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Phase II study of figitumumab in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck: clinical activity and molecular response (GORTEC 2008-02)

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Background: Preclinical studies suggest that insulin-like growth factor-1 receptor (IGF-1R) blockage could be a promising therapeutic target in squamous cell carcinoma of the head and neck (SCCHN). Therefore, we investigated the efficacy and toxicity of figitumumab, an anti-IGF-1R monoclonal antibody, in palliative SCCHN.

Patients and methods: Patients with palliative SCCHN progressing after platinum-based therapy were treated with figitumumab i.v. 20 mg/kg, every 3 weeks. The primary end point was the disease control rate at 6–8 weeks after treatment initiation. Tumor biopsies and plasma samples were collected before and after figitumumab administration to monitor the molecular response.

Results: Seventeen patients were included. Only two patients achieved stable disease at 6–8 weeks. Median overall survival and progression-free survival were 63 and 52 days, respectively. The main grade 3–4 adverse event was

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hyperglycemia (41%). Translational research showed that figitumumab downregulated IGF-1R at the surface of tumor cells with activation of the epidermal growth factor receptor (EGFR) pathway, as shown by the upregulation of p-EGFR in tumor cells ($P = 0.016$), and an increase in the plasma level of tumor growth factor- α ($P = 0.006$).

Conclusion: Figitumumab monotherapy has no clinically significant activity in unselected palliative SCCHN.

Key words: figitumumab, head and neck cancer, insulin-like growth factor receptor, phase II study, targeted therapy

introduction

Over 500 000 people per year worldwide will develop squamous cell carcinoma of the head and neck (SCCHN). Despite treatment, recurrence and distant metastases will occur in > 50% of cases. Patients with tumors not amenable to surgery or radiotherapy are considered incurable. First-line palliative treatment consists of platinum-based chemotherapy in combination with a monoclonal antibody targeting the epidermal growth factor receptor (EGFR) [1]. However, the median overall survival (OS) remains low at 10–11 months [2, 3]. No standard of care has been defined for patients ineligible for chemotherapy or with disease progression after platinum-based therapy and their prognosis is dismal [4–6].

The insulin-like growth factor-1 receptor (IGF-1R) plays an important role in cell growth, proliferation and differentiation and is often overexpressed in SCCHN [7–9]. Jun et al. [10] found that IGF-1R overexpression is correlated with poor survival in advanced-stage patients, suggesting that IGF-1R inhibition could be a relevant target in SCCHN. Promising results with IMC-A12, an anti-IGF-1R human monoclonal antibody, were documented in SCCHN cell lines and tumor xenografts [11].

Figitumumab is a fully human monoclonal antibody IgG2 subtype targeting the IGF-1R. Phase I studies established the recommended dose as 20 mg/kg administered every 3 weeks, with some patients experiencing stable disease (SD) and encouraging further investigation [12–14].

This study aimed to assess the efficacy and toxicity of figitumumab monotherapy in recurrent SCCHN patients. The primary end point was the rate of disease control at 6–8 weeks after treatment.

patients and methods

study design

This study was an open-label multicenter phase II study. Eligible patients received i.v. figitumumab monotherapy (20 mg/kg every 3 weeks) until disease progression or unacceptable toxicity.

inclusion and exclusion criteria

Eligible patients were required to have histologically or cytologically proven recurrent SCCHN, Eastern Cooperative Oncology Group performance status (ECOG PS) zero to two, disease not amenable to curative treatment and at least one measurable lesion according to the RECIST. Progressive disease (PD) (i) after platinum- or taxane-based therapy given for palliation or (ii) within 6 months after platinum-based chemoradiation or (iii) in the first-line palliative setting (if patients were considered ineligible for chemotherapy) was also required. Patients needed to have adequate organ function, absolute neutrophil count $> 1000/\text{mm}^3$, hemoglobin ≥ 8 g/dl, platelet count $> 75\,000/\text{mm}^3$, serum creatinine ≤ 1.5 the upper limit of

normal (ULN), total bilirubin ≤ 1.5 ULN (except for Gilbert's syndrome patients) and alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) < 2.5 ULN.

Patients were excluded if they had nasopharyngeal carcinoma, known brain metastases, previous malignancy (with the exception of basal cell carcinoma of the skin or preinvasive carcinoma of the cervix), uncontrolled diabetes or other serious illness or medical conditions. Patients were also ineligible if they had received previous anti-IGF-1R therapy, more than two prior lines of chemotherapy in the palliative setting and radiation therapy or surgery or investigational drugs within 4 weeks of the study. Previous administration of anti-EGFR therapy was allowed.

The clinical and translational parts of the study were approved by the independent ethics committee and the Belgian and French health authorities and conducted in accordance with the Declaration of Helsinki (October 2000). Written informed consent was obtained for each patient. It was prospectively planned to perform translational research and patients gave their informed consent for repeated biopsies and plasma collection.

study end points and outcome

The primary end point was the rate of disease control, defined as either complete response (CR), partial response (PR) or SD according to RECIST criteria at 6–8 weeks after treatment. Secondary end points were toxicity, progression-free survival (PFS) and OS.

