Pharmacodynamic Trial of Nimotuzumab in Unresectable Squamous Cell Carcinoma of the Head and Neck: A SENDO Foundation Study

Federico Rojo1,2,6, Elías Gracias7, Nadia Villena1,3, Teresa Cruz7, Josep Maria Corominas1,2,4, Irene Corradino9, Mercedes Cedeño8, Clara Campas1,3, Marta Osorio7, Normando Iznaga10, Beatriz Bellosillo1,2,4, Ana Rovira1,3, Silvia Marsoni9, Pere Gascon5, Sergio Serrano1,2,4, Cristina Sessa10, Tania Crombet8, and Joan Albanell1,3,4

Abstract

Purpose: To assess the pharmacodynamic effects of nimotuzumab, an anti–epidermal growth factor receptor (EGFR) monoclonal antibody with intermediate affinity for the receptor, in skin and tumor tissues from head and neck cancer patients.

Experimental design: Pharmacodynamic study in patients with advanced squamous cell carcinoma of the head and neck, unsuitable for chemoradiotherapy, enrolled in a single-center trial. Patients received 8 weekly infusions of nimotuzumab. The first nimotuzumab infusion was administered 1 week before starting radiation, whereas the remaining doses were administered concomitantly with irradiation. Paired biopsies were taken from skin and primary tumors, before (pretherapy) and 1 week (on single-agent therapy) after first infusion. Immunohistochemistry was conducted to assay the effects of nimotuzumab on total and phosphorylated EGFR, phosphorylated extracellular signal-regulated kinase 1/2 (p-ERK1/2), p-AKT, and proliferation (Ki-67).

Results: Nimotuzumab was well tolerated and there was no evidence of skin rash. Objective response was achieved in 9 of 10 patients. The pharmacodynamic assays showed inhibition of p-EGFR in both skin and tumor (P = 0.042 in skin and P = 0.034 in tumor). No significant changes in p-ERK1/2, p-AKT, or Ki-67 were detected in skin. In addition, lymphocytic infiltrates, folliculitis, or perifolliculitis were not observed. In tumor samples, there was an upregulation of p-AKT (P = 0.043), a reduction in proliferation index (P = 0.012), and a nonsignificant trend toward a decrease of p-ERK1/2 (P = 0.091).

Conclusions: The pharmacodynamic data confirmed the ability of nimotuzumab to decrease EGFR phosphorylation. Downstream effects were observed in tumor cells but not in skin, a finding that may help to explain the lack of skin rash in patients treated with nimotuzumab. Clin Cancer Res; 16(8); 2474–82. ©2010 AACR.

Nimotuzumab (also known as h-R3) is a humanized anti–epidermal growth factor receptor (EGFR) monoclonal antibody (mAB) that was obtained by complementarity-determining regions grafting of a murine mAB to a human framework. Nimotuzumab binds to domain III of the extracellular region of the EGFR, within an area that overlaps with both the surface patch recognized by cetuximab and the binding site for EGF, while still allowing the receptor to adopt its active conformation, hence warranting a basal level of signaling (1–3). Nimotuzumab exhibits different pharmacokinetic properties when compared with other anti-EGFR antibodies. At the dose levels associated with systemic clearance saturation, nimotuzumab exhibits a more prolonged half-life and a higher area under the curve (4). Clinical trials with this antibody, involving >4,000 patients worldwide, have shown evidence of efficacy in the treatment of patients bearing advanced epithelial-derived tumors with low toxicity and without provoking skin rash, even when it was used alone or in combination with radiotherapy (5–9). At present, nimotuzumab is approved for therapeutic use in cancer treatment in several countries.

The field of EGFR as a clinically relevant target is increasingly growing. Several EGFR antagonists have been approved in various clinical settings (10–12), including antibodies against extracellular domain of the receptor, such as cetuximab, panitumumab, or nimotuzumab, and...
small tyrosine kinase inhibitors such as erlotinib and gefitinib. However, specific adverse events have been described for EGFR-targeting agents. Skin rash occurs in the majority of patients treated with these agents and might cause a decrease in the quality of life as well as treatment interruption (13, 14). The only, and challenging, exception is nimotuzumab. Although the pharmacokinetic properties of nimotuzumab might underlie the lack of skin toxicity, we felt that there was a need to prove that this antibody was able to inhibit EGFR phosphorylation in patient tissues. For this reason, the present pharmacodynamic study was conducted in patients with advanced head and neck cancer who are not suitable to receive chemotherapy. The pharmacodynamic activity of a single dose of nimotuzumab was evaluated on EGFR as well as on extracellular signal-regulated kinase 1/2 (ERK1/2), AKT, and Ki67, both in skin and tumor tissue. Following this window-of-opportunity pharmacodynamic study, patients were continued on nimotuzumab and radiation therapy. Clinical activity and safety were also evaluated.

