

A Phase II Pharmacodynamic Study of Preoperative Figitumumab in Patients with Localized Prostate Cancer

Kim N. Chi, Martin E. Gleave, Ladan Fazli, et al.

A Phase II Pharmacodynamic Study of Preoperative Figitumumab in Patients with Localized Prostate Cancer

Kim N. Chi^{1,2}, Martin E. Gleave¹, Ladan Fazli¹, S. Larry Goldenberg¹, Alan So¹, Christian Kollmannsberger^{1,2}, Nevin Murray², Anna Tinker², and Michael Pollak³

Abstract

Purpose: Activation of the insulin-like growth factor 1 receptor (IGF-IR) is implicated in prostate cancer development and progression. This study evaluated biologic and clinical effects of figitumumab, a fully human monoclonal IGF-IR antibody, in patients with localized prostate cancer.

Experimental Design: Eligible patients received figitumumab 20 mg/kg intravenously every 3 weeks for 3 cycles followed by prostatectomy. The primary endpoint was IGF-IR expression inhibition as assessed by immunohistochemistry.

Results: Sixteen patients were accrued. Median age was 63 years, median prostate-specific antigen (PSA) was 7.2 µg/L (range, 2.5–35), clinical stage was T1 in four patients and T2 in 12 patients, Gleason score ≤7 or >7 in 15 and one patients. Two patients received only 1 cycle (patient choice and grade III hyperglycemia). A PSA decline from baseline of ≥25% and ≥50% occurred in 15 (94%) and 5 (31%) of patients. Mean figitumumab concentration was 350.4 µg/mL (range, 26.3–492.8) in plasma and 51.3 µg/g (range, 27.4–79.6) in prostate tissue. Compared with pretreatment biopsies, IGF-IR expression decreased in the prostatectomy specimens in 14 of 16 patients. The mean IGF-IR immunohistochemistry visual score was 2.1 (SD = 0.6) in biopsy and 1.1 (SD = 0.5) in prostatectomy specimens ($P < 0.0001$). Androgen receptor expression was also decreased and there was a trend for a decrease in downstream IGF-IR signaling components.

Conclusions: Figitumumab is biologically active in prostate cancer. PSA declines in treatment-naive patients were observed, potentially mediated by IGF-IR effects on androgen receptor expression. These results support the clinical relevance of IGF-IR signaling in prostate cancer and justify further clinical trials.

Introduction

The insulin-like growth factor (IGF) axis is composed of 2 peptide growth factors (IGF-I and -II), 2 transmembrane receptors (IGF-IR and -IIR), 6 IGF-binding proteins (IGFBP-1 to -6), and IGFBP proteases. IGFs are synthesized primarily in the liver and have effects on protein and carbohydrate metabolism but also regulate cellular processes of proliferation, differentiation, and apoptosis (1). These later attributes have resulted in the IGF axis being associated with a critical role in the development of a number of malignancies including prostate cancer (2). IGF-IR is a transmembrane receptor tyrosine kinase that is widely expressed in human tissues. Binding of IGF ligands induces conformational changes of IGF-IR and activation of its intrinsic

intracellular tyrosine kinase activity. The activated receptor induces recruitment of the insulin receptor substrates (IRS) 1 and 2, which in turn activates the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3-kinase (PI3K)/Akt intracellular signaling pathways, leading to cellular proliferation and apoptosis inhibition.

High blood concentrations of IGF-I has been associated with a risk of prostate cancer in meta-regression analysis (3), and high plasma IGF-I and low IGFBP-3 has been associated with more advanced stages of prostate cancer (4, 5). In human primary prostate cancers, IGF-IR, IGF-I, and -II have all been reported to be increasingly expressed compared with normal prostate tissue (6–8) and is also increased in advanced and metastatic disease (9, 10). In the TRAMP model, prostate cancer incidence is substantially reduced in IGF-I-deficient mice, whereas organ-specific overexpression of IGF-IR increased prostate neoplasia (11). In pre-clinical studies, the IGF axis appears to be upregulated in bone metastases (12) and IGF-I has been showed to accelerate tumor growth (13), promote migration through PI3K/AKT (14), and be involved with angiogenesis through VEGF (15). An increasing body of evidence has linked activation of the IGF axis with castrate-resistant progression of prostate cancer including via ligand-independent

Authors' Affiliations: ¹Vancouver Prostate Centre; ²BC Cancer Agency, Vancouver, British Columbia; and ³McGill University, Montreal, Quebec, Canada

Corresponding Author: Kim N. Chi, BC Cancer Agency, 600 West 10th Avenue, Vancouver, BC V5Z 4E6, Canada. Phone: 604-877-6000; Fax: 604-877-0585; E-mail: kchi@bccancer.bc.ca

Translational Relevance

This study of preoperative administration of single-agent figitumumab, an insulin-like growth factor 1 receptor (IGF-IR) antibody, to patients with prostate cancer undergoing prostatectomy shows biologic activity with decreased IGF-IR expression in prostate cancers. This effect was also associated with a decrease in prostate cancer androgen receptor expression and a prostate-specific antigen decline in the majority of patients. These novel data support the hypothesized role of the importance of IGF signaling in prostate cancer development and progression and justifies further clinical trials targeting this pathway.

activation of the androgen receptor (16) and increases in IGF-I (17), IGF-IR (10), IGFBP-2 (18), and IGFBP-5 (19).

