Cancer Therapy: Preclinical

FGFR3 Is Overexpressed Waldenström Macroglobulinemia and Its Inhibition by Dovitinib Induces Apoptosis and Overcomes Stroma-Induced Proliferation

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Abstract

Purpose: There is no standard of therapy for the treatment of Waldenström macroglobulinemia (WM), therefore there is a need for the development of new agents. Fibroblast growth factor receptor 3 (FGFR3) was shown to play a major role in several types in cancer. Dovitinib, an inhibitor of FGFR3, was effective in hematologic malignancies. In this study, we tested FGFR3 as a therapeutic target in WM and tested the effect of dovitinib on cell proliferation and apoptosis of WM cells in the context of BM microenvironment.

Methods: The expression of FGFR3 in WM cells was tested using immunofluorescence and flow cytometry. Cell signaling in response to stimulation with FGF3 and stromal cells, and its inhibition by dovitinib was performed using immunoblotting. Cell survival and cell proliferation were assessed by MTT and BrdU assays. Apoptosis was measured by detection of APO-2.7 and cleavage of caspase-3 using flow cytometry. Cell cycle was performed by PI staining of cells and flow cytometry. The combinatory effect of dovitinib with other drugs was analyzed using CalcuSyn software. The effect of dovitinib was tested in vivo.

Results: FGFR3 was overexpressed in WM cells and its activation induced cell proliferation. Inhibition of FGFR3 by dovitinib decreased cell survival, increased apoptosis, and induced cell cycle arrest. Inhibition of FGFR3 by dovitinib reduced the interaction of WM to bone marrow components, and reversed its proliferative effect. Dovitinib had an additive effect with other drugs. Moreover, dovitinib reduced WM tumor progression in vivo.

Conclusion: We report that FGFR3 is a novel therapeutic target in WM, and suggest dovitinib for future clinical trial the treatment of patients with WM. Clin Cancer Res; 17(13); 4389–99. ©2011 AACR.

Introduction

Fibroblast growth factor receptor 3 (FGFR3) is a member of the FGFR family which consists of an extracellular region, composed of 3 immunoglobulin-like domains, a single hydrophobic membrane-spanning segment and a cytoplasmic tyrosine kinase domain (1). The extracellular portion of FGFR3 interacts with FGF3, setting in motion a cascade of downstream signals, ultimately influencing mitogenesis and differentiation (2). FGFR3 was shown to play a major role in several types in cancer; it was shown to mediate growth and neoplasia in colorectal (3), bladder (4), and oral (5) cancers.

Point mutations in specific FGFR3 domains can be found in the translocated allele t(4;14)(p16.3;q32) found in 15% to 20% of multiple myeloma patients, and have been proven to be oncogenic. These mutations produce a constitutively activated receptor, which shows dimerization and autophosphorylation even in the absence of ligand (6). However, in many cases FGFR3 expression was not related to chromosome abnormality. FGFR3 was overexpressed in human myeloma cell lines without a t(4;14) translocation (7), and about 25% of multiple myeloma tumors and cell lines without a t(4;14) translocation do not express FGFR3 (8).

Waldenström macroglobulinemia (WM) is a low-grade non-Hodgkin lymphoma, characterized by the presence of abnormal lymphoplasmacytic cells producing high levels of IgM immunoglobulins (9). There is no standard of therapy for the treatment of WM, and there are no FDA-approved therapeutic agents for the specific treatment of WM (10). Most treatments were originally derived from other lymphoproliferative diseases including multiple myeloma and chronic lymphocytic leukemia (11). Therefore, there is a need for the development of novel chemotherapeutic agents that specifically target deregulated signaling pathways specifically present in WM.

Dovitinib, a benzimidazole-quinolinone, is a potent inhibitor of wild-type and mutant FGFR3 and other RTKs (12). Dovitinib was effective in hematologic malignancies

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including AML (13), CML (14), as well as colon cancer (15) and other solid tumors (16). Moreover, in multiple myeloma cells from t(4;14) patients with high expression of FGFR3, dovitinib inhibited downstream extracellular signal transduction induced by FGFR3, which was associated with cytotoxic response, and was effective in a xenograft model of FGFR3 multiple myeloma (12, 17).

In this study, we aimed to test FGFR3 as a therapeutic target in WM and to test the effect of dovitinib on cell proliferation and apoptosis of WM cells in the context of BM microenvironment.

