PERGAMON

European Journal of Cancer 37 (2001) 2169–2177

European Journal of Cancer

www.ejconline.com

The relationship of epidermal growth factor receptor levels to the prognosis of unresectable pharyngeal cancer patients treated by chemo-radiotherapy

N. Magné[a,d], X. Pivot[b,*], R.-J. Bensadoun[a], E. Guardiola[b], G. Poissonnet[c], O. Dassonville[c], M. Francoual[d], J.-L. Formento[d], F. Demard[c], M. Schneider[b], G. Milano[d]

[a]*Department of Radiotherapy, Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06189 Nice Cedex 2, France*
[b]*Department of Medical Oncology, Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06189 Nice Cedex 2, France*
[c]*Department of Head and Neck Surgery, Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06189 Nice Cedex 2, France*
[d]*Department of Oncopharmacology, Centre Antoine Lacassagne, 33 Avenue de Valombrose, 06189 Nice Cedex 2, France*

Received 6 February 2001; received in revised form 4 June 2001; accepted 8 August 2001

Abstract

The aim of this study was to analyse prognostic factors for time to treatment failure (TTF) and overall survival (OS) in patients with unresectable cancer of the pharynx. A twice daily (b.i.d.) radiotherapy with concomitant cisplatin-5-fluorouracil chemotherapy was administered to 77 consecutive patients (68 males, 9 females; median age: 56 years). The studied factors were: age, gender, tumour differentiation, tumour volume, initial hemoglobin level, karnofsky index (KI), primary tumour location, T, N, epidermal growth factor receptor (EGFR) level in the tumour (fmol/mg protein). KI and EGFR level were significant predictors in a multivariate analysis for TTF ($P=0.004$ and $P=0.0001$) and OS ($P=0.004$ and $P=0.0001$). In order to select subgroups with different outcomes, a stratification of patients was performed based on the EGFR value: patients with tumour EGFR levels <35 fmol/mg protein, between 35 and 275 fmol/mg protein and >275 fmol/mg protein had 95%, 51% and 16% 3 year OS rates, respectively (log rank test; $P=0.0001$). Interestingly, for patients exhibiting a complete response (CR) after concomitant b.i.d. chemo-radiotherapy, patients with EGFR levels <35 fmol/mg protein were all alive at 3 years; in contrast, there was only 70 and 13% 3 year survival rates for patients with EGFR tumour levels between 35 and 275 fmol/mg protein and above 275 fmol/mg protein, respectively. EGFR determination appears to be a powerful prognostic parameter in unresectable pharyngeal cancer patients treated by concomitant chemo-radiotherapy.

Keywords: Head and neck cancer; Prognostic factor; Chemo-radiotherapy; b.i.d. radiotherapy; Epidermal growth factor receptor

1. Introduction

Up to one-third of patients with squamous cell carcinomas of the head and neck present unresectable tumours due to extensive locoregional involvement. These patients carry a poor survival with approximately 10% surviving at 3 years when they are treated by radiotherapy alone [1]. In an attempt to improve their survival, fractionation of the radiation therapy has evolved from a once-daily treatment to hyperfractionation and/or accelerated fractionation. Thus, in terms of locoregional control, an improvement of approximately 15% has been obtained with these new radiotherapy modalities [2]. Nevertheless, none of these radiotherapy regimens have been shown to significantly impact upon patient survival. This fact has served as the impetus for the investigation of treatments based on concomitant chemo-radiotherapy.

In three major randomised clinical trials conducted in advanced head and neck cancer patients, the concomitant chemo-radiotherapy arm had a significant superiority in terms of survival when compared with patients in the radiotherapy alone arm [3–5]. From these

* Corresponding author. Tel.: +33-4-9203-1022; fax: +33-4-9203-1046.
E-mail address: xavier.pivot@cal.nice.fnclcc.fr (X. Pivot).

0959-8049/01/$ - see front matter
PII: S0959-8049(01)00280-5

studies, the increase in 3-year survival rates for those treated by chemo-radiotherapy compared with radiotherapy alone is considered to be approximately 20%. A recent meta-analysis has confirmed that chemotherapy administered concomitantly with radiotherapy had an absolute significant 5-year survival benefit of 8% [6]. In our centre, we have introduced for head and neck cancer patients, the strategy of combining hyperfractionated radiotherapy with cisplatin and 5-fluorouracil [7,8]. This first pilot phase II study confirmed the feasibility of this regimen which is now under evaluation in a multicentre randomised study in France. However, despite the high response rates, the loco-regional control rate and the survival rate at 3 years obtained with chemo-radiotherapy ranged between 35 and 55%.