Thus, targeting the IGF axis is an attractive concept as a treatment for prostate cancer. Preclinical studies have supported this approach using a variety of IGF pathway inhibitors (20–22). In both castrate-sensitive and -resistant prostate cancer models, activity has been observed with IGF-IR-specific monoclonal antibodies which induced tumor cell apoptosis, cell-cycle arrest, and downregulation of ligand-independent, androgen-regulated gene expression (23).

Figitumumab is a fully human IgG2 antibody with high affinity for the IGF-IR. Figitumumab induces IGF-IR downregulation by promoting receptor internalization and degradation. No figitumumab-related dose limiting toxicities were identified at doses up to 20 mg/kg in phase I studies and dose-dependent IGF-IR downregulation on granulocytes and increases in circulating IGF-I have been observed (24, 25). We undertook this preoperative study to evaluate the effects of figitumumab in prostate cancer cells directly. Treatment of men with figitumumab before surgery permits evaluation of the prostatectomy specimens for changes in expression of IGF-IR and related proteins allowing for proof of principle determination of biologic activity, ascertainment of figitumumab concentrations within target tissues, and gain insights into the downstream effects of IGF-IR inhibition and IGF biology in human prostate cancer.

Patients and Methods

Eligibility criteria

To be eligible, patients had to have biopsy confirmed adenocarcinoma of the prostate and previously untreated, with radical prostatectomy already decided as the planned primary treatment. Patients had to have a minimum of 2 positive biopsies and at least one of the following: serum prostate-specific antigen (PSA) >10 µg/L, Gleason score 7 to 10, and/or Gleason score 6 cancer and ≥3 biopsies positive. Other inclusion criteria were an Eastern cooperative oncology group performance status 0–1, white blood cell count of $\geq 3.0 \times 10^9/L$, hemoglobin ≥ 100 g/L, platelets

$\geq 100 \times 10^9/L$, and normal serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and creatinine. Patients on heparin or coumadin anticoagulation were ineligible. The study was approved by the Institutional Review Board and all patients provided written informed consent.

Treatment and follow-up plan

Figitumumab 20 mg/kg was delivered as a 2.5-hour intravenous infusion. Each course of treatment was composed of 3 injections of figitumumab given every 21 (± 3) days followed by prostatectomy within 7 days of the last figitumumab dose. There were no dose adjustments. Treatment was discontinued in the event of any serious adverse reaction. Patients did not receive any androgen deprivation or nonsteroidal antiandrogen therapy. Patients were assessed with a history and physical examination, and laboratory tests (complete blood count, serum creatinine, electrolytes, bilirubin, AST, ALT, bilirubin, γ -glutamyltransferase, glucose, PSA and testosterone, IGF-I, IGF-II, and C-peptide) at baseline, before each figitumumab infusion, and postoperatively (at 1–2 weeks and 3, 6, and 12 months). Blood samples were taken at baseline and presurgery for measurement of IGF-IR on circulating granulocytes by quantitative flow cytometry.

Plasma and tissue figitumumab concentrations

Figitumumab plasma and prostate tissue concentrations were quantitatively determined using a validated ELISA (ALTA Analytical Laboratory). The plasma sample was collected preoperatively (within 24 hours). Approximately 1 cm³ of the prostatectomy specimen was collected and immediately frozen on dry ice. All samples were stored at -80°C until analysis.

Tissue analyses

Immunohistochemistry staining was conducted by Ventana autostainer model Discover XT (Ventana Medical System) with enzyme-labeled biotin streptavidin system and solvent resistant DAB Map kit by using androgen receptor (sc-816), IGF-IR (sc-713), and phospho-IGF-IR (sc-101703) antibodies (Santa Cruz Biotechnology Inc.); and phospho-AKT/Ser473 (3787), phospho-p44/42 MAPK (Erk1/2; 4376), and phospho-S6 (5364) antibodies (Cell Signaling Technology Inc.). Using a categorical compositional scoring method, the sum of intensity level multiplied by percentage of cells at each intensity level was calculated. Values on a 4-point scale were assigned to each immunostain. Descriptively, 0 represented no staining by any tumor cells; 1 represented a faint or focal, questionably present stain; 2 represented a stain of convincing intensity; and 3 a stain of strong intensity. The overall score was determined as follows: overall score = [(% cells with visual score 1) \times 1] + [(% cells with visual score 2) \times 2] + [(% cells with visual score 3) \times 3]. Quantitative digital image analysis was conducted with Image-Pro Plus version 4.5.1.22 (Media Cybernetics). FISH was conducted on formalin-fixed, paraffin-embedded prostate tissue using a commercial

