EXPERT REVIEWS

Molecular biology of squamous cell carcinoma of the head and neck: relevance and therapeutic implications

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More than 90% of all head and neck cancers are squamous cell carcinoma. Despite advances in the management of patients with this disease, the survival rate has not been significantly improved. Several mechanisms of carcinogenesis have been elucidated and molecular targeted agents seem to be promising therapeutic tools. Cetuximab, a monoclonal antibody inhibitor of the EGF receptor, improves survival rates in association with radiotherapy in advanced squamous cell carcinoma of the head and neck (SCCHN) or in palliative disease, and is nowadays the only targeted agent approved in this indication. Other targeted agents are also clinically relevant to the treatment of different malignancies, including SCCHN. This article focuses on the major molecular pathways implicated in SCCHN carcinogenesis and provides an overview of their therapeutic implications.

Keywords: angiogenesis • clinical implications • epidermal growth factor • hepatocyte growth factor • insulin-like growth factor • molecular pathways • risk factors • squamous cell carcinoma of the head and neck • targeted therapy

Worldwide, more than 500,000 people will develop head and neck cancer each year. The pathogenic role of alcohol and tobacco is well known. Despite a decrease in smoking, the incidence of squamous cell cancer of the head and neck (SCCHN) over the last decade has remained stable, with the exception of cancer of the oropharynx, where the increase in frequency may be attributed to the pathogenic role of the human papillomavirus (HPV). The treatment of SCCHN depends on the site and stage of the tumor, and can include surgery, radiotherapy and chemotherapy. Currently, the choice of treatment is based on the anatomy and morphology of the tumor rather than on its biology. Molecular predictors of response or resistance to treatment are severely lacking. The availability of such predictors would enable more appropriate treatment choices, hence the importance of improving our knowledge of the molecular pathogenesis of these tumors. This article will describe the molecular pathways implicated in SCCHN tumorigenesis as well as their relevance. The clinical implications for the use of targeted therapies in SCCHN will also be discussed.

Cancer is caused by a progressive accumulation of genetic modifications, leading to the inactivation of tumor-suppressor genes or the activation of proto-oncogenes, the latter of which express proteins that are altered in quantity or quality.

Risk factors

Tobacco is the most important causative factor for SCCHN. Tobacco smoke, and in particular some of its components such as benzo(a)pyrene, can induce structural DNA damage. Host factors, including genetic variations of systems implicated in DNA damage correction or enzymes dedicated to metabolize these toxins, can also play also a role in individual sensitivity to tobacco carcinogens. The induced damage may be repaired through the nucleotide excision repair (NER) or the base excision repair (BER) systems, the latter enabling the removal of a single base pair when a cytotoxic mutation is detected [1]. Sequence variations in *NER*/*BER* genes could explain interindividual variations to tobacco toxins [2–4]. Numerous reports of gene polymorphisms of both repair systems are detailed in the literature. Some studies suggest

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that polymorphisms on the cytochrome P450 group, which render the enzyme more active, could explain why some individuals have a higher risk of developing SCCHN when exposed to tobacco [4–6]. The same problem can occur when polymorphisms of detoxifying enzymes, such as glutathione *S*-transferase (GSP) or uridine diphosphate–glucuronosyl transferase (UDPGT), decrease their protective activity.

Betel quid chewing is a popular habit in India and Southeast Asia where the incidence of oral SCC is the highest in the world. Areca nut extract (ANE), the major component of betel quid, has been documented to induce negative oxygen species, and consequently to cause genetic damage. ANE usage is tightly linked to oral cancer; however, the details of carcinogenesis remain unclear.

Alcohol also increases the risk of upper aerodigestive tract cancer. The effect of alcohol is unknown, but current thinking suggests that it may be due to increased mucosal permability, liver damage and/or immune suppression. Chronic alcohol exposure results in the increased activation of carcinogens by cytochrome P450. On the other hand, the accumulation of acetaldehyde and other alcohol metabolites could affect gene transcription or have mutagenic effects [7].

Other risk factors have been reported: Plummer–Vinson syndrome, chronic infection with syphilis, long-term immunosuppression, poor oral hygiene and ill-fitting dentures. Rosenquist reported that poor oral hygiene, inadequate dental status and malfunctioning complete dentures were independent risk factors in oral and oropharyngeal squamous cell carcinoma [8].

HPV-related tumors

High-risk HPV is an important etiological factor for cancer of the oropharynx and is responsible for its increasing incidence. A meta-analysis showed that 26% of SCCHN contain HPV DNA and that more than 50% of all oropharyngeal cancers are related to HPV infection, mainly HPV16 (95%) [9,10]. HPV-positive and -negative tumors are different entities based on differences in their clinical and molecular behaviors [10]. Patients with HPV-positive oropharyngeal cancer are younger, have a better performance status, are less addicted to alcohol and tobacco, and have had more sexual partners than those with HPV-negative cancer [11]. The clinical presentation is also slightly different and frequently involves a small primary tumor with large nodal involvement.

In HPV-positive tumors, malignant transformation begins with inactivation of the *p53* tumor-suppressor gene by HPV protein E6, explaining why there is a lower incidence of *p53* mutations found in HPV-positive tumors compared with HPV-negative tumors. E7, the second HPV protein, inactivates the retinoblastoma tumor-suppressor protein (pRb). $P16^{ink4a}$ (*CDKN2A*) is a cyclin-dependent kinase inhibitor that blocks pRb phosphorylation and cell cycle progression at the G1 to S check points. Loss of p16 expression is common in HPV-negative SCCHN. By contrast, in HPV-positive tumors p16 is overexpressed due to the loss of negative feedback induced by inactivation of *Rb* by HPV E7 [12,13]. p16 overexpression is now generally considered to be a surrogate marker for HPV-induced SCCHN and is easily detected by immunochemistry, such as in cervical cancer.

The diagnosis of a HPV-positive tumor has important clinical implications. Weinberger and colleagues showed that both overall survival (OS) and disease-free survival (DFS) were better in patients with HPV-positive and high p16 levels than in those with or without HPV and low levels of p16 [14]. Tumors that were p53-positive or HPV-negative had a worse prognosis [15]. Compared with the molecular marker group with the best prognosis ($p16^+/p53^-$/HPV high-risk patients), the $p16^-/p53^+$/HPV-negative group had the lowest OS (84 vs 60%; hazard ratio [HR]: 4.1) and disease-specific survival (86 vs 66%; HR = 4.0). Gillison and colleagues also reported data on HPV status and survival outcomes in oropharyngeal cancer in the Radiation Therapy Oncology Group (RTOG)-0129 study [16]. This study compared standard fractionated radiotherapy plus cisplatin (100 mg/m² on days 1, 22 and 43) with accelerated fractionated radiotherapy plus cisplatin (100 mg/m² on days 1 and 22). Tumor HPV status was determined by HPV16 *in situ* hybridization for 323 out of the 433 patients in this trial. A total of 206 patients (64%) were HPV-positive. p16 immunochemistry test results and HPV status were highly correlated (96% of HPV-positive patients overexpressed p16), confirming that p16 is an adequate surrogate marker for HPV infection. In this same study, the 2-year OS was 87.9 and 65.8% for patients with HPV-positive and -negative tumors, respectively.

