

# Reactivation of p53 by MDM2 Inhibitor MI-77301 for the Treatment of Endocrine-Resistant Breast Cancer

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## Abstract

Endocrine therapy has been highly effective for the treatment of estrogen receptor–positive breast cancer, but endocrine resistance develops in a significant proportion of patients. In an effort to develop novel therapeutic strategies for the treatment of endocrine-resistant breast cancer, we have evaluated a potent and specific MDM2–p53 interaction inhibitor, MI-77301, which has been advanced into clinical development, for its therapeutic potential and mechanism of action *in vitro* and *in vivo* in WHIM9 and WHIM18 patient-derived xenograft (PDX) models. Both WHIM9 and WHIM18 PDX models exhibit estradiol-independent tumor growth and are resistant to fulvestrant, a highly

effective and selective estrogen receptor degrader (SERD). MI-77301 activates wild-type p53 in WHIM9 and WHIM18 cells *in vitro* and in xenograft tumor tissues *in vivo*, and it effectively induces upregulation of p21 and cell-cycle arrest *in vitro* in both models. Although fulvestrant fails to inhibit tumor growth in either of the xenograft models, MI-77301 is highly effective in inhibition of tumor growth at a well-tolerated dose schedule. This study provides a preclinical rationale for evaluation of MI-77301 or other MDM2 inhibitors as a new therapeutic strategy for the treatment of endocrine-resistant breast cancer retaining wild-type p53. *Mol Cancer Ther*; 15(12); 2887–93. ©2016 AACR.

## Introduction

Selective estrogen receptor modulators (SERM), selective estrogen receptor degraders (SERD), and aromatase inhibitors (AI) are part of endocrine therapies for the treatment of estrogen receptor (ER)–positive breast cancer. Although these therapies have proven to be highly effective, their usefulness is limited by the development of *de novo* and acquired resistance (1, 2). A number of resistance mechanisms have been identified, including deregulation of various components of the ER pathway itself, alterations in cell-cycle and cell survival signaling pathways, and activation of escape pathways that can provide tumors with alternative proliferation and survival stimuli (1, 2). For example, increased expression or signaling

of growth factor receptor pathways, especially the EGFR–HER2 pathway, has been associated with both experimental and clinical resistance to endocrine therapy (3–5) and has led to the development of clinical treatment strategies combining an endocrine therapy with a targeted agent that blocks the EGFR/HER2 pathway (6, 7). In addition to dysregulation in growth factor receptor pathways, alterations in the cyclin-CDK-RB (cyclin-cyclin dependent kinase-retinoblastoma protein) pathway, which lead to loss of control of the cell cycle, are common in human breast cancer (8–11). A specific CDK4/CDK6 inhibitor, PD 0332991 (palbociclib), has been observed in preclinical studies to be particularly effective against ER-positive breast cancer cell lines (12), including cell lines resistant to endocrine therapy, and shows synergy with tamoxifen (12). Recent clinical data have shown that the combination of palbociclib with letrozole, an AI, has greatly improved progression-free survival in ER-positive metastatic breast cancer over letrozole alone (13). This was the basis for an accelerated FDA approval of the palbociclib–letrozole combination for the treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer as an initial endocrine-based therapy for metastatic disease (14).

Despite these great advances in development of new therapeutic strategies to overcome endocrine resistance, there is still a need to develop new approaches for the treatment of ER-positive, metastatic breast cancer, particularly endocrine-resistant breast cancer. Although the median disease-free survival time with the combination treatment of palbociclib and letrozole in ER-positive metastatic breast cancer is greatly improved over treatment with letrozole alone, it remains only 20 months (13).

Recently, a panel of patient-derived xenograft (PDX) models, referred as "Washington University Human in Mouse (WHIM)"

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lines, were developed from breast cancer patients with poor-prognosis, treatment-resistant disease (15). These WHIM lines were characterized by whole-genome sequencing and were compared with originating tumors from patients, which showed that structural and copy-number aberrations were retained with high fidelity in these models (15). These WHIM lines therefore provide a set of excellent models with which to evaluate novel therapeutics for the treatment of breast cancer patients with poor-prognosis, treatment-resistant disease.

