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A phase I trial of the IGF-1R antibody Cixutumumab in combination with temsirolimus in patients with metastatic breast cancer

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Abstract

The mammalian target of rapamycin (mTOR) plays a critical role in promoting tumor cell growth and is frequently activated in breast cancer. In preclinical studies, the antitumor activity of mTOR inhibitors is attenuated by feedback up-regulation of AKT mediated in part by Insulin-like growth factor type 1 receptor (IGF-1R). We designed a phase I trial to determine the maximum-tolerated dose (MTD) and pharmacodynamic effects of the IGF-1R antibody Cixutumumab in combination with temsirolimus in patients with metastatic breast cancer refractory to standard therapies. A 3 + 3 Phase I design was chosen. Temsirolimus and Cixutumumab were administered intravenously on days 1, 8, 15, and 22 of a 4-week cycle. Of the 26 patients enrolled, four did not complete cycle 1 because of disease progression ($n = 3$) or comorbid condition ($n = 1$) and were replaced. The MTD was determined from the remaining 22 patients, aged 34–72 (median 48) years. Most patients (86 %) had estrogen receptor positive cancer. The median number of prior chemotherapy regimens for metastatic disease was 3. The MTD was determined to be Cixutumumab 4 mg/kg and temsirolimus 15 mg weekly. Dose-limiting toxicities (DLTs) included mucositis, neutropenia, and thrombocytopenia. Other adverse events included grade 1/2 fatigue, anemia, and hyperglycemia. No objective responses were observed, but four patients experienced stable disease that lasted for at least 4 months. Compared with baseline, there was a significant increase in the serum levels of IGF-1 ($p < 0.001$) and IGFBP-3 ($p = 0.019$) on day 2. Compared with day 2, there were significant increases in the serum levels of IGF-1 ($p < 0.001$), IGF-2 ($p = 0.001$), and IGFBP-3 ($p = 0.019$) on day 8. A phase II study in women with metastatic breast cancer is ongoing.

Keywords

Cixutumumab; Temsirolimus; Metastatic breast cancer; IGF-1R; mTOR

Introduction

Aberrant activation of the phosphatidylinositol-3-kinase (PI3K) pathway occurs frequently in breast cancer as a result of a variety of mechanisms. For example, mutation in *PIK3CA* (the p110 α subunit of PI3-Kinase) is detected in 25–40 % of breast cancers [1–4], and loss of PTEN expression has been reported to occur in up to 50 % of breast cancers [5]. Other

genetic events include mutations in *AKT1* [6] and amplification of upstream receptor tyrosine kinases, such as *HER2* or downstream signaling molecules such as *S6K1* [7, 8]. The mammalian target of rapamycin (mTOR), the central mediator of the PI3K pathway, has therefore generated significant interest as a potential therapeutic target in breast cancer. Rapamycin analogs bind to FKBP12 and disrupt the rapamycin sensitive mTOR-Raptor complex to impair the production of proteins important for cell-cycle progression and angiogenesis. The rapamycin analogs are clinically available for the treatment of renal cell cancer and pancreatic islet cell tumor. The combination of everolimus and exemestane was FDA approved for the treatment of breast cancer patients with hormone receptor positive tumors that have been previously treated with nonsteroidal aromatase inhibitors based on the results of BOLERO-2 demonstrating an increase in progression-free survival over exemestane alone [9]. However, several other studies of mTOR inhibitors in the treatment of breast cancer have been disappointing [10–12].

A potential mechanism of resistance to mTOR inhibitor therapy is the induction of AKT phosphorylation, which is frequently observed in both preclinical and clinical studies [13–17]. Data indicate that the up-regulation of AKT activity is dependent on Insulin-like growth factor (IGF)/Insulin like growth factor type 1 receptor (IGF-1R) signaling [14, 15, 18, 19]. In preclinical studies, exogenous IGFs completely reversed the antiproliferative effects of rapamycin [14, 18, 20], while inhibition of IGF-1R, either by a small molecule inhibitor or an IGF-1R antibody such as Cixutumumab, abrogated the induction of AKT activity and enhanced the antiproliferative effect of rapamycin [14, 15, 18, 19].