Maxwell and colleagues specifically examined the interaction between tobacco and HPV status in terms of disease recurrence in patients treated with chemoradiation. Patients were categorized as never, former or current tobacco users. Never-tobacco users with HPV-positive SCCHN carcinoma were shown to have a lower risk of disease recurrence compared with HPV-positive current tobacco users ($p = 0.038$) [17].

Recently, a new vaccine has been introduced for the prevention of cervical cancer in young girls. The scientific community waits to see whether this vaccine will have any effects in the incidence of oropharyngeal cancer in both females and males.

In conclusion, HPV-positive oropharyngeal cancer seems to be a separate disease with a better prognosis and higher sensitivity to radiation therapy and chemotherapy. However, a recent meta-analysis demonstrated that observed improved survival for HPV-positive SCCHN was specific to the oropharynx, whereas there was no difference in survival between HPV-positive and non-oropharyngeal patients [18]. Fakhry *et al.* prospectively evaluated the association of tumor HPV status with therapeutic response and survival among a series of patients treated by induction chemotherapy and followed by concomitant chemoradiation. Consistently with restrospective data on patients with oropharyngeal SCCHN, tumor HPV status was strongly associated with therapeutic response and survival [19]. Larger samples are needed to more thoroughly evaluate the possibility of confounding by smoking and other variables.

In future clinical trials, patients should be stratified based on their HPV status and tobacco abuse, given that patients with HPV-positive oropharyngeal cancer who are also current smokers have a higher risk of disease recurrence. It is still too early, however, to change current practice and to treat patients with HPV-positive tumors with less intensive treatment(s) outside the clinical trial setting.

Genetic cancer syndromes

Some genetic cancer syndromes will predispose patients to developing SCCHN. Fanconi's anemia is an autosomal recessive DNA repair disorder known for its risk of developing lymphoreticular malignancies owing to germline mutations in the caretaker genes *FAA*, *FAD* and *FCC*. It also carries the risk of developing a second primary of the tongue, piriform sinus or postcricoid area [20]. Patients with Bloom syndrome are at risk of developing SCC of the tongue and larynx owing to mutations in the helicase genes [21]. Xeroderma pigmentosum (XP), a disorder of the XP genes in the NER system, can cause second primaries of the oral cavity and potential skin malignancies.

Tumorigenesis/carcinogenesis

Squamous cell cancer of the head and neck is induced by a number of successive genetic alterations that progressively transform the normal squamous epithelium to hyperplasia, dysplasia, carcinoma *in situ* and, finally, invasive cancer. Loss of heterozygosity at 9p21 and 3p are early events occurring in hyperplasia and mild dysplasia, and can be found in more than 60% of patients with SCCHN [22].

FHIT and *CDKN2A* are tumor-suppressor genes located at the 3p14.3 and 9p21 loci, respectively. 3p14.3 deletion leads to deregulation of cell signal pathways including NF-κB, Akt-Survivin and SRC [22,23]. 9p21 deletion can deregulate p16 and p14arf, both cell cycle regulators, by affecting the function of the p53 and pRb pathways through inactivation of *CDKN2A*.

Premalignant head and neck lesions harbor *p53* mutations in more than 50% of the tumors, suggesting that these mutations are also an early carcinogenic event in SCCHN. *p53* is a tumor-suppressor gene mapped on chromosome 17p13 and induces apoptosis or cell cycle arrest in case of cellular stress. Later important events in tumor progression include amplification at 11q13 and 3q. The 11q13 region includes the cyclin D1 (*CCND1*), cortacine (*CTTN*) and Fas-associated protein with death domain (*FADD*) genes, resulting in cell cycle deregulation and migration.

One of the genes located at 3q23 is *ATR*. This gene induces differentiation, aneuploidy and eliminates radiation-induced G1 arrest. Another important region for cancer growth is the 3q26.3-qter region, which amplifies, among others, *PIK3CA*, a key molecular mediator implicated in many of the downstream signals from tyrosine kinase membrane receptors, including the EGF receptor (EGFR) [24].

Klussmann and colleagues described the genetic differences between HPV-related and -unrelated tumors of the oropharynx [25]. They found that chromosomal alterations and amplifications were significantly lower in HPV-positive compared with HPV-negative oropharyngeal cancer. 3q26.3-qter gain was frequent in these tumors (>60%) irrespective of the HPV status. This last alteration was correlated with advanced-stage HPV-negative tumors. HPV-negative tumors have more chromosomal alterations, more loss of 3p, 5q, 9p, 15q and 18q, more amplifications at 11q13, and fewer 16q losses and Xp gains compared with HPV-positive tumors. 16q loss, predominantly identified in HPV-related tumors, was a strong indicator of favorable outcome. The high occurrence of 16q loss in HPV-positive tumors suggests that the *FRA16D* site located at 16q23.2 is a HPV integration site that might lead to 16q DNA loss.

Specific pathways & clinical implications

EGF receptor

The EGFR is a transmembrane glycoprotein commonly expressed in many normal tissues. It is a member of the HER tyrosine kinase receptor family composed of four different receptors (EGFR/c-erbB-1, c-erbB-2/HER-2/neu, c-erbB-3/HER-3 and c-erbB4/HER-4), all of which are transmembrane proteins with tyrosine kinase activity. The EGFR has an extracellular domain, which provides a ligand-binding site for multiple ligands. EGF, TGF-α and amphiregulin (AR) are specific ligands of the EGFR, while β-cellulin (BTC), heparin-binding EGF (HB-EGF) and epiregulin (EPR) are less specific ligands that bind EGFR and ErbB4. Upon ligand fixation, EGFR homodimerization or heterodimerization with another HER receptor occurs, leading to the activation of the intracellular tyrosine kinase. This stimulates kinase signal transduction pathways involved in tumor proliferation, apoptosis, angiogenesis and cell migration/invasion [26]. Downstream signaling through the Ras/Raf/Mek/Erk pathway controls gene transcription, cell proliferation and cell cycle progression, while the PI3K/protein kinase B (PI3K/Akt) pathway stimulates numerous antiapoptotic signals in the cell (Figure 1). SRC tyrosine kinase, phospholipase-Cγ, protein kinase C (PKC) and signal transducer and activator of transcription (STAT) activation have also been documented [27]. EGFR is overexpressed in up to 90% of all SCCHNs [26], and high expression levels of EGFR and its ligand TGF-α are associated with decreased DFS and OS [28]. EGFR can also be located in the nucleus and is correlated with poor clinical outcomes in patients with squamous cell carcinoma of the oropharynx [29]. Nuclear localization of the EGFR is correlated with increased expression of cyclin D1, inducible nitric oxide synthase, Aurora A kinase and B-Myb, leading to increased cell cycle progression and proliferation [30–32].

