

Use of the Humanized Anti-Epidermal Growth Factor Receptor Monoclonal Antibody h-R3 in Combination With Radiotherapy in the Treatment of Locally Advanced Head and Neck Cancer Patients

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Authors' disclosures of potential conflicts of interest are found at the end of this article.

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A B S T R A C T

Purpose

To evaluate safety and preliminary efficacy of the humanized anti-epidermal growth factor receptor monoclonal antibody h-R3 in combination with radiotherapy (RT) in unresectable head and neck cancer patients. Secondary end points were the measurement of h-R3 serum levels and the assessment of the potential mechanisms of antitumor effect on patient biopsies. Anti-idiotypic response to h-R3 was assessed. To predict pharmacologic effect, a mathematical model for antibodies recognizing antigens expressed in tumors and normal tissues was built.

Patients and Methods

Twenty-four patients with advanced carcinomas of the head and neck received six once-weekly infusions of h-R3 at four dose levels in combination with RT. Pretreatment tumor biopsies were obtained to evaluate epidermal growth factor receptor expression as an enrollment criterion. Second biopsies were taken to evaluate the proliferative activity and angiogenesis in comparison with the pretreatment samples. Patient serum samples were collected to measure h-R3 levels and anti-idiotypic response.

Results

The combination of h-R3 and RT was well tolerated. Antibody-related adverse events consisted in infusion reactions. No skin or allergic toxicity appeared. Overall survival significantly increased after the use of the higher antibody doses. Immunohistochemistry studies of tumor specimens before and after treatment revealed that antitumor response correlated with antiproliferative and antiangiogenic effect. One patient developed antibodies to h-R3. The mathematical model predicted that the maximum difference between the area under the curve in tumors and normal tissues is reached when the antibody has intermediate affinity.

Conclusion

h-R3 is a well-tolerated drug that may enhance radiocurability of unresectable head and neck neoplasms.

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INTRODUCTION

During the last 10 years, epidermal growth factor receptor (EGFR) has become a widely explored target for anticancer therapy.¹⁻⁴ Elevated levels of EGFR are associated with malignant transformation of squamous cells and are observed in epithelial tumors, particularly in head and neck squamous cell carcinomas (SCCHN).^{5,6} There is a relationship between

EGFR expression and tumor cell proliferation, metastases, and radiation resistance.⁷⁻⁹

The blockade of growth factors or their receptors has emerged as a therapeutic strategy to sensitize tumors to ionizing radiation, which is highly appealing for tumors such as SCCHN, in which radiotherapy (RT) has been the main traditional therapeutic approach.^{10,11}

Several EGFR antagonists have been investigated in the clinical setting, including

antibodies (IMC-C225, ABX EGF, EMD 72000) and small tyrosine kinase inhibitors (Iressa, ZD1839, AstraZeneca, Wilmington, DE; Tarceva, OSI-774, OSI Pharmaceutical, New York, NY).^{12,13} However, many objective responses to anti-EGFR drugs have been short-lasting and severe acne-like skin rash has been a common toxicity,¹⁻³ often leading to treatment interruption. These drawbacks concern the drugs, not the target, and therefore there is a need for exploring new anti-EGFR agents.

h-R3 is a humanized monoclonal antibody (mAb) that was obtained by complementarity-determining regions grafting of a murine mAb (ior egf/r3) to a human framework.¹⁴ In the preclinical studies, h-R3 demonstrated a remarkable antiproliferative, pro-apoptotic, and antiangiogenic effect.¹⁵ Apart from other anti-EGFR antibodies, the parental antibody of h-R3 was generated from BALB/c mice immunized with a purified placenta fraction enriched in EGFR.¹⁶

h-R3 exhibits different pharmacokinetic properties when compared to other anti-EGFR antibodies. At the dose levels associated with systemic clearance saturation, h-R3 exhibits a more prolonged half-life and a higher area under the curve (AUC).¹⁷

The first clinical trial where 12 subjects received single doses of h-R3 demonstrated its low toxicity. Here we show the results of a phase I/II trial in 24 advanced SCCHN patients designed to evaluate if the antibody was safe in combination with RT and if it showed any evidence of efficacy at the range of doses already shown to correspond to zero order elimination.

