A Novel Antiandrogen, Compound 30, Suppresses Castration-Resistant and MDV3100-Resistant Prostate Cancer Growth In Vitro and In Vivo

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Abstract
Resistance to antiandrogen drugs, like MDV3100, occurs in patients with castration-resistant prostate cancer (CRPC). Thus, preventing or treating antiandrogen resistance is a major clinical challenge. We identified a novel antiandrogen, Compound 30, and compared its efficacy with MDV3100. We found that Compound 30 inhibits androgen receptor (AR) activity in LNCaP cells, C4-2 cells, as well as MDV3100-resistant cell lines. Compared with MDV3100, Compound 30 treatment induces greater reduction in AR, prostate-specific antigen (PSA), and AR transcriptional activity, and prevents AR nuclear translocation in AR-sensitive LNCaP cells. Compound 30 has antiproliferative effects in LNCaP cells, castrate-resistant C4-2 cells, and those resistant to MDV3100. Compound 30 was equally as effective as MDV3100 in reducing tumor volume and PSA in vivo. More importantly, Compound 30 is effective at inhibiting AR activity in MDV3100-resistant cell lines and significantly prevented tumor growth and PSA increases in mice bearing MDV3100-resistant xenografts. Together, our data show that Compound 30 strongly inhibited AR activity and suppressed castration-resistant LNCaP growth as well as MDV3100-resistant cell growth in vitro and in vivo. These data provide a preclinical proof-of-principle that Compound 30 could be a promising next generation anti-AR agent, especially in the context of antiandrogen-resistant tumors. Mol Cancer Ther; 12(5); 567–76. ©2013 AACR.

Introduction
Prostate cancer is the most common male cancer in North America and second leading cause of cancer-related deaths (1). Androgens drive prostate cancer carcinogenesis and progression, regulating gene and signaling networks that promote cell survival through binding with the androgen receptor (AR), a ligand-responsive transcription factor. Androgen deprivation therapy (ADT), consisting of castration combined with an antiandrogen, blocks the growth-promoting effects of androgens and activates apoptosis in prostate cancer tumor cells (2), prolonging survival for patients with prostate cancer (3). Despite high initial response rates, remissions following ADT are temporary due to the emergence of castration-resistant prostate cancer (CRPC), where AR reactivation occurs and tumors grow in the presence of low levels of androgens. AR activation remains a central mechanism driving CRPC progression (4), involving variable combinations of AR gene amplification, increased AR sensitivity, promiscuous AR-binding mutants, altered expression of coregulators, ligand-independent activation by oncogenic signaling pathways, and increases in androgen biosynthesis (5–8). These mechanisms likely work in concert to drive CRPC; hence, targeting the AR remains a critical component of novel CRPC therapies (9, 10).

ADT prevents AR activation by reducing the levels of androgen production through castration or by targeting the AR directly using AR inhibitors. Limited efficacy of antiandrogens like bicalutamide has led to the development of more potent AR-targeting compounds. MDV3100 is a second-generation AR inhibitor that has significant antitumor effects both in vitro and in vivo (11). A phase I/II trial in patients with metastatic CRPC showed MDV3100 treatment decreased serum prostate-specific antigen (PSA) by 50% and reduced or stabilized metastatic disease (12). Furthermore, the phase III AFFIRM trial (13), testing MDV3100 in postdocetaxel patients with CRPC, showed an overall survival advantage and a 37% reduction in mortality risk (14). Despite these results, MDV3100 treatment does not lead to complete CRPC regression, as progression and death from drug-resistant disease occurs in most patients (14). Therefore, the development of MDV3100-resistant tumors in CRPC represents a
significant challenge in the treatment of advanced prostate cancer. As such, identifying novel compounds with activity in MDV3100-resistant tumors is critical to improve the future of prostate cancer therapy.

