Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor


*Human Oncology and Pathogenesis Program, #Bioinformatics Core, Genomics Core, Memorial Sloan-Kettering Cancer Center, New York, NY 10065; #Department of Radiation Oncology, David Geffen School of Medicine, University of California, Los Angeles, CA 90095; and Howard Hughes Medical Institute, Chevy Chase, MD 20815

This contribution is part of the special series of Inaugural Articles by members of the National Academy of Sciences elected in 2010.

Contributed by Charles L. Sawyers, August 23, 2010 (sent for review June 14, 2010)

Androgen receptor (AR) splice variants lacking the ligand binding domain (ARVs), originally isolated from prostate cancer cell lines derived from a single patient, are detected in normal and malignant human prostate tissue, with the highest levels observed in late stage, castration-resistant prostate cancer. The most studied variant (called AR-V7 or AR3) activates AR reporter genes in the absence of ligand and therefore, could play a role in castration resistance. To explore the range of potential ARVs, we screened additional human and murine prostate cancer models using conventional and next generation sequencing technologies and detected several structurally diverse AR isoforms. Some, like AR-V7/AR3, display gain of function, whereas others have dominant interfering activity. We also find that ARV expression increases acutely in response to androgen withdrawal, is suppressed by testosterone, and in some models, is coupled to full-length AR (AR-FL) mRNA production. As expected, constitutively active androgen receptor splice variants lacking the ligand binding domain-targeted antiandrogens, such as MDV3100, or by selective siRNA silencing of AR-FL, indicating that the growth-promoting effects of ARVs are mediated through AR-FL. These data indicate that the increase in ARV expression in castrate-resistant prostate cancer is an acute response to castration rather than clonal expansion of castration or antiandrogen-resistant cells expressing gain of function ARVs, and furthermore, they provide a strategy to overcome ARV function in the clinic.

Androgen receptor (AR) contains an N-terminal transactivation domain (encoded by exon 1), the DNA binding domain (exons 2 and 3), a short hinge region (exon 4), and the C-terminal ligand binding domain (LBD; exons 4–8) where the androgenic ligands testosterone and dihydrotestosterone bind (1). When bound by androgens, AR undergoes a conformational change that permits nuclear translocation, DNA binding, and regulation of AR target genes (2). AR signaling is required for development of the normal prostate (3) and prostate cancer progression, even in the end stage of castration-resistant disease (4).

Previous studies have identified up to seven different AR splice variants lacking the LBD (ARVs), all isolated from the CWR22R system (5), that share the common structural feature of an N terminus encoded by exons 1/2 or exons 1/2/3 of AR followed by variable C-terminal sequences (cryptic exons) originating from introns 2 or 3 (6–8). All these ARVs are purported to have constitutive, ligand-independent activity. Using isoform-specific RTPCR, one variant designated AR-V7 (7) or AR3 (8) (hereafter called AR-V7) has been detected in other human prostate cancer cell lines and xenografts as well as normal and malignant human prostate-tissue samples. AR-V7 levels are generally higher in castration-resistant vs. androgen-dependent tumors, and AR-V7 expression in early-stage prostate cancer has been associated with a worse prognosis after radical prostatectomy. Based on evidence that AR-V7 can enhance growth of androgen-dependent xenografts in castrated mice (8), it has been proposed that ARVs can function as drivers of castration resistance (6–8). Here, we show a greater diversity of ARVs than previously appreciated that have different activities, ranging from constitutively active to dominant negative. In addition, we show that ARV expression is negatively repressed by androgen. Some ARVs promote castration resistance or anchorage-independent growth, but they do so by acting through full-length AR (AR-FL) and not independently. These findings have implications for whether and how ARVs cause castration resistance and for strategies to overcome their gain of function properties.

