JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 1019–1028
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Received October 21, 2007
Accepted March 20, 2008
DOI 10.1211/jpp.60.8.0008
ISSN 0022-3573

Epidermal growth factor receptor inhibitors for radiotherapy: biological rationale and preclinical results

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Acknowledgement: D. Zips and M. Baumann received honoraria from Merck AG and AstraZeneca. Research grants by Schering AG and Boehringer Ingelheim Austria were given to D. Zips, M. Krause and M. Baumann. This work was supported by the German Research Council (DFG Ba1433-5, Ba1433-6) and was partly presented at the ARR Annual Meeting in Belfast 2007. We gratefully acknowledge the kind help of Dr Charles Gillham (Trinity College, Dublin, Ireland) with the revision of this manuscript.

Abstract

Blocking the epidermal growth factor receptor (EGFR) represents a role model for a successful biological targeting approach to improving outcomes after radiotherapy. This review summarizes data from several local tumour control experiments in which EGFR inhibitors were combined with radiation in FaDu human squamous cell carcinomas xenografted into nude mice. BIBX1382BS is an oral bioavailable inhibitor of the intracellular tyrosine kinase domain of EGFR. It was administered in different experimental settings: concurrent with fractionated radiotherapy, following completion of irradiation, and in the period between surgery and adjuvant irradiation. Despite beneficial effects on tumour growth, in none of these experimental settings did BIBX1382BS improve local tumour control. In contrast, cetuximab (Erbitux), an IgG1 monoclonal antibody against the extracellular ligand-binding domain of EGFR, improved local tumour control when given concurrently with radiation. Results from a series of local tumour control experiments designed to elucidate the underlying mechanisms of cetuximab suggest that multiple radiobiological mechanisms might contribute to the observed effects: decreased number of clonogenic tumour cells, increased cellular radiation sensitivity, decreased repopulation and improved reoxygenation of clonogenic tumour cells during the combined treatment. In summary, the data suggest that different classes of EGFR inhibitors may have a different potential to improve local tumour control after fractionated irradiation.

Role of radiotherapy in oncology

Cancer is an important public health and socioeconomic problem in Europe. Each year 2.9 million new cases are diagnosed and 1.7 million patients die from the disease (Boyle & Ferlay 2005). Surgery, chemotherapy and radiotherapy are the main modalities for cancer treatment. Radiotherapy is involved in curing about 50% of cancer (Bamberg et al 1996). It may be given alone or in combination with surgery and/or chemotherapy. In clinical practice, radiotherapy with curative intent is given in 30–35 daily fractions over 6–7 weeks. Despite considerable progress in radiation oncology treatment, results for many tumour types, such as lung, head and neck and brain, remain poor. Distant metastases and locoregional tumour progression are the main reasons for treatment failure. The clinical relevance of local or distant relapse varies between the different malignant diseases. For advanced, inoperable head and neck cancer treated with radiotherapy, locoregional progression is the leading cause of treatment failure and cancer-related death, whereas distant metastases are less frequent. Consequently, increased locoregional control rates by optimization of radiotherapy often result in improved overall survival figures (Pignon et al 2000; Bonner et al 2006; Bourhis et al 2006). There are similar data even for tumour types with a high risk of distant metastases, such as lung (Saunders et al 1999; Turrisi et al 1999) and breast cancer (Overgaard et al 1997; Ragaz et al 1997). Taken together, locoregional tumour control is important for quality of life as well as for patient survival. Improving locoregional control is therefore a major task for experimental and clinical radiation oncology. Areas of intense radiation research over the last decade have included modified fractionation, integration of chemotherapy, high-precision beam delivery and biological targeting.