In this study, we tested the antancer activity of the novel antiandrogen, Compound 30 (15), in in vitro and in vivo models of prostate cancer. Compound 30 was developed by optimizing AR ligand-binding efficiency of aryl-oxy tetramethylcyclobutyl lead compounds identified in a high-throughput cell-based screen for pure, nonsteroidal, AR antagonists (15). In that study, Compound 30 was shown to more potently inhibit AR activity than bicalutamide and had significant antitumor effects in a mouse model of CRPC (15). Likewise, we found that Compound 30 potently inhibited AR activity in LNCaP and C4-2 cells, and was as efficacious as MDV3100 in preventing tumor progression and PSA increases in a mouse model of CRPC. Importantly, using our newly developed model of MDV3100 resistance, we showed that Compound 30 has antiproliferative and AR-targeting effects in an MDV3100-resistant LNCaP cell line established from MDV3100-resistant tumors. Overall, our results suggest that Compound 30 may be a viable treatment for CRPC that is resistant to AR pathway inhibitors.

Materials and Methods

Cell culture

AR-positive LNCaP and C4-2 cells were provided by Dr. Leland W. K. Chung (Cedar Sinai, Los Angeles, CA) tested and authenticated by whole-genome and whole-transcriptome sequencing on Illumina Genome Analyzer IIx platform in July 2009. They were maintained in RPMI-1640 supplemented with 5% FBS (Invitrogen Life Technologies). MR49F cells from MDV3100-resistant LNCaP and MDV3100-resistant tumors were maintained in RPMI-1640 with 5% FBS and 10 μmol/L MDV3100.

Reagents and antibodies

The synthetic androgen R1881 was from PerkinElmer, Compound 30 was supplied by Pfizer Inc., and customized synthetic MDV3100 was purchased from Shanghai Haoyuan Chemexpress. AR, PSA, and cyclin D were purchased from SantaCruz Biotechnology, PARP (Cell Signaling Technology), vinculin (Sigma Chemical Co.), and Ki67 (Lab Vision Corporation).

Cell growth assay

Cells were treated with various doses of compounds or dimethyl sulfoxide for the indicated amount of time, and growth rates were assessed with a crystal violet assay (described previously; ref. 16). Each assay was done in triplicate.

Reverse transcription and quantitative PCR analysis

Total RNA was extracted from cultured cells after 48 hours of treatment using TRizol reagent (Invitrogen Life Technologies, Inc.). Quantitative real-time PCR (qRT-PCR) was conducted as described previously (17).

Transient transfection and luciferase assay

LNCaP and MR49F cells were cotransfected with 0.6 μg of probasin luciferase reporter and 0.6 μg of control Renilla luciferase plasmids per 10 cm plate using Lipofectin (Invitrogen). Cells were treated with different compounds for 1 hour before treatment with 1 nmol/L of R1881. Luciferase activity was measured as previously reported (18).

Immunoblotting analysis

Total proteins were extracted as previously reported using radioimmunoprecipitation assay buffer (17). A total of 30 μg of total proteins were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Western blot analyses were conducted as previously reported (17).

Immunofluorescence

LNCaP and MR49F cells were cultured in 12-well plates containing coverslips and RPMI +5% charcoal stripped serum (Invitrogen Life Technologies) for 48 hours. Cells were then treated for 2 hours with indicated concentrations of compounds followed by 1 nmol/L of R1881 treatment for 15 minutes. Immunofluorescence was conducted as previously described (18).

Cell-cycle analysis

Cells were incubated in the absence or the presence of different concentrations of MDV3100 or Component 30 and were stained with propidium iodide. Cell-cycle population was analyzed by flow cytometry as previously reported (17).

Assessment of in vivo tumor growth for castration-resistant LNCaP xenografts and MDV3100-resistant xenograft transplantation

A total of 1 × 10⁶ LNCaP cells with 0.1 mL Matrigel (Becton Dickinson Labware) were inoculated in bilateral flanks of 6- to 8-week-old male athymic nude mice (Harlan Sprague Dawley Inc.). Tumor volume, body weight, and serum PSA levels were measured weekly. When serum PSA levels reached more than 50 ng/mL, castration was conducted. When PSA recovered to precastration levels, mice were randomized into 3 treatment groups; vehicle, 10 mg/kg of MDV3100 or 25 mg/kg of Compound 30 and treated daily.