In addition to having a potential role in mediating resistance to rapamycin treatment, IGF/IGF-1R signaling is a crucial component of normal growth and is essential for malignant transformation and the development of breast cancer [21, 22]. IGF-1R is the primary response mediator for both IGF-1 and IGF-2. Binding of IGFs to IGF-1R activates the receptor tyrosine kinase activity which triggers a cascade of downstream signalings especially the PI3K pathway [23] mediated by the insulin receptor substrate (IRS-1) and Ras-Raf-MAPK signaling [24]. IGFs are potent mitogens for breast cancer proliferation [25–29]. Constitutive activation of IGF-1R causes rapid appearance of mammary tumors [30], and inhibition of IGF-1R suppresses xenograft growth of breast cancer cells in athymic mice [31, 32]. In addition, IGF-1R over-expression is common in breast cancer cell lines and fresh tumor biopsies [27, 33, 34]. These results justify the evaluation of IGF-1R as a therapeutic target in breast cancer.

Cixutumumab (*ImClone, Inc.*) is a fully humanized monoclonal antibody that binds to the IGF-1R with high affinity ($K_d = 0.04$ nM) and blocks ligand binding to the receptor. Treatment with Cixutumumab has been associated with down-regulation of the IGF-1R levels and IGF-1R phosphorylation, inhibition of downstream signaling, induction of apoptosis, decrease in cell proliferation [35, 36], and inhibition of in vivo xenograft tumor growth [35]. Importantly, Cixutumumab has been shown to abrogate AKT activation that was induced by mTOR inhibition in breast cancer cell lines.

Patient population and methods

Eligibility and enrollment

This study enrolled women 18 years of age or older with a histologically confirmed diagnosis of metastatic or locally recurrent breast cancer that was either measurable or evaluable. Any number of prior therapies was acceptable. Additional eligibility criteria included Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0–1, fasting serum glucose <120 mg/dL, and adequate organ function. Exclusion criteria included diabetes requiring oral hypoglycemics or insulin, uncontrolled brain metastases, current use of hormonal therapy, investigational agents, herbal preparations, enzyme-inducing anti-epileptic drugs or CYP3A4 inducers, systemic anticancer therapy within 3 weeks or radiation within 2 weeks before registration; prior treatment with agents targeting IGF-1R/IGF or PI3K/Akt/mTOR pathway, known hypersensitivity reactions to macrolide antibiotics, known HIV positivity, and uncontrolled intercurrent illnesses. Women who were pregnant or breast feeding were not eligible.

Participating institutions obtained study approval from their respective institutional review boards and had filed assurances with the Department of Health and Human Services. Written informed consent was required for enrollment.

Study treatment

Patients were pretreated with 25–50 mg of diphenhydramine administered intravenously (i.v.) or by mouth (po) approximately 15–30 min before starting temsirolimus as a 30-min i.v. infusion followed by Cixutumumab as a 60 min i.v. infusion on days 1, 8, 15, and 22 of a 28-day cycle. Treatment was continued until disease progression, unacceptable adverse events, patient decision to withdraw from the study, or inability to continue treatment.

Management of toxicity

Temsirolimus was to be held, and Cixutumumab continued for grade 3 neutropenia or grade 2 thrombocytopenia until absolute neutrophil count reached >1,000/mcL and platelet count >100,000/mcL, at which time temsirolimus was to be administered at next lower dose level (reduction by 5 mg) with Cixutumumab continued at the same dose. Temsirolimus was to be discontinued, and Cixutumumab continued for grade 2 pneumonitis. If symptoms continued or worsened, then Cixutumumab was also to be discontinued. Temsirolimus was to be discontinued for grade 4 and held for grade 3 or intolerable grade 2 stomatitis until symptoms resolved to grade 0 or 1, at which time, those who had had a grade 3 stomatitis were resumed with temsirolimus at one dose-level reduction (dose level –1), and those who had grade 2 stomatitis could be resumed with temsirolimus at the same dose or one dose lower (dose level –1). Cixutumumab was to be held for grade 3 stomatitis until symptoms resolved to grade 0 or 1, at which time Cixutumumab could be restarted at the same dose.

