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Review

The efficacy and toxicity of EGFR in the settings of radiotherapy: Focus on published clinical trials

Nicolas Magné[a,*], Cyrus Chargari[a], Pierre Castadot[b], Mithra Ghalibafian[a], Jean-Charles Soria[c], Christine Haie-Meder[a], Jean Bourhis[a], Eric Deutsch[a]

[a]Department of Radiotherapy, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94 805 Villejuif, France
[b]Department of Radiotherapy, Institut Jules Bordet, Bruxelles, Belgique
[c]Department of Medical Oncology, Institut Gustave Roussy, Villejuif, France

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ABSTRACT

Basic research in solid malignant tumours has led to a wealth of knowledge about this disease process and about novel ways to more effectively target our therapies. Laboratory research continues to identify novel therapeutic targets and moreover, clinical research is identifying effective new treatment regimens. Many preclinical studies in this area have targeted the epidermal growth factor receptor (EGFR) signalling pathway to increase radiosensitivity. The *in vitro* rationale for targeting EGFR and concurrent ionising radiation is well established, but to date, rare clinical data could provide proof-of-principle. Here we report all the different published clinical trials focusing on efficacy and toxicity in order to clarify and to summarise the present state-of-the-art of this particularly promising combination in solid tumour management.

1. Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane protein which was implicated in the progression of many solid malignant tumours. Its activation induces some transduction pathways inside the cell, and contributes to many cellular processes such as cell proliferation, inhibition of apoptosis and angiogenesis.[1,2] Small molecules inhibiting tyrosine kinase (TKI) function or monoclonal antibodies (MoAbs) directed against EGFR could block all these cellular functions and enhance the antitumour activity of ionising radiation.[3–5] Several mechanisms have been advocated. Anti-EGFR therapies increase the proportion of cells in G1 cell cycle, and decrease the proportion in the S phase, which are, respectively, the more radiosensitive and radioresistant phases. Considering that RT usually arrests cells in the G_2 phase of the cell cycle, combined effects of EGFR inhibition and RT on two distinct cell cycle checkpoints (G_1 and G_2) might prove formidable for the cancer cell to withstand.[6]

Another potential mechanism is the possible impact on apoptosis factors and on DNA repair following radiation.[7,8] Stimulation of EGFR signalling inhibits apoptosis, and blockade of EGFR signalling promotes apoptosis. The promotion of RT-induced apoptosis in the face of EGFR downregulation would also represent an important mechanistic feature reflecting EGFR inhibitor action at the cellular level.[9] A mechanistic lead regarding the propensity of EGFR inhibitors to enhance RT-induced response derives from preliminary data

* *Corresponding author:* Tel.: +33 1 42 11 42 66; fax: +33 1 42 11 52 08.
E-mail address: nicolas.magne@igr.fr (N. Magné).
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suggesting an inhibitory influence of EGFR inhibitors on DNA damage repair after radiation exposure.[10,11] Recent studies suggested the possibility of a direct antiangiogenic effect of EGFR on endothelial cells.[8] A mechanistic linkage has been established, confirming downregulation of vascular endothelial growth factor (VEGF) at the level of mRNA after exposure to EGFR inhibitors,[12,13] suggesting that downregulating EGFR signalling would thereby downregulate angiogenic processes through the inhibition of VEGF signalling. Combining EGFR targeted therapies with chemotherapy (CT)[14–19] or radiotherapy (RT)[20,21] is a promising therapeutic approach based on more conventional strategies. The occurrence of cross-resistance might be infrequent since the cellular targets of CT and/or RT and EGFR inhibitors are different.

Whilst potentially improving the local control, the combination of RT and targeted therapies is subject to considerable uncertainty concerning its potential toxicity. In addition, it was advocated that EGFR targeting could increase CT- or RT-induced long-term toxicity. Thus, fatal adverse events such as interstitial lung disease were reported in pivotal studies with EGFR inhibitors.[22]

For the clinician, this translates into more complex decisions when selecting the best treatment for each patient and particularly so in the combination of EGFR targeted therapies and RT. Management of side-effects and quality of life are and will continue to be important considerations during this decision process. One must take care that the synergistic effect obtained by such a combination in terms of efficacy does not also add to toxicity. The *in vitro* rationale for targeting EGFR is well established, but rare clinical data can provide proof of principle. The aim of this study is to report all the published clinical trials of EGFR-targeted therapies in combination with RT, particularly focusing on efficacy and toxicity in order to clarify and to summarise the present state-of-the-art of this particularly promising combination in solid tumour management.

2. Methods

An English-language literature search was conducted to identify only published clinical studies assessing the efficacy and toxicity effects of EGFR blockade with the main goal to establish a comparison between tyrosine kinase inhibitors and monoclonal antibodies therapies. Data for this review were identified by searches of Medline and Cancerlit. The search terms 'EGFR', 'tyrosine kinase inhibitor', 'monoclonal antibody', 'clinical trial', 'phase I', 'phase II', 'phase III' and 'radiotherapy' were used. References identified from within retrieved articles were also used. There was no limitation on the year of publication, and no abstract forms were included. Only published articles were taken into consideration.

3. Results

3.1. *Tyrosine kinase inhibitors (TKI)*

Tyrosine kinase inhibitors are small molecules given orally that target EGFR. By blocking the intracellular ATP-binding site, phosphorylation cannot be completed, thereby inhibiting the signal cascade that activates growth and proliferation factors. In contrast to MoAbs, TKIs induce neither EGFR internalisation nor degradation, and thus do not decrease the level of EGFR protein.[4] All the data concerning combining TKI with RT in clinical trials are summarised in Table 1.