Materials and Methods

Reagents

RTKs inhibitor dovitinib was obtained from Novartis. Recombinant FG3 was purchased from R&D. Antibodies against FGFR3, p-FGFR3, pRAF, pERK1/2, pSTAT3, PARP, caspase-3, caspase-9, Cyclin-D3, CDK2, P27, pRb, pFAK, pSRC, pAKT, α-tubulin, and β-actin used for Western blotting were purchased from Cell Signaling Technologies.

Cells

The WM cell line (BCWM1) and IgM-secreting low-grade lymphoma cell lines (MEC-1) were used in this study. The BCWM1 was a kind gift from Dr. Treon (Dana-Farber Cancer Institute, Boston, MA). MEC-1 was a kind gift from Dr. Neil Kay (Mayo Clinic). Both cell lines were obtained from Dr. Treon (Dana-Farber Cancer Institute, Boston, MA). MEC-1 was a kind gift from Dr. Neil Kay (Mayo Clinic). Both cell lines were purchased from Cell Signaling Technologies.

In some cases, cells were preactivated with FGF3, serum, or other agents. For immunoblotting, cells were incubated for 6 hours with increased doses of dovitinib (0–2,500 nmol/L, for 6 hours). In some cases, cells were treated with increased concentrations of dovitinib (0–2,500 nmol/L, for 6 hours).

Immunoblotting

To test the effect of FGF3 on cytoskeletal signaling in WM, BCWM1, and MEC-1 cells (5 × 10^6) were serum-starved for 3 hours and then stimulated with FGF3 (50 ng/mL) for 0, 5, 30, and 60 minutes. In some cases, cells were treated with increased concentrations of dovitinib (0–2,500 nmol/L, for 6 hours). In some cases, cells were preactivated with FGF3, serum, or blocking antibody against FGFR3 (0, 1, and 10 μg/mL, for 6 hours). In some cases, cells were treated with increased concentrations of dovitinib (0–2,500 nmol/L, for 6 hours).

Cell viability test

Primary CD19+ cells from WM patients and normal subjects, BCWM1 and MEC1 cells (0.5 × 10^6 cell/mL) were
cultured with increased doses of dovitinib (0–2,500 nmol/L for 24 hours. Cell growth was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Chemicon International) dye absorbance.

**Apoptosis assay**

BCWM1 and MEC1 cells (0.5 × 10⁶ cell/mL) were cultured with increased doses of dovitinib (0–2,500 nmol/L) for 24 hours. Cells were treated with PerCP-Cy5.5–APO-2.7 for 30 minutes on ice, washed, and analyzed by flow cytometry. Alternatively, cells were fixed with formalin for 20 minutes at RT, permeabilized with 0.5% Triton-X-100 for 30 minutes at RT and treated with FITC conjugate of anti-cleaved-caspase-3, washed and analyzed by flow cytometry. In some cases, cell were cocultured with BMSC, and treated with or without of bortezomib, melphalan, and dexamethasone.

**Cell cycle analysis**

BCWM1 and MEC1 cells (0.5 × 10⁶ cell/mL) were cultured with increased doses of dovitinib (0–2,500 nmol/L) for 24 hours. Cells were fixed with 70% ethanol, washed, RNA was degraded by RNAase, DNA was stained with 5 μg/mL propidium iodide (SigmaChemical), and cells were analyzed by flow cytometry.

**Proliferation assays**

BCWM cells were treated with increasing concentrations of dovitinib for 24 hours, and cell proliferation was detected using BrDU assay. To test the role of FGFR3 on WM cell proliferation BCWM-1 and MEC-1 were cultured with or without FGF3, or with or without coculture with BMSCs, with increasing concentrations of dovitinib. For coculture experiments, a confluent monolayer of BMSCs in 96-well plate wells was obtained by seeding 1 × 10⁶ cells/well overnight. The next day, BCWM-1 cells (3 × 10⁵/well) were cultured with or without BMSCs cells for 24 hours.

**The effect of dovitinib on adhesion of WM cells to BM components**

Fibronectin-coated plates, or a confluent monolayer of HUVEC, or BMSCs cells generated by plating 1 × 10⁵ cells/well in 96-well plates overnight were used. BCWM-1 and MEC-1 cells (1 × 10⁶ cells/mL) were serum-starved for 3 hours, prelabeled with Calcein-AM, treated with increasing doses of dovitinib or blocking antibody for FGFR3 for 3 hours and then added to the plates with fibronectin, BMSCs, or HUVEC cells for 1 hour at 37°C. Nonadherent cells were washed, and adherent cells were detected by measuring the fluorescence intensity in the wells using a fluorometer (Eₘ/Eᵢ = 485/520 nm).