Side-effects were recorded according to the National Cancer Institute–Common Toxicity Criteria (NCI–CTC; version 3). PFS was defined as the time interval between the date of inclusion and the date of disease progression or the date of death. OS was defined as the time interval between the date of inclusion until death or until the date of last follow-up. The objective response rate (ORR) was calculated according to RECIST criteria [15]. The relative dose intensity of figitumumab was calculated as being equal to the dose intensity divided by the planned dose intensity, multiplied by 100.

Translational research was only carried out on paired biopsies and plasma samples.

pretreatment evaluation and follow-up

Pretreatment examinations were carried out within 2 weeks before the start of treatment and included complete history, physical examination, head and neck fibroscopy, chest and abdominal computed tomography (CT), cervical imaging by magnetic resonance or CT and 12-lead electrocardiogram. Laboratory tests included creatinine, total protein, albumin, bilirubin, lipase, amylase and electrolytes (glucose, sodium, potassium, chloride, phosphorus, magnesium, uric acid). Imaging and laboratory tests were repeated every 6–8 weeks. Imaging for tumor evaluation was centrally reviewed.

blood and tissue samples

Specific tumor biopsies were taken at two different time points during the study: (i) baseline samples (B0) within the 2 weeks preceding the first figitumumab administration and (ii) 2 h after the end of the second infusion of figitumumab (C2). These tumor biopsies were carried out only for the translational research and took according to a well-defined manual of operation described in the study protocol. At each time point, one

biopsy was stored in RNeasy Lysis Buffer* for up to 1 week at 4°C and then frozen at –80°C, and another biopsy was fixed in 4% formalin and embedded in paraffin. Plasma (3 ml) was collected at the same time points as the biopsy samples and stored at –80°C.

plasma analyses

Plasma was assayed for EGFR ligands using 'sandwich' enzyme-linked immunosorbent assay (ELISA) kits for the epidermal growth factor (EGF; R&D Systems, DE00, Abingdon, UK), tumor growth factor- α (TGF- α ; R&D Systems, DTGA00), insulin growth factor binding protein-3 (IGFBP-3; R&D Systems, DGB300) and Serum Amyloid A (SAA; Invitrogen and KHA0011, Life Technologies Europe, Gent, Belgium).

Growth hormone (GH) and insulin were dosed automatically by chemiluminescent immunoassays. GH was measured using a Unidel Dxl analyzer* (Beckman Coulter Inc., Villepinte, France) and insulin and IGF-1 were measured with the Liaison* system (Diasorin, Brussels, Belgium).

Immunohistochemistry (IHC) was carried out on 4- μ m paraffin-embedded tumor sections using the following antibodies: Ki67, phospho-p44/42 MAPK (p-erk1/2), IGF-1R β antibody, pan-Akt, phospho-Akt, EGFR and phospho-EGFR (pEGFR) (supplementarydataA, available at *Annals of Oncology* online). Expression was subsequently quantified using an optical microscope at $\times 400$ magnification by measuring the staining intensity and the number of positive cells expressed as a percentage of the complete tissue section. In each case, a histoscore with a potential range of 0–300 was calculated as follows: Histoscore = (% weakly stained cells) + (% moderately stained cells) $\times 2$ + (% strongly stained cells) $\times 3$ [16].

To insure that the different antibodies were correctly scored, all slides were assessed in a blinded fashion and reviewed by another independent observer with similar results.

RNA extraction, gene expression profiles, reverse transcription (RT-PCR) and quantitative real-time PCR

RNA (TriPure Isolation Reagent; Roche, Vilvoorde, Belgium and Qiagen RNeasy Micro Kit; Qiagen, Venlo, The Netherlands) was extracted from the paired biopsies (pre- and post-figitumumab infusion) of eight patients. One patient was excluded from this analysis due to the absence of tumor cells in the corresponding IHC-stained biopsy. After checking the concentration (Nanodrop) and quality (Agilent Bioanalyzer 2100) of the extracted RNA, RNA samples of high quality (RNA integrity number > 5) were selected for further investigation. We carried out gene expression profiles on 3/7 patients. 100 ng of RNA was amplified and labeled using the Affymetrix GeneChip* 3[2] IVT Express Kit. RNA was hybridized to Affymetrix GeneChip HG-U133 Plus 2.0, stained on an Affymetrix Gene Chip* Fluidics Station 450 and scanned in the GeneChip* Scanner 3000. Quality control was carried out using Affymetrix GCOS software. The genes that were up- or downregulated more than two times between the pre- and post-figitumumab biopsies for the three patients were selected. To further clarify the potential role of these selected genes, quantitative RT-PCRs (qRT-PCRs) were carried out using the RNA stock of the seven patients (including the three involved in the microarray study) from whom paired biopsies were available.