Materials and Methods

**Trial design.** A single-center phase Ib clinical trial was done in patients newly diagnosed to have histologically documented advanced (unresectable) locoregional squamous cell carcinoma of the head and neck (SCCHN), not suitable for chemoradiotherapy and candidates for radical radiotherapy. Other inclusion criteria were age ≥18 years, an Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy longer than 6 months, absolute neutrophil count ≥1.5 × 10⁹/L, platelet count ≥100 × 10⁹/L, serum creatinine level less than or equal to the upper limit of normal, and bilirubin level less than 2 times the upper limit.

The study was designed as a proof-of-concept biomarker trial, both in skin and head and neck tumor specimens, to test whether nimotuzumab affected EGFR phosphorylation and downstream events. Of note, there are as yet no guidelines on pharmacodynamic studies for sample size calculations (15). Prior pharmacodynamic studies with EGFR-targeting agents have been done in series from close to 10 to more than 60 patients (16–18). The number of patients for the present study was 10. Our goal was to explore whether nimotuzumab was able to affect EGFR phosphorylation while minimizing the number of patients that underwent serial biopsies. The consensus was that if no inhibition of EGFR was observed, then nimotuzumab schedules for phase II/III studies should be reconsidered and additional pharmacodynamic studies should be planned. The study was approved by the Ethics Committee of the National Institute of Oncology and Radiobiology (Havana, Cuba) and by the National Regulatory Authorities of Cuba, the Center for State Control of Quality of Drugs (Havana, Cuba). Written informed consent was obtained from all patients. The clinical trial was carried out under the principles embodied in the Declaration of Helsinki. Pharmacodynamic assays were done at Hospital del Mar, and this portion of the study was approved by the Ethics Committee of IMIM-Hospital del Mar.

**Treatment.** Ten patients were sequentially allocated in two cohorts of five, at two dose levels of nimotuzumab (200 and 400 mg). Each patient received 8 weekly infusions of antibody. Two infusions were administered before starting radiotherapy, and the six subsequent doses were administered concomitantly with radiotherapy. Nimotuzumab was administered i.v. in 250 mL saline solution. Ionizing radiation (cobalt 60) was delivered in doses of 2 Gy once daily, 5 days a week for 6 weeks (total dose of 6,600 cGy). Planning and simulation were done on the basis of recent computed tomography scans. Lateral opposed portals encompassing the primary tumor and the neck area were used. During treatment, the fields were reduced to treat the areas of macroscopic disease with the maximum dose. At a total dose of 5,000 cGy, only the primary tumor and the metastatic nodes were treated. Spinal cord dose was limited to 4,500 cGy. The planned overall treatment time was 6 to 7 weeks.

**Clinical evaluation.** Assessments were done at baseline and every 4 weeks. This included physical exam and complete blood count for clinical laboratory test. Additionally, computed tomography scans were done at baseline and every 3 months until 1 year to assess the clinical response according to response evaluation criteria in solid tumors (RECIST). RECIST dictates confirmation of the response no less than 4 weeks after the criteria for response are first met. Accordingly, longer intervals may be appropriate, although they might reduce the probability of confirming a response or a stable disease. All responses were confirmed in the following assessment done by computed tomography scan at the 12-week interval. Toxicity was
assessed according to National Cancer Institute Common Toxicity Criteria v3.0 (NCI-CTCv3). Criteria for stopping antibody administration included voluntary withdrawal, unmanageable toxicity, or worsening of the patient condition.

**Biopsy samples.** Paired skin punch biopsy specimens and incisional primary tumor biopsies from the 10 recruited patients were obtained; one pre-nimotuzumab treatment and another at day 8 (on-therapy sample) before the second antibody administration. This 1-week window of opportunity was used to assess the effects of single-agent nimotuzumab and to avoid further delays in radiation therapy. All samples were fixed in 4% buffered formalin solution for 24 hours before being dehydrated and paraffin embedded. Representative tissue sections were stained by hematoxylin and eosin to evaluate morphology.

