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"Gefitinib and Radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck"

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“Gefitinib and Radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck ”

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LIST OF PUBLICATIONS

This dissertation is based upon the following publications:

F.Caponigro, G.Franchin, V.Ravo, G.Silvestro, B.Morrica, **C.Romano**, E. Minatel, S. Pepe.
A Phase I-II Trial of Gefitinib (Iressa) and Radiotherapy In Patients With Locally Advanced Inoperable Squamous Cell Carcinoma of The Head And Neck
European Journal Of Cancer, Supplements Vol 3, Issue 2, 2005, Pag. 313

F.Caponigro, **C. Romano**, A. Milano, R. Solla , G. Franchin, V. Adamo, E. Mari, B. Morrica, S.Pepe A phase I/II trial of gefitinib and radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck
Anticancer Drugs 2008; 19:739-744

ABSTRACT

Introduction: Gefitinib, an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, induces growth arrest in squamous cancer cell carcinoma of head and neck (SCCHN) cell lines mainly by blocking cells in G1 and preventing them from entering the cell cycle. Clinical studies have demonstrated the activity of gefitinib monotherapy in SCCHN. Preclinical studies have shown that the combination of radiotherapy (RT) and drugs interfering with the EGF pathway may result in radiosensitization in squamous cell carcinomas that over express EGFR.

Purpose: Two different doses of gefitinib, administered along with standard radiation therapy, were tested in locally advanced inoperable head and neck cancer who have neiver received radiotherapy or chemotherapy or undergone surgery for head and neck carcinoma, with the aim of finding the maximum tolerated dose and assessing the toxicity and activity of the combination.

Patients and methods: The standard "3+3" design was used for the phase I study. Radiation therapy was given according to conventional dose and schedule. Gefitinib dose escalation was stopped if more than a third of patients of a given cohort had dose limiting toxicity (DLT).

Results: DLT was observed in 3 out of 4 patients treated at the dose of 500 mg and included grade 3 stomatitis in 3 patients and grade 3 liver toxicities in 1 patient. The dose level of 250 mg was recommended for the phase II study. Six confirmed objective responses were observed among 16 patients. Four patients had a complete response, which was confirmed in three cases; eight patients had a partial response, which was not confirmed in six patients. Stable disease and disease progression were observed in one and three patients, respectively. Median duration of response was 5.4 (range: 1–21) months. The observed stable disease lasted 7.4 months. The median progression free-survival was 6.7 months (95% CI: 4.5–12.1) and the median OS was 8.5 months.

Conclusion: Our results do not support further trials with gefitinib and radiation therapy, according to our schedule, in this patient population. Integration of gefitinib within chemoradiotherapy regimens and combination with other biological therapies may represent the next challenge.

1. BACKGROUND

1.1 Head and Neck Cancer

Head and neck cancer (HNC) is a common neoplasm with an increasingly high incidence, particularly in the elderly population. Approximately 36,500 new cases of head and neck cancer was be diagnosed in the United States in 2003 and that 11,000 American deaths will result from head and neck malignancies. While head and neck cancer accounts for only 3% of all new cancer cases and 2% of all cancer deaths in the United States annually, it is the fifth most common malignancy worldwide (Jemal et al. 2003) and the third most prevalent cancer after breast and colorectal cancers, accounting for 6% of the individuals with a cancer diagnosis. Squamous cell carcinoma represents the most common histology and tobacco and alcohol are the primary etiologic agents in these cancers. Also genetic susceptibility and various infectious agents can be implicated (Diaz et al. 2003)

Data from the Surveillance, Epidemiology and End Results (SEER) program report also the majority of cancers are from the tongue (21%), gum and other mouth sites (15%), tonsil (11%), and salivary-gland (10%). (SEER-http://seer.cancer.gov/publications/survival/surv/head_neck.pdf). Figure 1 display a rapidly decreasing slope in relative survival until sometime between 18 and 36 months followed by a leveling off for any head and neck cancer sites.

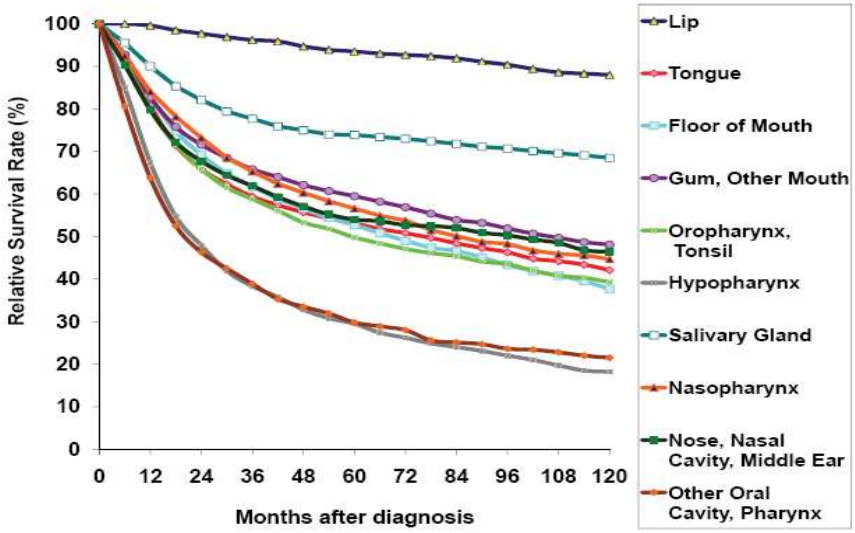

Fig.1: Cancer of the Head and Neck: Relative Survival Rate (%) by Primary Site, Ages 20+, 1988-2001

Patients with lip cancer had the best prognosis, with 5-year relative survival approximately 94%. On the other hand, cancers of the hypopharynx (5-year relative survival rate 30%) and “other cancers of the oral cavity and pharynx” (5-year relative survival rate 30%) have the worst prognoses in terms of relative survival rates**.**

Although early-stage head and neck cancers (especially laryngeal and oral cavity) have high cure rates, over 60% of HNC patients present with advanced disease.

Cure rates decrease, of course, in locally advanced cases, whose probability of cure is inversely related to tumor size and even more so to the extent of regional node involvement. In most cases HNC is diagnosed as unresectable locally advanced disease whose five-year survival is < 10%. In fact, despite improvements in diagnosis and local management, long-term survival rates have not increased significantly over the past 40 years and are among the lowest worldwide of the major cancers (Reuter et al. 2007).

Treatment for early stage disease involves usually surgery and radiation therapy (RT). The treatment of locoregionally advanced disease has evolved gradually from surgery as the mainstay of treatment to radiotherapy as the principal treatment (Kramer et al. 1987). More recently, additional benefit has been obtained with altered-fractionation-radiotherapy (i.e. accelerated fractionation or hyperfractionated radiotherapy) and with radiotherapy combined with chemotherapy (chemoradiotherapy) either in the definitive setting or following surgery, according to each center's expertise (Garden et al. 2004) (Cohen et al. 2004).

Combined chemoradiotherapy has represented the mainstay of treatment over the last decades, but, disappontingly, the patient outcome has not substantially improved. The value of chemoradiotherapy is, however, counterbalanced by increased and often prohibitive toxicity, particularly among patients with coexisting medical conditions and decreased performance status.

Although altered radiation fractionation and chemoradiotherapy had a favorable impact for advanced head and neck cancer patients, the outcome of patients presenting with stage III-IV HNC is still poor, with 5-year actuarial survival rates fluctuating between 30% and 40% in most trials (Licitra et al 2004). Squamous-cell carcinoma of the head and neck (SCCHN) remains a challenging clinical problem, due to the persisting high rate of local and distant failure, as well as the occurrence of second primaries.

Patients affected by SCCHN also face tremendous impacts on quality of life after definitive therapy. Despite marked advances in reconstructive surgery and rehabilitation, intensity-modulated radiotherapy (IMRT) and conservation approaches, patients continue to have significant functional deficits.

These compelling problems are responsable for the emerging importance of treatment of HNC.

Recent research efforts have attempted to exploit biologic differences that may exist between normal and malignant cells, to develop tumor-specific therapies. During the past decade, intense research has initiated a new era of cancer treatment, that of molecular therapeutics. Promising preclinical studies have prompted the development of clinical trials testing EGFR inhibitors as single agent therapy or in combination with conventional cytotoxic therapy, with response rates lower than anticipated in the advanced disease setting.

The potential advantages of EGFR-TKIs include ease of administration and no issues with infusion reactions.

Monotherapy trials seem to show similar response rates and survival rates between EGFR antibodies and EGFR-TKIs in metastatic, chemotherapy-refractoryHNC. It is unlikely that a clinical trial will directly compare the two approaches with RT.

The Epidermal growth factor receptor (EGFR) is a prime target for new anticancer therapy, with a broad range of inhibitors currently under investigation (Baselga 2001).

1.2 EGFR pathway and its role in HCN

It was in 1980 that Cohen et collegues (1980) managed to purify the Epidermal growth factor receptor (EGFR), 15 years after the initial isolation of its ligand, EGF (Cohen 1965). EGFR is a transmembrane glycoprotein of 170 kDa, encoded by a gene located on chromosome 7p12 (Davies et al. 1980). It belongs to the ErbB receptor family (EGFR or Her-1, Her-2, Her-3, and Her-4). These receptors are composed of an extra-cellular ligand-binding domain, a hydrophobic transmembrane segment, and an intracellular tyrosine kinase domain.

In normal cells, the expression of EGFR ranges from 40,000 to 100,000 receptors per cell (Carpenter et al. 1979). In squamous cell carcinoma of head and neck EGFR and its ligand, TGF-α, are overexpressed in 80–90% of cases; the corresponding magnitudes of increase are 1.7-fold ($P = 0.005$) and 1.9-fold ($P = 0.006$) respectively, when compared to controls (Grandis and Tweardy 1993). The nature of the protein overexpression is thought to result from enhanced transcription, with no apparent change in mRNA stability; gene amplification has been observed less frequently. TGF-α is participating in an autocrine-signalling pathway in transformed, but not in normal mucosal epithelial cells (Grandis et al 1998).