Interestingly, a much higher percentage of these WHIM lines contains a wild-type p53 status than previously established breast cancer cell lines. Although only the MCF-7 and ZR-75-1 cell lines retain a wild-type p53 status among a large number of human breast cancer cell lines established in the past, approximately one third (7/22) of the WHIM lines contains wild-type p53 (15). This suggests the possibility that reactivation of wild-type p53 could be an effective therapeutic strategy for the treatment of a significant proportion of poor-prognosis, treatment-resistant breast cancer patients. To test this possibility, we evaluated the therapeutic potential and mechanism of action of MI-77301 (also known as SAR405838; ref. 16), a potent and specific MDM2 inhibitor currently in clinical development, using two WHIM lines, WHIM9 and WHIM18, which contain wild-type p53. MI-77301 binds to MDM2 with a high affinity ( $K_i = 0.88$  nmol/L) and blocks both the interaction of MDM2 with p53 and the MDM2-mediated p53 degradation, leading to activation of wild-type p53 in cells (16). WHIM9 has a highly overexpressed, wild-type ESR1 and WHIM18 contains an ESR1-YAP1 fusion gene (15). Both these WHIM lines show estradiol-independent growth in mice and are unresponsive to treatment with fulvestrant, a highly potent SERD that has been used as a second-line treatment for hormone receptor-positive metastatic breast cancer.

Our data show that MI-77301 effectively activates p53 *in vitro* and *in vivo* and completely inhibits tumor growth in both models without any signs of toxicity. This suggests that MDM2 inhibitors should be clinically evaluated as a new therapeutic strategy for the treatment of endocrine-resistant breast cancer containing wild-type p53.

## Materials and Methods

### Chemical synthesis of MI-77301

MI-77301 (SAR405838) was synthesized using a procedure similar to that used for MI-888 (17) and its purity, assessed by HPLC is >95%.

### WHIM models, cell growth, and cell cycle

WHIM9 and WHIM18 models were developed at the Washington University School of Medicine, St. Louis, MO. Tumors were passaged in SCID mice for pharmacodynamics and efficacy experiments.

For *in vitro* experiments, tumors were harvested from mice bearing xenograft tumors and cultured in nutrient-rich media for 3 to 5 passages.

Cell growth inhibition activity was determined in a water-soluble tetrazolium (WST)-based assay. Cell-cycle analysis was performed by flow cytometric analysis of DNA content after PI staining, with cell clumps, doublets and subdiploid cells excluded from the analysis.

### Western blotting

For western blot analysis in cells, the following primary antibodies were used: PUMA $\alpha/\beta$  (sc-374223) and MDM2 (SMP-14, sc-965) from Santa Cruz Biotechnology; p53 (DO-1, OP43) and MDM2 (OP46) from Millipore; p21 (DCS60) and Caspase-3 (8G10) and PARP (46D11) from the Cell Signaling Technology. For tumor tissues, the following antibodies were used: p53 (OP43; Millipore), MDM2 (sc-965; Santa Cruz Biotechnology), p21 (556431; BD Biosciences), PARP (9542; Cell Signaling Technology), caspase-3 (AAP-113; Stressgen Bioreagents) and HRP-conjugated GAPDH (sc-5778; Santa Cruz Biotechnology).

### Immunohistochemistry staining

For immunohistochemistry (IHC), the following antibodies were used: p53 (OP43; EMD Millipore) and cleaved caspase-3 (9664; Cell Signaling Technology). p53 was detected by the VECTOR Red Alkaline Phosphatase Substrate Kit (AK-5100, Vector) and cleaved caspase-3 was detected with a diaminobenzidine (DAB) tetrahydrochloride substrate using a DAB/buffer system (Sigma).

### *In vivo* pharmacodynamic and efficacy experiments

For pharmacodynamics studies, when tumors reached a mean of 400 mm<sup>3</sup>, 3 mice per group were treated with vehicle control or a single dose of the drug *via* oral gavage, sacrificed at the time point indicated, and tumor tissue was harvested for analyses.

