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# Targeting the Spleen Tyrosine Kinase with Fostamatinib as a Strategy against Waldenström Macroglobulinemia

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## Abstract

**Purpose:** Waldenström macroglobulinemia (WMG) is a lymphoproliferative disorder characterized by good initial responses to standard therapeutics, but only a minority of patients achieve complete remissions, and most inevitably relapse, indicating a need for novel agents. B-cell receptor signaling has been linked to clonal evolution in WMG, and Spleen tyrosine kinase (Syk) is overexpressed in primary cells, suggesting that it could be a novel and rational target.

**Experimental Design:** We studied the impact of the Syk inhibitor fostamatinib on BCWM.1 and MWCL-1 WMG-derived cell lines both *in vitro* and *in vivo*, as well as on primary patient cells.

**Results:** In WMG-derived cell lines, fostamatinib induced a time- and dose-dependent reduction in viability, associated with activation of apoptosis. At the molecular level, fostamatinib reduced activation of Syk and Bruton's tyrosine kinase, and also downstream signaling through MAPK kinase (MEK), p44/42 MAPK, and protein kinase B/Akt. As a single agent, fostamatinib induced tumor growth delay in an *in vivo* model of WMG, and reduced viability of primary WMG cells, along with inhibition of p44/42 MAPK signaling. Finally, fostamatinib in combination with other agents, including dexamethasone, bortezomib, and rituximab, showed enhanced activity.

**Conclusions:** Taken together, these data support the translation of approaches targeting Syk with fostamatinib to the clinic for patients with relapsed and possibly even newly diagnosed WMG. *Clin Cancer Res; 21(11); 2538–45. ©2015 AACR.*

## Introduction

Signaling through the B-cell receptor (BCR) occurring through both antigen-dependent and -independent mechanisms appears to play an important role in the pathobiology of several common B-cell malignancies. BCR aggregation results in phosphorylation of the Igα (CD79a) and Igβ (CD79b) immunoreceptor tyrosine-based activation motifs (ITAMs) catalyzed by members of the Src family of kinases (SFK), such as Lyn. Phosphorylated ITAM residues then serve as docking sites for the spleen tyrosine kinase (Syk), and binding results in a conformational change that facilitates exposure of tyrosines 348 and 352 for phosphorylation by SFKs, as well as Syk autophosphorylation. Later association with other signaling intermediates, such as Shc, Bruton's tyrosine kinase (BTK), phospholipase C γ 2, and PI3K, results in downstream activation of signal transduction pathways crucial to lymphoma pathobiology. Among these are the proliferation-associated MAPKs such as p44/42, and the survival-associated protein kinase B/Akt (1, 2).

Waldenström macroglobulinemia (WMG) is diagnosed in the presence of a lymphoplasmacytic B-cell lymphoma involving the bone marrow and a serum IgM monoclonal protein (3). Though this disease typically has an indolent clinical course, its presenting features can include symptomatic anemia, thrombocytopenia, hepatosplenomegaly, and lymphadenopathy, among others, and currently available therapies are not curative. At the molecular level, recent studies have identified the L265P mutation of myeloid differentiation primary response gene 88 (*MYD88*) as a commonly recurring abnormality in WMG patients (4–8). This mutation contributes to disease pathobiology through activation of NF-κB signaling (4), as well as of BTK (9), implicating a role for BCR signaling. Indeed, previous studies had linked BCR signaling to clonal evolution in WMG (10). These findings led to translation of the BTK inhibitor ibrutinib (11) to the clinic for patients with relapsed and/or refractory WMG. In this setting, ibrutinib showed significant antitumor activity (12), with a response rate of 81%, though no complete remissions were noted.

With this validation of BCR signaling as a target in WMG, we considered the possibility that other intermediates could be attractive as well. We focused in particular on Syk given the availability of fostamatinib, a specific and clinically relevant (13) Syk inhibitor and previous findings showing that *Syk* was

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Novel therapies are needed for Waldenström macroglobulinemia (WMG) because the currently available standard and novel agents induce high overall response rates but few patients achieve complete remission, and none are cured of this lymphoproliferative disorder. The current study validates use of the Spleen tyrosine kinase inhibitor fostamatinib as an attractive strategy using cell lines and primary samples *in vitro*, as well as a xenograft model *in vivo*. Since fostamatinib is currently undergoing clinical testing, our data provide a rationale for translation of this drug into trials targeting patients with relapsed and/or refractory, and possibly even newly diagnosed WMG.

overexpressed in primary patient cells (14). In the current report, we present data showing the activity of fostamatinib against preclinical models of WMG both *in vitro* and *in vivo*, and dissect some of its mechanisms of action.

## Materials and Methods

### Reagents

Fostamatinib disodium (FosD; R935788) and bortezomib were purchased from Selleck Chemical, and stock solutions were prepared in PBS and DMS (Fisher Scientific), respectively. Rituximab was purchased from the MD Anderson Pharmacy, while dexamethasone was from Sigma-Aldrich, with stock solutions made in PBS and ethanol (Fisher Scientific), respectively.

