Molecular markers of response and resistance to EGFR inhibitors in head and neck cancers

Carol Box\(^1\), Miriam Zimmermann\(^1\), Suzanne Eccles\(^1,2\)

\(^1\)Tumour Biology and Metastasis Team, Division of Cancer Therapeutics, McElwain Laboratories, The Institute of Cancer Research, Cotswold Road, Belmont, Sutton, Surrey, SM2 5NG, UK, \(^2\)Cancer Research UK Cancer Therapeutics Unit

TABLE OF CONTENTS

1. Abstract
2. Introduction
   2.1. Characteristics of squamous cell carcinoma of the head and neck (SCCHN)
      2.1.1. The SCCHN microenvironment
   2.2. Receptor tyrosine kinase (RTK) therapeutic targets in SCCHN
      2.2.1. The EGFR family in SCCHN: validation as targets
3. First generation tyrosine kinase inhibitors (TKI) of EGFR
   3.1. TKIs as SCCHN therapies
      3.1.1. Molecular indicators of response to TKIs
   3.2. Resistance to TKIs: markers and mechanisms
      3.2.1. EGFR-mediated
      3.2.2. Receptor switching
      3.2.3. Non-receptor-mediated
   3.3. Therapeutic strategies to overcome resistance
4. Next generation inhibitors of ERBB signalling
   4.1. EGFR family irreversible TKIs
   4.2. Multi-targeted inhibitors
   4.3. HSP90 inhibitors
5. Therapeutic antibodies targeting the EGFR
   5.1. Cetuximab: an anti-EGFR monoclonal antibody (mAb) approved for therapy in SCCHN
   5.2. Additional EGFR therapeutic antibodies and new applications in SCCHN
   5.3. Markers of response and resistance to monoclonal antibodies
6. Summary and perspectives
7. Acknowledgements
8. References

1. ABSTRACT

Receptor tyrosine kinases (RTK) are key targets for novel cancer therapeutics since they activate multiple oncogenic signalling pathways. Also, they are inherently ‘druggable’ due to their small ATP-dependent kinase domains (inhibitable by small molecules) and cell surface location which renders them accessible to monoclonal antibody-based therapies. The epidermal growth factor receptor (EGFR) is overexpressed in the majority of SCCHN cases and this review focuses primarily on the progress made in targeting the EGFR for the therapy of SCCHN by both small molecules and antibody-based therapies. We then discuss the overlapping and distinct molecular markers of response, innate or acquired resistance to each modality, and how these may be overcome. We also consider other RTKs overexpressed in this disease that may impact on responses and/or provide additional targets for combination therapy.

2. INTRODUCTION

2.1. Characteristics of squamous cell carcinoma of the head and neck (SCCHN)

SCCHN is the 6\(^{th}\) commonest cancer with more than 500,000 new patients diagnosed per year worldwide (1). It is generally an aggressive disease associated with significant morbidity and a high mortality rate (approximately 50% at 5 years (2)) due to spread to regional lymph nodes, development of distant metastases, lack of effective treatments and therapy-resistant recurrences. The majority of epithelial head and neck cancers are squamous cell carcinomas (SCCs) which encompass tumours arising in the oral cavity (including the lips, mouth and tongue), pharynx (naso-, oro- and hypopharynx), larynx and paranasal sinuses (3).

The most important independent risk factors for SCCHN are tobacco and alcohol consumption as well as
EGFR inhibitors in head and neck cancer

human papillomavirus (HPV) 16/18/33 infections with the degree of risk varying between tumour sites (4). The rising incidence of HPV-related cancers has changed the epidemiology and demographics of SCCHN with an increase in the proportion of oropharyngeal tumours occurring in a younger patient population of higher socioeconomic status (5). It is now well established that HPV-positive patients respond better to current standard treatment regimens than HPV-negative patients and consequently have a much better prognosis. Stimulation of the host immune response together with expression of the HPV oncoproteins E6 and E7 changes the molecular landscape of SCCHN, e.g. by elevation of the tumour suppressor p16INK4A (6), thus also influencing response to molecularly targeted therapies.

2.1.1. The SCCHN microenvironment

Increasing attention is being paid to the microenvironment of cancers with the recognition that interactions with the host can significantly influence their biological behaviour and responses to therapy (7-8). The bulky nature of most advanced SCCHN is a major contributory factor to treatment failure. First, the delivery of drugs is limited due to poor vascularisation resulting in suboptimal and heterogeneous drug concentrations (9). Secondly, these inefficiencies in the tumour blood supply lead to hypoxic regions (10-11) which are strongly associated with insensitivity to both chemo- and radiotherapy (12). Cellular responses to hypoxia are mediated primarily via the transcriptional complex HIF-1 (hypoxia inducible factor 1) which consists of heterodimers of the hypoxia-responsive HIF-1alpha and constitutively expressed HIF-1beta. HIF-1 binds to hypoxia response elements (HREs) in DNA and leads to the transcription of target genes which regulate processes such as metabolism, survival, angiogenesis, migration and invasion; all of which can contribute to tumour progression (13). Thus, adaptation of tumour cells to the hypoxic microenvironment promotes progression via multiple mechanisms. Therapeutic agents targeting pathways upregulated in hypoxia could therefore be useful in the treatment of (particularly advanced) SCCHN and it has become increasingly apparent that treating a tumour ‘holistically’ - addressing both underlying genetic drivers and epigenetic/microenvironmental factors - holds the most promise for future success.

2.2. Receptor tyrosine kinase (RTK) therapeutic targets in SCCHN

RTKs have emerged as prominent targets for novel therapeutics due to both their position at the head of multiple cell signalling cascades and to the frequency of their overexpression and/or hyperactivation in most tumours. Undoubtedly the most widely studied growth factor receptor in SCCHN is the epidermal growth factor receptor (EGFR) whose overexpression in the majority of SCCHN cases was first described over 25 years ago (14). Nevertheless, given the so far disappointing results of most clinical trials with single agent EGFR inhibitors (particularly small molecules (15)), we will also consider other RTKs overexpressed in this disease that may impact on responses and/or provide additional targets for combination therapy.

Of these, vascular endothelial growth factor receptor (VEGFR) is the most established therapeutic target with a variety of tyrosine kinase inhibitors (TKIs) and an inhibitory VEGF antibody (bevacizumab) approved as drugs for cancer treatment. VEGFRs are expressed on the surface of vascular and lymphatic endothelial cells (and also on some tumour cells) where they regulate angiogenesis, a key requirement of malignant progression (16). Overexpression of VEGFRs and their ligands has been well documented in SCCHN and linked to lymph node metastasis (17) and poor prognosis (18). In particular, the hypoxic nature of advanced SCCHN yields high levels of VEGF-A via HIF transcriptional activity (16). Bevacizumab has not been evaluated as a monotherapy in SCCHN, but its ability to enhance the effects of chemotherapy/chemoradiation has been tested in clinical trials (19-20) (see Table 1 and http://www.clinicaltrials.gov). Several pan-VEGFR kinase inhibitors such as semaxanib, sorafenib and sunitinib also inhibit various other kinases including PDGFR, KIT and RAF and these have been tested in SCCHN both as single agents and in combinatorial approaches (16). Most recent results indicate that although well tolerated, these inhibitors show limited activity in SCCHN (21-23). The future for VEGFR as a therapeutic target in SCCHN may lie with a new generation of inhibitors, e.g. SKLB1206, BMS-690514 and XL647, which co-target the EGFR and are currently in early stages of development and yet to be tested clinically in SCCHN (Table 1).

The insulin-like growth factor-1 receptor (IGF-1R) is frequently overexpressed in SCCHN and is associated with the development of second primary tumours (24). It activates RAS-MAPK and PI3K-AKT signalling pathways to drive tumour growth and cell survival and is therefore another potential therapeutic target in SCCHN (25-26). The anti-IGF-1R antibody cixutumumab caused tumour regression in SCCHN xenografts (25), however in the recently published results of a phase II clinical trial, a second antibody, figitumumab, showed no benefit in unscreened patients progressing after cis-platinum (27). Targeting IGF-1R may nevertheless prove useful in combination with other targeted therapies due to its role in resistance to both cytotoxic drugs and targeted agents, as discussed later.