PTEN/CEP 10 dual-color probe (Vysis, Abbott Laboratories). PTEN copy number was determined by counting the number of probe signals in 200 nonoverlapped, intact, interphase nuclei in each sample, of which 100 are tumor nuclei and 100 are nontumor nuclei as controls. Hemizygous PTEN deletion was defined as greater than 30% (mean \pm 2 SD in the nontumor nuclei controls) of nuclei containing one PTEN signal in the presence of CEP 10 signals. Homozygous PTEN deletion was defined as greater than 20% of nuclei containing no PTEN signals in the presence of CEP 10 signals (26).

Statistical considerations

The primary endpoint of this study was the biologic response rate, defined as the proportion of patients with inhibition of IGF-IR expression as determined by immunohistochemical analysis of the prostatectomy specimen. The rate of positive expression of IGF-IR in human prostate cancer is reported at 90% to 100% with a mean immunohistochemical score of 1.4 to 2.2 (of 3; ref. 10). A biologic response rate of >30% staining inhibition was considered of interest. Using a single stage design, a total of 14 evaluable subjects were planned to be enrolled. Patients not completing 3 cycles of protocol therapy were to be replaced. The alpha level of the design was 0.05 and the power 0.9. Differences in pharmacodynamic parameters from pretreatment to posttreatment specimens were evaluated using a paired *t* test. Intergroup comparisons were compared using a Student *t* test for categorical variables. All reported *P* values are 2-sided.

Results

Patient characteristics

Sixteen patients were accrued from July 2008 to October 2009. Baseline characteristics are listed in Table 1.

Characteristic	Median (range)	Number of patients
Age, y	63 (50–71)	
PSA, ng/mL	7.2 (2.1–35)	
Clinical stage		
T1		5
T2		11
Pathologic stage		
T2		7
T3		9
Biopsy Gleason score		
6		1
7		15
9		1
Prostatectomy Gleason score		
7		11
8		3
9		2

Table 2. Related adverse events

Adverse event	Grade, <i>n</i>		
	I	II	III
Hyperglycemia	0	0	1
Hypersensitivity reaction	1	1	0
Skin rash/dryness/pruritis	3	0	0
Fatigue	3	0	0
Myalgia	2	0	0
Nausea	1	0	0
Diarrhea	1	0	0
Nausea	1	0	0
Flu-like syndrome	1	0	0
Bowel urgency	1	0	0
Alopecia	1	0	0
Dry eyes	1	0	0

Treatments administered and adverse events

Fourteen patients received all planned 3 cycles of therapy. Two patients received only 1 cycle: one patient requested to be removed from protocol therapy, another patient develop Common Toxicity Criteria for Adverse Events (version 3.0) grade III hyperglycemia. This latter patient had a preexisting history of type II diabetes on oral hypoglycemic medications and required the temporary addition of insulin to establish glycemic control. Otherwise, patients only experienced grade I and II adverse events (Table 2). There were no postoperative complications. The median time from last dose to surgery was 6 days (range, 2–7) for patients receiving all 3 cycles of figitumumab. The 2 patients who received only 1 cycle of therapy had surgery 41 and 62 days postfigitumumab dosing.

Serum PSA and testosterone

Median PSA at baseline was 7.2 μ g/L (range, 2.5–35) which declined to 4.45 μ g/L (range, 1.1–13) preoperatively. Fifteen patients (94%) experienced a PSA decline of \geq 25%, and 5 (31%) patients had a PSA decline of \geq 50%. A waterfall plot of maximal percentage PSA decline by patient is shown in Fig. 1. Mean serum testosterone at baseline was 13.7 nmol/L (range, 5.1–21; SD = 3.6). After cycle 1 of figitumumab, serum testosterone decreased in all patients by approximately 50% to a mean level of 7.2 nmol/L (range, 3.1–11.0; SD = 2.5; *P* < 0.0001) and remained at that level for the duration of treatment, although no patient had a decrease to castrate concentrations (defined as 1.7 nmol/L). Serum testosterone recovered to baseline levels by 6 months (mean testosterone, 13.8; range 8.7–19; SD = 3.2).

Figitumumab concentrations

In plasma, the mean preoperative figitumumab concentration was 350.4 μ g/mL (SD = 142.8, N = 13). Considering only patients who received all 3 cycles, the mean concentration was 406.1 μ g/mL (SD = 46.7, N = 11). The figitumumab concentration in prostate tissue was roughly 8-fold lower at 51.3 μ g/g (SD = 13.6, N = 14).