### Tissue culture and patient samples

The BCWM.1 cell line derived from a patient with WMG (15) was from Dr. Steven Treon (Dana Farber Cancer Institute; Boston, MA). These cells are $CD5^-$, $CD10^-$, $CD19^+$, $CD20^+$, $CD23^+$, $CD27^-$, $CD38^+$, $CD138^+$, $CD40^+$, $CD52^+$, $CD70^+$, $CD117^+$, $cIgM^+$, $cIgG^-$, $cIgA^-$, ckappa$^-$, and clambda$^+$ (15), and harbor the *MYD88* L265P mutation but have a wild-type C-X-C chemokine receptor type 4 (*CXCR4*; ref. 16). MWCL-1 WMG cells were from Dr. Stephen Ansell (The Mayo Clinic; ref. 17), and are $CD3^-$, $CD19^+$, $CD20^+$, $CD27^+$, $CD38^+$, $CD49D^+$, $CD138^+$, $cIgM^+$, and $\kappa^+$. They also harbor the *MYD88* L265P mutation and a wild-type *CXCR4* (16), but have a *TP53* missense mutation at exon 5 (V143A, GTG>GCG; ref. 17). BWCM-1 cells were propagated in RPMI-1640 media (Life Technologies) supplemented with 2 mmol/L L-glutamine (Invitrogen) and 10% FCS (Hyclone), while MWCL-1 cells were grown in similarly supplemented IMDM (Life Technologies). All cultures also contained 100 U/mL penicillin and 100 μg/mL streptomycin (Mediatech). Cell line authentication was performed by the MD Anderson Characterized Cell Line Core Facility using short tandem repeat DNA fingerprinting with the AmpFlSTR kit (Applied Biosystems). Primary cells were obtained from patients undergoing bone marrow aspiration after they had provided informed consent in compliance with the Declaration of Helsinki according to an Institutional Review Board-approved protocol. $CD20^+$ primary cells were purified by positive selection using magnetic-activated cell sorting with $CD20^+$ microbeads (Miltenyi Biotec).

### Cellular viability assays

Proliferation and viability assays were performed using the WST-1 reagent as previously described (18, 19). Briefly, cells were plated in triplicate and exposed to the conditions noted in the text, and premixed WST-1 cell proliferation reagent (Clontech Laboratories) was added and used according to the manufacturer's specifications. Conversion of WST-1 to the formazan dye was measured at 650 nm using a Victor 3V plate-reader (PerkinElmer Life Science), and viability data were prepared using GraphPad Prism version 6 showing the mean ±

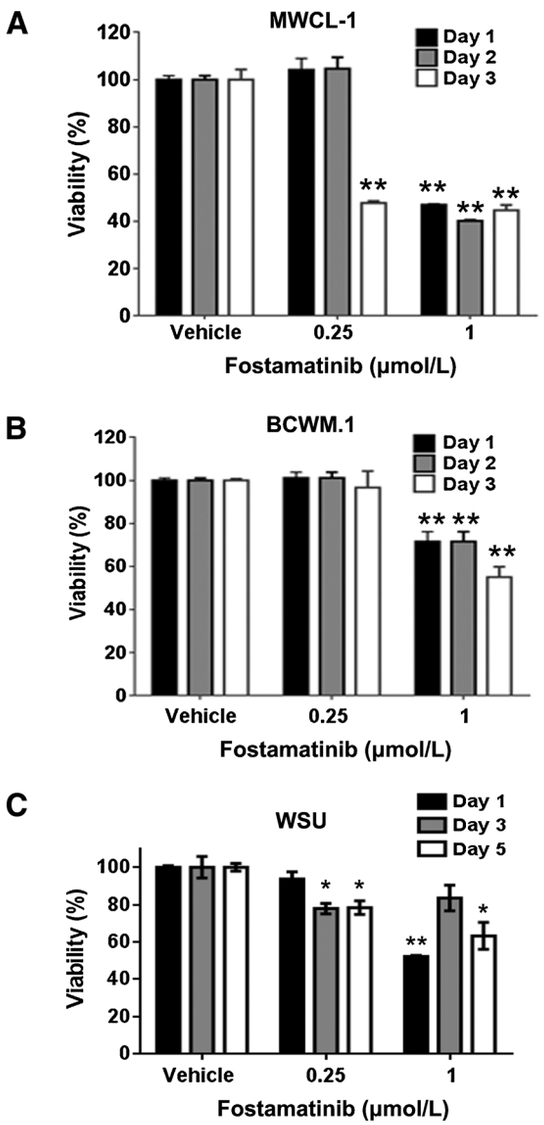


**Figure 1.**
Fostamatinib reduces viability in WMG cells. MWCL-1 (A), BCWM.1 (B), and WSU cells (C) were seeded in 96-well plates at 10,000 cells per well and treated with vehicle, 0.25, or 1 μmol/L concentrations of fostamatinib for 24, 48, or 72 hours, as indicated. Cell viability was assayed using the tetrazolium reagent WST-1, and data are representative of one of two independent experiments, each performed in triplicate, and are presented as mean ± S.D. A ** indicates statistical significance at a level of $P < 0.01$, while * indicates a $P < 0.05$, compared with vehicle-treated controls using the Student *t* test.



