Clonal Architecture of *CXCR4* WHIM-like Mutations in Waldenstrom Macroglobulinaemia

Lian Xu[1*], Zachary R. Hunter[1,2*], Nicholas Tsakmaklis[1], Yang Cao[1,2], Guang Yang[1,2], Jiaji Chen[1], Xia Liu[1,2], Sandra Kanan[1], Jorge Castillo[1,2], Yu-Tzu Tai[2,3], James L. Zehnder[4], Jennifer R. Brown[2,5], Ruben D. Carrasco[2,5],Ranjana Advani[6], Jean M. Sabile[6], Kimon Argyropoulos[7], M. Lia Palomba[7], Enrica Morra[8], Alessandra Trojani[8], Antonino Greco[8], Alessandra Tedeschi[8], Marzia Varettoni[9], Luca Arcaini[9], Nikhil M. Munshi[2,3], Kenneth C. Anderson[2,3], and Steven P. Treon[1,2].

[1]Bing Center for Waldenström's Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA, USA; [2]Department of Medicine, Harvard Medical School, Boston, MA, USA; [3]Lipper Center for Multiple Myeloma, Dana Farber Cancer Institute; [4]Department of Pathology, Stanford University Medical Center; [5]Department of Medical Oncology, Dana Farber Cancer Institute; [6]Department of Medical Oncology, Stanford University Medical Center. [7]Department of Medical Oncology, Memorial Sloane Kettering Cancer Center; [8]Hematology Unit, Niguarda Hospital, Milan, Italy; [9]Department of Molecular Medicine, University of Pavia; Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. *Contributed equally to this work.

Abstract

CXCR4WHIM somatic mutations are distinctive to Waldenstrom Macroglobulinaemia (WM), and impact disease presentation and treatment outcome. The clonal architecture of *CXCR4WHIM* mutations remains to be delineate. We developed highly sensitive AS-PCR assays for detecting the most common *CXCR4WHIM* mutations in WM. By combined AS-PCR and Sanger sequencing, *CXCR4WHIM* mutations were identified in 44/102 (43%), 21/62 (34%), 2/12 (17%), and 1/20 (5%) untreated WM, previously treated WM, IgM MGUS and MZL patients, respectively, but no CLL, MM, non-IGM MGUS patients or healthy donors. Cancer cell fraction analysis in WM and IgM MGUS patients showed *CXCR4S338X* mutations were primarily subclonal, with highly variable clonal distribution. Combined AS-PCR and Sanger sequencing revealed multiple *CXCR4WHIM* mutations in many individual WM patients including homozygous and compound heterozygous mutations validated by deep RNA sequencing. The findings show that *CXCR4WHIM* mutations are more common in WM than previously revealed, and are primarily subclonal supporting their acquisition after MYD88L265P in WM oncogenesis.

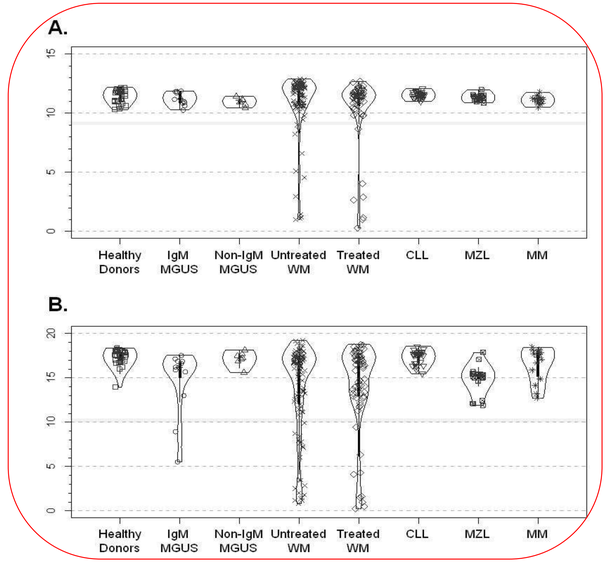
Baseline characteristics

N=	102	62	12
Age (years)	62 (range 33-88)	63 (range 44-86)	69 (range 56-82)
Gender (M/F)	58/44	48/15	6/6
Serum IgM (g/L)	26.7 (range 2.7-86.3)	36.1 (range 7.4-83.9)	3.97 (range 1.4-16.4)
Hemoglobin (g/L)	117 (range 48-155)	105 (range 82-138)	134 (range 119-163)
Serum β_2-microglobulin (mg/L)	2.9 (range 1.0-9.5)	3.9 (range 1.3-14.2)	1.9 (range 1.7-3.4)
Adenopathy (≥ 1.5 cm)	34 (33.3%)	37 (58.7%)	0 (0%)
Splenomegaly (≥15 cm)	15 (14.7%)	7 (11.1%)	0 (0%)
Bone Marrow Involvement (%) by IHC	40 (range 5-95)	60 (range 3-95)	0 (0%)
MYD88 L265P positive	97 (95.1%)	55 (89%)	6 (50%)