The primary end point was safety of h-R3 when used at multiple doses in combination with radiation. Secondary end point was the preliminary evaluation of the clinical response related to the drug concentration in blood and the assessment of the potential mechanisms of antitumor effect on patient biopsies. Anti-idiotypic response to h-R3 was measured.

Additionally, as the complex interaction between antibodies (at different dosages and with different affinities) and tissues containing different amounts of receptors is difficult to guess intuitively, we built a mathematical model valid for those antibodies recognizing antigens ubiquitously expressed in normal tissues and in tumors, where the binding to the tumor and normal tissues depends on the receptor expression, the administered dose, the affinity constant, and the mAb half-life.

PATIENTS AND METHODS

This was a single-center phase I/II clinical trial, in which patients were sequentially allocated in cohorts of three. As this was the first h-R3 combination trial—in which safety was the primary end point—the starting dose was suboptimal according to pharmacokinetics. Each patient received six once-weekly infusions of h-R3 at the following levels: 50, 100, 200, and 400 mg in combination with RT, the standard treatment for unresectable SCCHN at the

moment of protocol design. Total cumulative doses were 300, 600, 1,200, and 2,400 mg, administered by intravenous infusions, diluted in 250 mL of sodium chloride, over 30 minutes. The weekly doses of h-R3 were administered before RT, without premedication. Two patients interrupted radiation and antibody therapy within the first weeks, impeding the evaluation of antitumor response. Two additional subjects were recruited to complete response evaluation.

After finishing accrual, the protocol was amended to include five new patients in the two highest dose groups to further evaluate the relationship between the antitumor response and serum antibody levels.

Ionizing radiation (cobalt 60) was delivered in doses of 2 Gy once daily, 5 days per week (total dose of 60 to 66 Gy). Planning and simulation were performed 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 50 Gy, only the primary tumor and the metastatic nodes were treated. Spinal cord dose was limited to 45 Gy. The planned overall treatment time was 6 to 7 weeks.

After finishing radiation plus h-R3, patients with residual macroscopic disease at the primary site had complete excision of the remaining tumor. Neck dissection was permitted for patients with residual cervical adenopathy or for neck nodes > 3 cm before RT at the discretion of the surgeon.

The protocol was approved by the ethical committee of the Institute of Oncology and by the Regulatory Agency. Written informed consent was obtained from all patients.

Eligibility

Patients with histologically documented advanced (unresectable) locoregional SCCHN who were candidates for radical radiotherapy were recruited. EGFR overexpression in primary tumors was mandatory for enrollment. Other eligibility criteria were: age > 18 years, an Eastern Cooperative Oncology Group performance status of 0 or 1, absolute neutrophil count $\geq 1.5 \times 10^9/L$, platelet count $\geq 100 \times 10^9/L$, serum creatinine level \leq the upper limit of normal, and bilirubin level less than two times the upper limit.

Main exclusion criteria were: prior RT or chemotherapy, concurrent active cancer, class III or IV cardiac failure according to the New York Heart Association classification, any uncontrolled intercurrent illness, and pregnancy or lactation.

Pretreatment and Treatment Evaluation

Baseline studies included physical examination, complete hematologic and biochemical profile, chest radiography, and computed tomography scan. Laboratory tests were performed every 2 weeks during RT and every 4 weeks thereafter. Toxicity was graded according to National Cancer Institute's Common Toxicity Criteria (version 2.0), and the Radiation Therapy Oncology Group morbidity scoring system.

A standard head and neck examination was performed monthly during the first year, and every 3 months thereafter. Any suspected locoregional recurrence underwent biopsy and radiographic studies were performed as clinically indicated. Tumor response was classified according to the Response Evaluation Criteria in Solid Tumors.¹⁸

Survival was calculated from the first date of treatment until death and was analyzed using the Kaplan-Meier method and the log-rank test.