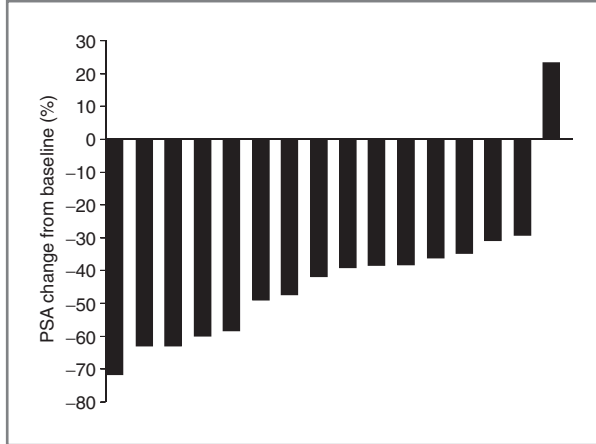


Figure 1. Waterfall plot of greatest percentage of PSA decline from baseline (columns represent individual patients).

Pharmacodynamic studies

Serum IGF levels and granulocyte expression of IGF-IR.

Mean serum IGF-I at baseline was 196.2 ng/mL (SD = 41.8) which increased significantly by approximately 5-fold to 952.8 ng/mL (SD = 246.7, $P < 0.001$) after figitumumab. C-Peptide also increased significantly by 2-fold [mean baseline = 3.7 ng/mL (SD = 2.0), preoperatively = 8.5 (SD = 3.7), $P = 0.001$] as did serum IGF-II levels to a lesser extent [mean baseline = 1,540.4 ng/mL (SD = 311.5), preoperatively = 1,963.1 ng/mL (SD = 314.7), $P < 0.001$]. The mean percentage change in granulocyte expression of IGF-IR was -34% (SD = 21; range, $+17\%$ to -54% ; $P = 0.0001$) as determined by quantitative fluorescence.

Biopsy and prostatectomy tissue assessments. IGF-IR expression was determined in biopsy and matched prostatectomy specimens (N = 16; Fig. 2). The mean immuno-

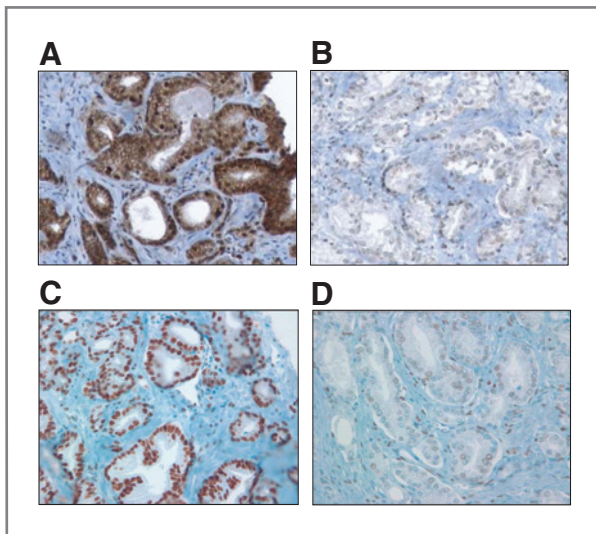


Figure 2. Representative sections from pretreatment prostate biopsy and posttreatment prostatectomy specimens for IGF1R (A, biopsy; B, prostatectomy) and androgen receptor (C, biopsy; D, prostatectomy) expression by immunohistochemistry.

histochemistry visual score was 2.1 (SD = 0.6) in the core biopsy specimens and 1.1 (SD = 0.5) in the prostatectomy specimens ($P < 0.0001$; Fig. 3). The mean percentage of cells that had 0 or 1 staining (no staining or faint or focal, questionably present stain) was 19.4% (SD = 26.2%) in the biopsy specimens and 74.4% (SD = 28.5%) in the prostatectomy specimens ($P < 0.0001$). Automated image analysis produced similar results with a positive IGF-IR staining area of 23.4% (SD = 14.3%) in the biopsy samples and 4.5% (SD = 5.8%) in the prostatectomy specimens ($P = 0.0002$; Fig. 3). Fourteen patients (88%) had a decrease in IGF-IR staining in the prostatectomy specimen and 1 patient had "stable" staining (visual overall score 2.0 in biopsy and 2.1 prostatectomy tissue). Phospho-IGF1R expression was lower in the prostatectomy specimens than in the biopsy specimens (N = 16) by mean visual overall score [0.6 (SD = 0.7) vs. 1.2 (SD = 0.7) respectively, $P = 0.0294$] and by percentage cells with no staining [64.4% (SD = 34.0%) vs. 16.9% (SD = 23.9%) respectively, $P = 0.0001$; Fig. 3].