Binding to EGFR by its natural ligands, mainly EGF or transforming growth factor alpha (TGF-α) results in a conformational change in the receptor, which promotes homodimerization with other EGFR molecules or heterodimerization with other HER family members (especially Her-2); dimerization results in subsequent autoactivation of the tyrosine kinase from the intracellular domain of the receptor. This process will activate an intracellular signalling pathway, leading to the inhibition of apoptosis, activation of cell proliferation and angiogenesis (Roskoski et al. 2004), as well as an increase in metastatic spread potential like detailed in Figure 2 (Harari and Huang 2000) were are shown the major signalling pathways downstream of c-erbB-receptors Binding of specific ligands (e.g. TGF-a) may generate homo- or heterodimeric complexes resulting in conformational changes in the intracellular EGFR kinase domain, which lead to autophosphorylation and activation. Consequently, signalling molecules, including growth factor receptor-bound protein-2 (Grb-2), are recruited to the plasma membrane. Activation of several signalling cascades is triggered predominately by the RAS-to-MAPK and the PI-3K/Akt pathways, resulting in enhanced tumour growth, survival, invasion and metastasis.

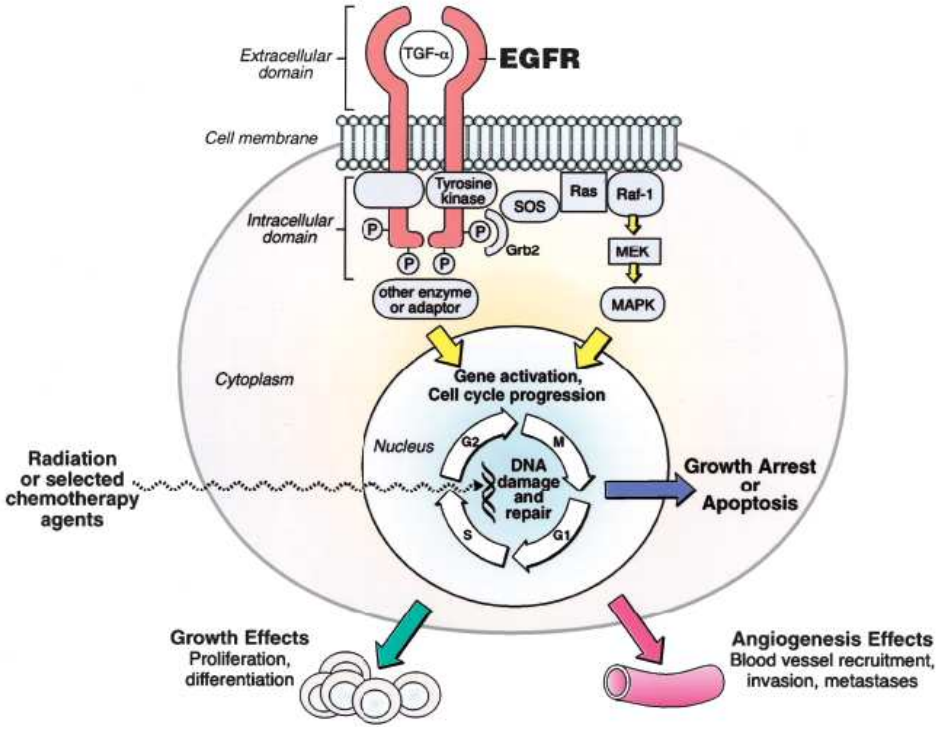

Figure 2: Role of EGFR in signal transduction and tumor progression

Simplified schematic illustration of the EGFR system depicting EGFR, mitogen-activated protein kinase signal transduction cascade to the nucleus, and stimulation of cell cycle machinery

EGFR overexpression is an early event in SCCHN carcinogenesis; it is already present in "healthy" mucosa (field cancerization) from cancer patients, when compared to healthy controls; this overexpression will increase steadily in parallel to observed histological abnormalities, from hyperplasia to invasive carcinoma, through dysplasia and in situ carcinoma (Rubin Grandis et al. 1996)

The epidermal growth factor (EGF) and its receptor (EGFR, ErbB-1, or HER-1) were not only shown to play an influential role in cellular growth and differentiation in healthy tissues, but also in tumorigenesis and the progression of malignant disease (Arteaga 2003).

EGFR is overexpressed in approximately 90% of HNC and has been associated with worse prognosis, thereby providing a stroge rationale for clinical investigation of EGFR-targeting drugs in HNC (Santini et al. 1991).

1.3 Targeting the EGFR in HNC

There is strong evidence that supports targeting of the EGFR in cancer therapy: the EGFR is frequently overexpressed and/or abnormally activated in tumours including SCCHN, colorectal cancer, glioblastoma, or non-small-cell lung cancer. Particularly in SCCHN, multivariate analyses have shown EGFR levels to be an independent predictor of poor outcome (Rubin Grandis et al. 1996) (Ang et al. 2002). Moreover, early studies with anti-EGFR monoclonal antibodies directed against the EGFR were shown to be of clinical benefit (Bonner et al. 2000).

Although there are several potential strategies for targeting the EGFR, only monoclonal antibodies (mAbs) and the low molecular weight tyrosine kinase inhibitors (TKI) are in the most advanced stages of clinical development and will, therefore, be the focus of our attention.

EGFR targeting can be considered as one of the most exploited approaches to a rational targeted therapy of cancer, and it can be basically achieved by two complementary therapeutic strategies .

The first one targets the extracellular domain of the receptor with monoclonal antibodies; binding of the antibody to the EGFR prevents activation of the receptor by endogeneous ligands through competitive inhibition; it also results in internalization and degradation of the antibody-receptor complex, downregulating EGFR expression. Cetuximab or C225, has been the first EGFR-targeting agent to be used in combination with radiotherapy in HNC. Bonner and

collegues (2006) demonstrated in a phase III randomized trial that concomitant radiation (RT) therapy plus cetuximab, improved locoregional control, disease-free survival, and overall survival in locally advanced NHC patients.

The second strategy targets the intracellular domain of the receptor with low-molecular-weight tyrosine kinase inhibitors (gefitinib or ZD 1839 or Iressa®; erlotinib, or OSI-774 or Tarceva®), competing with adenosine triphosphate (ATP) for its binding site on the intracellular domain of EGFR inhibiting EGFR-specific tyrosine kinases (Baselga and Arteaga. 2005).

These two classes of anti-EGFR agents did not meet the expectations in clinical practice when used in monotherapy, resulting more often in a cytostatic than a cytotoxic effect (Raben et al. 2004).

Combining EGFR inhibitors with conventional chemotherapy provided disappointing results so far (Burtness et al. 2005).

1.4 The radiobiological rationale

The effect of radiation on tumor-cell proliferation has been extensively studied in the setting of RT of the head and neck. Accelerated repopulation, a condition of enhanced cellular proliferation after exposure to ionising radiation, appears to be responsible, at least in part, for radioresistance of head and neck cancers. Preclinical evidence suggests that EGFR has an important role in the proliferative response to ionizing radiation, counteracting the toxic effects of RT.

Most preclinical and clinical studies demonstrated a lower local control after radiation therapy in tumors overexpressing EGFR (Baumann and Kraus 2004).

Mechanisms of activation may be diverse, including increased EGFR expression (Schmidt-Ullrich et al. 1994) but one key mechanism involves probably ligand-stimulated activation. RT is able indeed to activate early the transduction signalling pathway of EGFR, through radiation-induced release of TGF-α, one of the EGFR ligands (Dent et al. 1999).

The inhibition of radiation-induced activation of the EGFR signalling pathway is one of the factors explaining the observed synergy between RT and EGFR inhibition; an increase in radiosensitivity through this pathway was demonstrated in vitro (Tofilon et al. 2003). It has to be reminded that, at this point, no clear relationship has been demonstrated between EGFR expression (at least as measured by immunohistochemistry) and the level of radiation sensitization achieved with anti-EGFR. We are unable as well to identify tumors that are radioresistant by virtue of EGFR signalling, and are thus likely to become radiosensitized by EGFR inhibitors (Sartor 2004).

A former study in 140 patients with primary laryngeal squamous-cell carcinoma showed that the 5-year survival rate was 81% for patients with EGFR non-expressing tumors, compared with 25% for patients with EGFR-expressing tumors ($P < 0.0001$) (Maurizi et al. 1996) These results were also confirmed by others (Demiral et al. 2004). A retrospective study (Ang et al. 2002) evaluated the EGFR expression in 155 patients with stage III-IV SCCHN accrued in the control arm of RTOG 9003 study, andreceived exclusive external beam RT (70 Gy in 7 weeks). A detectable expression of EGFR was found in 148/155 patients (95%), with a wide range in interindividual variability. In this study, EGFR expression was found to be independent from tumor stage or initial nodal involvement; in multivariated analysis, it showed to be an independent pronostic factor of overall survival ($P = 0.006$) and of disease-free survival ($P = 0.003$), as well as a robust pronostic factor of locoregional relapse ($P = 0.002$) but not of distant relapse ($P = 0.50$).

If quantitative evaluation of EGFR by immunohistochemistry has emerged so far as a convenient and promising marker for clinical outcome correlation, a more accurate reflection of the "activity state" of EGFR signalling status might be provided by the phosphorylated or "activated" forms of EGFR downstream signalling molecules like phosphorylated MAPK, phosphorylated AKT or Stat-3 (Albanell et al. 2001) (Hambek et al. 2005). They are currently actively evaluated as potential surrogate markers of EGFR signalling in clinical therapeutic trials.

1.5 Gefitinib

Gefitinib (ZD1839, Iressa; AstraZeneca) is a potent and seletive inhibitor of EGFR tyrosine kinase in vitro, inhibits the growt of a wide range of human tumors xenografts in nede mice in a dose-dependent and reversible manner. (Barker et al. 2001) (Stamos et al. 2002). It is a quinazoline analogue (showed in Figure 3) and was approved by the FDA on May 2003 as a third line therapy for non-small cell lung cancer that is unresponsive to cisplatin and docetaxel (Cohen et al. 2003). Gefitinib was the first orally available EGFR tyrosine kinase inhibitor (TKI), to undergo clinical evaluation in human cancer and works as a potent and specific EGFR tyrosine kinase inhibitor (EGFR-TKI) by competing with adenosine triphosphate for the binding site on the intracellular domain of the receptor and by noncompetitively inhibiting epidermal growth factor peptide ligand

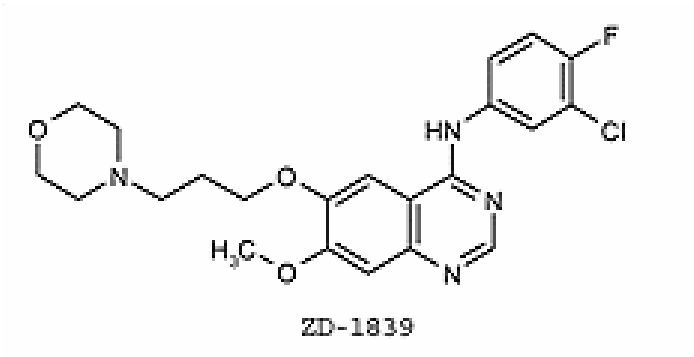

Figure 3: Molecular structure of gefitinib

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A preclinical rationale for gefitinib use in HNC has been provided by Di Gennaro et al (2003). In their studies gefitinib induced growth arrest in HNC cell lines by inhibiting EGFR-mediated signalling; cell cycle kinetic analysis demonstrated that gefitinib induced a delay in cell cycle progression and a G1 arrest together with a partial G2/M block; this was associated with increased expression of both $p27^{KIP1}$ and $p21^{CIP1/WAF1}$.

