



Understanding radiotherapy and its potential for use in novel combination trials

Head and neck cancer (squamous cell)

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Incidence and background

Squamous cell carcinoma of the head and neck (SCCHN) is the fifth most common neoplasm worldwide, with an estimated global incidence of more than 500,000 cases per annum (1). The UK incidence was 7745 cases per year in 2006 (2). SCCHN presents formidable challenges for therapeutic intervention and these are often secondary to the vulnerability of critical anatomical structures in the head and neck region to tumour- or treatment-induced damage. Such damage, irrespective of the aetiology, can be associated with significant structural, cosmetic and functional deficits that negatively impact patients' quality of life.

As with many solid cancers, securing locoregional disease control is of central importance when managing patients with SCCHN. Failure to achieve this goal leads to persistent or recurrent disease at the primary tumour site or in regional lymph nodes. In addition, patients can develop metastatic disease, either as a consequence of spread from the primary tumour before the initial diagnosis or from treatment-resistant persistent/recurrent locoregional disease.

Both of these clinical scenarios (persistent/recurrent locoregional or metastatic disease) represent extremely difficult management problems. Salvage treatment of the former situation is possible, but in practice this is frequently unsuccessful – especially in patients in whom macroscopic disease is evident at or within 6 months of the end of initial chemoradiotherapy – and usually entails very significant acute and long-term morbidity. Systemic metastatic disease may be palliated by cytotoxic chemotherapy, biological agents or low-dose radiotherapy, but it remains incurable with a median survival of approximately 6–9 months. As a consequence of these considerations, the primary objective of most current clinical studies in newly diagnosed SCCHN is to improve locoregional tumour control through radiation dose-escalation, pharmacological intervention or both.

Stage distribution at presentation and indications for radiotherapy

Early stage disease

Early stage (stage I and II) SCCHN accounts for 30–40% of cases, with expected long-term disease free survival rates ranging from 60% to 90% (3). For a patient with limited disease, the factors that may influence the selection of treatment modality (external beam radiotherapy vs brachytherapy vs surgery) include: primary tumour site, age, co-morbidity, occupation, preference and quality of life following treatment. Surgery is widely seen as the treatment of choice for early stage oral cavity and paranasal sinus tumours. In oropharyngeal (tonsil, base of tongue), hypopharyngeal and laryngeal cancers, primary radiotherapy is generally preferred, as surgery is often linked to greater levels of functional impairment and decreased quality of life (3). Previous attempts to conduct randomised phase III trials that directly compare radiotherapy vs surgery have largely been unsuccessful due to extremely poor rates of patient accrual. This situation will not change in the foreseeable future and, therefore, it is highly likely that current practices will continue along the lines of perceived standards-of-care without further testing in clinical trials.

Locally advanced disease

Locally advanced (stage III and IVA, IVB) SCCHN accounts for around 60–70% of all presentations (3). This stage grouping contains a heterogeneous mix of tumours as determined by the T and N stage of disease. Thus, patients with T1 and T4 primary tumours can share the same stage grouping, depending on the status of the cervical nodes. The T stage of the primary lesion has a significant bearing on the potential for resection of the tumour, which, in turn, influences patient outcomes. There are no definitive published criteria for what constitutes ‘resectable’ or ‘unresectable’ disease. Therefore, trial design should always include a clear statement on the basis for patient selection relative to their potential resectability.

Locally advanced potentially resectable disease

Approximately 50% of patients with SCCHN present with locoregionally advanced disease that is potentially resectable and have a projected 5-year overall survival (OS) in the range of 40–50% with surgery and postoperative (chemo) radiotherapy (3). As with early stage disease, just because a locally advanced tumour is technically resectable, this does not mean that this is the treatment of choice. Concerns about poor post-surgical functional outcomes and the risks of leaving microscopic residual disease that will require adjuvant post-operative (chemo)radiation often dictate that patients are better treated with primary non-surgical modalities.