Results
Identification of Structurally Diverse ARVs in Human and Murine Prostate Cancer Models. The family of nuclear hormone receptors undergoes extensive splicing that yields multiple, functionally diverse variants (9, 10). To determine if similar diversity exists for AR, we selected two other prostate cancer cell lines for analysis, the human cell line VCaP (11) and the murine cell line Myc-CaP (12), because we routinely detected lower molecular weight AR protein isoforms (ranging from ~60–80 kDa to 110 kDa for AR-FL) in lysates from these models. To identify the molecular structure of these smaller isoforms, we performed 3′ RACE followed by standard subcloning and Sanger sequencing and additionally, in the case of VCaP, by next generation RNA-Seq.

Sequencing of AR mRNA transcripts in VCaP, primed from the border of exons 2/3, identified the known ARVs AR-V1 and AR-V7 (7) as expected as well as four additional isoforms [numbered AR-V8 through AR-V11 using the nomenclature of Hu et al. (7)] (Fig. L4 and Table S1). The AR isoforms were detected by next generation sequencing platforms (454 and SOLiD) as well as by conventional subcloning and Sanger sequencing and additionally, in the case of VCaP, by next generation RNA-Seq.

www.pnas.org/cgi/doi/10.1073/pnas.1012443107
previously appreciated, although cloning of complete cDNAs is needed to fully characterize the molecular anatomy of these variants. Of note, an exon skipping 4/8 variant was also isolated independently from another xenograft model (called ARV567es) while this work was under review (13).

In murine Myc-Cap cells, we recovered four ARVs, which we labeled mAR-V1–4 (Fig. 1C and Table S2). Only mAR-V2 and mAR-V4 were pursued further, because isoform-specific knockdown experiments validated that these two isoforms encode the most abundant truncated ARVs (Fig. 1D). Structurally, mAR-V2 resembles the human isoforms AR-V1 and AR-V7 with retention of AR exons 1–3 but differs with the unique C-terminus containing only a single additional amino acid. mAR-V4 is distinct, because exons 1–4 are retained before the addition of unique C-terminal sequence. mAR-V4 is structurally analogous to the recently described human ARV567es (13). Unlike the human ARVs, the unique C-terminal sequences of mAR-V2 and mAR-V4 are not derived from murine AR introns. Rather, these sequences map outside the murine AR gene to distal regions on the X chromosome (Fig. 1C).

We do not know the structural basis for the generation of these isoforms at the genomic level, but the fact that Myc-CaP cells have AR gene amplification (also seen in ~30% of castration-resistant human prostate cancer) raises the possibility of intrachromosomal gene rearrangements.

**ARV Expression Is Correlated with AR-FL mRNA Levels and Induced by Castration.** Analysis of AR-V7 expression across a range of human prostate tissues has shown low levels in normal prostate and higher levels in castration-resistant prostate cancer samples. In both settings, AR-V7 transcript levels are substantially lower than those of AR-FL. The increase in ARV expression observed with advanced disease could occur through selection of more malignant or castration-resistant subclones or because of hormone-dependent regulation of ARV expression. To distinguish between these possibilities, we examined the kinetics of ARV expression in the VCaP and LuCaP35 human prostate cancer xenograft models, both of which are known to express ARVs. No ARV protein expression (using an N-terminal AR antibody) was detected in VCaP tumors grown in intact mice. However, a substantial increase in both ARV and AR-FL expression was detected just 2 d postcastration, reaching a maximum at 14 d (Fig. 2A and B). Remarkably, this