Cixutumumab and temsirolimus were both to be held for grade 3 hyperglycemia or symptomatic glucose ≥300 mg/ml. Cixutumumab was to be discontinued if the patient developed a grade 3 allergic reaction or acute infusion reaction to the agent. Temsirolimus and Cixutumumab could be continued or be held at the physician's discretion for other grade

2 AEs depending on the nature of the AE. All treatments were discontinued for other grade 4 toxicities. Both agents were to be held for other grade 3 toxicities until symptoms resolved to grade 0 or 1 with a one dose-level reduction when treatment was resumed.

Patient evaluations

Within 14 days before study registration, before each treatment cycle and 4 weeks after completion of treatment, patients underwent a complete physical exam, blood chemistries, toxicity assessments (CTCAE v3.0), and research blood draws. Imaging studies for disease status were performed within 28 days before registration, and at the end of every other treatment cycle. Complete blood counts were taken and fasting blood glucose was measured weekly during treatment. Treatment response was evaluated according to RECIST v1.1 in patients with measurable disease.

Statistical considerations

A 3 + 3 phase I clinical trial design was chosen where the MTD was defined as the highest dose tested where at most one of the six patients developed a dose-limiting toxicity (DLT) during the first cycle of treatment. DLT was defined as any of the following adverse events judged to be possibly, probably or definitely related to the study agents: grade 4 hematologic toxicity; hyperglycemia that could not be controlled with diabetic mediation; any grade 3 non-hematologic toxicity (except asymptomatic medically manageable laboratory abnormalities that did not result in a treatment delay); dose reduction or treatment delay of greater than 1 week during first treatment cycle.

Dose escalation

The first cohort of 3–6 patients was to receive temsirolimus at 25 mg and Cixutumumab at 3 mg/kg (dose level 1). If fewer than 2 DLTs occurred, subsequent cohorts of 3–6 patients were to receive temsirolimus at 25 mg and Cixutumumab increased to 5 mg/kg (dose level 2) and then 6 mg/kg (dose level 3). If the first dose level was not well-tolerated, the next cohort of three patients was to be treated with temsirolimus lowered to 20 mg with the Cixutumumab remaining at 3 mg/kg (dose level –1).

Two of the three patients were treated at dose level 1, and the two patients who were treated at dose level –1 developed DLT. As such, the protocol was amended so that the next cohort of patients would be treated with temsirolimus at 15 mg and Cixutumumab 3 mg/kg (dose level –2). If the combination was well-tolerated, then subsequent cohorts of 3–6 patients were to receive temsirolimus at 15 mg with Cixutumumab increased in 1 mg/kg increments to a maximum of 6 mg/kg (dose levels: –2A, –2B, and –2C, respectively). No inpatient dose escalation was allowed.

Peripheral blood collection and measurement of serum levels of IGF-1, IGF-2, and IGFBP-3

Blood for serum and plasma was collected on day 1 before study drug administration, day 2 (24-h post completion of drug administration), and day 8 (before study drug administration). Serum IGF-1, IGF-2, and IGFBP-3 levels were analyzed at the Core Laboratory for Clinical Studies at Washington University School of Medicine. Serum IGF-1 and IGFBP-3 levels were measured using the solid-phase, enzyme-labeled Chemiluminescent immunometric

assay on the Immulite 1000 System supplied by Siemens Medical Solutions. Serum IGF-2 levels were determined using the BioVendor ELISA kit read on the BioTek ELx800 plate reader.

Results

Study course

Between October 1, 2008 and January 15, 2011, 26 Caucasian women were enrolled onto this trial at three institutions (Mayo Clinic Rochester, University of Chicago, and Washington University in St Louis). Four patients were not included in the determination of the MTD; three progressed before the completion of their first cycle of treatment, and one discontinued therapy during cycle 1 because of wisdom tooth pain unrelated to the study drug. The demographics and tumor characteristics of the remaining 22 patients are presented in Table 1. The median age was 48 (range 34–72) years, with ECOG PS 0 ($n = 12$) or 1 ($n = 10$). Twenty-one patients had hormone receptor positive disease. The median number of chemotherapy regimens for the treatment of metastatic breast cancer was three.