Concept of biological targeting in radiotherapy

The aim of curative radiotherapy is to sterilize all clonogenic tumour cells. Clonogenic tumour cells represent a small subpopulation of all malignant cells in a tumour and when they survive following therapy they may lead to recurrence. Whether a tumour can be cured by ionizing radiation depends to a large extent on the number and radiation sensitivity of clonogenic tumour cells. Intuitively, total radiation dose is the most important treatment parameter that affects local control probability. Unfortunately, in many clinical situations, the toxicity of surrounding normal tissue prevents the delivery of a radiation dose necessary to sterilize all clonogenic tumour cells. Although recent advances in accurate radiotherapy beam delivery and supportive care have allowed some degree of dose escalation, this is not considered the ultimate solution to the problem. In the future, biological innovations incorporated within the high-tech environment of modern radiotherapy are expected to improve local tumour control (Baumann 2006). For biological approaches, it is worth noting that in addition to the number and radiation sensitivity of clonogenic tumour cells, other factors contribute to the radiotherapy response. These include the capacity of clonogenic tumour cells to repopulate, to reoxygenate and to recover from sublethal damage during fractionated irradiation. Cell cycle effects might also play a role. Understanding these factors led to important alterations in the delivery of radiotherapy such as modified fractionation. More recently, there is an increasing understanding about the underlying molecular mechanisms of repopulation, reoxygenation, cell cycle control and recovery. Cross-talk via released proteins and cognate receptors among malignant and non-malignant tumour cells such as fibroblasts, endothelial cells and immune cells plays an important regulative role. Ideally, the understanding of the molecular basis of radiation resistance leads to the identification of molecules driving these processes and, subsequently, to pharmacological compounds that can be used for specific targeting. Theoretically, even a small decrease in the number of surviving clonogenic cells induced by biological targeting compounds may result in a relevant increase in local tumour control after high-dose irradiation. This is due to the fact that radiation itself reduces the number of clonogenic cells by several logs and that recurrences after high doses of radiation stem from a few surviving clonogenic tumour cells (reviewed in Krause et al 2006). Moreover, radiation can be modified in temporal and spatial distribution. Therefore, radiation oncology can be considered as an optimal environment to integrate biological targeting into curative concepts in cancer treatment. The proof of this concept has been demonstrated by using inhibitors of the epidermal growth factor receptor (EGFR) signalling in head and neck cancer, which represents a role model for modern, biologically optimized radiotherapy (Baumann 2006; Baumann et al 2007).

EGFR

The EGFR (ErbB1, HER) is a member of the ErbB family of receptor tyrosine kinases including ErbB2 (HER2/*neu*), ErbB3 (HER3) and ErbB4 (HER4). Natural ligands of the EGFR include the epidermal growth factor, the transforming growth factor α, epiregulin, betacellulin, amphiregulin and heparin-binding EGF-like growth factor. Upon ligand binding, receptor activation results in receptor dimerization, phosphorylation of intracellular tyrosine residues of the receptor itself (autophosphorylation) and other proteins. Numerous cytoplasmic signalling cascades, including the RAS–RAF–MAPK and PI3K/AKT pathways, are activated, eventually leading to the modification of proliferation, differentiation, apoptosis, migration, angiogenesis and adhesion (Yarden & Sliwkowski 2001). Besides the cytoplasmic signalling, a nuclear EGFR pathway contributes to the downstream effects of receptor activation (Figure 1; reviewed in Lo & Hung 2006). Both pathways can be activated independently from ligands by cellular stress such as ionizing radiation. This indicates that, in addition to the physiological role of the EGFR system to mediate cell–cell contacts in an autocrine and paracrine manner to regulate tissue homeostasis in the growing and adult organism, it also functions as a cell protection system (Yarden & Sliwkowski 2001; Lo & Hung 2006). EGFR is often over-expressed and constitutively activated in cancers of epithelial origin. For example, EGFR is expressed in almost all squamous cell cancers of the head and neck region. Gene amplification, activating mutations as well as up-regulated autocrine loops by increased release of ligands (Mendelsohn & Baselga 2006), makes the EGFR system a significant molecular component potentially involved in all six hallmarks of cancer (Hanahan & Weinberg 2000): self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, tissue invasion and metastasis, sustained angiogenesis and unlimited replicative potential. Consequently, targeting EGFR represents a rational approach for anticancer therapy. Two therapeutic strategies have been used successfully for suppressing EGFR signalling: small molecule inhibition of cytoplasmic tyrosine kinase (TK) domains and antibody targeting of the extracellular region of receptors.

EGFR and resistance to irradiation

High levels of EGFR expression have been correlated with a poor prognosis in patients with head and neck cancer treated with radiotherapy (Ang et al 2002; Bentzen et al 2005; Eriksen et al 2005). Interestingly, poor survival of patients with high-EGFR tumours was due to poorer locoregional tumour control but not distant metastasis rate (Ang et al 2002), suggesting that EGFR expression indicates radiation responsiveness of the tumour but not its metastatic potential. The relationship between high EGFR expression and poor outcome after chemoradiation has also been observed for cancers of the nasopharynx (Chua et al 2004), oesophagus (Gibson et al 2003), uterine cervix (Pillai et al 1998) and rectum (Giralt et al 2005), as well as for gliomas (Chakravarti et al 2004).

Subgroup analysis of outcome data from patients with head and neck cancer suggest that EGFR might be involved in repopulation, an important mechanism of resistance to radiotherapy (reviewed in Steel 2002; Baumann et al 2003a) during fractionated irradiation (Bentzen et al 2005; Eriksen et al 2005). Briefly, while each fraction of irradiation reduces the number of clonogenic tumour cells (depopulation), proliferation

