Targeting IGF-IR with Ganitumab Inhibits Tumorigenesis and Increases Durability of Response to Androgen-Deprivation Therapy in VCaP Prostate Cancer Xenografts

Cale D. Fahrenholtz, Pedro J. Beltran, and Kerry L. Burnstein

Abstract
Prostate cancer is the most commonly diagnosed malignancy in men. While tumors initially respond to androgen-deprivation therapy, the standard care for advanced or metastatic disease, tumors eventually recur as castration-resistant prostate cancer (CRPC). Upregulation of the insulin-like growth factor receptor type I (IGF-IR) signaling axis drives growth and progression of prostate cancer by promoting proliferation, survival, and angiogenesis. Ganitumab (formerly AMG 479) is a fully human antibody that inhibits binding of IGF-I and IGF-II to IGF-IR. We evaluated the therapeutic value of ganitumab in several preclinical settings including androgen-dependent prostate cancer, CRPC, and in combination with androgen-deprivation therapy. Ganitumab inhibited IGF-I–induced phosphorylation of the downstream effector AKT and reduced proliferation of multiple androgen-dependent and castration-resistant human prostate cancer cell lines in vitro. Ganitumab inhibited androgen-dependent VCaP xenograft growth and increased tumor-doubling time from 2.3 ± 0.4 weeks to 6.4 ± 0.4 weeks. Ganitumab blocked growth of castration-resistant VCaP xenografts for over 11.5 weeks of treatment. In contrast, ganitumab did not have appreciable effects on the castration-resistant CWR-22Rv1 xenograft model. Ganitumab was most potent against VCaP xenografts when combined with complete androgen-deprivation therapy (castration). Tumor volume was reduced by 72% after 4 weeks of treatment and growth suppression was maintained over 16 weeks of treatment. These data suggest that judicious use of ganitumab particularly in conjunction with androgen-deprivation therapy may be beneficial in the treatment of prostate cancer.

Introduction
Prostate cancer is the most commonly diagnosed cancer in men and is the second leading cause of cancer-related deaths in men each year (1). Androgen-deprivation therapy is and has been the gold standard of care for advanced or metastatic prostate cancer for decades. While this treatment strategy initially shows benefit, eventually tumors recur as castration-resistant prostate cancer (CRPC) for which there are limited treatment options with only modest survival benefit.

The insulin-like growth factor receptor type I (IGF-IR) and native ligands, IGF-I and IGF-II, are dysregulated in a variety of cancers including prostate cancer (2–5). Signalings through the IGF-IR plays an important role in many cellular processes including: mitogenesis, apoptosis, proliferation, and angiogenesis (Supplementary Fig. S1). The downstream effects of IGF-IR activation include PI3K/AKT and MEK/ERK signaling cascades (6, 7). Elevated levels of serum IGF-I, the most potent activator of IGF-IR, has been correlated with an increased risk of prostate cancer (8, 9). IGF-I and IGF-II are increased with progression to castration-resistance in vivo (10). In clinical specimens, IGF-IR expression is elevated in primary prostate cancer versus benign prostatic epithelium (11). Studies with an IGF-IR receptor inhibitor, the antibody cixutumumab (formerly IMC-A12), showed positive results against LuCaP 35 prostate cancer xenografts alone and in conjunction with androgen-deprivation therapy or docetaxel (12–14). A recently completed phase II clinical trial with figitumumab (formerly CP-751,871), an immunoglobulin G (IgG)2 IGF-IR antibody inhibitor that blocks IGF-I binding and promotes receptor internalization, in patients with localized prostate cancer showed a decrease in serum levels of prostate-specific antigen (PSA; refs. 15, 16). These studies support further investigation of inhibition of IGF-IR signaling with ganitumab in robust models of human prostate cancer.