Treatment course and toxicities

Table 2 provides a summary of dose levels tested. Three patients were treated at the starting dose of 25 mg of temsirolimus with 3 mg/kg of Cixutumumab. Two of these three patients developed DLTs, namely, a grade 2 oral mucositis requiring treatment delay of greater than 7 days. Enrollment was then opened to dose level –1 (temsirolimus at 20 mg and Cixutumumab at 3 mg/kg) where the first two patients treated developed grade 3 AEs. One experienced grade 3 neutropenia requiring a dose reduction. The other patient was hospitalized with grade 3 anorexia, fatigue, and dyspnea that were initially considered to be related to treatment (DLT) but, after further evaluation and follow-up, these were recognized as related to disease progression when a bone scan revealed disease progression and the symptoms worsened after treatment discontinuation. This information did not become available until after three patients had been enrolled onto the next lower dose (Dose level –2: temsirolimus at 15 mg and Cixutumumab at 3 mg/kg).

One of the first three patients on dose level –2 developed grade 3 oral mucositis, but none of the three additional patients enrolled developed a DLT. Three patients were then enrolled at dose level –2A (temsirolimus at 15 mg and Cixutumumab at 4 mg/kg). As none of these patients developed a DLT, enrollment to dose level –2B (temsirolimus at 15 mg and Cixutumumab at 5 mg/kg) was opened. One of the first three patients enrolled developed grade 2 thrombocytopenia requiring a treatment delay of more than 7 days, and one of the three additional patients enrolled at this dose level developed grade 3 neutropenia requiring a treatment delay of more than 7 days. Dose escalation was halted. Three additional patients were enrolled onto dose level –2A, and none developed a DLT. Thus, dose level –2A is considered to be the MTD in patients with pretreated metastatic breast cancer.

Table 2 provides the DLTs as well as the severe (grade 3 and higher) toxicities reported to be possibly, probably, or definitely related to study drug over the entire course of treatment for the 22 patients evaluable for MTD. There were no grade 4 or 5 toxicities.

Efficacy and patient outcome

All patients have discontinued treatment. There were no clinical responses by RECIST criteria. Four patients (assigned dose levels: 2, 2A, 2A, and 2B) experienced disease stabilization lasting at least 4 months. The reasons for treatment discontinuation included disease progression ($n = 19$), excessive toxicity ($n = 2$), and refusal ($n = 1$) (Table 3).

Serum levels of IGF-1, IGF-2, and IGFBP-3

To evaluate target inhibition by Cixutumumab, serum levels of IGF-1, IGF-2, and IGFBP-3 were tested on day 1 before study drug, on day 2, and on day 8 for patients treated on dose levels -2, -2A, and -2B (Table 4 and Fig. 1). We hypothesized that IGF-IR blockade by Cixutumumab would disrupt the negative feedback regulation of pituitary growth hormone secretion and therefore increase hepatic production of IGFs and IGFBP3. We found the percent increases in IGF-1 and IGFBP-3 on day 2 from pretreatment levels as well as the percent increase in IGF-1, IGF-2, and IGFBP-3 on day 8 from pretreatment levels to be significant. Moreover, percent change in IGF-2 levels on day 2 from pretreatment levels were significantly greater among those who maintained stable disease for at least 4 months relative to those who did not (median percent change: 14 % vs. 2 %; rank sum p value = 0.028).

Discussion

In this phase I study, the MTD of Cixutumumab in combination with temsirolimus in patients with metastatic breast cancer was defined as Cixutumumab 4 mg/kg and temsirolimus 15 mg administered intravenously weekly. The most frequent hematologic toxicities with the combination therapy were grade 2 anemia, neutropenia, and thrombocytopenia. The most frequent non-hematologic AEs were grade 2 mucositis, hyperglycemia, fatigue, and anorexia. Mucositis, neutropenia, and thrombocytopenia were dose limiting. There were no grade 4 or higher AEs. Most of the toxicities were manageable. At the MTD, the AEs were mild to moderate, and no DLT was observed. Because of theoretical concerns about hyperglycemia, we monitored glucose levels weekly during the first cycle, but no grade 3+ hyperglycemia was observed.

