PERGAMON

European Journal of Cancer 37 (2001) S16–S22

European Journal of Cancer

www.ejconline.com

The EGFR as a target for anticancer therapy—focus on cetuximab

J. Baselga*

Medical Oncology Service, Hospital General Universitari Vall d'Hevron, Paseo Vall d'Hebron 119-129, 08035, Barcelona, Spain

Received 7 March 2001; accepted 25 June 2001

Abstract

The anti-epidermal-growth-factor-receptor (EGFR) monoclonal antibody cetuximab specifically binds to the EGFR with high affinity, blocking growth-factor binding, receptor activation and subsequent signal-transduction events leading to cell proliferation. Preclinical studies, both *in vitro* and *in vivo*, have shown that cetuximab enhances the antitumour effects of chemotherapy as well as radiotherapy by inhibiting cell proliferation, angiogenesis and metastasis and by promoting apoptosis. As of June 2000, 526 patients with advanced solid tumours were treated with cetuximab in phase I/II clinical trials. Analysis of the results of three phase I trials showed that cetuximab has non-linear pharmacokinetics, with saturation of drug-elimination pathways occurring at doses between 200 and 400 mg/m^2. Adverse-event data for 239 patients across most of the completed or ongoing phase I–III trials indicated that the antibody was generally well tolerated. Cetuximab has been evaluated both alone and in combination with radiotherapy and various cytotoxic chemotherapeutic agents in a series of phase I/II studies that primarily treated patients with either head and neck or colorectal cancer. Although not a primary objective of these studies, clinical responses to cetuximab were observed in many patients who had previously failed chemotherapy and/or radiotherapy or were otherwise unlikely to achieve a therapeutic outcome. Based on these promising results, additional phase II and phase III trials are currently underway in head and neck and colorectal cancer.

Keywords: Antibodies; Monoclonal; Antineoplastic agents; Chimeric proteins; Cisplatin; Colorectal neoplasms; Drug; head and neck neoplasms; Receptor, Epidermal growth factor

1. Introduction

Decades of research investigating the molecular basis of cancer have produced a new generation of promising therapies designed to target specific molecular processes that promote tumour growth and survival. One of the first important milestones in the development of these novel antitumour agents was the concept of therapy based on inhibiting activation of the epidermal growth factor receptor (EGFR). The EGFR is a transmembrane receptor tyrosine kinase stimulated by growth factors, such as transforming growth factor (TGF)-α or EGF, that bind to the extracellular domain of the receptor (reviewed in Ref. [1]; Fig. 1a). Ligand binding induces receptors to dimerise and activates the intracellular kinase domain present on each receptor, resulting in phosphorylation of tyrosine residues on each member of the receptor pair. Signalling complexes then form in the cytoplasm and activate gene transcription, which in turn induces responses such as cell proliferation. Ultimately, receptor–ligand complexes are internalised and the signal is terminated.

The concept of the EGFR as a therapeutic target developed from several key observations made both at the laboratory bench and in the clinic. First, preclinical studies showed that EGFR activation promotes multiple tumorigenic processes, stimulating proliferation, angiogenesis and metastasis as well as protecting cells from apoptosis (reviewed in Ref. [2]). In addition, Sato and colleagues [3] found that monoclonal antibodies (MAbs) directed against the EGFR inhibited EGF-induced cell proliferation. Finally, clinical evaluations showed that many different types of solid tumours exhibit elevated levels of EGFR and/or its ligands, both of which are often associated with aggressive disease and poor patient outlook [4].

This has led to the development of a number of anti-EGFR strategies that target different components of the EGFR signalling network or cells that express EGFRs.

* Tel.: +34-93-274-6077; fax: +34-93-274-6059.
E-mail address: baselga@hg.vhebron.es (J. Baselga).

0959-8049/01/$ - see front matter
PII: S0959-8049(01)00233-7

For example, tyrosine-kinase inhibitors block signal transduction by inhibiting the intrinsic kinase activity of the EGFR, while ligand–toxin conjugates activate EGFRs and then, when the ligand and its toxic cargo are internalised, kill EGFR-expressing cells. Antisense approaches are also being developed to inhibit the synthesis of growth factors or their receptors. This review focuses on one of these therapeutic strategies—using anti-EGFR MAbs to block EGFR function.