Reverse transcription was carried out using a Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen), a dNTP Mix (ABgene; Fisher Scientific, Tournai, Belgium) and an oligo-dTmix (Roche, Vilvoorde, Belgium). The following predesigned TaqMan gene expression assays and TaqMan Gene Expression Master Mix were obtained from Applied Biosystems: baculoviral IAP (inhibitors of apoptosis protein) repeat containing 3 (BIRC3 or cIAP2) (Hs00154109_m1), SAA (Hs00761940_s1),

matrix metalloproteinase 3 (MMP3) (Hs00968306_g1) and SERPINE1 (Hs01126604-m1).

Human glyceraldehyde 3 phosphate dehydrogenase (GAPDH) (AB Applied Biosystems, NM_002046.3, Life Technologies, Gent, Belgium) was used as an internal control as previously described [17]. qRT-PCR was carried out in a 25 μ l total reaction mixture using an MYIQ (Bio-Rad, Nazareth Eke, Belgium) Thermal Cycler. The thermal cycling conditions were 50°C for 2 min followed by 95°C for 10 min, 95°C for 15 s and 60°C for 60 s. The last two steps were repeated for 40 cycles. Real-time PCR was carried out in duplicate for each gene. We present the qRT-PCR data by Δ Ct values of the studied genes relative to GAPDH.

The fold change ($2^{-\Delta\Delta C_t}$) in gene expression in each paired sample was calculated using the formulas: gene expression Δ Ct = average gene expression Ct minus average GAPDH Ct; gene expression $\Delta\Delta$ Ct = gene expression Δ Ct tumor after cycle 2 minus gene expression Δ Ct tumor pretreatment.

statistical methods

Success was defined as a CR, PR or SD at 6–8 weeks. The Simon optimal one-sample two-stage testing procedure was used with the following hypotheses: $P_0 = 0.10$, $P_1 = 0.25$, $\alpha = 0.15$ and $\beta = 0.10$ [18]. According to these hypotheses, 37 patients needed to be recruited to the trial. At least 6/37 patients were required to achieve SD or PR at 6–8 weeks after treatment for the study to meet its primary end point. The trial was to be discontinued if less than two successes were observed after 17 patients had been treated. Kaplan–Meier analyses were used to calculate PFS and OS probabilities.

For IHC, qPCR and ELISA, a Wilcoxon signed rank test was used to compare paired biopsies. Survival curve probabilities were calculated using the Kaplan–Meier method.

All statistical analyses for IHC assays were conducted on the NCSS Data Analysis Program and the difference was considered significant when $P < 0.05$.

results

patient characteristics

Nineteen patients from two Belgian and three French centers were screened between January 2009 and August 2009, and of these, 17 were eligible for the trial (Figure 1).

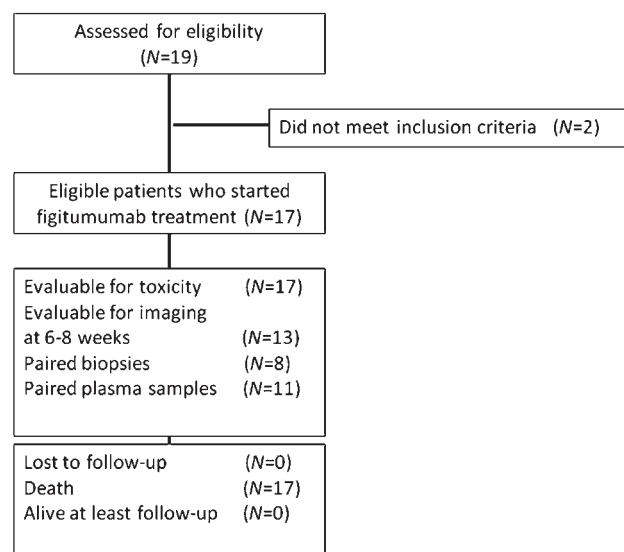


Figure 1. Consort diagram.

Baseline characteristics are described in supplemental Table S1 (available at *Annals of Oncology* online). All patients had been previously exposed to platinum-based chemotherapy.

dose intensity and acute adverse events

The relative dose intensity of figitumumab was 90%. Adverse events are shown in supplemental Table S2 (available at *Annals of Oncology* online). The main grade 3–4 toxic effects were

hyperglycemia (41%), infection (29%), asthenia (24%), anorexia (24%), modifications of ASAT and ALAT (12%), anemia (6%) and tumor bleeding (6%).

efficacy

According to RECIST criteria, two patients had SD at week 6 despite an increase in the sum of the largest diameters of the target lesions (+6% and +7%, respectively) (Figure 2). Four patients with rapid clinical PD were unable to be evaluated at

