Targeting receptor tyrosine kinases and their signal transduction routes in head and neck cancer

J. J. Cruz, A. Ocaña, E. Del Barco & A. Pandiella*

Servicio de Oncología, Hospital Universitario de Salamanca, Centro de Investigación del Cáncer; Instituto de Biología Molecular y Celular del Cáncer, CSIC-Universidad de Salamanca, Spain

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Squamous cell carcinoma of the head and neck (SCCHN) is the sixth most common cancer in the world. At present several therapeutic approaches, including surgical removal, chemotherapy and radiotherapy, are used. Yet a significant number of patients relapse, often with metastases. In an attempt to improve treatment of SCCHN new targeted therapies are emerging. Among them special interest has been devoted to agents that act on the epidermal growth factor receptor (EGFR) and other receptor tyrosine kinases, or the signal transduction routes used by these receptors to induce tumour cell proliferation. Such treatments include monoclonal antibodies and small molecule inhibitors of either the intracellular tyrosine kinase activity of these receptors or relevant signalling intermediates. Here we review the biological bases of these new targeted treatments, with special emphasis on the clinical results that point to an implementation of these drugs into the therapeutic armamentarium against SCCHN.

Key words: head and neck cancer, receptor tyrosine kinases, targeted therapies

introduction

Head and neck cancer is a generic term that includes different cancer types arising in different areas such as oral cavity, oropharynx, hypopharynx and larynx. The most common type is squamous cell carcinoma of the head and neck (SCCHN) that has been considered the sixth most common cancer in the world, with approximately 600,000 new cases per year [1]. SCCHN is an epithelial cancer that arises in the mucosa of the aerodigestive tract. More than 50% of newly diagnosed patients with SCCHN are not cured and will relapse locally or at a distant site, and 10% of newly diagnosed patients with SCCHN present with distant metastases. Recurrent and/or metastatic SCCHN patients have a poor prognosis, with a median survival no longer than 1 year [2, 3].

At present the established treatment of SCCHN includes different therapeutic approaches. For the locoregional disease surgery and/or radiotherapy are the standard therapeutic treatments [2]. On the other hand, the treatment of locoregionally advanced SCCHN has evolved gradually from surgery as the mainstay of treatment, to radiotherapy as the principal treatment [4–8]. More recently additional benefit has been obtained with altered-fractionation radiotherapy (accelerated fractionation or hyperfractionated radiotherapy), and with radiotherapy combined with chemotherapy (chemoradiotherapy) [8, 9]. The value of chemoradiotherapy is, however, counterbalanced by increased and often prohibitive toxicity, particularly among patients with coexisting medical conditions and decreased performance status [8]. Another controversial point in this situation is the role of induction chemotherapy administered before a locoregional treatment. Related to this a recently reported trial has shown that the administration of induction chemotherapy followed by chemoradiotherapy can be more active than chemoradiotherapy alone in unresectable locally advanced head and neck cancer [10].

For the treatment of recurrent and/or metastatic SCCHN, therapeutic options include re-irradiation and salvage surgery and chemotherapy, with best supportive care for patients unable or unwilling to undergo treatment. Palliative chemotherapy has demonstrated survival advantages over best supportive care, and the most commonly used agents are cisplatin and carboplatin, generally in combination regimens with infusional fluorouracil or a taxane [2, 3].

Taking all these data into consideration, there is clearly a therapeutic need for new active agents for the treatment of patients with SCCHN, particularly patients with recurrent or metastatic disease.

The efforts to understand the molecular bases of tumour genesis/progression in SCCHN led to the identification of alterations in certain signal transduction routes linked to the control of cell duplication [11]. This fact, together with the development of therapies that target these signalling routes, offers a promising scenario for the future treatment of SCCHN. An example of this is the targeting of a membrane receptor, the epidermal growth factor receptor (EGFR), which is frequently present in SCCHN and is involved in the regulation of cell
proliferation. Treatments aimed at reducing the function of this receptor have resulted in clinical benefit in SCCHN [12–14]. In this review we will focus on the novel therapeutic opportunities offered by the increase in the knowledge of the molecular pathologic bases of SCCHN, with special emphasis on treatments that target specific proteins involved in sustaining tumoral cell proliferation/survival.