3.1.1. *Gefitinib (ZD1839, Iressa®)*

Amongst TKIs, ZD1839 is indicated as daily oral monotherapy for the continued treatment of patients with locally advanced or metastatic non-small cell lung cancer (NSCLC) after failure of CDDP-based and docetaxel CT. Phase I clinical trials assessed ZD1839 tolerability and pharmacokinetics in solid tumour patients. ZD1839 proved to be suitable for once-daily oral administration and had a favourable toxicity profile. Based on these phase I data, 250 and 500 mg/day doses were selected for use in the further clinical development.[23–25] In a phase I clinical trial, the addition of ZD1839 to RT and gemcitabine (GMZ) was evaluated in 18 patients with locally advanced pancreatic carcinoma.[26] Six patients were initially treated with RT plus concomitant ZD1839 (250 mg/day) alone without GMZ. Successive cohorts of patients received from 100 to 200 mg/m^2/week of GMZ with ZD1839 (250 mg/day) and RT. RT was 3D conformal and was delivered with 1.8 Gy fractions, 5 d per week with a total dose of 45 Gy. Then, ZD1839 was continued after RT until disease progression. Maurel and colleagues revealed no dose-limiting toxicities (DLTs) and common toxicities were mild neutropaenia, asthenia, diarrhoea, cutaneous rash and nausea/vomiting. One patient experienced grade 3 necrotising vasculitis during the ZD1839 monotherapy.[26] Fifteen of 18 patients were evaluated for response. One patient presented with a partial response, 7 with stable disease and 7 with progressive disease. With a median progression free survival and overall survival of 3.7 and 7.5 months, respectively, the combination of ZD1839, RT and GMZ has acceptable toxicity but a moderate efficacy.

Czito and colleagues reported another phase I trial divided into 2 locations assessing the combination of ZD1839, capecitabine and concomitant RT (3D conformational, 50 Gy/28 fractions) firstly in patients with localised pancreatic cancer ($n = 10$) and secondly in rectal cancer ($n = 6$).[27] Dose limiting toxicity (DLT) was seen in 6 of 10 patients in the pancreatic study and 2 out of 6 patients in the rectal study. The primary DLT in both locations was diarrhoea. In the pancreatic study, 7 patients were enroled at level 1 (capecitabine 650 mg/m^2/bid, ZD1839 250 mg/m/d) and 3 patients at level 2, (capecitabine 825 mg/m^2/bid, ZD1839 250 mg/d). At level 1, 2 patients suffered from grade 3 secondary events (nausea, vomiting, diarrhoea). At level 2, 2 patients experienced grade 3 nausea, vomiting, diarrhoea, dehydration and one patient developed an arterial thrombus requiring emergent femoral bypass and distal amputation. At level 3, one patient experienced grade 4 diarrhoea and grade 3 cardiac toxicities. All rectal cancer patients were enroled at level 1. One experienced grade 4 diarrhoea, and another developed thrombosis of the left superficial femoral artery, then died from aspiration pneumonia. Levels of response were not sufficient. In fact, no objective response was reported in the pancreas cancer group and 2 partial responses were reported in the rectal cancer group. These data suggest that the combination of ZD1839,

Table 1 – Tyrosine kinase inhibitors against EGFR and radiotherapy in clinical trials

Trial	Phase	organs	pts	TKI		Chemotherapy	RT (Gy)	TKIpostRT	DLT	Grades 1–2 toxicities (%)	Grades 3–4–5 toxicities (%)	Recommended dose	Efficacy
Duffy et al.[27]	I	P	20	OSI774	100 mg/d	GMZ 40 mg/m^2/biw	50.4	Yes	No	ND	Lymphopaenia (100), diarrhoea (21), rash (14)	OSI774 100 mg/d	PR: 35% SD: 53% Median OS: 18.7 months
			6		125 mg/d				2		Lymphopaenia (100), neutropaenia (50), thrombopaenia (33), anaemia (17)		
Iannitti et al[26]	I	P	17	OSI774	level 1: 50 mg/d level 2: 75 mg/d level 3: 100 mg/d	GMZ 75 mg/m^2/w TXL 40 mg/m^2/w	50.4	Yes	No 3 pts 2 pts	Abdominal pain (12), nausea/ vomiting (11), fatigue (10), rash (5)	dehydration (17), diarrhoea (12), rash (6), thrombosis (18), hypersensitivity (12)	OSI 774: 50 mg/d	6 PR median OS: 14 months
Maurel et al.[20]	I	P	18	ZD1839 250 mg/d		GMZ 100 mg/m^2/w	45	Yes	No	Nausea(100), diarrhoea(60), rash (100)	No	ND	Median PFS: 3.7 months Median OS : 7.5 months
						GMZ 150 mg/m^2/w				nausea, diarrhoea (60), rash (50)	No		
						GMZ 200 mg/m^2/w				nausea (60), diarrhoea (60), rash (50)	Neutropaenia (60), Anaemia (30)		
Czito et al.[21]	I	P	10	ZD1839 250 mg/d		Level 1: capecitabine 650 mg/m^2/bid	50.4	No	No	Fatigue (60), rash (50), nausea (40)	Diarrhoea (50), 1 case of arterial thrombosis dehydration (20)	ND	7 SD, 3 PD
						Level 2: capecitabine 825 mg/m^2/bid			6 pts	Rash (30), anorexia(30)			
		R	6	ZD1839 250 mg/d		Level 1: capecitabine 650 mg/m^2/bid	50.4		2	Fatigue (15), rash (13), anorexia(12)	Diarrhoea (16), 1 death from arterial thrombosis	ND	2 PR, 2 SD, 1 PD
Chen et al.[22]	I	HN	23	ZD1839 250–500 mg/d		Cohorte I: intermediate-stage: no CT	70	Yes	No	Dermatitis (78), rash (78), xerostomia (74),	Radiation dermatitis (12.5), mucositis (62.5)	ZD1839 250-500 mg/d	3-year OS: 74% 3-year DFS: 61%
						Cohorte II: high-risk LAHNC: CDDP 30 mg/ m^2/w	72		2 pts	nausea (61), fatigue (65), diarrhoea (52)	Radiation dermatitis (13), mucositis (60), neutropaenia (47)	ND	
Stinchcombe et al.[24]	I	HN	23	ZD1839 250 mg/d		Induction CT, then: CBDP AUC2, TXL 45 mg/ m^2/w	74	No	No	ND	Oesophagitis (19.5), cardiac arythmia (9.5)	ND	Median PFS: 9 months Median OS: 16 months
Dobelbower et al.[28]	I	Oes	11	OSI774 50-150 mg/d		CDDP 75 mg/m^2, d8,36 5FU:1000 mg/m^2, d8-11 and 36–39	50.4	No	No	Diarrhoea (18), nausea (54), oesophagitis (32)	Rash (54), dehydration (27), nausea (9), lost weight (18), oesphagitis (18)	ND	ND
Krishnan et al.[29]	I	GBM	20	OSI774	100 mg/d 150 mg/d 200 mg/d	No	60	Yes	1 pt No	Rash (70), Diarrhoea (45), Nausea (35), Anorexia (35), Alopecia	Stomatis (15), Diarrhoea (5), Fatigue (5)	ND	Median PFS: 26 weeks.
			6		125 mg/d				2	(25), Fatigue (20)	Lymphopaenia (100), neutropaenia (50), thrombopaenia (33), anaemia (17)		

