review

Strategies to promote translational research within the European Organisation for Research and Treatment of Cancer (EORTC) Head and Neck Cancer Group: a report from the Translational Research Subcommittee

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Head and neck squamous cell cancer (HNSCC) is the sixth leading cause of cancer-related deaths worldwide. These tumors are commonly diagnosed at advanced stages and mortality rates remain high. Even cured patients suffer the consequences of aggressive treatment that includes surgery, chemotherapy, and radiotherapy. In the past, in clinical trials, HNSCC was considered as a single disease entity. Advances in molecular biology with the development of genomic and proteomic approaches have demonstrated distinct prognostic HNSCC patient subsets beyond those defined by traditional clinical–pathological factors such as tumor subsite and stage [Cho W (ed). An Omics Perspective on Cancer Research. New York/Berlin: Springer 2010]. Validation of these biomarkers in large prospective clinical trials is required before their clinical implementation. To promote this research, the European Organisation for Research and Treatment of Cancer (EORTC) Head and Neck Cancer Program will develop the following strategies—(i) biobanking: prospective tissue collection from uniformly treated patients in the setting of clinical trials; (ii) a group of physicians, physician—scientists, and EORTC Headquarters staff devoted to patient-oriented head and neck cancer research; (iii) a collaboration between the basic scientists of the Translational Research Division interested in head and neck cancer research and the physicians of the Head and Neck Cancer Group; and (iv) funding through the EORTC Grant Program and the Network Core Institutions Consortium. In the present report, we summarize our strategic plans to promote head and neck cancer research within the EORTC framework.
Key words: EGFR, head and neck cancer, HPV, translational research

introduction

In 2002, the crude incidence rates of carcinoma of the head and neck in Europe were 36/100 000/year in the male population and 7/100 000/year for women, whereas the corresponding mortality rates were 18 and 3/100 000/year. On the Europe scale, head and neck cancer accounts for 139 000 new cases per year. More than 90% of head and neck malignancies are squamous cell carcinomas [1]. In Europe, the relative survival rate for head and neck cancer patients was 72% at 1 year and 42% at 5 years in adults [2]. Five-year survival was higher in

women (51%) than in men (39%). The effect of age on survival is marked. Survival at 5 years was 54% for the youngest age-group (15–45 years) and 35% in the oldest group of patients (≥75 years) [2].

Head and neck squamous cell carcinomas (HNSCCs) represent a heterogeneous group of tumors in terms of the etiology, biology, and clinical behavior. In the past, clinical trials considered HNSCC as a single disease entity [3]. Genomic and proteomic approaches and molecular epidemiology studies have demonstrated that there is considerable heterogeneity among HNSCCs beyond that defined by traditional clinical–pathological factors [4, 5]. For example, the association of human papillomavirus (HPV) with a subset of oropharyngeal cancers (OPCs) elucidates how diversity in etiology affects tumor biology and clinical behavior. Tobacco and alcohol use

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account for the vast majority of HNSCCs. Recently, high-risk HPVs, especially type 16, have been implicated in the pathogenesis of a subset of HNSCC, especially those arising from the tonsillar oropharynx. Patients with HPV-positive tumors are estimated to have a 50%–80% reduction in risk of disease failure when compared with the HPV-negative patients [5–7]. HPV-positive HNSCCs are characterized by p16 positivity [4] and relatively fewer genomic abnormalities. From this point of view, HPV status should at least be included as a stratification factor into future randomized, controlled trials (RCT) and, in addition, it could be argued that separate RCTs are needed for HPV-associated HNSCCs. Phase II/III clinical trials that represent attempts to reduce toxicity burden with minimal risk for inferior tumor control in patients with HPV-positive HNSCCs are planned. A National Cancer Institute-sponsored State of the Science Meeting on Head and Neck Cancer and the HPV was convened on 9–10 November 2008 in Washington, DC [8]. In this meeting, the principles of trial design for HPV-positive patients were identified. A phase III non-inferiority trial was not considered feasible due to the large number of patients required. There was a near consensus that a large randomized phase II trial design with a standard control arm would be the most practical way to proceed. Therefore, it has now become clear that future clinical trials should take into account the molecular and clinical heterogeneity of the disease and include important biomarkers as stratification factors or predictive variables. This approach may spare good-prognosis patients the side-effects of unnecessary overtreatment suited for poor-prognostic subsets that will benefit more from a more aggressive approach.