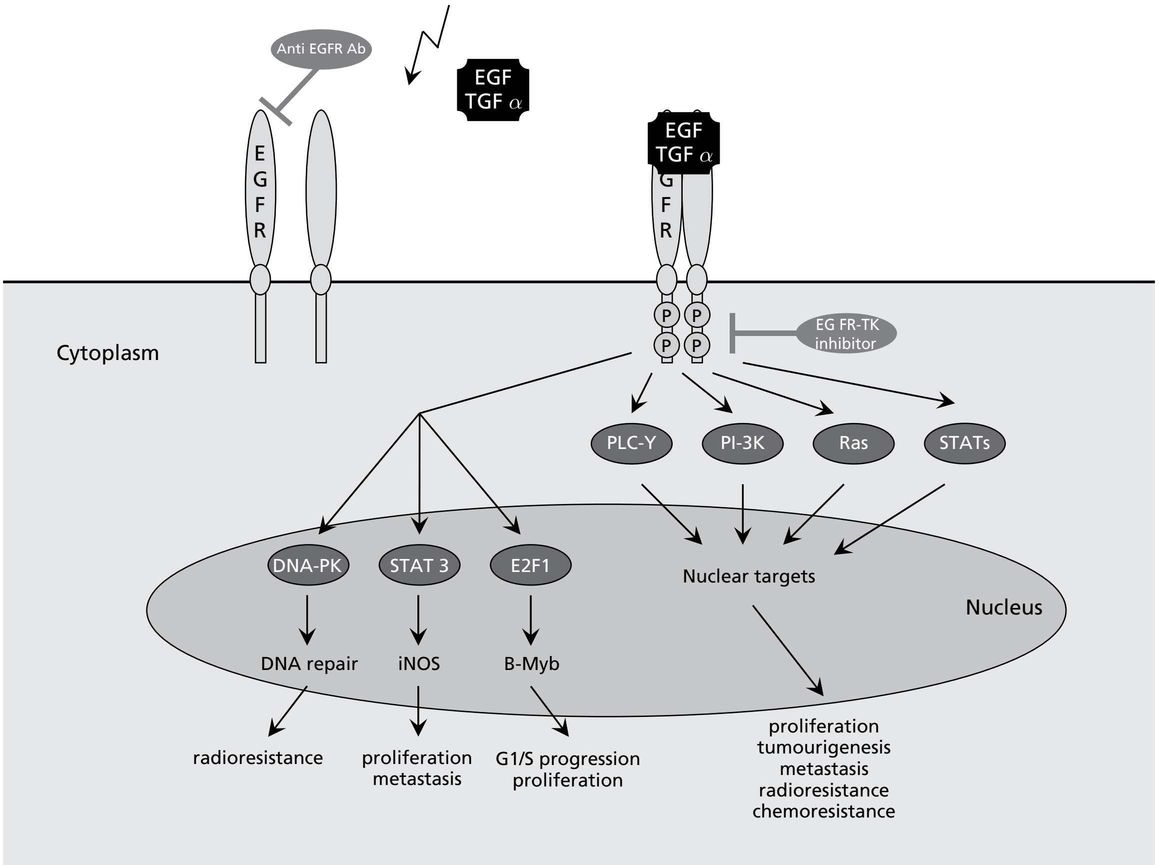

Figure 1 Overview of the epidermal growth factor receptor (EGFR) signalling pathways.

and/or decreased cell loss throughout the standard 6–7 weeks of fractionated radiotherapy increases the number of clonogenic tumour cells, thereby repopulating the tumour. Repopulation of clonogenic tumour cells is the most likely explanation for the so-called time factor of fractionated radiotherapy. As overall treatment time increases, a higher radiation dose is required to sterilize the tumour. Reducing the overall treatment time of fractionated radiotherapy in an attempt to counteract repopulation has been successfully evaluated. Interestingly, only patients with high EGFR expression experienced a benefit from shorter overall treatment times, suggesting a causative role of EGFR in repopulation (Bentzen et al 2005; Eriksen et al 2005). This is supported by preclinical data on human squamous cell carcinoma (hSCC) FaDu xenografted in nude mice (Petersen et al 2003). EGFR expression during fractionated irradiation was found to correlate with repopulation kinetics in FaDu hSCC, a tumour with a pronounced capacity to repopulate. In FaDu, repopulation kinetics follow a bi-phasic course with a low initial rate followed by a switch within 3 to 4 weeks of fractionated irradiation towards acceleration. This phase is preceded by significant reoxygenation (Petersen et al 2001). Around the time of the switch towards acceleration of repopulation, an up-regulation of EGFR, measured by immunohistochemistry, was observed. Of note, this correlation was not observed in hSCC tumour models with a low capacity to repopulate (Eicheler et al 2005). The proposed underlying molecular mechanism is that ligand-independent EGFR activation by ionizing irradiation leads to activation of mitogenic/proliferative pathways that mimic the effects of growth factors (Schmidt-Ullrich et al 1999). This mechanism has been also demonstrated for the most commonly expressed variant, EGFRvIII, which lacks a major proportion of the extracellular domain (Lammering et al 2004).