2. Cetuximab—a chimeric anti-EGFR MAb

Cetuximab (also known as C225) is a chimeric MAb that specifically binds to the EGFR with high affinity, preventing the ligand from interacting with the receptor (Fig. 1b). It has a higher affinity for the EGFR than

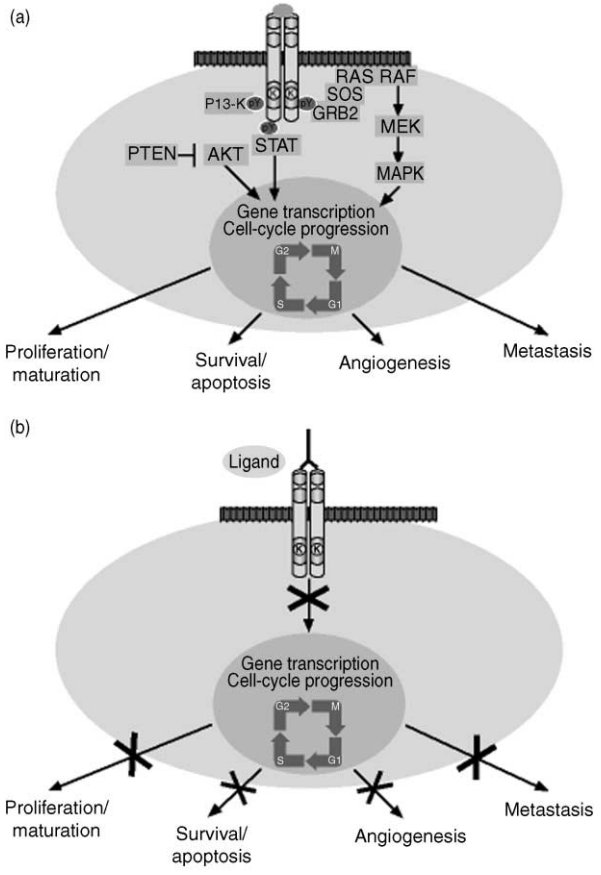

Fig. 1. Epidermal growth factor receptor (EGFR) signal transduction and cetuximab. (a) EGFR signal transduction is initiated when growth factor induces receptor dimerisation and phosphorylation. A transient signalling pathway composed of effector and adapter proteins forms in the cytoplasm and activates gene transcription. The EGFR signalling network then stimulates multiple cellular responses including proliferation, survival, angiogenesis and metastasis. MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inisitol-3-phosphate kinase. (b) The chimeric anti-EGFR monoclonal antibodies, cetuximab, binds to the EGFR with high affinity, blocking growth factors from both accessing the receptor and stimulating EGFR signal transduction pathways.

either TGF-α or EGF and effectively blocks ligand-induced EGFR phosphorylation [5]. Preclinical studies have shown that cetuximab also inhibits growth-factor-induced activation of the downstream mitogen-activated protein kinase (MAPK). There is a strong correlation between cetuximab concentrations sufficient to block MAPK activation and those that inhibit cell proliferation [6], raising the possibility that MAPK phosphorylation may serve as a useful pharmacodynamic marker in clinical studies.

In addition to preventing ligand from binding to the receptor, there is also evidence suggesting that cetuximab promotes receptor internalisation [7]. This may reduce the number of receptors available to interact with ligand on the cell surface. Recent studies with trastuzumab (Herceptin®, Roche), an antibody specific for the related HER2 receptor, have shown that trastuzumab stimulates both the internalisation and degradation of HER2 receptors [8]. It is possible that cetuximab has similar effects on the metabolism of EGFRs.

3. Cetuximab—mechanism of action

3.1. Cell-cycle progression

The antitumour activity of cetuximab has been attributed to several distinct mechanisms. Both cetuximab and M225, its murine progenitor, inhibit cell-cycle progression in many cell lines, causing cells to arrest in the G1 gap phase that occurs prior to DNA synthesis. An elegant series of experiments has shown that anti-EGFR antibody treatment causes an increase in the expression of the cell-cycle inhibitor $p27^{kip1}$ [9–11]. This in turn results in an increase in the formation of inhibitory $p27^{kip1}$–Cdk2 complexes which prevent cells from exiting the G1 phase of the cell cycle [9,10]. Similar antiproliferative effects have been observed *in vivo*, as cetuximab treatment led to an increase in $p27^{kip1}$ levels and a reduction in proliferating cell nuclear antigen (PCNA) expression in human tumour xenografts in nude mice [12].

3.2. Angiogenesis and metastasis

There is a growing body of data characterising cetuximab's anti-angiogenic properties, which were first reported by Petit and colleagues [13]. This group found that established A431 tumour xenografts treated with cetuximab displayed a significant decrease in the production of angiogenic factors, and these data have since been confirmed with additional tumour cell lines [14,15]. For example, in cultured transitional bladder carcinoma cells, cetuximab inhibited EGF-induced secretion of angiogenic factors in a dose-dependent manner. More pronounced effects were observed in the absence of