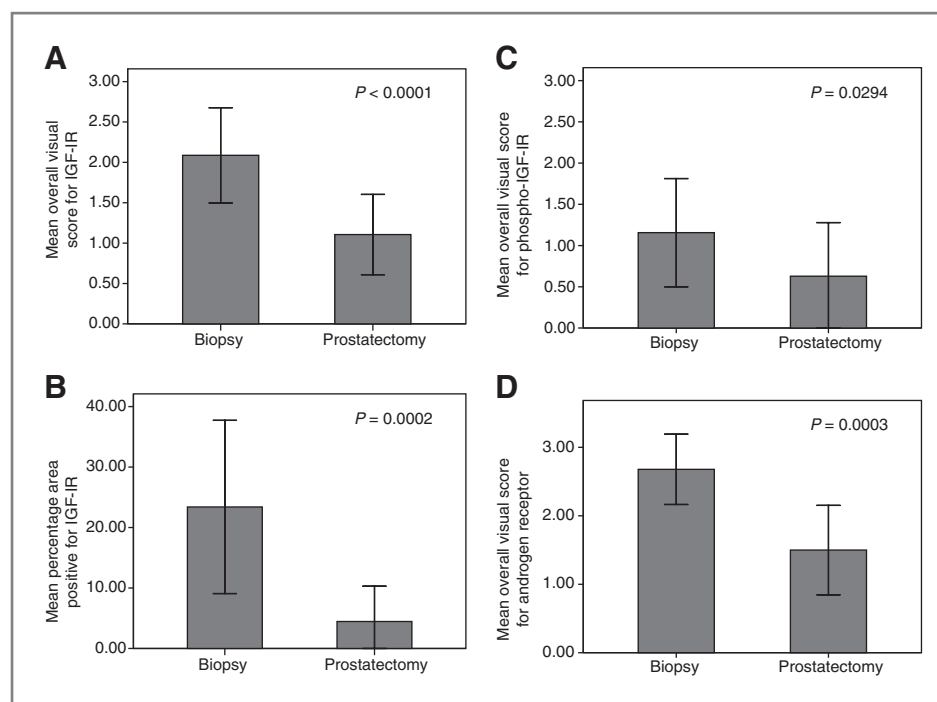
Downstream components of the IGF signaling axis were also evaluated with immunohistochemistry. Phospho-AKT visual overall score was not significantly different although there was a trend for more cells with negative staining in the prostatectomy specimens than in biopsy tissues [18.1% (SD = 34.1%) vs. 1.9% (SD = 7.5%) respectively, $P = 0.09$, N = 16]. Eleven matched specimens were available for phospho-p44/42 MAPK immunohistochemistry. There was a trend for a lower overall score for phospho-p44/42 MAPK in the radical prostatectomy specimens than in biopsy [1.1 (SD = 1.0) vs. 1.6 (SD = 0.8), $P = 0.092$] although the number of cells with no staining was higher statistically [55.5% (SD = 38.8%) in prostatectomy vs. 15.5% (SD = 27.0%) in biopsy, $P = 0.0279$]. Staining for phospho-S6 and Ki67 proliferation index (N = 13) was not different in the pre- and posttreatment tissues ($P = 0.87$ and $P = 0.24$, respectively).

Immunohistochemistry staining for androgen receptor was significantly decreased in the prostatectomy specimens compared with pretreatment biopsy (Fig. 2). Matched biopsy and prostatectomy specimens could be compared in 14 patients. Mean visual overall score was 2.7 (SD = 0.4) in the pretreatment biopsy specimen and predominantly nuclear, with a global decrease in staining with a mean visual overall score of 1.6 (SD = 0.6) in the prostatectomy tissues ($P = 0.0003$; Fig. 3). The mean percentage of cells with no staining for androgen receptor was 2.9% (SD = 6.1%) in the biopsy and 20% (SD = 20.4%) in the prostatectomy tissues ($P = 0.009$).

Nine patients had hemizygous PTEN deletions and 1 patient had a homozygous deletion (N = 16). Presence of a hemizygous or homozygous PTEN deletion did not appear to have any association with IGF-IR baseline or changes in expression, or PSA declines.

Only one patient appeared to have an increase in IGF-IR staining with the visual overall score being 1.5 in the biopsy and 1.9 in the prostatectomy specimen. Androgen receptor staining was also similar in the biopsy and prostatectomy specimen (visual overall score 1.6 for both). This patient

Figure 3. IGF-IR [visual score (A); automated image analysis (B)], phospho-IGF-IR (C), and androgen receptor (D) expression by immunohistochemistry from pretreatment prostate biopsy and posttreatment prostatectomy specimens (error bars represent ± 1 SD).



had a hemizygous PTEN deletion, figitumumab prostate tissue concentration above the mean (57.0 $\mu\text{g}/\text{mL}$), and experienced a decline in PSA by 63% from baseline. The one patient who did not experience a PSA decline (PSA increased by 22%) had a decrease in IGF1R staining score from 2.0 to 1.4, a decrease in androgen receptor staining from 3.0 to 2.2, a hemizygous PTEN deletion, and prostate tissue concentrations of figitumumab below the mean (33.4 $\mu\text{g}/\text{mL}$).