Inhibition of EGFR TK and fractionated irradiation

BIBX1382BS, a selective small molecule EGFR TK inhibitor (TKI), was used to test the hypothesis that its antiproliferative effects improve local tumour control of FaDu hSCC xenografted in nude mice, for example by inhibiting repopulation of clonogenic tumour cells during fractionated irradiation (Figure 2A; Baumann et al 2003b). FaDu expresses the EGFR, shows a ligand-independent activation of EGFR with a 2-Gy dose of radiation (abrogated by BIBX1382BS), and shows an up-regulation of EGFR expression during fractionated irradiation. As mentioned above, FaDu exhibits a pronounced capacity to repopulate during fractionated irradiation with a repopulated dose of about 20 Gy within 6 weeks, corresponding to one-third of the TCD50 (i.e. the radiation dose necessary to cure

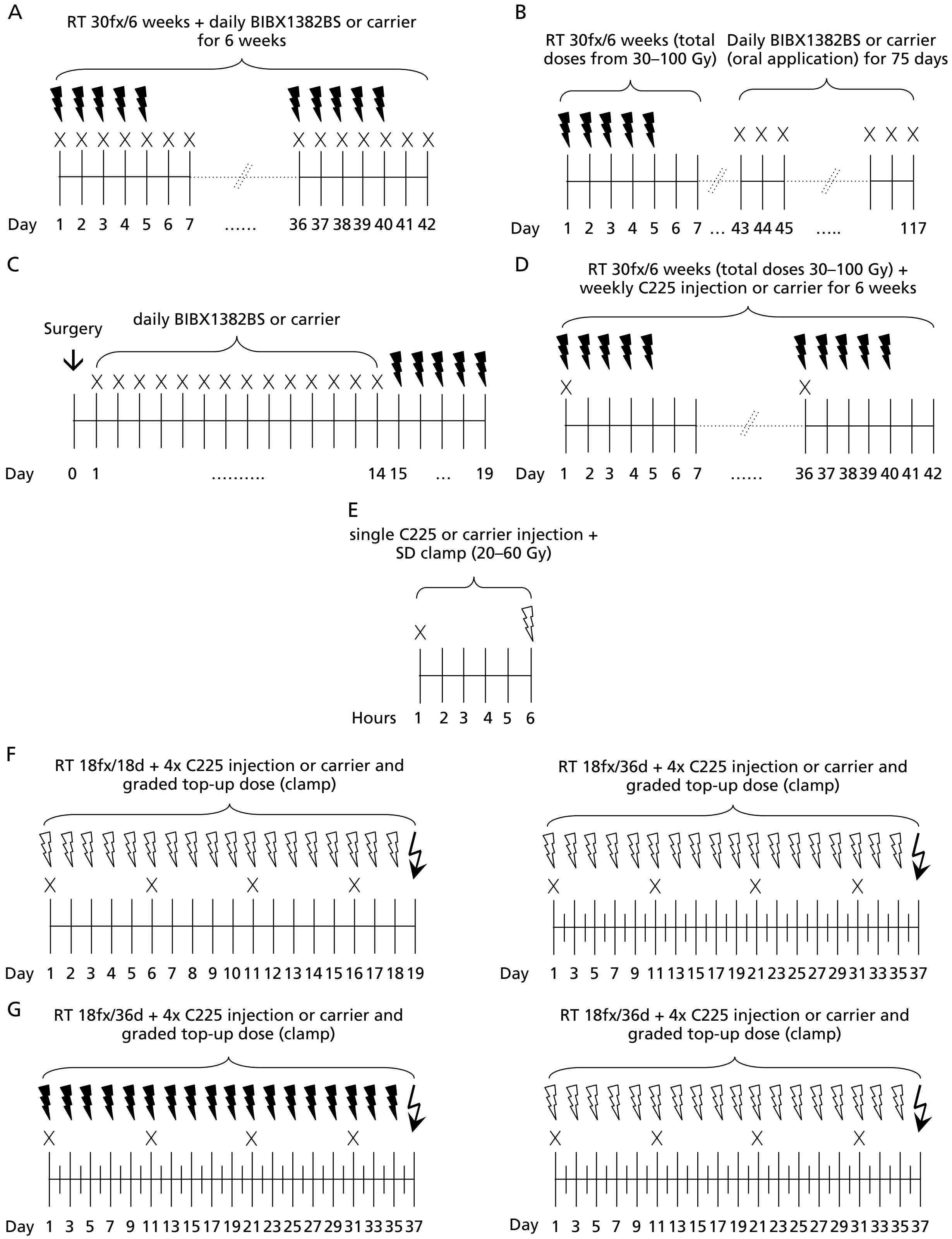

Figure 2 Schematic diagrams outlining treatment schedules. For further details see Table 1. For the combined treatments, irradiations were given under ambient conditions (⚡) or under clamped blood flow (ϟ). Administration of EGFR inhibitors is indicated by (X).

