM disorder (60/98; 61%) at a median of 46 months (4-442), longer in those with WM vs MGUS or NHL (51 vs 17 vs 13 months, p=0.023).

49% were symptomatic at cryoglobulinaemia diagnosis, with a trend towards greater proportion in those with MGUS/NHL vs WM (75%/60% vs 43%, p=0.0569). The most common symptoms were cutaneous/vasomotor symptoms (28%), neuropathy (21%), hyperviscosity (10%), arthralgia (7%) and renal involvement (2%).

At a median follow up of 2.4 years (0-9.4), 2-year overall survival (OS) was 89% (95% CI 80-94). Age was the only predictor of OS (HR 1.07, 95% CI 1.01-1.12, p=0.006). Fourteen patients required chemoimmunotherapy for cryoglobulinaemia at a median of 1 month (0-7) from diagnosis for cutaneous manifestations (5/14; 36%), cryoglobulinaemic glomerulopathy (2/14; 14%) or hyperviscosity (9/14; 64%). Independent predictors of cryoglobulinaemia-indicated treatment-free survival/death included cutaneous involvement (HR 1.48, 95% CI 1.21-7.7.92, p=0.018) and hyperviscosity (HR 5.21 95% CI 4.21-27.8, p<0.0001) on multivariate analysis.

Conclusions

This is the largest reported series describing the characteristics of IgM-associated type I cryoglobulinaemia. It is common amongst patients with clonal IgM disorders and approximately half of patients may be symptomatic. Distinct clonal populations are present in which type I cryoglobulinaemia may develop including an *MYD88*^{L265P} WM clone and *MYD88*-wild type CAD/anti-MAG clone. Treatment may be required selectively in those with hyperviscosity, cutaneous or renal involvement.

30.

Association of *MyD88* and *CXCR4* gain of function mutations exacerbates the "Waldenstrom-like" phenotype in mice

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Waldenström Macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized by bone marrow (BM) infiltration of clonal IgM+ cells with a phenotype ranging from activated B cells to plasma cells (PCs). *MYD88* gain of function mutations are found in 90% of cases and are associated in 30% of cases with a gain of function mutation of *CXCR4*. Patients with both mutations have higher BM infiltration and IgM titres. MYD88 is implicated in the signalling pathway downstream of Toll-like receptors (TLR) while CXCR4 is a chemokine receptor involved in different cellular processes including cell migration, proliferation, and differentiation. Despite progresses made in the last few years, how both mutations synergize is still unclear.
Previous results from our team on mice carrying an orthologous gain of function mutation of *Cxcr4* have shown an exacerbated humoral extrafollicular response with an aberrant accumulation of splenic and medullary IgM+ PCs. In parallel, mice expressing a gain of function mutation of *MyD88* presented a reduced lifespan with splenic expansion of IgM+ B cells.
To better understand how MyD88 and Cxcr4 may synergize to promote WM, we crossed mice bearing both gain of function mutations and compared WT mice to single and double mutant mice. The double mutant mice displayed enhanced splenomegaly associated with a reduced lifespan compared to single *MyD88* mutant animals. Moreover, young double mutant mice had higher serum IgM and higher number of IgM-secreting cells in the BM and spleen. Those characteristics were more marked in symptomatic old mice. Both *Cxcr4* and *MyD88* mutations were associated with enhanced B cell proliferation and with alteration of the molecular profile of PCs. Young and old double mutant mice also displayed B cell subsets dysregulation.
Altogether, by comparing these different mouse models we demonstrated that over-activated MyD88 and Cxcr4 signalling pathways synergize to promote a deleterious hyper-IgM syndrome through the regulation of PC differentiation and survival.

31.

A novel drop-off digital PCR assay for *CXCR4* mutations detection in IgM gammopathies: first data from the multicentric "BIOWM" trial of the Fondazione Italiana Linfomi (FIL)

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Background. In Waldenström's Macroglobulinemia (WM) and IgM gammopathy of uncertain significance (IgM-MGUS), *MYD88*^{L265P} and *CXCR4*^{S338X} are the most frequent mutations. Recent studies demonstrated that, in unselected bone marrow (BM) samples, allele specific polymerase chain reaction (AS-qPCR) was superior to next generation sequencing (NGS), in detecting *CXCR4*^{S338X}, while digital PCR (dPCR) was more sensitive than AS-qPCR for *MYD88*^{L265P}. **Aims.** 1) to test a novel drop-off dPCR assay for *CXCR4*^{S338X} mutations in WM and IgM-MGUS patients enrolled in the FIL multicentric BIOWM trial (NCT03521596), sponsored by the International WM Foundation/Leukemia and Lymphoma Society; 2) to compare dPCR with NGS; 3) to correlate the *CXCR4* mutational status with clinical features. **Methods.** DNA from 269 patients (203 WM, 66 IgM-MGUS) was tested by dPCR: 67 BM-CD19 selected (CD19+) cells,

202 BM and 267 peripheral blood (PB) unselected white blood cells (WBC). Allele frequency (AF) of *CXCR4* mutations at p.S338 locus (*CXCR4*^{MUT}) detected by the dPCR assay (sensitivity of 0,001%) was compared with NGS targeted resequencing data (median coverage 2369x). **Results**. 51/203 WM patients (25%) showed *CXCR4*^{MUT} in BM (median AF 2.7%, range: 0.2%-47%) and only 10 (5%) in PB (median AF: 0.6%, range: 0.2%-35%). 7/66 (11%) IgM-MGUS patients scored *CXCR4*^{MUT} in BM (median AF 0.65%, range: 0.2%-1.1%) and none in PB. All patients were screened for MYD88^{L265P}, too. (Fig.1). Interestingly, BM-WBC *CXCR4*^{MUT}*/MYD88*^{L265P} showed a correlation in mutational levels between the two mutations (R2=0.9), while no correlation was observed in CD19+ *CXCR4*^{MUT}*/MYD88*^{L265P} samples (R2=0.13) (Fig.2). Furthermore, dPCR vs NGS comparison was performed in 218 samples (123 CD19+, 95 BM) showing a substantial level of agreement between the methods: 87%, K-Cohen 0.69 in CD19+ and 88.4%, K-Cohen 0.53 in BM. dPCR-MUT/NGS-WT discordances were observed in 12 samples (AF≤0.1% by dPCR), while ddPCR-WT/NGS-MUT in 15 samples (mutations outside the p.S338X locus). Overall, clinical features of *CXCR4*^{MUT} vs *CXCR4*^{WT} patients did not differ significantly, except for lower haemoglobin levels (median 11 g/dl vs 13 g/dl, p<0.00001) and higher serum IgM monoclonal component (median 1.8 g/dl vs 1.3 g/dl, p=0.017), observed in mutated cases. 22/51 (43%) *CXCR4*^{MUT} WM treated at the time of enrolment (7 DRC, 9 BR, 3 other R-chemo and 3 single agents), showed higher disease and mutational burden compared to the *CXCR4*^{MUT} watch and wait (WW) patients. Indeed, median histologic BM infiltration was 80% vs 30% (p<0.00001), IgM monoclonal component was 4 g/dl vs 1.25 g/dl (p=0.005) and AF was 10% vs 1% (p=0.008), respectively. **Conclusions.** We here describe a new dPCR approach for CXCR4^{S338X} detection in BM, with no need for CD19+. We observed: 1) a lower AF in IgM-MGUS compared to WM; 2) suboptimal use of PB for CXCR4^{MUT} detection; 3) a concordant mutational level between CXCR4^{MUT} and MYD88^{L265P} only in unselected samples; 4) a correlation of CXCR4^{MUT} with low haemoglobin and high serum IgM component; 5) a statistically significant difference in BM involvement, serum IgM component and CXCR4^{MUT} level between patients treated and those in WW. A longer FU is needed to better clarify the clinical implications of CXCR4 mutations in our series.

Figure 1: CXCR4^{MUT} and MYD88^{L265P} mutational status in WM and IgM-MGUS by dPCR.