Signalling from the receptor tyrosine kinase MET (hepatocyte growth factor receptor) leads to increased cell proliferation, motility, invasion and metastasis (28). It is frequently upregulated and/or mutated in SCCHN, a cancer with a propensity for local invasion and metastasis (29). Several molecules targeting MET are in early stage clinical trials. Of note is the success of the kinase inhibitor cabozantinib (XL184) in the treatment of both soft tissue and bone metastases in castration-resistant prostate cancer (30). A striking therapeutic response was also observed in a subset of non-small cell lung cancer (NSCLC) patients (carrying an EML4–ALK fusion) to the MET-ALK kinase inhibitor crizotinib (31). Studies of MET inhibitors in SCCHN are not as advanced, but with molecules such as crizotinib showing promise in preclinical studies (32) and the ability of MET to transactivate other relevant RTK signalling pathways (29), clinical studies in SCCHN may be merited (Also see article by Goetsch et al.).
EGFR inhibitors in head and neck cancer

<table>
<thead>
<tr>
<th>Target</th>
<th>Expression &amp; prognosis</th>
<th>Drug(s)</th>
<th>Class of inhibitor</th>
<th>Additional target(s)</th>
<th>Associated therapy</th>
<th>Status</th>
<th>Ref. or trial no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPH</td>
<td>EPHA2 associated with angiogenesis &amp; survival(41)</td>
<td>dasatinib (Sprycel®, BMS-354825)</td>
<td>thiazole carboxamide</td>
<td>SFKs, ABL, KIT, PDGFR</td>
<td>none</td>
<td>ph II</td>
<td>(171)</td>
</tr>
<tr>
<td></td>
<td>EPHB4 survival &amp; invasion in vitro &amp; in vivo (39)</td>
<td>NVP-BHG712</td>
<td>pyrazolo pyrimidine</td>
<td>none</td>
<td>none</td>
<td>preclinical</td>
<td>(172)</td>
</tr>
<tr>
<td>FGFR</td>
<td>FGFR1 amplification (173); FGFR3b activating mutation (174); FGFR19 amplification (175)</td>
<td>FP-1039</td>
<td>FGFRI-Fc fusion protein (ligand trap)</td>
<td>none</td>
<td>none</td>
<td>preclinical</td>
<td>(34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RO4383596</td>
<td>pyrimido pyrimidone</td>
<td>VEGFR2, PDGFR</td>
<td>none</td>
<td>preclinical</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AZ12908010</td>
<td>unavailable</td>
<td>undisclosed</td>
<td>none</td>
<td>preclinical</td>
<td>(175)</td>
</tr>
<tr>
<td>IGF-1R</td>
<td>receptor &amp; ligand overexpression (176-177)</td>
<td>cixutumumab (JMC A12)</td>
<td>humanized IgG1</td>
<td>none</td>
<td>none</td>
<td>preclinical</td>
<td>(25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>figitumumab (CP751,871)</td>
<td>fully human IgG2</td>
<td>none</td>
<td>none</td>
<td>ph II</td>
<td>(27)</td>
</tr>
<tr>
<td>MET</td>
<td>mutation, copy number increase, receptor &amp; ligand overexpression (32, 54)</td>
<td>SU11274</td>
<td>pyrrole indolone</td>
<td>none</td>
<td>none</td>
<td>preclinical</td>
<td>(32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pyrrole indolone</td>
<td>none</td>
<td>none</td>
<td>preclinical</td>
<td>(32)</td>
</tr>
<tr>
<td>PDGFR</td>
<td>ligand overexpression (37)</td>
<td>imatinib (Gleevec®, STI571)</td>
<td>amionoprymidine</td>
<td>BCR-ABL, KIT</td>
<td>docetaxel</td>
<td>ph II</td>
<td>*0058877</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dasatinib</td>
<td>see above</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGFR</td>
<td>overexpression; ligand overproduction correlating with lymph node metastases &amp; reduced survival (116) and references within</td>
<td>bevacizumab (Avastin®)</td>
<td>humanized IgG to VEGF</td>
<td>none</td>
<td>CXR</td>
<td>ph II</td>
<td>(19-20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cediranib (AZD2171)</td>
<td>quinazoline</td>
<td>PDGFR, KIT</td>
<td>none</td>
<td>ph II</td>
<td>*00588770</td>
</tr>
<tr>
<td></td>
<td></td>
<td>motesanib (AMG706)</td>
<td>nicotinamide</td>
<td>PDGFR, KIT</td>
<td>RX</td>
<td>preclinical</td>
<td>(180)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>semaxanib (SU5416)</td>
<td>indolin-2-one</td>
<td>FLT3, FGR1, PDGFRβ</td>
<td>none</td>
<td>ph II</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sorafenib (BAY 43-9006, Nexavar®)</td>
<td>bi-aryl urea</td>
<td>RAF1, B-RAF, PDGFRβ, KIT</td>
<td>none</td>
<td>ph II</td>
<td>(23, 182)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sunitinib (Sutent®, SU11248)</td>
<td>indolimone</td>
<td>FLT3, CSF-1R, RET, PDGFRβ, KIT</td>
<td>none</td>
<td>ph II</td>
<td>(22, 183-184)</td>
</tr>
</tbody>
</table>

*Numbers refer to NCT trial numbers - see NIH website (http://www.clinicaltrials.gov) Abbreviations: L: lung, ph: clinical trial phase, Ref: reference, CX: chemotherapy, RX: radiotherapy, CXR: chemoradiotherapy

Mutations in FGFR (fibroblast growth factor receptor) genes are common in cancer (33) and have been reported in SCCHN. Alternatively, hyperactivated FGFR signalling is linked to receptor and ligand co-overexpression (34). Several approaches to inhibiting FGFR activity are in clinical development: (kinase inhibitors, antibodies and a ligand-trap fusion protein) and some of these have shown preclinical activity in SCCHN cell lines (34). The most advanced stage clinical trials have been with broad spectrum FGFR/VEGFR/PDGFR kinase inhibitors, e.g. ponatinib and dovitinib, whilst more FGFR-selective inhibitors, such as AZD4547 (AstraZeneca), BGJ398 (Novartis) and LY2874455 (Eli Lily), are in earlier stages of development. All await clinical testing in SCCHN.

Similar to FGFR, many of the kinase inhibitors targeting PDGFR (platelet-derived growth factor receptor) co-target additional RTKs, particularly BCR-ABL and KIT, with the most notable examples being dasatinib and imatinib, piloted in the treatment of chronic myeloid leukaemia (35-36). There are few reports describing PDGFR expression in SCCHN. Although high levels of PDGF have been found in patients’ sera, this did not correlate with clinicopathological stage (37). As such, it seems that the future of PDGFR in the therapy of SCCHN remains as a co-target of pan-RTK inhibitors until or unless more evidence of its function in this disease setting emerges.

Additional emerging targets for the therapy of SCCHN are the Eph receptors. Ephs comprise the largest family of RTKs and are unique in binding only membrane-bound ligands, the ephrins (38). Ephs and ephrins participate in bidirectional signalling contributing to a
EGFR inhibitors in head and neck cancer

variety of physiological processes and their expression in several types of cancer, including SCCHN, is well documented. For example, high levels of EphB4 in SCCHN correlate with higher stage and lymph node metastasis (39). EphA2 expression has been linked with early stages of SCCHN development (40) and with angiogenesis and survival (41). A chemical proteomics screen of a large panel of SCCHN cell lines led to the identification of EphA2 as critical for proliferation and survival and siRNA validated its potential as a novel therapeutic target (42). Therapeutic strategies for inhibiting Eph signalling include targeting the kinase domain with either established drugs, such as dasatinib (or novel, more selective compounds) or targeting the ephrin binding site. Although disruption of protein-protein interactions is challenging, it may be more successful for Eph than for other molecules due to unique features of the ephrin binding pocket that enable high affinity binding of small molecules (43).

2.2.1. The EGFR family in SCCHN: validation as targets

The epidermal growth factor receptor (ERBB1/HER-1) is the archetypal member of the ERBB family of transmembrane RTKs which also includes ERBB2 (Neu/HER-2), ERBB3 (HER-3) and ERBB4 (HER-4). They are generally activated (except for the orphan receptor ERBB2) by binding one or more ligands such as epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), amphiregulin (AR) or heregulin (HRG) (44). In cancers including SCCHN, one or more ligands are often overexpressed (45) either by host cells or tumour cells themselves (autocrine signalling). Ligand binding leads to homo- or hetero-dimerisation of the receptors, frequently with the preferred partner ERBB2, and activation of the intrinsic kinase domain. Phosphorylation of C-terminal tyrosine residues enables interactions with multiple cytoplasmic proteins generating an enormous diversity in downstream signalling regulating cell behaviour (44). Unusually the kinase domain of ERBB3 is only weakly catalytically active; however once transactivated ERBB3 can directly bind to and activate PI3K and, via AKT, controls many biological processes for tumorigenesis. These include protein translation, cell survival, nutrient sensing, metabolic regulation, and cell cycle control (46). Hypoxia or cellular stress (e.g. induced by cytotoxic therapies) can also activate the PI3K pathway via HIF and/or the mammalian target of rapamycin (mTOR) (47).