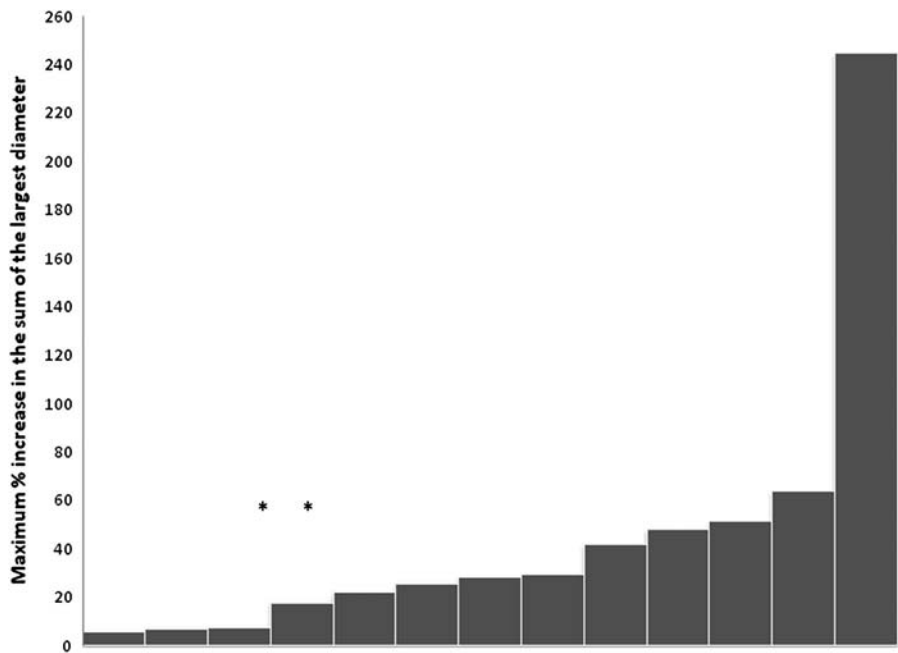


Figure 2. Maximum percentage modification in the sum of the largest diameters of assessable patients (centrally reviewed). Four patients had a maximum increase of <20% at 6 weeks. Two of these patients (*) developed new metastases at 6 weeks.

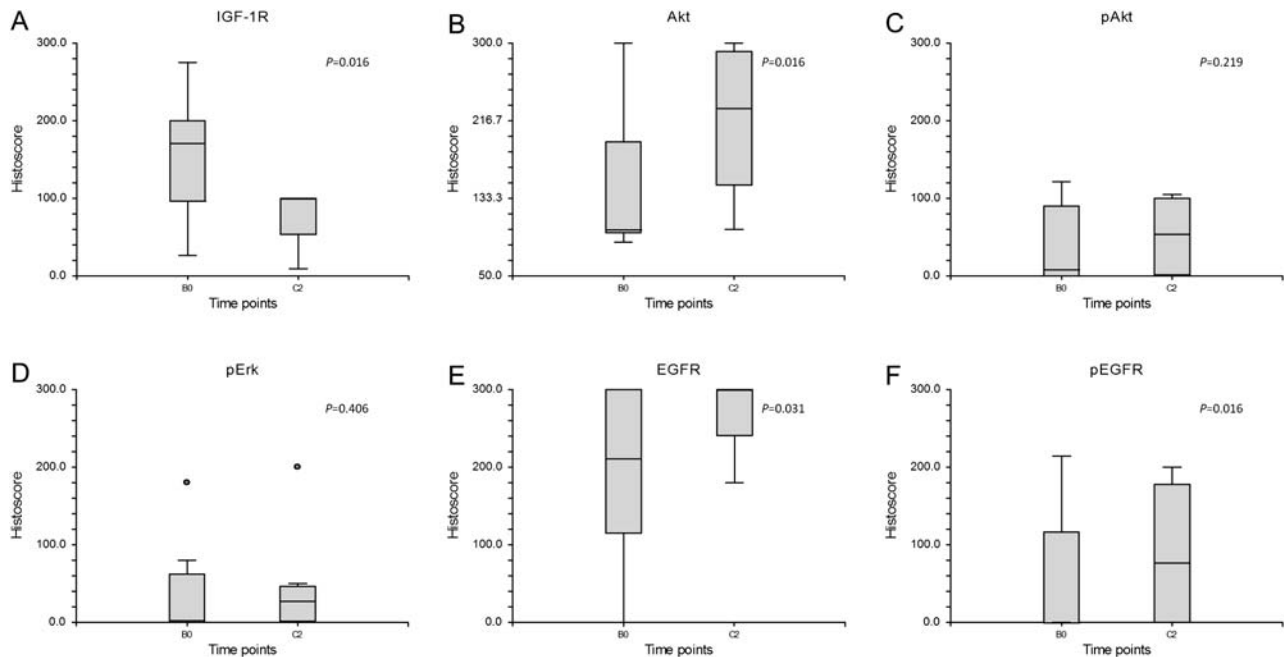


Figure 3A. Histoscore (box plot) before (B0) and after two cycles (C2) of figitumumab: IGF-1R (A), Akt (B), p-Akt (C), p-Erk (D), EGFR (E), p-EGFR (F).