receptor tyrosine kinases in SCCHN

Studies on receptor tyrosine kinases (RTKs) as therapeutic targets in SCCHN have focused mainly on the EGFR, although other candidates under preclinical and clinical evaluation are the receptor for the type I insulin-like growth factor (IGF-1R) and the receptor for the vascular endothelial growth factor (VEGF-R).

the epidermal growth factor receptor

The epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors [15]. This receptor family includes four related receptors: the epidermal growth factor receptor (EGFR/ErbB1), ErbB2 (HER2/neu), ErbB3 and ErbB4 [15–17]. ErbB receptors are composed of an extracellular ligand-binding domain, a transmembrane segment and an intracellular domain endowed with protein tyrosine kinase activity [15] (Figure 1). This activity allows the phosphorylation of intracellular proteins changing their signalling properties.

Studies carried out on several in vitro models have demonstrated that the mere presence of EGFR receptors is insufficient for their transforming capability. What is needed for their oncogenic potential is their activation. From the pathologic point of view, this is an important consideration, as often pathologic studies detect only the receptors but not the active receptors with signalling capability.

The mechanism of activation of the EGFR involves oligomerisation, i.e. interaction of the EGFR with another EGFR, or a cognate ErbB receptor [18]. It is worth mentioning that this type of activation applies to many other RTKs. When EGFR receptors get in close contact, their intracellular kinase domains are able to phosphorylate each other in tyrosine residues (Figure 1). In fact, the major substrate of the EGFR is the receptor itself. This tyrosine phosphorylation of their intracellular domains offers an attraction site for several intracellular signalling molecules that then interact with the tyrosine phosphorylated receptor. This may cause their tyrosine phosphorylation, with a change in their signalling properties, or may facilitate signalling by the mere relocalisation of these molecules to the plasma membrane, where they interact with other signalling intermediates.

In vivo, three major mechanisms of oligomerisation-mediated activation of the EGFR have been described: ligand binding, overexpression, or molecular alterations of the EGFR. Under physiological conditions, a variety of EGFR family ligands drive the formation of homo- or heterodimeric complexes among the four ErbB receptors [16, 19, 20]. Based on the oligomerisation model described above, and since the RTKs may freely move in the plasma membrane, it is expected that their random collisions activate the receptors [18]. However, what keeps checked RTK phosphorylation under normal conditions is the action of cellular phosphatases, expected to neutralise any attempts of increased activation of the RTKs. This model is supported by the fact that inhibition of the cellular phosphatase activity results in progressive tyrosine phosphorylation of RTKs, even in the absence of ligand [18]. The action of ErbB ligands is that of stabilising receptor dimers, facilitating their transphosphorylation. In SCCHN, expression of EGFR ligands has been described [21]. Indeed, high expression of TGFα in a cohort of patients with SCCHN has been associated with a worse prognosis [22]. Therefore, it must be kept in mind that even under conditions in which the receptor is not overexpressed, EGFRs may be stimulated and, therefore, translate proliferative signals, when ligands are available, either produced by the tumoral cell or the surrounding stoma. In this respect, it is worth mentioning that anti-EGFR antibodies have shown clinical benefit in colon carcinoma even in the absence of overexpressed EGFR [23]. From a more ample perspective, these important considerations have to be taken into account when selecting patients for anti-RTK therapies.

Another mechanism of activation of the EGFR is overexpression. This mechanism is particularly relevant in

Figure 1. Mechanisms of activation of receptor tyrosine kinases.
tumour cells. Receptor overexpression in the tumour may lead to ligand-independent receptor dimerisation (Figure 1). Considering the model that activation of the EGFR is due to receptor–receptor interactions, the higher the amount of receptor, the likelihood of spontaneous receptor–receptor collisions increases. Furthermore, the tyrosine phosphatase activity of the cell may become saturated when overexpression of the EGFR is high enough, provoking constitutive activation of the EGFR and the ensuing signalling pathways [18].

A third mechanism of activation of the EGFR is caused by molecular alterations of the receptor (Figure 1). These alterations are expected to provoke stabilisation of oligomeric structures even in the absence of ligand or receptor overexpression. Two pathologically relevant molecular alterations of the EGFR have been described: truncations and point mutations. Truncations of the extracellular N-terminal region of the EGFR have been described in glioblastomas and correlate with responses to EGFR inhibitors [24]. Point mutations have been firstly defined to have a clinical impact in lung cancer, in which they also associate with response to EGFR antagonists [25, 26].