Locally advanced unresectable disease

Patients with unresectable locally advanced SCCHN have a significantly worse prognosis with a 5-year OS of approximately 10–40% (4, 5). For patients with good performance status (PS; WHO 0–1), the standard treatment is concurrent cisplatin chemotherapy and radiotherapy. For patients with poor PS, the recommended treatment is radiotherapy with or without concurrent chemotherapy. The role of induction (neoadjuvant) chemotherapy (cisplatin, 5-FU or cisplatin, 5-FU, docetaxel) to bring about tumour shrinkage before definitive (chemo)radiotherapy is currently the subject of active investigation (6,7), but may become a standard-of-care during the next decade.

In addition, inhibition of the epidermal growth factor receptor (EGFR) with the monoclonal antibody cetuximab has been shown to lead to improvements in disease-free and overall survival when combined with radiotherapy in stage III/IV disease (8). Interestingly, in a follow-up publication, the authors reported that the occurrence of a prominent drug-induced skin rash was an important predictive biomarker of benefit from cetuximab (9). Patients in the mild and prominent rash groups had median OS values of 25.6 and 68.8+ months, respectively. The mechanistic basis for this result is unexplained and the authors of the report limit their discussion to the possibility of the skin reaction being a biomarker of an uncharacterised therapeutic immune reaction. Clearly this topic deserves significant further research in studies of cetuximab and other EGFR-targeted monoclonal antibodies. Much criticism has been levelled at the Bonner study on the basis of the lack of inclusion of cisplatin-based chemoradiotherapy as the 'standard' control arm. As a result, concomitant cetuximab plus radiotherapy has not been universally adopted. Preliminary results of the randomised phase III Radiation Therapy Oncology Group (RTOG) 0522 trial (cisplatin-based chemoradiation vs cisplatin-based chemoradiation plus cetuximab) are due in 2011 and should shed light on this matter.

Other humanised monoclonal antibodies (panitumumab, zalutumumab) and small molecule inhibitors (lapatinib, erlotinib, gefitinib) are currently being tested in this setting in randomised phase II/III studies in patients with locally advanced SCCHN.

Metastatic disease

Patients presenting with metastatic disease account for approximately 5–10% of new diagnoses of SCCHN. The aim of any treatment is palliative. Radiotherapy is delivered for the relief of symptoms and to achieve local disease stabilisation or control (10). Single/combination agent chemotherapy regimens are also used (11). The largest group of patients with metastatic disease comprises those who relapse systemically following treatment of the locoregional disease. Indeed, as a result of improvements in the efficacy of loco-regional treatment we are seeing increasing numbers of patients with systemic disease relapse.

Treatment modalities

Post-operative (chemo)radiotherapy

Post-operative radiotherapy is the standard of care for patients who are judged to be at high risk of local failure by pathological examination of the resection specimen. The high risk features are positive (<1 mm clearance) or close (1–5 mm) surgical margins or extracapsular extension in lymph nodes (12). The presence of multiple positive lymph nodes or perineural/lymphatic/vascular invasion in the tumour are relative indications for post-operative therapy that should be considered in concert with other features of the disease. RTOG 73-03 randomised patients with operable SCCHN to pre- (50 Gy) or post-operative RT (60 Gy). This study found a 12% improvement in locoregional control in the post-operative treatment group (13). In this group of patients who receive two treatment modalities, the 5-year OS is about 30% (14). The European Organisation for Research and Treatment of Cancer (EORTC) and RTOG performed clinical trials evaluating concurrent cisplatin-based chemotherapy and radiotherapy versus radiotherapy alone in the post-operative setting in patients with high risk factors for local recurrence as defined above (15,16). Both studies found improved locoregional control and disease free survival in the patients that received concurrent cisplatin with post-operative radiotherapy. Based on these two

studies, post-operative radiotherapy with concurrent cisplatin has become the standard of care for patients with high risk pathologic features (17). This setting represents a good opportunity in which to test novel targeted agents: (i) in combination with standard chemoradiotherapy in phase III studies powered to detect improvements in locoregional control and overall survival; and (ii) as a means of reducing treatment-induced toxicity by replacing cisplatin in studies powered to detect equivalence between standard chemoradiotherapy and radiotherapy plus the novel agent.