---

**Fig. 1.** Discovery of ARVs in additional prostate cancer models. (A and B) Next generation AR mRNA sequencing in VCaP. (A) Exon 3 truncation ARVs described here (blue) or previously (black) were initially identified by Sanger sequencing. 45S junctions supporting Sanger sequences were determined by TopHat in supervised mode (i.e., input of predetermined junctions). TopHat does not detect noncanonical splice sites (AR-V10) or exon runon (AR-V11). SOLiD coverage is represented on a log scale. The greatest number of SOLiD reads mapped to the native AR exons, consistent with the relative abundance of AR-FL in these cells. SOLiD specifically detected the unique AR-V11 sequence that was not identifiable using TopHat. (B) Unsupervised TopHat analysis of 454 junctions identifies putative ARVs distinct from exon 3 truncations involving exon skipping. A putative cryptic intron 5 exon is shown in red, with a large number of SOLiD reads relative to adjacent intron sequence. (C) Sequences of Myc-CaP ARVs were mapped to intergenic regions (dashed lines) of chromosome X (not drawn to scale) using the University of California, Santa Cruz (UCSC) Genome Browser (NCBI37/mm9 assembly). The genetic origins of mAR-V2 and mAR-V4–specific sequences are represented by blue or red boxes, respectively. Adjacent genes are shown with their physical position. The AR and AR-FL proteins are depicted with the native AR exons numbered (not drawn to scale). (D) Myc-CaP were transfected with siRNA against total AR (exons 1 and 3), mAR-V2, or mAR-V4 (four individual siRNA per ARV shown as a–d). A nontarget siRNA (N) was used as a negative control. Growth media was standard 10% FBS. Western blots were done at 24 h posttransfection.

---

### Table S2

<table>
<thead>
<tr>
<th>Gene Order</th>
<th>Pgr151</th>
<th>Eda2r</th>
</tr>
</thead>
<tbody>
<tr>
<td>XqC3</td>
<td>94.27–94.28</td>
<td>94.53–94.57</td>
</tr>
<tr>
<td>Gene Order</td>
<td>Ar (amplified)</td>
<td>Ophn1</td>
</tr>
<tr>
<td>XqC3</td>
<td>95.35–95.51</td>
<td>95.75–96.09</td>
</tr>
</tbody>
</table>

---

Watson et al.
increase was completely extinguished within 8 d of testosterone replacement. In contrast to VCaP, LuCaP35 xenografts expressed ARVs when grown in intact mice. Castration modestly increased AR-FL and ARV mRNA, but these increases did not result in obviously higher protein levels. As with VCaP, testosterone replacement down-regulated both AR-FL and ARV mRNA levels. Whereas ARV protein was no longer detectable, AR-FL protein levels were mostly unchanged, consistent with the well-established stabilization of AR-FL by androgen. In retrospect, these data are consistent with older literature documenting androgen-mediated negative feedback of AR-FL expression but now extended to ARV expression (14–17). The multiple ARV protein profile observed in VCaP tumors is also consistent with the complexity of ARV mRNAs detected by RNA-Seq.

Quantitative analysis of AR-V1 and AR-V7 mRNA levels relative to AR-FL in VCaP and LuCaP35 revealed that ARV levels range from 0.1% to 1.0% of AR-FL mRNA levels (Fig. 2). Extended analysis of VCaP tumors is also consistent with the complexity of ARV mRNAs detected by RNA-Seq.

Structurally Similar ARVs Have Distinct Biological Activity. Prior work has suggested that all ARVs lacking the LBD are ligand-independent, constitutively active isoforms. However, the ARV-specific siRNA experiments conducted in Myc-CaP cells under standard growth conditions of 10% FBS (Fig. 1D) revealed that expression of the AR-dependent probasin-Myc transgene was abolished by knockdown of mAR-V4 but not of mAR-V2. qRT-PCR confirmed that this decline in Myc expression was because of loss of probasin-Myc mRNA rather than endogenous mouse Myc (Fig. S1). Therefore, the low basal level of androgen present in 10% FBS was not capable of maintaining probasin-Myc expression after loss of mAR-V4, despite abundant levels of AR-FL (Fig. 1D). However, exogenous androgen supplementation restored AR-V4-mediated regulation of probasin-Myc, without a requirement for mAR-V4 (Fig. S2, day 3 lanes).