Several studies have described that high EGFR gene copy number is related to poor prognosis in SCCHN. Increased EGFR gene copy number has been reported in 10–58% of patients with SCCHN. This wide variation in results may be explained by the use of different detection methods (FISH or quantitative PCR), the heterogeneity of the SCCHN tissue origin and the different evaluation systems [33–36]. Nowadays, further investigation is warranted in SCCHN to determine if there really exists an association between disease prognosis, gene copy number and treatment efficacy with anti-EGFR therapy.

Blockage of the EGFR pathways can be achieved with monoclonal antibodies (MoAbs; i.e., cetuximab, panitumumab and zalutumumab) or with low-molecular-weight tyrosine kinase inhibitors (TKIs; i.e., gefitinib and erlotinib). The most investigated agent is cetuximab. Cetuximab is a chimeric IgG1 monoclonal antibody that specifically binds to the EGFR with high affinity, blocking ligand-induced EGFR phosphorylation. EGFR overexpression may be implicated in radioresistance. In irradiated cells, the EGFR is upregulated and can promote DNA repair

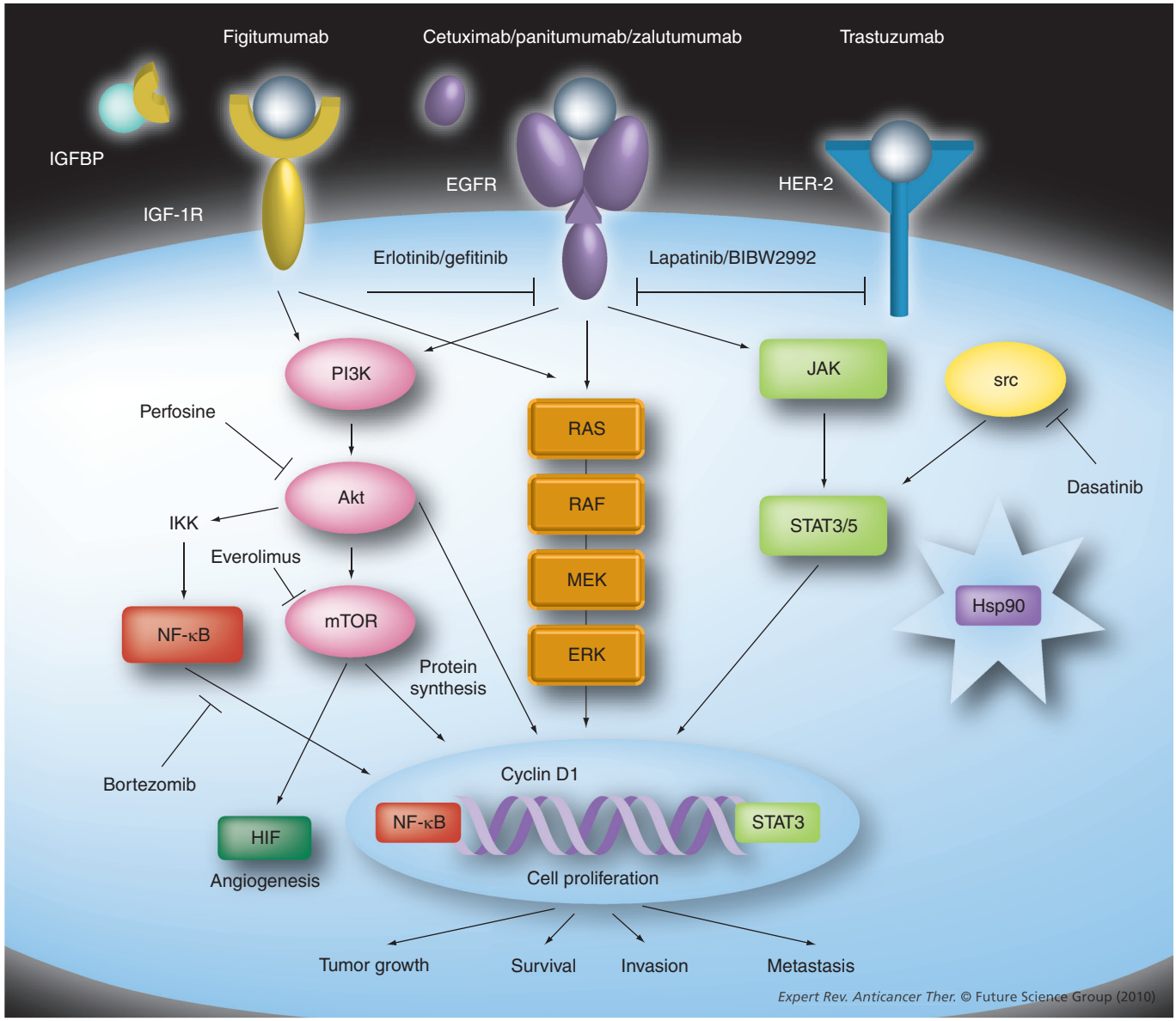

Figure 1. Molecular pathways of EGF receptor, IGF receptor and HER-2 and the principal targeted therapies investigated in clinical trials.
EGFR: EGF receptor; IGF-1R: IGF-1 receptor; IGFBP: IGF-binding protein.