EGFR is overexpressed in 90% of SCCHN and has been linked to malignant progression, resistance to radiotherapy and poor prognosis (48-49). Several groups have also reported increased EGFR expression in histologically-normal neighbouring tissue (50-51), suggesting a possible ‘field effect’ due to the initiating oncogenic driver(s). Overexpression of EGFR in SCCHN is frequently (10-30% cases) caused by EGFR gene copy number increases, either amplification at the 7p11 locus or polysoyomy (52-53). EGFR mutations in SCCHN are relatively rare with the exception of the constitutively active, ligand-independent EGFRvIII variant which has an in-frame deletion of exons 2–7 yielding a functional receptor with a truncated extracellular domain. Reports of EGFRvIII frequency in SCCHN vary widely with incidences ranging from 0 to 40% (52, 54) with similarly conflicting reports as to its impact on patient survival. It is clear, however, that EGFRvIII plays a significant role in resistance to anti-EGFR therapeutic antibodies (see below).

Whilst ERBB2 is also overexpressed in approximately 6% of SCCHN, its importance in this malignancy is uncertain with recent reports both of an association with poor prognosis (55) and of no relationship with outcome (56). Similarly, ERBB3 can be overexpressed in SCCHN (57) and has been found to correlate with reduced survival (58). There have been conflicting reports of ERBB4 expression in SCCHN (59) and its role is still under evaluation (60). Recently however high ERBB4 expressing SCC1 cells were found to proliferate rapidly in vitro and in vivo and were inhibited by a combination of cetuximab and an anti-ERBB4 antibody suggesting that ERBB4 may have value as a therapeutic target in SCCHN (61).

ERBB receptors are particularly implicated in cell motility and invasion (e.g. via activation of PLCgamma and/or the non-receptor kinase SRC (62-64)). Matrix metalloproteinases (MMP)-9 levels can be increased via JNK-1, which is often overexpressed in SCCHN, and EGFR activation has been correlated with MMP-9 expression and increased invasion in clinical cancers (59). EGFR can also mediate SCCHN invasion via the activation of STAT3, possibly via SRC (65-66). In addition, ERBB2 reportedly greatly promotes cell invasion via the activation of extracellular regulated kinase (ERK) (67). EGFR has been targeted by several agents such as gefitinib and erlotinib (TKIs) , cetuximab (monoclonal antibody), and lapatinib (dual EGFR/ERBB2 TKI) in SCCHN (15, 48) and we will now discuss progress and problems encountered to date.

3. FIRST GENERATION TYROSINE KINASE INHIBITORS (TKIs) OF EGFR

3.1. TKIs as SCCHN therapies

The first class of small molecules to successfully target EGFR are the quinazolines, exemplified by erlotinib and gefitinib, which compete with ATP binding to the kinase domain, resulting in reduced receptor autophosphorylation and signal transduction, cell-cycle arrest and inhibition of cell proliferation (24, 68-71) (Table 2). Although these agents have similar mechanisms of action, they differ in pharmacological properties. Since gefitinib accumulates in tumour tissue, but has lower plasma concentrations than erlotinib, it has been claimed that it can achieve target inhibition while causing less skin toxicity (72). There has been controversy over the optimal dose of gefitinib to be used in SCCHN. Single-arm Phase II studies suggested that 500 mg was more efficacious than 250 mg (the dose generally used in NSCLC) (73), and it is clear that experience in NSCLC (and determinants of sensitivity and resistance (see Section 3.2)) may not translate to SCCHN.

In most cases, responses to single agents in SCCHN have been limited and/or short-lived (15, 74),
hence combination therapies are being actively pursued (75). These include erlotinib, gefitinib or lapatinib in combination with radio- and/or chemotherapy or other targeted agents such as bevacizumab (Table 2). However, in some cases any benefit in efficacy is counterbalanced by treatment-related toxicities. A phase I/II study of erlotinib and bevacizumab in patients with recurrent or metastatic SCCHN assessed the MTD, toxicity and effects on disease progression. The response rate was 15% with four complete responses, which were consistent with the degree of pharmacodynamic changes in receptor phosphorylation observed. Although considered well-tolerated, three of the 46 patients experienced severe bleeding events (76). More recently, synchronous erlotinib, bevacizumab and concurrent chemoradiotherapy were trialled prospectively in locally advanced SCCHN. The regime was tolerated and efficacy compared favourably with historical controls, except for an increased risk of osteoradionecrosis. DCE-MRI was found to be useful in providing an early indication of response (77).

Lapatinib has been tested as a single agent and in combination with chemotherapeutic agents in SCCHN. In a panel of SCCHN cell lines, surprisingly, sensitivity to lapatinib did not correlate with expression levels of EGFR or ERBB2. Anti-tumour activity was observed in a xenograft model, which was enhanced by combination with paclitaxel, where there was evidence of increased apoptosis and anti-angiogenic effects (78). Clinically, lapatinib was tested as a short (2-6 weeks) treatment before chemo-radiotherapy in a Phase II trial of therapy-naïve patients with locally advanced SCCHN. Decreased cell proliferation (Ki67 staining) was seen, but no apoptosis. 17% of patients achieved an objective response and, of these, all had EGFR overexpression and 50% expressed ERBB2 (79). In a Phase II study, however, lapatinib - although well-tolerated - gave no benefit in recurrent/metastatic SCCHN regardless of

<table>
<thead>
<tr>
<th>Drug</th>
<th>Alternative name(s)</th>
<th>Developed by</th>
<th>Chemical class</th>
<th>Target(s)</th>
<th>Associated therapy</th>
<th>Status</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>erlotinib</td>
<td>OSI-774, Tarceva®</td>
<td>OSI, Genentech, Roche</td>
<td>anilinoquinazoline</td>
<td>EGFR</td>
<td>none</td>
<td>ph II</td>
<td>(185)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cisplatin</td>
<td>ph I/II</td>
<td></td>
<td>(186)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bevacizumab</td>
<td>ph I/II</td>
<td></td>
<td>(76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bevacizumab + CXR</td>
<td>ph II</td>
<td></td>
<td>(77)</td>
</tr>
<tr>
<td>gefitinib</td>
<td>ZD1839, Iressa®</td>
<td>AstraZeneca</td>
<td>anilinoquinazoline</td>
<td>EGFR</td>
<td>none</td>
<td>ph III</td>
<td>(87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CX</td>
<td>ph III</td>
<td></td>
<td>(187)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>ph II</td>
<td></td>
<td>(85-86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PF2341066</td>
<td>preclinical</td>
<td></td>
<td>(188)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>vorinostat</td>
<td>preclinical</td>
<td></td>
<td>(189)</td>
</tr>
<tr>
<td>lapatinib</td>
<td>Tykerb®, Tyverb®, GW-572016</td>
<td>GlaxoSmithKline</td>
<td>anilinoquinazoline</td>
<td>EGFR, ERBB2</td>
<td>none</td>
<td>ph II</td>
<td>(80)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>ph II &amp; ph III</td>
<td></td>
<td>(79)</td>
</tr>
<tr>
<td>vandetanib</td>
<td>Zactima®, ZEL 7135, ZD6474</td>
<td>AstraZeneca</td>
<td>anilinoquinazoline</td>
<td>EGFR, VEGFR2, RET</td>
<td>preclinical, ph I &amp; ph II</td>
<td>(134, 190)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>preclinical</td>
<td></td>
<td>(85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PF2341066</td>
<td>preclinical</td>
<td></td>
<td>(188)</td>
</tr>
<tr>
<td>NVP-AE788</td>
<td>none</td>
<td>Novartis</td>
<td>pyrrolopyrimidine</td>
<td>EGFR, ERBB2, VEGFR</td>
<td>none</td>
<td>preclinical</td>
<td>(129)</td>
</tr>
<tr>
<td>afatinib</td>
<td>Tomtovok®, Tovok®, BIBW2992</td>
<td>Boehringer Ingelheim</td>
<td>anilinoquinazoline</td>
<td>EGFR, ERBB2</td>
<td>none</td>
<td>ph II</td>
<td>(191)</td>
</tr>
<tr>
<td>canertinib</td>
<td>CI-1033</td>
<td>Pfizer</td>
<td>anilinoquinazoline</td>
<td>EGFR, ERBB2, B4</td>
<td>none</td>
<td>ph II (L)</td>
<td>(125)</td>
</tr>
<tr>
<td>dacotinib</td>
<td>PF-002998004</td>
<td>Pfizer</td>
<td>quinazoline</td>
<td>EGFR, ERBB2, B4</td>
<td>none</td>
<td>preclinical (L) &amp; ph II</td>
<td>(124, 192)</td>
</tr>
<tr>
<td>icotinib</td>
<td>Commana®, BPI-2009H</td>
<td>Beta Pharma</td>
<td>quinazoline</td>
<td>EGFR</td>
<td>none</td>
<td>preclinical (L)</td>
<td>(193)</td>
</tr>
<tr>
<td>neratinib</td>
<td>HKI-272</td>
<td>Pfizer</td>
<td>anilinoquinazoline</td>
<td>EGFR, ERBB2</td>
<td>none</td>
<td>preclinical (B)</td>
<td>(194)</td>
</tr>
<tr>
<td>CUDC-101</td>
<td>none</td>
<td>Curis</td>
<td>quinazoline</td>
<td>EGFR, ERBB2, HDAC</td>
<td>none</td>
<td>preclinical &amp; ph I</td>
<td>(195-197)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>ph I</td>
<td></td>
<td>(198)</td>
</tr>
<tr>
<td></td>
<td>SKLB1206</td>
<td>Sichuan University</td>
<td>purine</td>
<td>EGFR, ERBB2, B4</td>
<td>none</td>
<td>preclinical</td>
<td>(126)</td>
</tr>
<tr>
<td></td>
<td>BMS-690514</td>
<td>Bristol Myers Squibb</td>
<td>pyrrolotriazine</td>
<td>EGFR, ERBB2, VEGFR</td>
<td>none</td>
<td>preclinical (B, C, L, G)</td>
<td>(128)</td>
</tr>
<tr>
<td>XL647</td>
<td>EXEL-7647</td>
<td>Exelixis</td>
<td>quinazoline</td>
<td>EGFR, ERBB2, VEGFR2, 3, EPHB4</td>
<td>none</td>
<td>preclinical/ clinical (L)</td>
<td>(127)</td>
</tr>
</tbody>
</table>

