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Mavorixafor Disrupts the Crosstalk Between Waldenström's Macroglobulinemia Cells and the Bone Marrow Microenvironment Resulting in Enhanced Sensitivity to B-Cell–Targeted Therapies

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Background

- Waldenström's macroglobulinemia (WM) is a rare indolent B-cell lymphoma in which the bone marrow (BM) and lymphoid tissues are infiltrated by lymphoplasmacytic cells along with overproduction of immunoglobulin M (IgM).[1]
- More than 90% of patients with WM have mutations in the *MYD88* gene and 30% to 40% also show mutations in the carboxyl terminus of C-X-C chemokine receptor 4 (*CXCR4*).[1-3]
- Increased CXCR4 cell surface expression in WM cells and elevated levels of its ligand C-X-C motif ligand 12 (CXCL12) may prolong intracellular signaling via the CXCL12/CXCR4 axis and contribute to the homing and retention of WM cells in the BM.[3-5]
- In the BM, WM cells interact with the BM micro-environment via cell-to-cell contacts or indirectly through secreted factors (eg, interleukin 6 [IL-6]).[4-6] In preclinical models, the IL-6/signal transducer and activator of transcription-3 (STAT-3) axis contributed to immunoglobulin M (IgM) production and appeared to play a key role in the pathogenesis of WM.[5-9]
- Mavorixafor, an investigational, oral CXCR4 antagonist, is being evaluated in a clinical trial in combination with the Bruton tyrosine kinase (BTK) inhibitor ibrutinib in patients with WM caused by both *MYD88*^{L265P} and *CXCR4*^{WHIM} mutations (NCT04274738).[10]
- The effects of mavorixafor with ibrutinib and other B-cell–targeted therapies on WM cells harboring only the single mutation in *MYD88*^{L265P} and without a *CXCR4* mutation have not been evaluated.

Objective

- This study was designed to elucidate the crosstalk between WM cells and the BM stromal cell (BMSC)-based microenvironment and to explore the effect of mavorixafor in combination with different B-cell–targeted therapies in an *in vitro* WM/BMSC coculture model.

Methods

- WM cells (MWCL-1 cell line, *MYD88*^{L265P}*CXCR4*^{WT}) pretreated with mavorixafor and B-cell–targeted therapies, including BTK inhibitors (ibrutinib, zanubrutinib, evobrutinib, pirtobrutinib, and nemtabrutinib) or B-cell lymphoma 2 inhibitor (venetoclax), were cocultured with an established BMSC line (HS-27A).
- Crosstalk between WM cells and BMSCs, characterized by IL-6 production, IgM release, and CXCR4 expression, was measured after 48 or 72 hours of incubation.
- The effects of mavorixafor alone and with various B-cell–targeted therapies on calcium mobilization, cell migration, adhesion to BMSCs, and BMSC-induced drug resistance and IgM hypersecretion were also measured.
- Student 2-tailed *t* test and 2-way analysis of variance followed by Bonferroni post hoc test were used to calculate significance of differences between 2 independent groups and between multiple groups, respectively. Analyses were performed using GraphPad Prism 9 software (GraphPad Software).

Results

Coculture of WM cells with BMSCs increased IgM secretion, upregulated IL-6 production, and enhanced CXCR4 expression via IL-6R–janus kinase (JAK)–STAT3 signaling pathway