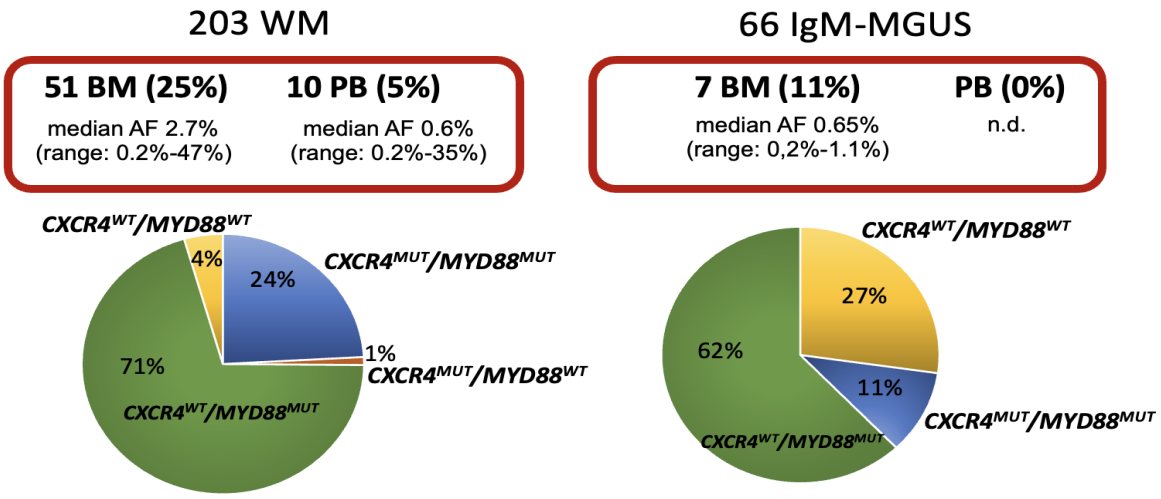
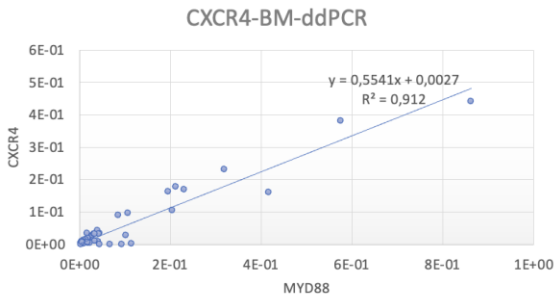
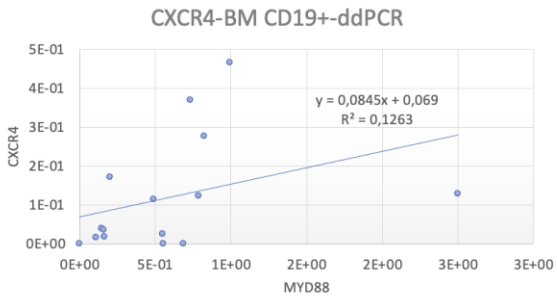


Figure 2. Correlation of mutational levels between $CXCR4^{MUT}$ and $MYD88^{L265P}$ in unselected BM and selected CD19+





32.

Asymptomatic IgM Monoclonal Gammopathy: Progression Risk and Survival Trends Of 915 Patients From A Multicenter Spanish Registry

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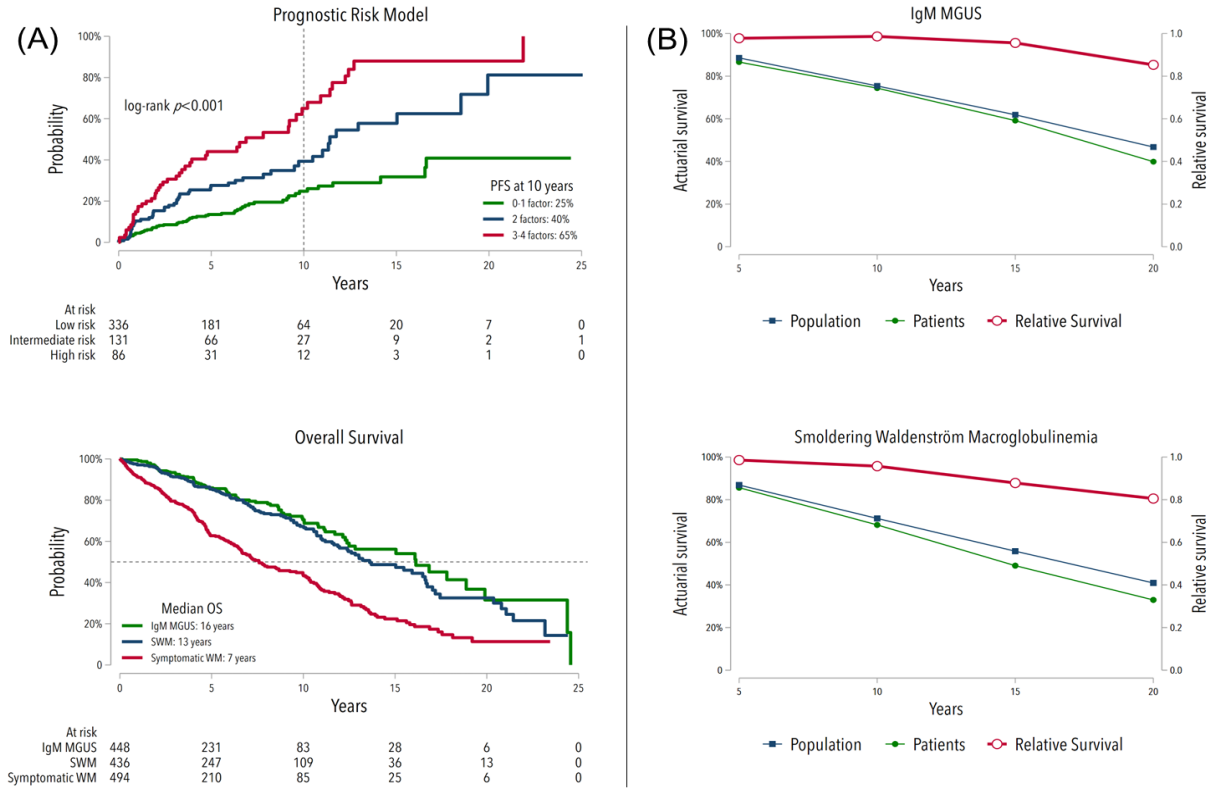
BACKGROUND: Waldenström macroglobulinemia (WM) is a lymphoplasmacytic lymphoma with a monoclonal IgM in serum. Early asymptomatic stages include IgM monoclonal gammopathy of undetermined significance (MGUS) and smoldering WM (SWM). While most IgM MGUS patients (pts) will not progress during follow-up, a greater proportion of SWM will do. Data is scarce regarding how IgM MGUS and SWM survival trends have evolved during the last decades. Here, we describe a comprehensive proposal to model progression risk and give insight into survival trends using retrospective data from the largest cohort of IgM MGUS and SWM pts in 13 Spanish hospitals.
METHODS: Progression-free survival (PFS) was calculated from the date of diagnosis to the date of last follow-up or disease progression (symptomatic WM, other non-Hodgkin lymphoma or AL amyloidosis). Overall survival (OS) was calculated up to death from any cause. To find the best model, we used a backward stepwise and lasso (least shrinkage and selection operator) logistic regression with continuous meaningful variables that predict progression at 10 years. We then categorized continuous data according to Liu and Youden methods. Relative survival (RS) was calculated using the Ederer II method.
RESULTS: 915 pts (462/453 IgM MGUS/SWM) were included. *MYD88* L265P (available in 465 pts) was detected in 107 (54%) IgM MGUS and 217 (81%) SWM pts. With a median follow-up of 6 years, disease progression was documented in 250 pts (16% IgM MGUS and 39% SWM). The incidence rate of progression per 100 person-years was 2.9 (95% confidence interval [CI] 2.4 – 3.7) and 7.0 (95% CI 6.1 – 8.2) in IgM MGUS and SWM, respectively. Low serum IgG (p=0.03), high IgM (p<0.01), high bone marrow (BM) infiltration (p=0.01), and low albumin (p<0.01) were selected using backward stepwise regression. Lasso regression was performed to select predictors of progression at 10 years, identifying also low IgA. We then chose the 4 best predictors between the two

methods (BM infiltration, IgM, decreased IgG/IgA, and albumin). To fit into an easy-to-use model, we categorized continuous data as meaningful for clinicians and were able to discriminate pts. 84% of IgM MGUS pts were categorized as low-risk, while 57% of SWM were intermediate or high-risk. There were only differences regarding OS between the asymptomatic stages and a series of symptomatic WM pts from the same hospitals ($p<0.01$) (Figure 1A).

