Response of the Insulin-Like Growth Factor (IGF) System to IGF-IR Inhibition and Androgen Deprivation in a Neoadjuvant Prostate Cancer Trial: Effects of Obesity and Androgen Deprivation

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Context: Prostate cancer patients at increased risk for relapse after prostatectomy were treated in a neoadjuvant study with androgen deprivation therapy (ADT) in combination with cixutumumab, an inhibitory fully human monoclonal antibody against IGF receptor 1 (IGF-IR).

Objective: A clinical trial with prospective collection of serum and tissue was designed to test the potential clinical efficacy of neoadjuvant IGF-IR blockade combined with ADT in these patients. The effect of body mass index (BMI) on response of IGF-IR/insulin components to IGF-IR blockade was also examined.

Design: Eligibility for the trial required the presence of high-risk prostate adenocarcinoma. Treatment consisted of bicalutamide, goserelin, and cixutumumab for 13 weeks before prostatectomy. Here we report on an analysis of serum samples from 29 enrolled patients. Changes in IGF and glucose homeostasis pathways were compared to control samples from patients in a concurrent clinical trial of neoadjuvant ADT alone.

Results: Significant increases were seen in GH ($P = .001$), IGF-I ($P < .0001$), IGF-II ($P = .003$), IGF binding protein (IGFBP)-3 ($P < .0001$), C-peptide ($P = .0038$), and insulin ($P = .05$) compared to patients treated with ADT alone. IGFBP-1 levels were significantly lower in the cixutumumab plus ADT cohort ($P = .001$). No significant changes in blood glucose were evident. Patients with BMIs in the normal range had significantly higher GH ($P < .05$) and IGFBP-1 ($P < 0.5$) levels compared to overweight and obese patients.

Conclusions: Patients with IGF-IR blockade in combination with ADT demonstrated significant changes in IGF and glucose homeostasis pathway factors compared to patients receiving ADT alone. In the patients receiving combination therapy, patients with normal BMI had serum levels of glucose homeostasis components similar to individuals in the ADT-alone cohort, whereas patients with overweight and obese BMIs had serum levels that differed from the ADT cohort. **(*J Clin Endocrinol Metab* 98: E820–E828, 2013)**

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Abbreviations: ADT, androgen deprivation therapy; BMI, body mass index; CV, coefficient of variation; IGFBP, IGF binding protein; IGF-IR, IGF receptor 1; N, normal; OB, obese; OV, overweight; PSA, prostate-specific antigen; RRP, radical retropubic prostatectomy.

Although lower grade prostate cancers respond well to primary therapy such as surgery or radiotherapy, Gleason grades 4+3 and higher commonly recur despite the initial treatment and account for most of the 30 000 deaths that occur from prostate cancer in the United States each year. Although androgen deprivation therapy (ADT) is the mainstay of therapy and is initially effective in more than 90% of men, subsequent development of resistance is inevitable as tumors adapt to the low T environment (1). This is the case even in men treated with the newest forms of antiandrogen therapy, abiraterone and MDV3100 (2–5). Various mechanisms have been proposed to contribute to the development of resistance to systemic androgen deprivation, including maintenance of intratumoral androgen levels, alterations in androgen receptor activity, and increased reliance on other growth-stimulatory signaling pathways (3, 6, 7). These mechanisms appear to be responsible for recurrence of prostate cancer weeks to months after initial ADT. However, mechanisms are also present that result in a more immediate bypass of ADT to allow cells to survive the initial insult of ADT as well as other treatments, eg, radiotherapy or taxanes (8–10). Potential mechanisms by which the IGF receptor 1 (IGF-IR) has been shown to bypass current therapies include stimulation of intracrine androgen synthesis, survivin signaling, and enhancement of androgen receptor nuclear localization by stabilizing microtubules (11–14).

The functional importance of IGF-IR signaling in response to ADT was established by preclinical treatment studies with the anti-IGF-IR antibody cixutumumab (IMC-A12). In a series of experiments, androgen-sensitive and androgen-insensitive human prostate cancer xenografts were implanted into immunocompromised mice, then treated with cixutumumab alone (15), combined with ADT (castration) (16), or combined with docetaxel chemotherapy treatments (17). Of these treatments, the most dramatic effect was seen when IGF-IR blockade was combined with ADT (16), which caused dramatic tumor regression to nearly undetectable levels and dramatic delays in time to tumor regrowth and was persistent for up to 12 weeks after conclusion of cixutumumab treatment. Treatment with cixutumumab resulted in ablation of IGF-I-dependent nuclear localization of androgen receptor, with or without ADT (16).

