Effect of a Low-Fat Diet Combined with IGF-1 Receptor Blockade on 22Rv1 Prostate Cancer Xenografts

Ramdev Konijeti1, Satomi Koyama3, Ashley Gray4, R. James Barnard4, Jonathan W. Said2, Brandon Castor2, David Elashoff3, Junxiang Wan3, Pedro J. Beltran6, Frank J. Calzone6, Pinchas Cohen3, Colette Galet1, and William J. Aronson1

Preclinical Development

Abstract

In preclinical models, both dietary fat reduction and insulin-like growth factor I receptor (IGF-1R) blockade individually inhibit prostate cancer xenograft growth. We hypothesized that a low-fat diet combined with IGF-1R blockade would cause additive inhibition of prostate cancer growth and offset possible untoward metabolic effects of IGF-1R blockade antibody therapy. Fifty severe combined immunodeficient mice were injected with 22Rv1 cells subcutaneously. Ten days postinjection, the animals were randomized to four groups: (i) high-fat diet + saline (HF); (ii) high-fat diet + IGF-1R blocking antibody, ganitumab (HF/Ab); (iii) low-fat diet + saline (LF); and (iv) low-fat diet + ganitumab (LF/Ab). After 19 days of treatment, the animals were euthanized, tumors were weighed, and serum was collected, and tumors were weighed. Tumor Ki67, Akt, and extracellular signal-regulated kinase (ERK) activation, serum insulin, IGF-I and TNF-α were measured. In vitro, ganitumab treatment inhibited growth and induced apoptosis in several prostate cancer cell lines. In vivo, tumor weights and volumes were unaffected by the different treatments. The LF/Ab therapy significantly reduced proliferation (Ki67) and ERK activation in tumors. The HF/Ab group had significantly higher serum insulin levels than the HF group. However, LF/Ab combination significantly reduced serum insulin back to normal levels as well as normalizing serum TNF-α levels. Whereas the combination of low-fat diet and IGF-1R blockade did not have additive inhibitory effects on tumor weight, it led to reduced tumor cell proliferation and a reduction in serum insulin and TNF-α levels. Mol Cancer Ther; 1–8. ©2012 AACR.

Introduction

Given the limited efficacy of current treatment regimens for advanced, castrate-resistant prostate cancer, there is strong interest in developing targeted therapies to treat men suffering from this disease (1, 2). Cancer cells are driven to proliferate by numerous pathways potentially limiting the efficacy of therapies targeting a single pathway. In addition, targeted therapies are often associated with adverse effects on the host requiring close monitoring, possible dose reduction of the therapeutic agents, and/or additional therapies (3). Preclinical and clinical studies investigating the use of low-fat diets as an "alternative therapy" for prostate cancer treatment have showed a potential role for inhibiting prostate cancer progression and improving the metabolic profiles of patients with prostate cancer (4–9). Dietary fat reduction inhibits cancer progression through a number of potential mechanisms including reduction in circulating insulin-like growth factor (IGF)-I levels and inhibition of the phosphatidylinositol-3-kinase (PI3K)-Akt pathway (10–13).

IGF-I is a peptide growth factor and a potent mitogen for the growth of androgen responsive and androgen-independent human prostate cancer cell lines. IGF-I plays a pivotal role in regulating cell proliferation, differentiation, and apoptosis (14, 15). IGF-I exerts its biological effects by binding to the IGF-1R and activating the PI3K/Akt and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways (10–12, 16). On the basis of the key role played by IGF-1 in the progression of prostate cancer as well as other malignancies including colon and breast cancer, strong interest exists in developing targeted therapies that inhibit the IGF-1 signaling pathway (17–19). An IGF-1 receptor (IGF-1R) antibody was previously found to decrease prostate cancer xenograft growth (20). Multiple biotechnology companies have developed monoclonal antibody therapies that target the IGF-1R. These antibodies are being investigated in clinical trials for the treatment of prostate cancer, both in the neoadjuvant setting (before radical prostatectomy) and in patients with metastatic, castrate-resistant prostate cancer (11). Potential
metabolic consequences of IGF-1R-targeted inhibition include elevation in blood glucose and insulin levels via feedback inhibition of the growth hormone IGF-I axis (21, 22).