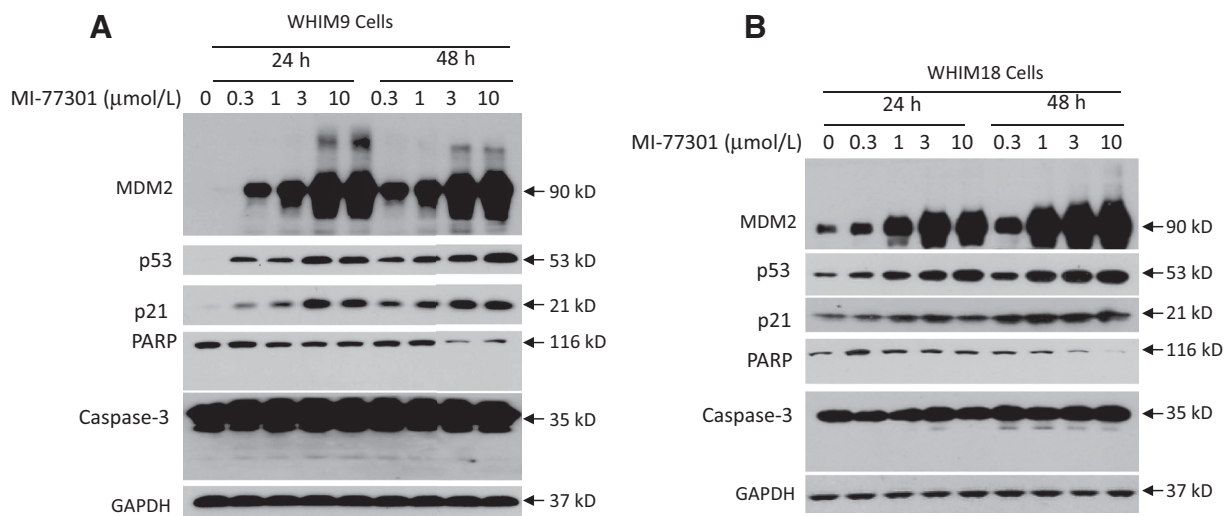
For *in vivo* efficacy experiments, when tumors reached 140 to 280 mm<sup>3</sup>, mice were randomized into groups of 8. MI-77301, or vehicle control (10% PEG400: 3% Cremophor: 87% PBS, or 2% TPGS:98% PEG200) was given orally once daily for the dose and duration indicated. Fulvestrant (FASLODEX injection 250 mg/5 mL) was injected subcutaneously at 0.1 mL volume (250 mg/kg) once per week for the duration indicated. Tumor sizes and animal weights were measured 2 to 3 times per week. Tumor volume (mm<sup>3</sup>) = (length  $\times$  width<sup>2</sup>)/2. Statistical analyses were done by two-way ANOVA and unpaired two-tailed *t* test, using Prism (version 6.0, GraphPad). All animal experiments were performed under the guidelines of the University of Michigan Committee for Use and Care of Animals.

## Results

### MI-77301 effectively activates p53 in WHIM9 and WHIM18 models *in vitro*

The co-crystal structure of MI-77301 complexed with human MDM2 protein shows that MI-77301 binds to the p53-binding pocket in the MDM2 protein, resulting in blocking the MDM2-p53 interaction. In turn, this blocking leads to inhibition of p53 ubiquitination by MDM2, causing accumulation and activation of wild-type p53 (16).

Although both WHIM9 and WHIM18 lines were found by sequencing to contain wild-type p53 (15), it was not known whether p53 is functional and can be activated by MDM2 inhibitors. We therefore evaluated the ability of MI-77301 to activate p53 in both WHIM lines *in vitro*. We harvested WHIM9 and WHIM18 xenograft tumors from mice, cultured the cells *in vitro* for 3 to 5 passages and then treated WHIM9 and WHIM18 cells with MI-77301, followed by western blotting to probe p53, MDM2 and p21 proteins. We also assayed PARP and caspase-3, two biochemical markers of apoptosis in both models. The results are provided in Fig. 1.



**Figure 1.**

MI-77301 activates p53 in both WHIM9 (A) and WHIM18 (B) cells and induces upregulation of p53, p21, and MDM2 proteins. WHIM9 or WHIM18 cells were obtained by culturing respective tumor tissues for 3–5 passages and treated with MI-77301 for 24 or 48 hours. Western blotting was performed to probe the levels of p53, p21, MDM2, PARP and caspase-3 using specific antibodies. GAPDH was used as a loading control.

In both cell lines, MI-77301 induces an increase of p53, MDM2, and p21 proteins in a dose-dependent manner. In WHIM9 cells, MI-77301 clearly increases the levels of p53 and MDM2 proteins at concentrations as low as 300 nmol/L and the level of p21 protein at a concentration of 1  $\mu$ mol/L at both 24 and 48 hours time points. In WHIM18 cells, MI-77301 at 300 nmol/L increases the level of p53 protein and at 1  $\mu$ mol/L increases the levels of p21 and MDM2 proteins at both 24 and 48 hours time points. MI-77301 also markedly reduces the level of full-length PARP at 48 hours time-point. However, MI-77301 has no effect on the level of cleaved PARP, and also has minimal effect in induction of caspase-3 activation in both cells. These data suggest that MI-77301 induces minimal or modest apoptosis in these two WHIM lines *in vitro*.

Thus, MI-77301 effectively activates the p53 pathway in a dose-dependent manner in both these WHIM lines.

#### MI-77301 inhibits cell growth and induces cell-cycle arrest in both WHIM lines

Because MI-77301 effectively activates p53 *in vitro* in both models, we next tested its ability to inhibit cell growth by a WST assay. The results are shown in Fig. 2A. MI-77301 effectively inhibits cell growth in both models and has  $IC_{50}$  values of 4.8 and 2.7  $\mu$ mol/L, in the WHIM9 and WHIM18 lines, respectively.