$$\begin{aligned}
 \text{A } \frac{dC_p(t)}{dt} &= -\frac{dC_p(t)}{dt} - D \left[\frac{C_p(t)}{V_p} - \left(\frac{C_{Tumor}(t) - C_{BTumor}}{V_T} \right) - \left(\frac{C_{TLiver}(t) - C_{BLiver}}{V_L} \right) - \left(\frac{C_{TSkin}(t) - C_{BSkin}}{V_S} \right) \right] \\
 \text{B } \frac{dC_{Tumor}(t)}{dt} &= D_{Tumor} \left[\frac{C_p(t)}{V_p} - \left(\frac{C_{Tumor}(t) - C_{BTumor}}{V_T} \right) \right] \\
 \text{C } \frac{dC_{TLiver}(t)}{dt} &= D_{Liver} \left[\frac{C_p(t)}{V_p} - \left(\frac{C_{TLiver}(t) - C_{BLiver}}{V_L} \right) \right] \\
 \text{D } \frac{dC_{TSkin}(t)}{dt} &= D_{Skin} \left[\frac{C_p(t)}{V_p} - \left(\frac{C_{TSkin}(t) - C_{BSkin}}{V_S} \right) \right]
 \end{aligned}$$

Fig 1. The four differential equations describe the changes in antibody concentration in (A) plasma, (B) tumor, (C) liver, and (D) skin, respectively, as a function of time (t), tissue volume (V), and a speed of clearance parameter (D) which was estimated from previous pharmacokinetic data. P, plasma; B, bound; dt, derivative of concentration through time.

h-R3 Levels

Serum was collected in the last 10 patients treated with 200 mg or 400 mg of h-R3. Antibody levels were measured using a solid phase immunoassay based on h-R3 binding to purified recombinant EGFR extracellular domain, followed by time-resolved detection using a dissociation-enhanced lanthanide fluoro-immuno assay (DELFA).¹⁹ Briefly, purified EGFR extracellular domain was bound to 96 well immunoassay plates. Then, human serum samples were added. Bound h-R3 was detected by addition of rabbit antihuman immunoglobulin, followed by Eu-N¹-labeled antirabbit immunoglobulin and measured by time-resolved fluorescence at 0.25 hours after addition of the DELFA enhancement buffer.¹⁹ Peak and media serum concentrations were calculated according the Wagner and Krueger-Thiemer definitions.

Anti-Idiotypic Response

Blood samples were collected at 0 and 7 days, and then monthly for up to 6 months to detect antibodies that react with the murine ior egf/r3 idiotype. A qualitative direct enzyme-linked immunosorbent assay (ELISA) was performed by coating the ELISA plates with ior egf/r3 (10 µg/mL), as previously described.¹⁷ Pretreatment samples of each patient were used as controls. The assay was considered positive when post-treatment/pretreatment ratio was higher than 2.

Immunohistochemistry

Pretreatment tumor biopsies were obtained for all patients in order to evaluate EGFR expression. A second biopsy was taken in nine of the 12 subjects accrued in the first trial set at week 3 to evaluate the proliferative activity and angiogenesis in comparison with the pretreatment samples. In the remaining three patients, there was no accessible tumor at the primary site on week 3. Biopsy specimens were snap frozen and stained with hematoxylin and eosin to confirm the presence of tumor tissue.

Immunohistochemistry was performed with the Vecstatin ABC kit (Vector Laboratories, Burlingame, CA). EGFR expression was determined using murine ior egf/r3 as primary antibody (dilution 1:200). EGFR expression was evaluated according to the following scoring system: 0 (no staining), 1+ (mild membrane staining in < 70% of the tumor cells), 2+ (moderate complete membrane staining in < 80% of the tumor cells), and 3+ (strong complete membrane staining in > 80% of the tumor cells).²⁰ Proliferation was evaluated after staining for Ki67 nuclear antigen,

using the rabbit polyclonal antibody NCLki67p (Novocastra Laboratories, New Castle, UK; dilution 1:1000). The percentages of Ki67 positive tumor cells were calculated by counting the number of brown-stained tumor nuclei per total number of cells in the selected microscope field at 200×. Blood vessels staining was carried using a rabbit antihuman/mouse von Willebrand Factor antibody (A0082, DAKO, Carpinteria, CA; diluted 1:500). Three high power fields were identified on each slide and microvessel density (MVD) was defined as the mean number of vessels on a 200× field.

Mathematical Model for the Kinetic Binding of the Anti-EGFR Antibodies

We developed a mathematical model composed of four differential equations (Fig 1) reflecting the behavior of a mAb in four compartments (plasma, tumor, liver, and skin). Initial concentrations were assumed to be zero for tumor, liver, and skin, and to be equal to peak concentration after infusion for plasma. C_T and C_B mean total and bound concentration in each time; bound concentration is a function of the antibody dissociation constant which was estimated to be 0.23 × 10⁻⁹ M. Numbers of EGFR per cell were assumed to be 10⁶ for the tumor and 10⁴ for the liver and skin. Antibody half-life was 240 hours.