**Combination of drugs**

The interaction between dovitinib and other anti-WM drugs was analyzed using the calcusyn software program (Biosoft). For analyses of combinations, different doses of each drug were used. Nevertheless, to make it clearer, only the most representative doses of each combination were included in the figures. Combination indexes (CIs) were generated with the calcusyn software for each set of combinations using the results obtained from each drug alone and in combination within the same experiment. CI < 1 indicates synergism, CI values = 1 indicated an additive effect, whereas values >1 indicates antagonism.

**The effect of dovitinib on tumor progression in vivo**

Ten SCID/bg mice were injected with 3 × 10⁶ BCWM-1 cells each at day 1 of the experiment. Mice were divided into 2 groups of 5 animals each. One group received a treatment 50 mg/kg of dovitinib once a day orally, whereas the second group received vehicle treatment once a day orally. The experiment was stopped after 8 days to determine the pharmacodynamic effect of dovitinib in vivo. The BM of these mice was extracted from both femurs, red blood cells were lysed, and the mononuclear cells were used for flow cytometry analysis. Cells were resuspended in blocking solution (2% BSA in PBS) for 30 minutes on ice; cells were then incubated with mouse-anti-human-CD19 antibody conjugated to PerCP-Cy5.5 (5 mg/mL). For apoptosis and FGFR3 experiments, cells were washed twice with blocking solution, fixed, and permeabilized using Cytofix/Cytoperm (BD Bioscience) according to manufacturer’s protocol, and incubated with mouse-anti-human-cleaved caspase-3 antibody conjugated to Alexa-Fluor-488 (5 mg/mL), and with unconjugated rabbit-anti-human phospho-FGFR3 antibody (5 mg/mL), for 1 hour on ice. Cells were then washed twice and incubated with rat-anti-rabbit secondary antibody conjugated to Alexa-Fluor-555, and analyzed using B-D Aria II cell sorter.

**Statistical analysis**

Results were reported as the mean ± SD for experiments done in 3 replicates samples and were compared by the Student’s t test. Results were considered significantly different for P values less than 0.05.

**Results**

**Expression of FGFR3 in WM cells**

First we tested the expression of FGFR3 on CD19+ cells from WM patients and normal subjects. We found that FGFR3 was overexpressed in WM patients compared to normal subjects (Fig. 1A). Moreover, we confirmed these results using flow cytometry analysis in an additional 6 bone marrow tumor samples of patients and 2 normal subjects, and in the cell lines BCWM-1 and MEC-1. The WM patient samples and the cell lines showed high expression of FGFR3, whereas the samples from normal subjects showed low expression of FGFR3 (Fig. 1B). Neither the patient samples nor the cell lines used had a t(4:14), data not shown. We then tested the activation of the receptor with FGF3, and found that the receptor was activated after 30 minutes as shown by phosphorylation of the receptor as well as activation of downstream pathways including...
pSTAT3, pRAF, and pERK1/2 proteins (Fig. 1C). Activation of pFGFR3 occurred early at 5 minutes, whereas maximum activation of the downstream signaling proteins occurred at 30 minutes.

The effect of dovitinib on survival and apoptosis of WM cells

We tested the effect of increasing concentrations of dovitinib on the survival of CD19+ cells isolated from WM patients (Fig. 2A), WM cell lines (Fig. 2B), and mononuclear cells from healthy donors (Fig. 2C). We found that dovitinib induced cell toxicity in both primary WM patients and cells lines with IC50 around 1 μmol/L, however, in mononuclear cells from healthy donors dovitinib had minimal effect up to 2.5 μmol/L.

Moreover, we tested the effect of increasing concentrations of dovitinib on the apoptosis in WM cells. We found that dovitinib induced apoptosis of WM cells after in a dose–response manner (Fig. 2D), in which we start to see the apoptosis started at 0.25 μmol/L (20%) and was highest at 2.5 μmol/L (40%). We further tested the cellular mechanism of apoptosis induction and we found that it induced PAPR starting at 0.25 μmol/L, and cleavage of caspase-3 and caspase-9 at 0.5 μmol/L, indicating both extrinsic and intrinsic apoptosis pathway activation in both BCWM-1 and MEC-1 cells (Fig. 2E).