Because MI-77301 robustly increases the level of p21, a key cell-cycle regulator, we determined its effect on cell-cycle progression by flow cytometry. MI-77301 was found to effectively induce  $G_2$  arrest in both cell lines (Fig. 2B).

#### MI-77301 activates p53 and reduces proliferation in xenograft tumor tissues

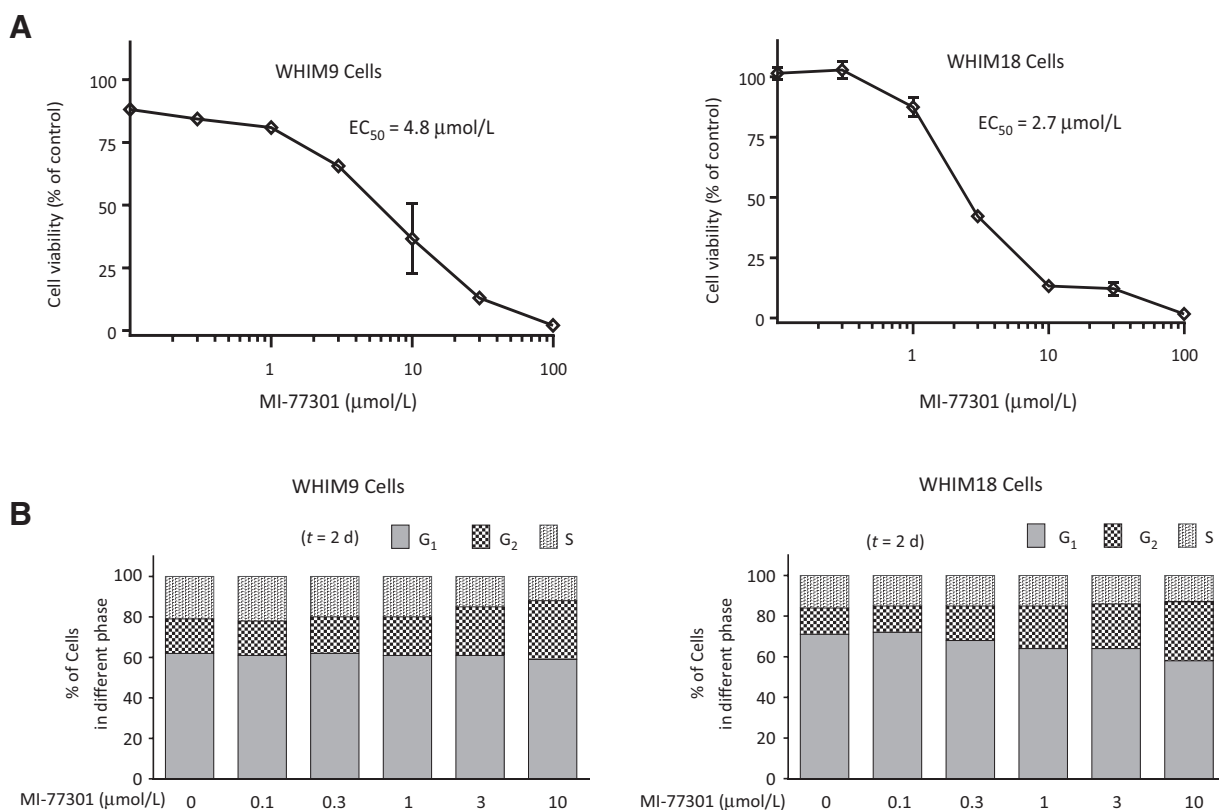
We next investigated the ability of MI-77301 to activate p53 in both WHIM9 and WHIM18 xenograft tumor tissues in mice. Because MI-77301 at 100 mg/kg has been shown to be highly effective in inhibition of tumor growth in multiple xenograft

models of human cancer cell lines (16), the same dose was used in our *in vivo* experiments. Mice bearing either WHIM9 or WHIM18 tumors were treated with a single, oral 100 mg/kg dose of MI-77301 and mice were sacrificed at different time points after the treatment to harvest tumors for Western blotting analysis.