*Numbers refer to NCT trial numbers - see NIH website (http://www.clinicaltrials.gov) CX: chemotherapy, RX: radiotherapy, CXR: chemoradiotherapy
EGFR inhibitors in head and neck cancer

whether patients had received prior treatment with an EGFR TKI (80) (Table 2).

3.1.1. Molecular indicators of response to TKIs

The limited success of EGFR TKIs in treating SCCHN is in stark contrast to their use in NSCLC where significant increases in survival have been achieved (81). The reason for this is linked to the identification of a subset of NSCLC patients with EGFR-activating mutations who are particularly responsive to EGFR antagonists (such as gefitinib and erlotinib) and have an improved outcome (reviewed in (82)). These gain-of-function somatic mutations/in-frame deletions (in exons 18-21) modify the ATP binding pocket of EGFR and enable better access, not only of ATP, but also the competitive TKI, resulting in increased sensitivity(53). It has also been suggested that such patients have a better prognosis overall, somewhat confounding interpretation of the efficacy of EGFR TKIs (83). However, these sensitising mutations are rare in SCCHN (detection rates 0 to 8%) depending somewhat on ethnicity and tumour sub-anatomical site: slightly higher mutation rates were found in Asians (84) or in tongue or tonsil cancers (85). Where mutations have been detected, no correlation with response has been seen (85). Clearly, EGFR sensitising mutations are not a useful biomarker for response to EGFR TKIs in SCCHN.

EGFR gene copy number, as determined by FISH (fluorescence in situ hybridisation) has also been proposed as a biomarker of response to TKI therapy in SCCHN. Reports are somewhat conflicting with Cohen et al. (86) concluding that high copy number associates with reduced survival but does not indicate response to EGFR TKIs whilst, in a larger phase III study, Stewart and colleagues observed an increased gefitinib response rate (14%) in EGFR FISH-positive compared with FISH-negative patients (5%) (87). Similarly, EGFR overexpression, possibly due to increased gene copy number, has been found to correlate with increased sensitivity to gefitinib in SCCHN cell lines (29) although this is yet to be confirmed in patients. Further exploration of EGFR’s potential as a response biomarker is enabled by the wide availability of phospho-specific antibodies. Such reagents have recently been utilised to demonstrate an association between high levels of phospho-Y1068 EGFR and reduced progression-free survival (88). Stransky and colleagues carried out whole exome sequencing of 74 SCCHN tumour-normal tissue pairs and identified mutations in NOTCH1, IRRF6 and TP63 genes, required for squamous differentiation, providing new insights into SCCHN biology which are likely to impact upon response to therapy (89). Alternative candidate biomarkers of response to EGFR TKIs in SCCHN are still being sought. In parallel studies efforts are focussed on trying to understand why so many EGFR-positive patients fail to respond to EGFR TKIs and to uncover mechanisms and biomarkers of resistance.

3.2. Resistance to TKIs: markers and mechanisms

Several different molecular mechanisms of innate and acquired resistance have been described in different tumour types including SCCHN (Figure 1).

3.2.1. EGFR-mediated

The discovery of EGFR ‘sensitising’ mutations hugely improved the TKI response rate in NSCLC, however around 30% of mutant NSCLCs either did not respond and the majority eventually acquired resistance to TKIs (81). This soon led to the discovery of the EGFR T790M ‘gatekeeper’ mutation, located in the receptor’s ATP-binding pocket, accounting for approximately 50% of therapy-resistant NSCLC relapses (90) and references within). The gatekeeper mutation has also been found in patients prior to gefitinib/erlotinib therapy and so may also contribute to primary resistance (reviewed in (82)).

The subcellular localisation of EGFR modulates its signalling and could be a determinant of response/resistance to TKIs. For example, in gefitinib-resistant breast cancer cell lines EGFR was localised to plasma membrane lipid rafts and disruption of the rafts with lovastatin overcame this resistance (91). An additive effect of lovastatin and an EGFR TKI (AG1478) in growth inhibition of SCC9 and SCC25 cells has previously been reported (92) suggesting that this mechanism may also be relevant to SCCHN. EGFR can also reside within the nucleus where it functions as a transcriptional co-activator and SCCHN patients with high levels of nuclear EGFR have a poor prognosis (93). In NSCLC, nuclear EGFR was found to upregulate transcription of the drug efflux pump BCRP (breast cancer resistance protein/ABCG2) thereby causing gefitinib resistance (94). Morris and colleagues analysed the genes of EGFR/PI3K pathway components and discovered that there was a frequent loss of PTEN, encoding an EGFR-inactivating phosphatase, in oral cancers. Furthermore, knockdown of PTEN reduced sensitivity to erlotinib in SCCHN or lung cancer cell lines (95) suggesting that PTEN has potential as a predictive biomarker of resistance to EGFR TKIs.

3.2.2. Receptor switching

One potential mechanism of resistance is persistently active signalling mediated via other EGFR family members such as ERBB2 or ERBB3. ERBB2 is overexpressed in a significant proportion of SCCHN, but the FDA-approved therapeutic mAb trastuzumab has not been extensively tested clinically in this disease. Preclinical studies suggest that trastuzumab can potentiate the effects of gefitinib or cetuximab (96) and may be worth further investigation. However dual EGFR-ERBB2 targeting can be achieved by lapatinib (see Section 3.1). High levels of phosphorylated ERBB2 and total ERBB3 were associated with resistance to gefitinib in SCCHN cell lines and co-treatment of gefitinib-resistant cells with pertuzumab, an anti-ERBB2 antibody, yielded significant growth inhibition (97). The ability of ERBB3 to activate the PI3K-AKT pathway is key to its role in resistance to both EGFR and ERBB2 TKIs. A recent report describes an antibody to ERBB3, MM121, which blocks its ligand-dependent phosphorylation and re-enabled gefitinib- or cetuximab-mediated growth inhibition of xenografts that were previously resistant (98).