Integrating these differential equations in time, concentration-curves were obtained for each tissue and for integrating again, and the AUC was obtained. AUC can be assumed as a surrogate of the pharmacodynamic effect of the antibody in that tissue.

RESULTS

Patient Characteristics

Twenty-four patients with advanced SCCHN were included. Patients (Table 1) received concurrent RT at doses from 60 to 66 Gy, depending on the response and toxicity (total cumulative dose of 60 Gy was only administered to three subjects). Tumor and lymph node distribution is presented in Table 2.

Toxicity

Eighteen of 24 patients developed mild or moderate h-R3-related adverse events (Table 3). Most common tox-

Characteristics	No. of Patients	%
Sex		
Male	21	87.5
Female	3	12.5
Age, years		
Median	57	
Range	45-78	
Race		
White	16	66.6
Black	8	33.3
Primary tumor site		
Base of tongue	6	25
Tonsil	8	33.3
Retromolar trigone	4	16.6
Soft palate	2	8.2
Buccal mucosa	1	4.1
Floor of mouth	1	4.1
Lower alveolar ridge	1	4.1
Pharyngeal wall	1	4.1
Tumor stage		
Stage III	8	33.3
Stage IV	16	66.6
Tumor differentiation		
Well differentiated	7	29.1
Moderately well differentiated	13	54.1
Poorly differentiated	3	12.5
Undifferentiated	1	4.1

iciencies were fever, hypotension, and tremors. One patient developed grade 3 somnolence after the first dose. No case of skin rash appeared. The most frequent radiation-associated toxicities were mucositis, dermatitis, and dysphagia (Table 4). Long-term effects included mild to moderate xerostomia.

Serum Levels of h-R3

Mean minimum and maximal serum steady-state concentration ranged from 19.20 to 75.61 $\mu\text{g/mL}$ and 39.02 to 147.12 $\mu\text{g/mL}$ for the doses of 200 and 400 mg, respectively. Trough levels of approximately of 34.6 $\mu\text{g/mL}$ and 81.9 $\mu\text{g/mL}$ were found for the doses of 200 and 400 mg.

	N0	N1	N2a	N2b	N2c	N3	Total
Tx	0	0	0	0	0	0	—
T1	0	0	0	0	0	0	—
T2	0	2	0	1	0	1	4
T3	4	2	0	2	2	2	12
T4	0	5	3	0	0	0	8
Total	4	9	3	3	2	3	24

NOTE. Twenty of 24 patients (83%) had either T3 or T4 at presentation. Abbreviations: T, tumor; N, lymph node.

Anti-Idiotypic Response

One patient treated with the highest dose developed antibodies to h-R3. This patient did not show any exacerbation of toxicity compared to the nonpositives and achieved a complete response.

Immunohistochemistry

EGFR expression was classified as 3+ in all patients. Before treatment, Ki67 staining ranged from 42% to 65% (median, 57%). Proliferative activity decreased at week 3; Ki67 staining ranged from 0% to 29% (median, 16%; Fig 2). Regarding blood vessel staining, tumor sections before treatment showed high vessel density. MVD ranged from 4 to 48 microvessels per field (median, 14 microvessels). On the third week after treatment, eight of 9 samples were characterized by a remarkable decrease in vascularity (Fig 2) and the MVD ranged from 3 to 15 microvessels per field (median, 7 microvessels). In one nonresponding patient, there was no evidence of decreased angiogenic activity. Neither EGFR expression nor Ki67 or blood vessel density was associated with tumor outcome. However, the numbers were too small to do meaningful multivariate analysis.

Clinical Response

In the first trial section, 12 patients were treated at doses from 50 to 400 mg. After the doses of 50 and 100 mg, two of six patients achieved complete response, while after doses of 200 and 400 mg, four of six patients had complete response. Another patient treated with 200 mg, who originally achieved a partial response, was rendered disease-free after the excision of the residual tumor, 6 weeks after radiation. One more patient that received the lowest dose had a partial response.