In addition, we tested the effect of dovitinib on the cell cycle of WM cells, and found that Starvation of the cells induced G1 arrest (66%), and when we added serum the cells started to cycle and the only 46% of the cells were in G1. The use of dovitinib induced G1 arrest in the cells and at concentrations of 1 μmol/L and higher the cells were arrested to values similar to starvation conditions (Fig. 2F). This was also correlated with the downregulation of proteins involved in the transition from G1 to S such as cyclin-D3, CDK2, and pRb, as well as upregulation of cell cycle inhibitory proteins such as P27. These effects were seen at 0.5 μmol/L but more profoundly at 1 μmol/L (Fig. 2G).

The effect of dovitinib on FGFR3-induced proliferation of WM cells

Activation of FGFR3 induced 30% increase in BCWM-1 and MEC-1 cell proliferation shown as DNA synthesis,
whereas 1 and 2.5 μmol/L of dovitinib inhibited the proliferative effect induced by FGF3 in a dose–response manner (Fig. 3A). These findings were in accord with the cell signaling data showing that dovitinib inhibited the activation of FGFR3 and its downstream target STAT3 in a dose–response manner starting from 0.25 μmol/L, as well as other downstream targets including RAF, and ERK starting at 0.5 μmol/L (Fig. 3B). In addition, dovitinib inhibited the cell cycle effect induced by activation of FGFR3; in which FGF3 rescued serum-starved cells from G1 arrest (G1 = 65%) and induced cell cycle to S-phase (G1 = 47%). Dovitinib reversed the effect FGF3

Figure 2. Dovitinib inhibits survival and cells cycle, and increases apoptosis of WM cells. We assessed the effect of dovitinib on survival of WM cells using MTT assay. Dovitinib inhibits survival of primary CD19+ cells from WM patients (A) and cell lines (B) with and IC50 of 1 μmol/L, while it did not inhibit the survival of mononuclear cells isolated from normal subjects (C). Moreover, increased concentrations of dovitinib induced apoptosis in WM cells in a dose–response manner, detected using APO-2.7 in flow cytometry (D). These results were confirmed by immunoblotting showing that dovitinib induced PARP, Caspase-3, and Caspase-9 cleavage in WM cells in dose–response manner (E). In addition, Dovotinib induced cell cycle arrest in G1phase detected by flow cytometry. (F) These results were confirmed by immunoblotting in which dovitinib inhibited cyclin-D3, CDK2, and pRb, and increased the cell cycle inhibitor P27 (G).
and induced G1-cell cycle arrest similar to the values achieved by serum-starvation at concentrations higher than 0.5 μmol/L. In addition, a sub-G1 population was detected indicating apoptosis of WM in a dose–response manner with dovitinib 1 μmol/L (Fig. 3C). These results were confirmed by the increased activation of cyclin-D3, pRB, and CDK2; and reduced the activity of the cell cycle inhibitor P27 (Fig. 3D). These effects were seen at 0.5 μmol/L but more profoundly at 1 μmol/L after 24-hour treatment.

The effect of dovitinib on adhesion of WM cells to BM components

We tested the effect of dovitinib on adhesion of BCWM-1 and MEC-1 to several components of the BM including fibronectin, representing the extracellular matrix (Fig. 4A), BMSCs (Fig. 4B), and endothelial (HUVEC) cells (Fig. 4C). We found that dovitinib inhibited the adhesion of WM cells to all the components of the BM in a dose–response manner, with 1 μmol/L of dovitinib inhibiting 40%, 50%, and 80% adhesion of BCWM-1 cells to fibronectin, BMSCs, and HUVEC, respectively, at 4 hours of treatment. These concentrations and durations were not sufficient to induce cell death in the BCWM-1 cells (data not shown). Moreover, these results were confirmed by showing the effect of dovitinib on adhesion-related kinases (SRC and FAK) when activated with the different components of the BM, and we found that it inhibited the activation of SRC and FAK induced by fibronectin (Fig. 4D), BMSCs (Fig. 4E), and endothelial cells (Fig. 4F), in a dose–response manner, whereas at 1 μmol/L the FGF3 effect was completely reversed.