Regarding survival trends, IgM MGUS had a RS slightly higher than that of the matched general population during the first 10 years of diagnosis, probably due to early access to public health, and then decreasing especially at 20 years. SWM had a RS lower than the general population from the first 10-15 years of diagnosis (Figure 1B).

CONCLUSION: We developed a new ready-to-use risk model for both IgM MGUS and SWM. Most IgM MGUS pts were low-risk, while SWM accounted for the majority of intermediate/high-risk groups. No differences were observed regarding OS between IgM MGUS and SWM. RS of IgM MGUS was higher in the first 10 years from diagnosis, while SWM showed always a decreasing RS.

Figure 1. **(A)** Prognostic risk model in asymptomatic IgM monoclonal gammopathy based on albumin (<3.5 g/L), serum IgM (≥10 g/L), bone marrow infiltration (≥20%), and immunoparesis (decreased IgG or IgA), and overall survival taking into account symptomatic Waldenström macroglobulinemia (WM) patients. **(B)** Relative survival in IgM monoclonal gammopathy of undetermined significance (MGUS) and smoldering Waldenström macroblobulinemia



33.

Does permanent dose reduction or temporary interruption affect outcome in ibrutinib-treated patients with Waldenstrom Macroglobulinemia?

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Although safe and effective in Waldenstrom Macroglobulinemia (WM), a proportion of patients will discontinue ibrutinib due to adverse events (AEs), especially in common practice where patients are unselected. Temporary treatment interruption (TTI) or permanent dose reductions (PDR) are commonly used in clinical practice to mitigate or prevent AEs or drug-to-drug interactions. The question whether TTI or dose reductions may affect treatment outcome and, if medically indicated, which of these strategies should be preferred, is still unexplored.

With this aim, we compared patients receiving ibrutinib and modifying treatment schedule, with those who received the BTKi without interruptions or reductions to understand the impact of TTI and PDR on patients' outcome.

In this multicentre study, we retrospectively analysed patients with relapsed/refractory WM treated with ibrutinib outside of clinical trials and identified 3 groups: patients temporarily interrupting ibrutinib for at least one day (GROUP A); patients permanently reducing ibrutinib (GROUP B); patients receiving ibrutinib without TTI or PDR (GROUP C). Patients in which both TTI and PDR were recorded have been incorporated in GROUP B as for those cases PDR resulted as the final event. We described the characteristics of the 3 groups and directly compared progression free survival (PFS) of: i) GROUP A vs GROUP C; ii) GROUP B vs GROUP C; iii) GROUP A vs GROUP B, first at univariate analysis, then at a multivariate model adjusted for age, Eastern Cooperative Oncology Group – Performance Status (ECOG-PS) and number of prior lines of therapy.

A total of 206 patients received ibrutinib from August 2016 to April 2022. Patients' characteristics are reported in table 1. GROUP A, GROUP B and GROUP C included 44, 40 and 122 patients, respectively.

GROUP A; TTI occurred with a median of 20 consecutive days (range 3-360). Ibrutinib was interrupted for: <8 days in 15 (34.1%); 8-14 days in 7 (15.9%); >14 days in 22 (50%). Overall, definitive discontinuation due to toxicity (tox-DTD) occurred in 4 cases (9.1%).

GROUP B; ibrutinib dosage was permanently reduced at: 280 mg in 25 (62.5%); 140 mg in 15 (37.5%). Five cases of tox-DTD (12.5%) were recorded.

Reasons for TTI and PDR are summarized in table 2. AEs leading to treatment schedule modifications resulted in at least an improvement in 19/22 (86.4%) patients of GROUP A and 24/30 (80%) of GROUP B (Table 2).

Both at univariate and multivariate analysis, no differences in PFS emerged when comparing GROUP A vs GROUP C and GROUP A vs GROUP B. GROUP B instead showed a significantly worse PFS compared to GROUP C at univariate analysis (*p .043*) that was independently confirmed at Cox proportional regression model (HR 11.1, CI 95% 1.45-85.86, *p .021*).

To our knowledge this is the first analysis on the impact of ibrutinib management strategies in patients with WM. In this series, permanent reduction, but not temporary interruption, emerged to be detrimental to PFS compared to patients not modifying treatment schedules. As per our data and differently from other lymphoproliferative diseases, this measure may compromise treatment outcome and should be avoided in WM.

Table 1. Characteristics of patients and disease at ibrutinib initiation

Characteristic	GROUP A N° 44 Value N° (%)	GROUP B N° 40 Value N° (%)	GROUP C N° 122 Value N° (%)
Median Age y (range) <75 y/≥75 y	74.1 (48.8-89.7) 26 (59.1)/18 (40.9)	74.1 (51.1-90.5) 17 (42.5)/23 (57.5)	65.5 (39.2-88.4) 96 (78.7)/26 (21.3)
Sex: Male/Female	27 (61.4)/17 (38.6)	25 (62.5)/15 (37.5)	86 (70.5)/36 (29.5)
ECOG-PS 0-1/>1	38 (86.4)/ 6 (13.6)	22 (55)/18 (45)	100 (82.0)/22 (18.0)
CIRS Median (range)	3 (0-15)	3 (0-18)	3 (0-12)

CIRS ≤6/CIRS*>6	38 (86.4)/6 (13.6)	30 (75)/10 (25)	104 (85.2)/18 (14.8)
CIRS3+	7 (15.9)	9 (22.5)	28 (23.0)
CIRS*>6 and CIRS3+	3 (6.8)	5 (12.5)	10 (8.2)
CrCl ml/min <50	 5 (11.4)	 9 (22.5)	 34 (27.9)
Pts with Cardio-Comorbidity	10 (22.7)	13 (32.5)	23 (18.9)
Pts with Hypertension	13 (29.5)	21 (52.5)	61 (50)
Pts with neuropathy	8 (18.2)	8 (20)	23 (18.9)
Pts with nephropathy	3 (6.8)	6 (15)	23 (18.9)
Median N° concomitant medications (range) **Pts with >3 concomitant medications**	3 (0-13) 17 (38.6)	3 (0-8) 17 (42.5)	3 (0-12) 54 (44.3)
Pts treated with CYP3A4 inhibitors/inducers	3 (6.8)	2 (5.0)	7 (5.7)
Pts treated with anticoagulants and/or antiplatelets	4 (9.1)	9 (22.5)	26 (21.3)
Revised-IPSS WM very low low intermediate high very high	 15/39 (38.5) 5/39 (12.8) 13/39 (33.3) 5/39 (12.8) 1/39 (2.6)	 5/32 (15.6) 7/32 (21.9) 15/32 (46.9) 5/32 (15.6) 0/32 (0)	 32/110 (29.1) 31/110 (28.2) 30/110 (27.3) 15/110 (13.6) 2/110 (1.8)
Prior Tx median (range) >1	1 (1-5) 16 (36.4)	1 (1-4) 23 (57.5)	1 (1-7) 51 (41.8)
MYD88 mutated	33/34 (97.1)	28/29 (96.6)	87/90 (96.6)
CXCR4 mutated	3/13 (23.1)	2/9 (22.2)	8/38 (21.1)
IgM (mg/dl) median (range)	2510 (115-5800)	2400 (152-6877)	2500 (151-6800)
Hb (g/dl) median (range)	9.8 (7-14.1)	9.8 (7.7-14.9)	9.8 (6.6-15.8)
Grade 3-4 neutropenia	3 (6.8)	3 (7.5)	15(12.3)