Given that dietary fat reduction seems to exert its anticancer effects, in part, through inhibition of the IGF-I axis and reduction of serum insulin levels, we hypothesized that combining dietary fat reduction with IGF-1R blocking antibody therapy would cause additive inhibition of prostate cancer progression and potentially offset the induction of insulin resistance by IGF-1R inhibition.

Materials and Methods

Animal husbandry and feeding protocol

Fifty male CB17 beige severe combined immunodeficient (SCID) mice (8 weeks old) were obtained from the UCLA Department of Laboratory Animal Medicine facility (accredited by the American Association for Accreditation of Laboratory Animal Care). The mice were housed 2 mice per cage. The cages were kept in a sterile and pathogen-free facility. Cages, bedding, and water were autoclaved before their use. The feeding receptacles were on top of the cages so that food intake could be monitored and new feedings given without opening the cage. Sterile technique was used whenever handling the cages, mice, and food. The experiments were approved by the UCLA Chancellor’s Animal Research Committee, and animals were cared for in accordance with institutional guidelines.

The diets were prepared and sterilized (by irradiation) by DYETS, Inc. The high-fat diet contained 43.3% calories from fat and the low-fat diet contained 12.4% calories from fat (Supplementary Table S1). Mice were fed ad libitum throughout the experiment. Mice were fed once a week. Feeding receptacles were filled with 50 g of fresh feed once weekly.

Cell culture

22Rv1, DU145, and PC-3 cell lines were obtained through the American Type Culture Collection (ATCC; Manassas, VA, USA) and grown according to ATCC guidelines. Each cell line was tested and authenticated by ATCC. The ATCC Cell Biology program uses state-of-the-art technology platforms for the authentication of cell lines by applying the growth curve to determine optimal growth conditions and the seed stock scheme when expanding cell lines to minimize passaging. ATCC Routine Cell Biology Program includes: certification that each cell line is negative for mycoplasma, bacteria, fungi contamination; confirmation of species identity and detection of possible cellular contamination or misidentification using cytochrome C oxidase I for interspecies identification and short tandem repeat analysis (DNA profiling) for intraspecies identification; conducting of additional test methods, such as cytogenetic analysis (G-banding, FISH), flow cytometry, and immunocytochemistry as well as consistent refinement of cell growth conditions as well as documentation systems, ensuring traceability.

All cell lines were used within 6 months after resuscitation. 22Rv1 is a human prostate carcinoma epithelial cell line derived from a xenograft that was serially propagated in mice after castration-induced regression and relapse of the parental, androgen-dependent CWR22 xenograft. 22Rv1 cells express PSA and the androgen receptor (23). 22Rv1 were grown in RPMI-1640 supplemented with 10% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco/Invitrogen). DU145 and PC-3 cells are human prostate carcinoma epithelial cell lines derived from brain and bone metastasis, respectively. These cell lines are androgen resistant and do not express prostate-specific antigen. DU145 and PC-3 cells were grown in DMEM and DMEM F-12K respectively, supplemented with 10% FBS and 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco/Invitrogen), respectively.

The Los Angeles Prostate Cancer 4 (LAPC-4) cell line (a generous gift from Drs Robert Reiter and Charles Sawyer) was developed at UCLA by direct transfer of cancer cells from a patient with advanced adenocarcinoma of the prostate into the subcutaneous tissue of severe combined immunodeficiency mice. LAPC-4 produces prostate-specific antigen (PSA), has a wild-type androgen receptor, and shows features of hormone-dependent growth and metastasis (24). LAPC-4 cells were authenticated in Dr Sawyer’s laboratory using cytogenetic analysis and assessing PSA expression as described by Klein and colleagues (24). LAPC-4 were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS, 1% penicillin/streptomycin, and 10 nmol/L R1881 (Perkin-Elmer Life Sciences) and were used less than 4 months after resuscitation.

In vitro studies

For individual experiments, cells were seeded at a final density of 1 × 10^5 cells/cm^2 (24-well) or 2 × 10^5 cells/cm^2 (96-well) in plates and grown to 80% or 50% confluence, respectively. Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C. 22Rv1, DU145, LAPC-4, and PC-3 were treated with 1 μmol/L ganitumab (AMG 479; Amgen, Inc.) for 24 and 72 hours.