Western blotting analysis showed that a single, oral dose of MI-77301 at 100 mg/kg induces clear accumulation of p53 protein at the 3 hours time point, with the effect peaking at the 6 hours time point and persisting for at least 24 hours (Fig. 3A). MI-77301 induces robust increase of both MDM2 and p21 proteins in the WHIM9 and WHIM18 xenograft tumor tissues. In the WHIM9 tumor, p21 increase is evident at the 3 hours time point and peaks at 6 hours but persists for 24 to 48 hours. In the WHIM18 tumor, p21 increase is minimal at 3 hours, but becomes very robust at 6 hours and continues to increase at 24 hours. For the MDM2 protein, the kinetics is very similar to that for p21 protein, with the exception that the increase in MDM2 protein is robust at 3 hours in both WHIM9 and WHIM18 tumors. Western blotting analysis further showed that MI-77301 markedly increases the protein level of PUMA, another p53 target gene product and also a potent proapoptotic Bcl-2 family protein in WHIM18 tumor tissues (Supplementary Fig. S1). MI-77301 treatment results in accumulation of cleaved PARP, but only a modest reduction of the level of full-length PARP and a minimal increase of the level of activated form of caspase-3 in the tumor tissues (Fig. 3A; Supplementary Fig. S1). These data suggest that MI-77301 induces modest apoptosis in the WHIM tumor tissues *in vivo*, consistent with our *in vitro* data (Fig. 2).

We further analyzed p53 and MDM2 accumulation by IHC staining in tumor tissues from mice treated with a single, oral, 100 mg/kg dose of MI-77301 (Fig. 3B). Strong p53 and MDM2 staining is observed in both WHIM9 and WHIM18 tumor tissues at 6 h but the staining becomes much weaker at the 24 hours time point. IHC staining of Ki-67, a marker of cellular proliferation, showed that a single dose of MI-77301 strongly suppresses cell

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**Figure 2.**

MI-77301 effectively inhibits cell growth and induces cell-cycle arrest in WHIM9 and WHIM18 cells. **A**, WHIM9 and WHIM18 cells were treated with MI-77301 for 4 days and cell viability was determined by a WST assay. **B**, WHIM9 and WHIM18 cells were treated with MI-77301 for 48 hours. Treated cells were stained by propidium iodide (PI) and cell-cycle analysis was performed by flow cytometry.

proliferation in both WHIM9 and WHIM18 tumor tissue for at least 24 hours.

#### MI-77301 demonstrates strong antitumor activity in both WHIM models

We tested the efficacy of MI-77301 in both WHIM9 and WHIM18 xenograft models in mice. In our previous study, we showed that MI-77301, daily administered at 100 mg/kg for 2 to 4 weeks demonstrated strong antitumor activity in multiple xenograft models of different types of human cancer without any signs of toxicity (16). We therefore tested the efficacy of MI-77301 at 100 mg/kg daily oral dosing for 3 to 4 weeks in both WHIM9 and WHIM18 models. The efficacy data in the two models are summarized in Fig. 4.

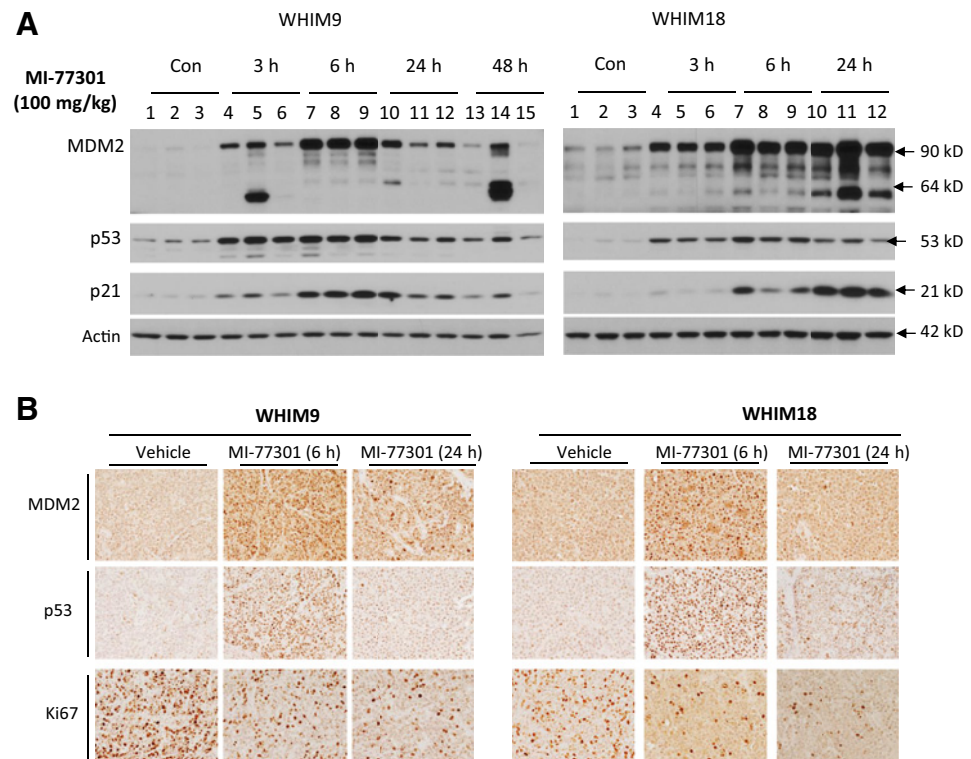
We found that MI-77301 demonstrates strong antitumor activity in both models. MI-77301 induces tumor regression by 48% (from an average tumor volume of 144 at the beginning to 75 mm<sup>2</sup> at the end of the treatment) and 20% (from an average tumor volume of 278 at the beginning to 222 mm<sup>2</sup> at the end of the treatment) at the end of 3-week treatment in the WHIM9 and WHIM18 models, respectively. Importantly, the antitumor effectiveness of MI-77301 is long-lasting in both models. In the WHIM9 model, on day 122 (25 days after the last dosing), the average tumor volume in MI-77301 group (197 ± 196 mm<sup>2</sup>) is still significantly smaller than that in the vehicle group (615 ± 344 mm<sup>2</sup>; *P* < 0.001). In the WHIM18 model, the average tumor