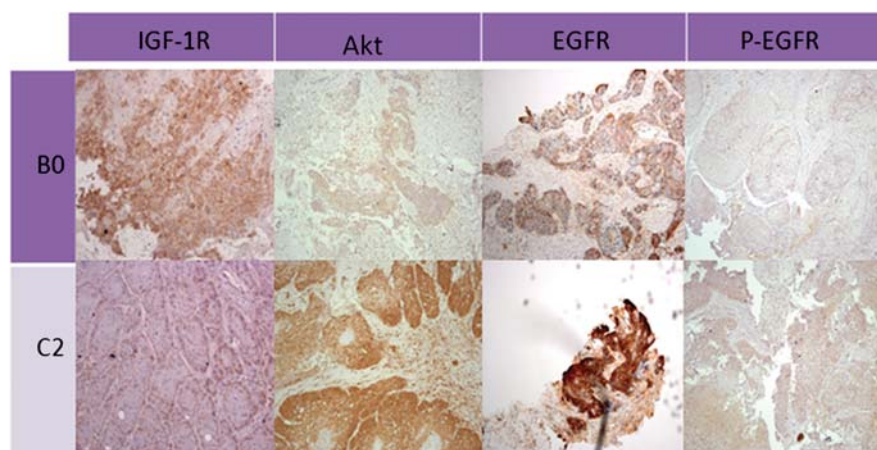


Figure 3B. Typical example of an immunohistochemistry staining with IGF-1R, Akt, EGFR and p-EGFR before (B0) and after two cycles of figitumumab (C2). EGFR, epidermal growth factor receptor; IGF-1R, insulin-like growth factor-1 receptor.

6–8 weeks and were considered to have PD as prespecified in the protocol. At 12 weeks, all patients had PD according to RECIST. At the interim analysis, median OS and PFS were low: 63 days [95% confidence interval (CI) 0–128 days] and 52 days (95% CI 27–77 days), respectively.

immunohistochemistry

Paired tumor biopsies were obtained from eight patients (Figure 3A and B). We first compared the level of expression of IGF-1R by histoscore between the paired biopsies. A decrease in IGF-1R expression was observed after two cycles of figitumumab ($P = 0.016$). Next, we investigated the downstream signaling pathways of IGF-1R. Surprisingly, figitumumab induced overexpression of Akt ($P = 0.016$), but upregulation of pAkt ($P = 0.219$) and pErk ($P = 0.406$) could not be demonstrated.

As the IGF-1R pathway has been shown to exhibit cross talk with other signaling pathways, we examined the potential interaction with the EGFR [19–22]. Upregulation of the EGFR ($P = 0.031$) and pEGFR ($P = 0.016$) was observed after figitumumab administration.

microarray and qRT-PCR

Fifteen perfectly matched genes were found to be up- or downregulated after figitumumab infusion (supplementary data B, available at *Annals of Oncology* online). From this list, we chose to investigate expression levels by qRT-PCR in genes implicated only in tumor growth and progression. We analyzed the messenger RNA levels of MMP3, plasminogen activator inhibitor type 1 (PAI1), cIAP2 and SAA using qRT-PCR. Relative RNA expression levels of SAA and cIAP2 were increased in the tumor biopsy samples obtained after figitumumab compared with baseline ($P = 0.023$ and 0.016 , respectively) but this was not so for MMP3 ($P = 0.531$) and PAI1 ($P = 0.531$) (Figure 4; data not shown).

plasma analyses

Paired plasma samples (pre- and post figitumumab) were obtained from 11 patients. As previously described with

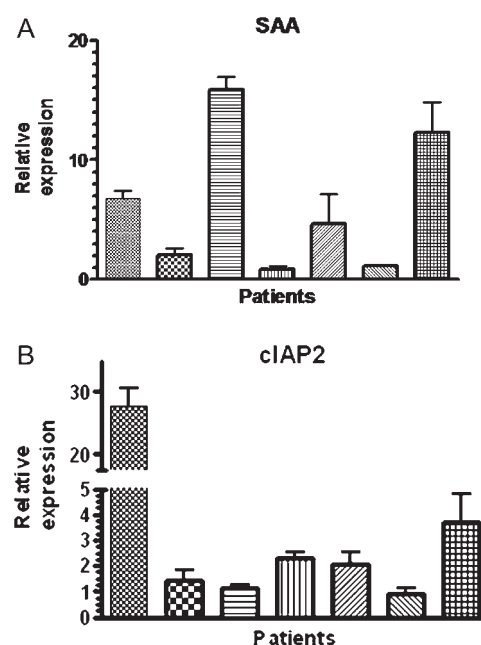


Figure 4. (A) Relative RNA expression levels of SAA in the tumor biopsy obtained after figitumumab compared with baseline samples in seven patients. (B) Relative RNA expression levels of cIAP2 in the tumor biopsy obtained after figitumumab compared with baseline samples in seven patients. SAA, Serum Amyloid A.