Early phase human clinical trials have also shown promise for a clinical response with the use of inhibitory monoclonal IGF-IR antibodies. In a phase II clinical trial, 16 patients were treated with figitumumab every 3 weeks for 9 weeks total before prostatectomy (18). Prostate-specific antigen (PSA) declines were noted in 15 of the patients, of which 5 were decreased more than 50%. In circulating white blood cells from these patients, phosphorylation of IGF-IR and AKT were both decreased, consistent with blockade of IGF-IR signaling. Cixutumumab has been tested as a single agent in men with castration-resistant prostate cancer (19) and in combination with mitoxantrone (20) in separate phase II studies. As a single agent, median time to progression ranged from 3.2 to 3.8 months, depending on administration regimen (19). Combined with mitoxantrone, for second-line therapy after docetaxel failure, median progression-free survival was 4.2 months (20). Figutumumab has also been tested in combination with docetaxel in a phase 1B study, which included 22 patients with castration-resistant prostate cancer, and found PSA declines in 12 patients of 30% or greater, and 9 had PSA declines of 50% or greater (21).

In this paper we present the results of IGF-IR blockade, using the fully human IGF-IR monoclonal antibody cixutumumab combined with ADT in a phase II neoadjuvant trial on serum components of the IGF and insulin systems. Importantly, we used as our control group patients undergoing ADT alone. In this study we demonstrate that obesity may play a role in the changes in insulin secretion and sensitivity after IGF-IR inhibition.

Patients and Methods

Experimental sample acquisition

Patient samples were obtained from 2 concurrent clinical trials. Eligibility for the first trial (NCT00769795) (cixutumumab) required the presence of high-risk prostate adenocarcinoma (at least one of the following: Gleason 8–10, PSA $\geq$ 20, stage T2c-T3, or predicted recurrence risk exceeding 50% by the Kattan nomogram). Patients were eligible for definitive surgical intervention with curative intent as well as not being treated with androgen-altering medications or substances. Type I diabetes or uncontrolled type II diabetes was excluded in light of the hyperglycemia adverse events seen as a class effect of IGF-IR blockade in clinical trials (22). After obtaining Institutional Review Board (IRB)-approved informed consent, treatment on this single-arm phase II neoadjuvant trial started with 1 week of oral bicalutamide (50 mg/d) followed by placement of a 12-week depot goserelin implant (10.8 mg sc), biweekly iv cixutumumab infusions (10 mg/kg), and continuation of the bicalutamide for 12 weeks before prostatectomy. Nonfasting blood samples were obtained before treatment initiation (Entry) and the day of (prior to) the first, third, and fifth infusions of cixutumumab as well as on the day of radical retropubic prostatectomy (RRP). Serum and plasma were obtained from the blood draws and kept at −80°C until assayed. Control samples (no IGF-IR inhibition) were obtained in a concurrent clinical trial (NeoADT) in which patients with intermediate-to-high-risk prostate cancer (Gleason 7–10, PSA $<$ 40, clinical stage T1-T3) were treated with neoadjuvant androgen deprivation (NCT00298155). Patients were treated with 1 week of oral bicalutamide (50 mg/d) lead-in followed by placement of a 12-week depot goserelin implant (10.8 mg sc). Three patients continued with bicalutamide, and 10 patients received bicalutamide plus dutasteride for 12 weeks before RRP.

Research blood samples were obtained at time points identical to cixutumumab.

Clinical assays

Serum PSA, T, and blood glucose were analyzed by CLIA-certified clinical pathology laboratories. Laboratory results were extracted from the medical record and entered into study-specific records with minimal identifiers. These data were then linked with the results of the experimental laboratory assays by IRB-approved personnel.

Experimental sample assays

Serum insulin (Invitrogen, Camarillo, California) and C-peptide of insulin (Diagnostic Systems Labs, Webster, Texas) were measured by ELISA per manufacturer's protocol. The mean intra- and interassay coefficients of variation (CVs) for insulin were 4.8% (SD = 0.6) and 8.1% (SD = 1.08), respectively. The mean intra- and interassay CVs for C-peptide were 3.3% (SD = 0.02) and 5.9% (SD = 0.04), respectively.