These survival rates demonstrated the need to identify patients who would need more aggressive or additional treatments. For squamous cell carcinomas of the head and neck, clinical prognostic factors such as primary stage, age and performance status have demonstrated a link with response rates and survival [9]. Numerous data has recently suggested that investigations of biological factors can provide relevant information about the tumour regarding not only the intrinsic biological aggressiveness of the tumour, but also its potential responsiveness to loco-regional curative treatment. These biological factors include labelling index, potential doubling time, cell-cycle time, tumour hypoxia, thymidylate synthase, dihydropyrimidine dehydrogenase, intratumoral neo-angiogenesis, p53 status and epidermal growth factor receptor (EGFR). Levels of particular interest are EGFR and p53 [10,11]. Over the past 20 years, comprehensive data have been accumulated to strongly support a role for EGFR and its ligands in tumour development and growth. We previously confirmed the high level of EGFR expression in head and neck squamous cell carcinoma [12] and demonstrated a survival impact for EGFR expression among a large population of patients with advanced head and neck cancer [13]. Of note, we recently compared the prognostic power of EGFR and p53 and found, through a multivariate analysis, that EGFR had a stronger impact on prognosis than p53 [14]. This led us to select EGFR as the factor to study. Most previously published studies concerned heterogeneous groups of head and neck cancer patients with variable localisations and receiving different treatments (cisplatin–5-fluorouracil induction chemotherapy or combined radio-chemotherapy with cisplatin–5-fluorouracil). This heterogeneity may have limited the clinical usefulness of the findings.

Thus, this study aimed to evaluate the prognostic impact of EGFR expression in a homogeneous group of patients with inoperable squamous cell carcinoma of the pharynx, all treated by the same twice daily (b.i.d.) chemo-radiotherapy.

2. Patients and methods

2.1. Patients

This study involved 77 consecutive patients treated in our institute from January 1992 to June 1999 with strictly unresectable squamous cell carcinoma of the oropharynx and the hypopharynx by a twice-a-day (b.i.d) continuous non-accelerated radiotherapy associated with a concomitant chemotherapy. Radiotherapy was given twice a day (two daily fractions of 1.2 Gy with a minimal 6-h interval between fractions) 5 days a week for 7 weeks, using ^{60}Cobalt or linear accelerator photons (4–6 MV). Two parallel opposed lateral fields were used, each being treated at each session. Doses were prescribed to the intersection point of the beams at midplane (International Commission on Radiological Units and Measurement (ICRU) point). The maximal permissible spinal cord dose was 40.8 Gy. Energy of electrons for the posterior boost to the spinal nodes ranged from 7 to 13 MeV (10–25 Gy total dose, depending on whether the treated region was invaded or not. At 57.6 Gy (48th fraction), fields were reduced to include the primary site only. The total dose was 80.4 Gy (67 fractions/46 days) for the orpharynx and 75.6 Gy (63 fractions/44 days) for the hypopharynx. Allowed dose variations across the tumour target volume in the central plane were limited to 10% of the prescribed dose. Irradiation of the supra-clavicular nodal regions was performed once a day with conventional fractionation (2 Gy/fraction) using an anterior supra-clavicular field. The total dose was 50 Gy for 5 weeks and complemented by direct electron beams to at-risk regions when necessary. A maximum of 3 courses of chemotherapy were given every 21 days. The chemotherapy included cisplatin 100 mg/m^2 intravenously (i.v.) followed by a 5-day continuous infusion of 5-fluorouracil (750 mg/m^2/day course 1, 430 mg/m^2/day courses 2 and 3). During the first day of radiotherapy, the administration of the first cycle of chemotherapy began, and the third course of chemotherapy was administered during the seventh week of radiotherapy or it was not given. Patients were hospitalised during the overall treatment time for enteral nutritional support and mucositis prevention. Feeding tube or percutaneous gastrostomy were systematically performed.

Staging of the primary tumour was performed according to the criteria of the American Joint Committee on Cancer Staging for head and neck cancer [15]. Karnofsky index (KI) was evaluated according to the criteria previously defined in Ref. [16].

2.2. Response to treatment

Because of a high level of locoregional acute inflammatory reactions, clinical response (tumoral, nodal and

global) was assessed 6 months after the completion of treatment. Complete response (CR) corresponded to the disappearance of all clinically visible or palpable lesions. Partial response (PR) was defined as tumour regression greater than 50%. Stable disease was defined as a reduction of tumour size of less than 50%, or an increase in tumour size of less than 25%. Progression was defined as a greater-than-25% increase in measurable disease or the development of new metastases.

2.3. EGFR determination

Tumour samples used in the study were part of the biopsy taken for diagnosis of the tumour. Average weight of the samples dedicated to the EGFR analyses was approximately 350 mg. All tissue samples were stored frozen in liquid nitrogen until the weekly analyses. EGFR was assayed according to a single point method described and developed in our laboratory [17]. Human recombinant [^{125}I]-labelled EGF (specific activity = 900–1400 Ci/mmol) and unlabelled recombinant human EGF were purchased from Amersham (Les Ulis, France). Specific binding was expressed as fmol of bound EGF/mg of membrane protein after a Scatchard plot analysis. For each series of EGFR assays, an internal standard (aliquot of a membrane preparation from human placenta) was used (mean = 281 fmol/mg protein; coefficient of variation = 11%; $n = 11$).