volume is 1582 ± 613 mm<sup>2</sup> in the vehicle group on day 88, whereas the average tumor volume reaches 1252 ± 110 mm<sup>2</sup> in the MI-77301 group on day 115, delaying the tumor growth by at least 27 days.

#### Combination of MI-77301 with fulvestrant in both WHIM9 and WHIM18 models

Fulvestrant is a SERD, which was approved by the FDA in 2002 as a new therapy for treatment of hormone receptor-positive metastatic breast cancer. Interestingly, a recent study showed that fulvestrant reduced the level of MDM2 in breast cancer cell lines through increased protein turnover (18). Because MI-77301 increases the level of MDM2 in these WHIM models *in vitro* and *in vivo*, we reasoned that the combination of MI-77301 and fulvestrant may further enhance the activation of p53, leading to a stronger antitumor activity but potentially more toxicity to animals. Therefore, we evaluated the combination of these two drugs in both WHIM models *in vivo* for efficacy and toxicity.

We observed that tumors in both models are resistant to fulvestrant alone, consistent with the clinical characteristic developed by the patients (15). Interestingly, the two models respond differentially to the combinational treatment. In the WHIM9 model, the combination of MI-77301 with fulvestrant has same antitumor activity as compared to MI-77301 alone. In contrast, in the WHIM18 model, although the combination does not have a better antitumor activity during the treatment period, it can

**Figure 3.**

MI-77301 induces upregulation of p53, MDM2, and p21 in WHIM9 and WHIM18 tumor tissues and suppresses cell proliferation in tumors. **A**, Mice bearing WHIM9 or WHIM18 xenograft tumors were treated with a single, oral dose of MI-77301 at 100 mg/kg. Tumors were harvested at 0, 3, 6, and 24 hours time points. Western blotting was performed to analyze the levels of p53, MDM2, and p21 proteins in tumor tissues. **B**, Immunohistochemistry staining was performed on WHIM9 and WHIM18 tumor tissue to determine expression of p53, MDM2, and Ki-67.

further delay the tumor growth after the treatment is stopped. For instance, on day 118 (30 days after the last dosing), the average tumor volume is  $1,632 \pm 709 \text{ mm}^2$  in MI-77301 alone group, whereas it is  $806 \pm 431 \text{ mm}^2$  in the combination group ( $P = 0.0136$ ).

Importantly, MI-77301 alone or combination with fulvestrant is well tolerated in both efficacy experiments and shows no signs of toxicity in animals, including no weight loss in animals (Supplementary Fig. S2). The lack of toxicity at the tested dose-schedule is also consistent with the observations for MI-77301 in our previous efficacy experiments in mice (16).

## Discussion

Reactivation of p53 by targeting the MDM2-p53 protein-protein interaction has been pursued as a novel cancer therapeutic strategy (19). Through intense research in the last decade, a number of highly potent, selective and orally active, small-molecule inhibitors are now in clinical development for cancer treatment (20, 21).

Although many human breast cancer cell lines have been developed in the past, essentially all the well characterized and widely used breast cancer cell lines, with the possible exception of the MCF7 and ZR-75-1 cell lines, harbor a mutated p53. The MCF7 cell line also has a high expression of MDMX (also called MDM4), a homolog of MDM2 protein, and we have found that selective MDM2 inhibitors are not very effective in the MCF-7 cell line (data not shown). The lack of proper model systems has thus limited proper evaluation of MDM2 inhibitors for their therapeutic potential in human breast cancer.