The European Organisation for Research and Treatment of Cancer (EORTC) Head and Neck Cancer Group (HNCG) has offered major contributions in the field of combined modality approaches in locally advanced disease [9–13]. These include (i) organ preservation programs in patients with operable tumors, comparing immediate surgery versus intended nonsurgical approaches (i.e. induction chemotherapy followed by radiation in good responders) or comparing the sequential approach (induction chemotherapy followed by radiation in good responders) versus the alternating approach (i.e. alternating chemotherapy and radiotherapy (RT) from the start in all patients); (ii) postoperative management of locally advanced tumors, comparing radiochemotherapy versus RT alone; and (iii) induction chemotherapy programs in locally advanced inoperable disease, comparing docetaxel (Taxotere®), cisplatin, and 5-fluorouracil (TPF) versus cisplatin and 5-fluorouracil (PF) induction regimens. Despite these advances, >50% of patients with locally advanced disease die of cancer. In addition, the majority of surviving patients suffer acute and late-treatment-related side-effects, mainly salivary dysfunction and speech and swallowing impairment. Advances in molecular biology have provided the tools to be used for more accurate prognostic patient classification, early detection, and toxicity prediction. Several prognostic and predictive biomarkers have been identified using genomic and proteomic approaches. The clinical application of these biomarkers for early detection, outcome, and toxicity prediction will improve survival and quality of life of patients with HNSCC. However, validation of these biomarkers in large prospective clinical trials is required before their clinical implementation. The EORTC-HNCG has developed strategies to promote patient-oriented laboratory research. This strategy article will summarize ongoing and planned clinical trials, outline planned translational research (TR) projects, and describe strategies to promote translational head and neck cancer research within the EORTC framework.

clinical trials in the EORTC-HNCG

Comprehensive information on ongoing clinical EORTC trials can be found at the EORTC Web site (www.eortc.be).

locally advanced setting

EORTC 24971/TAX323 phase III clinical trial was an RCT where patients with locally advanced (stages III and IV) HNSCC were randomized to receive either PF induction chemotherapy or TPF (docetaxel, cisplatin day 1, 5-fluorouracil continuous infusion days 1–5) [13] every 3 weeks for four cycles followed by RT alone. The results of this study revealed that locoregional control, as well as overall survival (OS), significantly improved with the TPF induction chemotherapy regimen, while quality of life was maintained and was even better in the TPF arm [13]. Based on the results of this important trial, TPF has now become the standard when chemotherapy is given for induction in patients with locally advanced HNSCC. The EORTC-HNCG plans to correlate tumor HPV DNA and p16 protein status with therapeutic response and survival. In addition, functional p53 status and β-tubulin expression status will be correlated with response to docetaxel to see whether these biomarkers have the potential to be used both as prognostic and as predictive factors.

Current clinical trials in HNSCC that combine chemotherapy with targeted agents provide unique opportunities to test hypothesis-driven TR questions. The ongoing EORTC 24061 study is a randomized phase II study in which cetuximab [chimeric immunoglobulin G1 monoclonal antibody targeting the epidermal growth factor receptor (EFGR)] is combined with TPF for induction. After this induction biochemotherapy regimen, patients with inoperable locally advanced HNSCC will be randomly assigned to receive cisplatin-based or carboplatin-based chemoradiation. A translational component of this study will correlate clinical outcomes with EGFR activation as determined by protein expression of EGFR and downstream signaling molecules in tumor biopsies taken at baseline. In addition, EGFR expression and downstream signaling will also be evaluated in the reacting skin and matched normal skin, in case of skin toxicity, with a comparison of data from patients who have not shown skin toxicity. For these translational studies, both tumor and skin biopsies are being collected.

postoperative setting

EORTC protocol no. 22071-24071, an intergroup study of the EORTC Radiation Oncology Group (ROG) and the EORTC-HNCG, which will be activated in 2010, is a multicenter phase III trial. A total of 800 patients with locoregionally advanced HNSCCs primarily treated with surgery and showing high-risk

features in the pathology specimen (i.e. positive or close surgical margins and/or extranodal spread) will be randomly assigned to receive postoperative cisplatin-based chemoradiation (current standard) or the same postoperative chemoradiation combined with panitumumab (a fully human immunoglobulin G2 monoclonal antibody targeting the EGFR). This study will include a number of TR projects. First, both paraffin-embedded and frozen tumor material, as well as blood and serum samples, will be collected and stored in a central biobank. This collected material can be used in the future for additional TR projects. Secondly, HPV DNA status and protein expression of biomarkers representing key molecules of the EGFR signaling pathway will be correlated with response to panitumumab in the entire patient population. Thirdly, the value of two predictive assays for treatment-related toxicity will be tested including individual intrinsic radiosensitivity by radiation-induced lymphocyte apoptosis, and the identification of single nucleotide polymorphisms (SNPs) associated with the development of normal tissue toxicities, to see whether these assays would (i) allow for identification of patients at risk for these side-effects, (ii) allow for subsequent selection of patients that are suitable candidates for preventive measures, and (iii) provide essential information for RT treatment optimization. Fourthly, 150 patients will be enrolled before surgery (in a so-called window study). In this pre-study cohort, gene expression signatures before and after the administration of a single panitumumab test dose will be correlated with 2-[fluorine-18]fluoro-2-deoxy-D-glucose–positron emission tomography (PET) response.