Serum levels of human GH were determined by commercial ELISA kit (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions similar to above. The detection limit of this assay is 25 pg/mL, and the intra- and interassay CVs are $< 5\%$ and $< 10\%$, respectively.

Serum levels of human IGF-I, IGF-II, IGF binding protein (IGFBP)-1, and IGFBP-3 were measured by specific in-house ELISA as previously described (23). Recombinant human IGF-I, IGF-II, IGFBP-1, and IGFBP-3 standards, monoclonal antibodies, and biotinylated polyclonal antibodies were purchased from R&D Systems. The IGF-I assay has a sensitivity of 0.1 ng/mL, and intra- and interassay CVs are < 6 and $< 8\%$, respectively. IGF-II has a sensitivity of 0.2 ng/mL, and intra- and interassay CVs are $< 10\%$. Before human IGF-I and IGF-II assays, serum samples were extracted with acid/ethanol. The human IGFBP-1 assay has a sensitivity of 0.1 ng/mL, and intra- and interassay CVs are $< 10\%$. The sensitivity of human IGFBP-3 is 0.3 ng/mL, and intra- and interassay CVs are $< 6\%$ and $< 8\%$, respectively. The absorbance was measured on a plate spectrophotometer (Molecular Designs, Sunnyvale, California) at 490 nm.

Results

Clinical characteristics of the study populations

The samples analyzed in this study were obtained in a neoadjuvant trial of the addition of cixutumumab treatment to standard combined ADT in patients with high-risk prostate cancer. Serum factors of the IGF pathway and glucose homeostasis were used to interrogate the effects of this combined treatment on IGF pathway homeostasis before, during, and after the treatment period.

The characteristics of these 2 populations are shown in Table 1. As can be seen, these populations are generally similar, except that patients treated with cixutumumab had higher median PSA levels (10.6 vs 5.2), and Gleason scores (4+4 vs 4+3). Clinical stages were similar (T2a vs T2b). These data are consistent with the intended target populations of these 2 studies.

Table 1. Study Patient Characteristics

	A12		NeoADT	
Characteristic	**n**	**Median**	**n**	**Median**
PSA		10.6		5.2
<10	13		11	
10–20	9		1	
>20	7		1	
Gleason sum		4 + 4		4 + 3
3 + 4	3		5	
4 + 3	4		5	
4 + 4	10		1	
4 + 5	12		2	
Clinical T stage		T2a		T2b
T1	9		2	
T2	12		8	
T3	8		3	
BMI, kg/m^2		28.9		28
18.5–24.9	4		3	
25–29.9	14		7	
≥ 30	11		3	

Effects of cixutumumab plus ADT therapy on serum IGF pathway homeostasis

The IGF-IR is found in tissues throughout the body (24, 25). Normal homeostasis of the IGF pathway relies on feedback inhibition by IGF-I signaling through the IGF-IR in the pituitary. Loss of this feedback inhibition would be predicted to affect homeostasis at multiple levels (26). As seen in Figure 1A, cixutumumab treatment results in loss of this feedback inhibition, leading to significantly increased circulating levels of GH, IGF-I, IGF-II, and IGFBP-3 in the cixutumumab cohort pretreatment vs during treatment (GH, 4.25-fold, $P < .0001$; IGF-I, 4.0-fold, $P < .0001$; IGF-II, 1.2-fold, $P = .0264$; and IGFBP-3, 1.7-fold, $P < .0001$) as well as compared to the NeoADT cohort during therapy (GH, 2.67-fold, $P = .0111$; IGF-I, 5.7-fold, $P < .0001$; IGF-II, 1.5-fold, $P = .0034$; and IGFBP-3, 1.9-fold, $P < .0001$). Together, these data confirm that cixutumumab significantly inhibits IGF-IR signaling.

Effects of cixutumumab plus hormonal therapy on glucose, insulin, and IGFBP-1

Blood glucose is an additional factor predicted to increase in the presence of elevated GH levels. As seen in Figure 1, there were no significant increases in blood glucose in the cixutumumab-treated patients, as a group, compared to pretreatment values or to the NeoADT cohort. Two of the cixutumumab patients, however, did experience clinically significant increases in their blood glucose levels and were placed on metformin; as is standard procedure, these 2 patients were taken off metformin before prostatectomy.