EGF stimulation in human tumour xenografts. In this study, the expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and interleukin (IL)-8 was correlated with microvessel density, and cetuximab therapy was associated with a decrease in angiogenic factors as well as a decrease in the number of microvessels [14]. There is also evidence indicating that cetuximab therapy inhibits tumour-cell invasion and metastasis. For example, Perrotte and colleagues [14] found that cetuximab therapy significantly inhibited lung metastasis in mice with established human tumour xenografts. While all of the control animals had lymph-node metastases and 40% had lung metastases, none of the cetuximab-treated animals had either type of metastasis. Cetuximab and similar antibodies directed against the external region of the EGFR have also been shown to inhibit the expression and activity of several matrix metalloproteinases (MMPs) that play a key role in tumour-cell adhesion, including the gelatinase MMP-9. Several studies have correlated this antibody-mediated decrease in MMP production with both a significant reduction in *in-vitro* tumour-cell invasion and the inhibition of tumour growth and metastasis in nude mice [16–18]. These inhibitory effects on the invasion, metastasis and angiogenesis of cancer cells may explain why cetuximab treatment is often more effective *in vivo* than *in vitro*.

3.3. Apoptosis

Cetuximab has also been shown to influence apoptosis. Cell survival is dependent, in part, upon the ratio of Bax, which promotes apoptosis, and Bcl-2, which protects cells from apoptosis [19]. Several reports have shown that treatment with cetuximab or similar anti-EGFR antibodies may alter the balance of Bax and Bcl-2 expression. In DiFi human colon carcinoma cells which express high levels of EGFR, cetuximab treatment induced programmed cell death [20]. In these cells, anti-EGFR antibodies caused an increase in the expression of Bax [21] and activated multiple apoptotic caspases [22]. Similarly, in a squamous-cell carcinoma (SCC) cell line, cetuximab treatment led to elevated Bax and decreased Bcl-2 expression and a corresponding increase in the frequency of apoptotic cells [11]. In addition, when cetuximab was applied to breast adenocarcinoma cells, an increase in the inactive phosphorylated form of Bcl-2 was observed [23]. Nevertheless, cetuximab treatment alone was not sufficient to induce cells to undergo programmed cell death and generally had cytostatic effects on cell growth in most of the tumour cell lines that have been studied to date [12]. In combination with several chemotherapeutic agents, however, cetuximab has been shown to increase the incidence of human tumour-cell apoptosis both *in vitro* and *in vivo* in a number of model systems [15,23–26]. It is not yet entirely clear whether the combination of radiotherapy and cetuximab similarly promote programmed cell death *in vivo*.

3.4. Enhancement of the antitumour effects of chemotherapy and radiotherapy

In addition to influencing cellular growth and survival, a number of preclinical studies have shown that cetuximab also potentiates the antitumour effects of chemotherapeutic agents. The synergistic effects of anti-EGFR antibody and chemotherapy (CTX) were first reported by Aboud-Pirak and colleagues [27]. In this study, the combination of anti-EGFR antibody plus cisplatin produced significantly greater growth inhibition of KB oral epidermoid carcinoma-cell xenografts in mice than either treatment alone. Subsequent studies have shown a similar enhancement of antitumour effects in a variety of cancer types. For example, one investigation [28] showed that the maximum tolerated dose of doxorubicin did not inhibit the growth of well-established A431-cell xenografts and that M225 treatment alone only partially reduced tumour growth. In contrast, combined treatment with doxorubicin and M225 produced a marked inhibition of growth, and tumours were eradicated in 40% of the animals [28]. Even more dramatic effects were reported by Fan and colleagues [29] who showed that the combination of M225 and cisplatin completely eradicated well-established A431 cell xenografts in 85% of animals. Furthermore, mice with regressed tumours remained tumour-free for over 6 months, indicating that the therapeutic benefits persisted long after cessation of treatment. In addition to cisplatin and doxorubicin, cetuximab has also been shown to enhance the anti-tumour activity of gemcitabine [15], docetaxel [23,30], paclitaxel [26] and topotecan [25]. Striking synergistic anti-tumour effects on human epidermoid cancer-cell xenografts have also been observed when cetuximab treatment is combined with radiotherapy (RTX; Fig. 2) [31,32].

4. Clinical studies

The efficacy of cetuximab in preclinical tumour models has led to the initiation of many clinical trials. As of June 2000, 526 patients with various tumour types had participated in clinical studies with cetuximab (Table 1). Early phase I dose-ranging trials demonstrated that cetuximab displays non-linear, dose-dependent pharmocokinetics that are not altered by the co-administration of cisplatin. Saturation of drug-elimination pathways occurred at doses between 200 and 400 mg/m^2, and the current recommended dose level is an initial loading dose of 400mg/m^2 followed by weekly maintenance infusions of 200 mg/m^2 [33].

Table 1
Summary of clinical experience with cetuximab

Clinical studies	Completed or ongoing studies[a]	Cancer	Patients treated[a]
Phase I/II, dose-ranging	8	Breast EGFR$^+$-tumours Lung Prostate SCCHN	132
Phase II/III	7	CRC Pancreatic Renal-cell SCCHN	394
Total	15		526

CRC, colorectal cancer; SCCHN, squamous-cell carcinoma of the head and neck; EGFR$^+$, epidermal growth factor receptor positive.
[a] As of June 2000.