Ganitumab (formerly AMG 479) is a fully human antibody (IgG1) against IGF-IR. Ganitumab reduces IGF-IR...
activation by binding the L2 domain (an extracellular leucine-rich domain that contributes to ligand binding) of IGF-IR, thereby preventing both IGF-I and IGF-II interaction with the receptor (17–19). Binding of ganitumab to IGF-IR also induces internalization and degradation of IGF-IR in vivo (18). Ganitumab does not interact with the closely related insulin receptor (INSR) but does inhibit hybrid IGF-IR/INSR receptors (18). Ganitumab has been studied in phase II trials for a variety of advanced tumors (20, 21). In phase I/ib escalation studies, ganitumab was generally well-tolerated when administered to patients with advanced solid tumors both as a single agent and in combination with either the multit kinase inhibitor sorafenib, either of the EGFR inhibitors panitumumab or erlotinib, or gemcitabine. These combination therapies as well as ganitumab as a single agent displayed promising anti-tumor effects in patients and resulted in few dose-limiting toxicities and generally mild adverse events including fatigue, nausea, vomiting, or chills (22, 23). To date, ganitumab has shown signs of efficacy against pancreatic cancer and Ewing’s sarcoma, yet there has been minimal evaluation of ganitumab for prostate cancer.

In this study, we tested the effect of ganitumab on several human prostate cancer cell lines both in vitro and in vivo. VCaP is an androgen-dependent human prostate cancer cell line that like approximately 50% of prostate cancers is wild-type for PTEN (a negative regulator for AKT signaling; refs. 24, 25). As observed in human prostate cancer, VCaP cells progress to castration-resistance in vivo (26, 27). VCaP also expresses the TMPRSS2:ERG fusion gene, which is seen in approximately 50% of primary prostate cancer specimens and may promote prostate cancer progression (28). Another androgen-dependent human prostate cancer cell line, LNCaP, does not express active PTEN (29). CWR-22Rv1 (22Rv1) and CWR-R1 are castration-resistant, PTEN wild-type and express both full-length androgen receptor (AR) as well as constitutively active AR splice variants, which lack the ligand-binding domain (termed AR-LBDs; refs. 30, 31). Using these established models of prostate cancer, we assessed the effectiveness of ganitumab alone and in conjunction with complete androgen-deprivation therapy (castration) as a treatment of androgen-dependent prostate cancer, advanced CRPC, and progression to CRPC. We show that ganitumab inhibits growth of both androgen-dependent and castration-resistant VCaP xenografts. Ganitumab does not affect growth of aggressive castration-resistant 22Rv1 xenografts. Finally, we found that ganitumab is highly effective against VCaP xenografts when combined with androgen-deprivation therapy.

Materials and Methods

Cell culture and chemical reagents

The human prostate cancer cell lines LNCaP.FGC (CRL 1740; batch F-11701) and CWR-22Rv1 (CRL-2505, batch 4484055) were obtained from American Type Culture Collection (ATCC). LNCaP and 22Rv1 cells were authenticated and certified by ATCC. ATCC ensures each cell line is negative for Mycoplasma, bacteria, and fungi contamination; confirms species identity; and conducts DNA profiling and cytogenetic analysis to authenticate each cell line. CWR-R1 cells were provided by Dr. Elizabeth Wilson (University of North Carolina, Chapel Hill, NC) in July 2011 and tested for prostate and cell line-specific characteristics including AR and AR splice variants (32, 33). VCaP cells were provided in March 2009 by Dr. Kenneth Pienta (University of Michigan, Ann Arbor, MI). VCaP cells were tested for prostate and cell line–specific characteristics including AR, TMPRSS:ERG fusion, and PSA. VCaP cells were negative for Mycoplasma, human T-lymphotropic virus, hepatitis (A, B, and C), and HIV. All cell lines were used within 6 months of resuscitation. VCaP, LNCaP, 22Rv1, and CWR-R1 cells were maintained as hybrid IGF-IR/INSR receptors (18). Ganitumab has been closely related insulin receptor (INSR) but does inhibit IGF-I and IGF-II interactions with the receptor (17–19). Binding of ganitumab to IGF-IR, thereby preventing both IGF-I and IGF-II interaction with the receptor (17–19). Binding of ganitumab to IGF-IR also induces internalization and degradation of IGF-IR in vivo (18). Ganitumab does not interact with the closely related insulin receptor (INSR) but does inhibit hybrid IGF-IR/INSR receptors (18). Ganitumab has been studied in phase II trials for a variety of advanced tumors (20, 21). In phase I/ib escalation studies, ganitumab was generally well-tolerated when administered to patients with advanced solid tumors both as a single agent and in combination with either the multit kinase inhibitor sorafenib, either of the EGFR inhibitors panitumumab or erlotinib, or gemcitabine. These combination therapies as well as ganitumab as a single agent displayed promising anti-tumor effects in patients and resulted in few dose-limiting toxicities and generally mild adverse events including fatigue, nausea, vomiting, or chills (22, 23). To date, ganitumab has shown signs of efficacy against pancreatic cancer and Ewing’s sarcoma, yet there has been minimal evaluation of ganitumab for prostate cancer.