In contrast to blood glucose levels, insulin and C-peptide were both significantly elevated after treatment but

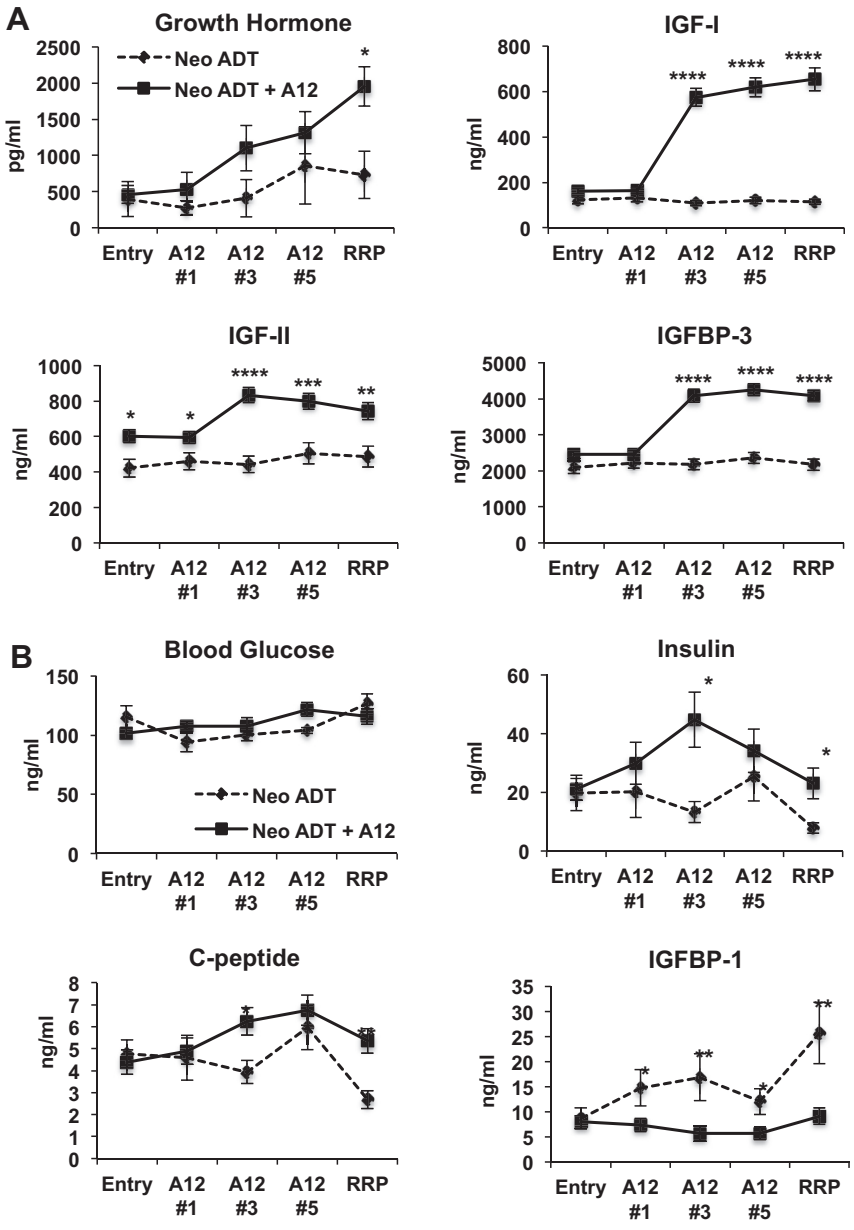

Figure 1. A, Effects of IGF-IR blockade and hormone therapy on IGF pathway homeostasis. Serum levels of GH, IGF-I, IGF-II, and IGFBP-3 were measured by ELISA. $*P < .05$, $**P < .01$, $***P < .001$, $****P < .0001$, NeoADT cohort compared to NeoADT plus A12 (cixutumumab) cohort. B, Effects of IGF-IR blockade and hormone therapy on glucose homeostasis and insulin activity. Serum levels of blood glucose, insulin, C-peptide, and IGFBP-1 were determined by ELISA. $*P < 0.05$, $**P < 0.01$, NeoADT cohort compared to NeoADT plus A12 cohort.