4.1. Safety and tolerability

In general, cetuximab appears to be well tolerated, both alone and in combination with CTX or RTX. As of June 2000, safety data were available for 189 patients treated with cetuximab, and drug-related adverse events (AEs) were usually mild to moderate. Grades 1–4 AEs were experienced by 62% of patients, with grades 3–4 AEs reported by 12% of patients. The most common AEs were asthenia (18%), fever (16%), nausea (16%) and acne (15%). Allergic reactions (4% grades 3–4) and acne-like rash (11% grades 3–4) were the most clinically relevant AEs reported. Allergic reactions occurred only during the first infusion. Patients with allergic reactions responded to standard treatments, and subsequent recurrences were controlled with prophylactic antihistamine therapy and increased infusion time. Acne-like rash is now considered to be an expected event, thought to be due to the presence of EGFRs in the epidermis. The rash is not dose-limiting and resolves fully upon cessation of treatment [34].

As cetuximab is a chimeric MAb, it has the potential to stimulate the production of human antichimeric antibodies (HACAs) which may interfere with therapy. However, HACAs were detected in only 3% (4/120) of patients, with neutralising antibodies present in 3 cases.

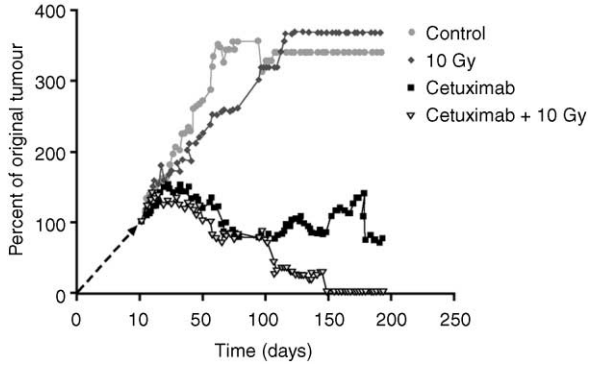

Fig. 2. Synergistic effects of anti-epidermal growth factor receptor (EGFR) antibodies plus radiation on A431 tumour xenografts. Human squamous carcinoma A431 cells were implanted subcutaneously in nude mice. Thirteen days later groups of 8–18 mice received either 10 Gy of ^{60}Co irradiation alone (◆), 10 Gy followed 4 h later by intraperitoneal injections of cetuximab (1 mg) with additional injections administered twice weekly for 4 weeks (▼), cetuximab injections only (■) or no treatment (●). Tumour surface area at each time point is shown as the median for each treatment group. Combination therapy with cetuximab and radiation was associated with a significant increase in the rate of tumour regression. Reproduced with permission from *Cancer Biother Radiopharm* [31].

Patients with detectable HACAs did not experience allergic or anaphylactic reactions, and HACA responses had no clinically limiting effect following weekly infusion of cetuximab [35].

4.2. Cetuximab combined with CTX—evidence of efficacy

More than 100 patients with squamous-cell carcinoma of the head and neck (SCCHN), colorectal cancer (CRC), non-small cell lung cancer (NSCLC) and androgen-independent prostate cancer have received cetuximab in combination with CTX in early phase I and II studies, and in many cases clinical responses have been observed in heavily pretreated patients (Table 2). For example, in a phase I study with advanced SCCHN patients, 67% (6/9) experienced clinical responses to cetuximab combined with cisplatin [36]. Although response to therapy was not a primary objective of this study, this evidence of efficacy—particularly in 3 patients who were previously treated with cisplatin—

Table 2
Response to cetuximab plus CTX

Study phase	Indication	Treatment	Response rates	Reference
Phase I	Advanced SCCHN and NSCLC	Cetuximab + cisplatin	58% (11/19) SD	Baselga and colleagues [33]
Phase I	Advanced SCCHN	Cetuximab + cisplatin	67% (6/9) PR + CR	Mendelsohn and colleagues [36]
Phase I/II	Androgen-independent prostate cancer	Cetuximab + doxorubicin	5% (1/19) SD; 5% (1/19) PR	Slovin and colleagues [38]
Phase II	Refractory SCCHN and CRC	Cetuximab + CTX	22% (14/63) PR + CR	Rubin and colleagues [37]; ImClone Systems Inc. data on file

CRC, colorectal cancer; CR, complete response; CTX, chemotherapy; NSCLC, non-small cell lung cancer; PR, partial response; SD, stable disease; SCCHN, squamous-cell carcinoma of the head and neck.

was promising and led to the initiation of several additional studies. Early phase II studies combining cetuximab with CTX have also yielded positive preliminary results. As of October 2000, 63 patients with SCCHN or CRC who had previously failed surgery, RTX and/or CTX received cetuximab plus either cisplatin or CPT-11. Of the patients with cisplatin-unresponsive SCCHN, 26% (6/23) experienced clinical responses (Fig. 3), and 20% (8/40) of patients with CPT-11-refractory CRC experienced partial or complete responses ([37]; ImClone Systems Inc., data on file). In total, 14 patients experienced clinical responses to cetuximab and a chemotherapeutic compound to which they had previously been resistant.