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In vitro AKT phosphorylation studies

VCaP and 22Rv1 were plated in medium containing 10% FBS. At approximately 70% confluence, medium was replaced with fresh medium supplemented with 5% charcoal-stripped serum and cells cultured for an additional 24 hours. Ganitumab (0–1,000 nmol/L) was added 90 minutes before treatment with 1 nmol/L IGF-I. Cells were harvested in radioimmunoprecipitation assay buffer (RIPA) buffer 30 minutes after IGF-I administration and immunoblotted.

In vitro proliferation assays

LNCaP, 22Rv1, or CWR-R1 cells were seeded in 24-well plates (BD Falcon; 2 × 104 per well) in RPMI/10% FBS. VCaP cells were seeded in 24-well plates (6 × 104 per well) in Dulbecco’s Modified Eagle’s Medium (DMEM)/10% FBS. The following day, cells were washed with PBS, and media supplemented with 2% FBS and ganitumab was added and incubated for 7 days. Cells were trypsinized (Cellgro), mixed with Trypan blue (Gibco), and live cells were counted using a hemocytometer. Data represent at least 3 independent experiments carried out in triplicate.

In vitro apoptosis studies

A total of 6.5 × 105 VCaP, 22Rv1, CWR-R1, or LNCaP were plated in the appropriate medium containing 10% FBS in 60-mm plates. The following day, cell monolayers were washed with PBS, and medium supplemented with 2% FBS and ganitumab was added and incubated for 7 days. Cells were trypsinized (Cellgro), mixed with Trypan blue (Gibco), and live cells were counted using a hemocytometer. Data represent at least 2 independent experiments.

Western blot analyses

Western blot analyses were conducted as previously described (32, 34). Antibodies against phospho-AKT (S473), total AKT, IGF-IR, and cleaved PARP were obtained from Cell Signaling, INSR, actin, and horseradish.

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peroxidase–conjugated secondary antibodies were obtained from (Santa Cruz). Densitometry was conducted using Adobe Photoshop CS3.

Xenograft studies
Studies involving animals were conducted in a manner approved by the University of Miami Animal Care and Use Committee. VCaP xenografts were established as previously described (32, 34). 22Rv1 cells (2 × 10^7) were injected subcutaneously into both hind flanks of castrated nude mice (Harlan) with Matrigel (BD Biosciences). Tumor volumes were assessed as previously described (34). Mice were euthanized when tumors reached a maximal volume of 1,000 mm^3 or 10 weeks posttreatment of androgen-replete VCaP xenografts; and 1,000 mm^3 or 17 weeks posttreatment of castration-resistant VCaP xenografts; and 1,000 mm^3 or 7 weeks posttreatment of castration-resistant 22Rv1 xenografts; upon which tumors were excised, and flash-frozen. Levels of circulating PSA were quantified from serum samples by an ELISA (Biocheck Inc.). Levels of IGF-IR, INSR, phospho-AKT, and total AKT were measured using either MSD multiplex assays (Meso Scale Discovery) as previously described (5, 18) or densitometry as described earlier. Ganitumab (Amgen Inc.) and control anti-streptavidin IgG1 antibody (Amgen Inc.) were diluted in sterile PBS and administered via intraperitoneal injections at a dosage of 300 μg twice weekly as specified.