Concurrent chemoradiotherapy

The addition of chemotherapy to radiotherapy was explored in a number of randomised trials during the 1960s through to the 1990s that sought to improve rates of OS and locoregional control compared to the rates obtained with radiotherapy alone. The Meta-Analysis of Chemotherapy in Head and Neck Cancer (MACH-NC) of 87 trials, combining in the experimental arm local-regional treatment and chemotherapy, showed that, whatever the sequencing of the two modalities, the addition of chemotherapy to radiotherapy yielded an improvement in survival, with an overall absolute benefit of 5% at 5 years (18). Concurrent chemoradiation was the most effective modality, with an 8% absolute 5-year survival benefit (18). Platinum-based regimens of chemotherapy were more effective than the others ($p < 0.01$). While the addition of chemotherapy to radiotherapy increases tumour responses and improves disease control, it also increases the incidence of mucositis (19). At present, cisplatin is regarded as the gold-standard agent for concomitant chemoradiotherapy regimens and it is unlikely that this situation will change in the foreseeable future. Therefore, studies assessing the potential for introducing novel agents as components of regimens for locally advanced SCCHN should include a cisplatin-based chemoradiotherapy control arm either in trials that seek to demonstrate improved outcomes through addition of a novel agent, or in protocols that test for equivalent anti-tumour effect with less toxicity when combining radiation with a targeted drug.

Altered fractionation radiotherapy

Radiation is commonly delivered as single daily fractions over the course of weeks (i.e. standard or conventional fractionation – 2 Gy/fraction). Some SCCHN have a high proliferation index and exhibit biologically aggressive behaviour. In addition, it has been suggested that the rate at which a tumour divides during a course of fractionated radiotherapy can increase, such that the tumour may show accelerated repopulation. To overcome this rapid growth, altered fractionation schemes such as hyperfractionation and accelerated fractionation have been evaluated. Hyperfractionation regimens deliver a larger total number of smaller radiotherapy fractions more than once a day (e.g. 1.15–1.5 Gy bid/tid) and exploit the difference in fractionation sensitivity between tumours and normal tissues. Tissues which exhibit toxicity from radiotherapy many months or years following treatment, such as the spinal cord, are particularly sensitive to the radiation dose per fraction as well as the total radiation dose. For this reason, a reduction in dose per fraction to 1.1–1.2 Gy allows a larger total dose to be administered with the twin goals of better tumour control and reduced late toxicity.

In the EORTC 22791 trial, 356 patients with SCCHN were randomised to receive conventional treatment or 80.5 Gy given in twice-daily fractions of 1.15 Gy per fraction over 7 weeks. Hyperfractionation resulted in an increase in tumour control from 40% to 59% with no increase in late normal tissue toxicity (20). Accelerated fractionation regimens attempt to reduce tumour proliferation by reducing the total treatment time without major reductions in fraction size. Studies of altered fractionation schemes in patients with SCCHN have found improvements in local control at the cost of higher toxicity compared

to conventional fractionation schemes. Fu et al. reported an 8% increase in loco-regional tumour control with a concomitant boost technique (1.8 Gy/fraction/day, 5 days/week and 1.5 Gy/fraction/day to a boost field as second daily treatment for the last 12 treatment days to 72 Gy/42 fractions/6 weeks) when compared with standard fractionation (70 Gy in 35 fractions) or accelerated radiotherapy with a 2-week treatment gap (21).

Recently a meta-analysis of hyperfractionated or accelerated radiotherapy as radical treatment in SCCHN compared trials which randomised patients between conventional radiotherapy (66–70 Gy in 2 Gy fractions for 5 days a week) and hyperfractionated or accelerated (or both) schedules. The results showed that there was a significant survival benefit with the so-called altered fractionation which corresponded to an absolute benefit of 3.4% at 5 years. This benefit was particularly pronounced for hyperfractionated RT, with an 8% benefit at 5 years (22).