The MTD for Cixutumumab and temsirolimus determined in this trial was lower than that reported by Naing et al. in patients with advanced solid tumors, in which the MTD (maximum-tolerated dose) for the combination was the single agent MTD of Cixutumumab (6 mg/kg) with the single agent MTD of temsirolimus (25 mg) [37]. The DLTs in both trials were mucositis, thrombocytopenia, and neutropenia. Both trials found mucositis and hyperglycemia to be among the most common AEs. A higher proportion of patients experienced metabolic complications such as hypertriglyceridemia and hypercholesterolemia, but fewer patients experienced fatigue and anorexia in the Naing study, while mucositis was more frequent and intolerable in our patient population. Both trials enrolled patients who were previously heavily treated. However, our patient population is restricted to women with metastatic breast cancer, whereas only 55 % of the patients were women and 21 % had breast cancer in the Naing study. In addition, patients were younger in our trial compared to those in the Naing study (median age: 48 vs. 53 years old). It is

possible that Cixutumumab potentiated the toxicities of temsirolimus more in patients with metastatic breast cancer (or female) than in those with other cancer types (or male). In contrast to the results of the current study, in which 8 of 22 patients (36 %; 7 grade 2 and 1 grade 3) experienced grade 2+ stomatitis, only 16 % of patients experienced grade 2 stomatitis in our previous phase II study of single agent temsirolimus (25 mg IV weekly) in patients with metastatic breast cancer [38]. Target inhibition by Cixutumumab was demonstrated by increases in the levels of IGFs and IGFBP3 following treatment, similar to what was observed in other studies of IGF-1R inhibitors [37, 39].

Our trial population was predominantly composed of patients with resistant ER+ breast cancers. The median number of prior chemotherapy regimens was 3 in the metastatic therapy setting. We did not observe any objective responses (by RECIST criteria) in this heavily pretreated unselected metastatic patient cohort, although prolonged stable disease lasting more than 4 months was observed in four of these patients.

IGF-1R as a therapeutic target in breast cancer is currently being evaluated in a number clinical trials, especially as part of strategies to overcome resistance to endocrine therapy for ER+ breast cancer and trastuzumab in HER2 positive disease [40, 41]. In this trial, we observed a prolonged disease stabilization in one patient with ER+/HER2+ breast cancer, who was treated primarily with Cixutumumab alone since cycle 1 day 15 after temsirolimus was discontinued due to toxicity, demonstrating the potential activity of IGF-1R inhibition in this tumor type. Recent preclinical data suggested that inhibition of both IGF-1R and IR (insulin receptor) may be necessary and that combination treatment with hormonal therapy may be synergistic [42,43]. A phase 2 study of a tyrosine kinase inhibitor of both IGF-1R and IR, BMS-754807 combined with letrozole or BMS-754807 alone is being conducted in hormone receptor-positive breast cancer with acquired resistance to nonsteroidal aromatase inhibitors (NCT01225172).

Clinical trials combining mTOR inhibitors and endocrine agents have shown efficacy in the treatment of metastatic endocrine-resistant ER+ breast cancer [9, 44], leading to the approval of the combination of everolimus and exemestane in this setting. However, it remains a challenging task to identify predictors of response to mTOR inhibitors in breast cancer [38, 45] despite preclinical data suggesting loss of PTEN, mutations in *PIK3CA*, or over-expression S6K1 or phosphorylation of AKT (all markers of PI3K pathway signaling activation) as potential candidates [46–49]. A recent report identified *TSC1* loss of function mutation as a predictor of response to everolimus in bladder cancer [50]. Whether *TSC1* mutation predicts sensitivity to mTOR inhibitors in breast cancer remains to be tested, but the incidence of *TSC1* mutation in breast cancer is low, having been reported in only 3 out of 512 samples tested in the COSMIC data base (<http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TSC1#histo>). Although preclinical data suggested that ER targeting is needed for mTOR inhibitors to be most effective [51], this concept remains to be tested in clinical trials. The small sample size and heterogeneity of patients enrolled in this phase I study limited our ability to assess the anti-tumor activity of the combination of temsirolimus and Cixutumumab. A phase II study in patients with metastatic breast cancer, who have had at least one but no more than two chemotherapy regimens for the treatment of metastatic breast cancer is currently ongoing (NCT00699491).