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SD. Experiments were performed in duplicate on different days, and results from one representative dataset are presented in the text.

### Apoptosis and cell-cycle analysis

Induction of apoptosis in cells treated with fostamatinib was detected by staining with an antibody to Annexin V and with ToPro3 (both from Life Technologies). FACS was then performed using a Fortessa FACS machine (Applied Biosystems). Cell-cycle analysis was similarly performed after staining with 1 μmol/L propidium iodide (Sigma-Aldrich).

### Western blotting

Cells were harvested, washed, and lysed with 1× lysis buffer (Cell Signaling Technology) containing 1× Protease and 1× Phosphatase inhibitor mixtures (Roche Diagnostics). Lysates were then sonicated and clarified by centrifugation, and protein concentrations were determined using the DC Assay Kit (Bio-Rad). A total of 50 μg of protein boiled in denaturing buffer (3% dithiotreitol, 0.1 mol/L Tris-HCl, pH 6.8, 4% sodium dodecylsulfate, 0.2% bromophenol blue, 20% glycerol) was separated by gel electrophoresis, and transferred onto nitrocellulose membranes. These were blocked with Tris-buffered saline + 0.01% Tween 20 containing 5% non-fat milk, and then exposed to primary and secondary antibodies. Protein abundance was visualized using the Enhanced Chemiluminescence Kit according to the manufacturer's specification (Pierce Scientific) with autoradiography film (Eastman Kodak). The images were scanned and densitometry of the protein bands was performed using Image J Software (NIH; Bethesda, MD) with normalization to β-actin as a loading control. Primary antibodies used included those against phospho-Tyr525/526 Syk (#12081), phospho-Tyr323 Syk (#2715), and total Syk (#2712), phospho-Tyr323 BTK (#2715) and total BTK (#3533), phospho-Ser117/Ser221 MAPK kinase (MEK; #9154) and total-MEK (#9122), phospho-Thr202/Tyr204 p44/42 MAPK (#4370) and total p44/42 MAPK (#9102), and phospho-Ser473 Akt (#4058) and total AKT (#9272), all of which were from Cell Signaling Technology. Loading controls were provided using an anti-β-actin antibody (A1978) from Sigma-Aldrich.

### Xenograft model

Experiments were performed in accordance with procedures and protocols approved by the MD Anderson Cancer Center Animal Care and Use Committee. Six-week-old non-obese diabetic mice with severe combined immunodeficiency (NOD.Cg-*Prkdc*$^{scid}$ *Il2rg*$^{tm1Wjl}$/SzJ) from Jackson Laboratories were injected subcutaneously with $1 \times 10^7$ MWCL-1 cells with Matrigel (BD Biosciences). Seven days after tumor formation, they were injected intraperitoneally with either PBS, or this