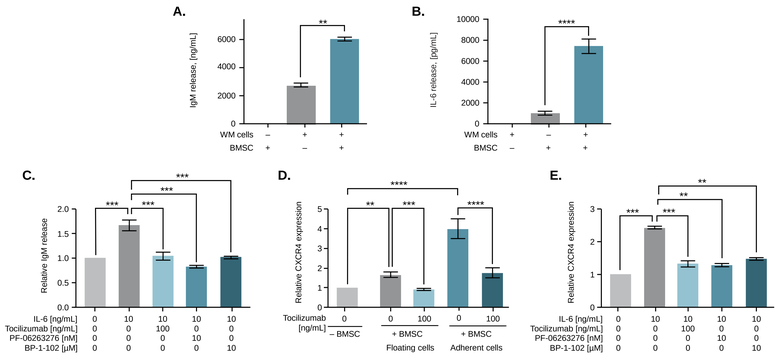

Figure 1. BMSC-derived IL-6 increases IgM release and CXCR4 cell surface expression in WM cells via IL-6R-JAK-STAT3 signaling. For coculture experiments, HS-27A BMSCs were cultured in plates until 90% confluence and MWCL-1 cells were plated at a density of ≈2 × 10^5 cells/mL. IgM **(A)** and IL-6 **(B)** release in MWCL-1 WM/HS-27A BMSCs coculture model. Relative IgM release in the presence of exogenous IL-6 +/- IL-6R antibody tocilizumab, pan-JAK inhibitor PF-06263276, or STAT3 inhibitor BP-1-102 **(C)**, relative CXCR4 expression in MWCL-1 cells cocultured with HS-27A BMSCs +/- tocilizumab **(D)** or pretreated with tocilizumab, BP-1-102, or PF-06263276, followed by exogenous IL-6 stimulation **(E)** were also measured. *P* values <.05 were considered statistically significant and set as follows: **—*P*<.01; ***—*P*<.001; ****—*P*<.0001.

BMSC-induced resistance of WM cells to B-cell–targeted therapies

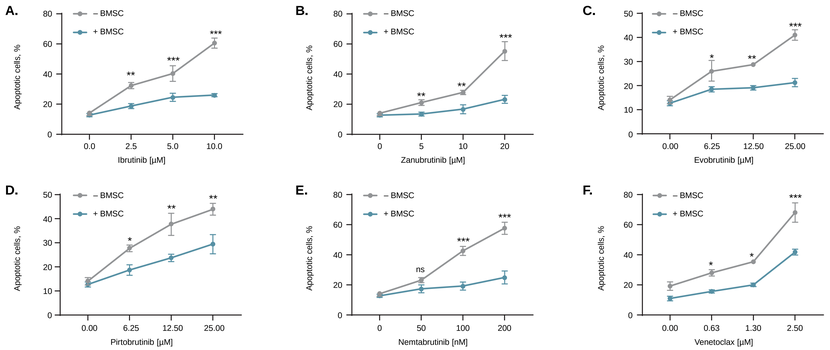

Figure 2. Apoptosis of WM cells treated with B-cell–targeted therapies with or without BMSC coculture. Apoptosis of MWCL-1 cells with or without coculture with HS-27A BMSCs in response to ibrutinib **(A)**, zanubrutinib **(B)**, evobrutinib **(C)**, pirtobrutinib (LOXO-305) **(D)**, nemtabrutinib (ARQ-531) **(E),** and venetoclax **(F)**. *P* values <.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<.05; **—*P*<.01; ***—*P*<.001.

Mavorixafor alone inhibited CXCL12-induced Ca^{2+} mobilization and caused disruption of WM cell migration and adhesion to BMSCs

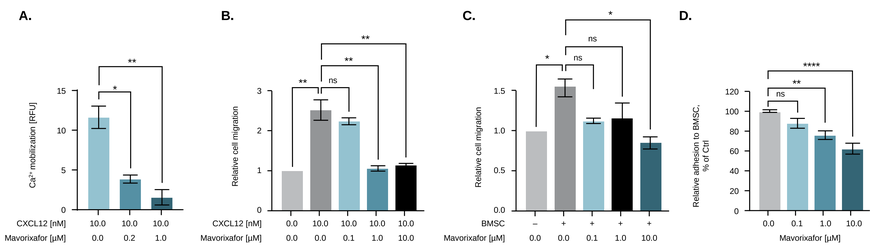

Figure 3. Ca^{2+} mobilization, cell migration, and adhesion of WM cells to BMSC with or without mavorixafor pretreatment. Effects of increasing concentrations of mavorixafor on CXCL-12-induced Ca^{2+} mobilization in MWCL-1 cells **(A)**, relative migration of MWCL-1 cells toward CXCL12 **(B)**, or toward HS-27A BMSCs **(C)** and adhesion to BMSC cells **(D)**. *P* values <.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<.05; **—*P*<.01; ****—*P*<.0001.