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Fig. 1. Serum levels of IGF-1, IGFBP-3, and IGF-2 samples were collected on 15-mg temsirolimus dose levels with Cixutumumab at 3 mg/kg (DL-2), 4 mg/kg (DL-2A), and 4 mg/kg (DL-2B) at the indicated time points

Table 1

Patient and tumor characteristics

Dose level ^a	1 (n = 3)	-1 (n = 1)	-2 (n = 6)	-2A (n = 6)	-2B (n = 6)
Median age in years (range)	35 (34–40)	56	48 (36–65)	51 (43–63)	49 (43–72)
ECOG performance status					
0	3	1	3	2	3
1	0	0	3	4	3
ER/PR/HER2 status					
ER+ or PR+/HER2+	2	1	0	1	3
ER+ or PR+/HER2–	1	0	6	5	2
ER– and PR–/HER2+	0	0	0	0	1
No. prior chemotherapy regimens in metastatic setting					
0–1	0	0	1	3	2
2–5	3	1	4	3	4
6 or more	0	0	1	0	0
No. prior hormone therapy regimens in metastatic setting					
0–1	3	1	2	3	4
2–5	0	0	4	2	1
6 or more	0	0	0	1	1
Presence of visceral metastasis	3	1	6	6	6

^a Please refer to Table 2 for doses of temsirolimus and Cixutumumab

Cycle 1 dose-limiting toxicities and other severe toxicities reported during treatment by dose level

Table 2

Dose level	Temsirolimus (mg)	Cixutumumab (mg/kg)	No. enrolled/N.evaluable	Dose-limiting toxicities (cycle 1)	Other grade 3 or higher toxicities reported during treatment course ^a
1 ^b	25	3	3/3	Grade 2 oral mucositis with treatment delay >7 days (n = 2)	None
-1	20	3	2/1	Grade 3 neutropenia requiring dose reduction (n = 1)	Grade 3 leukopenia (n = 1) Grade 3 lymphopenia (n = 1)
-2	15	3	7/6	Grade 3 oral mucositis (n = 1)	Grade 3 leukopenia (n = 1) Grade 3 lymphopenia (n = 1) Grade 3 anorexia (n = 1) Grade 3 ALT (n = 1)
2A ^c	15	4	6/6	None	Grade 3 leukopenia (n = 1) Grade 3 thrombosis
-2B	15	5	8/6	Grade 2 thrombocytopenia with treatment delay >7 days (n = 1) Grade 3 neutropenia with treatment delay >7 days (n = 1)	Grade 3 ALT (n = 1)

Four patients were not evaluable for MTD since they did not complete cycle 1 treatment because of documentation of disease progression (dose level -1; n = 1; dose level -2B: n = 2), and wisdom teeth problems requiring medical attention (dose level -2; n = 1)

^a Adverse Events possibly, probably, or definitely related to treatment, with the highest grade provided for each patient. Not including those reported as a DLT

^b Starting dose level

^c MTD

Table 3

Patient course

Dose level	No. cycles	Reason off treatment	PFS ^a (days)	OS ^b (months)
1	1, 2, 2	prog; prog; prog	29, 54, 56	17.7; 24.0+; 12.0
-1	2	prog	56	11.3+
-2	1, 1, 1	prog; ae; ae;	37, 37+, 317,	24.0+, 18.1+, 15.4+,
	4, 4, 6	prog; prog; prog	114, 119, 164	19.4, 8.2+, 18.7+
-2A	2, 2, 2,	prog; prog; prog;	32, 50, 56,	1.7, 12.1, 18.7,
	4, 5, 6	prog; refusal; prog	109, 140+, 166	13.1+, 4.6+, 24.0+
-2B	2, 2, 2	prog; prog; prog	47, 53, 54,	24.0+, 3.6, 11.1
	2, 2, 6	prog; prog; prog	55, 55, 163	3.2, 8.9+, 10.9

prog disease progression, *ae* adverse events^a CT scans were required after every 2 cycles of treatment^b Patients were to be followed until death or a maximum of 2 years

Table 4

Serum IGF-1, IGF-2, and IGFBP-3 (DL -2, -2A, and -2B)

Percent change	<i>n</i>	Median (%)	25–75th percentile	Sign-rank test <i>p</i> value
IGF-1				
Day 2 relative to day 1	15	↑46	30 to 66 %	<0.001
Day 8 relative to day 2	13	↑149	127 to 177 %	<0.001
IGF-2				
Day 2 relative to day 1	12	↑5	–1 to 14 %	NS
Day 8 relative to day 2	12	↑13	6 to 22 %	0.001
IGFBP-3				
Day 2 relative to day 1	15	↑14	4 to 19 %	0.019
Day 8 relative to day 2	13	↑47	33 to 63 %	<0.001