CIRS, cumulative illness rating scale; CrCL, Creatinine Clearance; Tx, therapy; Pts, patients

	GROUP A	GROUP B
REASONS LEADING TO IBRUTINIB MODIFICATION n. (%)		
Tot.	44 pts	40 pts
Toxicity	22 (50%)	30 (75%)
Invasive procedures	16 (36.4%)	0 (0%)
Drug interactions	2 (4.5%)	5 (12.5%)
Other	4 (9.1%)	5 (12.5%)
OUTCOME OF THE EVENT AFTER IBRUTINIB MODIFICATION* n. (%)		
Tot.	22 pts	30 pts
Completely resolved	8 (36.4%)	10 (33.3%)
Improved	11 (50%)	14 (46.7%)
Stable	3 (13.6%)	6 (20%)
Worsened	0 (0%)	0 (0%)

Table 2. Main reasons for TTI and PDR and outcomes of the adverse events after TTI and PDR.
*Referring only to TTI and PDR due to toxicity

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Zanubrutinib plus Ixazomib and Dexamethasone for newly diagnosed symptomatic Waldenström macroglobulinemia: a prospective, phase II study

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Introduction:
Lymphoplasmacytic lymphoma/ Waldenström Macroglobulinemia (LPL/ WM) is a rare B-cell malignancy and constitutes 1%-2% of non-Hodgkin lymphoma. Because of the low incidence of this disease, there is wide heterogeneity in the approaches and regimens for the symptomatic patients. In recent years, BTK inhibitors have performed good efficacy for WM patients. The response rate of single-agent for newly diagnosed or relapsed/refractory patients is up to 80-90%. However, the deep remission rate of BTK inhibitor is low, and the rate of very good partial response (VGPR) is only 20-30%. Ixazomib, a proteasome inhibitor that can inhibit NF-kB signaling pathway activation is also widely used in WM patients. Previous clinical studies have shown that the response rate of ixazomib combined with rituximab and dexamethasone in newly diagnosed WM is up to 96%, and the VGPR rate is 15%. How to further improve the depth of remission is the key to enhance the efficacy of WM patients. Herein, we performed a phase 2 investigator-initiated clinical trial (NCT04463953) to evaluate the efficacy and safety of the combination of zanubrutinib, ixazomib, and dexamethasone (ZID) in newly diagnosed symptomatic WM patients. **Methods:** Patients received ID (Ixazomib 4 mg orally days 1, 8, 15; dexamethasone 20 mg orally days 1,2, 8,9, 15,16) induction therapy in 28-day cycle for up to six cycles. After induction therapy, maintenance therapy was conducted every three months. Zanubrutinib was administered 160 mg twice a day in the whole treatment period and discontinued in the last month, ending with ID regimen. Treatment duration will be maximum twenty-four months. The primary endpoint was the deep remission (≥VGPR) rate after six courses of induction therapy.
Results: From Jun 2020 to Feb 2022, twenty patients were enrolled in this study. The clinical characteristics were described in Table 1. One patient did not complete all six cycles induction study, and therefore the efficacy analysis was performed on nineteen patients. After induction therapy, eight patients achieved VGPR, ten patients achieved partial remission (PR), and one patient had minor response (MR). The overall, major, and very good partial response rates were 100%, 94.7%, and 42.1%, respectively. Median onset-to-treatment (MR or better) time and optimum reaction time was 1.1 months (range: 0.7-3.1) and 3.7 months (range: 0.9-17.1), respectively. After treatment, all patients recovered normal hemoglobin levels and two (10.5%) patients achieved MRD negative marrow response by flow cytometry. Spleen volume and lymph node size returned to normal in six (85.7%) and two (25.0%) patients. The median follow-up time was 12.3 months (range: 5.4-23.4), all patients are alive. One patient experienced disease progression after cessation of treatment for 2.5 months. Before progression, the patient had received a total of twenty-one months treatment and achieved a PR response. Two patients experienced an IgM rebound after cessation of treatment for one and four months, respectively, but they were both asymptomatic and had no indication for secondary treatment. Prior to that, they received treatment for 19 and 21 months, respectively, and both achieved VGPR responses. Insomnia was the most frequently reported grade 1-2 adverse event (n=5; 25%). Serious adverse reactions (grade ≥3) were observed in two patients (10%) including rash and neutropenia in each.
Conclusion: The zanubrutinib plus ixazomib and dexamethasone regimen has a high rate of deep remission in newly diagnosed symptomatic WM patients with limited adverse events. The study is ongoing and further results will be continuously released.

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RESULTS OF A PHASE 2 EXPANDED ACCESS STUDY OF ZANUBRUTINIB IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA

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Background: Bruton tyrosine kinase (BTK) inhibition is an emerging standard of care for Waldenström macroglobulinemia (WM). Zanubrutinib (BGB-3111) is a second-generation BTK inhibitor designed to maximize BTK occupancy and minimize off-target inhibition of TEC- and EGFR-family kinases. Zanubrutinib was recently approved by the United States Food and Drug Administration, Health Canada, and the European Union at a dose of 320 mg once daily (QD) or 160 mg twice daily (BID) for the treatment of adult patients with WM. BGB-3111-216 (NCT04052854) is a single-arm, expanded access study of zanubrutinib for treatment-naïve patients who are unsuitable for standard chemoimmunotherapy or patients with relapsed/refractory WM.

Aims: To provide real-world experience with zanubrutinib in patients with WM.

Methods: Eligible patients with treatment-naïve or relapsed/refractory WM were assigned to receive zanubrutinib at a dose of 320 mg QD or 160 mg BID. The primary endpoint was the number of patients enrolled/treated and the number of enrolling sites. Secondary endpoints of safety and efficacy included selected treatment-emergent adverse events (TEAEs), disease response (overall response rate and very good partial response or better), progression-free survival, and overall survival. Response was evaluated by investigator assessment according to the 6th International Workshop on WM (*Br J Haematol.* 2013;160(2):171-6) every 6 months at minimum. The study was closed by the sponsor in July 2021, and active patients were transitioned to commercial zanubrutinib via a patient-assistance program.

Results: Fifty patients with WM (17 treatment naïve; 33 relapsed/refractory), were enrolled from December 2019 to June 2021 across 10 academic and community medical centers in the United States. At study entry, median age was 72 years, 54% had intermediate-risk disease, 40% had high-risk disease, and median number of prior therapies for patients with relapsed/refractory disease was 2. Median treatment exposure was 9.2 months (range, 1.4-20.0). Thirty-eight (76%) patients had ≥1 TEAE, and 36 (72%) experienced ≥1 TEAE of special interest (**Table**). Grade ≥3 TEAEs of special interest included hypertension (8%), infection (8%), atrial fibrillation or flutter

(2%), neutropenia (2%), and second primary malignancy (2%). No new safety signals were observed. In the 41 patients with ≥1 response evaluation, overall response rate was 85.4% (35; 95% CI: 70.8, 94.4), and major response rate was 73.2% (30; 95% CI: 57.1, 85.8). Overall, 39.0% achieved a best overall response of very good partial response (16; 95% CI: 24.2, 55.5). Of the 4 patients who achieved a best overall response of progressive disease, 3 had IgM values that met partial response criteria before the first 6 months response assessment. Progression-free survival and overall survival were immature due to short follow-up, and the median was not met.
Conclusion: The results of this real-world, expanded-access study were consistent with those of the established zanubrutinib profile in WM and other B-cell malignancies when administered as monotherapy at a daily dose of 320 mg orally (either as 160 mg BID or 320 mg QD) in patients with intermediate or high-risk relapsed/refractory or treatment-naïve WM.