Discussion

In this study, the preoperative administration of figitumumab allowed us to evaluate figitumumab concentrations in plasma and target cancer tissue and define changes in the IGF-IR pathway in all enrolled patients. Figitumumab concentrations in prostate tissues were roughly 87% lower than in concurrently sampled plasma, but still exceeded concentrations associated with activity in tumor xenografts (EC_{50} 15 $\mu\text{g}/\text{mL}$; ref. 20). After figitumumab treatment, we observed significant decreases in IGF-IR expression and a trend for decreasing downstream signaling pathway components, showing the biologic activity of figitumumab in target cancer tissue. Previous studies with figitumumab have shown effects on IGF-IR expression on circulating granulocytes, which were also seen in this study. We also observed increasing serum levels of IGF-I and IGF-II, reflecting compensatory endocrine responses to receptor downregulation, and increasing serum C-peptide levels, indicative of increased insulin production, reflecting another compensatory response related to insulin resistance that arises secondary to elevations in growth hormone caused by IGF-I receptor blockade (27).

While blockade of IGF signaling may directly influence cell survival in a manner independent of steroid hormone signaling, there is also complex crosstalk between androgen and IGF signaling that may be relevant to our findings. IGF-IR signaling has been associated with the expression and activation of the androgen receptor through Foxo1, β -catenin, and PI3K/AKT even in the presence of androgens (16, 28, 29). In line with those *in vitro* findings, we observed a marked decrease in androgen receptor expression associated with figitumumab treatment. This effect of IGF-IR targeting on androgen receptor expression by prostate cancer cells may represent a mechanism of antitumor activity underlying the high proportion of the patients that experienced a PSA decline on this study. At the systemic level, serum testosterone was observed to decrease significantly on figitumumab treatment, a finding consistent with prior research associating IGF-I signaling and Leydig cell testosterone production (30). This may also account for some of the PSA declines observed in our study, although the decrease in testosterone was relatively modest and not to castrate levels. Recently, insulin has been shown to increase *de novo* steroidogenesis in prostate cancer cells (31) although *in vivo* studies have shown no effects of IGF-IR blockade on intratumoral androgen levels (32).

The degree of PSA declines observed in our study of patients without prior or concurrent hormone therapy is in contrast to results with IGF-IR targeting in patients with castration resistant prostate cancer. Cixutumumab, another fully human monoclonal antibody against IGF-IR, has been tested as a single agent in patients with castration-resistant prostate cancer (CRPC), but clinical activity was modest with only 3 of 31 patients experiencing a PSA reduction

(33). Preclinical data suggest that IGF-IR inhibition results in differential effects in androgen-dependent and -independent models, with induction of apoptosis in the former whereas only cell-cycle arrest in the latter (23), perhaps explaining in part the differences in PSA declines seen in this study of men with treatment-naïve, castrate-sensitive disease. Furthermore, the development of castration resistant disease has been associated with increased androgen receptor expression including by gene amplification (34) and it is possible that IGF-IR signaling effects on androgen receptor expression may be less relevant in that context. Thus, further development of IGF-IR-targeted agents in prostate cancer may be best directed to patients with early, castrate-sensitive disease. A randomized phase II study being conducted by the Southwest Oncology Group evaluating cixutumumab in metastatic castration-naïve patients is underway and will test the hypothesis whether the addition of IGF-IR inhibition therapy improves the efficacy of hormone therapy (clinicaltrials.gov identifier NCT01120236). Given the role of persistent androgen receptor signaling in CRPC, rational combination strategies of IGF-IR targeting with next generation hormone therapy such as abiraterone acetate (35) and MDV-3100 (36) should also be explored.

A limitation of this study is the lack of a randomized untreated control group, as the inherent differences in the tissue collection process between biopsies and radical prostatectomy specimens could potentially have an effect on gene expression. However, IGF-IR immunohistochemistry results in the pretreatment biopsies on this study was consistent with IGF-IR staining in prostatectomy specimens in previously published reports, which supports the robustness of these results. There was also inconsistency between pharmacodynamic effects and PSA declines including one patient who had a PSA increase despite decreased IGF-IR and androgen receptor expression. Although this discrepant observation could be an indication of a cancer that is driven independently of IGF-IR signaling, these results could also be a reflection of the small sample size, brief duration of

study treatment, the semiquantitative immunohistochemistry evaluation, and limitations of PSA as a clinical endpoint.