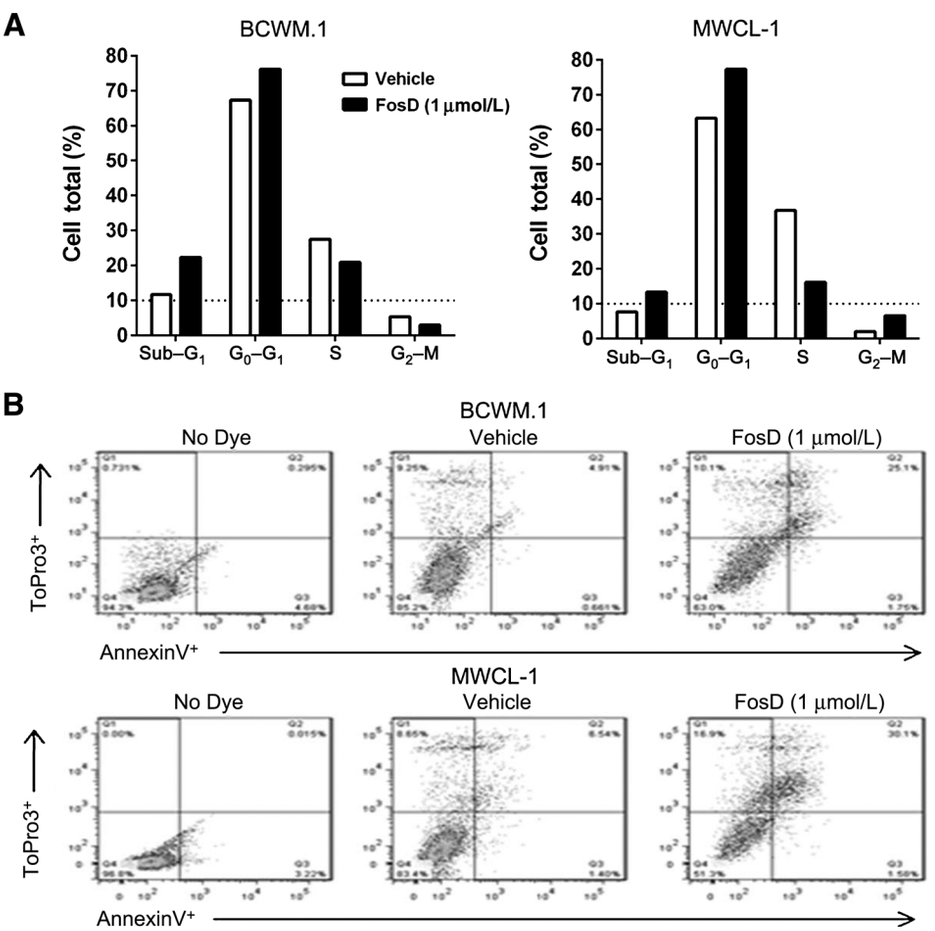


**Figure 2.**
Fostamatinib induces cell-cycle arrest and apoptosis. BCWM.1 and MWCL-1 cells treated with either vehicle or 1 μmol/L of fostamatinib for 24 hours were examined for their cell-cycle profile (A) by propidium iodide staining followed by analysis with flow cytometry of 10,000 events. Apoptosis was assessed after these same conditions (B) by staining cells with an antibody to Annexin V and the nuclear stain ToPro3. The "No Dye" panels contain cells that have been exposed to Annexin V staining buffer but not to either Annexin V or ToPro3.



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vehicle with fostamatinib to a final concentration of 80 mg/kg thrice weekly. The rate of growth was determined by measuring the tumor size twice weekly for a total of 45 days with calipers using the formula for an ellipsoid sphere: $(L \times W^2)/2 = mm^3$.

### Statistical analysis

The *t* test or ANOVA, or their corresponding nonparametric methods (Wilcoxon rank-sum test or Kruskal–Wallis test), were used to detect differences for continuous variables between groups (20). Generalized linear regression models (21) were used to study the tumor growth over time in the xenograft model. Autoregressive (20) covariance structure was used to account for inter-mouse variability and the longitudinal nature of the data. An interaction between treatment and time is assessed to test the heterogeneity of slopes, that is, the tumor growth rate. A two-sample *t* test was used to compare the differences of tumor volume between the two groups at each time point. The transformation of logarithm to the base 2 of the tumor volume was used in the analyses to satisfy the normality assumption of the models, and Bonferroni multiplicity adjustment was applied for multiple comparisons. SAS version 9.2 (SAS), R 2.80, and S-Plus version 8.04 (TIBCO Software, Inc.) were used to carry out the computations for all analyses.

## Results

### Fostamatinib induces apoptosis of WMG-derived cell lines

To determine whether Syk inhibition could be valuable as an approach against WMG, we examined the activity of fostamatinib against WMG-derived cell lines. Using an assay that measures the metabolism of WST-1 by mitochondrial succinate-tetrazolium reductase, we found that BCWM.1 cells exposed to fostamatinib showed a time- and concentration-dependent decrease in viability (Fig. 1A). This was especially notable at 1 μmol/L of fostamatinib, which reduced viability by up to 45% after three days. To confirm these findings, we studied MWCL-1 cells, which were also sensitive to fostamatinib at 1 μmol/L, with a 53% to 60% reduction in viability after 1 to 3 days of exposure (Fig. 1B), though 0.25 μmol/L showed activity as well after 3 days. Notably, these concentrations are well within those achieved by patients in phase I and pharmacokinetics studies of fostamatinib (22), where peak concentrations as high as 6 μmol/L were observed, suggesting that they are physiologically relevant.

Cell-cycle analysis of BCWM.1 cells exposed to fostamatinib compared with vehicle controls revealed an increase in cells at $G_0$–G1 from 67% to 76% (Fig. 2A, left), and fewer cells were seen in S phase (27%–21%). Similarly, MWCL-1 cells treated with fostamatinib tended to accumulate at $G_0$–$G_1$ (63%–77%; Fig. 2A, right), with a reduction in the fraction in S phase (37%–16%). In both models, fostamatinib increased the proportion of cells with a sub-$G_1$ DNA content, which rose from 12% to 22% in BCWM.1 cells, and from 8% to 13% in MWCL-1 cells, suggesting the activation of programmed cell death. To examine for the latter, cells were stained with an antibody to Annexin V and with the nuclear dye ToPro3, and analyzed by cell sorting. Compared with vehicle-treated BCWM.1 cells (Fig. 2B, top), fostamatinib increased the proportion that were Annexin $V^+$ from 4.6% to 26.9%, whereas in MWCL-1 cells, this increased from 7.9% to 31.6% (Fig. 2B, bottom). At higher drug concentrations, fostamatinib induced increased levels of apoptosis. Fostamatinib concentrations of 0.1, 1, 5, and 10 μmol/L induced staining with Annexin V in 18.5%, 24.1%, 28.2%, and 34.8% of BCWM.1 cells, respectively (Supplementary Fig. S1A), and in 17.5%, 21.4%, 30.4%, and 57.0% of MWCL-1 cells, respectively (Supplementary Fig. S1B). These findings indicate that Syk inhibition reduced viability in WMG-derived cell lines through a combination of cell-cycle accumulation at $G_0$–$G_1$ and activation of apoptosis.