As mentioned above, activation of the EGFR protein tyrosine kinase results in the recruitment and phosphorylation of several intracellular substrates. A major downstream signalling route of the EGFR family is the RAS–RAF mitogen-activated-protein-kinase (MAPK) pathway [27] (Figure 2). Activation of RAS initiates a multistep phosphorylation cascade that leads to the activation of the MAPKs extracellular signal-regulated kinases 1 and 2 (ERK1/2). ERK1/2 regulate transcription of molecules that are linked to cell proliferation, survival and transformation [28]. Another important target in EGFR signalling is phosphatidylinositol 3-kinase (PI3K) and its downstream protein-serine/threonine kinase AKT [29–31]. AKT transduces signals that trigger a cascade of responses from cell growth and proliferation to survival and motility [32].

targeting the EGFR in SCCHN

There is strong evidence that supports targeting of the EGFR in cancer therapy: the EGFR is frequently overexpressed and/or abnormally activated in tumours including SCCHN, colorectal cancer, glioblastoma, or non-small-cell lung cancer [22, 33]. Moreover, early studies with anti-EGFR monoclonal antibodies directed against the EGFR were shown to be of clinical benefit [34–36]. Particularly in SCCHN, multivariate analyses have shown EGFR levels to be an independent predictor of poor outcome [22, 33].

Although there are several potential strategies for targeting the EGFR, only monoclonal antibodies (mAbs) and the low molecular weight tyrosine kinase inhibitors (TKI) are in the most advanced stages of clinical development and will, therefore, be the focus of our attention.

monoclonal antibodies against the EGFR

As shown in Table 1, several mAbs that recognise the extracellular, ligand-binding region of the EGFR have been described. Below we indicate the clinically relevant studies performed with them.

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**Figure 2.** Signalling by receptor tyrosine kinases, and site of action of inhibitory compounds.
cetuximab

Cetuximab is a mAb that binds to the ectodomain of the EGFR with high affinity, competes with ligand binding and blocks ligand-induced activation of the receptor [34–36]. In addition, cetuximab induces antibody-mediated receptor dimerisation, resulting in receptor downregulation and this effect may be important for its growth-inhibitory capacity [35]. Cetuximab is highly specific, as it only interacts with the EGFR, but not with other ErbB receptors. Cetuximab has demonstrated antitumor activity in mice models [36]. In these experiments cetuximab potentiated the antitumoral activity of several chemotherapy drugs such as cisplatin, paclitaxel or 5-fluorouracil [37, 38]. This synergistic interaction could also be observed in combination with radiotherapy [39].

In the first phase I study, cetuximab was well tolerated and the main adverse effects were related to allergic and dermatologic reactions [40]. The dose recommended for further phase II/III studies was an initial loading dose of 400–500 mg/m², followed by 250 mg/m² weekly. These phase II/III trials will be discussed below.

As radiotherapy is a standard therapeutic modality in the treatment of SCCHN, and in preclinical models cetuximab was synergistic with this approach, the combination of both modalities was tested [39]. A phase I study showed that this combination was well tolerated, and with promising activity [41]. Recently, a confirmatory phase III clinical trial with 424 patients with locoregionally advanced SCCHN were randomly assigned to treatment with high-dose radiotherapy alone, or high-dose radiotherapy plus weekly cetuximab. The median duration of locoregional control was 24.4 months among patients treated with cetuximab plus radiotherapy, and 14.9 months among those given radiotherapy alone. There was a significant increase in the median duration of overall survival that was 49.0 months among patients treated with combined therapy, compared with 29.3 months among those treated with radiotherapy alone. Radiotherapy plus cetuximab significantly prolonged progression-free survival. The regimen was well tolerated, with the exception of acneiform rash and infusion reactions. The incidence of grade 3 or greater toxic effects, including mucositis, did not differ significantly between the two groups [12].

Following the observation that the combination of cisplatin plus cetuximab was safe, different clinical trials were performed to test this approach [13, 14, 40]. In a phase III study in metastatic or recurrent SCCHN, 117 patients were randomised to receive cisplatin plus cetuximab, or cisplatin plus placebo. Median PFS was 2.7 months for the control arm and 4.2 months for the experimental arm. Median overall survival was 8.0 months and 9.2 months, respectively. Objective response rates were 10% and 26%. The toxicity was similar in both groups, except for the cutaneous rash associated with cetuximab [42] (Table 2).