The fact that mAR-V2 and mAR-V4 differed in their ability to regulate probasin-Myc suggests that individual ARVs may not be functionally equivalent, despite similar structural features. Therefore, we directly compared the biological function of three ARVs truncated after exon 3 (AR-V1, AR-V7, and mAR-V2) that differ only in the amino acid sequence encoded by the C-terminal extension and a fourth ARV (mAR-V4), which represents the class of ARVs with truncation after loss of mAR-V4, despite abundant levels of AR-FL (Fig. 1D). These ARVs are also the most abundantly expressed alleles in the human and murine models studied to date. After transient transfection of
expression plasmids into Cos-7 cells, all four ARVs were robustly expressed at levels equal to or higher than AR-FL (Fig. 3B), but they differed in their subcellular localization. AR-V7 and mAR-V4 were nuclear, whereas AR-V1 and mAR-V2 were cytoplasmic (Fig. 3C). We next measured their transcriptional activity in prostate cancer cells, choosing the AR-negative DU145 cell line to avoid potential confounding effects of endogenous AR-FL. Using the AR-dependent luciferase reporter vector (4× ARE-Luc) as a readout, the activities of AR-V7 and mAR-V4 were comparable with hormone-induced AR-FL activity, whereas AR-V1 and mAR-V2 were only modestly above background (Fig. 3D). These data establish that some, but not all, ARVs can function in isolation (without AR-FL) to activate AR reporter constructs and that this correlates with nuclear localization.

Curiously, the level of ARV protein expression, particularly for the constitutively nuclear isoforms AR-V7 and mAR-V4, was reduced relative to AR-FL in stable lines, despite equal or greater levels of ARV mRNA expression (Fig. S3A). This pattern was seen in DU145 (Fig. 3E), LNCaP (Fig. 4A), and Myc-CaP (Fig. 4B) cells stably transduced with ARVs. Treatment with the proteasome inhibitor MG132 increased the level of some ARVs, providing some evidence that ARVs may be less stable (Fig. S3B). However, some but not all tumors derived from LNCaP sublines expressed abundant levels of ARV protein (Fig. 5B), suggesting that other variables may be at play. Further work is required to understand the complex relationship of ARV mRNA and protein levels.

Gain of Function ARVs Require AR-FL. The antiandrogen MDV3100 has shown impressive clinical activity in end-stage, castration-resistant prostate cancer and is currently under evaluation in a phase III registration trial (18, 19). MDV3100 acts on AR-FL by directly binding the LBD; therefore, constitutively active ARVs such as AR-V7 and mAR-V4 could, in theory, confer resistance to MDV3100. We tested this directly in the castration-resistant LNCaP xenograft model, which we had previously shown to be sensitive to MDV3100 (18). Remarkably, MDV3100 blocked the growth of both GFP and AR-V7–expressing LNCaP xenografts equivalently (Fig. 5A). The sensitivity of LNCaP/AR-V7 tumors cannot be explained by loss of AR-V7 expression, because moderate to high AR-V7 expression was present in all but one of the MDV3100-treated LNCaP/AR-V7 tumors (Fig. 5B). To explore this unexpected effect of MDV3100 treatment in a more controlled experimental setting, we turned to a highly quantitative soft-agar assay. Consistent with the transcriptional output and xenograft studies, only the constitutively nuclear ARVs (AR-V7 and mAR-V4) conferred increased anchorage-independent growth (Fig. 5 C and D). Interestingly, coexpression of AR-V1 with AR-V7, which is observed in human xenografts and tumors, completely abrogated the gain of function conferred by AR-V7, indicating that AR-V1 is likely a dominant negative variant. AR-V7 inhibition by AR-V1 was not because of loss of AR-V7 expression in the double-variant line (Fig. 4I). As in the xenograft assay, MDV3100 completely blocked the gain of function conferred by AR-V7 and mAR-V4. The growth suppression by MDV3100 in agar is specific, because colony formation by AR-negative DU145 cells was unaffected.