TKI: tyrosine kinase inhibitor, P: Pancreas, R: rectum, HN: head and neck, LAHNC: locally advanced head and neck cancer, Oes: oesophagus, GBM: glioblastoma, CT: chemotherapy, OSI774: erlotinib, ZD1839: gefitinib, 5FU: 5 fluorouracile, GMZ: gemcitabin, CDDP: cisplatin, CBDP: carboplatin, AUC: area under the curve, TXL: paclitaxel, CT: chemotherapy, DLT: dose limiting toxicity, pts: patients, CR: complete response, PR: partial response, OS: odd survival, PFS: progression-free survival, DFS: disease-free survival, LRC: locoregional control, SD: stable disease, PD: progressive disease, PR: partial response, RITC: residual isolated tumour cells, ND: no data.

capecitabine and RT results in significant improvement of toxicity without a potential benefit for the patients.

Chen and colleagues published results establishing the safety and toxicity profile in locally advanced squamous cell head and neck cancer treated with concomitant radiation (72 Gy) and escalating doses of daily ZD1839 (250 or 500 mg/d; cohort 1) in a phase I clinical trial.[28] Patients with high-risk disease received the same combination plus weekly cisplatin (CDDP, 30 mg/m^2, cohort 2). After the completion of RT, patients received maintenance ZD1839 at 250 mg/d for a period of up to 2 years. No DLT was observed in patients treated in cohort 1 at either 250 or 500 mg of ZD1839 daily with concomitant boost RT. In patients receiving CRT and ZD1839, DLT included one grade 4 diarrhoea and one grade 4 neutropaenic fever. The profile of acute toxicity during concurrent ZD1839 and CRT was consistent with the toxicity profile previously reported with more than grade 3 mucositis in the range of 43% to 77%.[29] Eight of 15 patients who started maintenance ZD1839 (53%) experienced grade 1 to 2 acne-like skin rash and diarrhoea without any occurrence of grade 3 or 4 toxicity. ZD1839 (250 or 500 mg daily) was well tolerated with concomitant boost RT or concurrent RT with weekly CDDP.

Stinchcombe and colleagues investigated the tolerability of ZD1839 (250 mg/d) for unresectable stage III NSCLC in combination with 3D-CRT.[30] After induction chemotherapy (carboplatin AUC5, irinotecan 100 mg/m^2 and TXL 175 mg/m^2 on days 1 and 22) with pegfilgrastim support, patients received concurrent CRT (carboplatin AUC 2, and TXL 45 mg/m^2/w) and ZD1839 (250 mg/d) with 3D-RT. Amongst 23 patients initiating the concurrent CRT, 20 patients completed therapy to 74 Gy. The primary toxicities of concurrent CRT were grade 3 esophagitis (19.5%) and cardiac arrhythmia (atrial fibrillation) (9.5%). In terms of efficacy, the median PFS and OS were 9 months (95% CI: 7–13 months) and 16 months (95% CI: 10–20 months), respectively. Despite its low toxic profile, this treatment modality demonstrated disappointing survival results.

Combined modality strategies should be carefully assessed with cautious management of toxicity. These first clinical data combining gefitinib and RT in gastrointestinal and in head and neck cancers showed a potential benefit in terms of efficacy which was counterbalanced by toxicities. In light of these issues, preclinical investigations of gefitinib as a radiosensitiser are warranted with particular attention to toxicity. Efficient clinical research is ongoing to determine the place of TKIs in association with RT. In HNSCC, a randomised phase 2 trial is ongoing (CARISSA), that will assess gefitinib in combination with CDDP-based postoperative chemoradiation.

3.1.2. *Erlotinib (OSI774, Tarceva®)*

Erlotinib was the first EGFR-inhibitor to demonstrate an increase in survival in phase III trials in patients with advanced non-small cell lung cancer. Daily oral OSI774 monotherapy is indicated for the second-line treatment of patients with locally advanced or metastatic NSCLC. In combination with GMZ, another current indication is first-line treatment of patients with locally advanced, unresectable or metastatic pancreatic cancer. OSI774 has been currently investigated in combination with CRT in a number of malignancies. Hidalgo and colleagues reported a phase I clinical study with OSI774 with adverse events including mild fatigue, rash, diarrhoea and nausea.[31] The authors concluded that the recommended dose for disease-directed studies of OSI774 administered orally once daily, continuously at uninterrupted schedule is 150 mg/day.

Administered concomitantly with CRT, OSI774 has shown potential activity and a minor toxicity. Iannitti and colleagues determined the MTD of OSI774 with concurrent CRT for 17 patients with locally advanced pancreatic cancer and gathered preliminary data on maintenance OSI774 after CRT in a phase I trial.[32] Patients received a combination of GMZ 75 mg/m^2 weekly and paclitaxel (TXL) 40 mg/m^2 weekly during RT (50.4 Gy, 28 fractions of 1.8 Gy per fraction). OSI774 was administered over 3 dose levels (50–100 mg/d) with CRT, then all patients received 150 mg/d maintenance until disease progression. At OSI774 dosages $\geqslant$75 mg/d concomitantly with CRT, the DLTs were diarrhoea, dehydration, rash, myelosuppression, and small bowel stricture. The median survival of the 13 patients with locally advanced disease was 14.0 months and 6 out of 13 (46%) had a partial response. From these results, the maximum tolerated dose (MTD) of OSI774 with GMZ, TXL and concurrent CRT is 50 mg/d. As monotherapy, full dose maintenance OSI774 (150 mg/d) was well tolerated.