The role of altered fractionation in combination with systemic therapy is still being defined, especially in light of the observation that pure hyperfractionation regimens with dose escalation confer the same absolute OS benefit as concomitant chemoradiotherapy (22). Both RTOG and EORTC have conducted randomised trials to compare accelerated fractionation to standard fractionation in combination with concurrent cisplatin chemotherapy (RTOG0129 and EORTC22962), and the results are awaited. Importantly, however, the potential for exploiting altered fractionation regimens in studies of novel agents was highlighted by the results of the phase III trial of radiation plus cetuximab in which patient benefit was greatest in those treated with a concomitant boost schedule (8,9). Subsequent studies of EGFR-targeted monoclonal antibodies (cetuximab in RTOG-0522, panitumumab in NCT00820248, zalutumumab in DAHANCA19/NCT00496652) in patients with unresected disease have all included altered fractionation as part of the radiotherapy design. Therefore, this issue needs careful consideration in future trial design of novel agents plus radiotherapy, but it is important to appreciate that the lessons of EGFR-targeted therapies will not necessarily apply to other classes of drugs.

Insights from head and neck cancer biology

The genetic alterations associated with SCCHN are numerous and appear to include a variety of cell signalling pathways. In the majority of cases, lifetime environmental exposure to carcinogens, such as tobacco and alcohol, plays an important role in causing DNA damage. As with other tumours, the multi-step accumulation of separate genetic events is thought to lead to the development of SCCHN. However, in recent years a new subtype of SCCHN that is more prominently related to infection with the human papilloma virus (HPV) has emerged. Early indicators suggest that these tumours are driven by different underlying molecular mechanisms and this may make them suitable candidates for clinical studies involving novel targeted therapies.

EGFR pathway

Epidermal growth factor receptor (EGFR), a member of the ErbB/HER family of receptor tyrosine kinases, has a major role in the biology of SCCHN (23). There are four family members: EGFR or c-erbB-1, c-erbB-2/neu/HER-2, c-erbB-3/HER-3 and c-erbB-4/HER-4. The receptors consist of a glycosylated extracellular ligand-binding domain, a hydrophobic transmembrane component and an intracellular domain with tyrosine kinase activity. SCCHN very commonly (>90%) usurps normal EGFR-mediated cell signalling pathways to obtain a growth and survival advantage over neighbouring normal tissues. Three

main mechanisms are at the heart of the ability of SCCHN to deregulate normal EGFR signalling: (i) tumours synthesise and release growth factor ligands that stimulate their own receptors (autocrine signalling) and those of their neighbours (paracrine signalling); (ii) tumours alter the number, structure or function of cell surface receptors such that they are more likely to transmit growth signals (even in the absence of the cognate ligand); (iii) they deregulate the signalling pathways downstream of the receptor so that it is constitutively active. SCCHN is a prime example of EGFR-driven oncogenesis, since this signalling pathway can account for all of the malignant features of the disease. Overexpression of EGFR correlates with treatment resistance, tumour spread and poor survival. The consequences of ligand binding, receptor dimerisation and activation and subsequent intracellular signalling provide mechanistic explanations for many of the features that characterise SCCHN.

In contrast to certain tumour types in which amplification or mutation of the EGFR gene is implicated, overexpression of the protein without gene amplification is the dominant process in SCCHN. Several studies have shown links between EGFR overexpression and head and neck cancer oncogenesis and progression. High levels of tumour EGFR expression are associated with worse prognosis and resistance to chemotherapy and radiotherapy (24). Two EGFR targeting strategies are currently being investigated in pre-clinical and clinical settings: the use of monoclonal antibodies (mAbs) and small-molecule tyrosine kinase inhibitors (TKIs).

In patients with locally advanced disease, cetuximab (a mAb targeting EGFR) plus radiotherapy significantly improved the median duration of locoregional control and OS compared with radiotherapy alone (6). Importantly, the authors reported no significant exacerbation of radiotherapy-induced adverse events in the group of patient treated with the mAb – although those findings have recently been called in to question following the more widespread use of this agent. The RTOG-0522 phase III trial of cisplatin-based chemoradiotherapy with or without cetuximab in >900 patients with stage III/IV pharyngeal and laryngeal cancers has now closed to recruitment. Initial reports of the data are expected in late 2011 and are likely to have a major role in shaping the further development of this field.

