Mavorixafor Enhances Efficacy of Bruton's Tyrosine Kinase Inhibitors by Overcoming the Protective Effect of Bone Marrow Stroma on Tumor Cells in Waldenström's Macroglobulinemia

Abstract 427

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Background

- Waldenström's macroglobulinemia (WM) is a rare B-cell malignancy characterized by monoclonal immunoglobulin M (IgM) hypersecretion and invasion of B cells in the bone marrow (BM) and lymphoid tissues.[1,2]
- >90% of WM cases show mutations in *MYD88*, and 30%–40% show mutations also in the carboxyl terminus of CXCR4.[2-4]
- The CXCR4/CXCL12 axis is crucial for the homing and retention of WM cells in the BM.[4]
- A clinical trial evaluating the efficacy of the oral CXCR4 antagonist, mavorixafor, in combination with the Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, in WM patients with $MYD88^{L265P}$ and $CXCR4^{WHIM}$ mutations is currently ongoing (NCT04274738).[5]
- The effects of mavorixafor with ibrutinib and other BTK inhibitors on WM cells harboring only the single MYD88 mutation and with wild-type (WT) CXCR4 ($MYD88^{L265P}$ without $CXCR4^{WHIM}$ mutation) have not been evaluated.

Objectives

- This study was designed to test the ability of mavorixafor to sensitize WM cells carrying $MYD88^{L265P}$ with WT CXCR4 ($CXCR4^{WT}$) to BTK inhibitors in a WM/BM stromal cell (BMSC) coculture model.
- The effects of mavorixafor on Ca^{2+} mobilization, cell migration, and adhesion of WM cells to BMSC were also measured.

Methods

- WM cells (MWCL-1 cell line, $MYD88^{L265P}CXCR4^{WT}$) pretreated with mavorixafor and/or BTK inhibitors (ibrutinib, zanubrutinib, evobrutinib, pirtobrutinib [LOXO-305], nemtabrutinib [ARQ-531]) were cocultured with established BMSCs (HS27a cells).
- Cell viability, apoptosis, and IgM release were measured after 72 hours.

Results

Coculture with BMSCs increases CXCR4 expression and IgM release

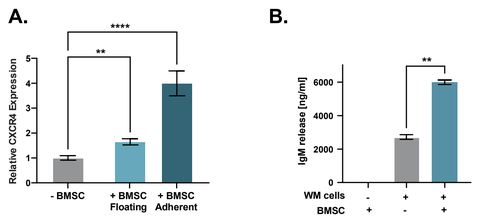

Figure 1. BMSC coculture effects on CXCR4 expression on WM cells and IgM release. Relative CXCR4 surface expression **(A)** and IgM release **(B)** in the presence of BMSC coculture. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; **—*P*<0.01; ***—*P*<0.001; ****—*P*<0.0001. ANOVA test for multiple comparisons was used. ANOVA, analysis of variance.

Results (cont'd)

BMSC-induced resistance of WM cells to BTK inhibitors

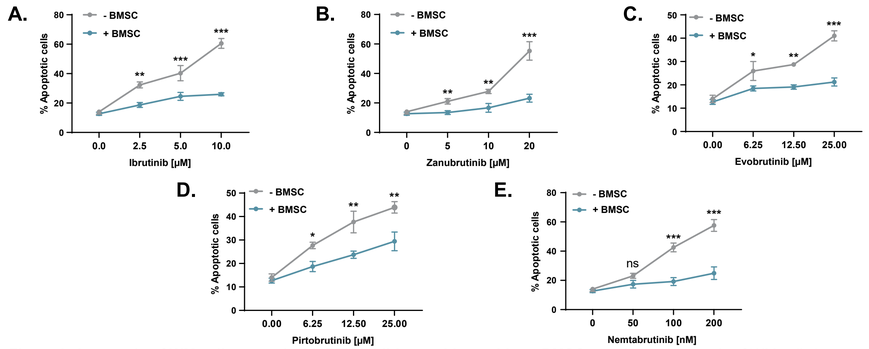

Figure 2. Apoptosis of WM cells treated with BTK inhibitors with or without BMSC coculture. Apoptosis of WM cells in response to ibrutinib **(A)**, zanubrutinib **(B)**, evobrutinib **(C)**, pirtobrutinib (LOXO-305) **(D)**, and nemtabrutinib (ARQ-531) **(E)** with or without BMSC coculture. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001. ANOVA test for multiple comparisons was used.