Duffy and colleagues assessed the toxicity and safety profile of OSI774 for locally advanced pancreatic cancer when administered over 2 dose levels (100–125 mg/d) concurrently with lower doses of GMZ (40 mg/m^2 twice weekly) and RT (50.4, Gy, 28 fractions, of 1.8Gy per fraction).[33] Then, patients received maintenance weekly GMZ 1000 mg/m^2 (on days 1 and 8 of a 21 d cycle) and daily OSI774 for four cycles. Three patients were treated at OSI774 100 mg/d without limiting toxicity. Two of six patients at OSI774 125 mg had dose-limiting toxicities, including neutropaenia and thrombocytopaenia causing both dose delay and elevated liver enzymes. The MTD for OSI774 in combination with twice weekly GMZ-based CRT was 100 mg/d. Amongst 20 patients treated at 100 mg/m^2, 53% had stable disease and 35% had partial response. With a median OS for all patients of 18.7 months, OSI774 100 mg/d in combination with GMZ-based CRT seemed to be a well tolerated dose regimen, providing encouraging results in terms of efficacy. However, there was one grade 5 toxicity occurrence with an acute gastrointestinal bleed. Although an oesophagogastroduodenoscopy revealed gastric and duodenal ulceration secondary to invasion of tumour, an attribution to treatment could not be excluded.

In patients with squamous or adenocarcinoma of the oesophagus, Dobelbower and colleagues evaluated the safety and tolerability of OSI774 delivered at 150 mg/day with concurrent 5-fluorouracil (5FU), CDDP, and thoracic RT in a phase I trial.[34] Patients received either 50, 100 or 150 mg oral OSI774 daily beginning on the first day of RT (3 patients in each dose cohort), concurrent CDDP (75 mg/m^2 iv, days 8 and 36) and 5FU (1000 mg/m^2 i.v., days 8–11 and 36–39) given concomitantly with a 50.4 Gy thoracic RT, delivered at 1.8 Gy/day, 5 d/week. OSI774 with concurrent CRT was well tolerated at 50, 100 and 150 mg/day. The major toxicities were diarrhoea (grade 1 = 18%, grade 2 = 18%), skin rash (grade 4 = 54.5%), nausea (grade 1 = 18%, grade 2 = 54%, grade 3 = 9%) and dehydration (grade 3 = 27%). All patients experienced oesophagitis during treatment (grade 1 = 55%, grade 2 = 32%, grade 3 = 9%,

grade 4 = 9%). Most toxicities encountered were grade 1–2 diarrhoea, grade 1 skin rash, grade 1–3 nausea and grade 3 dehydration. Half of the patients developed grade 4 skin toxicity.

Up to 40% of glioblastoma multiforme (GBM) have EGFR gene-amplification, associated with resistance to RT. In 2006, Krishnan and colleagues designed a multicentre phase I trial to evaluate the toxicity and MTD of OSI774 plus RT therapy in patients with GBM.[35] After stratification according to the use of enzyme-inducing anticonvulsants (EIACs), 19 patients underwent a resection or biopsy, then were treated with OSI774 for 1 week before concurrent OSI774 and 60 Gy of RT. Then, patients were treated with maintenance OSI774 until progression. The OSI774 dose was escalated in cohorts of 3 starting at 100 mg/day. The highest dose level was 150 mg/day OSI774 for patients not on EIACs, and 200 mg/day for patients on EIACs. MTD was not reached in either group. Besides, treatment was tolerated without major toxicity defined as grade 4 or 5. The most frequent toxicities (all grade 1 or 2) were rash (70%), diarrhoea (45%), nausea (35%), anorexia (35%), alopecia (25%), and fatigue (20%). The only grade 3 or higher toxicities were stomatitis (15%), diarrhoea (5%), and fatigue (5%). With a median follow-up of 52 weeks, progression was documented in 16 patients, and 13 deaths occurred. OSI774 was well tolerated with concurrent RT, but treatment was stopped early in 4 patients because of toxicity developed after the assessment period during maintenance OSI774. Furthermore, a patient died from an acute myocardial infarction 1 week after initiation of OSI774 treatment.

In each of the previously mentioned trials, combination therapy using erlotinib and RT was shown to be safe with a minimal increase in toxicity compared with the conventional treatment regimen except for cutaneous rash (up to 50% grade 4 side-effects). Unlike in other studies, development of a rash did not seem to predict better outcomes.[36] It might be due to a consequence of a small number of included patients. Erlotinib should now be subject to further assessment in combination with temozolamide concurrently with and subsequent to RT, in order to take into account promising results reported with cytotoxic chemotherapy in patients with GBM.[37] Considering that a hallmark of cancer is aberrant cross-talk between pathways, blocking an overexpressed receptor tyrosine kinase may not always inhibit cell growth. Several irreversible inhibitors have been specifically assessed in preclinical development. CI-1033 is a small molecule TKI that is a pan ErbB irreversible inhibitor, thus differing from ZD1839 and OSI-774. Preclinical data suggested that irreversible TKIs (such as CI-1033) might increase the effectiveness of RT.[38] These preliminary results are worthy of further development.

3.2. *Monoclonal antibodies (MoAbs)*

MoAbs block ligand EGF from binding to the extracellular domain of the EGFR. By this action, the receptor is internalised, leading to receptor down-regulation at the cell surface. The receptor is prevented from autophosphorylation and activation; therefore, downstream signalling is inhibited.[4] All the different data concerning MoAbs with RT in clinical trials are summarised in Table 2.

3.2.1. *Cetuximab (C225, Erbitux®)*

Amongst MoAbs, C225 is currently given intravenously as a weekly injection in the treatment of advanced colorectal cancers, in combination with irinotecan or, in head and neck cancer, in combination with CDDP or RT. As early as 2001, Robert and colleagues evaluated in a phase I trial the combination of C225 with RT in patients with advanced squamous cell carcinoma of the head and neck, and reported impressive response rates.[39] Sixteen patients were treated in 5 successive treatment schedules. Three patients entered at each dose level of C225 and received conventional RT (70 Gy, 2 Gy/day) and the final 3 patients received hyperfractionated RT (76.8 Gy, 1.2 Gy bid). C225 was delivered as a loading dose of 100 to 500 mg/m^2, followed by weekly infusions of 100 to 250 mg/m^2 for 7 to 8 weeks. The most commonly reported adverse events were fever, asthenia, transaminase elevation, nausea and skin toxicities (grade 1 to 2 in most patients). Skin toxicity outside of the RT field was not strictly dose dependant; however, grade 2 or higher events were observed in patients treated with higher dose regimens. There was one patient with a grade 4 allergic reaction. Fifteen of 16 patients achieved an objective response (13 complete and 2 partial remissions). C225 has a long half-life, lending itself to convenient weekly administration in these clinical studies. Twelve patients who had high levels of EGFR expression and tumours easily accessible for repeated biopsies (before therapy, 24 h after first C225 infusion, 24 h before third C225 infusion) were entered at 3 different dose levels of C225 with a fixed dose of CDDP. EGFR-tyrosine kinase activity was significantly reduced after C225 infusion, and EGFR/C225 complexes were also detected at higher doses of C225. Due to these pharmacological results, standard treatment entails a loading dose of 400 mg/m^2 at week 1, followed by a maintenance dose of 250 mg/m^2 weekly for further clinical trials.