2.4. Statistical analysis

Time to treatment failure (TTF) was calculated from the end of treatment to the progressive disease. Patient overall survival (OS) was calculated from the time of pathological diagnosis to the time of death or last event. Estimates of survival probability (TTF and OS) were obtained using the Kaplan–Meier non-parametric method [18]. Prior to implementation, variables considered for the model were carefully examined to determine those which may be closely correlated in order to eliminate redundant ones. The following variables were studied: age, gender, primary tumour site, KI (60–70% versus 80–100%), level of EGFR in the tumour was analysed as a continuous variable, tumour classification (T2 versus T3 versus T4), and node status (N0 + N1 versus N2 + N3), tumour differentiation (well differentiated versus poorly differentiated), number of chemotherapy courses (2 versus 3), tumour volume (continuous variable) and initial haemoglobin level (continuous variable). Logistic regressions were performed to analyse the impact of variables on response. Cox proportional hazards regression model was used to detect the impact of factors on TTF and OS. Differences in survival duration between subgroups were analysed using a two-sided log-rank and Wilcoxon test. Statistical significance was set at $P < 0.05$. Statistical analyses were carried out using JMP software (SAS Institute, Cary, NC, USA).

3. Results

Patients characteristics are given in Table 1. There were 68 males and 9 females with a median age of 56 years. All patients had a stage IV tumour.

The presence of measurable levels of EGFR in the tumour was found in all of the explored tumours. The levels varied widely from 2 to 2671 fmol/mg protein with a median at 103 fmol/mg protein, and Q1, Q2, Q3 quartiles were 35, 103, 275, respectively. An analysis to search for the relationship of EGFR levels with several factors was performed. No significant relationship was found between age, gender, tumour locations, T, N, tumour differentiation, tumour volume and tumour EGFR levels.

Treatment compliance was obtained in 74 patients and three early deaths related to acute toxicity occurred. 2 patients received only one cycle of chemotherapy due to early death, 57 patients received two cycles, and 18 patients received three cycles. The number of cycles administered was not related to factors such as age,

Table 1
Patients' characteristics

Parameter	No.
Total number of patients	77
Male/female	68/9
Median age (years) (range)	56 (42–73)
Haemoglobin level (median/range; g/l)	128 (110–147)
Karnofsky index	
60–70%	13 (17%)
80–100%	64 (83%)
Primary tumour location	
Oropharynx	41 (53%)
Hypopharynx	16 (21%)
Panpharynx	20 (26%)
Tumour volume (mm^3)	58 210 (3420–99 000)
TNM classification	
Tumour	
T2	6 (8%)
T3	14 (18%)
T4	57 (74%)
Node	
N0	12 (16%)
N1	10 (13%)
N2a	5 (6%)
N2b	19 (25%)
N2c	20 (26%)
N3	11 (14%)
Stage IV	77 (100%)

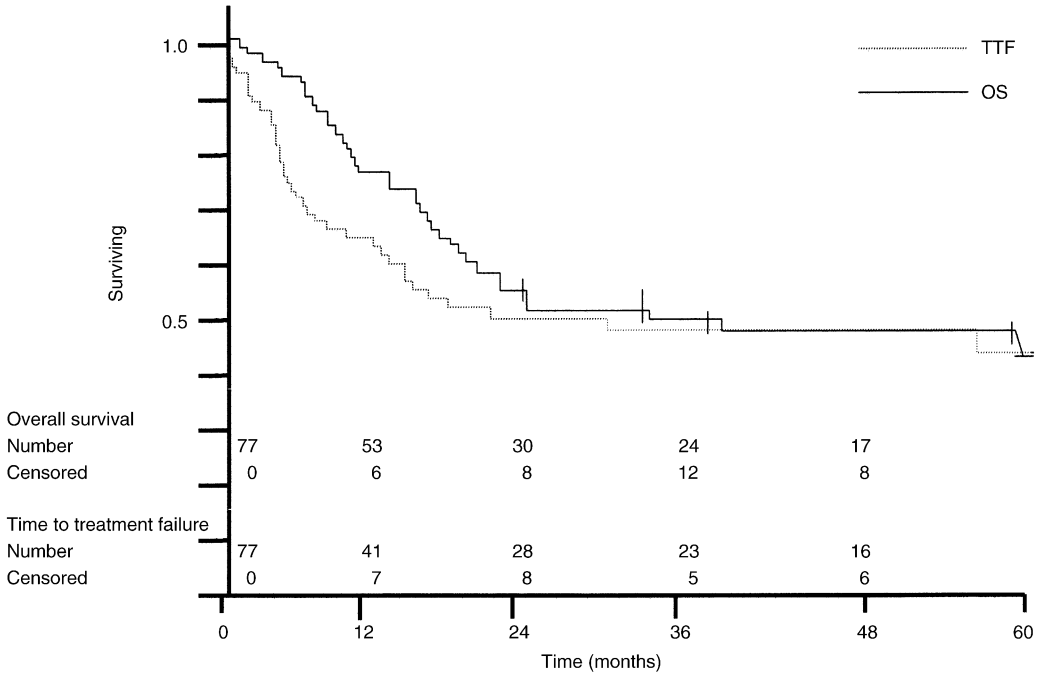


Fig. 1. Time-to-treatment failure (TTF) and overall survival (OS) curves.

gender, KI. Duration of the chemoradiotherapy treatment ranged between 46 and 75 days (median: 54 days).