In summary, using preoperative administration of figitumumab, we were able to document that a dose of 20 mg/kg administered every 3 weeks resulted in plasma and tissue concentrations sufficient to lead to biologic effects as estimated by pharmacodynamic endpoints. Clinical activity was also observed, with a high proportion of patients having PSA declines. This supports the hypothesized role of IGF signaling in prostate cancer development and progression and justifies further clinical trials targeting this pathway.

Authors' Contributions

Conception and design: K.N. Chi, N. Murray, M.N. Pollak

Development of methodology: K.N. Chi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.N. Chi, M.E. Gleave, S.L. Goldenberg, A. So, C. Kollmannsberger, N. Murray, A.V. Tinker

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.N. Chi, M.E. Gleave, S.L. Goldenberg, A. So, C. Kollmannsberger, N. Murray

Writing, review, and/or revision of the manuscript: K.N. Chi, M.E. Gleave, S.L. Goldenberg, A. So, C. Kollmannsberger, N. Murray, A.V. Tinker, M.N. Pollak

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.N. Chi, N. Murray

Study supervision: K.N. Chi

Immunohistochemical evaluation of the target biomarker: L. Fazli

Patient accrual: N. Murray

Grant Support

The study was supported by a grant-in-aid from Pfizer.

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.

References

- O'Brien MF, Watson RW, Fitzpatrick JM. Insulin-like growth factor I and prostate cancer. *Urology* 2001;58:1-7.
- Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, et al. Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* 1998;279:563-6.
- Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* 2004;363:1346-53.
- Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, Pollak M, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst* 2002;94:1099-106.
- Shariat SF, Lamb DJ, Kattan MW, Nguyen C, Kim J, Beck J, et al. Association of preoperative plasma levels of insulin-like growth factor I and insulin-like growth factor binding proteins-2 and -3 with prostate cancer invasion, progression, and metastasis. *J Clin Oncol* 2002;20:833-41.
- Cardillo MR, Monti S, Di Silverio F, Gentile V, Sciarra F, Toscano V. Insulin-like growth factor (IGF)-I, IGF-II and IGF type I receptor (IGFR-I) expression in prostatic cancer. *Anticancer Res* 2003;23:3825-35.
- Ouban A, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. *Hum Pathol* 2003;34:803-8.
- Liao Y, Abel U, Grobholz R, Hermani A, Trojan L, Angel P, et al. Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. *Hum Pathol* 2005;36:1186-96.
- Hellawell GO, Turner GD, Davies DR, Poulosom R, Brewster SF, Macaulay VM. Expression of the type 1 insulin-like growth factor receptor is up-regulated in primary prostate cancer and commonly persists in metastatic disease. *Cancer Res* 2002;62:2942-50.
- Krueckl SL, Sikes RA, Edlund NM, Bell RH, Hurtado-Coll A, Fazli L, et al. Increased insulin-like growth factor I receptor expression and signaling are components of androgen-independent progression in a lineage-derived prostate cancer progression model. *Cancer Res* 2004;64:8620-9.
- DiGiovanni J, Kiguchi K, Frijhoff A, Wilker E, Bol DK, Beltran L, et al. Deregulated expression of insulin-like growth factor 1 in prostate