Alternatively, a kinase switch mechanism involving EGFR transactivation by RTKs such as insulin-
like growth factor-1 receptor (IGF-1R) or c-MET can overcome EGFR dependence. Amplification of MET can promote signalling in gefitinib-resistant lung cancer cells via ERBB3-dependent activation of PI3K (99). Similarly, increased MET copy number was found in 13% SCCHN tumours (32) and MET expression was found to correlate with sensitivity to gefitinib in vitro (29). Signalling via IGF-1R was upregulated in gefitinib-resistant SCCHN cell lines (100-101) and treatment with an IGF-1R TKI (AEW541 or PQ401) re-sensitised cells to gefitinib. This provides a rationale for using IGF-1R inhibitors to overcome resistance to EGFR TKIs in SCCHN patients but has yet to be tested clinically.

3.3.3. Non-receptor-mediated

Resistance may be due to signalling pathways being ‘short-circuited’ by mutations in downstream signalling molecules, rendering upstream RTK activation redundant. Resistance to EGFR inhibitors due to K-RAS mutation is well documented in colorectal carcinoma (102-103) and also in NSCLC (104-105). However, K-RAS mutations are rare and H-RAS mutations more common in SCCHN [18-22%; (106-107)] and the latter therefore more likely to play a role.

Alterations in the PI3K-AKT signalling pathway downstream of EGFR have also been implicated in resistance to EGFR TKIs. A variety of mechanisms including mutations in PIK3CA (causing constitutive activation of the PI3K p110alpha catalytic subunit) and loss/inactivation of the phosphatase PTEN, a negative regulator of PI3K activity, have been found in multiple tumour types (e.g. lung and breast) resulting in EGFR-independence and resistance to inhibitors such as gefitinib or erlotinib (108-109). PIK3CA mutations are present in approximately 8% of SCCHNs (60) whilst PTEN mutations are seldom detected (89). Both of these mechanisms may also be responsible for escape from lapatinib-mediated inhibition of ERBB2 in breast cancer (110) and may account for some inevitable cases of resistance when the drug is used in SCCHN. Expression of the translation-initiation factor e1F4E, downstream of PI3K and AKT, has been suggested as a surrogate biomarker of pathway activation (111).
EGFR inhibitors in head and neck cancer

Epithelial-mesenchymal transition (EMT) plays a role in cancer progression and may be important in the development of resistance. Characterised by loss of E-cadherin (and thus reduced cell-cell adhesion) and an increase in vimentin expression, there have been several reports linking EMT with intrinsic resistance to EGFR TKIs. Examples of potential mediators include cortactin, a cytoskeletal protein, and deltaEF1 (delta-crystallin enhancer binding factor 1, an E-cadherin repressor), which have been independently associated with resistance to gefitinib (112) and erlotinib (113), respectively, in SCCHN. Sequist et al. reported that EMT was concurrent with acquired resistance to erlotinib in 2/37 NSCLC tumours with no other known resistance mechanisms, suggesting that EMT, by itself, may be a cause of acquired resistance. By contrast, Maseki et al. reported emergence of EMT in an SCCHN cell line that acquired resistance to gefitinib and concluded that altered ubiquitin-mediated degradation of EGFR, rather than EMT per se, was the mechanism of resistance (114). Thus, further investigations are required to clarify the causal and directional relationship between EMT and resistance. Expression of the adhesion molecule EMP-1 (epithelial membrane protein-1) was found to associate with both intrinsic and acquired resistance to gefitinib in NSCLC patients (115). The authors postulated that EMP-1, integrin signalling circumvented the requirement for EGFR in these patients; however, there have been no subsequent descriptions of this resistance mechanism and a report of downregulation of EMP-1 transcription in SCCHN (116) suggests that this is not likely to be a useful biomarker of EGFR TKI resistance in this context.

Clearly there are many potential routes for a tumour to become resistant to EGFR TKIs and the underlying molecular mechanisms identified in certain tumour types, such as NSCLCs, are not necessarily applicable to SCCHN. It should also be noted that, following a break from therapy, EGFR T790M TKI-resistant NSCLCs may become re-sensitised to the TKI concurrent with apparent loss of the T790M mutation (90) indicating that acquired resistance mechanisms may be transient and dependent upon continued drug exposure to maintain selective pressure (117). This also highlights the potential need for repeated tumour biopsies during therapy and that efforts should be focussed on finding biomarkers which are either detectable in blood or are non-invasive, such as magnetic resonance imaging modalities (118). Using multiplex bead-based assays, Byers and colleagues analysed the serum of 32 SCCHN patients for expression of 38 cytokine and angiogenic factors (CAFs). They identified an 8-protein signature (including VEGF and interleukins-4 and -8) that associated with shorter time to progression and which has the potential to predict (particularly HPV-negative) patients with a higher risk of recurrence (119). This demonstrates the potential of blood biomarkers for patient stratification and could easily be extended to look for biomarkers of therapeutic response/resistance especially as this approach would enable longitudinal studies of biomarker expression without the need for repeated biopsy of solid tumours.

4. NEXT GENERATION INHIBITORS OF ERBB SIGNALLING

4.1. Irreversible EGFR family TKIs

Potential advantages of irreversible TKIs over first generation reversible inhibitors are that blockade of signalling may be longer-lived, many have higher affinity and also inhibit more than one EGFR family member (120). The irreversible dual EGFR/ERBB2 TKI afatinib (BBW 2992) has shown activity against cetuximab-resistant cells (121) and clinical efficacy in some advanced NSCLC trials, extending time before tumour progression (122). However, no benefit in overall survival was seen in the LUX-Lung1 trial of advanced, metastatic NSCLC patients who had failed erlotinib and/or gefitinib plus chemotherapy (123). Afatinib is currently being trialled in SCCHN. Dacomitinib (PF-00299804) is an irreversible pan EGFR/ERB inhibitor active in EGFR vIII mutant and EGFR TKI-resistant cell lines and xenografts. A recent phase II trial of first-line treatment in recurrent and/or metastatic SCCHN achieved its primary endpoint with an 11% partial response rate and 63% stable disease with a manageable toxicity profile (124). Canertinib (CI-1033) is an orally active irreversible pan-ERBB TKI designed to inhibit a unique cysteine residue in this receptor family, effectively suppressing signalling via all homo- and heterodimers (125). It not only inhibits downstream signalling, but invokes receptor ubiquitination, endocytosis and thus downregulation of RTK expression. It has shown activity in multiple human tumour xenografts, but is not being pursued in NSCLC and has not yet been trialled in SCCHN. Similarly, neratinib (HKI-271) is active against ERBB2 as well as EGFR L858R, T790M and vIII mutations and exon 19 deletions. However, in Phase II trials in NSCLC, toxicity limited the dose that could be administered and results were disappointing (120) (Table 2).

4.2. Multi-targeted inhibitors

SKLB1206 is a novel, orally available reversible inhibitor of EGFR that is not only potent against the wild-type receptor, but also activating and T790M mutants. It further inhibits ERBB2, ERBB4 and VEGFR2 and has shown efficacy in multiple xenograft tumour models with anti-angiogenic effects in vitro and in a zebrafish embryo assay (126). Similarly, XL-647 is an oral TKI which targets EGFR, ERBB2, VEGFR and EphB4. In a small phase II trial of NSCLC patients who had relapsed on gefitinib or erlotinib, however, there was only a 3% response rate, and those with a T790M mutation had a significantly worse progression-free survival (127). Interestingly, however, EGFR mutant NSCLC treated as first-line with XL647 and which then relapsed, retained a degree of sensitivity to erlotinib. This intriguing finding suggests that agents with apparently similar modes of binding to the EGFR kinase domain may select for different mechanisms of resistance (128). Another agent that can inhibit both EGFR and VEGFR2 is NVP-AEE788. This agent was active in orthotopic SCCHN xenografts and potentiated the effects of paclitaxel, revealing decreased microvesssel density and increased apoptosis (129). Vandetanib targets primarily VEGFR2, EGFr and RET. VEGFR2 is expressed on some tumour cells and thus represents a direct and indirect (via...
EGFR inhibitors in head and neck cancer

angiogenesis) therapeutic target in cancer. Vandetanib has shown activity in SCCHN cell lines (130) and also restored their sensitivity to cisplatin and radiation in vitro and in vivo (131). Several Phase I and II trials are underway in SCCHN (Table 2). Signalling pathways downstream of RTK have also been targeted in SCCHN, for example using inhibitors of SRC family kinases (dasatinib, saracatinib) and RAF/MEK (sorafenib, sunitinib) (70).