Table. BGB-3111-216 Analyses

Adverse events of interest, n (%) **(Safety population)**	**160 mg BID (n=41)**	**320 mg QD (n=9)**	**Overall (n=50)**
≥1 TEAE of special interest	31 (75.6)	5 (55.6)	36 (72.0)
Grade ≥3	7 (17.1)	1 (11.1)	8 (16.0)
Hypertension	4 (9.8)	0 (0.0)	4 (8.0)
Infection	3 (7.3)	1 (11.1)	4 (8.0)
Atrial fibrillation or flutter	1 (2.4)	0 (0.0)	1 (2.0)
Neutropenia	1 (2.4)	0 (0.0)	1 (2.0)
Second primary malignancy	1 (2.4)	0 (0.0)	1 (2.0)
Best overall response by investigator assessment, n (%) **(Efficacy evaluable population)**	**160 mg BID (n=33)**	**320 mg QD (n=8)**	**Overall (n=41)**
Very good partial response	13 (39.4)	3 (37.5)	16 (39.0)
Partial response	12 (36.4)	2 (25.0)	14 (34.1)
Minor response	4 (12.1)	1 (12.5)	5 (12.2)
Stable disease	1 (3.0)	1 (12.5)	2 (4.9)
Progressive disease	3 (9.1)	1 (12.5)	4 (9.8)
Very good partial response or complete response	13 (39.4)	3 (37.5)	16 (39.0)
Major response rate	25 (75.8)	5 (62.5)	30 (73.2)
Overall response rate	29 (87.9)	6 (75.0)	35 (85.4)

BID, twice daily; QD, once daily; TEAE, treatment-emergent adverse event.

36.

Long follow-up in WM patients; prognostic factors and clinical significance

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INTRODUCTION

Waldenstrom's Macroglobulinemia (WM) is an incurable, low-grade B-cell lymphoma. About half of the patients will not need treatment at diagnosis. However, factors that will affect initiation of treatment are of interest. Asymptomatic WM (AWM) is an entity that fulfills the diagnostic criteria of WM, but has no treatment indication. Because of its scarcity and the long follow-up needed, novel prognostic markers are needed.

PATIENTS AND METHODS We retrospectively studied 161 patients diagnosed with WM. Patients' medical records were retrieved after patients' informed consent was obtained and relevant findings were collected at diagnosis. We examined factors affecting the overall survival (OS) and the time from diagnosis to time requiring treatment, using multiple cut-offs proposed by the various Clinical Prognostic Models for WM (IPSS, SWOG, Mayo Clinic, French Group). Statistical analysis was performed using SPSS v. 26 software.

RESULTS Patients' median age was 65 years (37-90), and 59% were men. The median follow-up was 77 months (5-354) and median time to treatment (TTT) was 17 months (6-222). Eighty-six patients were asymptomatic at diagnosis (AWM) with 29 (34%) of them evolving into symptomatic disease over years, of whose approximately half of them (16 patients) having progressed into the first two years. The highly significant variables for OS in the whole cohort were the following; IgM levels ≥7000 mg/dL (p=0.038), b2-microglobulin (β2MG) ≥ 4 mg/dL (p<0.0001), albumin ≤ 3.5 mg/dL (p<0.0001), bone marrow (BM) lymphoplasmacytic infiltration ≥ 50% (p=0.009), age ≥70 (p=0.001), hemoglobin (Hgb) ≤ 11 mg/dL (p=0.002), platelets (PLTs) ≤ 140 K/μL (p=0.008), hepatosplenomegaly (p=0.027), free-light chain ratio (FLCR) ≥ median value (2,79) (p=0.033). The presence of lymphadenopathy showed a tendency of statistical significance (p=0.07). Applying the existing prognostication systems, only the IPSS and the French's Group produced statistically significant results (p<0.000 and p=0.001 respectively), with 40% and 33% 5-year OS in the high-risk groups respectively. However, applying the SWOG prognostication system revealed a 52% 5-year OS in the high-risk group, that was not statistically significant (p= 0,564). Only four patients in our cohort, who all succumbed within 10 years after the initial diagnosis, had all the high-risk factors, in the application of the Mayo prognostication system. In univariate analysis statistically significant shorter TTT was observed in patients with abnormal levels of LDH {LDH ≥ upper normal limit (UNL)} (p=0.052), FLCR ≥ 10 (p=0.02), BM lymphoplasmacytic infiltration ≥ 50% (p=0.001), β2MG ≥ 4 mg/dL (p=0.001), monoclonal IgM protein ≥ 4500 mg/dL (p=0.032) and hypo-gammaglobulinemia (p=0.044). Utilizing multivariate analysis, the combination of the four following parameters was statistically significant for TTT; LDH ≥ UNL, BM infiltration ≥50%, FLCR ≥ 10 and b2MG ≥ 4 mg/dL.

CONCLUSION WM is an indolent, yet incurable disease, with numerous therapeutic options when needed. Thus, improving the prognostication of its course and the time of treatment initiation, will optimize therapeutic choices and provide better outcomes for our patients. A four-parameter system (BM infiltration, FLCR, LDH, β2-MG), to predict the risk of asymptomatic disease to rapidly evolve and require treatment, is proposed.

37.

Autoimmune manifestations in a large series of Waldenstrom's Macroglobulinemia patients.

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INTRODUCTION

Waldenström's Macroglobulinemia (WM) is an indolent B-cell lymphoma characterized by monoclonal IgM secretion. Autoimmune phenomena are a known complication of WM and occur as a result of autoantibody activity of the monoclonal IgM. These autoantibodies could be cold agglutinins, mixed cryoglobulins, and antineural components that lead to hemolytic anemia, mixed cryoglobulinemia, and peripheral neuropathy, respectively[1]. The aim of the present study was to report the incidence of these phenomena and describe the patients' clinical characteristics. PATIENTS AND METHODS We retrospectively studied 170 patients diagnosed with WM, from 1977 to 2022. Patients' medical records were retrieved after patients' informed consent was obtained and relevant findings were collected at diagnosis. Patients with WM and autoimmune manifestations were defined as Group 1 while the remainder cohort as Group 2. Statistical analysis was performed using SPSS v. 26 software. RESULTS The 18 patients (10,5%) with autoimmune disorders presented at a median age of 67 years, 60% were men and 40% were women. Monoclonal Ig type was IgM-kappa in 41%, IgM-lamda in 28% and biclonal in 11%. Applying the IPSS, none of these patients had >3 adverse risk factors. Five patients (3%) had AIHA with positive Direct Antiglobulin Test at diagnosis. Four patients (2,3%) had positive cryoglobulins test, along with symptomatic disease (mostly skin involvement). Seven patients (4,1%) had peripheral neuropathy with positive anti-Myelin-associated-Glycoprotein antibodies. Two patients (1,2%) had autoimmune thrombocytopenia. All the aforementioned manifestations were present at the initial diagnosis, apart from one case of AIHA, which was developed after the administration of Fludarabine, and one case of ITP which occurred later in the disease course, but was the only indication for treatment. In Group 1, only 2 patients had lymphadenopathy and 2 had splenomegaly, compared to Group 2 (21%, 13,9% respectively). The median bone marrow infiltration was equal in both groups (40%). The median value of IgM was significantly lower in Group 1 (750 mg/dL versus 2300 mg/dL in Group 2). Notably two patients had severely increased free-light chains (FLC) (>1000 mg/dL) and almost half of them (8/18, 47%) presented an FLC-ratio >10, versus 21% in Group 2. Overall survival was not statistically different between the two groups. ($p = 0.466$). Fifteen patients received rituximab in combination with glucocorticoids (13 as monotherapy, 1 with cyclophosphamide, 1 with bortezomib and 1 with chlorambucil), two patients received monotherapy with chlorambucil and one received high-dose of dexamethasone along with IVIG. Despite the appropriate treatment, none of the patients achieved long-lasting remission of the autoimmune disorder. Specifically, most patients with AIHA and cryoglobulinemia, who received considerable doses of corticosteroids, succumbed due to opportunistic infections. CONCLUSION Due to the scarcity of WM and the fact that weak autoimmune responses could be suppressed by the initial treatment, true prevalence of the autoimmune phenomena in WM could be underreported. In our group of WM and autoimmune manifestations, lower IgM levels and a FLCR >10 were clinical characteristics of significant value and need further evaluation.