research areas of the HNCG Translational Research Subcommittee

human papillomavirus

In addition to cervical cancer, the most widely acknowledged HPV-associated malignancy, HPVs, especially type 16, are implicated in the development of a subset of OPCs, particularly in individuals that lack the traditional risk factors of tobacco and alcohol abuse and mainly restricted to tonsillar OPCs. Surveillance, Epidemiology, and End Results data have demonstrated a rise in the annual incidence of base-of-tongue and tonsil cancers by 2.1% and 3.9%, respectively, from 1973 to 2001 among white individuals aged 20–44 years, whereas the incidence at other sites declined [14, 15] An increased incidence of sexual behaviors associated with viral transmission through this period, as shown by the increase in herpes simplex virus 2 seroprevalence over this time period, has been implicated for this change in demographics. Licitra et al. [16] reported on the incidence and survival outcomes of HPV-related and HPV-unrelated HNSCC sites from 15 European (Sweden, Austria, Slovenia, Scotland, Wales, Poland, Germany, the Netherlands, Switzerland, Italy) population-based cancer registries including 29 265 adult patients with cancer diagnosed in the period from 1988 to 2002. Incidence rates of HNSCCs increased more for HPV-related than HPV-unrelated cancer subsites. Three-year survival rates improved more in HPV-related than in HPV-unrelated cancer anatomic subsites. Along with epidemiological

evidence, experimental evidence also supports a causal role of HPV in a subset of head and neck cancers. Rampias et al. [17] showed that repression of HPV E6 and E7 oncogene expression leads to apoptosis and restoration of p53 and pRb tumor suppressor pathways in oropharyngeal squamous carcinoma cells.

HPV-associated HNSCCs are associated with better prognosis compared with stage-matched HPV-negative ones in the majority of studies [5, 6, 18–23]. HPV positivity confers a 60%–80% reduction in risk of death from cancer relative to similarly treated HPV-negative tumors. Licitra et al. [18] found in a retrospective series of 90 patients with OPC treated primarily with surgery that HPV-positive status significantly affects OS ($P = 0.0018$), incidence of tumor relapse ($P = 0.0371$), and second primary tumors ($P = 0.0152$). It has also been shown that organ preservation strategies may be more successful in HPV-associated OPCs compared with HPV-negative ones [24]. It is important to emphasize, however, that at present the contribution of the different therapeutic choices to the survival benefit observed for the HPV-positive patient remains unclear. In fact, literature data indicate that when HPV-positive patients are treated similarly to age- and stage-matched HPV-negative patients, the survival benefit observed is independent of the type of therapy administered. Therefore, patients with HPV-positive tumors may unnecessarily receive treatments that significantly increase morbidity. Treatment deintensification in the HPV-positive subgroup remains an important research question. Clinical trials that stratify to more or less intense therapy based on HPV status are only now being undertaken.

Weinberger et al. [5] identified p16 protein expression by immunohistochemistry (IHC) as a surrogate marker for biologically and clinically relevant HPV infection. HPV DNA detection by itself in HNSCC does not prove a causal association. Only transcriptionally active HPV DNA is biologically and clinically relevant in the causation of HNSCC. They sought to determine the incidence and clinical implications of biologically relevant HPV16 infection in a cohort of 107 patients with oropharyngeal squamous cell cancers treated primarily with RT or surgery followed by postoperative RT at Yale University [5]. HPV16 DNA viral load was determined by real-time PCR. In addition, they constructed a tissue array composed of these tumors and studied expression of p53, pRb, and p16 proteins using a quantitative *in situ* method of protein analysis [automated quantitative analysis (AQUA)]. They hypothesized that among HPV16-DNA-positive cases, p16 expression status would determine the biologically relevant ones. Their results delineated three tumor classes with distinct molecular and clinical features based on HPV16 DNA presence and p16 expression status: HPV16 negative/p16 nonexpressing (class I), HPV16 positive/p16 nonexpressing (class II), and HPV16 positive/p16 expressing (class III) oropharyngeal tumors. OS in class III was 79% compared with the other two classes (20% and 18%, $P = 0.0095$). Disease-free survival rates for the same classes were 75% versus 15% and 13% ($P = 0.0025$), respectively. The 5-year local recurrence was 14% in class III versus 45% and 74% in the other two classes ($P = 0.03$). Only patients in class III had significantly lower p53 and pRb expression ($P = 0.017$ and 0.001, respectively). Multivariate

survival analysis confirmed the prognostic value of the three-class model. They demonstrated that only the HPV16-positive/p16-expressing tumors fit the cervical carcinogenesis model and these are the ones associated with a more favorable prognosis.