IGF-1R inhibition [23], we found that the plasma levels of IGF-1, IGFBP-3, GH and insulin were significantly increased after figitumumab administration (Figure 5A).

To further analyze the potential activation of the EGFR pathway in response to figitumumab treatment, we compared the plasma levels of some EGFR ligands before and after figitumumab using ELISAs. The level of TGF- α was upregulated ($P = 0.006$) after figitumumab therapy, whereas that of EGF was not (Figure 5B). According to the results of SAA in the gene expression analysis, SAA was also found in plasma and was upregulated ($P = 0.016$) after figitumumab in all tested patients apart from one patient who had SD at week

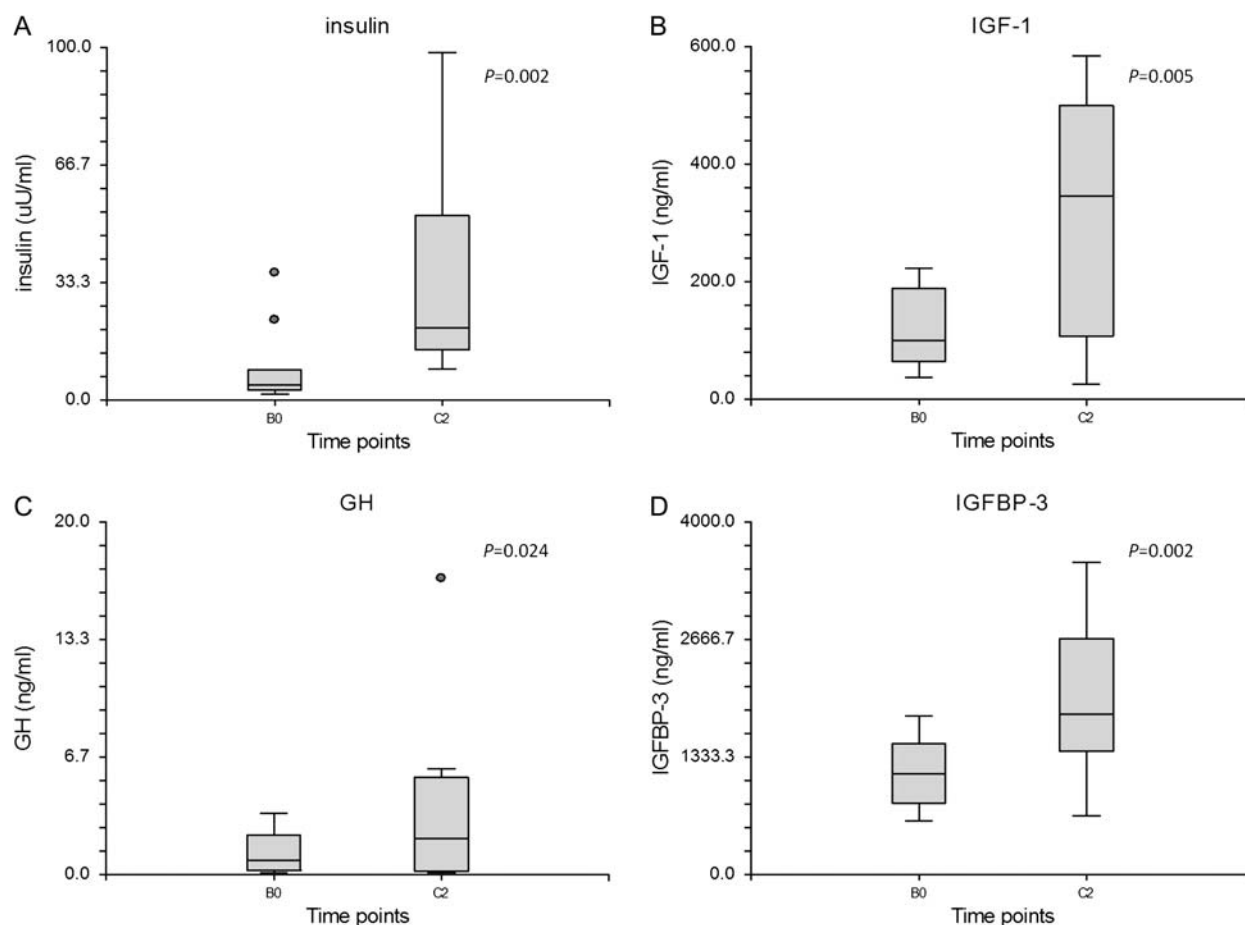


Figure 5A. Plasma levels (box plot) before (B0) and after figitumumab (C2) of insulin (A), IGF-1 (B), growth hormone (C) and IGFBP-3 (D).