Generation of MDV3100-resistant tumors

MDV3100-resistant LNCaP xenografts were excised and transplanted to castrated mice treated with 10 mg/kg/daily of MDV3100. When tumors reached 2,000 mm³, xenografts were harvested and serially transplanted into castrated male mice receiving 10 mg/kg/daily of MDV3100. Third-generation transplanted tumors were used as MDV3100-resistant xenografts in this study. When PSA levels reached more than 50 ng/mL, mice were randomized into 3 groups; (1) stop MDV3100 dosing and switch to vehicle, (2)
continuance of MDV3100, and (3) stop MDV3100 and switch to Compound 30.

**Generation of MDV3100-resistant MR49F cells**

Third-generation MDV3100-resistant xenograft tumors were harvested into RPMI +10% FBS. Adherent cells were maintained in RPMI +5% FBS + 10 μmol/L MDV3100.

**Immunohistochemistry**

A tissue microarray was constructed using a manual tissue microarrayer (Beecher Instruments Inc.). Immunohistochemical staining was conducted as we previously reported. All comparisons of staining intensities were made at ×200 magnification.

**Statistical analysis**

Data were analyzed statistically using a 1-way ANOVA test, with \( P < 0.05 \) considered to indicate significance. Student \( t \) test (2 sided) was used to evaluate statistically significant differences in all experiments. Results are expressed as mean ± SE with at least 3 biologic replicates. \( P < 0.05 \) was considered significant.

**Results**

**Compound 30 is more potent than MDV3100 in inhibiting AR transcriptional activity**

The antiandrogen, Compound 30, is more potent than the first-generation antiandrogen bicalutamide both *in vitro* and *in vivo* (15); however, it has not been compared with new-generation antiandrogens. Here, we explored the efficacy of Compound 30 (chemical structure is shown in Fig. 1A) compared with the new-generation antiandrogen, MDV3100, on AR pathway activity, proliferation, and apoptosis in the prostate cancer cell line LNCaP. We found that Compound 30 decreased AR transcriptional activity, measured by probasin luciferase activity (Fig. 1B, top left), in a dose-dependent manner and with greater potency compared with MDV3100 [probasin 50% effective concentration (EC50) = 12.5 and 20 μmol/L, respectively; Supplementary Table S1]. Similar data were observed using PSA luciferase (Fig. 1B, bottom left). Decreased AR activity correlated with a decrease of AR at both mRNA (Fig. 1B, top right) and protein (Fig. 1C) levels, and these decreases were exaggerated in comparison with those induced by MDV3100, especially at the 10 μmol/L dose. In accordance with our PSA luciferase data, we observed a dose-dependent decrease in PSA protein expression in LNCaP cells treated with Compound 30 (Fig. 1C), and Compound 30 induced an approximate 70% decrease in PSA mRNA levels at 0.1 μmol/L, compared with an approximate 30% decrease induced by MDV3100 at the same dose (Fig. 1B, right bottom). Finally, we found that like MDV3100, Compound 30 also inhibited AR nuclear translocation, as shown by fluorescence microscopy (Fig. 1D). Interestingly, we observed that although treatment of LNCaP cells with MDV3100 also reduced AR protein expression, AR mRNA levels were dramatically increased by treatment with 1 or 10 μmol/L MDV3100. This may be a compensatory mechanism to upregulate AR protein production after MDV3100 treatment that was not observed with Compound 30.