Overall survival significantly increased after the use of doses of 200 or 400 mg in comparison with lower doses ($P = .03$). With a median follow-up from treatment beginning to the closeout date of 45.2 months (range, 41.5 to 48.1 months), the median survival for 50 and 100 mg treated patients was 8.60 months, while the median survival of the patients receiving 200 and 400 mg was 44.30 months. The 3-year survival rate was 16.7% for subjects treated with the two lowest doses and 66.7% for the patients treated with 200 and 400 mg.

After the protocol was amended, 10 new patients were treated with 200 or 400 mg. In this setting, nine subjects achieved objective response and five (two at 200 and three at 400 mg) had complete response. Another patient treated with 200 mg had a surgery of the residual primary lesion, 6 weeks after irradiation, reaching a sustained disease remission. Since the additional 10 subjects were included after the protocol was amended, this patient set has a shorter follow-up time. Finally, 14 (87.5%) of 16 patients achieved objective response and nine had complete responses at 200 or 400 mg dosage levels. Two additional patients underwent

Table 3. h-R3-Related Adverse Events

Adverse Events	50 mg (n = 3)		100 mg (n = 3)		200 mg (n = 8)		400 mg (n = 8)	
	Grade 1/2	Grade 3	Grade 1/2	Grade 3	Grade 1/2	Grade 3	Grade 1/2	Grade 3
Fever	—	—	2	—	3	—	3	—
Hypotension	—	—	2	—	1	—	4	—
Tremors	—	—	1	—	5	—	4	—
Myalgia	—	—	1	—	2	—	2	—
Headache	—	—	—	—	2	—	3	—
Somnolence	—	—	—	—	1	—	—	1
Disorientation	—	—	—	—	1	—	1	—
Chills	—	—	—	—	—	—	1	—
Hematuria	—	—	—	—	1	—	1	—
Vomiting	—	—	—	—	—	—	1	—
Abnormal serum creatinine	—	—	—	—	—	—	1	—
Elevated ALT	—	—	—	—	—	—	1	—
Anemia	—	—	—	—	—	—	2	—

NOTE. Adverse events were more frequent at the highest dose level.

successful salvage surgery, resulting in 11 (68.75%) complete responses.

At present, the overall mean survival is 22 months; eight patients remain alive and seven are disease-free. One surviving patient had a distant metastasis (lung), whereas 11 patients died as a result of locoregional progression. Three additional subjects died because of unrelated causes while in complete remission of the head and neck neoplasia.

In this small sample, no correlation was found between the tumor/lymph node stage and the rate of locoregional progression. However, 10 of the 11 patients who experienced locoregional failure had T3 or T4, while three subjects had N2 or N3.

Mathematical Modeling for the Kinetic Binding of Anti-EGFR Antibodies

The fact that h-R3 in combination with RT elicited similar response in advanced SCCHN compared with what

is published for other anti-EGFR antibodies, but at lower doses and without skin toxicity was surprising, especially taking into account that h-R3 has less affinity ($K_D = 10^{-9}$ M) than IMC-C225 ($K_D = 10^{-10}$ M) for the EGFR.

Therefore, we wanted to check if, at least in theory, this difference in binding affinity could be a plausible hypothesis for a different biodistribution of the antibodies between tumor and normal tissues.

The model predicted that there is a dependence between the effect and the antibody pharmacodynamic, where intermediate affinity mAbs (10^{-8} and 10^{-9} M) will have the maximum effect (high tumor uptake and low uptake in normal tissues), while lower affinity antibodies would have little tumor uptake and higher affinity mAbs would induce a rapid uptake by normal tissues reducing again the therapeutic index (Fig 3). Although the prediction looks counterintuitive, the model foresees that the

Table 4. Radiotherapy-Related Adverse Events

Adverse Events	50 mg (n = 3)		100 mg (n = 3)		200 mg (n = 8)		400 mg (n = 8)	
	Grade 1/2	Grade 3	Grade 1/2	Grade 3	Grade 1/2	Grade 3	Grade 1/2	Grade 3
Dysphagia	1	1	2	—	3	2	6	—
Mucositis	1	2	3	—	7	1	2	2
Radiation dermatitis	—	1	—	—	6	—	4	2
Laryngitis	—	1	1	—	1	—	2	—
Mouth dryness	—	—	—	—	3	—	3	—
Nausea	—	—	—	—	1	—	1	—
Vomiting	—	—	—	—	2	—	—	—
Headache	—	—	—	—	—	—	1	—
Otalgia	—	—	—	—	1	—	—	—
Anorexia	—	—	—	—	—	—	1	—
Asthenia	—	—	—	—	1	—	—	—

NOTE. No grade 4 adverse events were detected. Seven of 24 patients (29%) developed grade 3 radiation adverse events.