4.3. Cetuximab and RTX—evidence of efficacy

Promising results have also been obtained from clinical trials with cetuximab and RTX in SCCHN. In a recent phase I study, patients with advanced unresectable disease were treated with either conventional or hyperfractionated radiation combined with weekly doses of cetuximab (100–400 mg/m^2 loading dose and 100–250 mg/m^2 maintenance dose). Although all of the 15 evaluable patients had EGFR$^+$ tumours, which are associated with poor prognosis in SCCHN [39], complete responses were experienced by 87% (13/15) of patients, and the remaining patients (2/15) experienced partial responses [40].

Although caution should be used in drawing conclusions based on retrospective analysis, in the absence of data from comparative phase III studies it is useful to evaluate the response to cetuximab therapy with the data that is currently available. The results of the cetuximab and RTX study described above [40] compare favourably with those of previous trials that treated SCCHN patients with conventional or hyperfractionated RTX alone (Fig. 4a) [41–43]. Of the studies shown in Fig. 4(a), the patients participating in the cetuximab trial had the poorest prognosis, as all of the patients had unresectable disease and the majority (80%) of patients had oropharyngeal carcinomas [40]. The study reported by Zakotnik and colleagues [42] contained the most similar patient population in terms of size and the incidence of oropharyngeal carcinoma and unresectable disease. However, only 31% (10/32) of the patients treated with RTX alone achieved complete responses. In contrast, 87% (13/15) of the patients who received cetuximab plus RTX experienced complete responses, suggesting that cetuximab may enhance the efficacy of RTX. When other response parameters, such as 2-year disease-free survival rates were examined, the combination of cetuximab and RTX also compared well with historical controls (Fig. 4b).