In a phase II study, Baselga et al. [14] studied the efficacy of cetuximab in combination with platinum-based chemotherapy in metastatic and platinum-refractory patients. The response rate was 10% with a disease control rate of 53%. The most common cetuximab-related adverse events were skin reactions, particularly an acne-like rash [14]. A similar study was performed by Herbst et al. [13] with 132 patients testing cetuximab with cisplatin after progression or stabilisation to cisplatin/paclitaxel or cisplatin/fluorouracil. The authors concluded that the relative contribution of cetuximab would be better defined in a single-agent trial. In this respect, one trial studied the role of cetuximab monotherapy in patients with metastatic or recurrent SCCHN previously treated with platinum-based chemotherapy. Patients were treated with cetuximab monotherapy and when progression was evidenced, a platinum treatment was associated. The regimen was well tolerated, and grade 3 and 4 toxicity was rarely observed. The overall objective response rate was 16.5% and the disease control rate was 53.4% [43] (Table 3).

In combination with other chemotherapeutic drugs, a phase I study with 52 patients studied the role of cetuximab as a first-line treatment in the metastatic or recurrent SCCHN in combination with cisplatin or carboplatin plus 5-fluorouracil [44]. The overall response rate was 48.9% and the disease control rate was 83.0%. The regimen was well tolerated, but the toxicity observed in the patients treated with the combination that included cisplatin was higher [44].

The combination of cetuximab with carboplatin in patients with recurrent or metastatic, platinum-resistant nasopharyngeal carcinoma has been explored in one study. The overall response rate was 11.7%, with 11.7% partial responses and 48.3% stabilisations. The median overall survival was 8 months and the median time to disease progression was 3 months. The regimen was well tolerated, cutaneous rash, nausea and vomiting were the more common toxic effects observed [45].

Recently, a retrospective evaluation of these clinical trials has been reported. In the study, a total of 330 platinum-refractory patients with recurrent or metastatic SCCHN treated with

### Table 1. Monoclonal antibodies designed to target the ErbB family in SCCHN

<table>
<thead>
<tr>
<th>Agent</th>
<th>Characteristic</th>
<th>Target</th>
<th>Tumour type</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetuximab</td>
<td>Chimeric</td>
<td>EGFR</td>
<td>SCCHN, colon, NSCLC</td>
<td>Phase II–III [12–14]</td>
</tr>
<tr>
<td>EMD-7200</td>
<td>Humanised</td>
<td>EGFR</td>
<td>SCCHN, ovarian, colon, cervix</td>
<td>Phase II [48]</td>
</tr>
<tr>
<td>h-R3</td>
<td>Humanised</td>
<td>EGFR</td>
<td>SCCHN</td>
<td>Phase II [49]</td>
</tr>
</tbody>
</table>

### Table 2. Efficacy data of cetuximab in SCCHN chemotherapy-naïve metastatic patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Regimen</th>
<th>ORR</th>
<th>TTP (months)</th>
<th>OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burtness et al. [42]</td>
<td>117</td>
<td>cisplatin versus cetuximab</td>
<td>10</td>
<td>2.7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26</td>
<td>4.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>

ORR, overall response rate (%); TTP, time to treatment progression (months); OS, overall survival (months).
Herbst et al. [13] 132 Cetuximab
Baselga et al. [14] 96 Cetuximab

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</tr>
</thead>
<tbody>
<tr>
<td>Baselga et al. [14]</td>
<td>96</td>
<td>Cetuximab + platinum</td>
<td>10</td>
<td>85 days</td>
<td>183 days</td>
</tr>
<tr>
<td>Herbst et al. [13]</td>
<td>132</td>
<td>Cetuximab + platinum</td>
<td>20 (PD/1)</td>
<td>4.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Trigo et al. [43]</td>
<td>103</td>
<td>Cetuximab monotherapy</td>
<td>16.5</td>
<td>85 days</td>
<td>175 days</td>
</tr>
</tbody>
</table>

ORR, overall response rate (%); TTP, time to treatment progression (months); OS, overall survival (months). PD/1: progressive disease cohort 1.