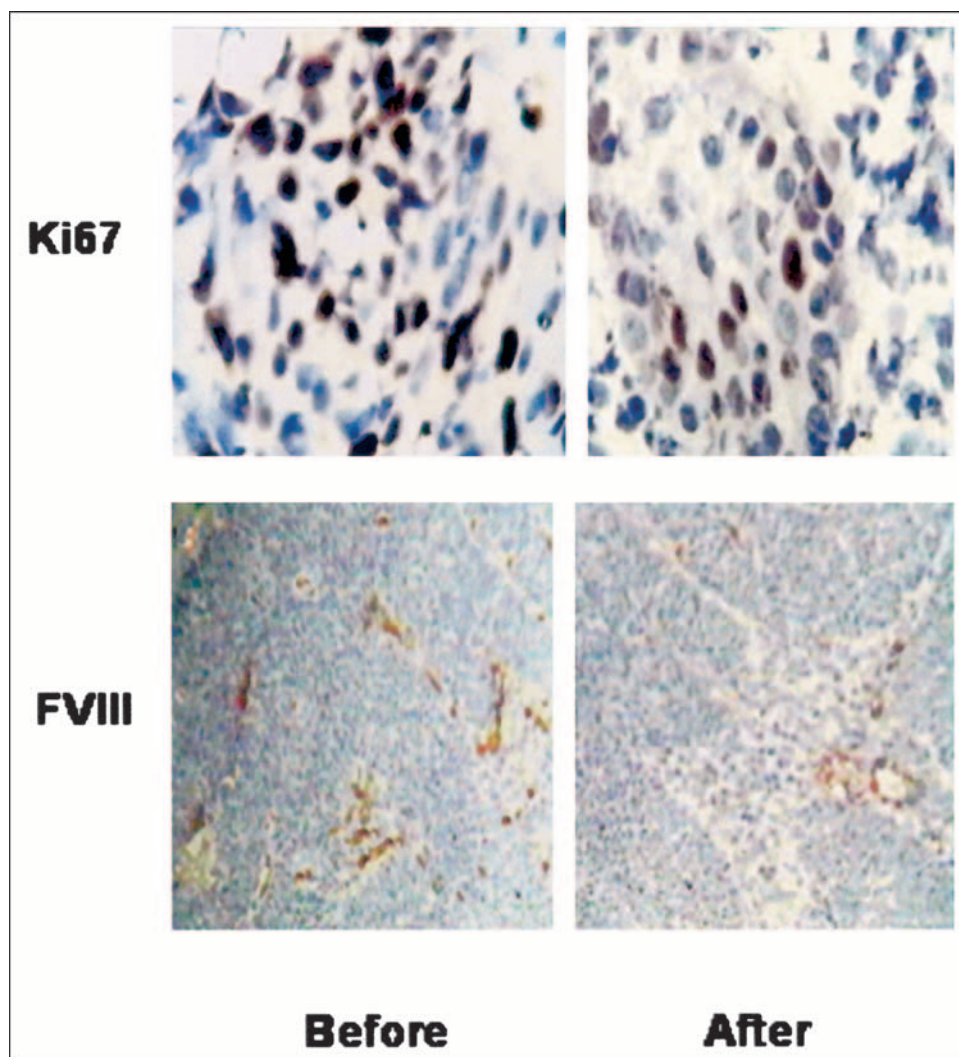


Fig 2. Expression of selected markers (Ki67 and FVIII) in tumor biopsies of a patient treated with h-R3 and radiotherapy before and on-week 3 (on-therapy).

maximum difference between the AUC for the tumor and the normal tissues is reached when the antibody has intermediate affinity.

DISCUSSION

For many years, RT was the conventional treatment for unresectable SCCHN.^{10,21} However, survival was poor¹¹ and many clinical trials evaluating altered fractionation schemes or chemoradiotherapy are now being conducted.²¹⁻²⁵ Here we report the results of an exploratory trial designed to evaluate the safety and preliminary activity of a new anti-EGFR antibody.