Results
Ganitumab inhibits IGF-IR signaling in androgen-dependent and castration-resistant prostate cancer cells in vitro
To assess the potential use of ganitumab for treatment of both androgen-dependent and CRPC, we determined whether androgen-dependent VCaP and castration-resistant 22Rv1 were sensitive to IGF-I stimulation. Serum deprivation decreased AKT phosphorylation, whereas IGF-I treatment induced phosphorylation of AKT, a downstream effector of IGF-I/IGF-IR signaling in both VCaP and 22Rv1 cell lines (Fig. 1A and B). Administration of ganitumab reduced IGF-I–induced phosphorylation of AKT in both cell lines. These results indicate that ganitumab inhibited IGF-I/IGF-IR signaling in both androgen-dependent and CRPC cells.

Ganitumab inhibits growth of androgen-dependent and castration-resistant prostate cancer in vivo
To assess the effect of ganitumab on prostate cancer cell proliferation, we administered ganitumab to several prostate cancer cell line models in vivo. VCaP cells are wild-type for PTEN and androgen-dependent; 22Rv1 and CWR-R1 are castration-resistant, express wild-type PTEN and full-length AR as well as constitutively active AR splice variants; LNCaP do not express active PTEN and are androgen-dependent. The proliferation of VCaP, 22Rv1, CWR-R1, and LNCaP cells was inhibited by ganitumab to varying degrees (Fig. 1C–F). VCaP cells were the most sensitive to ganitumab. Ganitumab increased the level of cleaved PARP, a late-stage marker of apoptosis, in VCaP cells but had no effect on 22Rv1, CWR-R1, or LNCaP cell lines (Fig. 1G). We chose an androgen-dependent (VCaP) and a castration-resistant (22Rv1) model for further studies in vivo.

Ganitumab inhibits androgen-dependent prostate cancer growth in vivo
We used androgen-dependent VCaP xenografts to evaluate ganitumab treatment in a more physiologic setting. VCaP human prostate cancer cells were injected subcutaneously into intact mice. When tumors reached an average volume of 260 to 320 mm^3, mice were randomized into 2 groups and treatment with ganitumab or control antibody at a dose of 300 μg twice weekly via intraperitoneal injections commenced. The ganitumab dosage was based on safety and efficacy shown in previous mouse xenograft studies (5, 18). Ganitumab inhibition of tumor growth was observed beginning 1 week after treatment initiation. Growth inhibition was maintained as shown by the tumor-doubling time, which was strikingly increased from 2.3 ± 0.4 weeks in control-treated mice to 6.4 ± 0.4 weeks in ganitumab-treated mice (Fig. 2B).

The clinically relevant biomarker PSA was also assessed throughout the duration of the experiment. The PSA gene is regulated by AR and circulating PSA is used to assess biochemical recurrence in patients (35). Serum PSA doubling time was increased from 1.6 ± 0.5 weeks in control-treated mice to 4.1 ± 0.9 weeks ganitumab-treated mice (Fig. 2C), which paralleled changes in tumor volume. Levels of IGF-IR were significantly decreased in ganitumab-treated mice compared with control-treated mice, and INSR levels were unaffected by ganitumab treatment (Fig. 2D–F). Ganitumab had no effect on levels of AR (Supplementary Fig. S2A). These data show that ganitumab slows the growth of this androgen-dependent prostate cancer model.