**small molecule tyrosine kinase inhibitors**

Low molecular weight TKIs are small molecules that compete with ATP for the place where this nucleotide binds to the intracellular tyrosine kinase of the EGFR [15]. Two TKIs of the EGFR are in clinical development and they have been evaluated in SCCHN: gefitinib (ZD1839, Iressa; AstraZeneca, London, UK) and OSI-774 (OSI-774, formerly known as CP-358, 774, Tarceva; Genentech, San Francisco, CA).

**gefitinib**

Preclinical studies with gefitinib have shown antitumour activity in a variety of cultured tumour-cell lines and in human tumour xenografts, both as a single agents and in combination with chemotherapy [50, 51].

Phase I studies have demonstrated that daily administration of gefitinib is safe, with dose-dependent pharmacokinetics but with a high degree of interpatient variability [52–55]. Gefitinib significantly suppressed EGFR phosphorylation, inhibited MAPK activation, reduced keratinocyte proliferation and increased p27 levels and apoptosis [54].

Using gefitinib as a first or second-line treatment in patients with metastatic or recurrent SCCHN, Cohen et al. [56] showed modest activity with a response rate of 10.6% and a disease control rate of 53%; with a median time to progression and survival of 3.4 and 8.1 months, respectively. In this study, the development of skin toxicity was a statistically significant predictor of response and improved outcome. On the other hand, the same regimen but with doses of 250 mg showed less activity, reaching 1.4% of partial responses. After these results, the authors concluded that a dose–response relationship may exist for this agent in SCCHN [57].

The results from the study performed by Wheeler et al. confirm the efficacy of gefitinib in recurrent SCCHN [58]. As a first-line treatment, a clinical benefit of 45% was observed and 25% in previously treated patients. In this study no association between rash and clinical response was detected. The median time to disease progression was 3 months and median survival was 6 months [58].

As several preclinical studies have shown the synergistic activity between gefitinib and several cytotoxic drugs, such as cisplatin and 5-fluorouracil, further clinical studies were performed [59]. In this respect, a recent phase II study has evaluated the combination of gefitinib with docetaxel and cisplatin in patients with metastatic or recurrent SCCHN. An overall response rate of 62.3% (37.3% complete response and 25% partial response) was observed with a median progression-free survival of 5.1 months [60].

Novel antibodies against the extracellular domain of the EGFR are in clinical development. The EMD 72000 is an example of a humanised mAb currently in clinical development. This mAb has been evaluated recently in a patient with SCCHN showing a good toxicity profile. The main toxicity was related to cutaneous reactions [48].

The h-R3 is another mAb that has been evaluated in combination with radiotherapy in a phase I study. It has demonstrated a good toxicity profile with a promising activity. The main toxicity was related to infusion reactions. In these studies, contrary to what happens with other anti-EGFR monoclonal antibodies cutaneous rashes were not observed [49]. The results of a phase II study with or without radiotherapy are pending.

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The combination of gefitinib with chemotherapy and radiotherapy has been evaluated by Cohen et al. in patients with locally advanced SCCHN [61]. The treatment schedule consisted of the administration of two cycles of induction chemotherapy with carboplatin and paclitaxel, followed by concomitant administration of gefitinib, 5-fluorouracil and hydroxyurea. Treatment with gefitinib was administered for 2 years. The regimen was well tolerated, in terms of activity, and 88% of complete responses were observed [61].

Preclinical data have shown that the combined treatment with gefitinib and cetuximab result in a synergistic effect on cell proliferation and in superior inhibition of EGFR-dependent signalling and induction of apoptosis [62].

erlotinib

Erlotinib (OSI-774, formerly known as CP-358, 774, Tarceva; Genentech, San Francisco, CA) is an orally-available quinazoline that is a selective inhibitor of the EGFR. A phase I study with increasing daily doses of erlotinib demonstrated that the maximum tolerated dose was 150 mg/day. Similar to gefitinib, erlotinib inhibited EGFR-dependent processes in skin and tumour biopsies [63].

A phase II study with 115 patients with recurrent or metastatic SCCHN showed a 4.3% objective response and a median overall survival of 6.0 months [64]. A subgroup analyses revealed a significant difference in overall survival favouring patients who developed at least grade 2 skin rashes versus those who did not, whereas no difference was detected based on HER1/EGFR expression [64].