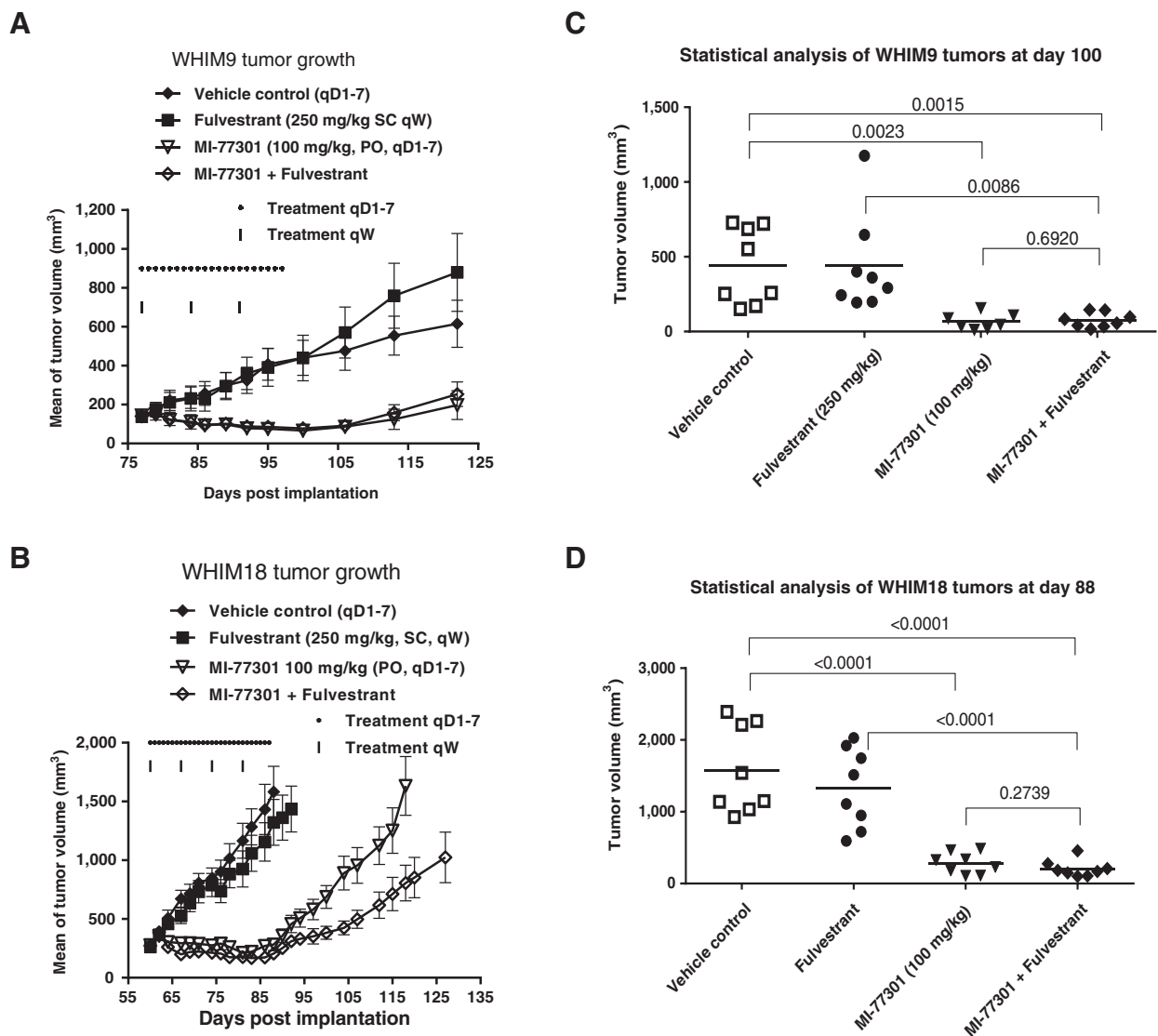
Recently, a large number of PDX breast cancer models, termed the Washington University Human in Mouse (WHIM) lines, have

been successfully developed from fresh tumor tissues obtained from poor-prognosis, treatment-resistant, breast cancer patients (15). These models have been extensively characterized by whole-genome sequencing and compared with the originating tumors from patients (15). It was found that these PDX tumors retain the structural and copy-number aberrations in the originating breast tumors with high fidelity, and are thus excellent models with which to evaluate novel therapeutic agents. Among the 22 PDX models characterized, 7 models contain wild-type p53. Of these, WHIM9 and WHIM18 exhibit estradiol-independent tumor growth in mice and are resistant to fulvestrant (15). Hence, WHIM9 and WHIM18 are excellent models for testing the therapeutic potential and mechanism of action of MDM2 inhibitors as a new therapeutic strategy.