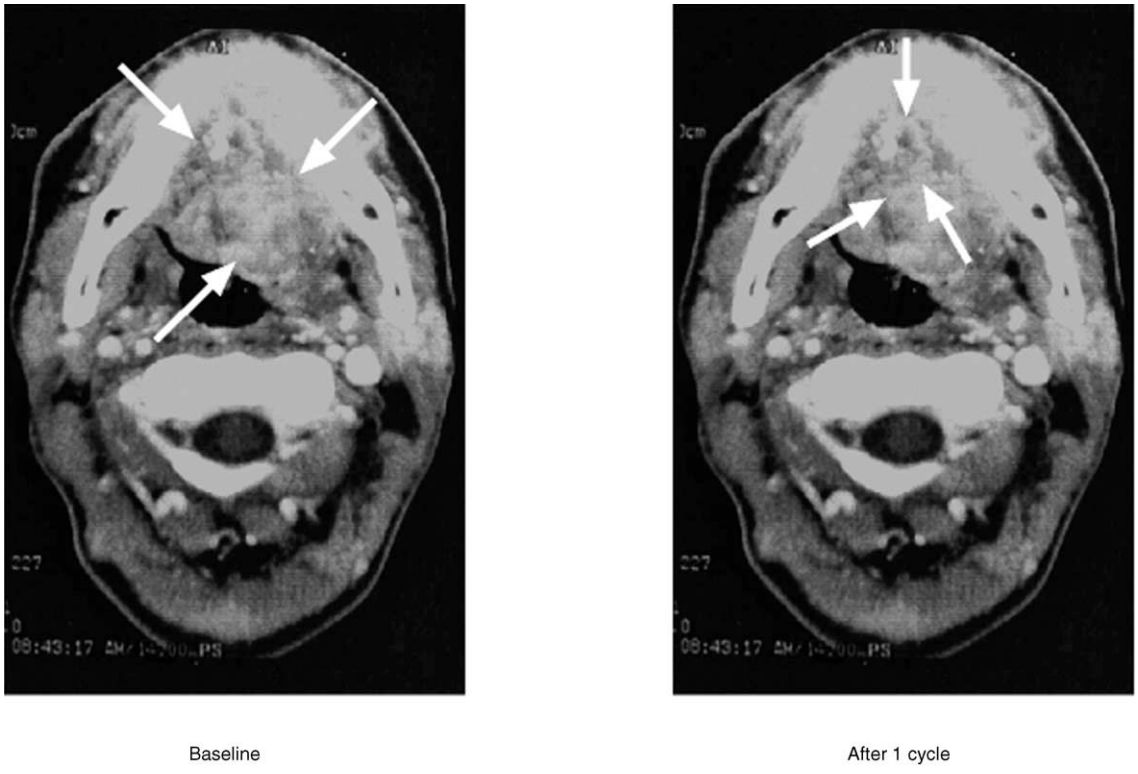

Fig. 3. Response to cetuximab plus cisplatin in squamous cell carcinoma of the head and neck (SCCHN). This patient with recurrent SCCHN had previously received surgery, radiotherapy (RTX), cisplatin, 5-fluorouracil (5-FU) and paclitaxel. After one 7-week cycle of cetuximab (400 mg/m^2 loading dose followed by weekly 250 mg/m^2 maintenance doses) plus cisplatin (100 mg/m^2 every 3 weeks), the patient experienced a partial response. Reproduced with kind permission from J. Mendesohn.

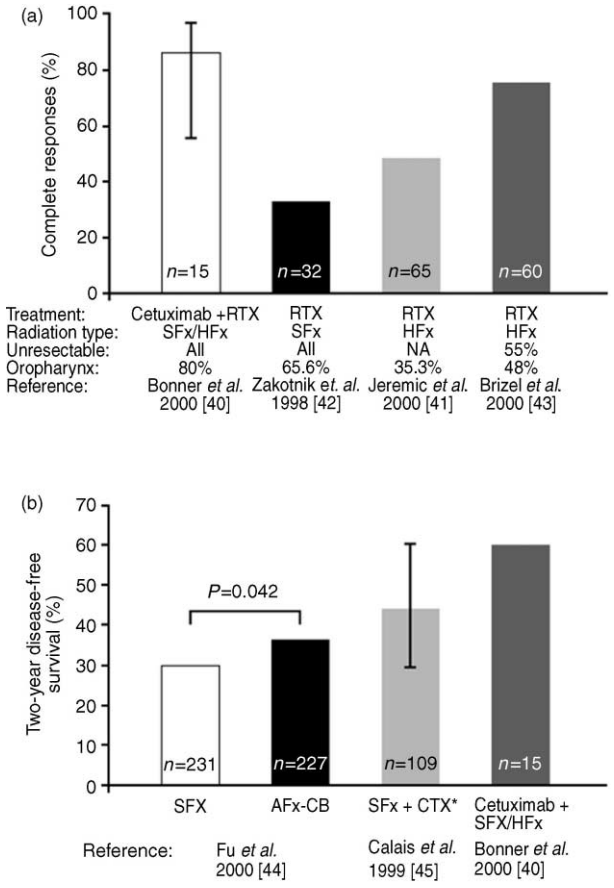

Fig. 4. Cetuximab plus radiotherapy (RTX) in squamous cell carcinoma of the head and neck (SCCHN)—response and survival rates compared with historical controls. (a) The complete response rate to cetuximab plus standard-fractionation (SFx) or hyperfractionation (HFx) RTX compared with past studies with RTX alone. Based on these encouraging results, phase III studies have been initiated to directly compare responses to cetuximab plus RTX versus RTX alone in SCCHN. Bar = confidence interval. NA, not available. (b) The 2-year disease-free survival rates of SCCHN patients treated with cetuximab plus RTX compare favourably with those of previous studies with SFx or accelerated fractionation with concomitant-boost (AFx-CB) RTX alone. *3-year disease-free survival (bar = 95% confidence interval).

5. Conclusion

Phase I/II trials with cetuximab have produced promising clinical results in SCCHN and CRC, particularly when cetuximab was administered in combination with CTX or RTX. Remarkably, complete responses were even observed in heavily pre-treated patients with particularly poor prognoses ([36,37,40]; ImClone Systems Inc., data on file]). In the light of these results, the clinical development of cetuximab is continuing with a number of phase II and III studies designed to determine whether cetuximab enhances the efficacy of conventional CTX or RTX in patients with SCCHN and CRC.