In addition to cetuximab, ongoing phase III clinical trials of humanised mAb are open to recruitment. Panitumumab is being tested (NCT00820248) in patients with stage III/IV oral, pharyngeal and laryngeal SCCHN who receive treatment with conventionally fractionated radiotherapy and three doses of concomitant cisplatin or modestly accelerated radiotherapy and 3-weekly mAb. Zalutumumab is undergoing evaluation (DAHANCA19/NCT00496652) in patients with stage I–IV larynx and pharynx SCCHN who receive 6 fractions of radiotherapy and one dose of cisplatin per week with or without the addition of the mAb. The primary and secondary endpoints of the studies will include progression-free and overall survival, locoregional control and toxicity and quality of life endpoints.

Small molecule TKIs have not been tested as extensively as mAb in patients with SCCHN. Even so, phase I/II studies of chemoradiation combined with gefitinib (25), erlotinib (26) or lapatinib (27) have been reported. The latter agent is a dual tyrosine kinase inhibitor that targets both EGFR and c-erbB2. A randomised ‘window of opportunity’ phase 0 study has been completed with this drug, as has a randomised phase II study with chemoradiation. Lapatinib is currently undergoing phase III evaluation in the context of post-operative chemoradiation in patients with high risk features after surgical treatment of stage III/IV head and neck cancer. One of the attractions of small molecule TKIs is oral bioavailability, which allows out-patient treatment and facilitates testing of maintenance regimens.

PI3K/Akt/mTOR pathway

Phosphatidylinositol 3-kinases (PI3Ks) are a family of signalling enzymes which regulate a variety of important cellular functions, including growth, cell cycle progression, apoptosis, migration, metabolism and vesicular trafficking (28,29). Mutations in the gene encoding the catalytic subunit of PI3K, PIK3CA, are frequently seen in SCCHN, especially in pharyngeal cancers (30). These mutations can contribute to PI3K/Akt-mediated carcinogenesis in head and neck cancer (31). The PI3K/Akt pathway regulates various cellular functions, several of which are involved in the most important mechanisms of radioresistance: intrinsic radioresistance; tumour-cell proliferation; and hypoxia (32). Activation of the PI3K pathway may also mediate resistance to chemotherapy and novel agents such as EGFR inhibitors.

The PI3K family can be divided into three classes of enzyme according to structure and substrate specificity. In the context of SCCHN, the class I enzymes play a major role and represent potential therapeutic targets. Their major products, phosphatidylinositol 3,4-bisphosphate (PI3,4P₂) and phosphatidylinositol 3,4,5-trisphosphate (PIP₃), are the key lipids responsible for mediating cellular responses to growth factor or cytokine stimulation, integrin ligation and signalling via integrin-linked kinase (ILK) or focal adhesion kinase (FAK) (33). Class I PI3Ks are heterodimeric proteins comprising regulatory and catalytic subunits of which there are four isoforms: p110 α , p110 β , p110 δ (Class IA) and p110 γ (Class IB).

PI3K is downstream of receptor tyrosine kinases (RTK) in the membrane that are phosphorylated in response to growth factor binding. Class IA isoforms are activated by RTK (e.g. EGFR, ErbB3, Met, platelet-derived growth factor receptor [PDGFR], vascular endothelial growth factor receptor [VEGFR], insulin-like growth factor receptor [IGF-1R]), but p110 γ is activated by G-protein coupled receptors. In SCCHN, the overexpression of EGFR and its heterodimerisation with the other erbB family members indicates that PI3K may readily be activated. The Met oncogene, which encodes hepatocyte growth factor receptor, is upregulated in up to 60% of SCCHN and expression has been linked to cisplatin resistance, potentially through its ability to activate the PI3K survival pathway. The best described downstream target of PI3K is protein kinase B (PKB)/Akt which has three isoforms: Akt1, Akt2 and Akt3. Akt, in turn, phosphorylates multiple proteins that regulate a number of cellular responses. In particular, Akt is a key cell survival protein through its ability to regulate apoptosis by inactivating the pro-apoptotic protein Bad, the pro-death caspase 9 and Forkhead transcription factors. In addition, Akt activates IKK (I κ B kinase) a positive regulator of NF κ B and Mdm2 survival proteins. Another important substrate of Akt is the mammalian target of rapamycin (mTOR). Phosphorylated mTOR activates p70 S6 kinase which enhances mRNA translation and drives cell growth by activating the ribosomal protein S6 and elongation factor 2 (eF2).