half of the tumours). BIBX1382BS resulted in a clear-cut inhibition of cell doubling time in-vitro (factor 4.9; 5 μM BIBX1382BS) and tumour volume doubling time in-vivo (factor 2.6; daily 50 mg kg^{-1} BIBX1382BS). The apparent underlying mechanism of the antiproliferative effect of BIBX1382BS in FaDu is a decrease of the growth fraction and of the proportion of cells in S phase. Apoptosis in FaDu remained low and no evidence for direct cytotoxicity by the EGFR TKI was found. Only after prolonged incubation at high doses of BIBX1382BS was radiosensitization observed in-vitro. Antiproliferative effects of BIBX1382BS in irradiated FaDu tumours were comparable with unirradiated tumours in-vivo. The tumour growth delay caused by irradiation was 2.3 days Gy^{-1} in BIBX1382BS-treated tumours and control tumours. Despite the clear-cut antiproliferative effects of BIBX1382BS, concomitant BIBX1382BS (50 mg kg^{-1}) and irradiation with 30 fractions over 6 weeks did not significantly alter local tumour control (Table 1). Thus, the hypothesis that the EGFR TKI BIBX1382BS improves local tumour control by inhibiting repopulation of clonogenic cells in FaDu tumours during fractionated irradiation was rejected. Several reasons may explain this unexpected finding. (i) Pharmacokinetic problems during the 6-week course of irradiation. It is possible that tumour sub-volumes containing radioresistant clonogenic tumour cells were not reached by the compound. (ii) Repopulation of clonogenic FaDu tumour cells in-vivo is not regulated by cell proliferation but by a decreased cell loss due to the improved microenvironment during the course of fractionated irradiation. This mechanism of repopulation in FaDu is supported by the observation of temporal coincidence of repopulation and reoxygenation (Petersen et al 2001). Moreover, manipulation of cell loss did affect repopulation capacity in FaDu tumours in-vivo (Hessel et al 2003). (iii) Although BIBX1382BS inhibited repopulation, it may have induced radioresistance via other mechanisms such as increased hypoxia, improved recovery from sublethal damage or increased cellular radioresistance. Tumour hypoxia was not evaluated in the experiment, however the marked drop in proliferation induced by BIBX1382BS would probably result in decreased oxygen consumption and thereby reduced tumour hypoxia. The in-vitro results on clonogenic cell survival after irradiation do not support that BIBX1382BS increases cellular radioresistance (including potential effects via cell cycle alterations) or recovery of sublethal damage. (iv) The molecular downstream machinery renders FaDu resistant to modification of cellular radiation sensitivity by EGFR inhibition (Toulany et al 2005a, 2005b, 2006). However, this does not apply to the antiproliferative effects of BIBX1382BS that are clearly present in-vitro and in-vivo. Importantly, the experiment analysed whether the antiproliferative effect of BIBX1382BS could be used to improve local tumour control by, for example, inhibition of repopulation. (v) BIBX1382BS has a limited effect on clonogenic FaDu tumour cells as compared with non-clonogenic cells. In fact, the observed discrepancy between the outcome measured by tumour growth delay and local tumour control supports this theory. Tumour volume, measured in order to determine growth delay, relies largely on the bulk of non-clonogenic cells,

Table 1 Epidermal growth factor receptor (EGFR) inhibition combined with irradiation: summary of local tumour control experiments performed with FaDu human squamous cell carcinoma growing in nude mice

EGFR inhibitor	Experiment	Endpoint	Control (95% confidence limits)	EGFR inhibitor (95% confidence limits)	*P* value	Reference
BIBX1382BS (tyrosine kinase inhibitor)	30 fx/6 weeks with concomitant BIBX1382BS (Figure 2A)	TCD50	63.6 Gy (55;73)	67.8 Gy (60;77)	$P = 0.5$	(Baumann et al 2003b)
	Adjuvant BIBX1382BS after 30 fx/6 weeks (Figure 2B)	TCD50	66.1 Gy (59;73)	67.9 Gy (61;75)	$P = 0.9$	(Krause et al 2004)
	BIBX1382BS in the time interval between surgery and irradiation with 5 fx/5 days (Figure 2C)	TCD50	25.4 Gy (18;33)	30.5 (24;37)	$P = 0.25$	(Krause et al 2007)
C225 (cetuximab, Erbitux; monoclonal antibody)	30 fx/6 weeks with concomitant C225 (Figure 2D)	TCD50	73.0 Gy (64;82)	63.1 Gy (57;69)	$P = 0.01$	(Krause et al 2005a)
	Single-dose irradiation (clamp) with C225 (Figure 2E)	TCD50	56.3 Gy (50;62)	46.0 Gy (41;51)	$P < 0.01$	(Krause et al 2005a)
	18 fx under clamp conditions over 18 or 36 days with concomitant C225 (Figure 2F)	Repopulated dose	19.7 Gy	13.8 Gy	$P > 0.05$*	(Krause et al 2005b)
	18 fx under ambient or clamp conditions over 36 days with concomitant C225 (Figure 2G)	Reoxygenated dose	24.2 Gy	31.3 Gy	$P > 0.05$*	(Krause et al 2005b)