Gefitinib radiosensitize tumor cells by a variety of mechanisms, including reduction in the proportion of cells in the radioresistant S phase by inducing G0/G1 cell cycle arrest, inhibition of RT-induced damage repair, and induction of apoptosis (Herbst and O'Reilly 2003). Gefitinib has been shown to inhibit repair of RT-induced DNA double-strand breaks and enhanced radiosensitivity in HNC cells. (Shintani et al 2003).

EGFR expression levels in head and neck cancer cell lines correlated with increased RT resistance (Akimoto et al. 1999).(Shintani et al. 2003). In xenograft tumor models, gefitinib in combination with RT resulted in synergistic growth inhibition. (Ochs, 2004). Al-Hazzaa et al(2005) observed a significant decrease in the percentage of surviving cells on treatment with gefitinib and cisplatin (CDDP) compared with CDDP or gefitinib alone in a human HNC cell line. Gefitinib applied before RT and before and/or during CDDP/fluorouracil improved the cytotoxic effect in HNC cell lines. As several preclinical studies have shown the synergistic activity between gefitinib and several cytotoxic drugs, such as cisplatin and 5-fluorouracyl, further clinical studies were performed (Magne et al. 2002).

Thus, combining gefitinib with RT or chemoradiotherapy showed cooperative effects in preclinical studies and warranted clinical investigation in

patients with locally advanced HNC patiemts (Ciardiello et al. 2000) (Sirotnak et al. 2000)

Phase I studies indicated that gefitinib monotherapy was well tolerated, generally with mild, manageable, and reversible adverse effects at doses up to 600 mg/d. The dose used is 250-500 mg / day in a single administration that is far below the maximum tolerated dose of 700 mg. The most frequent drug-related adverse events were acne-like skin rash in 46% to 64% of patients and diarrhea in 47% to 55% of patients, reversible after discontinuation of treatment.(Baselga et al. 2002) (Herbst et al. 2002). Daily administration of gefitinib is safe, with dose-dependent pharmacokinetics but with a high degree of interpatient variability (Ranson et al. 2002), (Nakagawa et al. 2003). Gefitinib significantly suppressed EGFR phosphorylation, inhibited MAPK activation, reduced keratinocyte proliferation and increased p27 levels and apoptosis (Baselga et al. 2002).

The first clinical trial of gefitinib (500 mg/day) in recurrent or metastatic squamous cell HNC was run by Cohen (2003); a 10.6% response rate and a 53% disease control rate were reported; median progression free survival (PFS) was 3.4 months in this study, while median overall survival (OS) was 8.1 months A daily dose of 500 mg was well tolerated, with grade 1 to 2 skin rash in 48% of patients, grade 1 to 2 diarrhea in 42% of patients, and grade 3 diarrhea in 6% of the patients. In another phase II clinical and molecular trial, gefitinib at the dose of 500 mg was tested in 32 patients with recurrent squamous cell HNC. In cohort A (no previous chemotherapy) 3 partial response (PR) and 6 stable disease (SD) were observed out of 20 patients (clinical benefit = 45 %). In cohort B (one previous chemotherapy) 3 out of 12 patients achieved SD (clinical benefit 25%). Median duration of response was 6 months in the overall patient population; median time to progression was 3 months and median survival was 6 months. (Wheeler et al. 2005) The results of an expanded access program of gefitinib as palliative treatment in recurrent or metastatic HNC have been recently reported. Response rate was much lower than in the Cohen study (8%), while disease control was achieved in 36% of patients; median time to progression was 2.6 months, while median survival was 4.3 months (Kirbi et al. 2006).

The comparison of the 2 above studies indicated a substantially better outcome in the first, which can be partially explained by the different patient characteristics in the 2 studies.

Further efforts in the use of gefitinib in patients with recurrent/metastatic HNC included the use of a lower gefitinib dose (250 mg daily) (Cohen et al. 2005,), in keeping with prior studies in non-small cell lung cancer patients which showed similar efficacy for the 250 and 500 mg daily doses but better tolerability for the lower dose (Fukuoka et al. 2003) (Kris 2003). Unfortunately, this study showed that 250 mg gefitinib had less activity than 500 mg, with only one partial

response (giving an objective response of 1.4%), 33% stable disease rate, median progression free survival of 1.8 months and overall survival of 5.5 months.

Chen and collegue (2007) conduced a phase I dose-escalation study of gefitinib in combination with RT or chemoradiotherapy in patients with local advanced HNC to establish the safety and toxicity profile regarding this therapeutic strategy. Because EGFR overexpression seems to be important in HNC carcinogenesis and HNC patients have increased risk in developing a secondary primary tumor and distant metastasis, was studied also the safety, feasibility, and toxicity profile of protracted administration of gefitinib as maintenance therapy for a period of up to 2 years. Two patients had stable disease, and the rest (91%) had a clinical complete response in the primary tumor. With a median follow-up time of 26.3 months (range, 5 to 54.4 months, the estimated rates of 1-year Locoregional Control. Disease Free-Survival and Overall survival were 91%, 82%, and 87%, respectively; and at 3 years, these rates were 85%, 61%, and 74%, respectively. This study howed that gefitinib 500mg daily and definitive RT were well tolerated. The profile of acute toxicity during concurrent gefitinib and chemoradiotherapy was consistent with the toxicity profile reported in the larger chemoradiotherapy trials. Gefitinib does not seem to increase chemoradiotherapy-related mucositis and skin reaction.

Gefitinib monotherapy has undergone a phase III evaluation within a randomized trial of two different doses of gefitinib (250 and 500 mg/daily) and weekly methotrexate, given at the dose of 40 mg/m2. Final data of this study, which was run in patients with recurrent or metastatic squamous cell HNC, with the collaboration of our cancer center, have been recently preliminary presented , and no differences in overall survival, response rates, toxicities were observed among the 3 treatment arms (Simon et al. 2007). As for all biologic drugs, single agent studies have paved the way to combinations with other therapeutic modalities, among which radiation therapy. Following initial reports of enhanced radiation response with anti-EGFR therapies, the confirmation of improved local tumor control in animal model systems using median tissue culture dose experiments was reported examples of specific mechanisms for enhancement of radiation response include the capacity of EGFR inhibitors to abrogate radiation-induced phosphorylation of EGFR, to enhance radiation-induced apoptosis and attenuate radiation-induced expression of DNA repair proteins (Harari and Huang 2006).

To the best of our knowledge, this is the first study of gefitinib and radiotherapy, used togheter without concomitant chemoterapy, to be carried out in locally advanced inoperable squamous cell HNC.

2. AIMS OF THE STUDY

The aim of the present study was to clinically evaluate the combination of radiation therapy and an EGFR-tyrosine kinase inhibitor, such as gefitinib.

In the present trial, two different doses of gefitinib (250 and 500 mg daily), administered along with standard radiation therapy, were tested in locally advanced inoperable squamous cell HNC.

The primary objective of the dose finding part of the trial (phase I) is to identift the maximum totelated dose (MDT) of daily ZD1829 when used in combination with a standard radiotherapy regimen.

The primary objective of the main phase II part of this trial is to assess the activity of the selected dose of ZD1839 in combination with the standard radiotherapy regimen by estimating the ovarall response rate by estimating the complete response (CR) rate, the partial response rate (PR) , the duration of response, progression-free survival; overall survival.

The characterization of the safety profile and tolerability of gefitinib alone and of the combination was a further endpoint of the study.

3. MATERIALS AND METHODS

3.1 Patients selection

Eligibility criteria for study entry included male and female patients aged 18 to 75 years, with newly diagnosed, histologically-confirmed, inoperable, locally advanced squamous cell carcinoma of the head and neck (undifferentiated nasopharyngeal carcinoma was not allowed), who have neiver received radiotherapy or chemotherapy or undergone surgery for head and neck carcinoma, with at least one bidimensionally measurable target lesion according to Response Critera Evaluation in Solid Tumors (RECIST) ; WHO performance status (PS) 0 or 1; life expectancy of at least 3 months; adequate baseline organ function defined as absolute neutrophil count (ANC) $\geq$ 2000 x 10^9 /l, platelets $\geq$ 100,000 x 10^9 /l, bilirubin $\leq$ 1.5 mg/dl, serum transaminases $\leq$ 2.5 times the upper limit of reference range (ULRR) in absence of liver metastases or < 5 times the ULRR in the presence of liver metastases, serum creatinine < 1.25 times the ULRR.

Patients with a history of other co-existing malignancies or malignancies diagnosed within the last 5 years, with the exception of adequately treated cone-biopsed in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin, were also ineligible.

Pregnancy, lactation, uncontrolled infections, unstable systemic diseases, any evidence of clinically active interstitial lung disease and any unresolved chronic toxicity Common Toxicity Criteria (CTC) grade 2 were also exclusion criteria. Concomitant use of phenytoin, carbamazepine, barbiturates, rifampicin or St John's Wort was not allowed because this drugs induce CYP3A4 and may decrease levels of gefitinib.

The study was approved by the Ethics Committee of each participating center. Written informed consent was obtained from each patient before registration and may be discontinued from the trial treatment for withdrawal of informed consent, objective prgression of disease, dropouts, adverse events, ptotocol non-compliance or death.

The trial was performed in accordanse with principles of Good Clinical Practice.

The study was sponsored by AstraZeneca (study no. 1839IL/0070, ClinicalTrials.gov Identifier: NCT00233636).