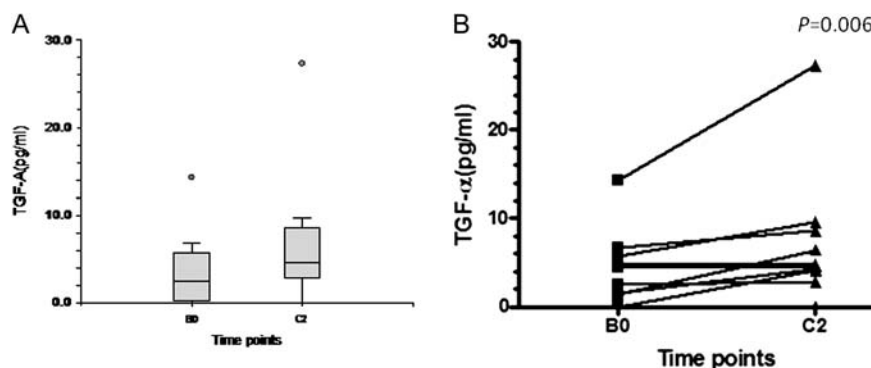


Figure 5B. Plasma levels of TGF-α before (B0) and after figitumumab (C2). Median value (box plot) (A) and individual values (B).

6. However, this patient was observed to have an increase in SAA while in progression at week 12 (Figure 5C).

discussion

We report on the first clinical trial investigating an IGF-1R inhibitor in SCCHN. Figitumumab monotherapy was found to have no significant clinical activity in unselected palliative SCCHN. Despite an increase in their target lesions, two patients achieved SD at 6–8 weeks according to RECIST but at

week 12, these patients had PD (RECIST). The median PFS (52 days) and OS (63 days) were extremely low compared with other single agent palliative studies carried out in the same setting [5, 24–26]. Therefore, the investigators decided to prematurely close the trial even though the statistical hypothesis would have allowed an additional 20 patients.

The baseline characteristics of our patients did not seem to explain the poor outcomes observed. Only three patients had ECOG PS two and only two had been previously treated with more than one chemotherapy regimen for palliation. Poor anti-

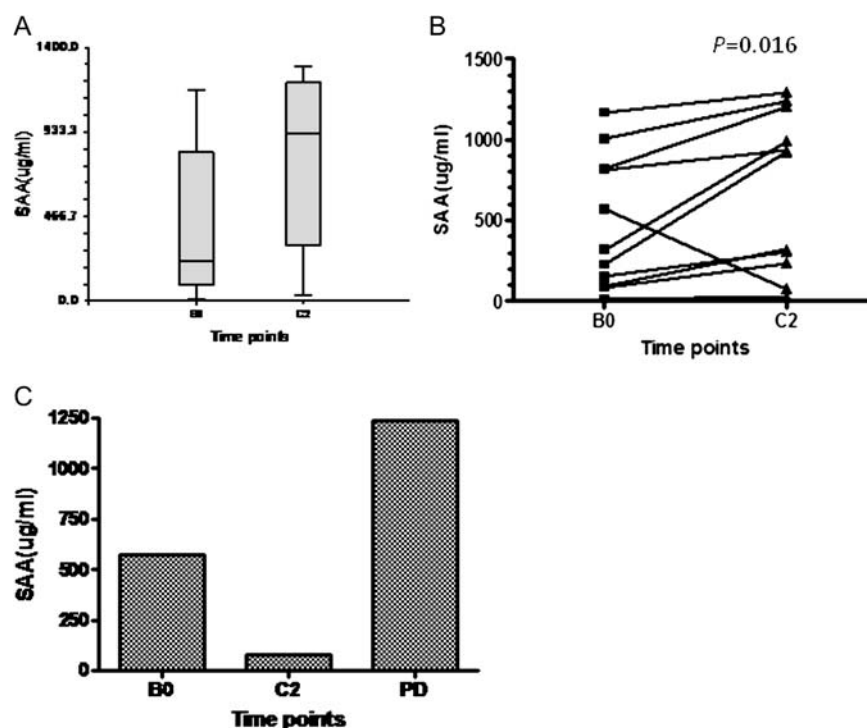


Figure 5C. Plasma levels of SAA before (B0) and after figitumumab (C2) for 11 paired samples. Median value (box plot) (A) and individual values (B). For one patient with SD at week 6, plasma samples B0, C2 and at PD were available (C). At SD, the patient had a decrease SAA level. At PD, the level an increase over the initial level was observed. IGFBP-3, insulin growth factor binding protein-3; PD, progressive disease; SAA, Serum Amyloid A; SD, stable disease; TGF- α , tumor growth factor-alpha.

IGF-1R clinical activity has, however, been documented in other tumor types [27]. In contrast, antitumor activity seems present in sarcomas and particularly in Ewing's sarcoma, where promising activity was detected in a phase I trial [14].