Mavorixafor alone inhibited CXCL12-stimulated Ca^{2+} mobilization and migration of WM cells and disrupted the adhesion of WM cells to BMSCs

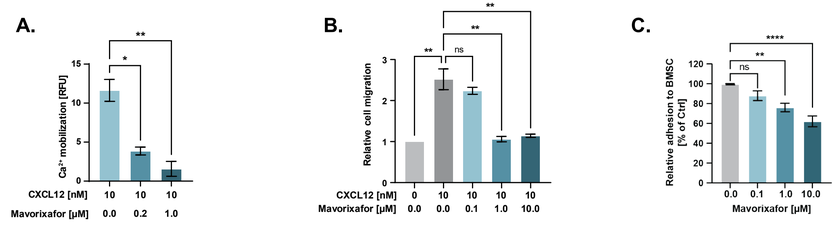

Figure 3. Ca^{2+} mobilization, cell migration, and adhesion of WM cells to BMSC with or without mavorixafor treatment. Effects of increasing concentrations of mavorixafor on Ca^{2+} mobilization **(A)**, cell migration **(B)**, and adhesion to BMSC cells **(C)**. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<0.05; **—*P*<0.01; ****—*P*<0.0001. ANOVA test for multiple comparisons was used.

Mavorixafor increased the sensitivity of WM cells to BTK inhibitors and decreased tumor cell viability in BMSC coculture model

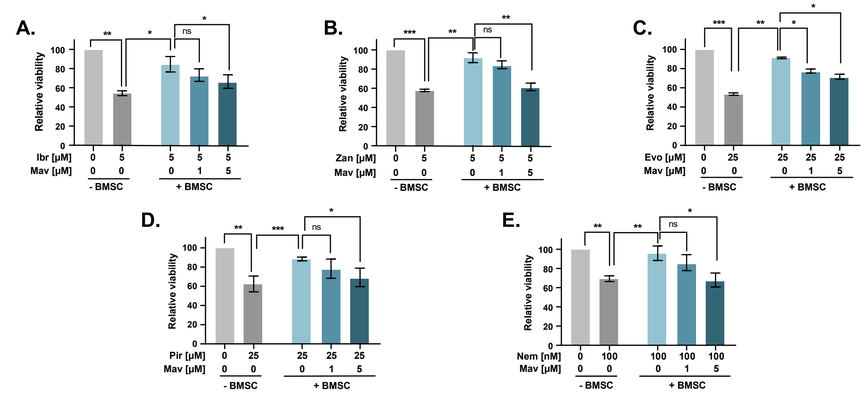

Figure 4. WM cell viability after treatment with BTK inhibitor ± mavorixafor with or without BMSC coculture. Viability of WM cells with or without coculture with BMSC in the presence of ibrutinib **(A)**, zanubrutinib **(B)**, evobrutinib **(C)**, pirtobrutinib (LOXO-305) **(D)**, and nemtabrutinib (ARQ-531) **(E)**, with or without mavorixafor treatment. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001. ANOVA test for multiple comparisons was used.