as well as the arrest of cells in S phase, a radioresistant phase [37–40]. Cetuximab may block the nuclear import of the EGFR, preventing activation of DNA repair mechanisms that protect cells from radiation- or chemotherapy-induced damage. This explains why some EGFR inhibitors demonstrate synergism with chemotherapy and radiotherapy in preclinical models [41]. Based on this strong background, cetuximab has been combined with radiation therapy and chemotherapy in clinical trials. A Phase I study showed that cetuximab could be safely administrated in combination with radiotherapy in patients with SCCHN [42]. The recommended dose in this study was a loading dose of 400 mg/m² and a weekly maintenance dose of 250 mg/m². Bonner and colleagues demonstrated in a Phase III study that cetuximab combined with radiotherapy improves locoregional control (median: 24.4 vs 14.9 months) and OS (median: 49 vs 29.3 months) compared with radiotherapy alone [43]. Patients with oropharyngeal tumors, early AJCC T stage (T1–3), treatment in the USA, concomitant boost, advanced AJCC N stage (N1–N3), high Karnofsky performance status (90–100%), male sex and age less than 65 years were factors associated with a potential increased benefit from cetuximab combined with radiotherapy versus radiotherapy alone. However, the trial was not powered for this subgroup analysis, and therefore these data should be interpreted with caution [44]. Based on the hypothesis that cetuximab and chemotherapy could act additively to reduce radioresistance, trials combining radiotherapy, chemotherapy and cetuximab have been initiated. Pfister and colleagues described an encouraging long-term survival (76% at 3 years) with this

triple combination, although toxicities were observed [45]. We eagerly await the results of the Radiotherapy Therapy Oncology Group (RTOG) Phase III study 0522 (NCT00265941 [201]), which compares chemoradiation with or without cetuximab.

Cetuximab has been also investigated in patients with incurable recurrent and/or metastatic disease. Vermorken and colleagues (EXTREME trial) showed that the addition of cetuximab to 5-fluorouracil (5-FU) and platinum-based therapy prolongs OS and progression-free survival (PFS) in this setting [46]. The median OS was prolonged by 36.5% (10.1 vs 7.4 months) and the median PFS by 70% (5.6 vs 3.3 months). These clinical benefits were achieved without any deterioration in quality of life [47].

Other trials investigated cetuximab as monotherapy or in combination with chemotherapy in cisplatin-refractory patients [48,49]. In these studies, cetuximab produced an overall response rate (ORR) of approximately 10%, but it is not clear whether this was due to the reversal of platinum resistance or to the action of cetuximab alone, because the ORR remained similar regardless of whether cetuximab was used alone or in combination with cisplatin. Importantly, the exact sequence and optimal timing of chemotherapy plus cetuximab has not been widely investigated. Recently, it has been shown that cisplatin-induced EGFR phosphorylation is a determinant of its cytotoxicity in head and neck cancer cells lines. Pretreatment with erlotinib blocked EGFR phosphorylation and degradation, therefore reducing cisplatin-induced cytotoxicity in the same cell lines. To avoid antagonistic effects and to optimize the antitumoral activity of both the chemotherapy and the anti-EGFR agent, this data suggests that EGFR inhibitors should not be used prior to chemotherapy administration [50]. These findings may have important implications for the design of future clinical trials.

Two other anti-EGFR MoAbs under development are panitumumab and zalutumumab. These MoAbs are fully humanized. Clinical trials are ongoing to test these molecules in palliative and curative settings. Machiels and colleagues tested zalutumumab in patients with incurable SCCHN resistant to platinum therapy. Patients were randomized between zalutumumab monotherapy and best supportive care (BSC). Methotrexate therapy was allowed in the BSC arm. The primary end point of the trial was OS. Although a median OS of 6.7 months was observed in the zalutumumab group compared with 5.2 months in the BSC group, the difference was not statistically significant ($p = 0.065$), despite a significant improvement in PFS ($p = 0.001$) [51].

Tyrosine kinase inhibitors, which bind intracellularly to EGFR tyrosine kinase and inhibit phosphorylation and downstream signaling pathways, have been also tested. The two main compounds are erlotinib and gefitinib. Phase II trials have demonstrated an ORR of between 1 and 15% in palliative patients [52]. Gefitinib has also been investigated in two Phase III trials in palliative patients but did not show any relevant clinical activity nor any survival benefit. The first trial compared gefitinib with methotrexate and the second investigated docetaxel plus gefitinib versus docetaxel plus placebo [53,54]. In a randomized Phase II trial, gefitinib has also been combined with cisplatin and radiation therapy in stage III–IV untreated, unresected and non-metastatic patients with SCCHN. In this study, presented orally by Grégoire at the Second International Conference on Innovative Approaches in Head & Neck Oncology (ICHNO) meeting in Barcelona during February 2009, gefinitib did not significantly improve the local control rate compared with placebo when given concomitantly with chemoradiotherapy or when given as maintenance therapy alone [55].

Nevertheless, both agents continue to be studied in early-stage as well as in advanced-stage SCCHN. Thomas and colleagues administrated erlotinib as neoadjuvant treatment in patients with resectable SCCHN [56]. They concluded that erlotinib was well tolerated and that tumor shrinkage was observed in 29% of patients. They also performed translational research and found that baseline p21waf expression was associated with a response to erlotinib [56]. The utility of this biomarker to select patients should be investigated further.

A prevention trial aimed at determining the ability of erlotinib monotherapy to reduce the incidence of oral cancer in high-risk patients is ongoing (NCT00402779). Translational research will also be performed in this study to determine the role of the EGFR and other biologic factors in the early pathogenesis of SCCHN.

Despite some encouraging results, only a minority of patients will benefit from cetuximab or other monoclonal antibodies. Therefore, understanding the primary and acquired evasive mechanisms of anti-EGFR therapy is a key strategy in the development of effective SCCHN treatments. In contrast to colon cancer, where *K-ras* mutations predict treatment resistance, little is known about predictive parameters of treatment resistance and/or efficacy in SCCHN [57]. EGFR-activating mutations, which have been linked to the efficacy of TKIs in lung cancer, do not appear to occur in SCCHN [58].

Epidermal growth factor receptor variant III, which lacks the ligand-binding domain, occurs in up to 40% of SCCHN and confers resistance to EGFR-targeted monoclonal antibodies in preclinical models but it has not been studied in prospective clinical trials. This receptor is only found in cancer cells and occurs following an in-frame deletion of exon 2–7, leading to independent ligand activation of the extracellular domain of the receptor [59]. Another potential reason for EGFR inhibitor resistance may be the constitutive activation of downstream signaling EGFR molecular pathways (Ras/Raf/MAPK and P13K/Akt/mTOR) induced, for example, by *Ras*-activating mutations, or *PTEN* alteration or PI3K-activating mutations. Another mechanism could be activation of the transduction signaling cascades of the EGFR by other tyrosine kinase receptors, such as c-Met or IGF-1 receptors, therefore bypassing EGFR inhibition [60]. Translational research, molecular investigation and imaging techniques to detect early response are therefore urgently needed in SCCHN in order to improve our understanding of the mechanisms of response or nonresponse to anti-EGFR therapy.