The ability of MDV3100 to reverse the growth advantage conferred by AR-V7 and mAR-V4 is unexpected, because both ARVs lack the MDV3100 binding site. To confirm that MDV3100 cannot directly inhibit either allele, we repeated the transcriptional output assays in AR-negative DU145 cells, and we saw no effect of MDV3100 on AR-V7 or mAR-V4 but dramatic suppression of AR-FL activity (Fig. S5). Because MDV3100 impairs the nuclear translocation efficiency of AR-FL (18), we considered the possibility of a similar effect on ARVs but saw no evidence that MDV3100 relocalized AR-V7 to the cytoplasm (Fig. 6A). In the absence of evidence for a direct effect of MDV3100 on ARVs, we...
hypothesized that MDV3100 inhibits AR-V7 or mAR-V4 indirectly by targeting AR-FL. Consistent with this model, siRNA directed against the AR C-terminus (designed to specifically target AR-FL) inhibited the growth of LNCaP/AR-V7 and LNCaP/mAR-V4 cells (Fig. 6B) to the same degree as control LNCaP/GFP cells. mRNA and protein-expression studies confirmed that the C-terminal–directed siRNA specifically knocked down AR-FL but not AR-V7 or mAR-V4 (Fig. 6C). In addition, expression of the AR-regulated genes TMPRSS2, PSA, and KLK2 were not maintained after selective AR-FL knockdown, despite the presence of AR-V7 or mAR-V4 (Fig. 6D).

We also examined the effect of ARVs on basal expression of three endogenous AR target genes. PSA expression was increased in LNCaP/mAR-V4, consistent with similar findings recently reported for LNCaP cells expressing ARV567es (13). However, basal PSA expression was decreased in LNCaP/AR-V7 cells (Fig. 6E). ARV expression did not alter the basal expression of TMPRSS2 and KLK2 (Fig. 6E). These data suggest that ARVs require AR-FL to activate endogenous target genes and that these effects vary in a gene- and variant-dependent manner.

Discussion

Earlier reports of ARVs in prostate cancer have suggested a general model, whereby these alleles are constitutively active and can substitute for the canonical androgen-dependent AR-FL signaling required for prostate cancer growth. If correct, ARVs could emerge as a primary cause of resistance to castration and/or conventional antiandrogen therapy directed at the LBD. By analyzing a greater range of prostate models, we reveal greater structural diversity of ARVs than previously appreciated as well as highly divergent biologic activity among ARVs that, on the surface, appear structurally similar. Unexpectedly, we find that biologically active ARVs require AR-FL to activate endogenous AR target genes and confer castration-resistant growth.

Currently, there is little insight into how ARVs are generated. Our studies comparing AR and AR-FL levels in several xenografts and patient samples establish that ARVs are expressed at much lower levels (~0.5–2.5%) relative to AR-FL. Furthermore, this ratio is generally maintained when AR-FL transcription is enhanced (by castration) or suppressed (by testosterone treatment). These data suggest that ARVs are likely generated through splicing errors that occur in normal or malignant tissues. Therefore, ARV levels would typically be dependent on AR-FL levels rather than a specific splicing defect that favors ARV production. This model does not rule out that other variables, such as structural alterations in the AR gene (amplification and mutation), may impact interaction of pre-mRNAs with the splicing machinery. Indeed, the atypical structure of the ARVs isolated from Myc-CaP cells could be a consequence of AR gene rearrangements that developed in conjunction with AR gene amplification. Point mutations in the AR gene affecting splice-recognition sites might also favor the production of certain ARVs.