The effect of dovitinib on the BMSC proliferative effect on WM cells

Figure 5A shows that BMSCs induce WM cell proliferation compared to WM cells alone, however dovitinib inhibited the proliferation of WM cells with and without stromal cells, indicating that it overcomes the resistance induced by stroma. These results were confirmed by showing that coculture of WM cells with stroma-induced activation of proliferative cell signaling pathways including phosphorylation of the FGFR3, STAT3, AKT, pathway, MAPK pathway as well as cell-adhesion–related proteins.
including Src and FAK (Fig. 5B). These effects were reversed by dovitinib in a dose–response manner, whereas at 1 mmol/L we found complete inhibition of FGFR3. In addition, dovitinib induced cell apoptosis of WM cells even in the presence of stromal cells in a dose–response manner as detected by APO-2.7 (Fig. 5C) and the increase in caspase-3 cleavage (Fig. 5D). At 1 mmol/L for 24 hours, dovitinib induced apoptosis in cells 0%, as well as caspase-3 cleavage (25% of MW cells). To further confirm that the previous effects were due to inhibition of FGFR3, a blocking antibody to inhibit FGFR3 on the WM cells was used. The effect of this inhibitory antibody on cell signaling in WM cells was tested, and showed that it inhibited proliferative signaling in WM cells that is induced by coculture with stromal cells, including reduction of pFGFR3, pSTAT3, pAKT, pRAF, and pERK (Fig. 5E). Moreover, inhibition of FGFR3 using a blocking antibody decreased the adhesion of WM cells to stromal cells (Fig. 5F).

**The effect of dovitinib in combination with other therapeutic agents**

We next examined the effect of increasing doses of dovitinib on the proliferation of WM cells when combined with other therapeutic agents including bortezomib, melphalan, and dexamethasone. Dovitinib led to inhibition of proliferation of WM cells alone or when combined with other therapeutic agents (Fig. 6A). Although dovitinib 0, 0.5, and 1 μmol/L with no other treatment induced 100%, 57%, and 29% proliferation of WM cells, the combination with bortezomib 5 nmol/L induced 76%, 47%, and 10%, the combination with melphalan 10 μmol/L induced 46%, 27%, and 14% and the combination with dexamethasone 25 nmol/L induced 68%, 34%, and 15% proliferation of WM cells, respectively. The combinations with bortezomib and dexamethasone were additive (CI close 1), the combination with melphalan was mildly synergistic (CI = 0.72 and 0.86 for 0.5 and 1.0 μmol/L of dovitinib, respectively; Fig. 6B). Similar results were obtained when testing the effect of the combination on apoptosis of WM. Dovitinib increased apoptosis detected by Apo-2.7 (Fig. 6C) and increased caspase-3 cleavage (Fig. 6D) in WM cell when treated in combination with bortezomib, melphalan, and dexamethasone compared to the effect of these drugs alone.

**The effect of dovitinib on tumor progression in vivo**

Tumor progression was detected as the percent of human CD19+ cell in the bone marrow. Figure 7A shows that treatment with dovitinib reduced tumor progression in the mice, in which the CD19+ cell in the BM of the dovitinib-treated group was 8.5% compared to 24.9% in the vehicle-treated group. To analyze whether dovitinib induces apoptosis in vivo, the cleavage of caspase-3 in CD19+ cells was detected. Figure 7B shows that dovitinib increased induction of apoptosis in CD19+ cells in the BM from 10.0% in the vehicle-treated group to 58.7% in combination with bortezomib, melphalan, and dexamethasone compared to the effect of these drugs alone.
the dovitinib-treated group. Moreover, the phosphorylation of FGFR3, as a target of dovitinib, was detected as a mean-fluorescence-intensity (MFI) of phospho-FGFR3 in CD19+ cells in the BM. The values were normalized to the MFI in the vehicle group. Figure 7C shows that the phosphorylation of FGFR3 receptor decreased to 60% in the dovitinib-treated group compared to the vehicle-treated group.

Discussion

WM is a lymphoplasmacytic lymphoma characterized by widespread involvement of the bone marrow. Despite different options of therapy, WM is still incurable (18). FGFR3 was identified as a therapeutic target in several types of cancer including hematologic malignancies. In this study, we investigated the FGFR3 as a therapeutic target in WM and to test the therapeutic potential of its inhibition by dovitinib.