References

1. Klapper LN, Kirschbaum MH, Sela M, Yarden Y. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. *Adv Cancer Res* 2000, **77**, 25–79.
2. Huang SM, Harari PM. Epidermal growth factor receptor inhibition in cancer therapy: biology, rationale and preliminary clinical results. *Invest New Drugs* 1999, **17**, 259–269.
3. Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J, Sato GH. Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. *Mol Biol Med* 1983, **1**, 511–529.
4. Salomon DS, Bradt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. *Critical Rev Oncol/Hematol* 1995, **19**, 183–232.
5. Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. *Clin Cancer Res* 1995, **1**, 1311–1318.
6. Baselga J, Cañadas M, Codony J, *et al*. Activated epidermal growth factor receptor: studies in head and neck tumors and tumor cell lines after exposure to ligand and receptor tyrosine-kinase inhibitors. *Proc Am Soc Clin Oncol* 1999 (abstr 2392).
7. Sunada H, Magun BE, Mendelsohn J, MacLeod CL. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. *Proc Natl Acad Sci USA* 1986, **83**, 3825–3829.
8. Klapper LN, Waterman H, Sela M, Yarden Y. Tumor-inhibitory antibodies to HER-2/ErbB-2 may act by recruiting c-Cbl and enhancing ubiquitination of HER-2. *Cancer Res* 2000, **60**, 3384–3388.
9. Wu X, Rubin M, Fan Z, *et al*. Involvement of p27Kip1 in G1 arrest mediated by an anti-epidermal growth factor receptor monoclonal antibody. *Oncogene* 1996, **12**, 1397–1403.
10. Peng D, Fan Z, Lu Y, DeBlasio T, Scher H, Mendelsohn J. Anti-epidermal growth factor receptor monoclonal antibody 225 up-regulates p27Kip1 and induces G1 arrest in prostatic cancer cell line DU145. *Cancer Res* 1996, **56**, 3666–3669.
11. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res* 1999, **59**, 1935–1940.
12. Mendelsohn J. Jeremiah Metzger Lecture. Targeted cancer therapy. *Trans Am Clin Climatol Assoc* 2000, **111**, 95–110.
13. Petit AMV, Rak J, Hung MC, *et al*. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor production by tumor cells in vitro and in vivo. *Am J Pathol* 1997, **151**, 1523–1530.
14. Perrotte P, Matsumoto T, Inoue K, *et al*. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* 1999, **5**, 257–265.
15. Bruns CJ, Harbison MT, Davis DW, *et al*. Epidermal growth factor receptor blockade with C225 plus gemcitabine results in regression of human pancreatic carcinoma growing orthotopically in nude mice by antiangiogenic mechanisms. *Clin Cancer Res* 2000, **6**, 1936–1948.
16. O-Charoenrat P, Rhys-Evans P, Modjtahedi H, Court W, Box G, Eccles S. Overexpression of epidermal growth factor receptor in human head and neck squamous carcinoma cell lines correlates with matrix metalloproteinase-9 expression and in vitro invasion. *Int J Cancer* 2000, **86**, 307–317.
17. O-Charoenrat P, Rhys-Evans P, Court WJ, Box GM, Eccles SA. Differential modulation of proliferation, matrix metalloproteinase expression and invasion of human head and neck squamous carcinoma cells by c-erbB ligands. *Clin Exp Metastasis* 1999, **17**, 631–639.