Apoptosis was assessed in cells growing on 24-well plates for 24 hours using Cell Death Detection ELISA PLUS for the determination of cytoplasmic histone-associated DNA fragments (Roche Applied Science) after the manufacturer’s instructions.

To assess cell growth, cells were seeded on 96-well plates, and allowed to attach overnight. Cells were incubated with 1 μmol/L IGF-1R blocking antibody (ganitumab) for 72 hours in serum-free media. CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega) was conducted according to manufacturer’s instructions. Mean ± SE values of the absorbance at 490 nm were plotted.

Experimental design

All mice were fed an HF diet for 2 weeks before being injected with 5 × 10^6 22Rv1 cells. On the day of subcutaneous injection, 22Rv1 were trypsinized and resuspended...
in serum-free RPMI-1640 at a concentration of $5 \times 10^5$ cells/0.1 mL of media. An equal volume of ice cold Matrigel (BD Biosciences) was added to the cells. 0.2 mL of the resulting solution containing $5 \times 10^5$ 22Rv1 cells was injected subcutaneously to the mice in the lateral flank. Mice continued consuming the HF diet for 10 days after injection, at which point they were randomized into 4 groups: (1) high-fat diet with intraperitoneal saline (HF, $n = 12$); (2) high-fat diet with intraperitoneal IGF-1R blocking antibody, ganitumab (Amgen, Inc.; HF/Ab, $n = 13$); (3) low-fat diet with intraperitoneal saline (LF, $n = 12$); and (4) low-fat diet with intraperitoneal ganitumab (LF/Ab; $n = 13$). Ganitumab antibody was administered at a dose of 20 mg/kg twice weekly and intraperitoneal saline was administered twice weekly. Mice were weighed and tumor dimensions were measured twice weekly. The tumor dimensions were measured using a caliper. Tumor volumes were calculated using the formula previously described: length $\times$ width $\times$ height $\times 0.5236$ (25). Nineteen days after initiation of treatment the mice were euthanized. Serum was collected by cardiac puncture and tumor tissue was harvested and weighed. Serum was stored at $-80^\circ$C. Tumor tissue was rinsed with saline. Half of the tumor was snap frozen in liquid nitrogen whereas the other half was fixed for 12 hours in 10% neutral buffered formalin and embedded in paraffin blocks for histological sections.

**Serum studies**

The levels of murine IGF-I, IGFBP-1, IGFBP-3, and murine growth hormone (mGH) were measured using in-house mouse-specific ELISA as previously described (13, 26, 27). The mouse IGF-1 assay has a sensitivity of 0.1 ng/mL and has no cross-reactivity with mouse IGF-II or human IGF-I. The intra-assay and interassay coefficient of variations are <10% in the range from 1 to 10 ng/mL. The mouse IGFBP-1 and IGFBP-3 assays have sensitivities of 0.2 ng/mL and no cross-reactivity with other IGFBPs or the human homologues. The intra-assay and interassay coefficient of variation are <6% and <8%, respectively, in the range from 1 to 6 ng/mL. Serum concentrations of mGH were determined using an RIA kit with a sensitivity of 0.02 ng/mL (National Hormone and Pituitary Program, Harbor-UCLA Medical Center, Torrance, CA, USA). Serum insulin and TNF-α were measured using LINCoplex technology with a multiplex assay kit (Millipore Corporation). The assay has no cross-reactivity with human insulin or human TNF-α. Interassay and intra-assay coefficients of variation are <12% and <5%, respectively.

**Immunohistochemistry**

Four-micrometer formalin-fixed tumor sections embedded in paraffin were stained with hematoxylin and eosin. Immunohistochemistry of representative tumor sections were conducted for Ki67 (#M7240; DAKO North America Inc.) and Pecam1/CD31 (#sc-1506; Santa Cruz Biotechnology Inc.) as previously described (28). Terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assays were done using the ApopTag Plus Peroxidase In Situ Apoptosis Kit (#S7101; Millipore), as previously described (28). A total of 200 cells were counted for each xenograft tumor and the number of either Ki67 or TUNEL-positive cells was scored by a single-blinded pathologist. The number of positive nuclei was expressed as a percentage by dividing the number of positive stained nuclei by the total number of cells counted. For Pecam1/CD31, the number of vessels were counted in five $20\times$ fields for each stained slide.