Erlotinib has also been investigated in combination with different cytotoxic drugs; for instance the combination of erlotinib with docetaxel has been tested with promising results [65]. Recently, a phase II study has evaluated the effect of erlotinib in combination with cisplatin and docetaxel [66]. Patients received docetaxel 75 mg/m² and intravenous cisplatin 75 mg/m² every 3 weeks, and erlotinib 150 mg by mouth daily. Partial responses were obtained in 14 of the 16 evaluated patients; the combination was well tolerated, and the more frequent toxic effects were diarrhoea, nausea and cutaneous rash. Nevertheless, a longer follow-up is necessary to study the overall response rate and survival [66].

A phase I clinical trial in combination with chemotherapy and radiotherapy has also shown promising results [67]. Patients received 70.2 Gy in 8 weeks and cisplatin 100 mg/m² every 3 weeks for three cycles, beginning both on day 8. Erlotinib was administered in a dose escalation (50 mg, 100 mg and 150 mg), beginning on day 1 and being continued until the end of the radiotherapy. All four patients evaluated had complete pathological responses. Five patients had clinical benefit after 7 days on erlotinib alone. The positive results presented in this study should be confirmed in prospective phase II studies [67].

Erlotinib has also been studied in combination with other targeted drugs such as bevacizumab, an antibody that neutralises the VEGF. Recently the preliminary results of a phase I/II study with erlotinib and bevacizumab in 51 patients with recurrent or metastatic SCCHN previously treated with chemotherapy has been reported. The combination of erlotinib, 150 mg/day, and intravenous bevacizumab at 15 mg/kg every 3 weeks, was well tolerated. The most frequent toxicity included cutaneous rash, diarrhoea and asthenia. Median progression-free survival in the phase II study was 127 days and overall survival of 226 days [68, 69].

the IGF-1R receptor

The receptor for the type I insulin-like growth factor (IGF-1R) belongs to the insulin receptor subfamily of RTKs [70]. IGF-1R is a heterotetrameric transmembrane receptor tyrosine kinase that is widely expressed in normal human tissues and is composed of two extracellular α-subunits and two transmembrane and intracellular β-subunits, linked by cysteine disulfide bonds [71]. The activation of the IGF-1R by IGF-1 provokes a decrease in p27 by targeting this cell cycle regulator to the ubiquitin/proteosome degradation machinery [72]. This action is mediated by the activation of the phosphatidylinositol-3-kinase (PI3K) pathway [72].

Recent descriptions indicate that the IGF-1R is expressed and activated in tissue samples from SCCHN patients [73]. Preclinical data have shown that the inhibition of this pathway reduces the tumour size [73]. Phase I clinical trials are ongoing testing antibodies to this receptor. Furthermore, small molecule IGF-1R specific antagonists have shown to be effective in controlling the proliferation of several tumoural cell types in culture and have in vivo action in xenograft models [74].

the receptors for VEGF and angiogenesis

Like most cancers, SCCHN is a tumour that requires blood supply. Tumour growth and the establishment of the metastases from the primary tumour are processes that depend on the formation of new vessels. With this in mind, different strategies have been developed in an attempt to target this process. Angiogenesis is a critical process in both normal physiological development and tumour biology. The best characterised factors involved in such a process are the vascular endothelial growth factors A and B (VEGF-A and VEGF-B) [75]. VEGF-A may bind to two different RTKs termed VEGFR-1 (Flt-1) and VEGFR-2 (KDR, or Flk-1), the latter being considered the major mediator of the actions of VEGF-A. The local concentration of VEGF-A is affected by the amount of oxygen. Thus, under hypoxic conditions, VEGF-A is up-regulated, and this stimulates endothelial migration, proliferation, and the creation of new vessels [75].

Interestingly, several growth factors, including VEGF, have been associated with a worse prognosis in patients with SCCHN [76]. The expression of VEGF and VEGFR-2 has been associated with a higher proliferation index and a worse survival in patients with SCCHN [76, 77]. These data indicate that the VEGF pathway can represent an interesting target for cancer therapy. A phase II study with SU5416, an inhibitor of the VEGF tyrosine kinase receptor showed clinical activity (20 patients valuable for response, one partial response, one minor response and three stable diseases) with an important toxicity (headache and/or myalgias) that produced dose reduction in half of the patients [78].
Another phase I study with bevacizumab, a monoclonal antibody against the VEGF, in association with chemoradiotherapy was shown to be active with a good toxicity profile [79]. David et al. [80] in a phase I study used the combination of bevacizumab with 5-fluorouracil, hydroxyurea and radiotherapy. The response rates averaged 75% of the treated patients and the regimen was well tolerated [80].