### Activation of Syk is reduced by fostamatinib

To determine the impact of fostamatinib on Syk, we used Western blotting with antibodies specific for phospho-Tyr525/526, which are residues in the activation loop of the Syk kinase domain (23). In BCWM.1 cells (Fig. 3A, left), fostamatinib reduced Syk activation in a concentration-dependent manner, with a 41% decrease in phospho-Syk after 1 μmol/L of this drug. A similar effect was seen in MWCL-1 cells, where fostamatinib reduced levels of phospho-Tyr525/526-Syk by up to 51% (Fig. 3A, right). Also, we examined the impact on phosphorylation of Syk at Tyr323, which provides a binding site for the Cbl ubiquitin ligase (24, 25), and is therefore thought to be inhibitory of Syk activity. Interestingly, fostamatinib also reduced levels of phospho-Tyr323-Syk in BCWM.1 cells (Fig. 3B, left) by up to 98% or more, and in MWCL-1 cells (Fig. 3B, right) by up to 75%. Finally, to determine the net effect on Syk activity of fostamatinib in these models, we examined the levels of phospho-Tyr223-BTK, a modification that occurs as a result of auto-phosphorylation after trans-phosphorylation at Tyr551 in the BTK activation loop (26, 27). Although both BCWM.1 (Fig. 3C, left) and MWCL-1 cells (Fig. 3C, right) had substantial phospho-Tyr223-BTK levels at baseline, fostamatinib reduced these dramatically by up to 95% and 59%, respectively. These findings indicate that fostamatinib

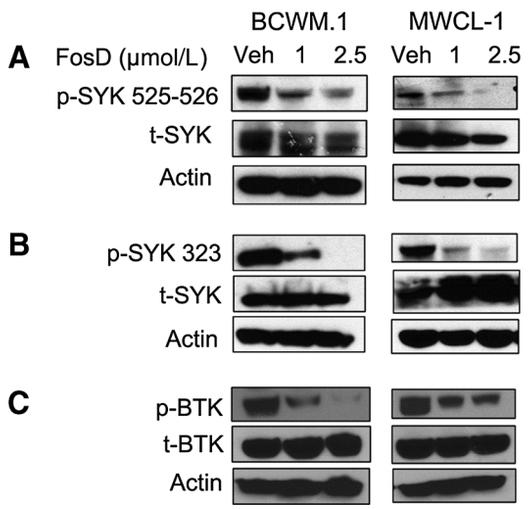


**Figure 3.**
Fostamatinib inhibits activation of Syk and BTK. BCWM.1 and MWCL-1 cells treated with either vehicle or fostamatinib at the indicated concentrations for 2 hours were harvested and lysed, and protein extracts were analyzed by Western blotting. The activation status of Syk (A and B) and BTK (C) was probed with phospho-specific antibodies using β-actin as well as the levels of total, non-phosphorylated Syk and BTK as controls. Data are representative of one of three independent experiments.



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did reduce Syk activity and the activation status of the downstream intermediate BTK, which is itself a key contributor to the pathobiology of WMG.

### Fostamatinib inhibits downstream signaling cascades

Given the role of BCR signaling in activating key downstream signal transduction cascades, we next looked at its impact on p44/42 MAPK. Vehicle-treated BCWM.1 (Fig. 4A, left) and MWCL-1 cells (Fig. 4A, right) had abundant levels of phospho-Ser117/Ser221 MEK. After exposure to fostamatinib, however, a dose-dependent decrease was seen, with a reduction in phospho-MEK levels by up to 96% or more in both model systems. This led to inhibition of signaling through p44/42 MAPK, where phosphorylation at Thr202/Tyr204 was reduced by up to 97% and 74% in BCWM.1 (Fig. 4B, left) and MWCL-1 cells (Fig. 4B, right), respectively. Finally, we looked at the impact of fostamatinib on Akt, a pathway whose activity has been described to influence homing and survival of WMG cells (28). Consistent with a role for BCR signaling in activating Akt, Syk inhibition with fostamatinib dramatically reduced phospho-Ser473 Akt levels by up to 95% to 98% in BCWM.1 (Fig. 4C, left) and MWCL-1 cells (Fig. 4C, right).