At the time of analysis, the median follow-up was 18 months (range: 1–97). CR was observed in 52 patients (62%), PR in 11 patients (15%), and PD in 17 patients (23%). The recurrences occurred as follows: locoregional, 20 patients (27%); metastasis, 14 patients (19%); and both, 4 patients (5%). 40 patients were alive at the last date of follow-up. Three-year survival rates for TTF and OS were 49.5 and 47%, respectively. Fig. 1 shows the survival curves.

Table 2 gives the results of the univariate and the multivariate analysis searching for factors related to response, TTF and OS.

Table 2
Results of the univariate and the multivariate logistic regression analysis of the search for factors predicting responses, time-to-treatment failure (TIF) and overall survival (OS)

Covariables	Response				Time-to-treatment failure				Overall survival			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	P	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*	r^2
Age	0.3	–	–	–	0.7	–	–	–	0.4	–	–	–
Gender	0.9	–	–	–	0.02	5	–	–	0.1	–	–	–
Primary tumour site	0.4	–	–	–	0.2	–	–	–	0.2	–	–	–
Karnofsky index (60–70%/80–100%)	0.004	8	0.01	4	0.0001	14	0.004	8	0.001	16	0.004	8
Haemoglobin level[a]	0.6	–	–	–	0.06	–	–	–	0.1	–	–	–
EGFR status[a]	0.02	5	–	–	0.0005	12	0.0001	32	0.0002	14	0.0001	36
Tumour volume[a]	0.7	–	–	–	0.07	–	–	–	0.1	–	–	–
Tumour differentiation (well/poorly)	0.8	–	–	–	0.9	–	–	–	0.8	–	–	–
Size (T2/T3/T4)	0.6	–	–	–	0.3	–	–	–	0.2	–	–	–
Nodal status (N0 + N1/N2 + N3)	0.9	–	–	–	0.05	4	0.4	–	0.04	5	0.3	–
Number of chemotherapy courses (2/3)	0.7	–	–	–	0.2	–	–	–	0.9	–	–	–

[a] Epidermal Growth Factor Receptor (EGFR) status, tumour volume and initial haemoglobin level were analysed as a continuous variable.

In the univariate analysis KI ($P=0.004$) and the EGFR level ($P=0.02$) were significant predictors of response. From the multivariate analysis, only KI remained a predictor of response ($P=0.01$). From the univariate analysis, the significant factors linked to TTF were: gender ($P=0.02$), KI ($P=0.0001$), EGFR level ($P=0.0005$), nodal status ($P=0.05$). By applying a multivariate analysis, KI ($P=0.004$) and EGFR ($P=0.0001$) remained as independent prognostic factors. KI ($P=0.001$), EGFR level ($P=0.0002$) and nodal status ($P=0.02$) were the significant factors for OS in the univariate analysis. In the multivariate analysis, KI ($P=0.004$) and EGFR level ($P=0.0001$) were independent significant prognostic factors for the length of OS.

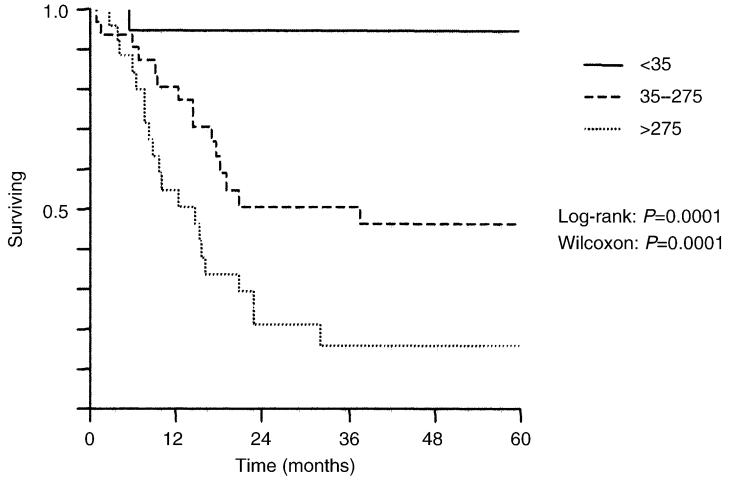
A stratification of patients was performed on the basis of EGFR cut-off values at the different quartiles. Patients with tumour EGFR levels <35 fmol/mg protein, between 35 and 275 fmol/mg protein, and >275 fmol/mg protein had a 3-year OS rate of 95, 51 and 16%, respectively (log-rank test: $P=0.0001$, and Wilcoxon test: $P=0.0001$). The relative risk of death was 3.7- and 7.14-fold higher for patients with an EGFR value between 35–275 fmol/mg protein and >275 fmol/mg protein than patient with a <35 fmol/mg protein EGFR level. Fig. 2 depicts the OS curves according to the three sub-groups.