Mavorixafor increased the sensitivity of WM cells to BTK inhibitors and increased apoptosis of tumor cells in BMSC coculture model

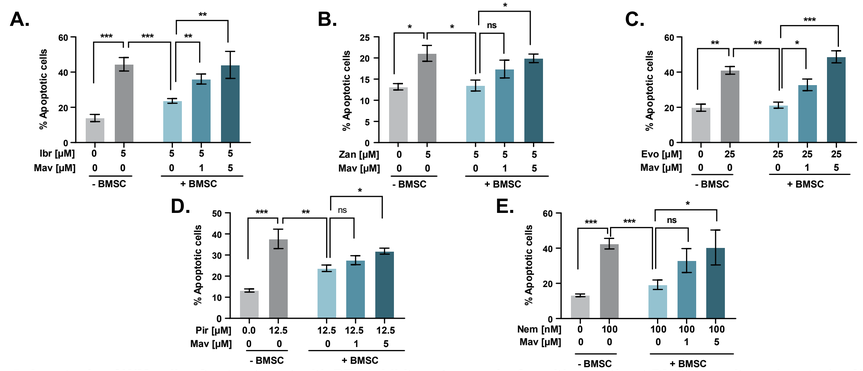

Figure 5. Apoptosis of WM cells after treatment with BTK inhibitors ± mavorixafor with or without BMSC coculture. Apoptosis of WM cells with or without coculture with BMSC in the presence of ibrutinib **(A)**, zanubrutinib **(B)**, evobrutinib **(C)**, pirtobrutinib (LOXO-305) **(D)**, and nemtabrutinib (ARQ-531) **(E)** with or without mavorixafor treatment. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001. ANOVA test for multiple comparisons was used.

Mavorixafor alone or in combination with BTK inhibitors reduces IgM hypersecretion

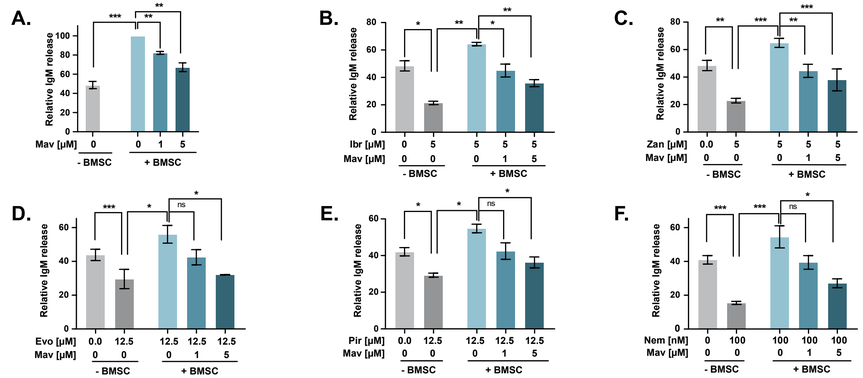

Figure 6. Relative IgM release from WM cells with BTK inhibitors ± mavorixafor with or without BMSC coculture. Relative IgM release of WM cells with or without coculture with BMSC **(A)** and in the presence of ibrutinib **(B)**, zanubrutinib **(C)**, evobrutinib **(D)**, pirtobrutinib (LOXO-305) **(E)**, and nemtabrutinib (ARQ-531) **(F)** with or without mavorixafor treatment. *P* values <0.05 were considered statistically significant and set as follows: ns, not significant; *—*P*<0.05; **—*P*<0.01; ***—*P*<0.001. ANOVA test for multiple comparisons was used.

Conclusions

- This is the first *in vitro* study to show that the protection of WM cells against BTK inhibitors conferred by BMSCs can be overcome by inhibition of the CXCL12/CXCR4 axis.
- These observations suggest a contribution of $CXCR4^{WT}$ upregulation to pathogenicity of WM cells carrying only the $MYD88^{L265P}$ mutation.
- Mavorixafor treatment enhanced the efficacy of not only ibrutinib, but all BTK inhibitors tested, supporting the greater potential of mavorixafor combinations as a therapeutic strategy in patients with WM.
- Further studies using additional WM cell lines and/or primary patient cells are warranted to support these findings in patients with WM, irrespective of their *CXCR4* mutational status.

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Disclosures

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

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