Mavorixafor enhanced sensitivity of WM cells to B-cell–targeted therapies in BMSC coculture model

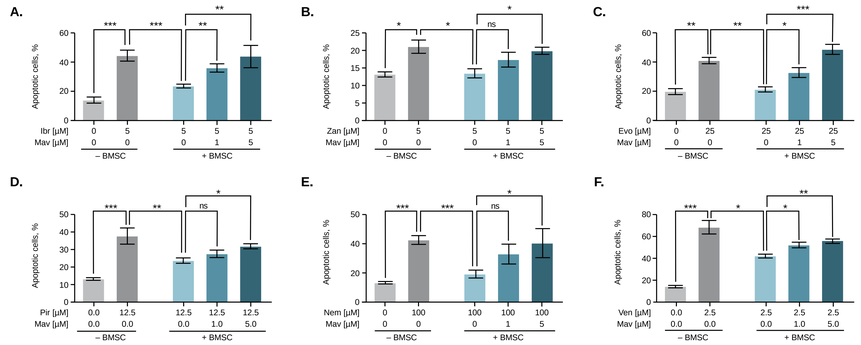

Figure 4. Apoptosis of WM cells after treatment with B-cell–targeted therapies ± mavorixafor with or without BMSC coculture. Apoptosis of MWCL-1 cells with or without coculture with HS-27A BMSCs in the presence of ibrutinib **(A)**, zanubrutinib **(B)**, evobrutinib **(C)**, pirtobrutinib (LOXO-305) **(D)**, nemtabrutinib (ARQ-531) **(E)**, and venetoclax **(F)** with or without mavorixafor treatment. *P* values <.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<.05; **—*P*<.01; ***—*P*<.001.

Mavorixafor inhibited BMSC-induced IgM hypersecretion when administered as a single agent or in combination with B-cell–targeted therapies

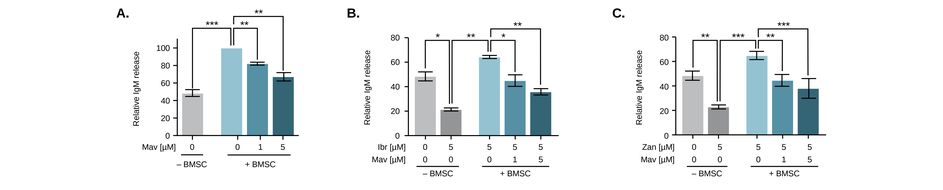

Figure 5. Relative IgM release from WM cells with B-cell–targeted therapies ± mavorixafor with or without BMSC coculture. Relative IgM release in MWCL-1 cells with or without coculture with HS-27A BMSCs **(A)** and in the presence of ibrutinib **(B)**, zanubrutinib **(C)**, evobrutinib **(D)**, pirtobrutinib (LOXO-305) **(E)**, nemtabrutinib (ARQ-531) **(F),** and venetoclax **(G)** with or without mavorixafor pretreatment. *P* values <.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<.05; **—*P*<.01; ***—*P*<.001.

Conclusions

- In this *in vitro* study, coculture of WM cells with BMSCs led to increased IgM secretion, IL-6 production, and enhanced surface expression of CXCR4 in WM cells by activation of the IL-6R–JAK–STAT3 pathway.
- These observations suggest a contribution of *CXCR4*^{WT} upregulation to pathogenicity of WM cells carrying only the *MYD88*^{L265P} mutation.
- This is the first *in vitro* study to show that the protection of WM cells against B-cell–targeted therapies conferred by BMSCs can be overcome by inhibition of the CXCL12/CXCR4 axis.
- Mavorixafor in combination with B-cell–targeted therapies disrupt the crosstalk between WM cells and BMSCs as shown by the enhanced effects on inhibition of BMSC-induced IgM hypersecretion and restored sensitivity of WM cells to B-cell–targeted therapies.
- Our study provides preliminary evidence for the potential use of mavorixafor alone or in combination with other B-cell–targeted therapies in the treatment of WM and potentially other lymphomas.
- Further studies using additional WM cell lines and/or primary patient cells are warranted to support these findings and the potential use of mavorixafor in patients with WM, irrespective of their *CXCR4* mutational status.

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Disclosures

HM, TK, TR, BM, SM, KZ, AT, and **CN** are current employees and/or have equity ownership of X4 Pharmaceuticals. **MT** is a former employee of X4 Pharmaceuticals and/or has equity ownership of X4 Pharmaceuticals.

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