For CRs, the OS rate at 3 years was 60% versus 26% for non-complete responders (log-rank test: $P=0.0003$, and Wilcoxon test: $P=0.0002$). Fig. 3 illustrates the OS curves of patients according to their response to treatment. Interestingly, among the patients with a CR after b.i.d. chemo-radiotherapy, all patients with EGFR levels <35 fmol/mg protein were alive without relapse at 3 years. In contrast, for CRs with an EGFR tumour level between 35 and 275 fmol/mg protein or an EGFR level >275 fmol/mg protein, the 3-year OS rate dramatically decreased to 70 and 13%, respectively (log-rank test: $P=0.0001$, and Wilcoxon test: $P=0.0001$). Fig. 4 illustrates the OS curves for complete responders according to their pretreatment EGFR tumour levels.

4. Discussion

Despite significant survival improvements in patients with unresectable head and neck squamous cell carcinoma due to the development of chemo-radiotherapy regimens, their survival remains rather poor with an approximately 50% 3-year OS rate [19,20]. The investigational regimen studied in our institution confirms this picture [7,8].

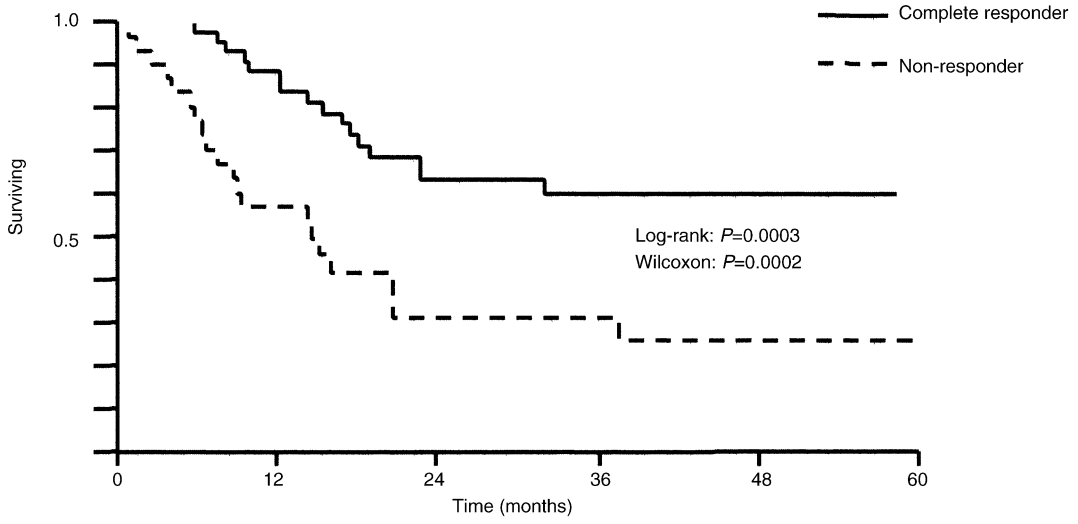
Identification of reliable tumour markers that reflect tumour aggressiveness could provide useful tools for choosing more or less aggressive chemo-radiotherapy regimens. Head and neck cancer illustrates the plethora of potential clinical and biological prognostic factors with parameters linked to cell kinetics [21,22] and apoptosis [23]. However, the most explored biological



Time in months	0	12	24	36	48
EGFR < 35 fmol/mg protein					
N	20	19	19	19	19
Censored	0	2	4	3	3
35 < EGFR < 275 fmol/mg protein					
N	37	27	19	18	17
Censored	0	3	4	1	5
EGFR > 275 fmol/mg protein					
N	20	13	6	3	2
Censored	0	1	0	1	1

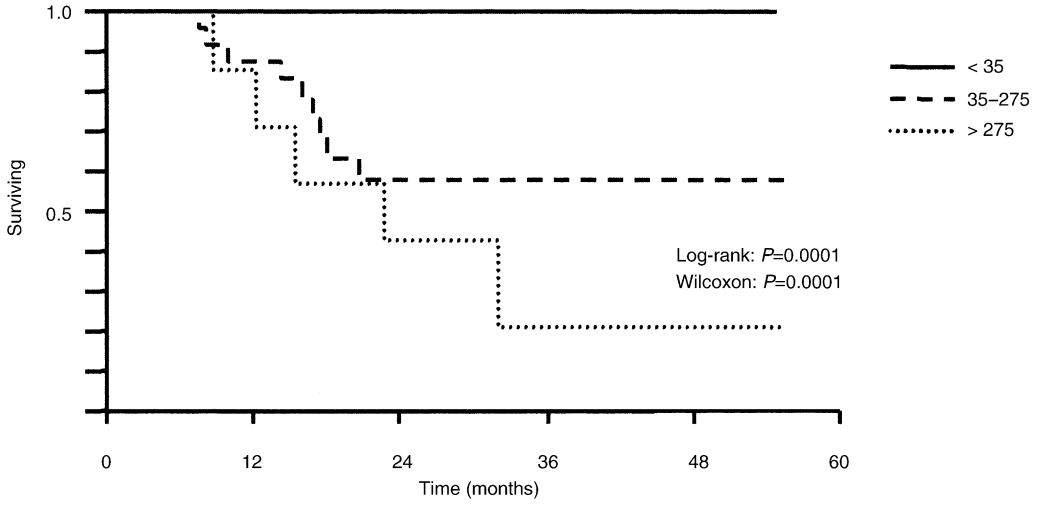
Fig. 2. Overall survival curves according to the three sub-groups of EGFR tumour levels.