By characterizing the biological properties of four different ARVs, we uncovered a correlation between constitutive nuclear localization and ligand-independent activity in transcription readouts and growth assays. The fact that only one of three ARVs truncated after AR exon 3 was nuclear (AR-V7) establishes the importance of the unique C-terminal sequence in defining the biologic activity of the resulting ARV. The nuclear localization sequence in AR-FL spans exons 3/4 (20, 21) and therefore, is partially deleted in all ARVs with exon 3 truncation. ARVs of the exon 3 class are, therefore, likely to be cytoplasmic unless the unique C-terminal sequence enables nuclear translocation. Of note, analysis of the C-terminal extension from AR-V7 (PredictNLS Online) did not reveal a predicted nuclear localization signal, and therefore, it is not clear how AR-V7 reaches the nucleus. Interestingly, immunostaining of AR-V7 has revealed cytoplasmic localization in many clinical samples, suggesting that additional variables regulate nuclear localization (8). In contrast, ARVs such as mAR-V4 and ARV567es that are truncated after exon 4 retain a complete nuclear localization signal and should be constitutively nuclear unless additional C-terminal sequence is inhibitory or functions as a cytoplasmic anchor or nuclear export signal.

The fact that nuclear ARVs require AR-FL to confer gain of function was unexpected, particularly because they are capable of activating AR reporter constructs in AR-negative cell lines. The pharmacologic (MDV3100) and genetic (AR-FL–specific siRNA knockdown) evidence presented here suggest that ARVs function upstream of AR-FL in models that most closely mimic the clinical scenario of combined AR-FL and ARV expression. Whereas the models studied here argue for sustained dependence of ARV-expressing castration-resistant prostate tumors on AR-FL (and

**Fig. 5.** Gain of function ARVs are not resistant to the antiandrogen MDV3100. (A) Precastrated mice were grafted with LNCaP/GFP or AR-V7 sublines (n = 6–7). When castrate-resistant tumors arose, the mice were treated with MDV3100. Fold-change tumor volume after treatment is plotted relative to the volume on day 0 of treatment (error bars = SEM). (B) Western blot of LNCaP/AR-V7 cell line (in vitro culture) or tumors from castrated mice treated for 25 d with MDV3100 or vehicle. (C and D) Anchorage-independent soft-agar growth in 10 μM MDV3100 for parental DU145 and stable LNCaP lines. (C) Images of representative plate quartile. (D) Mean total colony number (n = 3; error bars = SEM).
hence, sustained sensitivity to LBD-targeted drugs), it is possible that the combined, independent effects of AR-FL and ARVs are required for growth. Some castration-resistant prostate tumors may express high ARV at the expense of AR-FL, thereby potentially bypassing a requirement for AR-FL.

At a biochemical level, the simplest model to explain the AR-FL dependence is ARV/AR-FL heterodimer formation. Dimerization could occur through interaction of the AR-FL C-terminus with the N-terminal FxxLF motif in AR exon 1 (retained in all ARVs). These domains are already known to mediate intra- or intermolecular AR-FL N/C interactions (22, 23). ARV/AR-FL complexes have not, however, been detected in the CWR22Rv1 model by standard immunoprecipitation (8, 24). However, AR-FL and ARV were reported to form heterodimers when both were exogenously introduced into AR null cells (13). The much greater abundance of AR-FL relative to ARVs makes detection of complexes challenging, such that ARV-specific antibodies are needed to fully examine whether endogenous complexes are present.

Whatever the mechanism, the functional relationship between ARVs and AR-FL raises questions about the repertoire of AR target genes. One possibility is that ARVs simply substitute for hormones and activate an identical set of AR targets. However, transcriptome analysis after selective AR-FL or AR-V7 knockdown or comparing AR-FL to AR-V7 revealed distinct subsets of genes regulated by AR-FL, ARVs, or both (8, 13). Even among classic endogenous AR target genes, our data showed differential expression levels dependent on the particular gene and ARV present, providing evidence that the biology of ARVs is not wholly synonymous with that of androgen-stimulated AR-FL. This model of additional ARV target genes is consistent with our observation that AR-V7 and mAR-V4 stimulate soft-agar growth beyond that observed with AR-FL alone. Because this increase in growth remains dependent on AR-FL, we suspect that ARVs and AR-FL jointly drive growth by regulating a greater repertoire of targets than is regulated by AR-FL alone.