returned to pretreatment levels by the time of prostatectomy (Figure 1B). The elevations of insulin increased significantly by the third infusion compared to pretreatment (2.1-fold; $P = .02$) and to the NeoADT cohort (3.4-fold; $P = .02$). At the time of RRP, insulin levels were significantly higher in the cixutumumab cohort compared to the NeoADT cohort (2.9-fold, $P = .04$). Immunohistochemical staining of prostate tissue demonstrated no significant increase in insulin receptor staining in men treated with cixutumumab compared with the ADT-alone group (Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). C-peptide serum levels had similar elevations, with a significant increase at both the third and fifth infusions compared to pretreatment levels (1.4-fold, $P = .0309$; 1.5-fold, $P = .0096$) and to the NeoADT cohort at a time similar to the third infusion and at the time of RRP (1.6-fold, $P = .0255$; 2.0-fold, $P = .0038$).

The final serum marker of response to therapy was the IGFBP-1 protein, an insulin-regulated IGFBP. This protein interacts with IGF-I to modulate its activity at target tissues (27). IGFBP-1 levels in serum are negatively regulated by insulin, and thus IGFBP-1 is a measure of insulin sensitivity (27). Given the effects of cixutumumab treatment on circulating insulin, one would predict that IGFBP-1 levels would dramatically decrease upon cixutumumab treatment. However, only minimal decreases were seen by the third and fifth infusions (1.4-fold, $P = .2424$; 1.4-fold, $P = .2097$), and IGFBP-1 levels returned to pretreatment levels by the time of RRP. Interestingly, the levels of IGFBP-1 were significantly lower in patients on the cixutumumab study compared to the NeoADT cohort at all time points except entry (infusion 1, 2-fold lower, $P = .0232$; infusion 3, 3-fold lower, $P = .0054$; infusion 5, 2.1-fold lower, $P = .0164$; RRP, 2.8-fold lower, $P = .0011$). It should also be noted that the inverse was true when IGF-II levels were compared between the 2 studies; little increase was seen in the NeoADT cohort during treatment, whereas there were significant increases with cixutumumab treatment as discussed above.

In sum, changes in serum insulin, C-peptide, and glucose are all consistent with those effects predicted by effective IGF-IR blockade. The decrease in IGFBP-1 levels compared to the NeoADT cohort coupled with no significant change in glucose levels indicated that the changes in glucose contributed to cixutumumab are compensated for by an increase in insulin sensitivity based on suppression of IGFBP-1.

Serum T, PSA, and tumor volume and association with serum factors

Serum T and PSA levels were similarly decreased in both the cixutumumab and ADT-alone groups (Supplemental Figures 2 and 3). Both PSA and tumor volume were endpoints in the study. PSA levels are generally considered to reflect androgen signaling in prostate tissue because PSA is an androgen-regulated gene. Tumor volume, and specifically achieving a tumor volume of zero (pathological complete response), was the primary endpoint of the study. The 3-month treatment interval utilized in this study has been a standard duration of therapy used in multiple neoadjuvant studies before surgery and radiation, with no clear evidence that longer duration of therapy provides better clinical outcomes (28–31). PSA responses to the combination of cixutumumab and hormonal therapy achieved nadir PSA values at 0.1–3.5% of initial values. In absolute terms, nadir PSA values ranged from 0.02 to 2.25 ng/ml (median = 0.13). Using Pearson correlation coefficients with 95% confidence intervals, nadir PSA and tumor volume at the time of surgery were analyzed as continuous variables with tumor volume, serum T at surgery, insulin (peak and percentage change from baseline), and GH levels (peak and percentage change from baseline). There was no significant correlation of either PSA or tumor volume to any of these serum levels.

Association of body mass index (BMI) with IGF and glucose homeostasis markers

BMI and other factors associated with the metabolic syndrome and insulin resistance have been repeatedly linked to prostate cancer development, aggressiveness of disease, and even survival (32–35). To seek further understanding of this issue, the effects of BMI in this trial were evaluated. The patients were divided into the following groups based on their baseline BMIs: normal (18.5–24.9 kg/m^2); overweight (25–29 kg/m^2); and obese ($\geq$ 30 kg/m^2) (Table 2).