### Antitumor activity of fostamatinib

Since fostamatinib is clinically relevant in that it has been studied in patients with non-Hodgkin lymphomas (13), and is being evaluated in rheumatologic conditions (29), we next determined whether it could show antitumor activity. We developed an *in vivo* xenograft based on MWCL-1 cells in immunodeficient mice which grew steadily in the vehicle-treated cohort (Fig. 5A). In the fostamatinib-treated group, however, tumor growth was slower, and the difference between the two groups was different at a significance level of 0.0028 with adjustment of multiple comparisons (0.05/18 comparisons; 1 comparison at each of 18 time points). For example, the mean tumor volume of the control group on day 35 was larger than the one of the treatment group at a significance level of 0.0028 ($P$ value = 0.0002). Also, we examined CD20$^+$ cells isolated from bone marrow aspirates of

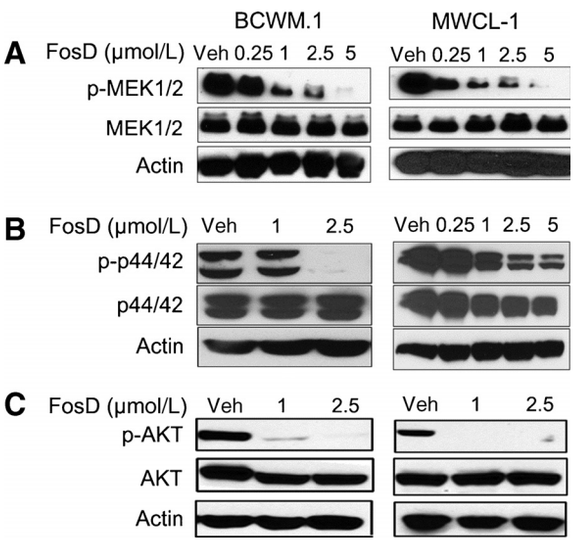


**Figure 4.**
Fostamatinib inhibits activation of the MAPK and Akt pathways. BCWM.1 and MWCL-1 cells treated as above were analyzed by Western blotting for the activation status of MEK, p44/42 MAPK, and Akt with phospho-specific antibodies as described earlier.

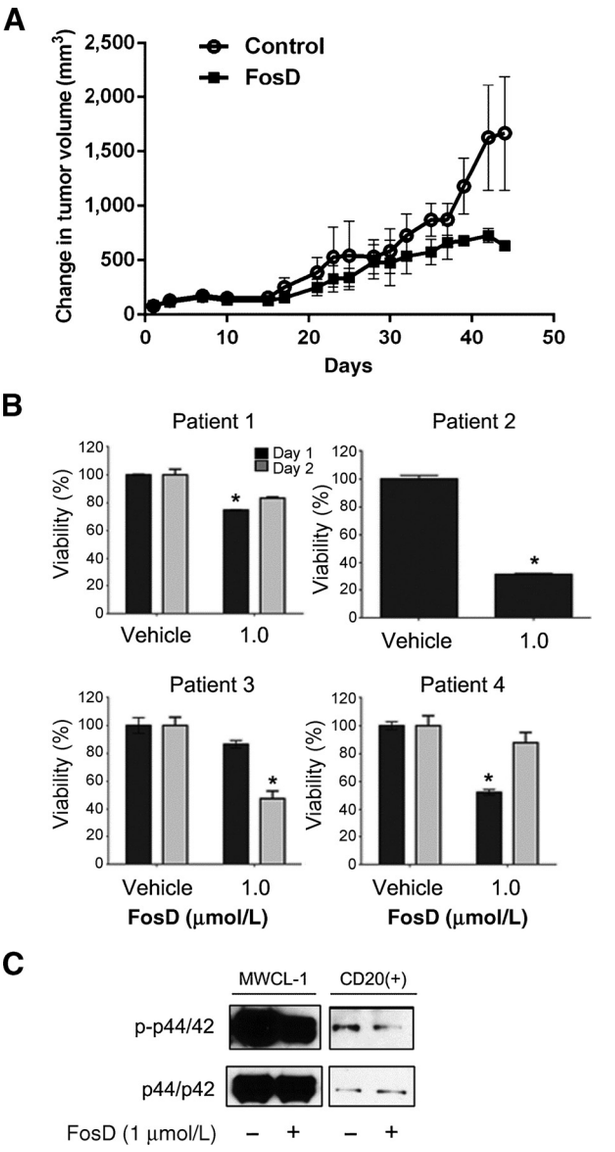


**Figure 5.**
Fostamatinib shows activity against an *in vivo* model and primary cells. Immunodeficient mice were injected subcutaneously with $1 \times 10^6$ MWCL-1 cells, and 7 days after implantation, they were randomized to receive vehicle or fostamatinib at 85 mg/kg daily (A). Tumor growth was monitored by caliper measurements and plotted against time. Primary cells from 4 patients with WMG purified using CD20 microbeads were then exposed to vehicle or 1 μmol/L of fostamatinib for 24 or 48 hours as indicated (B). Cell viability was measured by using the tetrazolium reagent WST-1, and all data points were normalized to the vehicle controls, which were arbitrarily set at 100%. Experiments were performed in triplicate, and data are presented as the mean ±S.D. A * indicates statistical significance at a level of $P < 0.05$ compared with vehicle-treated controls using the Student $t$ test. Whole protein cell lysates of cells from one patient were prepared (C) from the CD20$^+$ fraction and treated with fostamatinib or vehicle control for 2 hours. Western blotting with phospho-specific and total-p44/42 MAPK antibodies was then performed.