**Pharmacodynamic studies.** Pre- and on-nimotuzumab paired skin and tumor biopsies were assayed for the 10 patients. The antibodies used were anti-EGFR (mAb clone 2-18C9, Dako), anti-phosphorylated (activated) EGFR (p-EGFR, mAb clone 74, Chemicon), proliferation marker anti-Ki67 (mAb clone MIB-1, Dako), downstream signaling markers mitogen-activated protein kinase (ERK1/2, rabbit polyclonal p44/42 MAPK antibody, CST), phosphorylated ERK1/2 (rabbit polyclonal phospho-p44/42 MAPK Thr202/Tyr204 antibody, CST), protein kinase AKT (rabbit polyclonal antibody, CST), and phosphorylated protein kinase AKT (p-AKT, rabbit polyclonal phospho-AKT Ser473 antibody, CST). Lymphocytic infiltration in skin was studied by CD45 (mAb clone 2B11, Dako).

All markers were evaluated for percentage and staining intensity in target cells. Immunostaining was done using 3-μm tissue sections in the Dako-Link and Discovery XT platforms, as previously described (19). Briefly, after deparaffinization in xylene and graded alcohols, heat and enzymatic antigen retrieval was done. Endogenous peroxidase was blocked by immersing the sections in 0.03% hydrogen peroxide for 5 min. Slides were incubated with the primary antibodies, followed by incubation with the appropriate anti-immunoglobulin horseradish peroxidase–conjugated EnVision polymer (Dako) or peroxidase-conjugated OmniMap multimer (Ventana) to detect antigen-antibody complexes. Sections were then visualized with 3,3′-diaminobenzidine as chromogen and counterstained with hematoxylin. The same sections incubated with nonimmunized serum were used as negative controls, and sections from a breast human tumor with a known expression of the markers were stained as positive controls. Baseline (pretherapy) expression levels of EGFR, p-EGFR, ERK1/2, p-ERK1/2, AKT, p-AKT, and Ki67 were analyzed in skin and tumor biopsies and compared with expression levels on day 8. To score a cell as positive, membrane staining was required for EGFR; membrane staining for p-EGFR; cytoplasmic and nuclear staining for ERK1/2, AKT, and p-AKT; and nuclear staining for p-ERK1/2 and Ki67. CD45 was detected in the membrane of lymphoid cells. Each marker was assessed by two investigators (F.R. and M.C.), blinded to whether samples were pre- or on-treatment, and the average result was calculated. The histoscore (H-score) was determined by estimation of the percentage of cells positively stained with low, medium, or high intensity. The final score was determined after applying a weighting factor to each estimate and the following formula was used: H-score = (low %) × 1 + (medium %) × 2 + (high %) × 3; the results ranged from 0 to 300 (17, 18, 20). Staining intensity was estimated as follows: EGFR and p-EGFR expression was referred to staining in HT-29 and A431 cell lines, included as positive controls by the manufacturer (Dako). ERK1/2 and p-ERK1/2 staining in target cells was compared with stromal cells. Endothelial cells showed a strong (high) expression, whereas fibroblasts had intermediate expression. Low staining was considered as fainter than observed in fibroblasts. AKT and p-AKT staining levels were referred to stromal cells. Low staining was present in fibroblasts and endothelial cells. Intermediate staining was observed in interfollicular epidermis. High expression was defined as stronger than in normal skin. Ki67 was considered as a high-expression staining in all positive cells.

**Statistical analysis.** Statistical analyses were carried out using the SPSS Data Analysis Program, version 13.0 (SPSS, Inc.). All the pharmacodynamic parameters from paired pre- and on-therapy samples were analyzed using the Wilcoxon signed ranks test. Survival was calculated from the first date of treatment until death and was analyzed using the Kaplan-Meier method and the log-rank test. All statistical tests were conducted at the two-sided 0.05 level of significance.