The median pretreatment BMI (28.9 kg/m^2) in the cixutumumab population was similar to that in the NeoADT cohort (28.0 kg/m^2) (Table 1). Men were given 10 mg/kg of cixutumumab; thus, obese men received more overall drug than normal-weight men. Equal increases in IGF-I, IGF-II, or IGFBP-3 levels were detected in the 3 BMI groups (Supplemental Figure 4). Although GH levels increased in all groups, the most dramatic increase occurred in the obese BMI group (infusion 5, 11.3-fold, $P = .03$; RRP, 12.4-fold, $P = .01$) (Figure 2). However, the normal BMI group had significantly higher baseline and surgery levels of GH than the other 2 groups (baseline, ANOVA $P = .02$ with normal [N] vs obese [OB] at $P < .05$; surgery, ANOVA $P = .02$, with N vs overweight [OV] and N vs OB at $P < .05$). As predicted by the known association of BMI with insulin resistance, the overweight and obese cixutumumab patients had higher, trending toward significant, treatment levels of blood glucose, insulin, and C-peptide compared to the normal BMI patients (Figure 2). When compared to the ADT-alone cohort, overweight and obese cixutumumab patients had significantly higher levels of these factors (Supplemental Figures 5–7). IGFBP-1 is inhibited by insulin; thus, increased insulin levels should correlate with decreased levels of IGFBP-1. The overweight group showed a trend toward a significant decrease in IGFBP-1 in response to cixutumumab by the third infusion, but the normal and obese BMI patients had no significant change in IGFBP-1 levels. Entry point levels of IGFBP-1 were significantly higher in the normal and overweight cohorts than the obese group (2.5-fold, ANOVA $P = .02$; N vs OB and OV vs OB, $P < .05$) (Figure 2). By the third infusion, IGFBP-1 levels remained significantly

Table 2. Patient Characteristics by BMI Stratification

	Normal (18.5–24.9 kg/m^2)		Overweight (25–29.9 kg/m^2)		Obese ($\geq$ 30 kg/m^2)		
Characteristic	**n**	**Median**	**n**	**Median**	**n**	**Median**	***P* Value**
PSA		10.3		12.4		26.3	0.09 (ANOVA), 0.06 (overweight *vs* obese)
<10	1		9		3		
10–20	3		3		3		
>20	0		2		5		
Gleason sum		4 + 4		4 + 5		4 + 4	
3 + 4	0		1		2		
4 + 3	1		0		3		
4 + 4	2		5		3		
4 + 5	1		8		2		
Clinical T stage		T2b		T2a		T2a	
T1	0		5		4		
T2	3		6		3		
T3	1		3		4		