factors in head and neck cancer remain p53 [24–26] and EGFR [12,13,27–30]. This fact led us to recently compare their respective prognostic power and we found that, according to a multivariate analysis, EGFR remained the tumour parameter with the highest prognostic impact [14]. Thus, EGFR was studied in this study.



Time in months	0	12	24	36	48
Complete responder					
N	46	38	24	19	14
Censored	0	4	4	4	6
Non-responder					
N	31	17	7	6	3
Censored	0	2	5	0	2

Fig. 3. Overall survival curves according to their response to treatment.



Time in months	0	12	24	36	48
EGFR < 35 fmol/mg protein					
N	17	17	17	17	17
Censored	0	2	4	2	2
35 < EGFR < 275 fmol/mg protein					
N	21	16	10	8	4
Censored	0	2	0	2	4
EFGR > 275 fmol/mg protein					
N	8	7	3	1	1
Censored	0	0	0	1	1

Fig. 4. Overall survival curves for complete responders (CRs) according to their pretreatment EGFR tumour levels.

This study included a homogeneous population of 77 patients with inoperable stage IV squamous cell carcinoma of head and neck treated by an identical b.i.d. radio-chemotherapy regimen. In agreement with recent literature [31], we found that the tumour N-stage had a prognostic impact. However, T and stage did not emerge as prognostic factors in the present study. This is due to the fact that our series of patients was homogeneous with strictly unresectable head and neck cancer. Similarly, the tumour volume did not appear to be a significant factor possibly also due to the fact that a homogeneous population with a large unresectable tumour was used or to the sample size of the study [32]. Primary tumour localisation was not a prognostic factor, probably because all of the tumours were of pharyngeal origin. An impact for nutritional status on the survival of head and neck cancer patients has recently been suggested [33]. This study confirmed the influence of host-related factors, herein expressed by the KI, on the survival of patients treated by concomitant b.i.d. chemo-radiotherapy. The haemoglobin level is a well-established prognostic factor for patients with head and neck cancer treated by radiotherapy alone or by chemo-radiotherapy. However, this factor did not reach significance in the univariate analysis, probably due to the limited variations in the haemoglobin levels or due to the sample size [34].

EGFR tumour levels did not predict response to treatment according to a multiparametric logistic regression (Table 2). Two studies previously performed by Etienne and colleagues and by Dassonville and colleagues reached similar conclusions regarding the absence of a link between the level of EGFR in the tumour and the responsiveness to chemotherapy and/or radiotherapy. It appears that it serves no purpose to take into account EGFR tumour status in evaluating the potential activity of the initial treatment strategy. In contrast, the EGFR level was confirmed to be a highly relevant factor predicting the length of TTF and OS. We agree that it is always preferable to report a prognostic marker analysis using factors treated as continuous variables [35]. However, groups with prespecified cut-points (quartiles values) were examined in this study for reasons of clinical applicability of the EGFR determination. This use of an EGFR cut-off value based on quartile EGFR values showed a dramatic difference in terms of OS (log-rank test: $P = 0.0001$). Thus, 95% of patients were alive at 3 years when the EGFR level was lower than 35 fmol/mg protein versus 51 and 16% of patients alive at 3 years when the EGFR level was between 35 and 275 or higher than 275 fmol/mg protein, respectively.

Whatever the EGFR status, the non-CR patients had a poor prognosis [36]. The likelihood of achieving a complete tumour shrinkage is translated in terms of long survival. In this study, patients with a non-CR had a median OS of 14 months. In contrast, the median OS was not reached for patients achieving a CR. Interestingly, our results show that, among the CR patients, an EGFR level lower than 35 fmol/mg protein was translated into 100% of patients alive without recurrence at 3 years. In contrast, patients with a CR, but with high EGFR levels, had a statistically significant less favourable survival. This subset of patients may need additional treatments to improve their survival.

Interestingly, inhibition of EGFR is emerging as a valuable approach for anticancer therapy. The present results confirm the theoretical usefulness of this therapeutic approach in head and neck cancer. Currently, in this context, two treatment options are under clinical development: monoclonal antibodies and specific inhibitors of the EGFR tyrosine kinase enzyme. On the one hand, monoclonal antibodies, directed at the external domain of the EGFR, have been produced and have been shown to be active against human tumour xenografts in animals. Early clinical trials with a humanised murine chimeric monoclonal antibody to EGFR (C225, Cetuximab®) have shown significant clinical activity in combination with cisplatin or with radiotherapy [37–39]. On the other hand, a variety of inhibitors of the EGFR tyrosine kinase are currently under clinical investigation. These agents inhibit the autophosphorylation of the EGFR, and block the mitotic signal driven by EGFR activation. Among them, ZD1839 (Iressa™) has shown activity alone and in combination with cytotoxic drugs in a variety of cell lines and xenograft tumours and promising results have been obtained in early clinical studies [40].