Effect of ganitumab treatment on castration-resistant prostate cancer in vivo
To test whether ganitumab may be effective for CRPC, we used 2 xenograft models. VCaP xenografts are initially androgen-dependent but recur as castration-resistant xenografts after murine castration (27, 34). VCaP cells were injected into the flanks of intact severe combined immunodeficient mice (SCID) mice and mice were castrated when average tumor volumes reached 260 to 320 mm^3. Castrated mice with tumors averaging 538 mm^3 were randomized into 2 groups and treatment with ganitumab or continued control antibody was initiated. This experimental design allowed evaluation of ganitumab effectiveness for tumors that had recently progressed to castration-resistance.

Ganitumab halted tumor growth for the remainder of the experiment (11.5 weeks), whereas the tumors of the control-treated mice continued to grow rapidly (Fig. 3A). The ganitumab-treated mice showed decreased serum...
PSA relative to the control-treated mice at all comparable time points (Fig. 3B). Although tumor volume did not significantly increase in the ganitumab-treated mice, the serum PSA increased at later time points (15 weeks post-castration). Levels of IGF-IR were significantly decreased in ganitumab-treated mice compared with control-treated mice (Fig. 3C and D). Levels of INSR, AR, and ARD\textsubscript{LBD} were unaffected by ganitumab treatment (Fig. 3C and D and Supplementary Fig. S2B).

22Rv1 is a castration-resistant model of prostate cancer that grows readily in androgen-deprived environments including xenografts in castrated mice (30). 22Rv1 cells were injected into the hind flanks of castrated nude mice. Once tumors reached an average volume of 260 to 320 mm\textsuperscript{3}, treatment with ganitumab or control antibody commenced for 5 weeks. In this highly aggressive model of prostate cancer, ganitumab had no appreciable effect on tumor growth (Fig. 4A). There was no difference in weight between control and ganitumab-treated mice (Supplementary Table S1A). Protein levels of IGF-IR were significantly decreased in ganitumab-treated mice compared with control-treated mice (Fig. 4B and C). INSR, AR, and ARD\textsubscript{LBD} levels were not significantly changed after treatment with ganitumab (Fig. 4B and D and Supplementary Fig. S2C).

Ganitumab combined with androgen-deprivation is a highly effective treatment regimen

The standard of care for advanced or metastatic prostate cancer is androgen-deprivation therapy. Because ganitumab showed inhibitory effects on both androgen-dependent and castration-resistant VCaP prostate cancer xenografts, we tested whether combining ganitumab with androgen-deprivation therapy would show added...
benefits. VCaP cells were implanted into the hind flanks of SCID mice, mice were castrated when average tumor volume reached 260 to 320 mm$^3$. One week later, treatment with ganitumab or control antibody was initiated. A single dose of ganitumab resulted in an immediate decrease in tumor volume compared with control-treated mice after castration (Fig. 5A). Tumors of ganitumab-treated mice remained significantly smaller than those of control-treated mice throughout the remainder of the experiment (16 weeks). In addition to decreased tumor volume, combining androgen-deprivation therapy with ganitumab lengthened time to recurrence in the 17-week experiment. A, tumor volumes (mm$^3$) are shown relative to treatment initiation ($n = 3–4$ for control; $n = 4–8$ for ganitumab). B, tumor-doubling times for control- and ganitumab-treated mice are shown ($n = 4$ for control; $n = 8$ for ganitumab). C, serum PSA doubling times are shown ($n = 4$ for control; $n = 8$ for ganitumab). D, xenograft lysates were immunoblotted for IGF-IR, INSR, and actin for control-treated and ganitumab-treated tumors. Western blot analyses were quantified by densitometry and relative IGF-IR (E) and relative INSR (F) normalized to actin are shown. All data are displayed as SEM (‘P < 0.05; ‘‘P < 0.01; two-tailed Student t test).
experiment from 3.1 ± 0.7 weeks (control-treated) to 16.5 ± 0.7 weeks (ganitumab-treated) with recurrence defined as tumor volume surpassing that at time of castration as determined for each tumor individually (Fig. 5B). Serum PSA decreased to a greater extent with androgen-deprivation and ganitumab when compared with androgen-deprivation combined with control antibody (Fig. 5C). Between weeks 11 and 15, there was a sharp increase in serum PSA, which was not reflected in an increase in tumor volume. Ganitumab-treated mice lost a small proportion of body weight (~10%), whereas control-treated mice did not lose weight during the experiment (Supplementary Table S1B). Total levels of IGF-IR protein in xenografts were decreased in the ganitumab-treated mice compared with intact mice and control-treated mice at 2 and 17 weeks postcastration (Fig. 5D), which is consistent with previous studies in pancreatic cancer and Ewing’s sarcoma (5, 18). INSR levels remained unchanged under all conditions tested (Fig. 5E). Levels of phosphorylated AKT and total AKT were also unaffected by androgen-deprivation, ganitumab treatment, or combined androgen-deprivation and ganitumab treatment (Supplementary Fig. S3). AR and constitutively active splice variant AR3 (AR-V7) mRNA levels were increased in end-stage ganitumab-treated tumors (10 weeks postcastration) compared with control-treated tumors (17 weeks postcastration; Supplementary Fig. S4). These data show that combination of ganitumab and androgen-deprivation...
substantially decreased tumor growth and delayed progression to castration resistance.