Although both WHIM9 and WHIM18 tumor cells did not grow well in culture medium, we were able to culture the cells for several passages and tested the *in vitro* activity and mechanism of action of MI-77301. Our *in vitro* data demonstrate that MI-77301 is capable of effectively activating p53 in both models, as evident by the upregulation of p53, MDM2, and p21 proteins, indicating that these two WHIM models contain a functional p53. Our biochemical analysis and flow-cytometry data also show that MI-77301 is effective in induction of cell-cycle arrest *via* induction of p21. However, MI-77301 only has minimal or modest effect in induction of apoptosis in these WHIM models, as evident by the lack of robust cleavage of PARP and activation of caspase-3. Although we were able to obtain the  $IC_{50}$  values for MI-77301 in the cell growth inhibition assay *in vitro* in these two models, caution should be taken in interpreting the values since these cells do not grow well under the culture conditions.

Consistent with the *in vitro* data, a single, orally administered dose of MI-77301 at 100 mg/kg robustly activates p53 in both

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**Figure 4.**

MI-77301 strongly inhibits tumor growth in WHIM9 and WHIM18 xenograft models. **A** and **B**, Mice bearing the WHIM9 or WHIM18 xenograft tumor (one tumor per mouse) were treated with MI-77301 at 100 mg/kg, daily for 21 days via oral gavage, or fulvestrant subcutaneously at 250 mg/kg weekly for 3 weeks, or their combination. Treatment began when the tumor volume reached an average of 140 mm<sup>3</sup>. Tumor size of each mouse was measured using digital calipers every few days and graphed with prism software. **C** and **D**, Tumor volumes for WHIM9 on day 100 and WHIM18 on day 88 were graphed and compared among different groups by the *t* test with prism software.

WHIM9 and WHIM18 tumors in mice, with the effect persisting for at least 24 h. Based upon Ki-67 staining, MI-77301 is also effective in inhibition of proliferation of tumor cells *in vivo* but has modest effect on apoptosis induction based upon PARP cleavage and caspase-3 activation. Nevertheless, MI-77301 can effectively induce upregulation of PUMA in tumor tissues (Supplementary Information), suggesting a potential role of apoptosis induction in the antitumor activity of MI-77301.

*In vivo* efficacy experiments demonstrate that MI-77301 is highly efficacious in both WHIM models. MI-77301 induces partial tumor regression during the treatment in both models. Moreover, the antitumor activity still persists 3 to 4 weeks after cessation of the treatment. Importantly, treatment by single agent or the combina-

tion causes no obvious toxicity to animals evidenced by no loss of body weight and no behavior changes in tumor-bearing mice.

The WHIM9 line has a wild-type estrogen receptor and a high ER mRNA expression (15). The WHIM9 tumors exhibit estradiol-independent growth in mice and do not respond to fulvestrant treatment in our experiments. In this study, the combination of fulvestrant with MI-77301 fails to enhance the antitumor activity of MI-77301 in mice. In comparison, the WHIM18 contains an ESR1-YAP1 fusion gene, which lacks the ligand-binding domain of ESR1 (15). Previous analyses using MCF7 and T47D cells, which overexpress the ESR1-YAP1 fusion, showed that the fusion protein was not downregulated by fulvestrant in these cell lines and the cells were not responsive to fulvestrant. Consistently,

fulvestrant is not effective in inhibiting tumor growth in the WHIM18 model *in vivo*. Interestingly, although the combination of fulvestrant and MI-77301 is only modestly more effective in inhibition of tumor growth than MI-77301 alone, the combination strongly delays the tumor growth when the treatments were ceased. The mechanisms underlying the differential sensitivity to the combination in the two models remain unclear and need to be further investigated.

Taken together, our present study provides preclinical evidences that MDM2 inhibitors, such as MI-77301, may have a great therapeutic potential for the treatment of endocrine-resistant, human breast cancer retaining wild-type p53 status.

### Disclosure of Potential Conflicts of Interest

S. Wang has ownership interest (including patents) and is a consultant/advisory board member for Ascenta Therapeutics. No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

Conception and design: J. Lu, M.J. Ellis, S. Wang

Development of methodology: S. Li, M.J. Ellis, S. Wang

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Lu, D. McEachern, S. Li, M.J. Ellis  
 Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Lu, D. McEachern, S. Li, M.J. Ellis, S. Wang  
 Writing, review, and/or revision of the manuscript: J. Lu, D. McEachern, M.J. Ellis, S. Wang  
 Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. McEachern  
 Study supervision: S. Wang

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