EGFR appears to be a major prognostic factor in the population of patients with an unresectable head and neck cancer. The administrations of compounds like C225 (Cetuximab®) or ZD1839 (Iressa™) would be appropriate to target patients selected by their EGFR tumour expression and may represent a further refinement of the therapeutic strategy. These new therapeutic approaches represent an exciting and promising path for research aimed at improving the unfavourable prognosis of head and neck cancer patients.

References

1. Vokes EE, Weichselbaum RR, Lipman SM, *et al*. Head and neck cancer. *N Engl J Med* 1993, **328**, 184–194.
2. Fu KK, Pajak TF, Trotti A, *et al*. A radiation therapy oncology group (RTOG) phase III randomized study to compare hyperfractionation and two variants of accelerated fractionation to standard fractionation radiotherapy for head and neck squamous cell carcinomas: first report of RTOG 9003. *Int J Radiat Oncol* 2000, **48**, 7–16.
3. Brizel DM, Albers ME, Fisher SR, *et al*. Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. *N Engl J Med* 1998, **338**, 1798–1804.

4. Calais G, Alfonsi M, Bardet E, *et al*. Randomized trial of radiation therapy versus concomitant chemotherapy and radiation therapy for advanced-stage oropharynx carcinoma. *J Nat Cancer Inst* 1999, **91**, 2081–2086.
5. Wendt TG, Grabenbauer GC, Rodel CM, *et al*. Simultaneous chemo-radiotherapy versus radiotherapy alone in advanced head and neck cancer: a randomized multicenter study. *J Clin Oncol* 1998, **16**, 1318–1324.
6. Pignon JP, Bourhis J, Domenge C, Designé L. Chemotherapy added to locoregional treatment for head and neck squamous-cell carcinoma: three meta-analyses of updated individual data. *Lancet* 2000, **355**, 949–955.
7. Bensadoun RJ, Etienne MC, Dassonville O, *et al*. Concomitant B.I.D. radiotherapy and chemotherapy with cisplatin and 5-fluorouracil in unresectable squamous-cell carcinoma of the pharynx: clinical and pharmacological data of a French multicenter phase II study. *Int J Radiat Oncol Biol Phys* 1998, **42**, 237–245.
8. Marcy PY, Magné N, Bensadoun RJ, *et al*. Systematic percutaneous fluoroscopic gastrostomy for concomitant chemo-radiotherapy of advanced head and neck cancer: optimization of therapy. *Support Care Cancer* 2000, **8**, 410–413.
9. Regueiro CA, Aragon G, Millan I, Valcarcel FJ, de la Torre A, Magallon R. Prognostic factors for local control, regional control and survival in oropharyngeal squamous cell carcinoma. *Eur J Cancer* 1994, **30**, 2060–2067.
10. Veneroni S, Silvestrini R, Costa A, Salvatori P, Faranda A, Molinari R. Biological indicators of survival in patients treated by surgery for squamous cell carcinoma of the oral cavity and oropharynx. *Oral Oncol* 1997, **33**, 408–413.
11. Guerry M, Vabre L, Talbot M, *et al*. Prognostic value of histological and biological markers in pharyngeal squamous cell carcinoma: a case-control study. *Br J Cancer* 1998, **77**, 1932–1936.
12. Santini J, Formento JL, Francoual M, *et al*. Characterization, quantification and potential clinical value of the epidermal growth factor receptor in head and neck squamous cell carcinomas. *Head Neck* 1991, **13**, 132–139.
13. Dassonville O, Formento JL, Francoual M, *et al*. Expression of epidermal growth factor receptor and survival in upper aerodigestive tract cancer. *J Clin Oncol* 1993, **11**, 1873–1878.
14. Etienne MC, Pivot X, Formento JL, *et al*. A multifactorial approach including tumoural epidermal growth factor receptor, p53, thymidylate synthase and dihydropyrimidine dehydrogenase to predict treatment outcome in head and neck cancer patients receiving 5-fluorouracil. *Br J Cancer* 1999, **79**, 1864–1869.
15. American Joint Committee on Cancer. *Manual For Staging of Cancer*, 4th ed. Philadelphia, J.B. Lippincott, 1991.
16. Karnofsky DA, Burchenal JH. The clinical evaluation of chemotherapeutics in cancer. In MacLeod CM, ed. *Evaluation of Chemotherapeutic Agents. Symposium, Microbiology Section, New York Academy of Medicine*. New York, Columbia University, 1949, 191–205.
17. Formento JL, Francoual M, Formento P, *et al*. Epidermal growth factor receptor assay: validation of a single point method and application to breast cancer. *Breast Cancer Res Treat* 1991, **17**, 211–216.
18. Kaplan EL, Meier P. Non-parametric estimation for incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