Reversibility and durability of treatment with ganitumab combined with androgen-deprivation therapy

We evaluated the durability of combining androgen-deprivation therapy with ganitumab treatment by examining long-term ganitumab treatment. Reversibility of ganitumab treatment was also assessed. VCaP xenografts were established and mice castrated when tumors reached an average volume of 260 to 320 mm³. Treatment with ganitumab commenced 1 week after castration. Five weeks after castration, mice were randomized into 2 groups. One group remained on ganitumab (to evaluate durability of response) and ganitumab was discontinued in the other group (to evaluate reversibility of response).

In the ganitumab discontinued cohort, all tumors (n = 4) recurred within 11 weeks (Fig. 6A). Two of 5 tumors recurred in the long-term ganitumab treatment arm (Fig. 6B). Thus, while combination therapy with androgen-deprivation and ganitumab is highly effective, tumors recur after ganitumab cessation and some tumors recur during long-term ganitumab treatment.

Discussion

A major problem in the care of advanced prostate cancer is the lack of a durable response to androgen-deprivation therapy. Ganitumab is a fully human antibody that targets and inhibits IGF-IR by blocking ligand binding that has shown promising efficacy in the treatment of pancreatic cancer and Ewing’s sarcoma (20, 22). Here, we show that ganitumab represents a new therapeutic strategy for the treatment of prostate cancer.
particularly when combined with the standard of care, androgen-deprivation. Ganitumab effectively inhibited the IGF-IR signaling axis in multiple human prostate cancer cell lines by blocking IGF-I–induced AKT phosphorylation. Ganitumab also decreased proliferation in vitro, albeit to varying extents of both androgen-dependent and castration-resistant human prostate cancer cell lines. VCaP cells exhibited the highest sensitivity to ganitumab of all tested cell lines. Ganitumab increased apoptosis in VCaP cells but had minimal effects on cleaved PARP in LNCaP, CWR-R1, and 22Rv1 cells.

PTEN has been suspected to play a role in the efficacy of IGF-IR inhibitors as PTEN is a negative regulator of the PI3K/AKT pathway, which is major downstream effector of IGF-IR (7, 24). PTEN deficiencies lead to highly active, hyperphosphorylated AKT in the absence of exogenous signaling (36). PTEN is not expressed or is inactive in a significant number of prostate cancers (29, 37). The prostate cancer cell model LAPC-4, which harbors a wild-type PTEN, was recently shown to be insensitive to ganitumab in vitro (38). Harboring wild-type PTEN does not seem to be sufficient for sensitivity to ganitumab as also evidenced by a lack of response of 22Rv1 xenografts in castrated mice. LuCaP 35, a PTEN-null prostate cancer cell model, is inhibited through blockade of the IGF-IR signaling axis (12–14). These findings are in line with results.
from a recent phase Ib clinical trial in which PTEN status in advanced solid tumors did not correlate with response to ganitumab (23). Thus, the inhibitory effect of ganitumab does not seem to be solely due to blockade of the IGF-IR/AKT signaling cascade and PTEN status alone is unlikely to serve as a biomarker for ganitumab response.