**Results**

**Patients and clinical outcome.** Ten patients were included between October and November 2005. Median age was 63 years (range 53-73 years); there were eight males and two females; Eastern Cooperative Oncology Group performance status was 0 in six and 1 in four patients. All of them had squamous cell carcinomas: six well differentiated, three moderately differentiated, and one poorly differentiated. Three patients had stage III disease and seven had stage IV disease. Most of the patients had extensive tumor masses involving more than one anatomic site. Patients were not candidates to receive chemoradiotherapy; five had poor nutritional status, two had cardiovascular comorbidities, and three refused to receive chemotherapy.

Nine of ten patients achieved either complete or partial response. Out of the five patients treated at 200 mg, one had a complete response and four had a partial response. In patients treated with 400 mg, one had a complete response, three a partial response, and one stable disease. Four patients received methotrexate upon progression. With a median follow-up from treatment start to the closeout date of 33.7 months, the mean and median overall survival was 11.46 and 7.87 months, respectively. The mean and median survival for the 200 mg treated patients was 9.98 and 10 months, respectively, whereas the mean and median survival of the patients receiving 400 mg was 10.98 and 5.77 months, respectively. No outcome
differences were detected between dose groups (log-rank test $P = 0.7638$).

**Nimotuzumab and radiotherapy adverse events.** Mean and median nimotuzumab cumulative dose for the 200 mg cohort was 1,560 and 1,600 mg, and in the 400 mg cohort was 2,400 and 2,800 mg, respectively. Regarding radiotherapy, the median cumulative dose was 6,600 cGy. The antibody in combination with radiotherapy was generally well tolerated. Grade 3 severe adverse events related with the drug administration were not observed, including skin adverse events. Nimotuzumab-related side events consisted of fever (grade 1), chills (grade 1), and allergy (grade 2). Two severe adverse events (cardiac failure and respiratory disease) were detected in one patient and classified as unrelated with the antibody considering the previous medical history. Globally, four patients interrupted therapy: one voluntarily abandoned therapy after six doses, one for grade 2 allergic reaction after the first infusion, one due to worsening of the performance status after seven antibody administrations, and one discontinued treatment after a cumulative dose of 2,400 mg due to the previously described unrelated adverse event. Radiotherapy adverse events include mucositis, dry radiodermitis, dry mouth, and dysphagia.

**Pharmacodynamic effects in skin.** We and others have reported in studies with other EGFR-targeting agents and time points the presence of perivascular or peridendral lymphocytic infiltrates, neutrophils in underlying dermis, vacuolar degeneration, or presence of apoptotic keratinocytes (18, 20–22). In the present study, the morphologic evaluation of skin samples stained with hematoxylin and eosin revealed that nimotuzumab exposure did not induce modifications of epidermis maturation and thickness, or inflammatory changes, corroborated by the absence of CD45 staining (Fig. 1).

In skin samples, total EGFR staining was detected in keratinocytes of interfollicular epidermis. EGFR was also expressed in cells of the outer root sheath of the hair follicles, the sebaceous and eccrine glands, and in occasional endothelial cells of the dermal vessels. The phosphorylated/activated EGFR was detected in basal epidermal keratinocytes and follicular structures (18, 19, 23). Activated ERK1/2 was predominantly seen in a similar pattern as p-EGFR; that is, mainly in basal layers and partially colocalized with the Ki-67 proliferating marker. Total ERK1/2, AKT, and p-AKT were expressed diffusely in epidermis.

The pharmacodynamic analysis of skin samples (Fig. 2) showed that p-EGFR expression in keratinocytes was significantly inhibited in on-nimotuzumab samples [mean p-EGFR H-score: 106.9 ± 12.5 (SD) in pretherapy versus mean H-score: 60 ± 35.7 in on-therapy specimens; $P = 0.042$]. This reduction in p-EGFR skin expression was observed in all patients. The phosphorylation of downstream signaling molecules was not modified by nimotuzumab (p-ERK1/2 H-score: 34.5 ± 26.5 in pretherapy versus 33.5 ± 26.3 in on-therapy specimens, $P = 0.886$; p-AKT H-score: 100 ± 25.85 in pretherapy versus 110 ± 37 in on-therapy specimens, $P = 0.842$). A nonsignificant trend toward a reduction in proliferation (H-score: 48 ± 24.3 in

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**Fig. 1.** Hematoxilin and eosin (H&E, ×200) staining in paired skin biopsies from a representative patient. There were no differences in epidermis maturation, thickness, vacuolar degeneration, or apoptotic keratinocytes. No inflammatory infiltrates were observed. This feature was confirmed by CD45 immunostaining (diaminobenzidine, ×200), showing the same density of CD45-expressing mononuclear cells in the dermal vessels of pre- and on-nimotuzumab skin specimens.
pretherapy versus 36 ± 10.5 in on-therapy specimens, \( P = 0.084 \) was observed. No significant difference in the pharmacodynamic effects was seen between the two nimotuzumab doses.