**Western blot analysis**

Xenograft tumor tissues were lysed using 100 μL of RIPA buffer supplemented with an EDTA-free protease inhibitor cocktail and PhosSTOP tablets as recommended by the manufacturer (Roche Applied Bioscience) and clarified by centrifugation. Equal amounts of protein were separated on SDS gels and electrophoretically transferred to polyvinylidene fluoride membranes for Western blotting. The IGF-1R (#3027), Insulin Receptor (#3025), and PTEN (#9552) antibodies were purchased from Cell Signaling and used at a 1:1,000 dilution in 5% milk blocking solution. The Phospho-Ser<sup>473</sup>-Akt antibody (#4691) was purchased from Cell Signaling and used at a 1:1,000 dilution in 1% BSA blocking. The extracellular signal-regulated kinase (ERK)2 (#sc-154) and phospho-ERK1/2 (#sc-7383) antibodies were purchased from Santa Cruz and used at a 1:5,000 dilution in 5% milk and 1% BSA blocking solutions, respectively. All primary antibody incubations were done overnight at 4°C and followed by a constant 1-hour incubation at room temperature with a 1:2,000 dilution of the appropriate anti-mouse or anti-rabbit secondary antibody covalently coupled to horse-radish peroxidase (Bio-Rad Laboratories Inc., Cat. #172-1011 and #170-6515, respectively). All immune complexes in the Western blots were visualized using the Pierce ECL Western Blotting Substrate (ThermoScientific) and exposed to film. The quantitative analysis presented was done with ImageJ (29).

The phospho-ERK1/2 and phospho-Ser<sup>473</sup>-Akt Western blots were stripped using a mild stripping buffer (0.2 M glycine, 0.1% SDS, and 1% Tween 20; pH 2.2) for 10 minutes at room temperature. After blocking, the membranes were incubated with a rabbit anti-ERK2 antibody (Santa Cruz Biotechnology, Cat. #sc-154) at a 1:5,000 dilution in 5% milk overnight at 4°C for the phospho-ERK1/2 blot and with a rabbit anti-Akt (Cell Signaling, Cat. #4691) at a 1:1,000 dilution in 5% milk overnight at 4°C for the phospho-Ser<sup>473</sup>-Akt blot. The immunocomplexes were detected as described earlier. This allowed for correction of the phospho-ERK1/2 and phospho-Akt signals for the total amount of ERK1/2 and Akt protein present.

**Statistical analysis**

For in vitro studies, statistical analyses were conducted by using an unpaired nonparametric Mann–Whitney test. All other statistical analyses were conducted using
unpaired t tests or 1-way ANOVA followed by a Tukey post-hoc test using GraphPad Prism4 (GraphPad Software Inc.). In all cases, statistical significance was considered at P < 0.05.

Results
Sensitivity of prostate cancer cell lines to ganitumab
As shown in Fig. 1A, the IGF-1R blocking antibody ganitumab significantly inhibited 22Rv1 and DU145 cell growth compared with serum-free media (25% and 17% reduction, respectively). Ganitumab was also found to significantly induce apoptosis compared with serum-free media in 22Rv1 and DU145 (21% and 20%, respectively; Fig. 1B). Ganitumab treatment did not result in significant change in LAPC-4 or PC-3 cells growth (Fig. 1A) or apoptosis (Fig. 1B). Overall, the 22Rv1 cell line had the highest sensitivity to ganitumab treatment in terms of cell growth and apoptosis. Therefore, 22Rv1 cell line was chosen for the xenograft study.

Modulation of the IGF axis by IGF-1R blockade and low-fat diet
As shown in Fig. 2, mice receiving ganitumab had significant reductions in IGF-1R levels in 22Rv1 xenografts as measured by Western blot analysis. No significant change in insulin receptor levels was observed (Fig. 2A). Serum IGF-I and IGFBP-3 levels were significantly elevated in the HF/Ab and LF/Ab groups (Fig. 2B and C) relative to the HF control and LF control groups, respectively. The LF group had significantly lower serum IGF-I levels relative to all other groups (Fig. 2B). No changes were observed between the groups in circulating IGFBP-1 levels (Fig. 2D) or mGH levels (data not shown).