The case-control study ProtecT associated changes in the circulating IGF-I signaling axis that promote increased IGF signaling with prostate cancer detected through PSA screening. This large-scale study conducted in the United Kingdom showed that PSA-diagnosed prostate cancer is positively associated with circulating levels of IGFBP-2, IGFBP-3, and possibly with IGF-II but not with IGF-I (39). Thus, the ProtecT study provides support for the use of IGF-IR inhibitors, such as ganitumab, which inhibits binding of both IGF-I and IGF-II to IGF-IR, as a treatment of prostate cancer.

A recent phase II study of treatment-naive patients with localized prostate cancer showed that preoperative administration of figitumumab, an IGF-IR inhibitor, decreased serum PSA, and AR expression (15). Similarly, we found that ganitumab inhibited VCaP xenograft growth and decreased serum PSA under androgen-replete conditions. However, we did not observe differences in AR or AR splice variant levels in end-stage tumors of ganitumab-treated compared with control mice. VCaP cells express high levels of AR that do not seem to be regulated by IGF-IR signaling, but nonetheless ganitumab decreased PSA indicative of diminished AR activity. These data suggest that ganitumab may be clinically beneficial before androgen-deprivation therapy in the treatment of prostate cancer, although a role for AR is currently unclear.

Castration-resistant VCaP xenograft growth was also inhibited by ganitumab treatment. Ganitumab prevented significant increases in tumor volume in these castration-resistant xenografts throughout the duration of treatment (11.5 weeks). Because there are limited treatment options for established castration-resistant tumors, blocking IGF-IR with ganitumab may be an effective strategy in this setting.

Ganitumab treatment did not inhibit castration-resistant growth of 22Rv1 xenografts (Fig. 2) or growth of 22Rv1 tumors in intact (androgen-replete) mice (38). Thus, 22Rv1 cells exhibit de novo resistance to ganitumab in vivo. In contrast, VCaP xenografts were strikingly growth inhibited in the presence or absence of androgen in vivo. These cell lines are distinct. 22Rv1 are an aggressive CRPC line developed by serial passage of androgen-dependent CWR22 xenografts in castrated mice (30). 22Rv1 proliferate in the absence of serum (32), whereas VCaP cells rely on serum for growth and are initially androgen-dependent taking several weeks to achieve castration-resistance in vivo (34). VCaP express higher levels of IGF-IR compared with 22Rv1. VCaP and 22Rv1 also express different levels of AR (wild-type in VCaP, mutated in 22Rv1) and AR-LBDs. VCaP express higher levels of ERG than 22Rv1 and express the TMPRSS2:ERG fusion protein, seen in approximately 50% of prostate cancer specimens, whereas 22Rv1 do not contain the TMPRSS2:ERG fusion (40, 41). These data suggest that 22Rv1 and VCaP rely on different signals for survival and proliferation, which may underlie differential response to ganitumab. Thus, more mechanism-based research is needed to stratify patients based on molecular and genetic features of their prostate tumors.