18. Matsumoto T, Perrotte P, Bar-Eli M, *et al*. Blockade of EGF-R signaling with anti-EGFR monoclonal antibody (Mab) C225 inhibits matrix metalloproteinase-9 (MMP-9) expression and invasion of human transitional cell carcinoma (TCC) in vitro and in vivo. *Proc Am Assoc Cancer Res* 1998, **39**, 83.
19. Pepper C, Hoy T, Bentley DP. Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. *Br J Cancer* 1997, **76**, 935–938.
20. Wu X, Fan Z, Masui H, Rosen N, Mendelsohn J. Apoptosis induced by an anti-epidermal growth factor receptor monoclonal antibody in a human colorectal carcinoma cell line and its delay by insulin. *J Clin Invest* 1995, **95**, 1897–1905.
21. Mandal M, Adam L, Mendelsohn J, Kumar R. Nuclear targeting of Bax during apoptosis in human colorectal cancer cells. *Oncogene* 1998, **17**, 999–1007.
22. Liu B, Fang M, Schmidt M, Lu Y, Mendelsohn J, Fan Z. Induction of apoptosis and activation of the caspase cascade by anti-EGF receptor monoclonal antibodies in DiFi human colon cancer do not involve the c-jun N-terminal kinase activity. *Br J Cancer* 2000, **82**, 1991–1999.
23. Tortora G, Caputo R, Pomatico G, *et al*. Cooperative inhibitory effect of novel mixed backbone oligonucleotide targeting protein kinase A in combination with docetaxel and anti-epidermal growth factor-receptor antibody on human breast cancer cell growth. *Clin Cancer Res* 1999, **5**, 875–881.
24. Prewett M, Rockwell P, Rose C, Zuklys K, Goldstein NI. Altered cell cycle distribution and cyclin-CDK protein expression in A431 epidermoid carcinoma cells treated with doxorubicin and a chimeric monoclonal antibody to the epidermal growth factor receptor. *Molec Cell Differen* 1996, **4**, 167–186.
25. Ciardiello F, Bianco R, Damiano V, *et al*. Antitumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. *Clin Cancer Res* 1999, **5**, 909–916.
26. Inoue K, Shuin T, Hicklin DJ, *et al*. Paclitaxel enhances the effect of treatment of metastatic human transitional cell carcinoma with the anti-epidermal growth factor receptor monoclonal antibody C225. *Proc Am Assoc Cancer Res* 2000, **41**, 529 (abstr 3372).
27. Aboud-Pirak E, Hurwitz E, Pirak ME, Bellot F, Schlessinger J, Sela M. Efficacy of antibodies to epidermal growth factor receptor against KB carcinoma in vitro and in nude mice. *J Natl Cancer Inst* 1988, **80**, 1605–1611.
28. Baselga J, Norton L, Masui H, *et al*. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. *J Natl Cancer Inst* 1993, **85**, 1327–1333.
29. Fan Z, Baselga J, Masui H, Mendelsohn J. Antitumor effect of anti-epidermal growth factor receptor monoclonal antibodies plus cis-diamminedichloroplatinum on well established A431 cell xenografts. *Cancer Res* 1993, **53**, 4637–4642.
30. Prewett MC, Overholser J, Hooper A, Waksal H, Hicklin DJ. Anti-EGF receptor monoclonal antibody IMC-C225 combined with docetaxel inhibits growth of renal cell carcinoma tumors in a nude mouse model. *Proc Am Assoc Cancer Res* 2000, **41**, 811 (abstr 5149).
31. Saleh MN, Raisch KP, Stackhouse MA, *et al*. Combined modality therapy of A431 human epidermoid cancer using anti-EGFr antibody C225 and radiation. *Cancer Biother Radiopharm* 1999, **14**, 451–463.
32. Milas L, Mason K, Hunter N, *et al*. In vivo enhancement of tumor radioresponse by C225 anti-epidermal growth factor receptor antibody. *Clin Cancer Res* 2000, **6**, 701–708.
33. 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.
34. Cohen RB, Falcey JW, Paulter VJ, Fetzer KM, Waksal HW. Safety profile of the monoclonal antibody (MOAB) IMC-C225, an anti-epidermal growth factor receptor (EGFR) used in the treatment of EGFR-positive tumors. *Proc Am Soc Clin Oncol* 2000 (abstr 1862).
35. Khazaeli MB, LoBuglio AF, Falcey JW, Paulter VJ, Fetzer MK, Waksal HW. Low immunogenicity of a chimeric monoclonal antibody (MOAB), IMC-C225, used to treat epidermal growth factor receptor-positive tumors. *Proc Am Soc Clin Oncol* 2000 (abstr 808).
36. Mendelsohn J, Shin DM, Donato N, *et al*. A phase I study of chimerized anti-epidermal growth factor receptor (EGFr) monoclonal antibody, C225, in combination with cisplatin (CDDP) in patients (PTS) with recurrent head and neck squamous cell carcinoma (SCC). *Proc Am Soc Clin Oncol* 1999, 18 (abstr 1502).
37. Rubin MS, Shin DM, Pasmantier M, *et al*. Monoclonal antibody (MOAB) IMC-C225, an anti-epidermal growth factor receptor (EGFR), for patients (PTS) with EGFR-positive tumors refractory to or in relapse from previous therapeutic regimens. *Proc Am Soc Clin Oncol* 2000, (abstr 1860).
38. Slovin SF, Kelly WK, Cohen R, *et al*. Epidermal growth factor receptor (EGFr) monoclonal antibody (MoAb) C225 and doxorubicin (DOC) in androgen-independent (AI) prostate cancer (PC): results of a phase Ib/IIa study. *Proc Am Soc Clin Oncol* 1997, 16, 311a (abstr 1108).
39. Grandis JR, Melhem MF, Gooding WE, *et al*. Levels of TGF-a and EGFR protein in head and neck squamous cell carcinoma and patient survival. *J Natl Cancer Ins* 1998, **90**, 824–832.
40. Bonner JA, Ezekiel MP, Robert F, Meredith RF, Spencer SA, Waksal HW. Continued response following treatment with IMC-C225, an EGFr MoAb, combined with RT in advanced head and neck malignancies. *Proc Am Soc Clin Oncol* 2000 (abstr 5F).
41. Jeremic B, Shibamoto Y, Milicic B, *et al*. Hyperfractionated radiation therapy with or without concurrent low-dose daily cisplatin in locally advanced squamous cell carcinoma of the head and neck: a prospective randomized trial. *J Clin Oncol* 2000, **18**, 1458–1464.
42. Zakotnik B, Smid L, Budihna M, *et al*. Concomitant radiotherapy with mitomycin C and bleomycin compared with radiotherapy alone in inoperable head and neck cancer. *Int J Radiat Oncol Biol Phys* 1998, **41**, 1121–1127.
43. Brizel DM, Wasserman TH, Henke M, *et al*. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. *J Clin Oncol* 2000, **18**, 3339–3345.
44. 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 Biol Phys* 2000, **48**, 7–16.
45. 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 Natl Cancer Inst* 1999, **91**, 2081–2086.