A schematic diagram of the different treatment schedules is shown in Figure 2. fx, fractions of irradiation; TCD50, radiation dose necessary to control 50% of the tumours. *$P = 0.06$, combined effect of decreased repopulation and improved reoxygenation.

whereas local tumour control is solely dependent on clonogenic cells. In untreated FaDu tumours, only a very small proportion of all cells are clonogenic (Baumann et al 1990). Therefore, a differential effect of BIBX1382BS on non-clonogenic and clonogenic FaDu tumour cells would be compatible with the observed results. However, in-vitro investigations into the antiproliferative effects of BIBX1382BS on unirradiated and irradiated FaDu cells did not show differences between the response of non-clonogenic and clonogenic cells (Schlee et al 2003). This, of course, does not rule out the existence of a differential response in-vivo.

Overall, the specific EGFR TKI BIBX1382 given concurrent with fractionated irradiation of FaDu hSCC in-vivo resulted in prolonged tumour growth delay but did not improve local tumour control. The underlying mechanisms remain unclear. In line with our observations, other groups have found an increase in tumour growth delay when alternative EGFR TKIs such as ZD1839 (Iressa) or erlotinib (Tarceva) were combined with irradiation (Williams et al 2002; Solomon et al 2003; Chinnaiyan et al 2005; Sarkaria et al 2006; Feng et al 2007). However, in these experiments the effects on local tumour control, the most relevant experimental endpoint for curative radiotherapy, were not investigated (Krause et al 2003). Whether our observation of differences in outcome using tumour growth delay and local tumour control is a peculiarity of FaDu caused by its molecular profile or a more general phenomenon deserves further investigation. These are currently ongoing in our laboratory as part of a multi-institutional research project.

EGFR TKI can be combined with fractionated irradiation in different schedules. BIBX1382BS has been tested preclinically in FaDu tumours in-vivo (Krause et al 2004; Krause et al 2007) when given following incomplete resection and prior to postoperative irradiation (Figure 2c), and as adjuvant therapy after completion of fractionated irradiation (Figure 2b).

The concept of adjuvant inhibition of EGFR results from the assumption that after high-dose irradiation only a very small number of clonogenic tumour cells remain in the tumour. It was hypothesized that the remaining clonogenic tumour cells that lead to sustained survival and subsequent growth were EGFR-dependent. Adjuvant inhibition of EGFR signalling was expected to result in additional clonogenic cell inactivation, which in turn would give higher local tumour control rates. To test this hypothesis, FaDu tumours were irradiated with 30 fractions in 6 weeks and thereafter treated with daily BIBX1382BS (50 mg kg^{-1} bodyweight). Control tumours were only irradiated. Recurrent tumours after irradiation treated with BIBX1382BS grew at a lower growth rate than recurrences without BIBX1382BS treatment (Krause et al 2004). This implies that the growth of recurrent FaDu tumours after irradiation depends on EGFR signalling. However, the TCD50 values were not significantly different (Table 1). Thus, although the growth of recurrent FaDu tumours is EGFR-dependent, the clonogenic potential of survivors after irradiation is not affected by EGFR TKI BIBX1382BS.

Similar observations were made in an experiment to determine whether BIBX1382BS could improve local tumour control in the adjuvant setting (Krause et al 2007). FaDu tumours were incompletely resected microscopically and then treated with daily BIBX1382BS (1.2 mg for female animals and 1.5 mg for male animals) for 2 weeks followed by fractionated irradiation. As expected, at the time when irradiation was started, BIBX1382BS-treated tumours were much smaller than control tumours (factor 11). This difference in tumour volume would be expected to result in a difference in TCD50 of about 10 Gy and should have been detectable under the given experimental conditions. However, local tumour control rates after post-surgery irradiation were not significantly different between the experimental groups (Table 1). Immunohistochemical analysis performed in parallel showed no increase in hypoxia.

When interpreting the adjuvant experiments, it is worth noting that irradiation and BIBX1382BS were given sequentially. Therefore, the lack of direct interaction between both modalities does not explain why BIBX1382BS, despite its apparent antiproliferative effect, did not improve local tumour control. In fact, most of the arguments made above to explain the concomitant results do not apply here. The data from the adjuvant experiments suggest that non-clonogenic and clonogenic FaDu tumour cells respond differently to BIBX1382BS.