3.2 Trial design and treatment plan

This was a multicentre, open-label, non-comparative, two step phase I/II study, with a dose-finding period to determine the MTD of daily gefitinib administered in combination with a standard radiotherapy regimen, followed by a phase II study to evaluate the therapeutic activity of the combination of the selected dose of gefitinib with a standard radiotherapy regimen.

Gefitinib was administered once a day on a continuous basis.

The treatment was administered for a maximum of 12 months or until disease progression, unacceptable toxicity or withdrawal of consent. The minimum amount of follow-up was 12 months.

Radiotherapy with curative intent (a seven-to-eight-week course of treatment) was started concomitantly with gefitinib and was administered in daily fractions of 2.0 Gy. Wide treatment fields were planned to encompass the primary tumour and involved neck nodes and a total dose of 52.0 Gy was given to all patients. This was to be followed by a boost to the primary tumour bed, which was to receive at least 64.0 Gy.

Cohorts of three patients were treated with gefitinib at first dose level (250 mg). If one or two patients in the initial cohort had dose-limiting toxicity (DLT), three other patients were enrolled at the same level.

Dose escalation proceeded if no patients had DLT, which was defined as grade 3 to 4 haematologic, neurologic, cardiac (including prolungation of PR interval), lung, renal or hepatic toxicity; grade 3 to 4 weight loss with performance status deterioration; deterioration of visual acuity thought to be associated with gefitinib treatment, grade 3-4 skin toxicity outside the field of irradiation) or acute morbidity grades 3 to 4 in the radiation field, or gastrointestinal toxicity (stomatitis/oesophagitis or vomiting/diarrhea for more than 4 days despite aggressive symptomatic therapy) and grade 4 dysphagia.

The required fon a naso-gastric feed tube as a result of grade 3-4 stomatitis was considered a DLT.

Dose escalation was stopped if more than a third of patients of a given cohort had DLT.

The dose of gefitinib could be interrupted for a maximum of 14 days in the presence of grade 3 or 4 toxicity.

Once the adverse event (AE) decreased in severity to grade 1, the patient continued to take the assigned dose. If the AE resolved to grade 2, patients in the 500 mg cohort had their dose reduced to 250 mg, while patients in the 250 mg cohort were taken off study.

3.3 Patient evaluation

At enrollment, patients were evaluated by a complete history and physical examination, performance status recording, heart rate and blood pressure, complete blood cell (CBC) count, serum chemistries, urinalysis, electrocardiogram (ECG), chest X-ray, total body computed tomography (CT) scan. Other exams were performed only in the presence of clinical indication. Patients were monitored throughout treatment by clinical examination, toxicity assessment, CBC counts, biochemistry, concurrent illness or therapy, on day 1 of each week during radiotherapy. ECG and ophthalmic assessment was performed as clinically indicated.

During the combination trearment (Gefitinib plus Radiotherapy) visits were performed on days 1 to 5 of each week for aproximately 7 week, and the patients were monitored by clinical examination, toxicity assessment, CBC counts, biochemistry, concurrent illness or therapy.

During the treatment with single-agent gefitinib, the patients were monitored by clinical examination, toxicity assessment, CBC counts, biochemistry, concurrent illness or therapy, and tumour assessment 4 weeks after the end of radiotherapy and at every 8 week-intervals thereafter until trial closure. Response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST). (Therasse et al. 2000)

Responding or stable patients received additional treatment for a maximum of 12 months or until progression or unacceptable toxicities. National Cancer Institute (NCI) CTC were used to grade toxicity.

Patient with progressive didease, unacceptable toxicity or withdraw consent withdrawn.

Any ongoin trial treatment-releted toxicity or serious adverse events at withdrawal was monitored until resolution.

At the trial closure, all patients assessed for survival.

The trial flow chart is detailed in Figure 4.

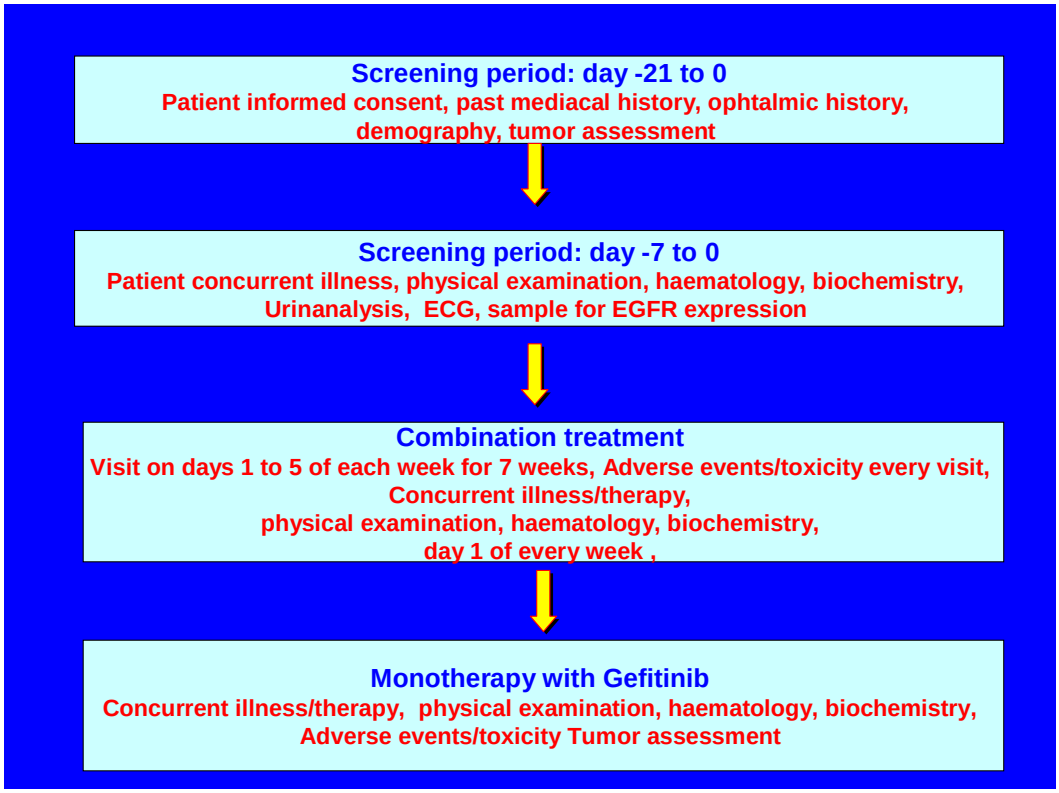


Figure 4. Trial flow chart

3.4 Statistical methods

The standard "3+3" design was used for the phase I study. Fleming's method was used to calculate the number of patients required in the phase II part of the study (13). A sample size of 28 patients receiving the MTD was to give more than 80% probability of rejecting a baseline response rate of 70% with an exact 5% one-sided significance test when the true response was at clinically relevant rate of 90%. The hypothesis that the response rate was equal to or less than the baseline was rejected if 23 or more responses were observed out of the 28 patients.

Objective Response rates (ORR) were summarised by proportions of patients responding by trial closure, intent to-treat population, together with a 95% confidence interval.

Progression free survival (PFS) was summarised by proportion of patients alive and profression-free with disease controlled at trial closure and was

calculated from the time of study entry until the first evidence of disease progression.

Overall survival (OS) was summarised by proportion of patients alive at trial closure and was calculated from the time of study entry to patient's death or last follow-up.

The distribution of time-to-event variables was estimated by the Kaplan–Meier method.

Duartion of response was summarised by median time from objective response to progression or death. Only patients who responded are included in this analysis.

4. RESULTS

4.1 Patients characteristic

Between July 2003 and March 2006, sixteen patients were enrolled in the study. Patients were ascertained also from subjects who referred to Dipartimento di Oncologia ed Endocrinologia at "Federico II" University in Naples, Italy.

The planned sample size was not reached due to the low accrual also for restrictive inclusion criteria.

Twelve patients were male, four patients female. Median age was 58.5 years (range 48-73). Performance status was 1 in the majority of patients. Hypopharynx was the most frequent site of primary tumor.

Extensive lymhnnodes involvement was present in the majority of patients, with distant lymhnnodes involvement in 3 patients

The characteristics of the eligible patients are detailed in table 1.

Table 1: Characteristics of eligible patients (total n.= 16)

Median Age (years) – range	**58.5 (48-73)**
Sex (M/F)	**12/4**
Perfomance status	
0	**5**
1	**11**
Primary site	
Hypopharynx	**7**
Oral cavity	**4**
Oropharynx	**3**
Neck not-otherwise specified	**1**
Parapharyngeal	**1**
Regional lymph nodes involvement	**9**
Distant lymph nodes involvement	**3**

4.2 Dose escalation results

Two dose levels were tested. No DLT occurred among the first 3 patients treated at 250 mg, so gefitinib dose was escalated to 500 mg.

Two patients had DLT among the first 3 patients treated at 500 mg; an additional patient treated at 500 mg had DLT; therefore, the accrual at the higher dose was stopped and further patients were treated at 250 mg.

In total, twelve and four patients were enrolled at dose level of 250 mg and 500 mg, respectively. DLT observed at the higher dose included grade 3 stomatitis in 3 patients and grade 3 liver toxicities in 1 patient.

The dose level of 250 mg was recommended for the phase II study.

The occurrence of adverse events represented the main cause of gefitinib interruption at both dose levels.

Patient decision, liver toxicity, lung toxicity and low-compliance were additional reasons for treatment interruption in one case each.

The median duration of gefitinib treatment was 100 days (range 36-272), and it was 27.4 % (range 9.9- 74.5%) of the maximum planned duration.

The median total given dose of radiotherapy was 69 Gy (range 50-104). Radiotherapy was given for a median of 8 (range 7-13) weeks, which was slightly more than expected, and mainly owing to the occurrence of toxicity.

4.3 Toxicity

Six patients died during the study (3 patients treated at 250 mg and 3 patients at 500 mg). In particular, two of these patients had a cardiovascular arrest; two other patients died of gastrointestinal toxicity (diarrhoea and dysphagia, respectively). The fifth patient passed away following an overwhelming sepsis, whereas the last patient died of an intratumoral hemorrhage.

None of these deaths was considered related to gefitinib by any single investigators, whereas dysphagia was considered likely to be related to radiation therapy.

Five serious adverse events (SAE) occurred in the subgroup of patients treated at 250 mg; three SAE were observed in the group of patients treated at 500 mg.