- epithelium leads to neoplasia in transgenic mice. *Proc Natl Acad Sci U S A* 2000;97:3455–60.
12. Rubin J, Chung LW, Fan X, Zhu L, Murphy TC, Nanes MS, et al. Prostate carcinoma cells that have resided in bone have an upregulated IGF-I axis. *Prostate* 2004;58:41–9.
 13. Kawada M, Inoue H, Masuda T, Ikeda D. Insulin-like growth factor I secreted from prostate stromal cells mediates tumor-stromal cell interactions of prostate cancer. *Cancer Res* 2006;66:4419–25.
 14. Marelli MM, Moretti RM, Procacci P, Motta M, Limonta P. Insulin-like growth factor-I promotes migration in human androgen-independent prostate cancer cells via the α v β 3 integrin and PI3-K/Akt signaling. *Int J Oncol* 2006;28:723–30.
 15. Stearns M, Tran J, Francis MK, Zhang H, Sell C. Activated Ras enhances insulin-like growth factor I induction of vascular endothelial growth factor in prostate epithelial cells. *Cancer Res* 2005;65:2085–8.
 16. Verras M, Sun Z. Beta-catenin is involved in insulin-like growth factor 1-mediated transactivation of the androgen receptor. *Mol Endocrinol* 2005;19:391–8.
 17. Nickerson T, Chang F, Lorimer D, Smeekens SP, Sawyers CL, Pollak M. *In vivo* progression of LAPC-9 and LNCaP prostate cancer models to androgen independence is associated with increased expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR). *Cancer Res* 2001;61:6276–80.
 18. Kiyama S, Morrison K, Zellweger T, Akbari M, Cox M, Yu D, et al. Castration-induced increases in insulin-like growth factor-binding protein 2 promotes proliferation of androgen-independent human prostate LNCaP tumors. *Cancer Res* 2003;63:3575–84.
 19. Miyake H, Nelson C, Rennie PS, Gleave ME. Overexpression of insulin-like growth factor binding protein-5 helps accelerate progression to androgen-independence in the human prostate LNCaP tumor model through activation of phosphatidylinositol 3'-kinase pathway. *Endocrinology* 2000;141:2257–65.
 20. Cohen BD, Baker DA, Soderstrom C, Tkalecic G, Rossi AM, Miller PE, et al. Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. *Clin Cancer Res* 2005;11:2063–73.
 21. Maloney EK, McLaughlin JL, Dagdigian NE, Garrett LM, Connors KM, Zhou XM, et al. An anti-insulin-like growth factor I receptor antibody that is a potent inhibitor of cancer cell proliferation. *Cancer Res* 2003;63:5073–83.
 22. Hailey J, Maxwell E, Koukouras K, Bishop WR, Pachter JA, Wang Y. Neutralizing anti-insulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells. *Mol Cancer Ther* 2002;1:1349–53.
 23. Wu JD, Odman A, Higgins LM, Haugk K, Vessella R, Ludwig DL, et al. *In vivo* effects of the human type I insulin-like growth factor receptor antibody A12 on androgen-dependent and androgen-independent xenograft human prostate tumors. *Clin Cancer Res* 2005;11:3065–74.
 24. Lacy MQ, Alsina M, Fonseca R, Paccagnella ML, Melvin CL, Yin D, et al. Phase I, pharmacokinetic and pharmacodynamic study of the anti-insulinlike growth factor type 1 Receptor monoclonal antibody CP-751,871 in patients with multiple myeloma. *J Clin Oncol* 2008;26:3196–203.
 25. Haluska P, Shaw HM, Batzel GN, Yin D, Molina JR, Molife LR, et al. Phase I dose escalation study of the anti insulin-like growth factor-I receptor monoclonal antibody CP-751,871 in patients with refractory solid tumors. *Clin Cancer Res* 2007;13:5834–40.
 26. Sircar K, Yoshimoto M, Monzon FA, Koumakpayi IH, Katz RL, Khanna A, et al. PTEN genomic deletion is associated with p-Akt and AR signalling in poorer outcome, hormone refractory prostate cancer. *J Pathol* 2009;218:505–13.
 27. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008;8:915–28.
 28. Fan W, Yanase T, Morinaga H, Okabe T, Nomura M, Daitoku H, et al. Insulin-like growth factor 1/insulin signaling activates androgen signaling through direct interactions of Foxo1 with androgen receptor. *J Biol Chem* 2007;282:7329–38.
 29. Manin M, Baron S, Goossens K, Beaudoin C, Jean C, Veysiere G, et al. Androgen receptor expression is regulated by the phosphoinositide 3-kinase/Akt pathway in normal and tumoral epithelial cells. *Biochem J* 2002;366:729–36.
 30. Wang G, Hardy MP. Development of leydig cells in the insulin-like growth factor-I (igf-I) knockout mouse: effects of igf-I replacement and gonadotropic stimulation. *Biol Reprod* 2004;70:632–9.
 31. Lubik AA, Gunter JH, Hendy SC, Locke JA, Adomat HH, Thompson V, et al. Insulin increases de novo steroidogenesis in prostate cancer cells. *Cancer Res* 2011;71:5754–64.
 32. Plymate SR, Haugk K, Coleman I, Woodke L, Vessella R, Nelson P, et al. An antibody targeting the type I insulin-like growth factor receptor enhances the castration-induced response in androgen-dependent prostate cancer. *Clin Cancer Res* 2007;13:6429–39.
 33. Higano C, Alumkal J, Ryan CJ, Yu EY, Beer TM, Chandrawansa K, et al. A phase II study evaluating the efficacy and safety of single agent IMC A12, a monoclonal antibody (MAb), against the insulin-like growth factor-1 receptor (IGF-IR), as monotherapy in patients with metastatic, asymptomatic castration-resistant prostate cancer (CRPC). *J Clin Oncol* 2009;27:5142.
 34. Linja MJ, Savinainen KJ, Saramaki OR, Tammela TL, Vessella RL, Visakorpi T. Amplification and overexpression of androgen receptor gene in hormone-refractory prostate cancer. *Cancer Res* 2001;61:3550–5.
 35. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 2011;364:1995–2005.
 36. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. *Lancet* 2010;375:1437–46.