Tumor volumes and final tumor weights
There was no significant difference in final tumor volumes or final tumor weights between the treatment groups (Table 1). At day 14 of the intervention, the mean tumor volume was significantly lower in the LF/Ab group compared with the HF group (Table 1). The animal weights were not significantly different between the groups.

Reduced proliferation and ERK activation in the LF/Ab group
Xenografts from the LF/Ab group had a 30% decrease in proliferation as measured by Ki67 immunostaining relative to the other groups (Fig. 3). Although there was no difference in tumor PTEN and phospho-Akt levels between the groups, phospho-ERK was significantly decreased in the LF/Ab tumors relative to the tumors in the HF and HF/Ab groups (Fig. 4A and B). There was no difference in apoptosis or angiogenesis between the groups (data not shown).

Metabolic effects of the low-fat diet and ganitumab treatment
The HF/Ab group had 57% higher serum insulin levels as compared with the HF control group (P < 0.05). However, combining a low-fat diet with IGF-1R antibody therapy reduced serum insulin levels significantly by 40% relative to the HF/Ab group (Fig. 5A, P < 0.05). Similarly, the LF/Ab group also had significantly lower mean serum TNF-α levels relative to the HF group (P < 0.05; Fig. 5B).

Discussion
Both IGF-1R blocking therapy and dietary fat reduction were previously found to inhibit prostate cancer xenograft growth (4, 6, 20). Given that dietary fat reduction seems to exert its anticancer effects, in part, through inhibition of the IGF-I axis, we hypothesized that combining dietary fat reduction with IGF-1R blocking antibody therapy would cause additive inhibition of prostate cancer progression. Similarly, given that dietary fat reduction was previously found to decrease serum insulin levels (4), we hypothesized that combining a low-fat diet with IGF-1R blocking...
therapy would offset the hyperinsulinemia associated with IGF-1R blocking therapy. In this study, neither dietary fat reduction, IGF-1 receptor blocking therapy, or combination therapy affected tumor weight nor tumor volume at the time of euthanasia. However, combining dietary fat reduction with IGF-1R blocking therapy resulted in decreased tumor proliferation and decreased ERK activation. Dietary fat reduction also offset the increased serum insulin observed with ganitumab antibody therapy.

Of the 4 cell lines tested in vitro, 22Rv1 and DU145 responded to ganitumab treatment with a significant decrease in cell growth and increase in apoptosis. The 22Rv1 cell line was chosen for the xenograft experiments because they responded similarly to the IGF-1R blocking therapy as previously described for the LNCaP cells (20). Dietary fat reduction also offset the increased serum insulin observed with ganitumab antibody therapy.

Table 1. Tumor volumes and weights

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor volume (mm³) at 14 d</th>
<th>Tumor volume (mm³) at sacrifice</th>
<th>Tumor weight at sacrifice (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>336.9 ± 53.7</td>
<td>450 ± 55.1</td>
<td>950.8 ± 138.4</td>
</tr>
<tr>
<td>HF/Ab</td>
<td>223.4 ± 40</td>
<td>333.4 ± 59.1</td>
<td>585 ± 81.1</td>
</tr>
<tr>
<td>LF</td>
<td>247.1 ± 28.6</td>
<td>366.7 ± 48.2</td>
<td>714.2 ± 4.1</td>
</tr>
<tr>
<td>LF/Ab</td>
<td>190.4 ± 36.6*</td>
<td>323.7 ± 39.4</td>
<td>594.2 ± 99.8</td>
</tr>
</tbody>
</table>