The overall incidence of treatment-induced serious adverse events was 9%. Sixty-eight adverse events, which are detailed in table 2, were considered linked to the combination of gefitinib and radiotherapy.

Table 2: Number of adverse events related to the combination of gefitinib and radiotherapy

Toxicities	Number of AEs	CTC Grade 1	CTC Grade 2	CTC Grade 3	CTC Grade 4
Stomatitis / Mucositis	14	6	4	4	--
Dysphagia	5	2	3	--	--
Diarrhoea	6	6	--	--	--
Vomiting	3	2	1	--	--
Anorexia	1	1	--	--	--
Fatigue	4	2	2	--	--
Anemia	1	--	--	--	1
Neutropenia	1	--	1	--	--
Fever	1	1	--	--	--
Cough	1	1	--	--	--
Skin toxicities	11	8	3	--	--
Edema	3	2	1	--	--
Worsening of general condition	1	--	--	1	--
Liver toxicities	7	3	--	4	--
Weight loss	4	--	4	--	--
Dysgeusia	1	--	1	--	--
Radiation dermatitis	2	2	--	--	--
Mouth dryness	1	1	--	--	--
Erythema	1	1	--	--	--

AEs: Adverse Events; CTC: Common Toxicity Criteria

Table 3 details grade 3 toxicities observed at the two dose levels.

Table 3: Grade 3- toxicities observed at the two gefitinib dose levels

Grade 3 toxicities	**Gefitinib dose level *250* mg**	**Gefitinib dose level *500* mg**
o **Atherosclerosis**	**1**	**0**
o **Mucositis**	**1**	**0**
o **Hepatic toxicities**	**3**	**1**
o **Stomatitis**	**0**	**3**
o **Worsening of general conditions**	**0**	**1**

Table 4 details grade 4 toxicities observed at the two dose levels.

Table 4: Grade 4 toxicities observed at the two gefitinib dose levels

***Grade 4* toxicities**	**Gefitinib dose level *250* mg**	**Gefitinib dose lelve *500* mg**
• **Anemia**	**1**	**0**
• **Hemorrhage**	**0**	**1**
• **Infection**	**0**	**1**
• **Cardiac arrest**	**1**	**1**
• **Hypercalcemia**	**1**	**0**

4.4 Response

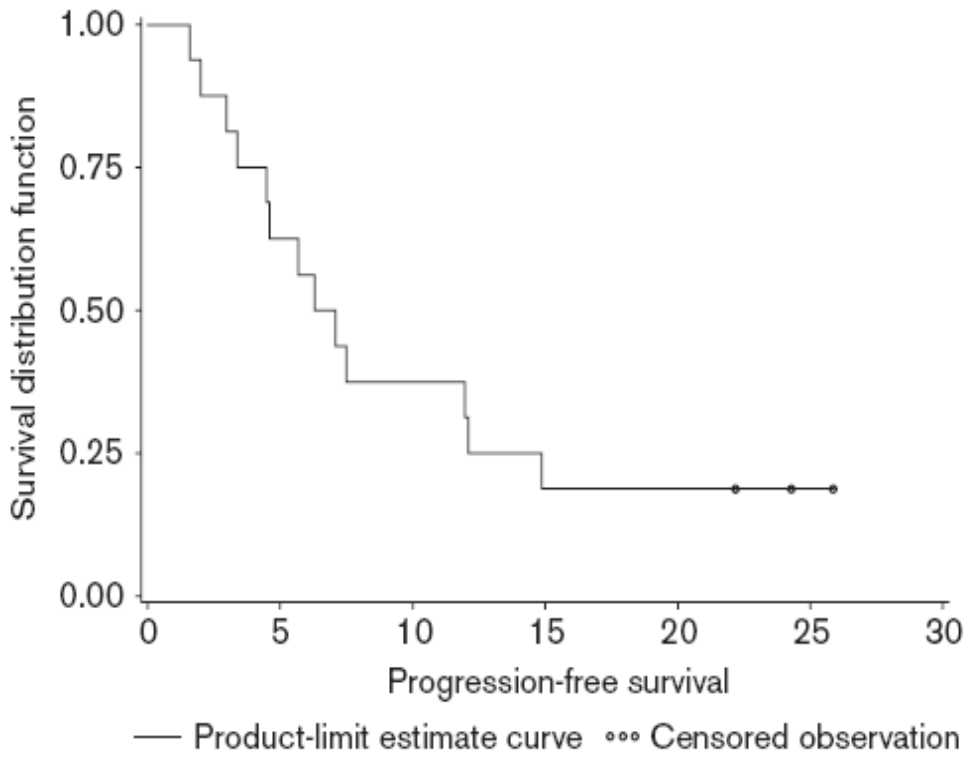
All sixteen patients were evaluable for response. The median duration of follow-up was 8.3 months (range 2-26 months). At the time of study closure, 11 patients had died and 5 were alive.

Four patients had a complete response, which was confirmed in 3 cases; eight patients had a partial response which was not confirmed in six. Stable disease and disease progression were observed in 1 and 3 patients, respectively. Median duration of response was 5.4 months (range 1-21 months). The observed stable disease lasted 7.4 months.

The median progression free survival was 6.7 months (95% CI:4.5-12.1 months

The Kaplan-Meier plots for PFS is shown in figure 5

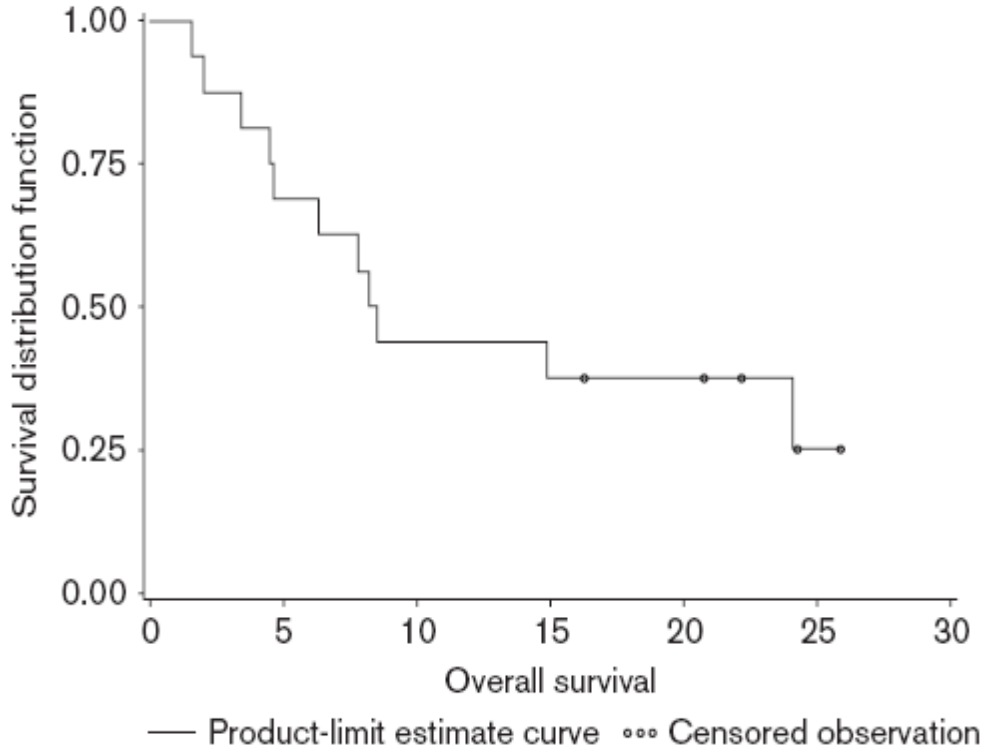
Figure 5: Kaplan-Meier plot of progression free survival



The overall survival was 8.5 months (95%CI: 4.6- not reached).

The Kaplan-Meier plots for OS is shown in figure 6

Figure 6: Kaplan-Meier plot of overall survival



5. DISCUSSION

For many years, radiotherapy has been an acceptable option for patients with locoregionally advanced head and neck cancer. More recently, chemoradiotherapy has been found to improve locoregional control or survival over that with radiotherapy alone in selected groups of patients. (Pignon et al. 2000) (Pignon et al. 2004)

Such combination regimens, however, are associated with high rates of severe and protracted mucositis and an increased need for nutritional support and invasive procedures for that purpose. as soon as late toxic effects, particularly swallowing dysfunction are also common. However, another phase III trial that evaluated chemotherapy combined with high-dose, fractionated radiotherapy, as compared with radiotherapy alone, found absolute increases in the duration of locoregional control and survival at two years of only 6 percentage points and 9 percentage points ($P > 0.10$ for both comparisons), respectively (Staar et al. 2001)

A considerable proportion of patients with head and neck cancer have reduced performance status or coexisting conditions, and these patients may be particularly prone to such adverse events. (Harari et al. 2003) (Denis et al. 2004) (Mittal et al. 2003) (Maguire et al. 2004). The generally greater toxicity of regimens of altered-fractionation radiotherapy places limits on the incremental improvements in efficacy gained by the addition of chemotherapy. In contrast, target therapy may make possible further gains in the efficacy of chemoradiotherapy regimens for head and neck cancer.

Although, to the best of our knowledge, no other trials of gefinitib and radiotherapy only have been carried out, gefitinib has been widely tested in combination with radiochemotherapy, chemotherapy and biological therapy. The rationale for this studies was sound and preclinical data strongly supported them (Harari and Haung 2006).

Ahmed and collegues (2007) have undertaken a study of gefitinib with a concurrent chemoradiation regimen followed by gefitinib adjuvant therapy in locally advanced disease. A very encouraging 91% complete response rate was observed in the study; estimated overall survival was 83% at 2 years, 73% at 3 years while toxicity was consistent with chemoradiotherapy trials.

Chen and collegue (2007) have recently published the data of a phase I trial of concurrent, daily gefitinib and radiation or chemoradiation for patients with locally advanced squamous cell HNC. Eligible patients were treated with daily gefitinib (250 or 500 mg) combined with either altered fraction radiation therapy alone or chemoradiotherapy in patients with intermediate or locally

advanced stage disease, respectively. Once the safety profile of gefitinib and radiotherapy was established, additional patients were accrued combining gefitinib with weekly cisplatin and concurred radiation therapy. The combination of gefitinib and radiotherapy was well tolerated at both gefitinib dose levels, with no significant increase in radiotherapy-induced toxicities. Increased toxicity was observed in patients also receiving chemotherapy, ald DLT included one grade 4 diarrhea and one grade 4 neutropenic fever. Fifteen patients started mantainance gefitinib, and eight (53%) experienced grade 1-2 acne-like skin rash and diarrhea, but no grade 3 or 4 toxicity occurred.