Collectively, the data presented here suggest that the role of ARVs in castration or antiandrogen resistance differs from classic models of drug-resistant alleles. Resistance to kinase inhibitors in diseases such as lung cancer, chronic myeloid leukemia, and gastrointestinal stromal tumor is often caused by mutations in the targeted kinase domain, present in a small fraction of tumor cells that gradually emerges under the selective pressure of kinase inhibitor therapy (25). On the surface, ARVs could function similarly. However, our studies reveal that ARVs are expressed within days of castration and disappear within days of androgen treatment, providing clear evidence of acute hormonal regulation. Therefore, ARV expression is more likely a reaction to hormone therapy rather than a driver of castration or antiandrogen resistance. Furthermore, multiple ARVs (typically AR-V1 and AR-V7) are simultaneously detected in patient tumors. The fact that AR-V1 dominantly inhibits AR-V7 provides further evidence that any role of ARVs in clinical castration resistance is likely to be complex. Careful annotation of ARV expression in prostate cancer patients after initiation of conventional or next-generation hormone therapy and at relapse should help clarify these issues. The fact that AR-FL is required for ARV function validates continued efforts to develop even better antiandrogens targeting the LBD.

Materials and Methods

Detailed materials and methods are provided in SI Materials and Methods. Primers designed for this study are listed in Tables S3–S8.

ARV Discovery. AR 3′ RACE PCR was performed using mRNA isolated from tumors growing in castrated mice. Tumors were collected from spontaneous, castrate-resistant Myc-CaP and 1d postcastration VCaP tumors.

Next-Generation Sequence Analysis. 454 reads were processed using the TopHat alignment algorithm (26) to identify splice junctions. Logarithmic SOLID read coverage was mapped to the AR locus, except for reads spanning splice junctions.

Plasmids and Cell Transduction. CDNA for mouse AR isoforms and human ARVs were cloned from Myc-CaP and 22Rv1, respectively, into Retro-X Q vectors (Clontech), as was EGFP. Human AR FL in pWZL/AR (27) was provided by William Hahn (Dana-Farber Cancer Institute, Boston). AR- or GFP-expressing stable cell lines were derived after pantropic retroviral infection (Clontech).
Reagents. The total AR primary antibody used in these studies was raised against an N-terminal epitope (N-20; Santa Cruz). AR-V7 specific antibody was kindly provided by Jun Luo (Johns Hopkins University, Baltimore). MDV3100 was synthesized at MSKCC, and R1881 was from PerkinElmer.

AR Reporter Assay. Cells were cotransfected in charcoal-stripped serum with 4x ARE-Luciferase and pRL-TK (Promega) at 10:1. Firefly activity was normalized to Renilla using Dual Luciferase Assay reagent (Promega).

Anchorage Independent Growth. In 0.1% DMSO vehicle, 10^4 DU145 or 10^5 LNCaP cells were suspended in soft agar with 20% FBS and 10 μM MDV3100. At 2–3 wk, colonies were stained with 0.5% crystal violet, imaged, and counted using GelCount (Oxford Optronix).

Tumorigenesis Assays. Human cells were injected s.c. into the flank of intact or castrated male C57 SCID mice (Taconic), whereas Myc-CaP was grafted into the mammary fat pad of intact male FVB mice (Taconic). Tumor-bearing mice (~500–1,000 mm^3) were treated by castration or oral 10 mg/kg MDV3100. Testosterone pellets were 12.5 mg per 90 d release (Innovative Research of America). All animal experiments were performed in compliance with the guidelines of the Research Animal Resource Center of the Memorial Sloan-Kettering Cancer Center.

ACKNOWLEDGMENTS. We thank Alan Hall (Memorial Sloan-Kettering Cancer Institute, New York) and Robert Vessella (University of Washington, Seattle), for kindly sharing reagents and equipment. This work was funded by the Howard Hughes Medical Institute, National Cancer Institute, and the Prostate Cancer Foundation.