4.3. HSP90 inhibitors

Heat shock protein (HSP) molecular chaperone inhibitors are of increasing clinical interest as ‘multi-targeted’ therapeutic agents. HSP90 is responsible for the correct folding, subcellular localisation and activation of multiple ‘client’ proteins, and it has been suggested that aberrant oncoproteins are particularly dependent on chaperones for their function (132). Chaperones are frequently upregulated in cancer (including SCCHN) (133) and especially so under conditions of microenvironmental cellular stress, such as hypoxia and nutrient deprivation (associated with aberrant tumour angiogenesis) or cytotoxic therapy. RTKs and many downstream kinases such as AKT, RAF and CDKs are key HSP90 client proteins, and others such as VEGFR2, VEGFR3, SRC, FAK, HIF-1alpha and MMPs play a key role in angiogenesis and invasion in SCCHN (133-134). Hence HSP90 inhibitors can simultaneously target multiple tumour and host oncogenic pathways and support networks. Preclinical studies have shown that chaperone inhibitors such as 17-AAG or NVP-AUY922 can have single agent activity in common solid tumours (135) and also potentiate the effects of radiotherapy in SCCHN (136). Second generation inhibitors with better pharmacological characteristics are now entering clinical trial in many solid cancers, and results are awaited with interest.

5. THERAPEUTIC ANTIBODIES TO THE EGFR

5.1. Cetuximab: an anti-EGFR monoclonal antibody (mAb) approved for therapy in SCCHN

Cetuximab (C225, Erbitux®) is an IgG1 mouse-human chimaeric mAb that targets the extracellular domain of EGFR, blocking ligand binding and enhancing receptor internalisation and degradation (137-138). An added benefit of many mAbs is their potential ability to induce antibody-dependent cellular cytotoxicity (ADCC) (139), but a disadvantage is that they may be less active in the presence of receptors that are ligand-independent or where there are mutated downstream signalling elements (such as KRAS) which can short-circuit the need for receptor activation. So far, of all the EGFR inhibitors tested, cetuximab is the only one with current FDA approval for SCCHN therapy. It has yielded objective clinical benefit as monotherapy in both metastatic and locally advanced SCCHN (68, 140). Several trials have established efficacy in SCCHN patients with platinum-refractory disease, although response rates are only of the order of around 10-13% in pre-treated patients (141). Cetuximab also improves responses when combined with radiotherapy (142) or chemotherapy, with an acceptable toxicity profile. In the EXTREME trial, cetuximab gave significantly improved survival, response rates, disease and symptom control compared with platinum-5-FU alone (140, 143) (Table 3).

5.2. Additional EGFR therapeutic antibodies and new applications in SCCHN

Panitumumab (ABX-EGF, Vectibix®) is a fully humanised anti-EGFR IgG2 mAb with similar direct effects to cetuximab (but less ADCC activity) which has yielded improved progression-free survival and/or overall survival in combination 1st-line palliative treatment with chemotherapy (144). The second generation fully human IgG1 zalutumumab (developed using phage display technology) reportedly binds EGFR domain III and locks the receptor in an inactive conformation, preventing dimerisation. It also mediates ADCC effectively and has the advantage of being weakly immunogenic. Zalutumumab has shown activity in Phase II-III trials (145-146) and was fast-tracked by the FDA for use in SCCHN patients who have failed standard therapies, but further trials are currently suspended. Nimotuzumab (h-R3) is a humanised mouse mAb that has been mainly trialled in oesophageal SCCs (alone or in combination with cytoreductive therapies), but is also in development for SCCHN (48). While both cetuximab and nimotuzumab reportedly inhibit both ligand-stimulated and -independent EGFR signalling, the effects vary with receptor density and antibody concentration. These properties may explain the reportedly differential clinical effects of the two mAbs (with fewer severe side-effects reported for nimotuzumab (147)). Nimotuzumab also gave a survival advantage in combination with radiotherapy or chemoradiotherapy in chemo-naïve EGFR-overexpressing SCCHN (148). Necitumumab is another fully human IgG1 EGFR domain III-specific antibody in preclinical development which has shown good PK properties and some activity in Phase I trials (149); Phase II trials in NSCLC in combination with chemotherapy are ongoing (Table 3).

In spite of the fact that EGFR is overexpressed in the majority of SCCHN, the unique specificity of mAbs and the added benefit (with appropriate isotypes) of host-mediated ADCC, many trials were considered to be underpowered and failed to reach their primary endpoint (141). MAbS have also been used in combination with TKIs and other agents, but cetuximab combined with the HDAC inhibitor bortezomib and radiotherapy gave unexpected early tumour progression associated with EGFR stabilisation, increased pro-survival signalling and cytokine induction (150).

In addition to the therapeutic deployment of mAbs (alone or in conjunction with chemo/radiotherapy) there has been extensive development of various engineered fragments and derivatives. Bispecific antibodies (bsAb), simultaneously targeting two oncoproteins, are proposed to increase specificity for cancer cells and limit the potential for ‘receptor switching’. This hypothesis was proven with a bsAb combining the variable regions of necitumumab and cixutumumab which simultaneously inhibited EGFR and IGF-1R signalling in vitro and showed efficacy in xenograft models (151-152). Further examples include MEH7945A (targeting EGFR+ERBB3), now in
Table 3. EGFR therapeutic antibodies used in SCCHN

<table>
<thead>
<tr>
<th>Drug</th>
<th>Developed by</th>
<th>Molecule type</th>
<th>Targets(s)</th>
<th>Associated therapy(s)</th>
<th>Status</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cetuximab (Erbitux®, IMC-C225)</td>
<td>Merck; ImClone; Bristol Myers Squibb</td>
<td>human-mouse chimeric IgG1</td>
<td>EGFR</td>
<td>none</td>
<td>FDA-approved for recurrent/metastatic SCCHN after platinum failure (199)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RX</td>
<td>FDA-approved for locally advanced SCCHN (142, 200)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CX</td>
<td>ph III (201-202)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>ph II &amp; ph III (203-204)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>bevacizumab</td>
<td>ph II (205) *00490565</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>sorafenib</td>
<td>ph I/II *00815295</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>hortezomib &amp; RX</td>
<td>ph I *150</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>cilengitide &amp; CX</td>
<td>ph I/II (206)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RAD001</td>
<td>preclinical (207)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R763</td>
<td>preclinical (208)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>dasatinib</td>
<td>preclinical &amp; ph II (164) *01488318</td>
<td></td>
</tr>
<tr>
<td>matuzumab (EMD 72000)</td>
<td>Merck Serono; Takeda</td>
<td>humanized mouse IgG1</td>
<td>EGFR</td>
<td>none</td>
<td>preclinical (209)</td>
<td></td>
</tr>
<tr>
<td>necitumumab (IMC-11F8)</td>
<td>Bristol Myers Squibb, Eli Lilly</td>
<td>fully human IgG1</td>
<td>EGFR</td>
<td>CX</td>
<td>ph III (L) (210)</td>
<td></td>
</tr>
<tr>
<td>nimotuzumab (b-R3, BIOMab EGFR, THERACIM)</td>
<td>CIM, Cuba; YM Biosciences</td>
<td>humanized mouse IgG1</td>
<td>EGFR</td>
<td>RX</td>
<td>ph II (211)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR/RX</td>
<td>ph Iib (148)</td>
<td></td>
</tr>
<tr>
<td>panitumumab (ABX-EGF, Vectibix®)</td>
<td>Amgen</td>
<td>fully human IgG2</td>
<td>EGFR</td>
<td>none</td>
<td>preclinical &amp; ph II (156) *00446446</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CX</td>
<td>ph III (212) *00460265</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RX</td>
<td>ph III *00820248</td>
<td></td>
</tr>
<tr>
<td>zalutumumab (HuMax-EGFr)</td>
<td>Genmab</td>
<td>fully human IgG1</td>
<td>EGFR</td>
<td>none</td>
<td>ph III (145)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RX</td>
<td>ph III *00496652</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CXR</td>
<td>ph III ongoing *00401401</td>
<td></td>
</tr>
<tr>
<td>DT-IgG</td>
<td>Emory University</td>
<td>recombinant human IgG</td>
<td>EGFR+VEGFR</td>
<td>none</td>
<td>preclinical (154, 214)</td>
<td></td>
</tr>
<tr>
<td>CONAN-1</td>
<td>Utrecht University</td>
<td>biparatropic nanobody</td>
<td>EGFR</td>
<td>none</td>
<td>preclinical (155)</td>
<td></td>
</tr>
<tr>
<td>MEHD7945a</td>
<td>Genentech</td>
<td>IgG</td>
<td>EGFR+ERBB3</td>
<td>none</td>
<td>preclinical (153, 215)</td>
<td></td>
</tr>
<tr>
<td>XGFR series</td>
<td>Roche</td>
<td>bispecific human IgG1</td>
<td>EGFR+IGFR</td>
<td>none</td>
<td>preclinical (L) (151)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers refer to NCT trial numbers - see NIH website (http://www.clinicaltrials.gov) CX: chemotherapy, L: lung, RX: radiotherapy, CXR: chemoradiotherapy

SCCHN clinical trials (153) and DT-IgG targeting EGFR and VEGF (154). Also, the ability of mAbs recognising different epitopes within EGFR itself has been exploited using phage display technology to generate high affinity biparatopic nanobodies. One example combines the specificity of cetuximab (which binds domain III) and matuzumab (recognising a region outside the ligand binding site which inhibits dimerisation) to generate CONAN-1, a potent receptor antagonist with in vivo therapeutic activity (155). A second approach involves replacing one Fab arm of the mAb with an immune effector arm that binds to a receptor such as CD3 and recruits T-cells to the tumour cells, but so far this has not been applied to EGFR mAbs (Table 3).