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patients with WMG, and found that fostamatinib was able to reduce viability in all of them (Fig. 5B), and this was associated with a decrease in p44/42 MAPK activation (Fig. 5C) in the one sample where sufficient cells were available to evaluate this by Western blotting.

**Combination regimens enhance anti-WMG effects**

Treatment of patients with WMG in either the front-line or relapsed and/or refractory setting often involves the use of multidrug regimens including corticosteroids, proteasome inhibitors, monoclonal antibodies, and alkylating agents (3, 30). To therefore see whether fostamatinib could be considered not just as a stand-alone therapy, but also as part of other regimens, combination studies were performed. Dexamethasone as a single agent showed modest to no significant activity against either BCWM.1 or MWCL-1 cells (Fig. 6A, left and right, respectively). Fostamatinib alone impacted upon viability in both cell lines, with an up to 28% reduction in MWCL-1 cells, for example. When the two were combined, however, enhanced activity was seen, especially in MWCL-1 cells, where an up to 80% decrease in viability was noted ($P < 0.01$). Proteasome inhibition with bortezomib showed activity and reduced viability by 36%, whereas fostamatinib with bortezomib reduced this further to 72% of controls in MWCL-1 cells ($P < 0.01$). Rituximab alone reduced viability by 25%, whereas an 84% reduction was seen with fostamatinib in combination with rituximab in MWCL-1 cells ($P < 0.01$). Finally, bendamustine was examined, and also reduced viability in both cell lines (Supplementary Fig. S2), with a reduction to 33% in MWCL-1 cells, which was enhanced further to 18% in combination with fostamatinib ($P < 0.01$).

It was also of interest in the context of combination therapy to determine whether different sequences of addition of some of these agents could provide enhanced activity. To examine this possibility, BCWM.1 and MWCL-1 cells were exposed either first to fostamatinib for 24 hours and then to fostamatinib with dexamethasone or bortezomib for another 48 hours, or to dexamethasone or bortezomib for 24 hours, followed by dexamethasone or bortezomib with fostamatinib for 48 hours. In BCWM.1 cells, there was an indication that addition of fostamatinib first led to a greater efficacy for the combination than if