Reference

1. *Bruno Bockorny, Jonessa A. Atienza, and Constantin A. Dasanu, 'Autoimmune Manifestations in Patients with Waldenström Macroglobulinemia', Clinical Lymphoma, Myeloma and Leukemia, 14.6 (2014), 456–59 <https://doi.org/10.1016/j.clml.2014.04.009>.*

38.

ASPEN Biomarker Analysis: Response to Bruton Tyrosine Kinase Inhibitor (BTKi) Treatment in Patients with Waldenström Macroglobulinemia (WM) Harboring *CXCR4*, *TP53,* and *TERT* Mutations

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Background: *MYD88*, *CXCR4,* and *ARID1A* are the most frequently mutated genes in WM (*Blood* 2014;123[11]:1637-1646), and the mutational status of *MYD88* and *CXCR4* impacts BTKi ibrutinib efficacy in WM (*N Engl J Med* 2015;372(15):1430-1440; *Blood* 2020;136(18):2038-2050). ASPEN is a randomized, phase 3 study comparing zanubrutinib with ibrutinib in patients with *MYD88*^{MUT} WM; patients with *MYD88*^{WT} WM received zanubrutinib.

Aims: To evaluate low frequency genetic alterations in patients with WM and their association with efficacy of ibrutinib and zanubrutinib (separately and pooled) in different subpopulations.

Methods: 190 patients with *MYD88*^{MUT} (98 zanubrutinib; 92 ibrutinib) and 20 patients with *MYD88*^{WT} (all zanubrutinib) had evaluable NGS results. NGS was performed on pretreatment bone marrow aspirates using a 152-gene panel with 0.25% sensitivity. Correlation between genetic alterations and treatment responses was analyzed by multivariate analyses.

Results: *CXCR4* (25.7%), *TP53* (24.8%), *ARID1A* (15.2%), and *TERT* (9.1%) were the most frequently mutated genes identified. *TP53*^{MUT} rates were similar between patients with *MYD88*^{MUT} and *MYD88*^{WT}. *TERT*^{MUT} was detected only in patients with *MYD88*^{MUT} (10% [19/190] mutation rate). *ARID1A*^{MUT} and *TERT*^{MUT} were associated with a higher rate of *CXCR4*^{MUT} and were more often detected in patients with *MYD88*^{MUT}.

In the pooled analysis of patients with *MYD88*^{MUT} WM, patients with *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} trended toward a lower very good partial response (VGPR) + complete response (CR) rate and a less favorable progression-free survival (PFS) than patients with the respective WT alleles (HR=1.32, 2.15, and 1.79, respectively) (Figure 1). The median time to response (VGPR+CR) also appeared longer in patients with mutant alleles.

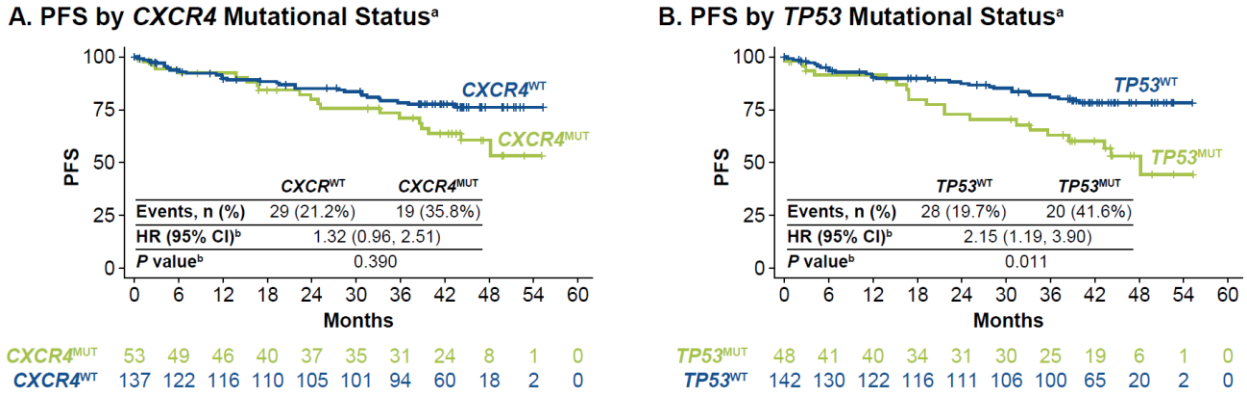
As shown in Table 1, among *CXCR4*^{MUT} subgroups, a lower major response rate (MRR) was observed in patients with *CXCR4*nonsense (*CXCR4*^{NS}; 53.8%; *P* = 0.135) compared with *CXCR4*frameshift (*CXCR4*^{FS}; 85.7%; *P* = 0.958) and *CXCR4*^{WT} (84.7%) receiving ibrutinib, whereas comparable MRR was observed across all subpopulations receiving zanubrutinib (85.7%, 73.7%, and 83.1%, respectively). The median PFS (months) in patients receiving ibrutinib by *CXCR4*^{NS}, *CXCR4*^{FS}, and *CXCR4*^{WT} mutational statuses was 39.8, 44.2, and not reached (NR), respectively, and NR in all subpopulations receiving zanubrutinib.

The VGPR+CR rate (13.6% vs 30.0%; *P*=0.202) and MRR (63.6% vs 85.7%; *P*=0.040) were lower in patients with *TP53*^{MUT} than *TP53*^{WT} with ibrutinib; VGPR+CR rate (34.6% vs 37.5%; *P*=0.636) and MRR (80.8% vs 81.9%; *P*=0.978) were similar between *TP53*^{MUT} and *TP53*^{WT} subgroups with zanubrutinib.

Among the 20 patients with *MYD88*^{WT}, 4 had *TP53*^{MUT}, with a lower MRR (50%), and none achieved VGPR or CR, compared with *TP53*^{WT} (63% MRR and 25% VGPR+CR).

Conclusion: In addition to *CXCR4*^{MUT} and *ARID1A*^{MUT}, *TP53*^{MUT} and *TERT*^{MUT} were detected at a high rate in the ASPEN study. *CXCR4*^{MUT}, *TP53*^{MUT}, and *TERT*^{MUT} were correlated with inferior response to BTKi therapy, and more patients with *CXCR4*^{MUT} were present in the zanubrutinib arm. Consistent with more potent inhibition of BTK, zanubrutinib demonstrated deeper responses in patients with *CXCR4*^{MUT} or *TP53*^{MUT} WM compared with ibrutinib, with more favorable response regardless of the mutational status.

Figure 1. Progression-Free Survivals in patients with *MYD88*^{MUT} WM by (A) *CXCR4* and (B) *TP53* Mutational Status



Data cutoff: October 31, 2021.

[a]Pooled analysis of patients with *MYD88*^{MUT} WM from cohort 1 including 98 treated by zanubrutinib and 92 treated by ibrutinib.

[b]HR and *P* values were estimated using a Cox regression model with *CXCR4 (*WT, FS, NS*), TP53 (*WT, MUT*)*, and *TERT (*WT, MUT*)* mutational statuses as covariates. WT is the reference group.

FS, frameshift; HR, hazard ratio; NS, nonsense; PFS, progression-free survival; WT, wild type.