MYD88/*CXCR4* mutation status

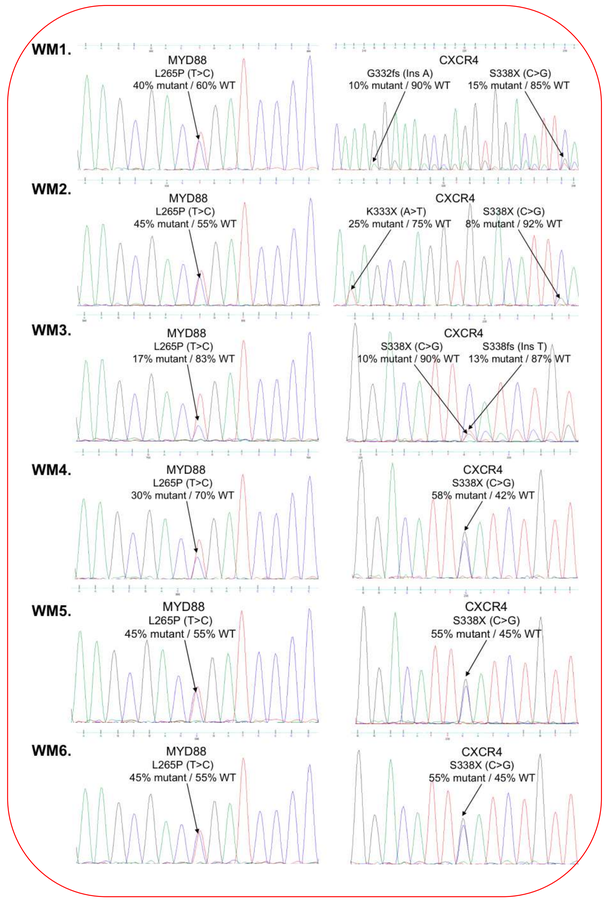
	(N=)	*MYD88* ^{L265P}	*CXCR4* ^{WHIM}
Healthy Donors	32	0 (0%)	0 (0%)
IgM MGUS	12	6 (50%)	2 (17%)
Non-IgM MGUS	7	0 (0%)	0 (0%)
Untreated WM	102	97 (95%)	44 (43%)
Treated WM	62	57 (92%)	21 (34%)
MZL	20	2 (10%)	1 (5%)
CLL	32	1 (3%)	0 (0%)
Multiple Myeloma	14	0 (0%)	0 (0%)

Real-time AS-PCR results



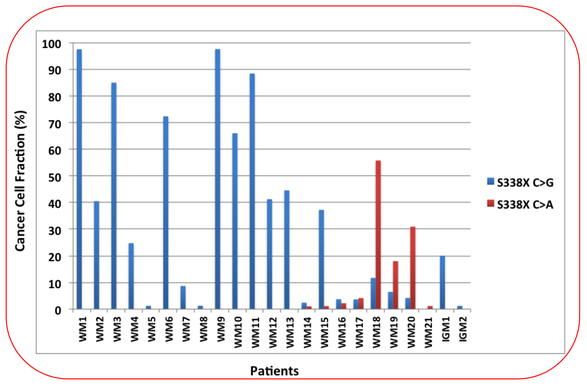
A. *CXCR4S338X C>A* and **B**. *CXCR4S338X C>G*.

Sanger sequencing results



Sanger traces from CD19-selected cells derived from BM aspirates of untreated WM patients showing compound heterozygous and homozygous *CXCR4WHIM* mutations.

Cancer cell fraction analysis



The cancer cell fraction for *CXCR4S338X C>A and/or C>G* expression relative to *MYD88L265P* is shown. *CXCR4S338X C>G* was expressed in patients WM1-WM20, and IGM MGUS 1 and 2; *CXCR4S338X C>A* was expressed in patients WM14-21, with both *CXCR4S338X C>G and C>A* present in WM14-20.

Summary and discussion

Our findings show that *CXCR4WHIM* mutations are primarily subclonal, with highly variable clonal distribution in WM patients. The subclonal existence of *CXCR4WHIM* mutations in WM, as well as IgM MGUS patients, supports that the acquisition of *CXCR4WHIM* mutations is likely to be an early oncogenic event, but follow acquisition of MYD88L265P. The presence of multiple *CXCR4WHIM* mutations in many WM patients may be indicative of targeted CXCR4 genomic instability and warrant further study.