First generation PI3K inhibitors, such as wortmannin and LY294002, are structurally unrelated, broad-spectrum inhibitors suitable only for laboratory research purposes. Both drugs compete with ATP for binding to the active site of p110. Wortmannin exerts irreversible inhibition through covalent binding, but is unstable, poorly water soluble and highly protein bound in serum. It also inhibits other kinases such as myosin light chain kinase, mTOR and DNA-protein kinase (DNA-PK). LY294002 (a synthetic compound based on the flavenoid quercetin) is more stable in solution but also exerts off-target effects (e.g. against casein kinase 2). More recently, novel classes of p110 isoform-selective PI3K inhibitors have been developed by a number of pharmaceutical companies. These agents have the potential to target

key components of the PI3K-Akt pathway to sensitise to both radiotherapy and chemotherapy without causing significant toxicity through broad-spectrum off-target effects.

Hypoxia modulation

The observation that hypoxic cells are more radioresistant than normoxic cells is one of the central principles of radiation oncology – a fact that is supported by a wealth of preclinical and clinical data. As a consequence, there have been many approaches aiming to modify the oxygenation of tumours in an attempt to enhance radiosensitivity. These studies have attempted: (i) to increase haemoglobin concentrations (blood transfusion, recombinant human erythropoietin [EPO]); (ii) to increase the pressure or concentration of inhaled oxygen (hyperbaric oxygen, ARCON [accelerated radiotherapy with carbogen and nicotinamide]; and (iii) to sensitise hypoxic cells selectively to the effects of radiation (hypoxic cell sensitisers, bioreductive drugs). The potential value of modifying hypoxia in SCCHN is strongly supported by a meta-analysis of 4250 patients from 27 trials (not including the studies of EPO), which demonstrates a 7% benefit in terms of locoregional control for patients treated with hypoxic modifying therapy plus radiation compared with radiation alone (34). However, despite this clear signal that hypoxia is an important target in SCCHN, none of the approaches detailed above is likely to be widely applicable in the clinic.

By using novel agents, it may be possible to target hypoxic cancer cells. One such example is provided by the poly (ADP-ribose) polymerase (PARP) inhibitor (ABT-888) that has been shown to have equivalent sensitiser enhancement ratios (SER) under both normoxic and hypoxic conditions (35). Therefore, future preclinical testing of novel putative radiosensitisers should include assays conducted under both hypoxic and normoxic conditions.

Angiogenic signalling pathway

In keeping with other solid tumours, the growth of SCCHN is intimately related to its ability to secure a blood supply. A small cluster of cancer cells can grow to 60–100 μm by deriving a supply of oxygen and nutrients by direct diffusion, but beyond this size any fledgling tumour must secure a dedicated blood supply. Cancers subvert the normal equilibrium between pro- and anti-angiogenic factors to turn on the 'angiogenic switch' and grow new tumour-associated blood vessels. The archetype of signalling in this system is provided by the interaction between vascular endothelial growth factors (VEGFs) and their receptors (VEGFR1, VEGFR2, VEGFR3) (36).

New tumour-associated blood vessels present a diverse array of potential therapeutic targets that may be exploited by oncologists. Novel agents may have the ability to switch off signalling through pro-angiogenic pathways or to switch on signalling through anti-angiogenic pathways. Both of these effects can be mediated either at the level of the ligand or its receptor. Alternatively, anti-vascular drugs may seek to destroy new tumour-associated blood vessels by exploiting differences between them and their counterparts in normal tissues. The relevance of the angiogenic phenotype of cancers to their susceptibility to radiation is complex and means that this is a complicated therapeutic target. For a microscopic tumour deposit, activation of the angiogenic phenotype is a quantum leap in its ability to grow and spread and, therefore, can only be viewed negatively by an oncologist. However, in large, established tumours, the presence of a good blood supply is an essential pre-requisite for radiosensitivity. Therefore, rather perversely, angiogenesis may be seen as beneficial by radiation oncologists. By pursuing this logic, drugs that target angiogenesis may be seen as detrimental to the