**Compound 30 potently inhibits cell proliferation and induces apoptosis**

Compound 30 was able to inhibit AR activity more potently than MDV3100 as measured by decreases in PSA and AR protein and mRNA expression. We found that Compound 30 was significantly more effective than MDV3100 in suppressing cell growth (Fig. 2A) in AR-positive LNCaP and its castration-resistant subtype, C4-2 cells. More importantly, Compound 30 was equally as efficacious in suppressing growth in both cell lines (IC50 = 1.86 μmol/L for LNCaP and 2.02 μmol/L for C4-2), whereas MDV3100 had a stronger antiproliferative effect in androgen-sensitive LNCaP cells compared with androgen resistant C4-2 cells (IC50 = 12.52 μmol/L for LNCaP and 34.90 μmol/L for C4-2; Fig. 2A and Supplementary Table S2). Reduction in cellular proliferation induced by MDV3100 and Compound 30 was associated with dose-dependent arrest in the sub-G0 phase of cell cycle as assessed by flow cytometry (Fig. 2B), and these data showed that at the 10 μmol/L dose, Compound 30 had a more substantial effect on cell-cycle arrest than MDV3100. Furthermore, because MDV3100 has been shown to induce PARP cleavage in VCaP cells, we investigated the effects of Cpd30 treatment on PARP cleavage in LNCaP cells compared with MDV3100. Importantly, only Compound 30 was able to induce PARP cleavage in LNCaP cells in a dose-dependent fashion (Fig. 2C).

**Compound 30 elicits anticancer activity in a castration-resistant *in vivo* model**

As Compound 30 showed potent antiproliferative activity *in vitro*, we sought to test its efficacy compared with MDV3100 in an LNCaP CRPC model *in vivo*. LNCaP cells were xenografted into athymic male nude mice and mice were castrated when serum PSA reached approximately 50 ng/mL. Treatment was initiated at the time when serum PSA reached precastration levels, which represents the hallmark of CRPC in the LNCaP model (19). Mice with CRPC tumors were randomized to 3 treatment groups: vehicle (8 mice), 10 mg/kg/day MDV3100 (13 mice), and 25 mg/kg/day Compound 30 (13 mice). As shown in Fig. 3A, both compounds significantly suppressed tumor growth (left) and decreased serum PSA (right) compared with vehicle controls. After 5 weeks of treatment, there were no significant differences between Compound 30 and MDV3100-treated tumor volumes or PSA (change from baseline was +488% in vehicle, +19% in MDV3100, and +7% in Compound 30, and serum PSA change from baseline was +619% in vehicle, −32% in MDV3100, and −27% in Compound 30). Waterfall plots of individual responses in the MDV3100-treatment group showed 5 (38%) animals had reduced tumor volume from baseline (Fig. 3B, left) and 9 (69%) animals had reduced PSA levels from baseline.
In the Compound 30-treated group, 6 (46%) animals had reduced tumor volume from baseline (Fig. 3B, left) and 10 (77%) mice had reduced PSA levels from baseline (Fig. 3B, right). Furthermore, staining for the cellular proliferation marker Ki67 in tumors from MDV3100- and Compound 30-treated mice showed similar reduction in Ki67 expression compared with vehicle control (Fig. 3D). However, because our in vitro data showed that Compound 30 had a greater effect on cell cycle repartition than MDV3100 (Fig. 2C), we further investigated the effects on cell-cycle progression by analyzing the expression of cyclin D protein, which is known as a G1 phase cyclin that regulates the entry of cells into G1, in Compound 30- and MDV3100-treated tumors. Consistent with our in vitro results, we observed lower cyclin D protein expression in 2 of 4 randomly selected Compound 30-treated tumors compared with those from MDV3100-treated mice (Fig. 3C).