Among clinical studies of gefitinib and chemotherapy, the combination of gefitinib with docetaxel and cisplatin has been tested, and a median progression free survival of 5.1 months has been reported (Arias de la Vega et al. 2007)).

More recently combination studies of other TKI, such as erlotinib and lapatinib, with radiation therapy have been started, but only very preliminary data are currently available.

Significantly different data have been observed with the combination of radiation therapy and cetuximab, a chimeric monoclonal antibody targeting EGFR. This combined approach has shown improved survival with respect to radiation therapy alone in a randomized phase III trial in patients with locally advanced disease, thus qualifying as a possible new standard in this subset of patients (Bonner et al. 2006). The improvements in outcome achieved with radiotherapy plus cetuximab, as compared with radiotherapy alone (absolute survival benefit, 10 percentage points at three years), compare favorably with the greatest increases in efficacy that have been demonstrated for chemoradiotherapy as compared with radiotherapy alone. (Pignon et al. 2004).

The success of combining cetuximab with RT led to the ongoing Radiation Therapy Oncology Group 0522 phase III trial comparing chemoradiotherapy with chemoradiotherapy and cetuximab.

Other monoclonal antibodies, such as panitumumab, a fully human monoclonal antibody anti-EGFR, have shown activity and are now under evaluation.

Like NSCLC, the SCCHN harbors the EGFR mutations which might be responsible for the clinical response of gefitinib in the SCCHN patients. To explore this possibility, a recent Asian study has analyzed EGFR in 41 HNC patients for the detection of somatic mutations by PCR-single-strand conformational polymorphism analysis. Three EGFR mutations (7.3%) were detected in exon 19. However, non significant association with histologic or demographic variables was observed, thus suggesting a different etiology of

EGFR mutations in HNC with respect to non-small cell lung cancer (Lee et al. 2005)

The issue of the occurrence of EGFR mutations and sensitivity to TKI in HNC has also been investigated by Cohen and collaborators (2005). This study has shown the rarity of EGFR kinase mutation in unselected cases of HNC in American patients.

Resistance to EGFR-targeted drugs may be related to abnormal activation of receptor downstream effectors which may render tumors insensitive to EGFR blockade. An intriguing area for investigation is the strategy of blocking two aspects of the same pathway. The combination of cetuximab plus gefitinib or erlotinib enhanced growth inhibition compared with either agent alone in a mouse xenograft model, suggesting that combined treatment with distinct EGFR inhibitory agents can augment the potency of EGFR signaling inhibitionThis concept may pave the way to clinical trials of combinations of EGFR-targeted drugs and downstream acting agents (Caponigro et al. 2006).

Cancer cells have an ability to harness diverse growth factors signalling pathways for cell survival. The existence of these escape mechanisms reinforces the need for combination of targeted therapies, among which combination of anti-EGFR therapies, and combination of therapies targeting EGFR and downstream effectors.

Matar (2004). have studied the effect of the combination of gefitinib and cetuximab in a panel of human cancer cell lines and in an EGFR-dependent human tumor xenograft model (A431). The combined treatment with the two agents resulted in a synergistic effect on cell proliferation, a greater inhibition of EGFR-dependent signalling and induction of apoptosis.

Clinical trials of combinations of EGFR-targeted drugs have recently started and the combination of gefitinib and cetuximab has proved feasible in HNC patients at the common dose of both agents, with hints of meaningful clinical activity .

A phase II, randomized, double-blind, placebo-controlled, seven-arm, multicenter study sponsored by AstraZeneca Pharmaceuticals has completed patient accrual and is currently under analysis. The primary objective of the study was to assess the additive effect of gefitinib 250 or 500mg (administered either concomitantly or as maintenance) with CDDP plus RT in terms of local disease control and disease-free survival rate at 2 years.

6. CONCLUSION

At the time of this trial drafting, radiotherapy was still the standard therapy in locoregional-disease. The role of chemotherapy was gradually, icreasingly acknowledged as effective when given concomitantly with radiation therapy
The aim of the present study was to clinically evaluate the combination of radiation therapy and gefitinib. The dose of 250 mg daily was selected for phase II. Grade 3 stomatitis was the main dose-limiting toxicity of the combination in keeping with a possible worsening of radiotherapy toxic effect induced by gefitinib or also by decreased performance status (PS was 1 in the majority of patients). Six patients died during the treatment and although none of these deaths was considered related to gefitinib, some concern is raised by this occurrence. Furthermore, response and survival data were disappointing.

A major limitation of the data is the lack of additional host-based prognostic factors and HNC consist of a heterogeneous collection of anatomic sites and cell types. Moreover, the planned sample size was not reached due to the low accrual, and hypopharynx was the most frequent site of primary tumor (the hypopharynx cancers have among the lowest relative survival rates of head and neck cancers with a 5-year relative survival rate about 30%). Moreover, extensive lymhnnodes involvement was present in the majority of patients, with distant lymhnnodes involvement in 3 patients

We conclude that our study does not support further trials with gefitinib and radiation therapy according to the present schedule, in this patients population. Appropriate integration of gefitinib within chemoradiotherapy regimens and combination with other molecular targeted approaches including other antireceptor therapies, receptor-downstream signaling transduction inhibitors, and targeted approaches interfering with other essential drivers of cancer, such as angiogenesis, may represent a rational way forward and strong efforts are worth pursuing in this setting.

Finally, well designed trials comparing this regimen with other forms of chemoradiotherapy are warranted. In the absence of these comparisons, physicians and patients should discuss the risks and benefits of each regimen on an individualized basis.

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Abstract 1087:	A phase III trial of gefitinib (IRESSA) and radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck (SCCHN)

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Background: Gefitinib, an orally active epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, induces growth arrest in SCCHN cell lines mainly by blocking cells in G1 and preventing them from entering the cell cycle. Clinical studies have demonstrated the activity of gefitinib monotherapy in SCCHN. Preclinical studies have shown that the combination of RT and drugs interfering with the EGF pathway may result in radiosensitization in squamous cell carcinomas that over express EGFR.
Methods: Pts with histologically confirmed, newly diagnosed, locally advanced inoperable SCCHN, never pretreated with surgery, chemotherapy or RT were enrolled into a phase I-II trial of gefitinib and RT. Two doses of gefitinib were tested (250 and 500 mg/day) in the dose-escalation phase and continued for up to 12 months; RT was administered concomitantly according to standard procedures (minimum of 52.0 grays; boost to the primary tumor site up to at least 64.0 grays). The recommended dose of gefitinib for phase II was determined by the dose-limiting toxicities (DLTs) observed during its combined administration with RT and for 2 weeks thereafter (phase I). Activity was evaluated 4 weeks after the end of the combined treatment and every 8 weeks thereafter, according to RECIST criteria.
Results: 12 pts (9 M, 3 F, median age 58) have been evaluated thus far. The most common primary tumor site was the hypopharynx (5 cases); TNM stage was IV A (10 pts) and IV B (2 pts); tumor grades were 1 (2 pts), 2 (6 pts) and 3 (4 pts). All pts completed the combined treatment according to the protocol. Total radiation dose was 60–74 grays. Overall best response was complete response in 3 pts, partial response in 5 pts, and unconfirmed partial response in 1 pt; 3 pts were not evaluable. Gefitinib-related grade 3 toxicities were mucositis (n = 1), liver toxicity (n = 1). RT-related grade 3 toxicities were stomatitis/mucositis (n = 5), general health deterioration (n = 1). Three pts died

during treatment with gefitinib alone (not considered treatment related). DLT occurred in 3 pts treated with gefitinib 500 mg (grade 3 stomatitis, 3 pts [RT-related]; grade 3 ALT increased, 1 pt [gefitinib-related]), and therefore 250 mg was selected as the recommended gefitinib dose for phase II.
Conclusion: Accrual is continuing in the phase II trial. More mature data will be presented.

A phase I/II trial of gefitinib and radiotherapy in patients with locally advanced inoperable squamous cell carcinoma of the head and neck

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Two different doses of gefitinib, administered along with standard radiation therapy, were tested in locally advanced inoperable head and neck cancer with the aim of finding the maximum tolerated dose and assessing the toxicity and activity of the combination. The standard '3 + 3' design was used for the phase I study. Radiation therapy was given according to conventional dose and schedule. Gefitinib dose escalation was stopped if more than one-third of patients of a given cohort had dose-limiting toxicity. Dose-limiting toxicity was observed in three of four patients treated at the dose of 500 mg, and included grade 3 stomatitis in three patients and grade 3 liver toxicities in one patient. The dose level of 250 mg was recommended for the phase II study. Six confirmed objective responses were observed among 16 patients. Our results do not support further trials with gefitinib and radiation therapy, according to our schedule, in this patient population. Integration of gefitinib within chemoradiotherapy regimens and combination with other biological therapies may represent the next challenge. *Anti-Cancer Drugs* 19:739–744 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: epidermal growth factor receptor, gefitinib, head and neck cancer, phase I/II study, tyrosine kinase inhibitor

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Introduction

Head and neck cancer (HNC) is among the most common cancers worldwide and squamous cell carcinoma represents the most common histology. In most cases, HNC is diagnosed as unresectable locally advanced disease whose 5-year survival is less than 10% [1]. Combined chemoradiotherapy has represented the mainstay of treatment over the last decades, but, disappointingly, patient outcome has not substantially improved.

Epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane glycoprotein receptor, which exerts multiple functions through an intrinsic tyrosine kinase activity, which is activated upon ligand binding. EGFR is overexpressed in approximately 90% of HNC and has been associated with worse prognosis, thereby providing a rationale for clinical investigation of EGFR-targeting drugs in HNC [2].