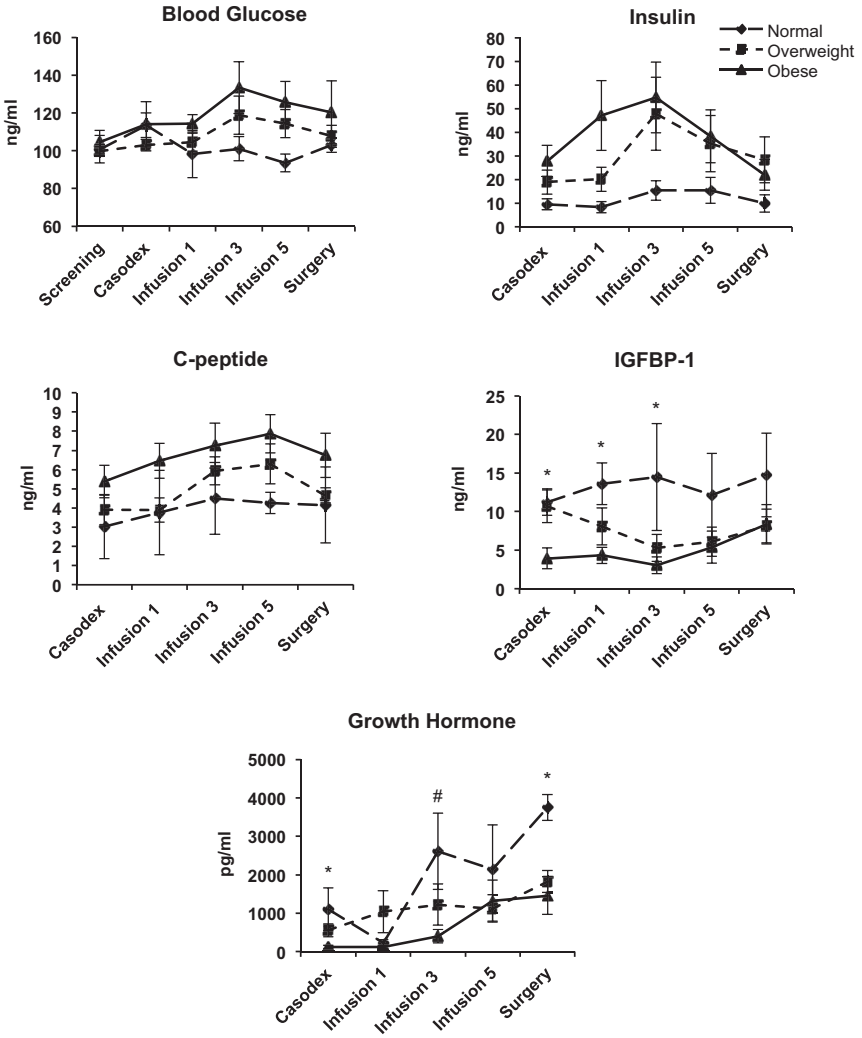

Figure 2. BMI affects serum components of the IGF and glucose homeostasis pathways. Patients were divided based on established BMI categories of normal weight (18.0–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (>30 kg/m^2). *$P < .01$, significant differences among all 3 groups using one-way ANOVA. #, Trending toward significance ($P = .06$). No changes in blood glucose, insulin, or C-peptide were seen in the normal-weight group. In contrast, overweight and obese patients trended toward increased levels of blood glucose (overweight, $P = .08$; obese, $P = .07$), insulin (overweight, $P = .09$; obese, $P = .11$), and C-peptide (overweight, $P = .07$; obese, $P = .07$) during treatment.

higher in the normal BMI group compared to the overweight and obese groups (2.7-fold and 3.5-fold, ANOVA $P = .03$, N vs OB $P < .05$). Increased levels of blood glucose after cixutumumab treatment trended toward significance in both the overweight and obese groups, but blood glucose levels were unchanged in the normal-weight men (Figure 2). These data are consistent with higher baseline circulating insulin and higher baseline insulin resistance in the obese BMI group.

Discussion

The role of the IGF pathway in cancer biology is well established, and the IGF pathway has been targeted with numerous investigational agents for anticancer therapy (25, 26, 36). Cixutumumab is a fully human monoclonal antibody that blocks signaling through the type I insulin-like tyrosine kinase growth factor receptor pathway and significantly enhances ADT to treat human prostate cancer xenografts in preclinical models (15–17). The current report examines the alterations that occur in the IGF system when IGF-IR is blocked in combination with ADT in men with treatment-naive prostate cancer. A panel of serum factors is described and employed to quantitate effects of perturbation of IGF pathway homeostasis. We also demonstrated for the first time that blood glucose, insulin, C-peptide, GH, and IGFBP-1 are all factors that showed differential changes in response to cixutumumab depending on BMI. Studying more patients undergoing cixutumumab plus ADT is necessary, however, to determine whether such changes are significantly correlated with BMI.

Numerous investigational agents targeting the IGF system in cancer are currently under clinical development. One fundamental limitation of failed clinical trials is the inability to determine the basis for the lack of efficacy. As strikingly demonstrated in the cases of the targeted agents gefitinib for lung cancer and panitumumab or cetuximab for colon cancer, the likelihood of efficacy is strongly predicted by the presence of epidermal growth factor receptor (gefitinib) or K-Ras (panitumumab and cetuximab) mutations, respectively (37, 38). This need for patient selection is likely inherent to the specificity of these targeted agents, compared to the less selective effects of traditional chemotherapy agents. In the case of the IGF-IR, because of well-defined endocrine feedback loops, the results of on-target effects and activation of associated components of the endocrine system must be assessed to fully understand the potential of this therapy. Thus, in an unselected population, it may not be possible to prove efficacy of targeted therapies, whereas highly significant effects are easily identified in appropriately selected patient subpopula-

tions (39). Altered insulin receptor signaling in vitro and pretreatment circulating levels of free IGF-I in vivo have been implicated in response to IGF-IR inhibition in sarcoma, lung cancer, and breast cancer (11, 40). However, no studies to date have identified whether these mechanisms occur in prostate cancer patients treated with IGF-IR inhibitors combined with androgen deprivation, and the clinical relevance of these data has not been confirmed in any cancer. This study is a demonstration of the power inherent to an integrated translational clinical trial design, providing the tools necessary to understand the effects of IGF-IR inhibition in the context of patients with prostate cancer receiving concurrent ADT.