Pfister and colleagues evaluated C225 combined with CRT in 22 patients with stage III or IV squamous cell head and neck cancers in a phase II trial.[40] Treatment included concomitant boost RT (1.8 Gy/d weeks 1 to 6; boost: 1.6 Gy 4 to 6 h later weeks 5 to 6; 70 Gy total to gross disease), CDDP (100 mg/m^2 intravenously for weeks 1 and 4), and C225 (400 mg/m^2 intravenously for week 1, followed by 250 mg/m^2 weeks 2 to 10). The severity of the expected, acute toxicities was typical of concurrent CDDP and RT alone. Grade 3 or 4 C225-related toxicities included acne-like rash (10%) and hypersensitivity (5%). Despite a 3-year overall survival rate of 76%, the study was closed for significant adverse events, including 2 deaths (one pneumonia and one unknown cause), one myocardial infarction, one bacteraemia, and one atrial fibrillation.

A phase III trial evaluating the addition of EGFR inhibitor was conducted by Bonner and colleagues in 424 patients with locally advanced squamous cell head and neck cancer (SCCHN), randomizing radiotherapy plus weekly C225 *versus* RT alone.[41] After a median follow-up of 54 months, overall survival was 55% at 3 years in the combined therapy arm *versus* 45% in the control arm ($p = 0.03$). Local control was also significantly improved at 50% *versus* 41% in the radiation alone arm. With the exception of acneiform rash and infusion related events, the incidence rates of severe (grades 3, 4 and 5) reactions were similar in the two treatment groups. Notably,

Table 2 – Monoclonal antibodies against EGFR and radiotherapy in clinical trials

Trial	Phase	Organs	pts	MoAb	Chemotherapy	RT (Gy)	MoAbpostRT	DLT	Grades 1–2 toxicities (%)	Grades 3–4–5 toxicities	Recommended dose	Efficacy
Hofzeinz et al.[37]	I	R	20	C225 400 mg/m^2 d1, 250 mg/m^2 d8,15,22,29	Level I (irinotecan 40 mg/m^2 + capecitabine 400 mg/m^2/bid)	50.4	No	No	Nausea (20), diarrhoea (60), asthenia (40) rash (60)	No	Ironotecan 40 mg/m^2 capecitabine 500 mg/m^2/bid	5 CR, 6 RITC
					Level II (irinotecan 40 mg/m^2 + capecitabine 500 mg/m^2/bid)			1 pt	Diarrhoea (70), rash (40)	Diarrhoea (20), ASAT elevation (10)		
					Level III (irinotecan 50 mg/m^2 + capecitabine 500 mg/m^2/bid)			2 pts	Diarrhoea (40), nausea (20), rash (20), asthenia (20)	Diarrhoea (40), nausea/vomiting (20)		
Rödel et al.[36]	I-II	R	60	C225 400 mg/m^2 w1, 250 mg/m^2 week2-9	oxaliplatin (50 mg/m^2 D1,8,22,29) + capecitabine (D1-14 and 22-35) at 3 levels (500, 650,825 mg/m^2/bid)	50.4	No	No	Diarrhoea (56), rash (92), allergic reaction (10), fever (21)	Diarrhoea (19), rash (4), 1 death of septicaemia and 1 death of cardiac failure	Capecitabine: 1650 mg/m^2 on days 1–14 and 22–35	CR: 9% SD: 53%
Machiels et al.[35]	I-II	R	40	C225 400 mg/m^2 d1, 250 mg/m^2/w	Level I: capecitabine 650 mg/m^2/bid	45	No	No	Rash (87), fatigue(57), diarrhoea (65), nausea	Anal/rectal pain (12.5), diarrhoea (15) cardiac ischaemia or thrombosis (5)	Capecitabine 825 mg/m^2/bid	CR = 5%
					Level II: capecitabine 825 mg/m^2/bid			No	(32)			
Robert et al.[32]	I	HN	16	C225 100-400 mg/m^2 D1, then 100-250 mg/m^2 weekly	No	70	No	No	Rash (80), allergic reaction (12), fever (60)	Allergic reaction (12), mucositis (68), rash (37)	400–500 mg/m^2 D1 followed by weekly dose of 250 mg/m^2	13 CR, 2 PR
Pfisteret al.[33]	II	HN	22	C225 400 mg/m^2 w1, 250 mg/m^2 week 2-9	CDDP 100 mg/m^2 w1 and w4	70	No	No	Rash (69)	Rash (22), hypersensitivity (5); 2 deaths, 1 myocardial infarction, 1 bacteraemia, 1 atrial fibrillation	Regimen not recommended	3y PFS: 56%,; 3y LRC: 71%, 3y OS: 76%
Crombetet al.[43]	I	HN	24	h-R3 weekly from 100 to 400 mg	No	60–66	No	No	Tremors (41), hypotension (28), myalgia (25), fever (33)	Somnolence (4)	ND	3y-OS increase for higher dose (66,7% for patients with 200-400 mg *versus* 16.7% for 50-100 mg, $p = 0.03$)
Bonneret al.[34]	III	HN	211	C225 400 mg/m^2 w1, then 250 mg/m^2/w concomitant, *versus* RT alone		70– 76.8	No	ND	Rash (70), dermatitis (63), mucositis (37), infusion reaction (12), fever (25),	Rash (17), mucositis (56), dermatitis (23), infusion reaction (3)	ND	benefit in PFS (HR for death 0.7, $p = 0.006$) and in OS (49 months *versus* 29.3, $p = 0.03$)
			213						rash (9), mucositis (42), dermatitis (72)	mucositis (52), dermatitis (18)		
Safranet al.[38]	I	Oes	60	C225 400 mg/m^2 w1, 250 mg/m^2 week 2–9	TXL 50 mg/m^2/w+ CBDP AUC2/w	50.4	No	5	Rash (28), dehydration (30), oesophagitis (30)	Rash (23), oesophagitis (15), hypersensitivity (5)	ND	CR = 27%