EGFR blockade by monoclonal antibodies combined with fractionated irradiation

The anti-EGFR monoclonal antibody C225 (cetuximab, Erbitux) binds specifically to the extracellular domain of the receptor, blocks receptor dimerization and causes receptor down-modulation by internalization of the antibody–receptor complex (Thomas & Grandis 2004; Li et al 2005). To test the effects of C225 on unirradiated FaDu tumours, mice were treated with 1 or 4 intraperitoneal injections of C225 (1 mg/injection) (Krause et al 2005a). This resulted in an inhibition of tumour growth by a factor of about 2. When given concurrent with irradiation, 1 mg of C225 was given weekly throughout a 6-week course of 30 fractions (Figure 2d). Tumour growth delay induced by C225 in irradiated tumours was comparable with unirradiated tumours (factor 1.4–2.7). The tumour growth delay per unit radiation dose was not significantly different between the tumours treated with C225 and controls (4.5 days Gy^{-1} vs 1.5 days Gy^{-1}, $P = 0.2$). Importantly, local tumour control after fractionated irradiation combined with C225 was greater than in tumours that were only irradiated (Table 1). TCD50 decreased from 73 Gy for controls to 63 Gy for tumours treated with C225 ($P = 0.01$), corresponding to a dose-modifying factor of 1.16. This was the first preclinical report demonstrating improved local tumour control in-vivo after a clinically relevant fractionated irradiation regimen with EGFR inhibition. These experimental findings are in line with the results from a Phase III clinical trial where C225 was given concomitantly during fractionated radiotherapy in patients with inoperable head and neck cancers (Bonner et al 2006). In this trial, local tumour control and overall survival after radiotherapy plus C225 was 10% higher than after radiotherapy alone. The implications for clinical practice are discussed elsewhere (Bernier & Schneider 2007). Radiobiological mechanisms underlying the effect of C225 on local tumour control after fractionated irradiation of FaDu hSCC were addressed in a series of experiments that are described and discussed in the following section.

Potential mechanisms of interaction

A variety of different mechanisms to explain the interaction between C225 and radiotherapy have been postulated, including increased apoptosis, inhibition of DNA repair, increased cellular radiosensitivity, decreased angiogenesis, independent cytotoxicity and inhibition of proliferation (reviewed in Milas et al 2000; Baumann et al 2007; Harari et al 2007). To investigate the interaction between irradiation and C225 in FaDu tumours in-vivo, a series of specifically designed local tumour control experiments were performed. For this, single-dose and fractionated irradiations were given.

In the first set of experiments, C225 was combined with single-dose irradiation under homogenous tumour hypoxia (Figure 2E; Krause et al 2005a). Homogenous hypoxia, or so-called clamp conditions, was achieved by placing a heavy clamp over the tumour-bearing leg of anaesthetized animals 2 min before and during irradiation. This experimental manoeuvre excludes the impact of varying tumour hypoxia on radiation response. In this experiment, C225 (1 mg/injection) was given once (6 h before irradiation) or 4 times (6 h before and 2, 5 and 7 days after irradiation). Control tumours of the same size were irradiated without C225. Local tumour control after C225 and irradiation was greater than after irradiation alone (Table 1). The dose-modifying factor was about 1.2, irrespective of the number of C225 injections. Local tumour control after single-dose irradiation under clamp conditions depends solely on two factors: the number of clonogenic tumour cells and their radiosensitivity. Potential effects on tumour hypoxia are excluded due to the irradiation under clamp conditions. Thus, C225 reduced the number of clonogenic cells and/or increased their cellular radiosensitivity. The lack of additional effect by the three injections after irradiation suggests that radiosensitization is the main mechanism of C225 in FaDu after single-dose irradiation under clamp conditions. Different molecular mechanisms of EGFR blockade leading to an increased cellular radiosensitivity as a result of impaired repair of radiation-induced DNA damage have been demonstrated by other groups. In A549 tumour cells, C225 inhibited the interaction of the internalized EGFR receptor complex with the DNA repair enzyme DNA-PK in-vitro (Dittmann et al 2005). In the same tumour cell line, blocking the EGFR-dependent PI3K/AKT pathway by BIBX1382BS resulted in decreased DNA repair in-vitro (Toulany et al 2006). However, C225's activity may, of course, be independent from interaction with irradiation cytotoxic effects on clonogenic FaDu tumour cells. This independent activity may lead to improved local tumour control in-vivo. As C225 has long-lasting receptor effects and the number of EGFR molecules per cell is finite, receptor saturation could explain why the additional injections provided no additional gain. Similar experiments were performed by Milas and colleagues on A431, a squamous cell carcinoma line with a very high EGFR expression: in contrast to FaDu, additional C225 injections following combined treatment with single-dose or fractionated irradiation and concomitant C225 gave higher local tumour rates than in tumours after concomitant C225/irradiation only (Nasu et al 2001; Milas et al 2007). Potential mechanisms that could result in radiation-independent tumour cell kill include direct and indirect effects. Direct cytotoxicity could result from induction of tumour cell apoptosis (Huang et al 1999). Inhibition of tumour angiogenesis and manipulation of the stem cell niche by EGFR inhibition might cause indirect or secondary cytotoxic effects on tumour cells (Milas et al 2000). In addition, immunological mechanisms such as the antibody-dependent cellular cytotoxicity (ADCC) might contribute to tumour cell death. Cetuximab has been shown to mediate ADCC against different cancer lines (Kawaguchi et al 2007; Kurai et al 2007). In breast cancer and lymphomas, ADCC mediated by therapeutic antibodies is a well-established mechanism of action considered to play an important role in response (Carter et al 1992; Reff et al 1994). Interestingly, ADCC would possibly explain the differential results we obtained when delivering EGFR TKI BIBX1382BS and monoclonal anti-EGFR antibody C225 in FaDu tumours in-vivo. Of course, modification of radiation response in-vitro by C225 cannot be explained by ADCC. Overall, further studies are necessary to clarify the role of radiosensitization and cytotoxicity as potential mechanisms of C225 on clonogenic survival after irradiation.