We have taken advantage of the homeostatic mechanisms of feedback in the IGF pathway to identify signs of IGF-IR blockade using a panel of serum assays. Treatment-induced increases in GH, IGF-I, IGF-II, and IGFBP-3 are all consistent with relief of normal feedback inhibition at the pituitary by cixutumumab. Although IGF-I, IGF-II, and IGFBP-3 increased to a similar extent regardless of nadir PSA or BMI, GH levels had the most dramatic increases in the obese BMI group due to the very low baseline levels of GH in this group. However, as reported by others (41), GH levels are typically lower in obese patients; thus, whereas the GH levels increased dramatically in this group, the peak GH level (1456 pg/ml at RRP; 117 pg/ml at baseline) was still significantly less than the peak level in the normal BMI group (3753 pg/ml at RRP; 1105 pg/ml at baseline). However, because IGF-I levels were increased equally in men regardless of BMI, until longer term outcome data are available, whether obese men would require an increase in IGF-IR antibody dose per kilogram cannot be determined. In the normal BMI group, insulin, C-peptide, IGFBP-1, and blood glucose levels were relatively unchanged and similar to the ADT-alone cohort. In the overweight men, increased insulin and C-peptide levels resulted in an appropriate decrease in IGFBP-1, implying that these men are insulin sensitive. However, in obese men, the failure of increased insulin and C-peptide to prevent a glucose rise is consistent with peripheral insulin resistance associated with obesity. Finally, IGFBP-1 secretion by the liver is normally inhibited by insulin (27). Interestingly, obese patients start with and maintain lower levels of both GH and IGFBP-1 and display higher baseline levels of both insulin and C-peptide, suggestive of a possible increased predisposition to greater induced insulin resistance. Differential hepatic insulin sensitivity has been suggested as a mechanism for maintenance of normal glucose levels, but differential effects of hepatic insulin-sensitive proteins such as IGFBP-1 are necessary to explain how insulin-resistant individuals such as the obese men in this study may have decreased insulin effects on glucose uptake but maintain suppressive effects on insulin-sensitive hepatic protein such as IGFBP-1 (42). Of note, the glucose data in this study were obtained from blood drawn at random times throughout the day and are certainly not fasting levels. This likely contributes to the variability and decreased statistical significance of the increases in glucose, insulin, and C-peptide. In sum, our serum factor data are an indirect but compelling argument that we are achieving effective IGF-IR blockade, but at the cost of inducing peripheral and hepatic insulin resistance in obese patients.

The PSA and tumor volume analysis demonstrated that there was no clear correlation between either of these readouts of clinical efficacy and measures of T. Given that nadir PSA of 0.2 or below has been proposed as a reflection of effective suppression of tumor growth, the lack of association between PSA nadir and tumor volume is somewhat surprising (43). This may potentially reflect a lack of correlation between serum PSA and tissue androgen-regulated signaling, which will require additional tissue-based analysis. Alternatively, other pathways may be more important than androgen signaling to determine tumor growth. Data from recent publications have shown that IGF-I can signal through the insulin receptor to mediate resistance to IGF-IR blockade in breast cancer and Ewing's sarcoma cell lines (40, 44, 45). Although we did not see increased insulin receptor protein expression in the prostates of men treated with cixutumumab compared with men on ADT alone, we cannot rule out increased signaling through the insulin receptor as a means of overcoming IGF-IR blockade in these men. However, because tumor volumes were not different between groups, this suggests that the higher insulin levels did not have a stimulatory effect on tumor growth. Furthermore, the changes we saw in insulin secretion were related to obesity and suggest that use of IGF-IR inhibition may need to take into account BMI as well as basal glucose homeostasis. Hormonal changes in response to the IGF-I receptor inhibitor cixutumumab also provide new information about the GH–IGF-I axis as well as glucose homeostasis and insulin and C-peptide. Importantly, all glucose and insulin parameters returned to baseline after cixutumumab was stopped.

Together, these data provide the first evidence to date of effective blockade of the IGF-IR via specific serum factors found in the glucose and IGF-I homeostasis pathways. These data provide a starting point for scientific inquiries with the potential to understand the mechanistic differences between responsive and resistant tumors to IGF-IR blockade, to significantly improve the therapeutic effects of these agents, and to improve selection of appropriate target patient populations.

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