*P < 0.05.
potentially be explained by the fact that the animals were fed ad libitum with 2 mice per cage to mirror normal human lifestyle and therefore did not receive isocaloric diets as previously described (4, 13), however, prior studies found dietary fat reduction inhibited LNCaP xenograft growth in mice fed ad-libitum (6). Because the animals were not housed 1 mouse per cage and we did not equalize caloric intake between the groups, we are unable to determine the total fat intake of the mice in each group—this represents a limitation of our study. In this study, dietary fat reduction induced a significant 20% decrease in serum IGF-I levels however did not result in a significant decrease in serum insulin or increase in serum IGFBP-1 as previously described (4, 13). LF group insulin and IGFBP-1 levels, although not significantly different than that of the HF group, showed a high level of variation (see error bar) between animals of the LF group. IGFBP-1 and Insulin are biomarkers of nutritional status thus are sensitive to caloric intake. This high variability was not observed in our previous study where the same diets were used in an isocaloric model (4). The lack of effect of monotherapy with dietary fat reduction or IGF-1R blockade suggests that the IGF-1 pathway may not be the main pathway supporting 22Rv1 xenograft growth. The cells may have developed a compensation mechanism, possibly through activation of the insulin receptor or the epidermal growth factor receptor as shown by others in vitro (31, 33). 22RV1 are androgen-dependent cells, therefore the androgen pathway may be overriding the potential inhibitory effects of the IGF-1R blockade and dietary fat reduction. Given that androgens are well known to be a growth factor for prostate cancer, we have previously measured serum testosterone levels in prior high-fat low-fat mouse studies to confirm that androgen levels do not differ between the groups. In a prior article from our group, changing dietary fat did not alter androgen levels (13). We have also found that changing dietary fat intake does not alter serum androgen levels in human trials (5). We also have showed that the low-fat diet used in an isocaloric model delayed conversion from androgen-sensitive to -insensitive prostate cancer and significantly prolonged survival of SCID mice bearing LAPC-4 xenografts (38). However, the androgen receptor expression levels were not measured in these studies. Given the importance of androgens in prostate cancer physiology, future preclinical and clinical trials with IGF-1R blockade and dietary modification should also incorporate measurements of androgens and androgen receptors.

Despite the lack of effect on xenograft progression with dietary fat reduction or with IGF-1R blockade, combining IGF-1R blocking therapy with dietary fat reduction resulted in a significant 35% reduction of malignant epithelial cell proliferation. These data suggest a potential benefit of combining a low-fat dietary intervention with IGF-1R blocking therapy to enhance efficacy in the treatment of prostate cancer. IGF-1R antibody therapy has been
well tolerated in phase I and phase II clinical trials, with hyperglycemia occurring in approximately 25% of subjects (22). It is hypothesized that hyperglycemia may result from elevated human growth hormone levels (resulting from IGF-1R antibody therapy), leading to insulin resistance in insulin target tissues (39). In this study, serum insulin levels were increased by 132% in the HF/Ab group as compared with the HF group. The serum insulin levels in the LF/Ab group were significantly lower (44% reduction) as compared with the HF group. The serum TNF-α levels were increased by 132% in the HF/Ab group as compared with the HF group. The serum TNF-α levels were increased by 132% in the HF/Ab group as compared with the HF group.

In summary, dietary fat reduction combined with IGF-1R antibody blockade resulted in decreased proliferation in prostate cancer xenografts and a reduction in serum insulin and TNF-α levels. Further preclinical and clinical trials are warranted to evaluate combining dietary fat reduction with IGF-I receptor blockade to enhance the efficacy and offset the metabolic consequences of antibody therapy directed against the IGF-1 receptor.

Disclosure of Potential Conflicts of Interest

P.J. Beltran is employed by Amgen as principal scientist. P.J. Beltran and F.J. Calzone have ownership interest (including patents) in Amgen. F.J. Calzone is a consultant/advisory board member for Amgen. No potential conflicts of interest were disclosed by the other authors.

Acknowledgments

The authors thank Colin McClean and his team for their expert assistance in the SCID mouse facility.

Grant Support

This work is supported by the National Cancer Institute (NCI) Grant No. P50CA92131 (W.J. Aronson), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grant No. 2P30DK063491 (P. Cohen), and the National Institute of Aging (NIA) Grant No. P01AG034906 (P. Cohen), and Wendy and Ken Ruby, President and Secretary of the Ruby Family Foundation (R. Konijeti).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 13, 2011; revised March 20, 2012; accepted April 17, 2012; published OnlineFirst May 4, 2012.

References


www.aacrjournals.org

Mol Cancer Ther; 2012

OF7

Downloaded from mct.aacrjournals.org on May 12, 2017. © 2012 American Association for Cancer Research.


Molecular Cancer Therapeutics

Effect of a Low-Fat Diet Combined with IGF-1 Receptor Blockade on 22Rv1 Prostate Cancer Xenografts

Ramdev Konijeti, Satomi Koyama, Ashley Gray, et al.

Mol Cancer Ther  Published OnlineFirst May 4, 2012.

Updated version  Access the most recent version of this article at: doi:10.1158/1535-7163.MCT-11-1003