Table1. Response Assessment by *CXCR4* and *TP53* Mutational Statuses in Patients With *MYD88*^{MUT} WM[a]

Patients with MYD88^{MUT} treated with ibrutinib					
	***CXCR4*^{WT} (n=72)**	***CXCR4*^{FS} (n=7)**	***CXCR4*^{NS} (n=13)**	***TP53*^{WT} (n=70)**	***TP53*^{MUT} (n=22)**
VGPR or better, n (%)[b]	22 (30.6)	0	2 (15.4)	21 (30.0)	3 (13.6)
OR (95% CI)	-	0.14 (0.00,3.23)	0.64 (0.13,3.08)	-	0.44 (0.12,1.55)
P value	-	*0.223*	*0.579*	-	*0.202*
Major response, n (%)[b]	61 (84.7)	6 (85.7)	7 (53.8)	60 (85.7)	14 (63.6)
OR (95% CI)	-	1.06 (0.10, 10.36)	0.33 (0.07,1.41)	-	0.29 (0.09,0.95)
P value	-	*0.958*	*0.135*	-	***0.040***
Time to VGPR or better Median (min, max), months	11.3 (2.0, 49.9)	-	31.3 (16.6, 46.0)	11.4 (2.0, 49.9)	24.9 (5.6, 46.9)
Time to major response Median (min, max), months	2.8 (0.9, 49.8)	7.0 (2.8, 41.5)	2.9 (1.2, 13.6)	2.9 (0.9, 49.8)	3.0 (1.0, 13.8)
PFS					
Events, n (%)	18 (25.0%)	4 (57.1%)	7 (53.8%)	18 (25.7%)	11 (50.0%)
Median, months[c]	NE	44.2	39.8	NE	44.2
HR (95% CI)[d]	-	2.08 (0.70,6.16)	3.39 (1.23,9.31)	-	2.36 (1.10,5.09)
P value[d]	-	*0.185*	***0.017***	-	***0.027***
Patients with MYD88^{MUT} treated with zanubrutinib					
	***CXCR4*^{WT} (n=65)**	***CXCR4*^{FS} (n=19)**	***CXCR4*^{NS} (n=14)**	***TP53*^{WT} (n=72)**	***TP53*^{MUT} (n=26)**
VGPR or better, n (%)[b]	29 (44.6)	5 (26.3)	2 (14.3)	27 (37.5)	9 (34.6)
OR (95% CI)	-	0.51 (0.16,1.66)	0.24 (0.04,1.26)	-	1.27 (0.46,3.52)
P value	-	*0.269*	*0.093*	-	*0.636*
Major response, n (%)[b]	54 (83.1)	14 (73.7)	12 (85.7)	59 (81.9)	21 (80.8)
OR (95% CI)	-	0.66 (0.18,2.36)	1.52 (0.25,9.01)	-	1.01 (0.29,3.47)
P value	-	*0.524*	*0.639*	-	*0.978*
Time to VGPR or better Median (min, max), months	6.5 (1.9, 42.0)	11.1 (2.8, 26.0)	10.3 (9.4, 11.1)	6.5 (1.9, 42.0)	11.1 (3.0, 26.0)
Time to major response Median (min, max), months	2.8 (0.9, 28.5)	2.9 (1.8, 49.8)	4.1 (1.0, 38.7)	2.8 (0.9, 49.8)	2.8 (1.0, 5.6)
PFS					
Events, n (%)	11 (16.9%)	4 (21.0%)	4 (28.5%)	10 (13.8%)	9 (34.6%)
Median, months[c]	NE	NE	NE	NE	NE
HR (95% CI)[d]	-	0.62 (0.17, 2.25)	0.67 (0.15,2.88)	-	2.20 (0.81, 5.98)
P value[d]	-	*0.473*	*0.598*	-	*0.120*

Data cutoff: October 31, 2021.

[a]Mutation determined by NGS; NGS results were available for 92 patients in the ibrutinib arm and 98 patients in the zanubrutinib arm.

[b]OR and *P* values were estimated using a logistic regression model with *CXCR4* (WT, FS, NS), *TP53* (WT, MUT), and *TERT* (WT, MUT) mutational statuses as covariates. WT is the reference group.

[c]Median PFS was estimated by Kaplan-Meier method.

[d]HR and *P* values were estimated using a Cox regression model with *CXCR4* (WT, FS, NS), *TP53* (WT, MUT), and *TERT* (WT, MUT) mutational statuses as covariates. WT is the reference group.

FS, frameshift; HR, hazard ratio; NE, not estimable; NGS, next-generation sequencing; NS, nonsense; OR, odds ratio; PFS, progression-free survival; VGPR, very good partial response; WM, Waldenström macroglobulinemia; WT, wild type.

Bold red text highlights *P* value <0.05.

39.

ASPEN: LONG-TERM FOLLOW-UP RESULTS OF A PHASE 3 RANDOMIZED TRIAL OF ZANUBRUTINIB VS IBRUTINIB IN PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA (WM)

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Background: ASPEN (NCT03053440) is a randomized, open-label, phase 3 study comparing zanubrutinib, a potent selective Bruton tyrosine kinase inhibitor (BTKi) designed to have greater affinity to BTK while minimizing off-target inhibition, with the first-generation BTKi ibrutinib in patients with WM. Here we present data with a median follow-up of 43 months.

Aims: To compare the efficacy and safety of zanubrutinib vs ibrutinib in patients with MYD88 mutant ($MYD88^{mut}$) WM and zanubrutinib in patients with wild-type MYD88 ($MYD88^{wt}$) WM.

Methods: In cohort 1, patients with *MYD88*^{mut} were randomized 1:1 to receive zanubrutinib 160 mg twice daily or ibrutinib 420 mg once daily. In cohort 2, patients with *MYD88*^{wt} received zanubrutinib 160 mg twice daily until progression. Randomization was stratified by *CXCR4* mutational status by Sanger sequencing and lines of prior therapy. The primary endpoint was very good partial response or better (VGPR + complete response [CR] rate).

Results: A total of 201 patients (102 zanubrutinib; 99 ibrutinib) were enrolled in cohort 1 and 28 in cohort 2. Baseline characteristics in cohort 1 differed between patients treated with zanubrutinib vs ibrutinib in *CXCR4* mutations by next-generation sequencing (32% vs 20%, or 33 of 98 vs 20 of 92 available samples, respectively) and patients aged >75 years (33% vs 22%, respectively). Median duration of treatment was 42 (zanubrutinib) and 41 months (ibrutinib), with 67% and 58% of patients remaining on treatment, respectively. The VGPR+CR rate by investigator was 36% with zanubrutinib vs 22% with ibrutinib (descriptive $P = 0.02$) in cohort 1, and 31% in cohort 2. One patient in cohort 2 obtained a CR. In cohort 1 patients with *CXCR4*^{WT} or *CXCR4*^{MUT}, VGPR+CR rates with zanubrutinib vs ibrutinib were 45% vs 28% ($P = 0.04$) and 21% vs 5% ($P = 0.15$), respectively. Median progression-free and overall survivals were not reached.

Consistent with less off-target inhibition, rates of atrial fibrillation, diarrhea, hypertension, localized infection, hemorrhage, muscle spasms, pneumonia, and adverse events (AEs) leading to discontinuation or death were lower with zanubrutinib vs ibrutinib (Table). Neutropenia (including grade ≥3) was higher with zanubrutinib (33.7%) vs ibrutinib (19.4%), although rate of grade ≥3 infection was lower with zanubrutinib (20.8%) vs ibrutinib (27.6%). AE incidence with zanubrutinib was similar across cohorts 1 and 2.

In patients treated with zanubrutinib in cohort 1, hemorrhage, neutropenia and infection prevalence decreased over time. Prevalence of infection was lower in patients treated with zanubrutinib vs ibrutinib. Annual prevalence analysis showed that atrial fibrillation remained ≤5% and hypertension remained stable with zanubrutinib, each with lower prevalence at all intervals vs an increasing trend with ibrutinib.

Consistently, exposure-adjusted incidence rates of atrial fibrillation/flutter and hypertension were lower with zanubrutinib vs ibrutinib (0.2 vs 0.8 and 0.5 vs 1.0 persons per 100 person-months, respectively; $P < 0.05$).

Conclusion: ASPEN is the largest phase 3 trial with head-to-head BTKi comparison in WM. At a median follow-up of 43 months, zanubrutinib was associated with higher VGPR+CR rates and demonstrated clinically meaningful advantages in long-term safety and tolerability vs ibrutinib.