MoAb: monoclonal antibody, R: rectum, HN: head and neck, Oes: oesophagus, GBM: glioblastoma, CT: chemotherapy, RT: radiotherapy, C225: cetuximab, h-R3: mimatuzumab, 5FU: 5 fluoro-uracile, GMZ: gemcitabin, CDDP: cisplatin, CBDP: carboplatin, AUC: area under the curve, TXL: paclitaxel, DLT: dose limiting toxicity, pts: patients, CR: pathological complete response, PR: partial response, OS: odd survival, PFS: progression-free survival, DFS: disease-free survival, LRC: locoregional control, SD: stable disease, PD: progressive disease, PR: partial response, RITC: residual isolated tumour cells, ND: no data.

C225 did not exacerbate the common toxic effects associated with radiotherapy of the head and neck, including mucositis, xerostomia, dysphagia, pain, weight loss and performance-status deterioration. C225 was associated with the development of acneiform rash in a majority of patients and hypersensitivity reaction in four patients. A major critique of this study is that none of the patients received CT, which is routinely used today in combination with RT in patients with SCCHN. However, it is proof of principle that EGFR inhibition can improve the efficacy of RT in patients, in terms of both local control and survival. There is currently a randomised trial underway (RTOG 0522) in which patients are randomised to RT and CDDP *versus* the same regimen plus C225.

In a phase I/II study, Machiels and colleagues assessed the safety and preliminary efficacy of C225 in the preoperative treatment of 40 patients with rectal cancer, receiving preoperative conformal RT combined with C225 and capecitabine.[42] The C225 therapeutic protocol was 400 mg/m^2 intravenous given 1 week before the beginning of RT followed by 250 mg/m^2/week for 5 weeks. Capecitabine was delivered for the duration of RT (650 mg/m^2 orally twice daily at first dose level; 825 mg/m^2 twice daily at second dose level). Four and 6 patients were treated at the first and second dose levels of capecitabine, respectively. No dose-limiting toxicity occurred. Thirty additional patients were treated with capecitabine at 825 mg/m^2 twice daily. The most frequent grade 1/2 side-effects were acneiform rash (87%), diarrhoea (65%), and asthenia (57%). Grade 3 diarrhoea was found in 15%. Three grade 4 toxic effects were recorded: one myocardial infarction, one pulmonary embolism, and one pulmonary infection with sepsis. Only two patients (5%) achieved a pathological complete response.

Another phase I/II trial with preoperative RT was recently conducted in rectal cancer by Rödel and colleagues, assessing the safety and efficacy of C225 combined with capecitabine and oxaliplatin (50 mg/m^2 on days 1, 8, 15, 22, 29).[43] At the lowest level dose of capecitabine (500 mg/m^2/bid), one patient experienced a grade 3 C225-related hypersensitivity reaction and 1 of the 3 patients in the first cohort developed grade 3 diarrhoea. Three additional patients were included without DLT. From these 2 phase I/II results, the recommended dose level of capecitabine was 1650 mg/m^2 on days 1–14 and 22–35. Forty eight patients were treated at the recommended dose level, with 9 patients (9%) developing grade 3–4 diarrhoea. Grade 3 acneiform rash was restricted to 4% of patients. However, two deaths occurred. One patient with dihydropyrimidine dehydrogenase deficiency died from septicaemia and consecutive multiorgan failure in the third week of neoadjuvant trreatment. The other patient died two weeks after the completion of the preoperative treatment of cardiac failure with no previous signs of cardiac toxicity. This trial failed to demonstrate an improved efficacy using triple drug therapy in terms of increased pathological complete response rate, with only 4 (9%) of the 45 operated patients achieving complete pathological tumoural response.

Hofheinz and colleagues conducted a phase I trial establishing the feasibility and efficacy of capecitabine and weekly irinotecan, combined with C225 and pelvic RT for patients with locally advanced rectal cancer.[44] Twenty patients received a dosing regimen of C225 (400 mg/m^2 on day 1 and 250 mg/m^2 on days 8, 15, 22, and 29) and escalating doses of irinotecan and capecitabine according to phase I methods: dose level I, irinotecan 40 mg/m^2 on days 1, 8, 15, 22 and 29 and capecitabine 400 mg/m^2/bid on days 1–38; dose level II, irinotecan 40 mg/m^2 and capecitabine 500 mg/m^2/bid; and dose level III, irinotecan 50 mg/m^2 and capecitabine 500 mg/m^2/bid. RT was given to a dose of 50.4 Gy (45 Gy plus 5.4 Gy). Surgical resection was scheduled 4–5 weeks after the completion of CRT. At dose level I, no DLT occurred. However, grade 3 diarrhoea affected 1 of 6 patients on dose level II. Two out of 5 patients treated at dose level III exhibited DLT (diarrhoea in 2 and nausea/vomiting in 1). A total of 10 patients were treated on dose level II and received a mean relative dose intensity of 100% of C225, 94% of irinotecan and 95% of capecitabine. After surgery, which was performed for all patients, five achieved a pathological complete remission and six had microfoci of residual tumour. However, 4 of the 5 patients with complete response had early stage T2 tumours. The combination of C225 and preoperative CRT does not seem to increase toxicity nor postoperative morbidity patients. Given the strong preclinical rationale to combine C225 with CRT in rectal cancer patients, results from the 3 described phase I/II trials are disappointing in terms of efficacy but should be investigated further.

A recently published phase II trial demonstrated the feasibility of the addition of C225 combined with CRT in localised oesophagogastric cancer.[45] Sixty patients received C225, paclitaxel (TXL), and carboplatin weekly for 6 weeks with 50.4 Gy radiation. Fourteen patients (23%) had grade 3 dermatologic toxicity consisting of a painful, pruritic acneiform rash on the face outside of the radiation field. The rates of grades 3 and 4 oesophagitis were 12% and 3%, respectively. Three patients had grade 3/4 C225 hypersensitivity reactions and were not assessable for response. Seventeen percent had a complete clinical response after CRT. Whereas dermatologic toxicity and hypersensitivity reactions were associated with the addition of C225, there was no increased toxicity in oesophagitis or other radiation-enhanced toxicity.