In a further set of experiments, fractionated irradiation was given to FaDu tumours with and without concomitant C225 (Krause et al 2005b). In order to analyse the effects of C225 on repopulation, irradiation with 18 fractions of 3 Gy under clamp conditions were given within either 18 or 36 days (Figure 2F). The fractionated treatment was followed by graded top-up doses under clamp conditions to obtain dose–effect relationships for local tumour control. C225 (1 mg/injection) was given 4 times during irradiation. Control tumours were irradiated without C225. Comparison of top-up TCD50 values after 18 and 36 days permits an estimation of the repopulated radiation dose. Effects of reoxygenation are excluded by this experimental design because all irradiations were given under clamp conditions. Consequently, different levels of tumour hypoxia during fractionated irradiation do not interfere with the results. In the control group (without C225) the TCD50 after 18 fractions in 18 days was 30.7 Gy, and after 18 fractions in 36 days was 50.4 Gy (repopulated dose: 19.7 Gy; Table 1). When C225 was given during fractionated irradiation, the repopulated dose was only 13.8 Gy, suggesting that C225 indeed inhibits repopulation. However, the difference in repopulation with and without C225 was not statistically significant. To investigate the effects of C225 on reoxygenation, 18 fractions of 3 Gy were given over 18 or 36 days under normal blood flow (so-called ambient conditions) and followed by top-up single-dose irradiation under clamp hypoxia (Figure 2F). The comparison of the top-up TCD50 values after fractionated irradiation under ambient and clamp conditions given within the same treatment time (18 or 36 days) allows estimation of the reoxygenated dose. For irradiations within 18 days, the reoxygenated dose was not significantly different between the control group and the C225-group (30.7 Gy vs 32.0 Gy). When fractionated irradiation was given within 36 days, the reoxygenated dose for the C225 group was higher than for the control group (31.3 Gy vs 24.2 Gy; Table 1), suggesting that C225 improves reoxygenation. However, this difference was not statistically significant. Taken together, the results suggest that C225 alters both repopulation and reoxygenation, but none of the effects reached statistical significance when evaluated separately. However, analysis of the whole data set revealed that the combined effect of improved reoxygenation and decreased repopulation was approaching statistical significance ($P = 0.06$). This result is in line with previous experiments where a complex interaction between repopulation and reoxygenation was found in FaDu tumours (Petersen et al 2001). Reoxygenation might be improved by C225 because the combined treated tumours shrunk faster than tumours that were only irradiated. A more rapid tumour regression might affect the microenvironment in terms of perfusion, oxygen and nutrient supply, which in turn could result in a lower amount of hypoxia. An alternative explanation comes from the preliminary observation that in unirradiated FaDu tumours, chronically hypoxic tumour cells show a higher EGFR expression than normoxic cells (Krause et al 2005b). From this, it could be speculated that C225 is predominantly active in chronically hypoxic tumour cells, theoretically resulting in a lower percentage of hypoxic cells.

In conclusion, the experimental data on FaDu hSCC suggest that C225 improves local tumour control via multiple radiobiological mechanisms: a decreased number of clonogenic tumour cells, increased cellular radiation sensitivity, decreased repopulation and improved reoxygenation. However, the quantitative contribution to the overall effect and the relationship between the different mechanisms are not completely understood. Furthermore, it is unclear whether our findings are exclusive to FaDu hSCC. Further studies in a panel of different tumour cell lines are ongoing. Additionally, combining inhibitors of multiple members of the EGFR family or other receptor kinases appears promising in order to combat redundancy and drug resistance (Williams et al 2004; Schutze et al 2007).

Summary

Biological targeting of the EGFR improves the results of curative radiotherapy. Different classes of EGFR inhibitors might differ in their potential to increase local tumour control after fractionated irradiation. Antiproliferative effects observed after EGFR inhibition during irradiation do not necessarily correlate with better local tumour control. Different mechanisms of interaction between EGFR inhibitors and irradiation contribute to the observed higher local tumour control rate after combined treatment. Further experimental studies are necessary to fully exploit the potential of EGFR inhibition in order to improve outcomes after radiotherapy.

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