Figure 6. Evaluation of durability and reversibility of combined ganitumab and androgen-deprivation therapy on VCaP xenografts. VCaP xenografts were established in intact mice, and mice were castrated when tumors reached an average volume of 260 to 320 mm³. Beginning 1 week after castration, mice were treated with ganitumab for 4 weeks. Mice were randomized into 2 groups and treatment with control antibody was initiated or ganitumab was continued. A, discontinuation of ganitumab after 4 weeks (5 weeks postcastration) resulted in recurrence of all VCaP tumors (n = 4; †, deceased mouse due to causes unrelated to treatment). B, long-term treatment with ganitumab resulted in recurrence in 2 of 5 tumors. (Average tumor volume is reflected in Fig. 5A.) C, quantification of recurrence after ganitumab discontinuation (reversibility) and long-term ganitumab treatment (durability) is shown.
In our preclinical study, ganitumab was most effective when combined with a more complete androgen-deprivation therapy. To date, no clinical trials have combined complete androgen-deprivation therapy (using abiraterone or the AR antagonist enzalutamide) with IGF-IR inhibition. On the basis of clinical trials, ganitumab is also suitable and well-tolerated alone and in combination with a variety of chemotherapeutic agents including the multi-kinase inhibitor sorafenib, EGF-R inhibitors panitumumab and erlotinib, or the nucleoside analog gemcitabine (23). In fact, the only patient with prostate cancer who participated in this study showed a partial response (~60% maximum change from baseline tumor size) in response to ganitumab plus gemcitabine.

PSA is a clinical biomarker used extensively to evaluate incidence and recurrence of prostate cancer. Recent studies have shown wide variability in PSA levels and suggest that PSA may not be suitable as a surrogate end point in clinical trials involving noncytotoxic drugs such as ganitumab (42). We found that PSA levels were increased with no parallel increase in tumor volume, AR expression, or ARALBD expression in ganitumab-treated mice harboring castration-resistant VCaP xenografts. Mice treated with both androgen-deprivation therapy and long-term ganitumab showed increased AR and AR3 mRNA levels compared with control-treated mice. However, our studies were only able to assess transcript levels in this setting, which are not necessarily indicative of protein expression or function (transcriptional activity). The molecular basis for the discrepancy between PSA levels and tumor volume in our preclinical models is not known but suggests that serum PSA may not be an ideal biomarker for clinical evaluation of recurrence during treatment with ganitumab.

Some VCaP xenografts acquired resistance to the combined treatment of ganitumab and androgen-deprivation (castration). Development of resistance to therapy is a major and common problem in cancer care. In models of Ewing’s sarcoma, upregulation of signaling by the closely related INSR is an important mechanism of resistance to ganitumab (5). However, we saw no increase in levels of INSR in any xenograft experiments or increased phospho-AKT1 levels when ganitumab was combined with androgen deprivation, thus it is unlikely that upregulation of INSR is a mechanism of resistance to ganitumab in either VCaP or 22Rv1 prostate cancer cell models. Because mTOR can potentiate compensatory pathways, coadministering ganitumab with an mTOR inhibitor may be effective in combating drug resistance. Further investigation into the mechanism of acquired resistance to ganitumab in prostate cancer is needed.

Our data suggest that ganitumab may be beneficial in the treatment of both androgen-dependent and some forms of CRPC. Importantly, ganitumab plus complete androgen-deprivation therapy was highly effective. Ganitumab is currently under investigation for the treatment of several tumor types either alone or in conjunction with targeted or cytotoxic agents. Here, we showed one example of de novo resistance to ganitumab with the CRPC model of 22Rv1 cells and evidence of acquired resistance to ganitumab using VCaP xenografts. The characteristics that give rise to de novo resistance and acquired resistance to ganitumab must be understood to identify patients with prostate cancer who will have the greatest likelihood of response and to develop strategies to prevent or abrogate acquired resistance to ganitumab therapy.

Disclosure of Potential Conflicts of Interest
P.J. Beltran has ownership interest (including patents) in Amgen Inc. K.L. Burnstein has a commercial research grant from Amgen Inc. No potential conflicts of interest were disclosed by the other author.

Authors’ Contributions
Conception and design: C.D. Fahrenholtz, P.J. Beltran, K.L. Burnstein
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