Figure 1. Compound 30 inhibits activity and expression of the AR.
A, chemical structure of Compound 30. B, left, 24 hours after transfection, LNCaP cells transfected with probasin (top left) or PSA (bottom left) luciferase and control Renilla plasmids were treated for 24 hours with various concentrations of Compound 30 or MDV3100. Cells were harvested and luciferase activity was determined. Data represent means of at least 3 independent experiments done in triplicate. B, right, LNCaP cells were treated with Compound 30 or MDV3100 for 24 hours and RNA was extracted and qRT-PCR was conducted to evaluate the expression of AR (top right) and PSA (bottom right). C, LNCaP cells were treated with Compound 30 or MDV3100 for 24 hours; AR and PSA were analyzed by Western blot analysis; vinculin was used as a loading control. D, LNCaP cells were maintained in androgen-deprived conditions for 24 hours and treated with 10 µmol/L Compound 30 or MDV3100 for 2 hours followed by addition of 1 nmol/L of R1881. After 15 minutes incubation, cells were fixed and AR localization was assessed by immunofluorescence imaging.
Compound 30 affects cell growth and AR transcriptional activity in vitro in MDV3100-resistant MR49F cells

Our in vivo experiment confirmed that both MDV3100 and Compound 30 had potent anticancer activity in CRPC tumors. However, we also observed that tumors treated with MDV3100 for 8 weeks or longer eventually resumed growth, which we interpreted as the establishment of MDV3100 resistance. Resistance to anticancer therapies is a common phenomenon observed in patients, therefore, we sought to develop a preclinical model of MDV3100-resistance. LNCaP-CRPC xenografts were treated with 10 mg/kg/day MDV3100 until tumor volume and PSA necessitated euthanasia and tumors were harvested and reimplemented in precastrated male nude mice subcutaneously (first generation transplant). Mice were continuously treated with MDV3100 to ensure maintenance of the resistant phenotype. This procedure was repeated to obtain second and third transplant generations. Third generation transplanted MDV3100-resistant xenograft tumors were harvested and single cell suspensions grown in in vitro culture under the constant pressure of 10 μmol/L MDV3100 to generate MDV3100-resistant MR49F cells (Supplementary Fig. S1). Results from immunoblotting analyses showed that MR49F cells expressed both AR and PSA protein, and their expression was not affected by MDV3100 treatment (Supplementary, Fig. S2).

As expected, MR49F cells showed a significant decrease in their response to MDV3100 treatment when compared with LNCaP parental cells, (IC50 = 12.52 μmol/L in LNCaP vs. 33.89 μmol/L in MR49F; Fig. 4A, left, Supplementary Table S3). In addition, MDV3100 was no longer able to reduce PSA expression, measured by Western blot analysis (Fig. 4C) or inhibit AR translocation from cytoplasm to nucleus upon androgen stimulation (Fig. 4D). Taken together, these results suggest that MR49F cells, which still express AR and PSA, resisted the effects of MDV3100 on AR activity.

Once MDV3100-resistant cells were established, we assessed the anticancer activity of Compound 30 on these cells. Importantly, although Compound 30 did not decrease AR protein expression in MDV3100-resistant tumors to the same extent as in LNCaP cells, reduction in AR expression was observed at higher treatment doses, and it profoundly reduced the expression of PSA in MR49F cells at as low a dose as 1 μmol/L (Fig. 4C). In addition, Compound 30 suppressed AR transcriptional activity in MR49F cells with greater potency to that obtained from LNCaP parental cells (EC50 = 0.23 μmol/L in MR49F and 2.0 μmol/L LNCaP cells; Fig. 4B, Supplementary Tables S1 and S4) and, more importantly, it was effective in its antiproliferative effects in MR49F cells (Fig. 4A right, Supplementary Table S5). Finally, AR nuclear translocation after androgen stimulation was inhibited by Compound 30 (Fig. 4D). Together, these results indicated that in the context of MDV3100 resistance modeled in MR49F cells, Compound 30 retained its ability to function as a potent AR antagonist and cell growth inhibitor.