Table

AE (all grade), % of treated patients	Cohort 1 Zanubrutinib (n=101)	Cohort 1 Ibrutinib (n=98)	Cohort 2 Zanubrutinib (n=28)
AE, grade ≥3	74.3	72.4	71.4
AE leading to discontinuation	8.9	19.4	14.3
Atrial fibrillation/flutter[a]	7.9	23.5	7.1
Diarrhea	21.8	34.7	32.1
Hemorrhage[a]	55.4	62.2	39.3
Major bleeding[b]	7.9	12.2	7.1
Hypertension[a]	14.9	25.5	10.7
Muscle spasm	10.9	28.6	14.3
Localized infection	1.0	11.2	7.1
Neutropenia[a]	33.7	19.4	21.4
Pneumonia	5.0	18.4	14.3
Infection,[a] all grade (grade ≥3)	78.2 (20.8)	79.6 (27.6)	82.1 (32.1)

[a]Grouped term.
[b]Includes grade ≥3 hemorrhage and central nervous system bleeding of any grade.
AE, adverse event.

40.

SARS-CoV-2 humoral responses following booster BNT162b2 vaccination in patients with B-cell malignancies

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Background: Patients with B-cell malignancies have suboptimal immune responses to SARS-CoV-2 vaccination and are a high-risk population for severe COVID-19 disease.
Methods: We evaluated the effect of a third booster BNT162b2 vaccine on the kinetics of anti-SARS-CoV-2 neutralizing antibody (NAbs) titres in patients with B-cell malignancies. Patients with Waldenström's macroglobulinemia (n=90), NHL (n=54) and chronic lymphocytic leukemia (n=49) enrolled in the ongoing NCT04743388 study and compared against matched healthy controls.
Results: All patient groups had significantly lower NAbs compared to controls at all time points. One month post the third dose (M1P3D) NAbs increased significantly compared to previous time points (median NAbs 77.9%, $p < 0.05$ for all comparisons) in all patients. The respective subgroup numbers for NAb ≥50% at 1MP3D were 81.3% for CLL, 60.6% for WM and only 35.3% for NHL. NAbs≥50% were seen in 59.1% of patients, 34.5% of patients with suboptimal responses post-second dose, elicited a protective NAb titre ≥50%. Active treatment, rituximab and BTKi treatment were the most important prognostic factors for a poor NAb response at 1MP3D; only 25.8% of patients on active treatment had NAbs ≥50%. No significant between group differences were observed. We also assessed the effect of BTK inhibitors in the WM group; At M1P3D, the median NAb titer was 39% in BTKi-treated vs. 96% in BTKi-untreated patients (p=0.003) and the percentage of patients with NAbs ≥50% was 43.8% and 76.5% respectively
Conclusion: Patients with B-cell malignancies have inferior humoral responses against SARSCoV-2 and booster dose enhances the NAb response in a proportion of these patients.

41.

Cell-free DNA is a reliable liquid biopsy to detect MYD88 and CXCR4 mutations in patients with IgM monoclonal gammopathies

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Background
The mutational status of the MYD88 and the CXCR4 genes may guide the current management of patients with Waldenström's macroglobulinemia (WM) and IgM monoclonal gammopathies. We have shown (Bagratuni et al. Leukemia 2018;32(12):2617-2625) that peripheral blood cell-free tumour DNA (cfDNA), also known as liquid biopsy, may constitute a feasible alternative method of evaluating the genomic landscape. Herein, we aimed to characterize the mutational status of WM/IgM MGUS patients by using peripheral blood plasma-derived cfDNA and using matched tumor DNA (tDNA) from BM-CD19+ selected cells, in order to determine whether cfDNA, can be used as an adjunct and/or alternative diagnostic tool in identifying the mutational profile of IgM monoclonal gammopathies.

Methods
Peripheral blood (10-12mL) was collected in EDTA tubes and DNA was extracted using the MagMax cell free DNA isolation kit. BM aspirates were collected at the same time with peripheral blood, and were processed for CD19 enrichment. The presence of L265P mutation was initially assessed by Allele-Specific PCR and then confirmed with direct sequencing. The presence of CXCR4 mutations was assessed with direct sequencing.

Results
207 patients were included in the study, whereas 150 had both tDNA and cfDNA informative samples. MYD88L265P mutation was detected in 135/150 patients (90%) in both tDNA and cfDNA; in 5 (3%) the mutation was seen in tDNA but not in cfDNA and 10 (7%) patients harbored the MYD88WT genotype both in tDNA and cfDNA. Thus, the overall concordance between tDNA and cfDNA for MYD88 genotype was 97% ($p<0.001$). Among patients with IgM MGUS, WM in remission and sWM /NDWM/relapsed (RR) WM, the concordance rates were 96% (27 out of 28 patients), 97% (36 out 37 patients) and 96% (76 out of 79 patients), respectively. The amount of cfDNA was not correlated with IgM levels ($p=0.717$), bone marrow invasion ($p=0.439$) or CD19+ yield. Inversely, the amount of CD19+ yield was associated with both IgM levels ($p=0.01$) and bone marrow invasion ($p<0.001$). The detection of the MYD88L265P in tDNA was associated with the CD19+ yield ($p=0.007$) and the IgM levels ($p=0.048$). The detection of the MYD88L265P in cfDNA was not associated with the IgM levels or the amount of the isolated cfDNA. The assessment of CXCR4 mutations in both tDNA and cfDNA was feasible in 131 patients. 15 patients (12%) had the same mutations in both paired samples. 11 patients (8%) showed discordant mutations between the tDNA and the cfDNA. Overall, the pathogenic mutation S338X was present in five patients and the L50X in one. 84 patients (64%) were characterized as wild-type both by tDNA and cfDNA analysis. The concordance rate between tDNA and cfDNA was 84% ($p<0.001$), which was consistent among patients with IgM MGUS (88%), WM in remission (82%), and sWM/NDWM/RRWM (85%).

Conclusion
Peripheral blood cfDNA is a reliable, minimally invasive approach for the detection of MYD88 and CXCR4 mutations in patients with IgM monoclonal gammopathies irrespective of the disease burden. We encourage the integration of cfDNA-guided endpoints in prospective studies in order to assess the evolving genomic dynamics during the disease course.

42.

CDK7 inhibition halts cell proliferation by targeting proliferative and metabolic vulnerabilities in waldenstrom macroglobulinemia and multiple myeloma.

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Cyclin dependent kinases (CDKs) are high value therapeutical targets owing to their important roles in regulating transcription and the cell cycle — two pathways commonly altered in cancer including waldenstrom macroglobulinemia (WM) and multiple myeloma (MM). Among CDKs, CDK7 uniquely bridges cell cycle and transcriptional control by activating other cell cycle CDKs and forming the general transcription factor TFIIH. However, the distinct role of CDK7 in regulating transcription and cell cycle progression has been hindered by the lack of selective CDK7 inhibitors. Here we elucidate the biological role of CDK7 and explore the functional consequence of its inhibition in WM and MM using chemical and genetic approaches, including a recently reported selective CDK7 covalent inhibitor YKL-5-124, and engineered systems for rapid CDK7 protein degradation (dTAG). We demonstrate that CDK7 inhibition elicits a strong therapeutic response in tumor cells blocking proliferation *in vitro* and driving tumor regression and prolonged survival *in vivo*.

CDK7 inhibition counteracts molecular hallmarks of deregulated cell cycle control at the G1/S checkpoint and selectively downregulates oncogenic E2F and MYC transcriptional programs, including glycolysis. CDK7 activity is indeed required for the expression of many components of the oncogenic MYC-regulated glycolytic cascade, with *HK2* and *LDHA* being the most affected genes. Importantly, in WM and MM patient tumor cell–derived RNA-seq data, elevated CDK7 gene expression correlates with expression of these genes.

We next assessed whether this translated into defects in glycolysis, glycolytic capacity, and glycolytic reserve. We employed a glycolysis stress test with a Seahorse analyzer using ECAR (extracellular acidification rate) as a measure of glycolytic activity observing a diminished glycolytic activity in tumor cells after CDK7 inhibition. Moreover, the inhibition of HK2 and lactate production by YKL-5-124 confers higher sensitivity to BRD4 inhibition and other agents, even in resistant models.

These data show that, through its role as a critical cofactor and regulator of MYC and E2F activity, CDK7 is a master regulator of the oncogenic cell cycle, transcription, and metabolism in WM and MM representing an attractive and therapeutically actionable molecular vulnerability.