Anti-IGF-1R treatments are well tolerated [28, 29]. The most frequent NCI-CTC grade 3–4 toxicity observed in our study was hyperglycemia, occurring in 41% of patients. This is higher than described in other studies where grade 1–2 hyperglycemia occurred in ~25% of cases [23]. Two of seven patients with hyperglycemia had a past history of controlled diabetes. The mechanisms that cause hyperglycemia are not well understood. One hypothesis is that the figitumumab-induced blockage of the insulin receptor/IGF-1R hybrids induces insulin resistance. Another hypothesis involves the dysregulation of the homeostatic mechanisms between the IGF-1R, IGF-1 and GH. The blockage of the IGF-1R leads to the inhibition of the hypoglycemic effect of IGF-1. In addition, elevated circulating levels of GH secondary to the IGF-1R inhibition lead to an increase in liver glucogenesis and insulin resistance [23]. These biological effects related to the anti-IGF-1R treatment were also observed in our patient cohort (Figure 4A).

Eight paired biopsies were obtained in our trial and these samples were used to evaluate potential treatment-resistance mechanisms. Figitumumab has been shown to promote downregulation of the IGF-1R by internalization and degradation via endosomes [30]. We consistently found that figitumumab downregulated IGF-1R expression in all our tested patients (Figure 3). We observed an absence of inhibition of the downstream molecular pathways with an

upregulation of AKT, leading to the hypothesis that this pathway may be indeed activated. However, this interpretation is limited by the technical difficulties that could occur with the detection of phosphorylated proteins by IHC.

As the PI3K/AKT/mTOR pathway is a shared downstream molecular pathway between the IGF-1 and EGF receptors, we hypothesized a resistance mechanism through the EGFR pathway. We found upregulation of EGFR and pEGFR as well as an increase in plasma TGF- α . Although never reported in a real clinical situation, our results are in line with some preclinical data [5, 14, 19–24, 26–32]. Combined blockage of IGF-1R and EGFR was more effective than blocking each one individually in SCCHN xenograft mouse tumor models [11]. These data, together with our findings, strongly suggest that combined inhibition of both the EGFR and the IGF-1R may be a promising approach. However, no antitumor activity was detected in 63/64 patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer treated with the anti-IGF-1R antibody IMC-A12 alone or in combination with cetuximab, suggesting that other resistance mechanisms are probably involved [27].

To better understand the molecular mechanisms involved, complementary DNA microarray and qRT-PCR were carried out on paired biopsies. Although interpretation of these analyses is limited due to the low number of patients, we detected an increase in cIAP2 and SAA gene expression in the post-treatment biopsies compared with the pretreatment biopsies. The IAPs are thought to inhibit cell death via the direct inhibition of caspases and procaspases [33]. IAPs have been described as being abnormally regulated in different

cancers and anti-IAPs were recently introduced as agents to overcome resistance to chemotherapy [34–36]. In preclinical trials, these agents also seem to be potent radiosensitizers in SCCHN [37]. Moreover, transcription of cIAP-2 and XIAP (X-linked IAP) is upregulated by the PI3K/AKT pathway [38]. SAA, a major apolipoprotein of high-density lipoprotein, is known to be an acute inflammatory phase protein i.e. also involved in cancer pathogenesis, where it seems to promote tumor development and accelerate tumor progression and metastasis [39]. In SCCHN, higher expression of SAA1 was detected in cancer samples compared with normal mucosa [40]. In addition, SAA also influences tumor invasion through the extracellular matrix by stimulating secretion of MMPs (MMP-1, MMP-2, MMP-3, MMP9) [41].

Altogether, our gene expression data and IHC results suggest that figitumumab monotherapy is not able to significantly block the PI3K/AKT and RAS/RAF/MAP pathways or inhibit the expression of genes involved in apoptotic, tumor proliferation or invasion processes.

In conclusion, figitumumab monotherapy has no clinically significant activity in unselected palliative patients with SCCHN.

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disclosure

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How valid are claims for synergy in published clinical studies?

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Background: Clinical trials evaluating drug combinations are often stimulated by claims of synergistic interactions in preclinical models. Overuse or misuse of the term synergy could lead to poorly designed clinical studies.

Methods: We searched PubMed using the terms ‘synergy’ or ‘synergistic’ and ‘cancer’ to select articles published between 2006 and 2010. Eligible studies were those that referred to synergy in preclinical studies to justify a drug combination evaluated in a clinical trial.

Results: Eighty-six clinical articles met eligibility criteria and 132 preclinical articles were cited in them. Most of the clinical studies were phase I (43%) or phase II trials (56%). Appropriate methods to evaluate synergy in preclinical studies included isobologram analysis in 18 studies (13.6%) and median effect in 10 studies (7.6%). Only 26 studies using animal models (39%) attempted to evaluate therapeutic index. There was no association between the result of the clinical trial and the use of an appropriate method to evaluate synergy ($P = 0.25$, chi-squared test).

Conclusions: Synergy is cited frequently in phase I and phase II studies to justify the evaluation of a specific drug combination. Inappropriate methods for evaluation of synergy and poor assessment of therapeutic index have been used in most preclinical articles.

Key words: clinical studies, isobologram, median effect, preclinical studies, synergy, therapeutic index

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