Apart from classical cytotoxic drugs, for targeted anti-cancer agents the maximum-tolerated dose does not represent the optimum biologic dose. Therefore in our trial, h-R3 dose was escalated up to the dosages shown to induce maximum EGFR targeting, according to our previous pharmacokinetic data. At this dose range, adverse events consisted

essentially in infusion reactions, which had also been reported after the use of high-doses of antibodies like trastuzumab,²⁶ rituximab,²⁷ alemtuzumab,²⁸ and gemtuzumab.²⁹ Similar reactions including asthenia, fever, and nausea had been found after IMC-C225 infusions.¹² Nevertheless, the most frequent toxicity after the use of other EGFR tyrosine kinase inhibitors, such as ZD1839 or Erlotinib, were skin rash and diarrhea.^{30,31} Likewise, in a meta-analysis involving 21 trials, 77% of patients treated with IMC-C225 were found to experience acneiform rash, while 2% had anaphylactic reactions.³² All patients treated with ABX-EGF, a high affinity antibody, experienced skin rashes after low doses (2.0 or 2.5 mg/kg).³³

This different (and favorable) toxicity profile of h-R3 deserves careful discussion. In comparison with IMC-C225, which is the anti-EGFR antibody most widely evaluated, h-R3 has four main differences: first, it is a humanized antibody with a larger proportion of human sequence; second, it has a lower magnitude affinity to EGFR than IMC-

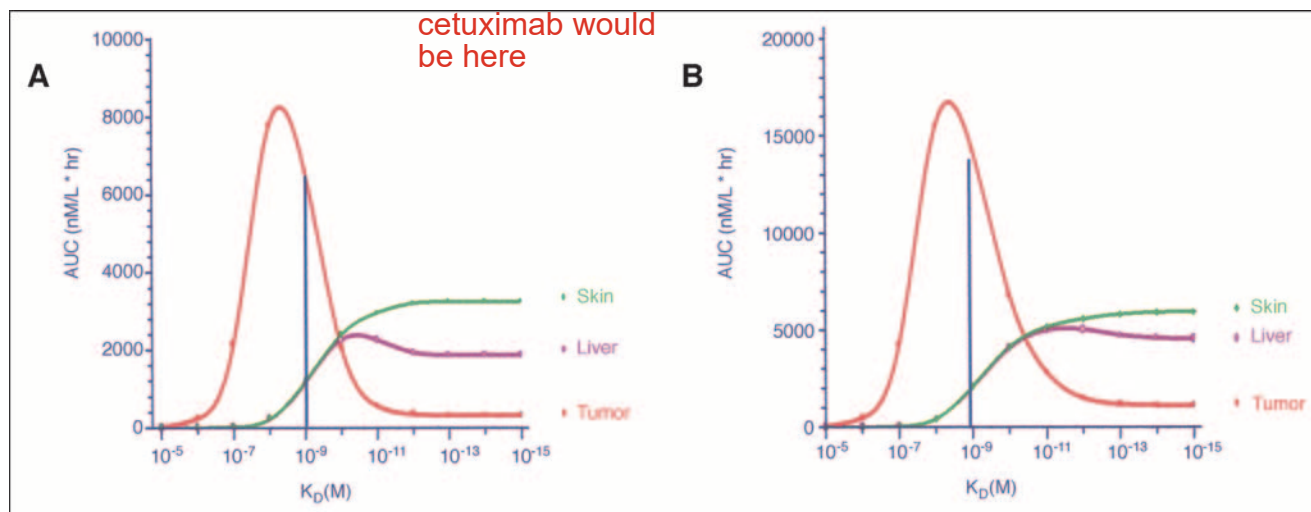


Fig 3. Dependence of the pharmacologic effect on the dissociation constant of the anti-epidermal growth factor receptor (EGFR) antibodies for (A) 100 mg dose and (B) 200 mg dose. Ordinate is the area under the curve (AUC) in nM/L*hr predicted by the mathematical model with different assumptions about the dissociation constant (K_D) of a putative anti-EGFR mAb. M, molar.

C225; third, it has been obtained by humanizing a murine antibody elicited against the EGFR of human placenta (not of cultured cells); and fourth, it has different pharmacokinetic properties.