Other members of the HER receptor family

C-erbB-2/HER-2-neu, c-erbB-3/HER-3 and c-erbB4/HER-4 are other members of the HER tyrosine kinase receptor family. The *HER-2/neu* gene encodes a transmembrane protein of 185 kDa. HER-2 has no ligand, but the intracellular part of this receptor

has tyrosine kinase activity. It can dimerize spontaneously or form heterodimers with other members of the EGFR family to activate some of the downstream signal-transduction pathways implicated in carcinogenesis. HER-3 does not have intrinsic tyrosine kinase activity but can be transphosphorylated by EGFR and HER-2/neu. HER-4, the fourth member of the family, encodes a 180-kDa transmembrane tyrosine kinase that can also form heterodimers with the other HER receptors.

The overexpression rates of HER-2, HER-3 and HER-4 in SCCHN have been reported by different groups. However, conflicting results exist and their value as a prognostic tool is still unclear. For example, HER-2 is overexpressed in 5–33% of patients with SCCHN, but contrary to gastric and breast carcinomas, where HER-2 overexpression or amplification is linked to a poorer prognosis and a decrease in OS, results in SCCHN are conflicting. This is probably due to the different methods used to detect the protein. Even if some studies have suggested that HER-2 overexpression may correlate with poorer DFS, larger studies using standardized methodology are needed to fully assess its prognostic significance in SCCHN [61,62]. Some studies have also shown that HER-3 could have a significant impact on survival, but again further studies are required to validate this theory [63]. The role of HER-4 has not been widely studied.

Although not formally demonstrated in SCCHN, primary or acquired resistance to anti-EGFR monoclonal antibodies may be due to the absence of the extracellular part of the receptor (EGFR variant III), as discussed earlier, or due to transactivation of the EGFR intracellular tyrosine kinase by other HER receptors such as HER-2 or HER-3 [64]. EGFR heterodimerization induces a stronger tumor-proliferation signal than EGFR homodimerization. Based on this background, new HER TKIs have been developed to bypass the potential mechanisms of resistance to EGFR monoclonal antibodies and the limited activity of gefitinib and erlotinib in SCCHN. Lapatinib is an oral small molecule that acts as a reversible inhibitor of both the EGFR and HER-2 tyrosine kinases, but it did not demonstrate any objective response when administered as monotherapy in head and neck cancer [65]. Lapatinib has also been tested in combination with chemoradiation and such trials are ongoing. BIBW2992 (an irreversible EGFR and HER-2 inhibitor) and pan-HER tyrosine kinase inhibitors such as PF00299804, which may inhibit the different HER receptors, have demonstrated interesting preclinical activity and are currently under investigation in SCCHN [66].

MET receptor

c-Met, the tyrosine kinase receptor for HGF, is overexpressed in a variety of tumors, including SCCHN, and is generally correlated with poorer prognosis. The overexpression concerns not only c-Met but also HGF paracrine secretion. Knowles and colleagues reported that approximately 80% of primary SCCHN tumors express HGF, c-Met or both [67]. c-Met activation stimulates downstream molecular pathways implicated in tumor growth, metastasis and angiogenesis. These pathways include MAPK, PI3K/A and STAT 3 pathways; matrix metalloproteinase (MMP)-7 and IL-8 expression are also involved [68–74].

Importantly, the HGF/c-Met pathway has been implicated in chemotherapy and targeted therapy resistance in SCCHN [75,76]. Akervall and colleagues identified the low expression of c-Met as a predictive factor for complete response in patients that received induction chemotherapy for a primary nondiploid SCCHN. Increased c-Met activation was also recently reported to be correlated with resistance to cetuximab and other EGFR inhibitors in SCCHN cell lines. The EGFR shares common molecular pathways with c-Met/HGF [77], and it is therefore possible that the HGF/c-Met pathway may cross-activate the EGFR signaling pathways downstream, bypassing EGFR inhibition by MoAbs or TKIs. Recently, it has been reported that overexpression of cortactin, a key regulator of dynamic actin networks, stabilized c-Met in SCCHN cell lines, enhanced HGF-induced mitogenesis and cell scattering, and led to gefitinib resistance [78]. In addition, amplification of c-Met may cause gefitinib resistance by driving ERBB3 (HER3)-dependent activation of PI3K in lung cancer [79]. Altogether, these data suggest that c-Met pathway activation can play a role in SCCHN tumor growth when therapeutic intervention inhibits the EGFR. These data also support the investigation of combining EGFR and c-Met inhibitors in the clinic.

Based on this background, c-Met inhibitors are very attractive targets for cancer treatment. Different approaches are currently being developed to inhibit the HGF/c-Met pathway and include small-molecule tyrosine kinase inhibitors and monoclonal antibodies that target either HGF or c-Met. Recently, Knowles *et al.* showed that PF-2341066, a c-Met TKI, can delay SCCHN tumor growth in a preclinical animal model [67]. Inhibition of c-Met in SCCHN patients therefore seems to be a very relevant treatment target and clinical trials are planned in this indication.

IGF-1 receptor

The IGF-1 receptor (IGF-1R) is a transmembrane heterotetramer receptor that consists of two α and two β subunits. Its ligands are both IGF-1 and IGF-2. After ligand binding to IGF-1R, two major downstream signaling cascades are activated: the PI3K/Akt/mTOR pathway and the Ras/Raf/MAPK pathway (Figure 1). The activation of both the Ras/Raf/MAPK and PI3K/Akt/mTOR pathways is similar to the downstream signaling described previously for EGFR activation. The IGF-2R binds essentially IGF-2, acts as a signal decoy and does not transduce the activation signal. IGF activity is regulated by IGF-binding proteins (IGFBPs) 1–6. The balance between these proteins and IGFs determines the level of activation of this molecular pathway in each cell. IGF-1R is 84% identical to the insulin receptor. The insulin receptor A (IR-A) isoform, expressed in fetal tissues and in malignant cells is, in particular, able to bind IGF-2 and to mediate survival and mitogenic activity [80].

The IGF-1R plays an important role in cellular growth and protects against cancer cell apoptosis, but also leads to cell proliferation, cell differentiation and tumor invasion [81]. Epidemiological studies demonstrated that increased circulating levels of IGF-1 are associated with an increased risk of developing malignancies [82]. Jun and colleagues explored the immunohistochemical expression of IGF-1R and IGFBP-3 in human tumor samples and concluded

that IGF-1R expression is frequent in SCCHN and is correlated with poor survival in advanced-stage patients [83]. These studies suggest that the IGF-1R is an interesting therapeutic target for cancer treatment.