Compound 30 has anticancer activity in MDV3100-resistant tumors in vivo

As described above, our in vitro studies indicated that Compound 30 was still effective in inhibiting AR activity in the context of MDV3100 resistance. Therefore, to test the efficacy of Compound 30 in suppressing MDV3100 tumor
growth in vivo, we implanted third generation MDV3100-resistant tumors into 34 mice under continuous treatment with 10mg/kg/day MDV3100. When tumors reached 300 mm$^3$ or approximately 50 ng/mL PSA, mice were randomly divided into 3 groups. At this time, MDV3100 treatment ceased, except for those animals assigned to MDV3100-treated group (11 animals). The other 2 groups received treatment with either vehicle (10 mice) or 25 mg/kg Compound 30 (13 mice; Fig. 5A). Consistent with the resistant nature of MR49F cells, we found that MDV3100-resistant tumors grew rapidly in the absence or presence of MDV3100, whereas those treated with Compound 30 showed significantly lower tumor volume and PSA. After 3 weeks of treatment, tumor volume change from baseline was 324% in vehicle-, 389% in MDV3100- and 138% Compound 30-treated groups (Fig. 5A, left), and serum PSA change from baseline was 389% in MDV3100- and 138% Compound 30-treated groups (Fig. 5A, right). Mice were euthanized when tumor volume exceeded 10% of their body weight and therefore at 3 weeks of treatment 1 mouse in vehicle-, 5 mice in MDV3100- and 2 mice in Compound 30-treated groups required euthanasia (Fig. 5B). Nevertheless, the group treated with Compound 30 survived significantly longer than vehicle and MDV3100 treatment groups (Fig. 5C). In addition, results from tumor immunohistochemistry (IHC) showed a marginal decrease Ki67 staining in Compound 30-treated tumors compared with vehicle and MDV3100-treated tumors (Fig. 5D) suggesting that Compound 30 had an impact on cell proliferation in vivo.

Discussion

Androgen ablation remains the most effective therapy for patients with advanced prostate cancer. Although most patients initially respond, progression to CRPC frequently occurs within 18 to 36 months. Blocking ligand binding to the AR is a complimentary approach that has been used clinically for nearly 3 decades. Although first generation antiandrogens, such as bicalutamide, do inhibit AR activity, they can induce mutated or overexpressed AR, which limits their clinical application in CRPC, where AR variants and overexpression occur frequently (20). In fact, even after castration, more than 80% of CRPC express AR and androgen-responsive genes (4, 21–23). Furthermore, it is well known that AR activity is a key driver of CRPC progression (24–26). Hence, identifying more effective AR pathway inhibitors in CRPC should enhance survival and slow disease progression.

Several new classes of AR pathway inhibitors are now in development and or approved for treatment of CRPC,
including more potent AR antagonists (e.g., MDV3100) and inhibitors of intratumoral steroidogenesis (e.g., abiraterone; refs. 13, 20, 27), as well as AR chaperone inhibitors (18, 28–30). MDV3100 is a potent, second-generation AR antagonist that binds to the ligand-binding domain of the AR with approximately 8-fold higher affinity than bicalutamide and impairs AR nuclear translocation and DNA binding (11). MDV3100 inhibits growth of CRPC xenografts and has efficacy in phase III clinical trials (31) and has been recently approved. However, despite its promising effects in some patients with CRPC, resistance to MDV3100 eventually occurred. The heterogeneity of responses of patients with CRPC to MDV3100 treatment and the eventual tumor progression, indicating MDV3100 resistance, suggest that AR targeting agents that are effective in MDV3100-resistant tumors will be required to treat CRPC.

In this study, we report for the first time that a new AR antagonist, Compound 30 (15), exhibits potent AR inhibition not only in CRPC, but also in MDV3100-resistant LNCaP derived cells in vitro and in vivo. In LNCaP cells, Compound 30 suppressed AR transcriptional activity and PSA expression levels in a dose-dependent manner. Similar to MDV3100 (11), Compound 30 prevented AR translocation to the nucleus, highlighting an important level of control over AR activity. However, Compound 30 also enhanced the reduction in AR protein expression compared with MDV3100, providing an alternative mechanism for reducing AR activity. The more modest reduction in AR protein expression induced by MDV3100 may be hindered by compensatory synthesis of AR, as evidenced by dramatically increased AR mRNA expression after MDV3100 treatment. Interestingly, this was not...
observed after treatment with Compound 30, possibly indicating an advantage of this drug over MDV3100. One possible mechanism for the downregulation of both AR mRNA and protein levels in Cpd30-treated cells may be a feed forward loop, whereby reduced levels of AR protein expression and translocation to the nucleus inhibit AR-dependent transcription factors in promoting the transcription and translation of AR.