Finally, mAbs can be exploited for the targeted delivery of cytotoxic payloads, most notably radioisotopes, as exemplified by 90Y labelled panitumumab in SCCHN xenografts (156). Also, an 188Re-labelled anti-E6 specific antibody has been used to target HPV-16 expressing SCCHN cells, opening up the possibility of novel therapies for these increasingly prevalent cancers (157).

5.3. Markers of response or resistance to monoclonal antibodies

Surprisingly perhaps, immunohistochemically determined expression levels of EGFR do not serve as generic predictive biomarkers of response to cetuximab (71), although some studies could be complicated by the fact that certain tumours (e.g. colon cancer) have high levels of downstream mutations. Copy number (assessed by FISH) showed a significant association with response to cetuximab and panitumumab in colon cancer (158), but not in SCCHN (159). The EGFRvIII mutation was found in 22/53 (42%) SCCHN tumours and was associated with better disease control in recurrent/metastatic disease irrespective of treatment (+/- erlotinib) (54). However, when it was transfected into SCCHN cells, EGFRvIII
EGFR inhibitors in head and neck cancer

decreased responses to cetuximab. The antibody is still able to bind to the mutant receptor, although internalisation is delayed (160). In cells in which acquired resistance to cetuximab was induced in vitro, a novel EGFR mutation was identified (S492R) and this was subsequently found in 2/10 patients who became refractory to treatment. Interestingly, the cells retained sensitivity to panitumumab, suggesting that some forms of resistance may be overcome by switching to a mAb with a different epitope specificity (161). Urepression of EGFR/HER ligands are also linked to resistance: tumour biopsies showing high expression of AR were less likely to respond to cetuximab-docetaxel treatment (162) and increased expression of HB-EGF due to downregulation of miR-212 was identified in SCCHN cells with acquired resistance (163).

EGFR internalisation followed by ubiquitin-mediated degradation (164) or nuclear distribution (165) has been linked to certain cases of cetuximab resistance. In addition, to the lack of accessibility of antibodies to these locations, nuclear EGFR regulates genes involved in G1/S transition. EGFR nuclear translocation has been linked to the activity of SRC family kinases and inhibitors such as dasatinib have been reported to increase membrane expression of EGFR and restore sensitivity to cetuximab (165). Interestingly, although nuclear EGFR is also associated with resistance to gefitinib, the underlying molecular mechanism may be different. In this case AKT phosphorylation of EGFR Ser229 (promoting nuclear localisation and transcriptional upregulation of efflux pumps) was implicated, with beneficial effects seen with pharmacological AKT inhibitors or siRNA (94).

The presence of alternative RTK (such as ERBBs, MET, IGF-1R) could also minimise the dependence of tumour cells on EGFR signalling. In patient-derived xenografts, activation of MET was identified as a key cause of primary resistance (166). Yonesaka identified a new mechanism of resistance in patients who became refractory to cetuximab mediated by amplification of ERBB2 or increased levels of the ERBB2/B3 ligand HRG (167). In addition to its role in resistance to EGFR TKIs, IGF-1R may also be involved in resistance to cetuximab as co-treatment of SCCHN xenografts with cetuximab and cixutumumab (IGF-1R antibody) produced an additive effect (25). Barnes et al. showed functional EGFR-IGF-1R heterodimerisation and reciprocal re-sensitisation of cixutumumab-resistant SCCHN cell lines by cetuximab (and the mTOR inhibitor rapamycin) has subsequently been demonstrated (168). The combination of cetuximab and an IGF-1R inhibitor will be tested clinically in two phase II trials (NCT01427205 and NCT00957853). Several mechanisms of resistance apply to both pharmacological inhibitors and mAbs: for example cells with mutations in KRAS, BRAF, HRAS and NRAS genes, activated SRC or PI3K pathways, and those with mesenchymal characteristics generally fail to respond well to either modality.

In experimental models, xenograft tumours that escaped anti-EGFR antibody therapy became refractory to further treatment and exhibited significantly enhanced growth associated with high levels of VEGF. Since inhibiting EGFR downregulates VEGF production, these data suggest that escape from angiogenic inhibition can contribute to anti-EGFR antibody resistance. This hypothesis was substantiated by the fact that cells transfected with the VEGF gene were more resistant than parental cells (169). Clearly, a serum or tissue ‘signature’ of response to EGFR inhibition would be extremely valuable. Arigiris et al. analysed the serum from patients with stage III-IVB SCCHN who had received cytotoxic chemotherapy plus cetuximab by Luminex® technology pre- and post-treatment and 14 analytes showed interesting changes. Of these VEGF and IL-6 (angiogenic cytokines) were associated with tumour response as measured by PET and PFS (170).

6. SUMMARY AND PERSPECTIVES

Given the prevalence of EGFR overexpression in SCCHN and the availability of highly active and specific inhibitors, clinical trial results of monotherapy with drugs or antibodies have been disappointing. This is due to the complex signalling networks in cancer cells (allowing escape from suppression of a single receptor) and the emergence of multiple mechanisms of acquired resistance. Definitive biomarkers of sensitivity and resistance have been slow to emerge, and often do not translate between different types of cancer, thus the population of SCCHN patients likely to benefit from these therapies is still unclear. Several candidate determinants of resistance have been identified, leading to rational combinations of therapies and some incremental increases in response. Also, we are now (to a degree) able to exclude some patients who are unlikely to respond. Nevertheless, it is likely that the next breakthroughs will come from advances in high-throughput DNA analysis technologies which will enable unbiased evaluation of large numbers of genes in large patient populations to further our understanding of this malignancy and its response to therapy.

7. ACKNOWLEDGEMENTS

CB and MZ were supported by grants from the Oracle Cancer Trust. SE is supported by The Institute of Cancer Research and Cancer Research UK grant number C309/A8274. We acknowledge NHS funding to the NIHR Biomedical Research Centre.

8. REFERENCES


4. EM Smith, LM Rubenstein, TH Haugen, M Pawlitza, LP Turek: Complex etiology underlies risk and survival in
EGFR inhibitors in head and neck cancer


EGFR inhibitors in head and neck cancer


EGFR inhibitors in head and neck cancer


71. ME Sharafinski, RL Ferris, S Ferrone, JR Grandis: Epidermal growth factor receptor targeted therapy of
EGFR inhibitors in head and neck cancer


73. EE Cohen, MA Kane, MA List, BE Brockstein, B Mehrotra, D Hsu, AM Mauer, C Pierce, A Dekker, EE Vokes: Phase II trial of gefitinib 250 mg daily in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck. *Clin Cancer Res*, 11(23), 8418-24 (2005)


83. SW Han, TY Kim, PG Hwang, S Jeong, J Kim, IS Choi, DY Oh, JH Kim, DW Kim, DH Chung, SA Im, YT Kim, JS Lee, DS Heo, YJ Bang, NK Kim: Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. *J Clin Oncol*, 23(11), 2493-501 (2005)


85. EH Tan, C Goh, WT Lim, KC Soo, ML Khoo, T Tan, DS Tan, MK Ang, QS Ng, PH Tan, A Lim, J Hwang, YH Teng, TH Lim, SH Tan, N Baskaran, KM Hui: Gefitinib, cisplatin, and concurrent radiotherapy for locally advanced head and neck cancer: EGFR FISH, protein expression, and mutational status are not predictive biomarkers. *Ann Oncol*, 23(4), 1010-6 (2012)