EGFR targeting can be considered as one of the most exploited approaches to a rational targeted therapy of cancer, and it can be basically achieved by either monoclonal antibodies directed against the extracellular domain of the receptor or by small molecules that act by inhibiting EGFR-specific tyrosine kinases [3]. Gefitinib was the first orally available EGFR–tyrosine kinase inhibitor (TKI) to undergo clinical evaluation in human cancer. A preclinical rationale for gefitinib use in HNC has been provided by Di Gennaro *et al.* [4]. In their studies, gefitinib induced growth arrest in HNC cell lines by inhibiting EGFR-mediated signaling; cell cycle kinetic analysis demonstrated that gefitinib induced a delay in cell cycle progression and a G_1 arrest together with a partial G_2/M block; this was associated with increased expression of both $p27^{KIP1}$ and $p21^{CIP1/WAF1}$. The first clinical trial of gefitinib (500 mg/day) in recurrent or metastatic squamous cell HNC was performed by Cohen *et al.* [5]; a 10.6% response rate and a 53% disease control rate were reported; median progression-free survival (PFS) was 3.4 months in this study, whereas median overall survival (OS) was 8.1 months. In another phase II clinical and molecular trial, gefitinib at the dose of 500 mg was tested in 32 patients with recurrent squamous cell HNC. In cohort A (no previous chemotherapy), three partial responses (PRs) and six stable diseases (SDs) were observed of 20 patients (clinical benefit = 45%). In

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cohort B (one previous chemotherapy), three of 12 patients achieved SD (clinical benefit = 25%). Median duration of response was 6 months in the overall patient population; median time to progression was 3 months, and median survival was 6 months. Importantly, no association between changes in c-myc or cyclin D1 gene expression and clinical benefit was observed [6]. The results of an expanded access program of gefitinib as palliative treatment in recurrent or metastatic HNC have been recently reported. Response rate was much lower than that in the Cohen study (8%), whereas disease control was achieved in 36% of patients. Median time to progression was 2.6 months, whereas median survival was 4.3 months [7]. The comparison of the two above studies indicated a substantially better outcome in the first, which can be partially explained by the different patient characteristics in the two studies. Further efforts in the use of gefitinib in patients with recurrent/metastatic HNC included the use of a lower gefitinib dose (250 mg daily) [8]; in keeping with earlier studies in nonsmall cell lung cancer (NSCLC) that showed similar efficacy for the 250 and 500 mg daily doses, but better tolerability for the lower dose [9,10]. Unfortunately, this study showed that 250 mg gefitinib had less activity than 500 mg, with only one PR (giving an objective response of 1.4%), 33% SD rate, median PFS of 1.8 months, and OS of 5.5 months [8]. Gefitinib monotherapy has undergone a phase III evaluation within a randomized trial of two different doses of gefitinib (250 and 500 mg/daily) and weekly methotrexate, given at the dose of 40 mg/m^2. Final data of this study, which was run in patients with recurrent or metastatic squamous cell HNC, have been recently presented, and no differences in OS, response rates, and toxicities were observed among the three treatment arms [11]. As for all biologic drugs, single agent studies have paved the way to combinations with other therapeutic modalities, among which is radiation therapy. After initial reports of enhanced radiation response with anti-EGFR therapies, the confirmation of improved local tumor control in animal model systems using median tissue culture dose experiments was reported [12]. Examples of specific mechanisms for enhancement of radiation response include the capacity of EGFR inhibitors to abrogate radiation-induced phosphorylation of EGFR, to enhance radiation-induced apoptosis, and to attenuate radiation-induced expression of DNA repair proteins [12].

Cetuximab, a chimeric monoclonal antibody targeting EGFR, has been the first EGFR-targeting agent to be used in combination with radiotherapy in HNC and positive results have been reported. [13].

In the present trial, two different doses of gefitinib (250 and 500 mg daily), administered along with standard radiation therapy, were tested in locally advanced inoperable squamous cell HNC with the main aim of finding the maximum tolerated dose (MTD) and assessing the activity of the combination by estimating the complete response (CR) rate, the PR rate, the duration of response, progression-free survival, and OS. The characterization of the safety profile of gefitinib alone and of the combination was a further endpoint of the study.

To the best of our knowledge, this is the first study of gefitinib and radiotherapy, used together without concomitant chemotherapy, to be carried out in locally advanced inoperable squamous cell HNC.

Patients and methods

Patient selection

Eligibility criteria for study entry included histologically confirmed, inoperable, and locally advanced squamous cell carcinoma of the head and neck (undifferentiated nasopharyngeal carcinoma was not allowed) with at least one bidimensionally measurable target lesion; age 18–75 years; WHO performance status (PS) 0 or 1; life expectancy of at least 3 months; and adequate baseline organ function defined as absolute neutrophil count $\geq 2000 \times 10^9$/l, platelets $\geq 100\,000 \times 10^9$/l, bilirubin ≤ 1.5 mg/dl, serum transaminases ≤ 2.5 times the upper limit of reference range (ULRR) in the absence of liver metastases or less than five times the ULRR in the presence of liver metastases, serum creatinine less than 1.25 times the ULRR. Previous surgery was not allowed. Patients were ineligible if they had received earlier radiotherapy or chemotherapy. Patients with a history of other coexisting malignancies or malignancies diagnosed within the last 5 years, with the exception of adequately treated cone-biopsed in-situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin, were also ineligible. Pregnancy, lactation, uncontrolled infections, unstable systemic diseases, any evidence of clinically active interstitial lung disease, and any unresolved grade 2 or higher Common Toxicity Criteria version 2.0 were also exclusion criteria. Concomitant use of phenytoin, carbamazepine, barbiturates, rifampicin, or St John's Wort was not allowed. The study was sponsored by AstraZeneca, Basiglio, Italy (study no. 1839IL/0070, Clinical Trials. gov Identifier: NCT00233636). The study was approved by the Ethics Committee of each participating center. Written informed consent was obtained from each patient before registration.

Treatment plan

This was a multicenter, open-label, noncomparative, two step phase I/II study, with a dose-finding period to determine the MTD of daily gefitinib administered in combination with a standard radiotherapy regimen, followed by a phase II study to evaluate the therapeutic activity of the combination of the selected dose of gefitinib with a standard radiotherapy regimen.

Gefitinib was administered once a day on a continuous basis. The treatment was administered for a maximum of 12 months or until disease progression, unacceptable toxicity or withdrawal of consent. The minimum amount of follow-up was 12 months. Radiotherapy was started concomitantly with gefitinib and was administered in daily fractions of 2.0 Gy according to the standard of each participating center.

Cohorts of three patients were treated with gefitinib at the first dose level (250 mg). If one or two patients in the initial cohort had dose-limiting toxicity (DLT), three other patients were enrolled at the same level. Dose escalation proceeded if no patients had DLT, which was defined as grade 3–4 hematologic, neurologic, cardiac, lung, renal, or hepatic toxicity; grade 3–4 weight loss with PS deterioration; and deterioration of visual acuity thought to be associated with gefitinib treatment, grade 3–4 skin or gastrointestinal toxicity, and grade 4 dysphagia. Dose escalation was stopped if more than one-third of patients of a given cohort had DLT. The dose of gefitinib could be interrupted for a maximum of 14 days in the presence of grade 3 or 4 toxicity. Once the adverse event (AE) decreased in severity to grade 1, the patient continued to take the assigned dose. If the AE resolved to grade 2, patients in the 500 mg cohort had their dose reduced to 250 mg, whereas patients in the 250 mg cohort were taken off study.

Patient evaluation

At enrollment, patients were evaluated by complete history and physical examination, PS recording, heart rate and blood pressure, complete blood cell (CBC) count, serum chemistries, urinalysis, ECG, chest radiograph, and total body computed tomography scan. Other exams were performed only in the presence of clinical indication. Patients were monitored throughout the treatment by clinical examination, toxicity assessment, CBC counts, biochemistry, concurrent illness, or therapy on day 1 of each week during radiotherapy. ECG and ophthalmic assessment were performed as clinically indicated.

During the treatment with single-agent gefitinib, the patients were monitored by clinical examination, toxicity assessment, CBC counts, biochemistry, concurrent illness or therapy, and tumor assessment 4 weeks after the end of radiotherapy and at every 8-week interval thereafter until trial closure. Response was assessed according to Response Evaluation Criteria in Solid Tumors. Responding or stable patients received additional treatment for maximum of 12 months or until progression or unacceptable toxicities. National Cancer Institute Common Toxicity Criteria version 2.0 were used to grade toxicity.

Statistical methods

The standard '3 + 3' design was used for the phase I study. O'Brien and Fleming's method [14] was used to calculate the number of patients required in the phase II part of the study. A sample size of 28 patients receiving the MTD was to give more than 80% probability of rejecting a baseline response rate of 70% with an exact 5% one-sided significance test when the true response was at clinically relevant rate of 90%. The hypothesis that the response rate was equal to or less than the baseline was rejected if 23 responses or more were observed of the 28 patients. Response rates were summarized by proportions together with a 95% confidence interval (CI). PFS was calculated from the time of study entry to the first evidence of disease progression; OS was calculated from the time of study entry to patient's death or last follow-up. The Kaplan–Meier analysis was used for evaluation of PFS and OS.

Results

Patient characteristics

Between July 2003 and March 2006, 16 patients were enrolled in this study. The planned sample size was not reached owing to the low accrual, which was likely because of the increased awareness that concomitant chemoradiotherapy was the best therapeutic option in this subset of patients. Twelve patients were males, four patients were females. Median age was 58.5 (range: 43–73) years. All patients had stage IV disease. PS was 1 in the majority of patients. Hypopharynx was the most frequent site of primary tumor. The characteristics of the eligible patients are summarized in Table 1.

Dose escalation results

Two dose levels were tested. No DLT occurred among the first three patients treated at 250 mg, so gefitinib dose was escalated to 500 mg. Two patients had DLT among the first three patients treated at 500 mg; an additional patient treated at 500 mg had DLT; therefore, the accrual at the higher dose was stopped and further patients were treated at 250 mg. In total, 12 and four patients were enrolled at dose level of 250 mg and 500 mg, respectively. DLT observed at the higher dose included grade 3 stomatitis in three patients and grade 3 liver toxicities in one patient.

The dose level of 250 mg was recommended for the phase II study. The occurrence of AEs represented the main cause of gefitinib interruption at both dose levels. Patient

Table 1 Characteristics of eligible patients (total $n=16$)

Median age (years) (range)	58.5 (43–73)
Sex (male/female)	12/4
Performance status 0	5
Performance status 1	11
Primary site	
Hypopharynx	7
Oral cavity	4
Oropharynx	3
Neck not otherwise specified	1
Parapharyngeal	1

decision (in two cases) and liver toxicity, lung toxicity, and low compliance (in one case each) were additional reasons for treatment interruption. The median duration of gefitinib treatment was 100 (range: 36–272) days, and it was 27.4 (range: 9.9–74.5%) of the maximum planned duration. The median total given dose of radiotherapy was 69 (range: 50–104) Gy. Radiotherapy was given for a median of 8 (range: 7–13) weeks, which was slightly more than expected, and mainly owing to the occurrence of toxicities.