radiation response through their activity in reducing tumour oxygenation. In fact, Jain and colleagues have proposed a more detailed model of this system, suggesting that by varying the dose and duration of exposure to anti-angiogenic therapies it may be possible to 'normalise' the vasculature and, hence, improve tumour sensitivity to radiation (37). Future clinical trials using agents that target ligands and receptors will need to address these issues by studying very carefully the effects of anti-angiogenic and anti-vascular agents on tumour blood and oxygen supply and the resulting effects on treatment outcome.

DNA damage response

Intrinsic radiosensitivity is related to the ability of the cell to detect DNA damage and effect its repair. At least five distinct pathways have been identified through which a cell can detect and repair DNA damage. These pathways are an essential part of protecting the integrity of the genome. They include: direct repair; mismatch repair (MMR); base excision repair (BER); nucleotide excision repair (NER); and double-strand break recombinational repair, which encompasses both non-homologous end joining (NHEJ) and homologous recombination (HR). Tumour suppressor genes, such as ataxia telangiectasia, TP53 and BRCA1, are involved in these pathways and mutations in these genes are associated with cancer predisposition.

Enhancing radiosensitivity by targeting DNA repair processes represents an important new treatment strategy, but it is one that carries significant risks. The clinical manifestations of naturally occurring syndromes in which DNA repair mechanisms are defective, such as ataxia telangiectasia, Fanconi anaemia, or Nijmegen breakage syndrome, serve to warn radiation oncologists of the injudicious use of DNA repair inhibitors. Recently, it has been appreciated that cancers may be more susceptible than normal cells to pharmacological modulation of DNA repair pathways through their reliance on one non-redundant repair pathway. This opens up the possibility of inducing cancer-selective 'synthetic lethality' with agents that block DNA repair through this essential pathway. Furthermore, this approach has great potential for enhancing the activity of DNA damaging agents such as chemotherapy and radiotherapy. DNA repair inhibitors, including PARP (38), Chk1 (39) and DNA-PK (40) inhibitors, have been developed and are beginning to enter the clinic in combination with cytotoxic chemotherapy. It is likely that drugs that target HR will show more intrinsic anti-tumour selectivity than those which target NHEJ, because of the restriction of HR to proliferating cells. Nonetheless, there is a real desire to test these drugs in carefully designed trials in combination with radiotherapy. SCCHN represents an important model system and it is likely that studies will be undertaken in this group of patients.

Human papilloma virus (HPV)-associated SCCHN

Recent research has demonstrated that HPV-positive and HPV-negative head and neck squamous cell carcinomas (HNSCC) are two distinct cancers with different etiologies (41). Human papillomavirus (HPV) is found in approximately 20% of all SCCHN, and as many as 50–85% of oropharynx cancers (42). The majority (approximately 90%) of HPV-associated SCCHN are caused by a single HPV genotype (HPV16) (43). HPV uses the replicative machinery of the host cell to make copies of its DNA. Since this only operates in dividing cells, HPV contains specific gene sequences encoding proteins that mediate cell cycle progression and avoidance of apoptosis: HPV E6 protein inactivates cellular p53 protein and HPV E7 mediates the degradation of the retinoblastoma tumour suppressor gene product (pRb). Importantly, neither of these cellular proteins is mutated in HPV-related SCCHN (44,45).

Weinberger et al classified SCCHN according to HPV negativity or positivity as follows: Class I – HPV negative, p53 mutated, p16 inactivated; Class II – HPV positive, p53 mutated, p16 inactivated; Class III – HPV positive, p53 wild-type (but inactivated by HPV E6), p16 not inactivated (46,47). Patients with Class III tumours have a significantly better prognosis, perhaps because of the different molecular pathogenesis of the disease (although the mechanistic basis of this is not understood). Patients with Class III tumours have improved response rates and progression-free, disease-specific and overall survival outcomes following radiotherapy alone, chemoradiotherapy or surgery with or without adjuvant therapy. As a consequence, all future studies of new agents in combination with radiation or chemoradiation will have to take account of HPV status as an inclusion/exclusion criterion or a stratification variable. In addition, strategies to generate anti-HPV immunity in patients treated for HPV-mediated SCCHN should also be explored as a means of consolidating treatment. One such approach, involving expression of HPV epitopes from a listeria monocytogenes bacterial platform, is already in clinical testing.