First we tested the expression of FGFR3 in WM cells. We found that it was overexpressed compared to normal CD19+ cells. Unlike multiple myeloma, WM neoplastic cells lack immunoglobulin heavy chain locus translocations (4, 14, 19). These findings are in agreement with previous reports showing that overexpression of wild-type FGFR3 in B lymphoid cells was oncogenic and cooperated with MYC to accelerate development of B-cell lineage neoplasms (20). FGFR3 was overexpressed in acute lymphoblastic leukemia and chronic myelogenous leukemia patients, whereas the expression of FGFR3 was
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not related with chromosome abnormality, suggesting that FGFR3 gene may be partially involved in tumorigenesis (21). The activation of FGFR3 in WM cells induced cell proliferation signaling pathways, which it a potential therapeutic target for inhibition of WM cells progression. Inhibition of FGFR3 with dovitinib decreased WM cell survival, increased apoptosis, and induced cell cycle arrest.

Figure 6. The effect of the combination of dovitinib with other drugs. A, dovitinib inhibited the proliferation of WM cells alone, and its combination with bortezomib, melphalan, and dexamethasone induced more inhibition of the proliferation. B, although the combination with bortezomib and dexamethasone had an additive effect (CI values close to 1), the combination with melphalan had a modest synergistic effect (CI = 0.7 and 0.8). Similarly, dovitinib induced apoptosis in WM cell whereas the combination with bortezomib, melphalan, and dexamethasone increase the apoptotic effect of dovitinib as shown in expression of APO-2.7 (C) and cleavage of Caspase-3 (D).

Figure 7. The effect of dovitinib on tumor progression in vivo. A, WM tumor progression in mice was detected as % of CD19+ cells in the BM taken from femurs of mice treated with dovitinib 50 mg/kg or vehicle and detected by flow cytometry. Dovitinib reduced the involvement of WM in the mouse BM from 24.9% in the vehicle-treated group to 8.5% of CD19+ cells. B, the number of apoptotic cells was measured using activation of caspase-3 in CD19+ cells in the mice treated with dovitinib versus control. The number of apoptotic cells in treatment group have 58.7% compared to the vehicle-treated group 10%. C, the effect of dovitinib on the activation of its target FGFR3 in CD19+ cells in the BM was analyzed using the level of phosphorylation of the receptor by detecting the mean-fluorescence-intensity (MFI) of pFGFR3 in CD19+ cells by flow cytometry and normalized to the average of MFI of the vehicle-treated group. Dovitinib reduced the activation (phosphorylation) of FGFR3 to 60% in the dovitinib-treated group compared to the vehicle-treated group.
WM is characterized by widespread involvement of the bone marrow in all patients, indicating homing and adhesion of the malignant cells to specific niches in the bone marrow, which provides a protective environment for the survival and proliferation of these cells (11, 22). Therefore, we tested the effect of FGFR3 and its inhibition on the interaction of WM cells with different BM components, and found that inhibition of FGFR3 reduced the adhesion of WM to extracellular matrix components (such as fibronectin), endothelial cells and BMSCs. These results were also confirmed by the inhibition of cell-adhesion–related cell signaling. Consequently, we found that inhibition of FGFR3 reversed the BMSCs-induced proliferation and restored the reduction of apoptosis in WM cells. These results are in agreement with previous studies showing that inhibition of the direct interaction of WM cells with BMSCs by inhibition of SRC kinase reversed the protective effect of the BMSCs in WM (18). Moreover, inhibition of FGFR3 by dovitinib had an additive effect with other drugs, including bortezomib, melphalan, and dexamethasone, in increasing the apoptosis and inhibiting the proliferation of WM cells in a dose–response manner. Finally, dovitinib inhibited FGFR3, increased apoptosis, and reduced proliferation of WM tumors in vivo.

In summary, we report that FGFR3 is a novel therapeutic target in WM, and that its inhibition with dovitinib decreased survival, induced apoptosis, overcame stroma-induced proliferation, and had additive effect with other therapeutic agents. These data provides a basis for future clinical trial using the FGFR3 inhibitor dovitinib for the treatment of patients with WM.

Disclosure of Potential Conflicts of Interest

I. M. Ghobrial is on the advisory boards of Millennium, Celgene, Novartis, and Onyx and receives research funding from Novartis, BMS, NovoXon, and Millennium. The other authors disclosed no conflicts of interest.

Author Contributions

A. K. Azab performed research, designed research, analyzed data and wrote the article. F. Azab performed research, designed research, and analyzed data. P. Miao performed research and analyzed data. P. Quang, A. M. Roccaro, A. Sacco, H. T. Ngo, Y. Liu, Y. Zhang, and B. L. Morgan analyzed data. I. M. Ghobrial designed the research and wrote the paper.

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