Cutaneous toxicity is commonly encountered with EGFR therapies and RT. In most of the published phase I/II clinical trials using C225, cutaneous side-effects are common. Those were investigated by Busam and colleagues in 10 patients who had cutaneous toxicity following treatment with C225.[46] Immunohistochemical and *in situ* hybridisation studies on skin biopsies were used to further characterise clinical reactions. The authors determined that the most common cutaneous reaction to C225 therapy was the development of an acneiform follicular eruption, which was most pronounced on the face, chest and upper back and typically manifested a week after the onset of treatment. The consistency of the morphology and timing of the clinical findings following monotherapy with C225 strongly suggested a direct biological effect of the antibody. Additional dermatological side-effects included focal areas of tender paronychial inflammation of toes and fingers and small aphtous ulcers of the oral mucosa. Serial punch biopsies of chest skin before and after eight days treatment revealed two main reaction patterns: a superficial dermal inflammatory cell infiltrate surrounding hyperkeratotic and ectatic follicular infundibula and a suppurative superficial folliculitis. Focal intraepidermal

acanthosis was found in two biopsies. Besides, immunohistochemical and *in situ* hybridisation studies on a subset of the biopsies showed an increase in the expression of p27 in epidermal keratinocytes after treatment with C225. These data support the concept that p27 plays a part in the *in vivo* regulation of follicular and epidermal homoeostasis by EGFR. However, as most acute adverse effects are associated with RT (xerostomia, mucositis, and local skin toxicity)[29], it remains difficult to isolate specific toxicity patterns of antiEGFR from phase I/II trials evaluating toxicity of anti EGFR combined with CRT.

3.2.2. *Perspectives for new MoAbs*

A step forward in the research on anti-EGFR-MoABs may be represented by humanised monoclonal antibodies, such as matuzumab (EMD72000), panitumumab (ABX-EGF) and mimatuzumab (h-R3). A phase I trial of EMD72000 in combination with TXL has been reported in 18 patients with EGFR-positive advanced non-small cells lung cancers, with only 23% objective response.[47] In a recent phase III trial, Van Cutsem and colleagues compared the activity of ABX-EGF plus best supportive care (BSC) to that of BSC alone in 463 patients with metastatic colorectal cancer with progressive disease after standard chemotherapy.[48] ABX-EGF significantly prolonged disease-free survival (HR: 0.54; 95% CI, 0.44 to 0.66, $p < 0.0001$). None of the patients had any grade 3-4 reaction. In addition, ABX-EGF is fully human and thus devoid of any antigenic effect.

Bier and colleagues reported results of a phase I study of EMD72000 in advanced SCC of the larynx and hypopharynx.[49] Nine patients received five administrations of EMD72000 in three consecutive ascending-dose groups (100 mg, 200 mg and 400 mg), followed by 4 weekly maintenance doses of half the loading doses, i.e. 50, 100 and 200 mg, respectively. EMD72000 was given as a 1-hour intravenous infusion, twice before and three times after surgery. Drug related adverse events were mainly grade 1–2, most of them being fever and a transient elevation of liver enzymes. In all patients, Cmax occurred within 1–3 h of the start of each EMD72000 infusion and after correction for dosage, appeared to be dose independent. In contrast, the elimination half-life ($t_{1/2}$) demonstrated dose dependency. In conclusion, EMD72000 was well tolerated in this small group of patients with advanced stage head and neck cancers. Pharmacokinetic data from this trial suggest the feasibility of conducting future studies with weekly EMD72000 doses of 200 mg. Although no data exists on the combination of ABX-EGF or EMD72000 with RT, previous results should stimulate further investigations for new MoAbs in combined modality studies.

Humanised monoclonal antibody h-R3 was obtained by grafting complementarity-determining regions of a murine mAb to a human framework, and it demonstrated in the preclinical studies a remarkable antiproliferative, pro-apoptotic, and antiangiogenic effect. Crombet and colleagues concluded from a phase I study that h-R3 is well-tolerated and may enhance radiocurability of unresectable head and neck neoplasms in 24 patients receiving six once-weekly infusions of h-R3 at four dose levels in combination with RT.[50] Antibody-related adverse events consisted in infusion reactions. The most frequent radiation-associated toxicities were mucositis, dermatitis and dysphagia. Overall survival significantly increased after the use of the higher antibody doses. The 3-year survival rate was 16.7% for subjects treated with the two lowest doses (50 and 100 mg) and 66.7% for the patients treated with 200 and 400 mg. Besides, immunohistochemistry studies of tumour specimens before and after treatment revealed that antitumour response correlated with antiproliferative and antiangiogenic effect.

4. Discussion

There is no strong rationale for indirectly comparing the MoAb trials with the TKI trials. Clinical trials included patients without staging and the diseases had very different natural histories, with various primitive tumours. There is no apparent distinction between TKIs and MoAbs regarding their propensity to trigger synergistic cytotoxic interactions with RT. However, the presence of EGFR ligands, especially EGF and TGF, may theoretically play opposite roles according to the type of EGFR targeting[4]. EGFR natural ligands may compete with the binding of MoAbs to receptor targets. They may also confer more dependency to the targeted cell through activation of EGFR pathway, and thus favour the activity of EGFR TKIs. Moreover, several mutations occuring in the tyrosine kinase domain of EGFR have been reported to activate EGFR pathways, thus increasing the activity of ZD2839. In contrast to ZD1839, the presence of EGFR-activating mutations is not correlated with sensitivity to this agent.[51] It would be of interest to compare the respective impact of MoAbs and TKIs on death receptor-mediated apoptosis. Both MoAbs and TKIs targeting EGFR exhibit cutaneous toxicity to which digestive toxicity can be added when TKIs are used. Pharmocokinetic variability appears to be more marked with TKIs than with MoAbs. This might be due to cytochrome p450 polymorphisms and the influence of associated treatments on this enzymatic pathway.[52] Besides, MoAbs activity is partially mediated by antibody-dependant cellular toxicity[53], which involves reactions mediated by immune cells, with strong impacts of polymorphism. This contributes to variability of MoAbs, compared with TKIs. Respective properties of TKIs and MoAbs are highlighted in Table 3.