EGFR inhibitors in head and neck cancer


94. WC Huang, YJ Chen, LY Li, YL Wei, SC Hsu, SL Tsai, PC Chiu, WP Huang, YN Wang, CH Chen, WC Chang, AJ Chen, CH Tsai, MC Hung: Nuclear translocation of epidermal growth factor receptor by Akt-dependent phosphorylation enhances breast cancer-resistant protein expression in gefitinib-resistant cells. J Biol Chem, 286(23), 20558-68 (2011)


98. B Schoebert, AC Faber, D Li, MC Liang, K Crosby, M Onsum, O Burenkova, E Pace, Z Walton, L Nie, A Fulgham, Y Song, UB Nielsen, JA Engelman, KK Wong: An ErbB3 antibody, MM-121, is active in cancers with ligand-dependent activation. Cancer Res, 70(6), 2485-94 (2010)


101. MJ Jameson, AD Beckler, LE Taniguchi, A Allak, LB Vanwagner, NG Lee, WC Thomsen, MA Hubbard, CY Thomas: Activation of the insulin-like growth factor-1 receptor induces resistance to epidermal growth factor receptor antagonism in head and neck squamous carcinoma cells. Mol Cancer Ther, 10(11), 2124-34 (2011)


107. AK Murugan, NT Hong, TT Cuc, NC Hung, AK Munirajan, MA Ikeda, N Taschida: Detection of two novel mutations and relatively high incidence of H-RAS
EGFR inhibitors in head and neck cancer

mutations in Vietnamese oral cancer. Oral Oncol, 45(10), e161-6 (2009)


114. SMaseki, K Ijichi, HTanaka, MFujii, Y Hasegawa, T Ogawa, SMurakami, E Kondo, HNakanishi: Acquisition of EMT phenotype in the gefitinib-resistant cells of a head and neck squamous cell carcinoma cell line through Akt/GSK-3beta/snail signaling pathway. Br J Cancer, 106(6), 1196-204 (2012)

115. AJain, CATindell, ILaux, JBHunter, JCurran, AGalkin, DEAfar, NAronson, SShak, RBNatale, DBAgus: Epithelial membrane protein-1 is a biomarker of gefitinib resistance. Proc Natl Acad Sci U S A, 102(33), 11858-63 (2005)


119. LAYeers, FC Holsinger, MS Kies, WN William, AKEl-Naggar, JJLee, J Hu, A Lopez, HT Tran, SYan, ZDu, KKAng, BS Gliisson, MgRaso, IlWistuba, JN Myers, WKhong, VPapadimitrakopolou, SM lipman, JVHymach: Serum signature of hypoxia-regulated factors is associated with progression after induction therapy in head and neck squamous cell cancer. Mol Cancer Ther, 9(6), 1755-63 (2010)


121. KMQuesnelle, JR Grandis: Dual Kinase Inhibition of EGFR and HER2 Overcomes Resistance to Cetuximab in a Novel In Vivo Model of Acquired Cetuximab Resistance. Clin Cancer Res, 17(18), 5935-44 (2011)


124. LLLiu, SJ Hotte, SLAurie, SSingh, EWinquist, SKLChia, EX Chen, KKK Chan, TWang, ITaylor, ARuiz-Garcia, CMormont, DSoulieres: Phase II trial of the irreversible oral pan-human EGFR inhibitor (HER) inhibitor PF-00299804 (PF) as first-line treatment in recurrent and/or metastatic (RM) squamous cell carcinoma of the head and neck (SCCHN). In: J Clin Oncol, 2011 ASCO Annual Meeting Proceedings, 29(Supplement 15), American Society of Clinical Oncology abstract nr 5561 (2011)


126. YPan, Y Xu, SFeng, SLuo, RZheng, J Yang, LWang, LZhong, HY Yang, BL Wang, Y Yu, JLiu, Z Cao, CX Wang, P Ji, Z Wang, X Chen, S Zhang, YQ Wei, SY
EGFR inhibitors in head and neck cancer


144. DA de Andrade, JP Machiels: Treatment options for patients with recurrent or metastatic squamous cell carcinoma of the head and neck, who progress after platinum-based chemotherapy. *Curr Opin Oncol*, 24(3), 211-7 (2012)

145. JP Machiels, S Subramanian, A Ruzsa, G Repassy, I Lifirenko, A Flygare, P Sorensen, T Nielsen, S Lisby, PM Clement: Zalutumumab plus best supportive care versus
EGFR inhibitors in head and neck cancer


163. H Hatakeyama, H Cheng, P Wirth, A Counsell, SR Marcom, CB Wood, PR Pohlmann, JGibert, B Murphy,
EGFR inhibitors in head and neck cancer


166. R Krumbach, J Schuler, M Hofmann, T Giesemann, HH Fiebig, T Beckers: Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur J Cancer, 47(8), 1231-43 (2011)


168. DH Shin, HY Min, AK El-Naggar, SM Lippman, B Glisson, HY Lee: Akt/mTOR counteract the antitumor activities of cixutumumab, an anti-insulin-like growth factor I receptor monoclonal antibody. Mol Cancer Ther, 10(12), 2437-48 (2011)


182. C Elser, LL Siu, E Winquist, M Agulnik, GR Pond, SF Chin, P Francis, R Cheikin, J Elting, A McNabola, D
EGFR inhibitors in head and neck cancer


187. A Argiris, M Ghebremichael, J Gilbert, B Burtness, A Forastiere: A Phase III randomized, placebo-controlled trial of docetaxel (D) with or without gefitinib (G) in recurrent or metastatic (R/M) squamous cell carcinoma of the head and neck (SCCHN): A trial of the Eastern Cooperative Oncology Group (ECOG). In: J Clin Oncol, 2009 ASCO Annual Meeting Proceedings, 27(Supplement 15), American Society of Clinical Oncology abstract nr 6011 (2009)


189. F Bruzze, A Leone, M Rocco, C Carbone, G Piro, M Caraglia, E Di Gennaro, A Budillon: HDAC inhibitor vorinostat enhances the antitumor effect of gefitinib in squamous cell carcinoma of head and neck by modulating ErbB receptor expression and reverting EMT. J Cell Physiol, 226(9), 2378-90 (2011)


198. TW Wong, FY Lee, S Emanuel, C Fairchild, Fargnoli, B Fink, A Gavai, A Hammell, B Henley, C Hilt, JT Hunt, B Krishnan, D Kukral, A Lewin, H Malone, D
EGFR inhibitors in head and neck cancer


206. JB Vermorken, J Guigay, R Mesia, JM Trigo, U Keilholz, A Kerber, U Bethe, M Picard, TH Brunnendorf: Phase I/II trial of cilengitide with cetuximab, cisplatin and 5-fluorouracil in recurrent and/or metastatic squamous cell cancer of the head and neck: findings of the phase I part. Br J Cancer, 104(11), 1691-6 (2011)


212. JB Vermorken, J Stohlmacher, I Davidenko, E Winquist, L Licitra, VR Pai, K Skladowski, CR Blajman, S Faivre, J Gansert: An analysis of safety in patients (pts) with recurrent and/or metastatic squamous cell carcinoma of the head and neck (R/M SCCHN) receiving chemotherapy (CT) with or without panitumumab (pmab) in a phase III clinical trial (SPECTRUM). In: J Clin Oncol, 2009 ASCO Annual Meeting Proceedings, 29(Supplement 15), American Society of Clinical Oncology abstract nr 6050 (2009)


EGFR inhibitors in head and neck cancer

Sliwkowski, G Fuh,C Eigenbrot: A two-in-one antibody against HER3 and EGFR has superior inhibitory activity compared with monospecific antibodies. Cancer Cell, 20(4), 472-86 (2011)

Abbreviations: ADCC - antibody-dependent cellular cytotoxicity; bsAb - bispecific antibody; mAb - monoclonal antibody; HDAC – histone deacetylase; MTD - maximum tolerated dose; PET - positron emission tomography; PFS - progression-free survival; RTK – receptor tyrosine kinase, TKI - tyrosine kinase inhibitor

Key Words: Epidermal Growth Factor Receptor, Squamous Cell Carcinoma, Head and Neck, Resistance, Response, Receptor Tyrosine Kinase, Inhibitor, Antibody, Review

Send correspondence to: Suzanne A. Eccles, Tumour Biology and Metastasis Team, CR-UK Cancer Therapeutics Unit, McElwain Laboratories, The Institute of Cancer Research, Cotswold Road, Belmont, Sutton, Surrey, SM2 5NG, UK, Tel: 44-0-20-8722-4210, Fax: 44-0-20-8722-4134, E-mail: sue.eccles@icr.ac.uk