**Pharmacodynamic effects in tumors.** It has been reported that the pharmacodynamic effects of EGFR-targeting agents in skin and tumor tissue are similar but not identical (20, 23). In one study, EGFR inhibition in breast tumor cells was more prominent than in skin in patients treated with gefinitib (23). For this reason, and taking into account the distinct affinity of nimotuzumab for the EGFR (3), we performed tumor biopsies in parallel to the skin biopsies. In all studied SCCHN patients, EGFR was detected in tumor cells. p-EGFR was detected in tumor cells mainly at undifferentiated areas and within the infiltrating edge. p-ERK1/2 and Ki-67 expression were also mainly

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**Fig. 2.** Pharmacodynamic effects of nimotuzumab in skin. A, bar graphs summarizing the pharmacodynamic changes, expressed as mean and SD. Significant downregulation in p-EGFR expression \( (P = 0.042) \) was observed in keratinocytes of interfollicular epidermis during nimotuzumab treatment. Downstream signaling was not modified \( (p\text{-ERK1/2}, P = 0.886; p\text{-AKT}, P = 0.842) \) and a nonsignificant trend toward a reduction in proliferation \( (P = 0.084) \) was observed. No significant changes were detected for total EGFR, ERK1/2, or AKT. B, representative pictures showing the staining patterns of EGFR, p-EGFR, p-ERK1/2, p-AKT, and Ki67 in epidermis from pre- and on-therapy skin samples (diaminobenzidine, ×400). Total EGFR was not modified. Phosphorylated/activated EGFR was detected at basal keratinocytes, and a reduction was noted after nimotuzumab treatment. p-ERK1/2 and Ki67 staining was observed at nuclei of keratinocytes, mainly at basal layer of epidermis. p-AKT was detected in the cytoplasm and nucleus of keratinocytes.
Fig. 3. Pharmacodynamic effects of nimotuzumab in SCCHN. A, bar graphs summarizing the changes on paired tumor biopsies (mean and SD). Significant downregulation in p-EGFR expression ($P = 0.034$) and proliferation ($P = 0.012$) were observed in tumor cells after nimotuzumab treatment. In addition, a significant upregulation of p-AKT expression ($P = 0.043$) and a nonsignificant trend toward a reduction in p-ERK1/2 ($P = 0.091$) were observed. No significant changes were detected for total EGFR, ERK1/2, or AKT. B, representative pictures for the expression of EGFR, p-EGFR, p-ERK1/2, p-AKT, and proliferation in pre- and on-therapy tumors, at two magnifications (×200 and ×400). Total EGFR was diffusely detected in the membrane of tumor cells, showing similar expression levels in pre- and on-therapy time points. Phosphorylated/activated EGFR was detected in most of tumor cells in pretherapy biopsies and nimotuzumab treatment significantly reduced the intensity and percentage of stained cells. Nuclear p-ERK1/2 was highly expressed in tumor cells and some stromal cells. Nimotuzumab reduced the proportion of tumor cells with nuclear p-ERK1/2 staining. p-AKT was observed in the cytoplasm and nucleus of tumor cells. Upregulation of p-AKT was observed following nimotuzumab exposure. Proliferation (Ki67) was reduced by nimotuzumab.
located in the infiltrating borders of the tumors, whereas p-AKT was diffusely present. Total ERK1/2 and AKT were broadly expressed in tumor cells.

The pharmacodynamic effects in paired tumor samples (Fig. 3) showed that nimotuzumab induced a modest but significant downregulation of p-EGFR (H-score: 243 ± 16.34 in pretherapy versus 180 ± 7.5 in on-therapy specimens, P = 0.034). Concomitantly, nimotuzumab reduced tumor cell proliferation (H-score: 195 ± 43 in pretherapy versus 150 ± 42.2 in on-therapy, P = 0.012) and showed a trend for p-ERK1/2 (H-score: 129 ± 32.9 in pretherapy versus 103 ± 19.2 in on-therapy, P = 0.091). Nimotuzumab significantly upregulated p-AKT expression (H-score: 105 ± 37.8 in pretherapy versus 140 ± 55.6 in on-therapy, P = 0.043), as described with other EGFR inhibitors (19, 23–25), suggesting feedback mechanisms on AKT in the presence of EGFR inhibitors. Expression levels of total proteins (EGFR, ERK1/2, and AKT) were not modified both in skin and tumor samples. No significant difference in the effects was observed between the two nimotuzumab dose groups.