As preclinical studies have shown that activation of the EGFR pathway can be implicated in anti-VEGF therapies, several studies are exploring the combination of anti-VEGF therapies and cetuximab or tyrosine kinase inhibitors [81]. As it has been pointed out previously, the results of a phase I/II study with erlotinib (150 mg/day) and bevacizumab (15 intravenous mg/kg every 3 weeks) in 51 patients with metastatic or recurrent SCCHN showed that the combination was well tolerated. The median time to disease progression was 3.8 months and median overall survival higher than 7 months [69].

### targeting signalling pathways downstream of RTKs

#### the Ras/Raf/MAPK pathway

Ras is a critical intermediate in the signal transduction pathways that mediate proliferative signals from the receptor tyrosine kinases [82]. The three main Ras proto-oncogenes include H-Ras, K-Ras and N-Ras, which encode four 21-kd proteins, as two K-Ras (K-Ras4a and K-Ras4b) have been identified [83, 84]. These Ras proteins are members of a large family of guanosine triphosphatases (GTPases) that play a role in signal transduction to the nucleus. Ras functions as a chemical switch, cycling between inactive guanosine diphosphate (GDP) bound and active GTP-bound states (Figure 2). This activation occurs in response to diverse stimuli including the activation of growth factor receptors such as the EGFR, or the IGF-1R. In its GTP-bound state, Ras activates several downstream effector pathways, particularly the Raf-1 serine-threonine kinase pathway. Raf-1 phosphorylates two mitogen-activated protein kinases, MEK1 and MEK2, which in turn phosphorylate the mitogen-activated protein kinases ERK1/2. Upon activation, ERK1/2 translocate to the nucleus where they phosphorylate several substrates including nuclear transcription factors [85].

Ras activation depends on the plasma membrane localisation of Ras, which requires Ras prenylation. Prenylation involves the covalent addition of either a farnesyl or a geranylgeranyl group to the conserved carboxy-terminal cysteine residue in the CAAX box [86]. The fact that Ras farnesylation is important for its action, together with the known role of Ras as an oncogene, led to efforts aimed at developing farnesyltransferase inhibitors (FTIs). It should, however, be mentioned that FTIs are not specific inhibitors of Ras because they also block the farnesylation of many other protein substrates of farnesyltransferase.

Related to the clinical development of FTIs, several trials are ongoing. A phase I study with CSH 6636 (Lonafarnib) administered before surgery has been conducted [87]. The study showed the inhibition of protein farnesylation. The regimen was well tolerated showing the feasibility of the oral dosing schedule. A phase II study in patients with locally advancing platinum refractory tumours reported no objective responses, although a high index of stabilisations was observed [88]. The regimen was well tolerated with a good toxicity profile. It should be mentioned that these studies were not based on criteria related to the presence of activated Ras, which is difficult to perform in pathological material.

Promising effectiveness of FTIs in lung cancer calls attention for its potential use in other neoplastic pathologies. The association of lonafarnib with taxanes in lung cancer has been studied in one phase II trial [89]. Of a total of 33 patients with non-small-cell lung cancer, partial responses and stable disease were observed in three (10%) and 11 patients (38%), respectively. Thus, 48% (14 out of 29) experienced clinical benefit. Median overall survival time was 39 weeks and the median disease progression-free survival time was 16 weeks. The combination of lonafarnib and paclitaxel was well tolerated with minimal toxicity [89].

#### the PI3K/AKT pathway

PI3K is a lipid kinase that also exhibits serine/threonine kinase activity [30, 90]. PI3K kinase is composed of a regulatory p85 subunit and a catalytic p110 subunit. Upon activation, PI3K phosphorylates membrane phosphatidylinositol-4,5-bisphosphate, which recruit a number of proteins with plekstrin homology and other lipid-binding domains to the cell membrane, where they can interact with their upstream regulators and downstream targets (Figure 2). One of these proteins is PDK1, a protein kinase that phosphorylates AKT. Phosphorylated AKT can then act on substrates involved in apoptosis, cell cycle regulation, protein synthesis and glycogen metabolism. PI3K pathway is implicated in signal transduction upon activation of growth factor receptors, including EGFR and IGF-1R.