Inhibition of SCCHN cell lines with a human monoclonal antibody of IGF-1R, IMC-A12, blocked cell proliferation, caused G1 cell arrest, blocked cell migration and inhibited anchorage-independent growth of SCCHN cells [84]. The same group also showed that the stimulation of SCCHN cells with either IGF or EGF resulted in IGF-1R and EGFR heterodimerization, but that only the IGF caused activating phosphorylation of both receptors [84]. Combined blockade of both the IGF-1R and the EGFR was more effective than blocking each one individually in a SCCHN xenograft mouse tumor model.

Anti-IGF-1R-targeting molecules are either anti-IGF-1R antibodies or IGF1-R TKIs. TKIs are less specific owing to the similarity of the tyrosine kinase domain between the IR and the IGF-1R. Schmitz and colleagues tested figitumumab, a fully human monoclonal antibody IgG2 subtype that specifically binds to the IGF-1R, in palliative SCCHN patients [85]. They found that figitumumab monotherapy has no clinically relevant activity. The most frequently observed toxicity was grade 3–4 hyperglycemia (41%). The mechanisms that cause hyperglycemia are not well understood but two hypotheses currently exist. One possible explanation involves the downregulation of the IGF-1R/IR heterodimers. Another hypothesis is that there is deregulation of a homeostatic mechanism involving IGF-1R regulation of the growth hormone and IGF-1 production, along with increased liver glucogenesis [86].

The MoAb IMC-A12 is currently undergoing clinical testing in SCCHN. In a Phase II trial, IMC-A12 is being evaluated as single agent and also in combination with cetuximab in patients with recurrent or metastatic SCCHN (NTC00617734).

Multiple study groups searched for predictors of response to IGF-1R treatment. In sarcoma and neuroblastoma cell lines, Huang and colleagues described factors of resistance to anti-IGF-1R treatment [87]. They concluded that crosstalk between multiple kinases (EGFR, Met and TGFBR2), expression of anti-apopototic genes (*Bcl-2*, *Bcl-XL* and *Api-2*) and overexpression of IGFBP-3/6 are implicated in resistance mechanisms, and that high levels of IGF-1, IGF-2 and IGF-1R are signatures for sensitive cell lines [87]. Mechanisms of resistance to erlotinib in non-small cell lung cancer (NSCLC) could also be explained by the formation of IGF-1R and EGFR heterodimers. These heterodimers activate the IGF-1R and its downstream mediators, and lead to the stimulation of EGFR synthesis via mTOR stimulation and to the formation of antiapoptotic surviving proteins [88]. The inactivation of the IGF-1R increases sensitivity to erlotinib.

Inhibition of angiogenesis

Angiogenesis is required for tumor growth and metastatic spread. Under hypoxic conditions, cancer cells produce and release multiple growth factors able to stimulate angiogenesis: VEGF, PDGF, FGF-β, TGF-β, PIGF and angiopoietin-2 [89–91]. VEGF is a key regulator of new blood vessel formation and its secretion is upregulated in many human cancers. VEGF binds to tyrosine kinase receptors VEGFR-1, -2 and -3. PDGFR-β is also an important receptor implicated in the initiation of pericyte proliferation (Figure 2). Pericytes stabilize blood vessels and provide survival signals to the endothelium [92]. Adding PDGF inhibition to VEGF blockade enhances endothelial cell apoptosis and blood vessel destruction [93,94]. Overexpression of the VEGF ligand has been observed in various tumor types and has been correlated with tumor development and/or poor prognosis. The majority of SCCHN overexpress the VEGF or the -2 and -3 receptors, making angiogenesis inhibition an attractive treatment target [95,96]. A meta-analysis involving 1002 patients showed that VEGF tumor overexpression evaluated by immunohistochemistry was associated with decreased survival [97].

Angiogenesis blockade is achievable through various mechanisms, and studies investigating angiogenesis inhibitors as single agents or in combination regimens are ongoing in SCCHN. The most clinically advanced molecules are bevacizumab, an anti-VEGF MoAb, and sorafenib or sunitinib. The latter two orally bioavailable molecules are multiple tyrosine kinase inhibitors that inhibit multiple tyrosine kinase receptors, including the VEGFR-2 and -3 receptors, and the PDGFR-β. Sorafenib has been investigated in two trials with modest clinical results. In the first study, only one patient (3%) had a confirmed partial response (PR) but the median PFS (4 months) and median OS (8 months) were encouraging [98]. The second trial included patients with SCCHN and nasopharyngeal cancers with stable disease observed in ten out of 26 patients (37%) [99].

Machiels and colleagues investigated sunitinib in a Phase II trial in patients with recurrent or metastatic SCCHN progressing after platinum-based therapy [100]. Limited activity was found in 19 patients (50%) who achieved stable disease (SD) at 6–8 weeks. A total of 12 of the 19 patients also had some degree of tumor shrinkage. The median PFS and OS were low at 2 and 3.4 months, respectively. Important complications were also observed, with a high incidence (16%) of grade 3–5 bleeds. Local complications, defined as the apparition or worsening of tumor skin ulceration and/or tumor fistula, were also recorded in 41% of the patients. Bevacizumab monotherapy has not been evaluated.

Angiogenesis inhibitors have also been tested in combination with inhibitors of the EGFR pathway based on preclinical studies, suggesting that inhibition of the VEGFR and EGFR pathways may be synergistic [101]. In addition, VEGF release has been implicated as a potential mechanism of resistance to anti-EGFR therapy. A Phase I/II study combining erlotinib and bevacizumab in patients with recurrent or metastatic disease showed that this combination was well tolerated, with an ORR of 15% and a median OS and PFS of 7.1 and 4.1 months, respectively [102].