Toxicity

Six patients died during the study as a result of AEs (three patients treated at 250 mg and three patients at 500 mg). In particular, two of these patients had a cardiovascular arrest and two other patients died of gastrointestinal toxicity (diarrhea and dysphagia, respectively). The fifth patient passed away after an overwhelming sepsis, whereas the last patient died of an intratumoral hemorrhage. None of these deaths was considered related to gefitinib by any single investigators, whereas dysphagia was considered likely to be related to radiation therapy.

Five serious AEs (SAEs) occurred in the subgroup of patients treated at 250 mg; three SAEs were observed in the group of patients treated at 500 mg. The overall incidence of treatment-induced SAEs was 9%. Sixty-eight AEs, which are detailed in Table 2, were considered linked to the combination of gefitinib and radiotherapy. Table 3 details grade 3 and 4 overall toxicities observed at the two dose levels.

Response

All sixteen patients were evaluable for response. The median duration of follow-up was 8.3 (range: 2–26) months. At the time of study closure, 11 patients had died and five were alive. Four patients had a complete response, which was confirmed in three cases; eight patients had a PR, which was not confirmed in six patients. SD and disease progression were observed in one and three patients, respectively. Median duration of response was 5.4 (range: 1–21) months. The observed SD lasted 7.4 months. The median PFS was 6.7 months (95% CI: 4.5–12.1) and the median OS was 8.5 months (95% CI: 4.6–not reached). The Kaplan–Meier plots for PFS and OS are shown in Figs 1 and 2, respectively.

Table 2 Number of adverse events related to the combination of gefitinib and radiotherapy

Toxicity	Number of AEs	CTC grade 1	CTC grade 2	CTC grade 3	CTC grade 4
Stomatitis–mucositis	14	6	4	4	0
Dysphagia	5	2	3	0	0
Diarrhoea	6	6	0	0	0
Vomiting	3	2	1	0	0
Anorexia	1	1	0	0	0
Fatigue	4	2	2	0	0
Anemia	1	0	0	0	1
Neutropenia	1	0	1	0	0
Fever	1	1	0	0	0
Cough	1	1	0	0	0
Skin toxicities	11	8	3	0	0
Edema	3	2	1	0	0
Worsening of general condition	1	0	0	1	0
Liver toxicities	7	3	0	4	0
Weight loss	4	0	4	0	0
Dysgeusia	1	0	1	0	0
Radiation dermatitis	2	2	0	0	0
Mouth dryness	1	1	0	0	0
Erythema	1	1	0	0	0

AEs, adverse events; CTC, Common Toxicity Criteria.

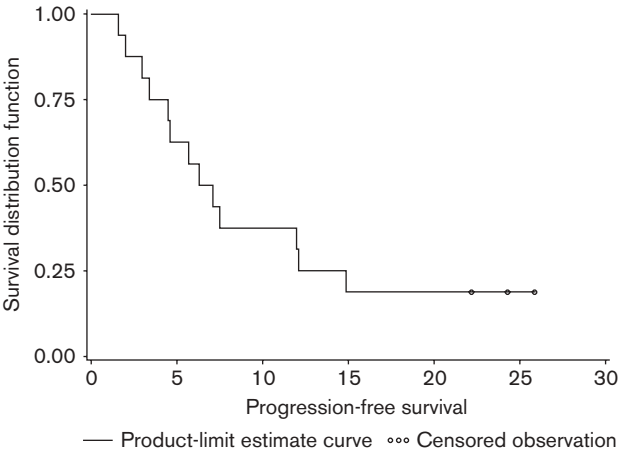
Discussion

The aim of this study was to clinically evaluate the combination of radiation therapy and an EGFR–TKI, such as gefitinib. The rationale for this study was sound and preclinical data strongly supported it [12]. The dose of 250 mg daily was selected for phase II. Grade 3 stomatitis was the main DLT of the combination in keeping with a possible worsening of radiotherapy toxic

Table 3 Grade 3–4 overall toxicities observed at the two gefitinib dose levels

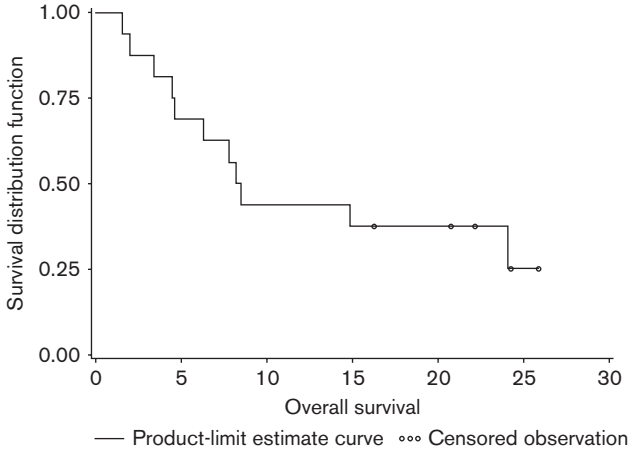
	Gefitinib dose level 250 mg	Gefitinib dose level 500 mg
Grade 3 toxicities		
Atherosclerosis	1	0
Mucositis	1	0
Hepatic toxicities	3	1
Stomatitis	0	3
Worsening of general conditions	0	1
Grade 4 toxicities		
Anemia	1	0
Hemorrhage	0	1
Infection	0	1
Cardiac arrest	1	1
Hypercalcemia	1	0

Fig. 1



The Kaplan–Meier plot of progression-free survival.

Fig. 2



The Kaplan–Meier plot of overall survival.

effect induced by gefitinib. Six patients died as a result of AEs and although none of these deaths was considered related to gefitinib, some concern is raised by this occurrence. Furthermore, response and survival data were disappointing. Although, to the best of our knowledge, no other trials of gefinitib and radiotherapy only have been carried out, gefitinib has been widely tested in combination with radiochemotherapy, chemotherapy, and biological therapy. Ahmed *et al.* [15] have undertaken a study of gefitinib with a concurrent chemoradiation regimen followed by gefitinib adjuvant therapy in locally advanced disease. A very encouraging 91% complete response rate was observed in the study; estimated OS was 83% at 2 years, 73% at 3 years whereas toxicity was consistent with chemoradiotherapy trials. Chen *et al.* [16] have recently published the data of a phase I trial of concurrent, daily gefitinib and radiation or chemoradiation for patients with locally advanced squamous cell HNC. Eligible patients were treated with daily gefitinib (250 or 500 mg) combined with either altered fraction radiation therapy alone or chemoradiotherapy in patients with intermediate or locally advanced stage disease, respectively. Once the safety profile of gefitinib and radiotherapy was established, additional patients were accrued combining gefitinib with weekly cisplatin and concurrent radiation therapy. The combination of gefitinib and radiotherapy was well tolerated at both gefitinib dose levels, with no significant increase in radiotherapy-induced toxicities. Increased toxicity was observed in patients also receiving chemotherapy, and DLT included one grade 4 diarrhea and one grade 4 neutropenic fever. Fifteen patients started maintenance gefitinib, and eight (53%) experienced grade 1–2 acne-like skin rash and diarrhea, but no grade 3 or 4 toxicity occurred. Among clinical studies of gefitinib and chemotherapy, the combination of gefitinib with docetaxel and cisplatin has been tested, and a median PFS of 5.1 months has been reported [17].

More recently, combination studies of other TKI, such as erlotinib and lapatinib, with radiation therapy have been started, but only very preliminary data are currently available. In particular, a phase I/II study of erlotinib with cisplatin and radiotherapy showed that the combination was safe and feasible [18], whereas results from an ongoing phase I study of lapatinib in combination with cisplatin and radiotherapy in locally advanced HNC demonstrated minor AEs and encouraging clinical activity [19].

Significantly different data have been observed with the combination of radiation therapy and cetuximab, a chimeric monoclonal antibody targeting EGFR. This combined approach has shown improved survival with respect to radiation therapy alone in a randomized phase III trial in patients with locally advanced disease, thus qualifying as a possible new standard in this subset of patients [13]. Other monoclonal antibodies, such as panitumumab, a fully human monoclonal antibody anti-EGFR, have shown activity and are now under evaluation.

A recent Asian study has analyzed EGFR in 41 HNC patients for the detection of somatic mutations by PCR-single-strand conformational polymorphism analysis. Three EGFR mutations (7.3%) were detected in exon 19. However, nonsignificant association with histologic or demographic variables was observed, thus suggesting a different etiology of EGFR mutations in HNC with respect to NSCLC [20]. The issue of the occurrence of EGFR mutations and sensitivity to TKI in HNC has also been investigated by Cohen *et al.* [21]. This study has shown the rarity of EGFR kinase mutation in unselected cases of HNC in American patients.

Cancer cells have an ability to harness diverse growth factors signaling pathways for cell survival. The existence of these escape mechanisms reinforces the need for combination of targeted therapies, among which are combination of anti-EGFR therapies and combination of therapies targeting EGFR and downstream effectors. Matar *et al.* [22] have studied the effect of the combination of gefitinib and cetuximab in a panel of human cancer cell lines and in an EGFR-dependent human tumor xenograft model (A431). The combined treatment with the two agents resulted in a synergistic effect on cell proliferation, a greater inhibition of EGFR-dependent signaling, and induction of apoptosis [22]. Clinical trials of combinations of EGFR-targeted drugs have recently started and the combination of gefitinib and cetuximab has proved feasible in HNC patients at the common dose of both agents, with hints of meaningful clinical activity [23]. Resistance to EGFR-targeted drugs may be related to abnormal activation of receptor downstream effectors, which may render tumors insensitive to EGFR blockade.

This concept may pave the way to clinical trials of combinations of EGFR-targeted drugs and downstream acting agents [24]. In particular, phase I studies of gefitinib and sorafenib [25] and of gefitinib and RAD-001 [26], respectively, have been run in NSCLC and are both showing preliminary hints of clinical activity.

We conclude that our study does not support further trials with gefitinib and radiation therapy according to the present schedule. Appropriate integration of gefitinib within chemoradiotherapy regimens and combination with other biological therapies may represent a rational way forward and strong efforts are worth pursuing in this setting.

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