We speculate that for h-R3, effective EGFR blockade in tumors can be achieved without causing deleterious effects on the skin, because the optimal dose is far lower than the toxic dose. In fact, the mathematical model that we have built predicts that there is an affinity window that can be exploited for EGFR antagonists, and that higher affinity is not necessarily the best. Other differences related to epitope specificity of the antibodies and to consequent changes in receptor signaling cannot be discarded to explain the lack of dermal reactions.

Recently, it had been proposed that skin rash is a surrogate marker of EGFR blockade given the association between survival and rash degree in patients treated with several EGFR inhibitors.^{34,35} Previously, Saltz et al³⁶ reported skin rash in 26 of 27 responding patients treated with IMC-C225 and irinotecan. However, skin rash also appeared in 74% of nonresponding patients.³⁶ Likewise, in several ZD1839 studies, the degree of pharmacodynamic effect and response was not associated with the development of skin toxicity.³⁷ Since the findings regarding skin toxicity and chance of response are still contradictory, it is probably too early to rely on skin toxicity as a surrogate marker of EGFR inhibition in the tumor. The search of new pharmacodynamic markers should continue and studies in tissue samples, when possible, could be helpful in that sense.

Beside the mAb favorable safety profile, radiation toxicity was low in comparison with aggressive chemoradiotherapy. The incidence of grade 3 or worse radiation adverse effects did not differ from the reported for the standard fractionation regimens.²¹

After repeated administrations, h-R3 trough levels matched with mAb doses that were established to be effective in the preclinical models.¹⁶ h-R3 trough levels were similar to those found after the use of IMC-C225 at the doses of 200/200 mg/m² (loading/maintenance) and 400/200 mg/m².¹² Likewise, Bier et al³⁸ reported that weekly administrations of a similar humanized anti-EGFR mAb (EMD 72000) at 200 mg, guarantee reasonable mAb serum concentrations in SCCHN patients.

Since according to our previous results, h-R3 doses above 200 mg were associated with EGFR maximum targeting, patients were grouped in two cohorts—50 and 100 mg (sub-optimal doses) and 200 and 400 mg (optimal doses)—to analyze the impact of the combination therapy. In the optimal dose cohort, the 3-year rate of overall survival was 67%. This figure compares favorably with the historic survival of patients treated with standard fractionated radiation and is similar to the survival rates achieved after the use of aggressive chemoradiotherapy, at the cost of a considerable higher toxicity. In a parallel study conducted in Canada with the same h-R3 antibody, 70% of the patients bearing locally advanced SCCHN achieved complete response after doses of 100 and 200 mg plus RT.³⁹ Long-term follow-up is now being conducted.

In a similar trial using IMC-C225 and radiation for locally advanced SCCHN, encouraging results were also obtained.¹² Thirteen of 15 patients achieved complete response. However, six responding patients relapsed with a median time to progression of 8 months. The actuarial 2-year disease-free survival rate was 65%. In the referred study, a higher cumulative antibody dose (eight to nine infusions) as well as a superior RT dose (70 to 76.8 Gy) was administered. Regarding toxicity, four patients developed allergic reactions.

The absence of hypersensitivity reactions after h-R3 treatment, probably in association with a low anti-idiotypic response, suggests the advantages of humanization.

On the basis of h-R3 trough levels in sera and the response and survival rates at 200 and 400 mg dose, it seems that 200 mg is the dose of choice for unresectable SCCHN.

Finally, this study provided initial information regarding the mechanisms of the antitumor effect of this RT antibody combination. Comparative pre- and post-treatment immunohistochemistry showed clear evidences of antiproliferative and antiangiogenic effects. Our preclinical data had suggested that h-R3 mediates its antineoplastic effect through signal transduction inhibition, which affects cell proliferation, angiogenesis, and cell survival.¹⁶ How much of these effects in the present trial could be attributed to the anti-EGFR antibody and how much to radiation

alone should be assessed in a comparative study between RT and a placebo versus RT plus h-R3. This study is ongoing in patients recruited in a double-blinded, randomized phase II trial. Pharmacodynamic studies in normal skin and tumor tissues after single mAb doses are also planned.

In summary, our preliminary results show that h-R3 is a well-tolerated drug that can enhance tumor radiocurability. The addition of h-R3 to the chemoradiotherapy regimens used at present might increase the response and survival rate without significantly potentiating toxicity, and therefore new trial designs should consider this three-agent approach.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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