In the coming age of using molecular targeted agents as strategies to improve results of RT, we can particularly select from strategies developed to interfere with “intrinsic” tumour signalling, such as anti-EGFR agents, or strategies interfering with the tumour microenvironment, such as anti-angiogenic approaches. Amongst those approaches, the monoclonal antibody cetuximab (C225, Erbitux®) remains the only example that has achieved development from preclinical data to a positive phase III trial leading to market approval.[41] In this trial, application of cetuximab during RT led to an improvement of local tumour control and survival compared to RT alone. Clearly, this trial is a milestone for the clinical use of EGFR inhibitors and for the principles of molecular targeting in radiation oncology. Therefore, to take full advantage of the potential of EGFR inhibitors in radiation oncology, detailed understanding of the underlying mechanisms of combined RT or CRT and EGFR inhibition both in tumours and in normal tissues is necessary. Preclinical studies should be considered as a prerequisite for optimising the success of clinical trials

Table 3 – EFGR targeting: similarities and differences

	MoAbs	TKI
Target	Extracellular domain of EGFR	ATP competitive inhibitor of intracellular TK
Mechanism	Competitive antagonism Independent of EGFR phosphorylation status	Inhibiting EGFR autophosphorylation Not strictly specific for EGFR
Variability	Few variability	More marked pharmocokinetic variability
Impact of polymorphisms	Strong impact: role of Ab-dependent cellular toxicity	Not documented
Impact of activating EGFR mutations	No impact	In favour: increasing the activity of ZD2839
Impact of EGFR natural ligands	In disfavour: may compete with MoAbs binding	In favour: confer more dependency to targeted cell through activation of EGFR pathway
Potential toxicity	Cutaneous, hypersensitivity, mucositis	Cutaneous, hypersensitivity, mucositis + digestive toxicity
Proof-of-principles	4 phase I clinical trials 3 phase I-II clinical trials 1 phase III in HNSCC	8 phase I clinical trial No phase III trial

MoAB: monoclonal antibody, Ab: antibody, TKI: tyrosine kinase inhibitor, TK: tyrosine kinase, HNSCC: head and neck squamous cell carcinoma.

in the interest of both patients and oncologists, thereby avoiding fruitless and sometimes toxic trials.

Effectively, patients treated with EGFR inhibitors frequently develop a rash characterised by inflammatory papules and pustules on the scalp, face, neck and upper trunk. A certain number of studies have even lead to the hypothesis that rash may be a clinical surrogate marker of favourable outcome.[46] Interestingly, in the work from Bonner and colleagues, a slight increase in the incidence of radiation dermatitis was observed in patients undergoing RT with concurrent administration of cetuximab (18 *versus* 23%) compared with those undergoing RT alone; the authors concluded that cetuximab did not exacerbate the common toxic effects of RT.[30] Several questions are still pending, such as the role of acneiform rash in radiation dermatitis and the reasons for the major difference in rash in the arm treated with RT plus cetuximab (1 *versus* 17%) and the slight difference of dermatitis radiation injury. It is also important to note that 8 patients have definitively stopped cetuximab due to a grade 3 acneiform rash. Recently, Budach and colleagues reported 2 cases of severe dermatitis injury of 5 patients treated by the same protocol as described by Bonner and colleagues.[54] Similarly, several case reports revealed severe acute cutaneous reactions, such as cutaneous ulcers, haemorrhagic dermatitis and epidermal necrosis[54–58]. These drug/radiation side-effects seem to be more frequent than the rates described by Bonner and colleagues.[48] In response to irradiation, activation of EGFR has been demonstrated in normal fibroblasts *in vitro*. The role of this change in EGFR transcription and protein in normal tissues is unclear. It could be speculated that increased EGFR protein levels could be involved in the regulation of radiation-induced repopulation processes in turnover tissues, which represents a dominant factor affecting radiation tolerance during fractionated irradiation.[59] Clearly, these kinds of side effects should be described early in the development of combination therapies. Otherwise, it becomes difficult to transpose very encouraging results from a phase III trial to the situation when a lot of teams must manage toxicities for their patients. In any case, the cetuximab example will remain a major achievement of drug development in the field of RT: a strong preclinical rationale leading to a clear-cut patient benefit. From the pharmaceutical point of view, this illustrates that combining a drug with RT can lead to fast market approval. The development of molecular targeted agents in combination with RT, presents challenges in the design of proof of principle and proof of efficacy studies. Although this calls for further thorough investigations, radiosensitisation by C225 seems partially dependant upon the presence of high EGFR levels. It will be necessary to better select patients who truly have the potential to benefit from anti-EGFR therapies. Other factors should be further explored, such as the importance of tumoural status in Kras mutations.[60] Finally, EGFR targeting should be assessed in RT settings for other primary tumours, for example, cervix cancer. Several studies confirmed the presence of EGFR expression in cervix cancer as compared with normal adjacent tissue.[61] Considering that tumours with high EGFR values are more radiosensitive than tumours with low EGFR values, there is now an emerging consensus that the combination of RT and EGFR targeting may play an important role in cervix cancer management.

5. Conclusion

Compared to reported data of literature on CRT, global toxicity of those trials does not seem to be increased. Nevertheless, as long as we have only one prospective trial and no long-term follow-up, it is important to maintain caution in new combined approaches. Till today, there are too few clinical data to support any strong conclusion regarding response rates or survival. However, it was demonstrated that EGFR inhibition can improve the efficacy of RT in HNSCC patients.[41] In contrast with C225, there are no proof of principle studies that demonstrated any benefit to adding ZD1839 or OSI774 to RT. Preliminary efficacy of EGFR modulators is encouraging, and further development of this targeted combined-modality paradigm is warranted. Given the strong preclinical rationale for combining EGFR inhibitors with RT, additional studies are crucial. However, phase I/II data and lack of long-term experience suggest that physicians should consider combined

modality approaches with caution, considering uncommon but potentially severe toxicity. A more thorough understanding of underlying mechanisms is required in order to optimise EGFR targeting in radiotherapy settings.

Conflict of interest statement

None declared.

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