Importantly, our in vivo studies pointed to an unmet application for the use of Compound 30 in the treatment of anti-AR–resistant CRPC. When we compared the antitumor activity of Compound 30 and MDV3100 in mice bearing castration-resistant xenograft tumors, we found both compounds significantly prevented increases in tumor volume and PSA. However, after long-term treatment with MDV3100 or Compound 30 (> 8 weeks), even well-controlled tumors recurred and PSA recovered, suggesting that resistance to both antiandrogens occurs. Indeed, despite efficacy of MDV3100 and its imminent approval for the treatment of CRPC (31), most patients treated with MDV3100 will eventually recur, providing clinical evidence for anti-AR resistance. In light of our data showing tumor recurrence with MDV3100, we developed a model of MDV3100 resistance in vivo and in vitro and were able to generate LNCaP-derived MDV3100-resistant xenograft tumors and cell lines that still expressed AR and PSA. We used these resistant cells to test the efficacy of Compound 30. Importantly, although our in vivo studies showed similar anticancer activity between Compound 30 and MDV3100 in CRPC xenografts, our in vitro results showed significant antiproliferative effects of Compound 30 in MDV3100-resistant cells. Accordingly, we found Compound 30 suppressed progression of MDV3100-resistant tumors in castrated mice and significantly prolonged survival in mice bearing MDV3100-resistant tumors. Furthermore, in vitro studies using the MR49F MDV3100-resistant cell line showed that Compound 30 effectively inhibited AR transcriptional activity and AR translocation to the nucleus, and potently suppressed cell proliferation.
Efficacy of Compound 30 in MDV3100-Resistant Prostate Cancer

Despite the positive effect of Compound 30 on MDV3100-resistant cells, we did observe indications of resistance to Compound 30 in both LNCaP and MR49F xenografts. This is not surprising, as the likelihood that a patient will become resistant to various treatments can be very high, depending on the therapy and type of cancer. Furthermore, we observed that in MDV3100-resistant cell lines, resistance to Compound 30 emerged 4 to 6 weeks after treatment. Although our data indicates that AR is still active, the emergence of tumors that are resistant to both Compound 30 and MDV3100 suggests that targeting AR with a drug against ligand binding domain provides only a short responding period before tumors become refractory to treatment. Possibly, targeting the AR with similar inhibitors, as in the case of MDV3100 and Compound 30, activates a resistance "memory" program, allowing cells to respond faster with survival mechanisms that are effective in opposing them from second-line therapy. However, given the maintained anticancer effects on MDV3100-resistant cells in vitro and significant increase in survival in vivo in Compound 30-treated mice with MDV3100-resistant tumors, Compound 30 may still be a viable alternative treatment offered to patients who fail MDV3100 therapy. In addition, our results do not exclude the possibility that Compound 30 may be more effective in some patients with CRPC as a second-line hormone therapy, or improve the efficacy of MDV3100 if used in combination with this drug. Future studies investigating the sequential and combinatorial use of these drugs are necessary.

In summary, our results suggest that Compound 30 is able to inhibit the continued AR activity that is associated with anti-AR resistance and may be a viable treatment strategy for MDV3100-resistant tumors in vivo. Furthermore, our data provide a preclinical proof-of-principle that novel androgens, while they may have similar efficacy against CRPC as other drugs, may be developed as an alternative in patients resistant to MDV3100.

Disclosure of Potential Conflicts of Interest
A. Zoubeidi has commercial research grant from Pfizer and is a consultant/advisory board member of Johnson and Johnson. No potential conflicts of interest were disclosed by the other authors.

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