Discussion

The goal of this study is to explore whether nimotuzumab, as a single agent, is able to inhibit EGFR phosphorylation and exert downstream effects in skin and tumor tissue. No serious adverse events related to nimotuzumab treatment and, in particular, no skin rash, were detected. However, we observed that nimotuzumab reduced EGFR phosphorylation both in skin and tumor. This effect was modest but significant after a single administration of the antibody. Previous nimotuzumab pharmacodynamic studies in tumor tissues in advanced SCCCHN patients showed evidence of antiproliferative and antiangiogenic effects (26); however, this finding had the limitation that on-treatment biopsies were obtained when patients were receiving radiotherapy and nimotuzumab and no skin biopsies were done. Furthermore, effects on EGFR and its signaling had not been previously assessed.

Skin reactions are common in patients receiving EGFR-targeted treatments other than nimotuzumab after few days of therapy, regardless of treatment type (i.e., small tyrosine kinase inhibitors or mAbs; refs. 22, 27–30). Furthermore, severe skin rash has been proposed as a surrogate marker of antitumor effects (13, 29). The induced inflammatory response is responsible for many of the signs and symptoms that are associated with skin toxicity (18, 31). An increase in the synthesis of chemokines has been reported in keratinocytes that are exposed to mAbs that block EGFR, which recruits inflammatory cells and induces cutaneous injury (22). Activation of genes involved in regulating the immune response subsequently releases more effector cytokines and amplifies chemokine production and leukocyte recruitment, leading to the development of skin toxicity (32).

Recently, recommendations from a task force on Methodology for the Development of Innovative Cancer Therapies have been published (15). One of the roles for biomarker studies was confirmation of target effect in a clinical setting where it might be correlated with observed (or lack of) therapeutic effect; it was mentioned that biomarker studies should be particularly considered in the absence of toxicity. In that work, no recommendations on statistical design were provided and, in fact, published studies with other EGFR-targeting agents have been based in samples sized from close to 10 to more than 60 (16–18, 33). In addition, no consistent relationship between the degree of EGFR inhibition and toxicity or activity has been reported to date (17, 18, 33). Further work is clearly needed to establish guidelines on pharmacodynamic study design.

Our results showed that EGFR phosphorylation was inhibited in skin cells after one dose of nimotuzumab. However, keratinocyte proliferation was decreased in a nonsignificant manner, and p-ERK1/2 and p-AKT were not modified. In addition, morphologic skin changes induced by other EGFR inhibitors (18, 23) were not observed. Studies with other anti-EGFR antibodies have shown downstream effects at time points such as 2 weeks, and patients treated with these agents may develop skin toxicities earlier than 2 weeks (22, 30, 33). At the same time, a number of EGFR-targeting agents have a relationship between rash and antitumor activity (30). It is possible that such relationship might be different for each agent, depending on pharmacokinetics, specificities, or immunogenicity. In fact, the antitumor activity of each agent is distinct. Of note, the degree of reduction of EGFR phosphorylation induced by EGFR-targeting agents enough to cause the inflammatory reaction that leads to the rash is yet unknown. Along this line, in a study on skin samples from patients treated with erlotinib, the degree of overall EGFR inhibition was significant but modest, and not observed in all patients despite a high percentage of skin rash (17). Similarly, in a study with gefitinib, the degree of EGFR inhibition was unrelated to toxicity (18, 33). In the present study, EGFR was inhibited modestly and not accompanied by significant downstream effect in skin. This latter observation suggests that the degree of EGFR inhibition achieved in skin was not sufficient to affect significantly downstream events, in contrast to what we observed in tumor specimens. No significant differences were detected between the two treatment cohorts, although the number of patients is too small to draw any conclusion on a possible dose effect.