19. Taylor SG, Murthy AK, Griem KL, *et al*. Concomitant cisplatin/5-FU infusion and radiotherapy in advanced head and neck cancer: 8-year analysis of results. *Head Neck* 1997, **19**, 684–691.
20. Vokes EE, Kies MS, Haraf DJ, *et al*. Concomitant chemoradiotherapy as primary therapy for locoregionally advanced head and neck cancer. *J Clin Oncol* 2000, **18**, 1652–1661.
21. Tomasino RM, Daniele E, Bazan V, *et al*. Prognostic significance of cell kinetics in laryngeal squamous cell carcinoma: clinicopathological associations. *Cancer Res* 1995, **55**, 6103–6108.
22. Pruneri G, Pignataro L, Carboni N, Buffa R, di Finizio D, Cesana BM, Neri A. Clinical relevance of expression of the CIP/KIP cell-cycle inhibitors p21 and p27 in laryngeal cancer. *J Clin Oncol* 1999, **17**, 3150–3159.
23. Homma A, Furuta Y, Oridate N, *et al*. Prognostic significance of clinical parameters and biological markers in patients with squamous cell carcinoma of the head and neck treated with concurrent chemoradiotherapy. *Clin Cancer Res* 1999, **5**, 801–806.
24. Mineta H, Borg A, Dictor M, Wahlberg P, Akervall J, Wennerberg J. P53 mutation, but not p53 overexpression, correlates with survival in head and neck squamous cell carcinoma. *Br J Cancer* 1998, **78**, 1084–1090.
25. Temam S, Flahault A, Périé S, *et al*. P53 gene status as a predictor of tumor response to induction chemotherapy of patients with locoregionally advanced squamous cell carcinomas of the head and neck. *J Clin Oncol* 2000, **18**, 385–394.
26. Cabelguenne A, Blons H, de Waziers I, *et al*. P53 alterations predict tumor response to neoadjuvant chemotherapy in head and neck squamous cell carcinoma: a prospective series. *J Clin Oncol* 2000, **18**, 1465–1473.
27. Grandis JR, Melhem MF, Barnes EL. Quantitative immunohistochemical analysis growth factor-alpha and epidermal growth factor receptor in patients with squamous cell carcinoma of the head and neck. *Cancer* 1996, **78**, 1284–1292.
28. Grandis JR, Melhem MF, Gooding WE, *et al*. Levels of TGF-alpha and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Inst* 1998, **90**, 824–832.
29. Maurizi M, Almadori G, Ferrandina G, *et al*. Prognostic significance of epidermal growth factor receptor in laryngeal squamous cell carcinoma. *Br J Cancer* 1996, **74**, 1253–1257.
30. Salomon DS, Bradt R, Ciardello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Crit Rev Oncol Hematol* 1995, **19**, 183–232.
31. Gamel JW, Jones AS. Squamous carcinoma of the head and neck: cured fraction and median survival time as fuctions of age, sex, histologic type, and node status. *Br J Cancer* 1993, **67**, 1071–1075.
32. Grabenbauer GG, Steininger H, Meyer M, *et al*. Nodal CT density and total tumor volume as prognostic factors after radiation therapy of stage III/IV head and neck cancer. *Radiother Oncol* 1998, **47**, 175–183.
33. Van Bokhorst-De Van der Schueren MAE, van Leeuwen PAM, Kuik DJ, *et al*. The impact of nutritional status on the prognoses of patients with advanced head and neck cancer. *Cancer* 1999, **86**, 519–527.
34. Dietz A, Rudat V, Conradt C, *et al*. Prognostic value of hemoglobin level for primary radiochemotherapy of head and neck carcinomas. *HNO* 2000, **48**, 655–664.
35. Altman DG, Lausen B, Sauerbrei W, Schumacher M. Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 1994, **86**, 829–835.
36. Carvalho AL, Salvajoli JV, Kowalski LP. A comparison of radiotherapy or chemo-radiotherapy with symptomatic treatment alone in patients with advanced head and neck carcinomas. *Eur Arch Otorhinolaryngol* 2000, **257**, 164–167.
37. Mendelsohn J, Shinn DM, Donato N, *et al*. Phase I study of chimerized anti-epidermal growth factor recptor (EGFR) monoclonal antibody, C225, in combination with cisplatin (CDDP) in patients (PTS) with recurrent head and neck and squamous cell carcinoma (SCC). *Proc Am Soc Clin Oncol* 1999 (abstr 1502).
38. Ezekiel MP, Bonner JA, Robert F, *et al*. Phase I trial of chimerized anti-epidermal growth factor receptor (anti-EGFR) antibody in combination with either once-daily or twice-daily irradiation for locally advanced head and neck malignancies. *Proc Am Soc Clin Oncol* 1999 (abstr 1501).

39. Baselga J, Pfister D, Cooper MR, *et al*. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J Clin Oncol* 2000, **18**, 904–914.

40. Ciardello F, Caputo R, Bianco R, *et al*. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa^{TM}), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin Cancer Res* 2000, **6**, 2053–2063.