Other potential targets

p53

As previously mentioned, disruption of the p53 pathway is one of the earliest events in SCCHN carcinogenesis. *p53* is a tumor-suppressor gene that maintains the integrity of the cellular genome. Its function may be abrogated by mutations of the *p53*

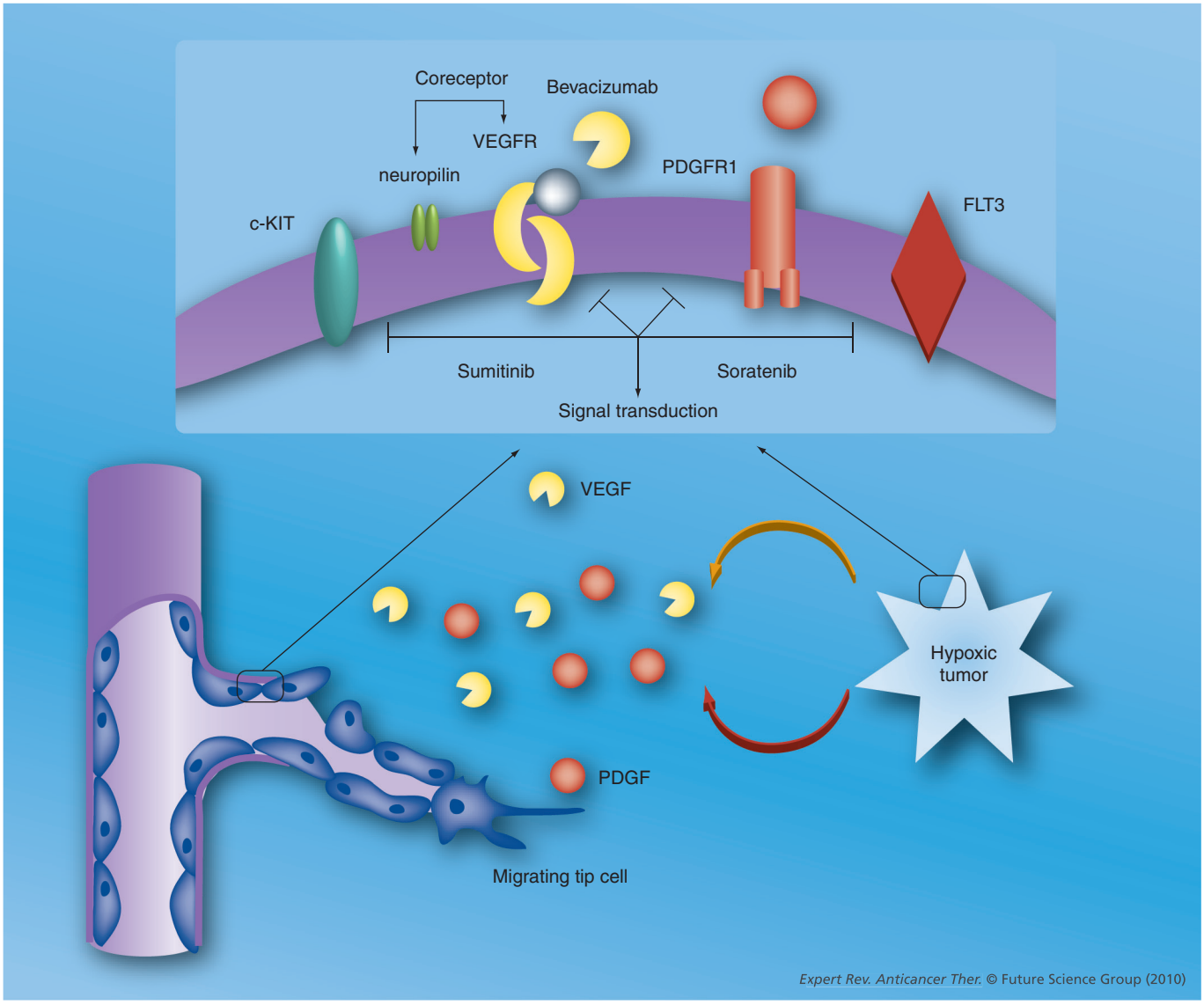

Figure 2. Tumor hypoxia leads to the production of multiple growth factors that stimulate receptors to initiate angiogenesis.
VEGFR: VEGF receptor.

gene, degradation of the p53 protein by the HPV E6 protein, and by the inactivation of p53 modulators [103]. The p53 pathway is disrupted in more than 50% of all human cancers and *p53* mutation occurs in more than 40% of all SCCHN [104].

Therapies using viral vectors to transport the *p53* gene into target cells have also been tested. Advexin (Ad)-p53 (NGN-201) is an adenovirus serotype 5 vector that contains a functional *p53* gene. In a Phase I trial, Ad-p53 was considered safe [105]. In a Phase III trial comparing Ad-p53 with methotrexate, no significant difference between the two groups could be demonstrated in the overall intent-to-treat population. The investigators observed that Ad-p53 was considerably more efficacious for patients with recurrent SCCHN and a favorable p53 profile (including patients with normal *p53* gene sequences or low-level p53 protein expression), than for patients with an unfavorable p53 profile (i.e., patients with high-level expression of mutated *p53* that can inhibit normal *p53* function). Median survival and median time to progression were 7.2 versus 2.7 months and 2.7 versus 1.4 months, respectively [106].

Src family kinases

Src is a member of the family of non-receptor tyrosine kinases (nRTKs). The normal cellular gene, *c-src*, regulates signals from multiple cell surface molecules, including integrins [107], growth factors [108] and G protein-coupled receptors [109]. SRC family kinases (SFKs) become active upon binding to several receptor tyrosine kinases. Cooperation between SFKs and the EGFR has been reported [110]. SFKs are highly activated in cetuximab-resistant cells and enhanced EGFR activation through HER3 and PI3K. Studies using dasatinib (an inhibitor of SFKs), ephrin A,

BCR-ABL, cKIT and PDGFR reversed resistance to cetuximab in these cells [110]. Koppikar and colleagues showed that SCCHN cell lines expressing active *c-src* have increased growth and invasion patterns. Combined treatment with gefitinib and an inhibitor of *c-src* (AZD0530) was more efficient than each agent individually [111].

Multiple studies evaluating the efficacy of dasatinib as monotherapy or in association with other molecules are ongoing on SCCHN patients. The first results of a Phase II trial with dasatinib monotherapy in recurrent and metastatic SCCHN showed significant toxicity with low clinical efficacy. We are still waiting for the pharmacokinetic results along with the tissue and blood biomarkers, as evaluated in this study [112].

Proteasome inhibitor (bortezomib)

The proteasome is implicated in the turnover of intracellular proteins, including those controlling cell signaling, survival and cell cycle regulation. Bortezomib selectively inhibits proteasome activity, which is required for the activation of the NF-κB and the degradation of components of the activator protein (AP)-1, and other oncologic pathways [113]. The activation of NF-κB and AP-1 signal-transduction pathways has been identified in SCCHN tumor progression [114,115].

Li and colleagues reported that the treatment of SCCHN cancer cells with bortezomib led to upregulation of the STAT-3 protein. This could suggest limited activity of bortezomib in this disease. Interestingly, the same group found that the effect of bortezomib could be further enhanced by the addition of a STAT3 inhibitor [116].