In tumor cells, nimotuzumab also significantly inhibited EGFR phosphorylation. Furthermore, there was a nonsignificant decrease of p-ERK1/2 and a significant reduction in the tumor cell proliferation. A significant upregulation of p-AKT, which might operate as a resistance mechanism to EGFR targeting, was also found in the neoplastic cells (19, 20, 23–25). These findings suggest a greater nimotuzumab effect in EGFR downstream events in tumor cells compared with skin. Although there are no differences in the way nimotuzumab recognizes EGFR in the tumor and skin cells, there are two potential explanations for a reduced effect in skin versus tumor.
(3). One is associated with the epitope recognized by nimotuzumab that allows a basal, ligand-independent signaling, whereas the second is related to the lower affinity of the antibody for EGFR compared with cetuximab or panitumumab.

Concerning the first mechanism, without ligand-induced activation, a very small fraction of the EGFR molecules adopts an energetically less favorable active conformation (34, 35). This ligand-independent EGFR activation occurs in tumor and normal cells (36, 37). A recent work, based on a computational approach (3), suggested that nimotuzumab would not interfere with this basal level of EGFR signaling. According to that report, the epitope recognized by nimotuzumab is displaced toward the COOH terminus of EGFR domain III, compared with the cetuximab binding site. In this position, nimotuzumab sterically interferes with EGF binding while permitting EGFR to adopt its ligand-independent active conformation (3).

A second mechanism for a greater effect in tumor cells relates to a reduced affinity of the antibody for the EGFR, implying that nimotuzumab might have an enhanced effect when the density of the receptor increases (8). In support of this view, nimotuzumab efficiently inhibited receptor phosphorylation in non–small cell lung carcinoma cells with moderate to high EGFR expression (7). Also, the antibody enhanced the in vitro and in vivo antitumor effects of radiation. On the contrary, nimotuzumab did not inhibit phosphorylation or improved radiosensitivity on those with low EGFR expression (7). In addition, several reports have shown similar antitumor effect between nimotuzumab and other anti-EGFR antibodies in tumors overexpressing EGFR. In human A431 squamous cell carcinoma model, both cetuximab and nimotuzumab showed similar capacity to inhibit EGFR phosphorylation and to induce either complement-mediated cytotoxicity or apoptosis (38). In A431 xenografts, the response was total tumor regression with either nimotuzumab or cetuximab (39). To further study the role of EGFR density, antibody-binding kinetics under monovalent and bivalent binding conditions were examined. These studies showed that when EGFR expression was high, cetuximab and nimotuzumab bound bivalently and accumulated to a similar degree on the cell surface. When EGFR density was low, high-affinity antibodies continued to interact strongly with the receptors. In contrast, nimotuzumab targeting was “density selective” and resulted in transient monovalent interaction in low-expression tissues (40). Taken together, both explanations seem to be consistent with a superior effect of nimotuzumab in EGFR-overexpressing cells compared with those with reduced levels.

The trial was not intended to evaluate the antitumor efficacy of the antibody plus radiation therapy. Patients included in the study were not candidates to receive chemoradiotherapy for poor nutritional status, cardiovascular comorbidities, or refusal to receive chemotherapy. An objective response was achieved in 9 of 10 patients; however, the median overall survival was less than 1 year. This poor survival might be attributed to advanced stage, comorbidities, contraindications for chemoradiation, use of once-daily fractionated radiotherapy instead of accelerated or hyperfractionated regimens, and premature interruption of the treatment in 4 of 10 patients due to worsening status. In a study in fit patients with advanced head and neck cancer, median survival of patients treated with nimotuzumab and radiation was 44.3 months and the 3-year survival rate was 66.7% (8). In another study, median overall survival time for 20 patients that completed six doses of antibody therapy was 43.4 months (41). In a third study, in 92 stage III or IVA SCCIN patients, those receiving nimotuzumab in combination with chemoradiotherapy or radiotherapy alone had a significant improvement of their outcome compared with the same therapy without nimotuzumab (42).

In summary, we provide evidence for the ability of nimotuzumab as a single agent to inhibit EGFR phosphorylation in both skin and tumor cells. Notably, EGFR downstream effects were observed in tumor cells but not in skin, a finding in line with the reported antitumor activity but no skin toxicity of nimotuzumab. This observation supports the concept of a wide therapeutic index for this antibody in EGFR-dependent tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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