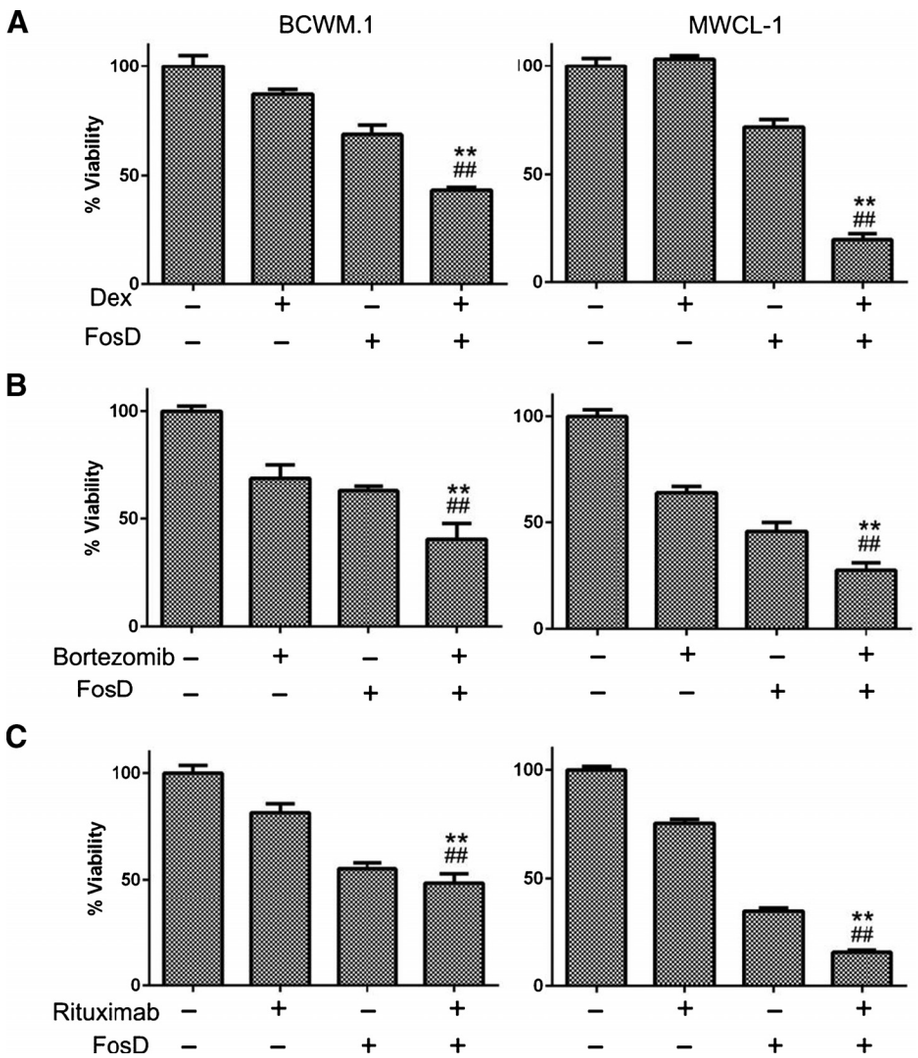


**Figure 6.**
Fostamatinib shows enhanced activity in combination with other agents. BCWM.1 or MWCL-1 cells were treated for 48 hours with vehicle, dexamethasone alone at 5 μmol/L, fostamatinib at 1.6 μmol/L, or with the combination with dexamethasone (A). Cell viability was determined with WST-1 as described above, and experiments were replicated five times on different days, with one representative set shown. **, $P < 0.01$ for the combination compared with the standard agent in each panel; ##, $P < 0.01$ compared with fostamatinib. BCWM.1 or MWCL-1 cells were also exposed to either single-agent bortezomib (5 nmol/L; B) or rituximab (0.1 mg/mL; C), and combinations with fostamatinib were examined as described above. Statistical differences were at $P < 0.01$ for all the panels except for BCWM.1 cells treated with rituximab and fostamatinib, in which the $P$ value was <0.05.



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dexamethasone (Supplementary Fig. S3A) or bortezomib (Supplementary Fig. S3B) was added first. For example, in the case of the dexamethasone combination, adding fostamatinib first reduced viability to 22% of controls, whereas use of dexamethasone first reduced viability only to 30% of controls ($P < 0.01$). However, these two sequences were equally effective in reducing the viability in MWCL-1 cells with fostamatinib and either dexamethasone or bortezomib. Together, these data support the possibility that enhanced activity could be seen by combining fostamatinib with some of the agents currently used in the clinic against this disease.

## Discussion

Recent studies of WMG have led to a greater understanding of the pathobiology of this disease, such as by identification of the L265P *MYD88* mutation in 90% or more of patients (4–8). Whole-genome sequencing has revealed that at least 10% of patients have somatic mutations in *CXCR4* and AT rich interactive domain 1A (SWI-like; *ARID1A*), while genes such as PR domain containing 2, with ZNF domain (*PRDM2*) and B-cell translocation gene 1, anti-proliferative (*BTG1*), among others, show frequent copy number abnormalities (31). A number of pathways have also been shown to play important roles in disease pathobiology, including, prominently, signaling through PI3K/Akt/mTOR (28, 32). This has led to the introduction of a variety of novel agents to therapy, including inhibitors of NF-κB (33–35), of histone deacetylases (36), and of mTOR (37), among others. Perhaps because of these novel approaches, outcomes of patients with WMG in at least some studies have been improving (38). However, other analyses have suggested only a modest improvement (39), and though this may be due in part to different levels of access to novel drugs, because a minority of patients achieve complete remission, newer therapies are needed in either case.

In the current study, we evaluated the potential utility of the Syk inhibitor fostamatinib in preclinical models of WMG. The rationale to consider that this could be of interest was based on the role of a number of BCR signaling-associated factors in disease pathobiology (4, 9), and earlier studies implicating Syk itself (10, 14). Consistent with this possibility, the Syk inhibitor fostamatinib at clinically relevant concentrations reduced the viability of WMG-derived cell lines (Fig. 1) by inducing cell-cycle arrest and apoptosis (Fig. 2). This was associated with decreased activation of Syk and downstream BTK (Fig. 3), as well as of signaling through p44/42 MAPK and Akt (Fig. 4). Finally, fostamatinib as a single agent was active against both a novel *in vivo* model of WMG and against primary cells (Fig. 5), and showed enhanced activity in combination with clinically relevant agents, including dexamethasone, bortezomib, and rituximab (Fig. 6).

Fostamatinib is the prodrug of R406, and is an oral ATP-competitive inhibitor which showed activity in several preclinical models, including both *in vitro* and *in vivo* against diffuse large B-cell lymphoma (40). Friedberg and colleagues performed a phase I/II study of fostamatinib, which identified a dose of 200 mg twice daily for phase II testing (13), with dose-limiting toxicities of neutropenia, diarrhea, and thrombocytopenia. Interestingly, although the overall response rate (ORR) in relapsed diffuse large B-cell lymphoma was only 24% (4/17), in patients with more indolent disorders, such as chronic lymphocytic leukemia/small lymphocytic lymphoma, the ORR was 55% (6/11), with a median progression-free survival of 6.4 months. These data, along with our current studies, provide a rationale to consider translating fostamatinib to the clinic for patients with WMG either alone, or in combination with other standard agents.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** I. Kuiatse, V. Baladandayuthapani, H.Y. Lin, C.C. Bjorklund, X. Zhang, R.Z. Orlowski
**Writing, review, and/or revision of the manuscript:** I. Kuiatse, H.Y. Lin, J.J. Shah, R.J. Jones, S.M. Ansell, S.P. Treon, R.Z. Orlowski
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