The Eastern Cooperative Oncology Group evaluated bortezomib in a randomized Phase II trial evaluating bortezomib monotherapy versus bortezomib plus irinotecan. The ORR of bortezomib monotherapy was 3% with stable disease observed in 23% of patients. The group concluded that the combination of bortezomib and irinotecan was toxic with disappointing activity [117].

mTOR inhibitor

Mammalian target of rapamycin is a serine/threonine protein kinase that regulates cell growth, cell proliferation, cell motility, protein synthesis and transcription [118]. The mTOR protein is activated through the PI3K/Akt pathway. As previously discussed, this pathway is supposedly implicated in SCCHN tumor growth and may be activated by various receptors (e.g., VEGFR, EGFR, HER2 and IGF-1R). Pathway inhibitors may fail due to the upregulation of escape pathways downstream from these receptors. Everolimus is an inhibitor of mTOR and is used to prevent graft rejection due to its immunosuppressive function. In preclinical trials, mTOR inhibitors have shown radiosensitizing capability and have restored sensitivity to chemotherapy, including cisplatin. Based on this theory, everolimus is worthy of investigation in SCCHN and clinical trials testing this agent in the palliative setting as monotherapy (NCT01051791), in association with cetuximab and cisplatin (NCT01009346) or in association with erlotinib (NCT00942734), are ongoing. Trials with curative intent are also ongoing, in which everolimus is being tested in combination with cisplatin and radiotherapy (NCT00858663, NCT01058408) or in combination with cisplatin and docetaxel as induction therapy (NCT00935961). Argiris *et al.* tested perifosine, an oral alkylphospholipid that inhibits Akt phophorylation, in palliative SCCHN. They could not detect a signifactive activity of perifosine in this disease [119].

Heat shock protein inhibitors

Heat-shock protein 90 (Hsp90) is a molecular chaperone for other client proteins. Hsp90 promotes conformational (shape) maturation of these client proteins and protects them from degradation [120].

Many of the clients are protein kinases or transcription factors involved in multiple signal-transduction pathways. They play critical roles in tumor cell growth and survival. Hsp90 inhibition causes degradation of the protein kinases and/or transcription factors, which, in turn, could increase tumor cell death in SCCHN cell lines [121]. Preclinical studies have shown that the ansamycin-based Hsp90 inhibitor, 17-allylamino-17-demethoxygeldanamycin, can enhance tumor cell sensitivity to radiation [122]. Unfortunately, this molecule is difficult to manage due to its poor solubility and cumbersome formulation. Yin and colleagues investigated the fully synthetic and bioavailable Hsp90 inhibitor, BIIB021, in a variety of SCCHN cell lines and tumor models, either as a single agent or in combination with radiation therapy. They concluded that this agent has strong anti-tumor activity in both settings, and that its ability to act as a radiosensitizer may be due to a reduction in radioresponse proteins, increased apoptosis and enhanced G2 arrest [123].

Conclusion & expert commentary

Even if standard treatment modalities such as radiotherapy, chemotherapy and surgery have shown significant improvements over the last few years, survival rates for patients with SCCHN remain stable. While alcohol and tobacco abuse or infection with HPV are considered the main culprits, other genetic damage may explain the interpatient variability seen in this tumor type.

More research and improvements in clinical trial design, to include biological samples for translational research, are urgently needed if we are to enhance our understanding of the molecular pathways implicated in the carcinogenesis of SCCHN. Future clinical trials should also stratify patients according to their HPV status given the different genetic modifications, physiopathology and overall outcome seen in patients with HPV-related pathology compared with those who are HPV negative.

Many relevant pathways are deregulated in SCCHN and represent important potential targets that need further investigating. Today, only cetuximab is US FDA approved for both palliative and curative treatment in patients with SCCHN. Despite encouraging results with this molecule, only a minority of patients will actually benefit from treatment. EGFR inhibition, together with an understanding of its evasion mechanisms, represents a key strategy in the development of effective SCCHN treatments. In contrast to colon cancer, where *K-ras* mutations predict treatment resistance, little is known about predictive parameters of treatment resistance and/or efficacy in SCCHN.

Concerning the effectiveness of these new targeted agents, the following points need to be considered: molecular pathways overlap and, therefore, a specific therapy may be considered to be ineffective. To determine the efficacy of targeted agents it could be better to evaluate molecular biology or functional imaging than conventional clinical outcome measures. Applications from translational research must be carefully considered in further clinical trials to detect specific biomarkers. However, the feasibility of conducting translational research is hampered by ethical considerations in obtaining iterative biopsies in palliative patients.

Because targeted agents are often investigated in unselected end-stage cancer patients, their efficacy is frequently limited: most patients have developed multifactorial resistance and are less likely to respond to new agents effectively. Better understanding of molecular pathways will help to design further clinical trials in a more selected population.

Five-year view

The multimodal standard curative treatment of locally advanced SCCHN includes radiation therapy and/or surgery and/or chemotherapy. Despite this aggressive approach, more than 50% of patients with SCCHN will relapse. Current treatment standards are unlikely to offer further improvement in outcome, as we have reached maximum tolerable toxicity levels in our patients. The therapeutic index may be improved by either replacing the chemotherapy with a targeted agent, so as to have less toxicity but with the same efficacy, or by introducing a new agent with no cross-toxicities, such as a molecular-targeted agent, with the aim of increasing treatment efficacy. In such a context, the identification of new relevant molecular targets is of utmost importance.

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Key issues

- Human papilloma virus (HPV)-related and unrelated tumors seem to be very different entities based on their clinical, genetic and molecular profile. Patients with HPV-positive oropharyngeal squamous cell carcinoma have a better prognosis than those with HPV-negative tumors.
- The EGF receptor (EGFR) is overexpressed in up to 90% of all squamous cell cancer of the head and neck (SCCHN), and high expression levels of the EGFR and its ligand TGFA are associated with decreased disease-free survival rates. In SCCHN, cetuximab plus radiotherapy improves overall survival compared with radiotherapy alone. However, further studies are needed to determine which subgroup of patients are able to benefit from cetuximab.
- Currently, there are no molecular predictors of anti-EGF receptor efficacy, and research is required to determine biomarkers that could optimize patient selection and predict therapeutic activity.
- Translational research, enhanced imaging techniques and molecular investigation, are needed to better understand the mechanisms of response or nonresponse to targeted therapy in SCCHN.
- Angiogenesis inhibitors have demonstrated modest activity in clinical trials but are sometimes associated with important adverse events such as fatal bleedings and disfiguring local complications (e.g., fistulas and ulcerations).
- Other relevant altered pathways in SCCHN are activated by c-Met and the IGF-1R. This may explain why there is some resistance to anti-EGFR treatments by cross-activation.

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