Therapeutic opportunities with radiotherapy in head and neck cancer

There are many therapeutic opportunities for testing novel targeted agents in patients with head and neck cancers. As reviewed above, the objectives/endpoints of these studies can be summarised as follows.

In patients with locally advanced disease (stage IV):

- To reduce locoregional recurrence and the development of metastatic disease
- To improve overall survival rates
- To decrease early and late toxicity
- To identify clinical outcomes in relation to patients' clinical, pathological characteristics to improve patient selection (stratified/personalised medicine).

In patients with intermediate stage disease (stage III):

- To decrease treatment intensity (e.g. replace concomitant cisplatin with targeted agent)
- To decrease early and late toxicity
- To improve functional outcomes.

In patients with HPV-positive disease:

- To decrease treatment intensity (e.g. replace concomitant cisplatin with targeted agent)
- To decrease early and late toxicity
- To improve functional outcomes
- To generate protective anti-HPV immunity.

In patients with recurrent disease:

- To increase treatment benefit from further radiation therapy with or without chemotherapy/targeted agents
- To increase survival in patients treated with palliative chemotherapy as a means of identifying agents that may be relevant for subsequent studies in combination with radiotherapy/chemoradiotherapy.

Within each of these clinical settings, there will be a need to develop a coherent programme of work involving orderly progress through phase I, II and III studies.

Phase I trials

These studies can be designed in patients being treated for palliation, with radiation alone or with chemoradiation. In the palliative setting, dose ranges between 20 Gy in 5 fractions and 36 Gy in 12 fractions (48) will facilitate examination of a number of drug dose levels and durations of treatment exposure. Patients with SCCHN frequently have disease that is amenable to biopsy as a means of generating biomarker data. Studies of novel agents combined with radical radiation therapy can be conducted in intermediate (stage III) disease and in patients >71 years of age with locally advanced disease (for whom concomitant chemotherapy is not indicated). Drug delivery schedules can also incorporate the use of so-called wash-in periods (during which window of opportunity biomarker analyses can be performed) and drug maintenance studies. Trial design can incorporate the use of 3D-conformal radiotherapy or intensity-modulated radiotherapy (IMRT) and conventional or altered fractionation schemes. In patients with locally advanced disease who are suitable for concomitant chemoradiation, trial design will be similar although greater caution in drug dose-escalation decisions are likely to be necessary. A minimum of 6 weeks of safety observation should be recommended in all patients in a single cohort before escalation to the next dose level is initiated. The endpoints of these studies will include safety and tolerability measures, with recommendation of a dose level for phase II study. In addition, exploratory biomarker and imaging analyses can be built in to add value to these trials.

Phase II/III trials

These studies will mirror the design of the phase I trials detailed above. Patient inclusion/exclusion or stratification based on HPV status of the tumour will be mandatory. Specific opportunities will include testing different fractionation schemes and the chance to examine formally the potential role of maintenance therapy as a means of reducing locoregional failure and/or systemic relapse. Study endpoints will include locoregional control or progression-free survival (primary) and response rate, disease-specific or overall survival and adverse events. In registration studies, the primary endpoint may need to be OS.

Specific agents of interest

Based on current evidence, the following agents would be of major interest for investigation in combination with radiotherapy:

1. Inhibitors of EGFR and other c-erbB family members, including both mAb and small molecule TKIs
2. Drugs that target the PI3K/Akt/mTOR pathway
3. Drugs that are capable of enhancing radiation responses in hypoxic and normoxic cells alike (eg PARP inhibitors).
4. VEGF and VEGFR inhibitors, including both monoclonal antibodies and small molecule inhibitors
5. DNA repair inhibitors, including PARP, Chk1 and DNA-PK inhibitors.

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