An important downstream substrate of AKT is the mammalian target of rapamycin (mTOR). mTOR is a kinase that phosphorylates proteins on serine or threonine residues [91–93]. mTOR regulates a wide array of cellular functions, including translation, transcription, mRNA turnover, protein stability, actin cytoskeletal organisation and autophagy [94]. PI3K/AKT pathway regulates mTOR indirectly through the phosphoinositide 3-kinase (PI3K) pathway. mTOR indirectly through the tuberous sclerosis products TSC1 and TSC2, which inhibit mTOR signalling by acting as GTPase-activating proteins for the Ras-related small G protein Rheb [95]. AKT can also phosphorylate Ser-2448 in mTOR suggesting that AKT may directly modulate the function of mTOR [96, 97].

Therapies directed to this pathway are in clinical development. For instance clinical trials with inhibitors of mTOR such as RAD001 or CC1779 are ongoing, and mature results to confirm their utility are still pending.

### drugs that indirectly affect RTK signalling, or other survival routes

A number of different drugs that affect signalling routes have recently been developed. Their mechanism of action is not direct, but rather affects the levels of these proteins by controlling their degradation or their biosynthesis. An important druggable target in this respect, which has been the subject of intense clinical development in other pathologies, is...
the proteasome [98]. The ubiquitin-proteasome pathway is one of the eukaryotic mechanisms responsible for protein degradation. It is critical for controlling signal transduction, transcriptional regulation, response to stress and control of receptor function. As expected from its general role in the control of the levels of multiple cellular proteins, inhibition of this cellular target results in an imbalance between cell proliferation and survival/apoptosis that finally results in cell death [99, 100]. Among interesting target proteins are IκB, an inhibitor of the nuclear factor-κappa β, p53, Bax or the cyclin-dependent kinase inhibitors p27 and p21.

Bortezomib (PS-341/Velcade) is the only proteasome inhibitor that has reached the clinical setting. In haematological malignancies, particularly in multiple myeloma, this inhibitor has proven clinical benefit and is approved for use [101]. Its activity and tolerability as a radiosensibiliser have also been evaluated with good results in patients with SCCHN that are candidates for the re-irradiation [102]. Several authors have investigated the administration of bortezomib and re-irradiation using an intermediate interruption of the treatment in patients with recurrent or metastatic SCCHN [103]. This approach, intermediate interruption, was performed in an intent to decrease toxicity, mainly hyponatraemia and hypotension, observed in the previous studies. The patients received intravenous bortezomib (0.6 mg/m² twice a week) and radiotherapy (1.8 Gy/day, until reaching the total dose of 60–72 Gy). As mentioned earlier, both therapies were suspended during 2 weeks at the middle of the planned treatment. Even though these preliminary results are encouraging, further evaluation of the efficacy of bortezomib in this field, in particular with other chemotherapeutic or biopharmaceutical agents, is required.

conclusions

The increased knowledge of the molecular alterations present in SCCHN has led to efforts to develop compounds that target these molecular alterations, or the signal transduction pathways affected. In this respect, since the EGFR is overexpressed in SCCHN, different strategies to target this activated receptor have reached the clinic. Among them, a monoclonal antibody termed cetuximab and two low-weight tyrosine kinase inhibitors (erlotinib and gefitinib) have shown promising results. For instance, in the treatment of locally advanced SCCHN, cetuximab increases the activity of radiotherapy. Also, in the metastatic setting, cetuximab increases the activity of chemotherapy compounds. Other drugs against the EGFR or other intracellular signalling pathways are in preclinical and clinical development. From all of these, anti-VEGFR therapies are promising agents, especially in combination with chemotherapy or other targeted drugs. Although these are important advances, different aspects are still pending to be defined. Important questions to be solved refer to the criteria for adequate patient selection for these targeted therapies, or the best way to increase the activity of these agents. In conclusion, during the last years, several compounds have reached the clinic with successful results. In the future, these therapies, and others to come, integrated in a rational way with standard treatments will probably improve the prognosis of SCCHN.

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references


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