These findings were confirmed in two large prospective studies [25, 26]. In the first study, patients with previously untreated stage III or IV head and neck squamous cell cancer were randomized to receive definitive RT concurrently with either cisplatin or cisplatin plus tirapazamine. Slides were available for HPV assay [in situ hybridization (ISH) HPV16/18] in 195 patients and for p16 in 186 patients, and for both in 173 patients. Fifty-four of 195 (28%) tumors were HPV positive, 107 of 186 (58%) p16 positive. HPV-positive tumors were associated with a better 2-year OS (94 versus 77%, $P = 0.007$) and a better failure-free survival (FFS; 86 versus 75%, $P = 0.035$) compared with HPV-negative tumors. Similarly, p16-positive tumors were associated with a better 2-year OS (92 versus 75%, $P = 0.004$) and FFS (87 versus 72%, $P = 0.003$) compared with the p16-negative ones. The second study was conducted by Radiation Therapy Oncology Group in the USA [26]. A correlative study was carried out to evaluate the association of tumor HPV status (THS) and survival in a randomized phase III trial comparing standard fractionation (FX) RT and cisplatin (100 mg/m^2, days 1, 22, and 43) with accelerated FX-RT and cisplatin (100 mg/m^2, days 1 and 22). THS for OPC was determined by HPV16 ISH. THS was evaluable for 73% (317 of 433) of OPC cases and 60.6% (55.2–65.9) were HPV16 positive. OS or progression-free survival (PFS) outcomes were similar for cases with and without HPV determination. After median follow-up of 4.4 years, cases with HPV-positive OPC had a better OS [$P < 0.0001$; 2-year 87.5% (82.8–92.2) versus 67.2% (58.9–75.4)] and PFS [$P < 0.0001$; 2-year 71.9% (65.5–78.2) versus 51.2% (42.4–59.9)]. Patients with HPV-positive OPC had a 59% reduction in risk of death [hazard ratio (HR) 0.41 (0.27–0.64)] and a 46% reduction in risk of progression or death [HR 0.54 (0.37–0.78)]. Ninety-six percent of HPV-positive tumors were p16 positive. p16 status was the most important prognosticator of outcome. p16 positivity conferred a 65% reduction in the risk of death, whereas HPV positivity conferred a 55% reduction in the risk of death. These results may be explained by the presence of other HPV subtypes that were not assessed in the study. Contrary to the findings of Sant et al. [2], in these two later studies most of the HPV-positive cases were p16 positive. This can be explained by the fact that HPV ISH, by detecting integrated HPV DNA, is a more specific assay than real-time PCR.

Smeets et al. [27] developed a detection algorithm for a biologically and a clinically meaningful HPV infection. The authors considered HPV E6 oncogene expression in frozen biopsies as a gold standard for biologically meaningful HPV infection and they tested the value of the following assays on formalin-fixed, paraffin-embedded (FFPE) tumor specimens and sera of 48 patients with HNSCC: HPV DNA general primer (GP) 5+/6+ PCR, viral load analysis, HPV16 DNA FISH detection, HPV16 E6 messenger RNA (mRNA) RT-PCR, and p16 immunostaining, and on corresponding serum sample detection of antibodies against the HPV16 proteins L1, E6, and

E7. Most suitable algorithm with 100% sensitivity and specificity appeared p16 immunostaining, followed by GP5+/6+ PCR on the p16-positive cases. Taken together, the incorporation of p16 IHC to the existing protocols for determination of HPV DNA presence may distinguish the transcriptionally active HPV-positive OPC.

To summarize, the current evidence supports the conclusion that THS is an important and independent prognostic factor for OS and disease-specific survival for HNSCC. p16 protein status is also an important and independent predictor of OS and disease-specific survival for HNSCC. Future study design and data analysis should acknowledge the unique natural history and prognosis of this patient subgroup and incorporate HPV DNA and p16 protein status into the next generation of clinical trials. The EORTC-HNCG plans to retrospectively evaluate the TAX323 study cohort for tumor HPV and p16 status in association with treatment outcome per study arm. This retrospective analysis will provide us with important information that will be validated in the setting of a prospective three-arm trial (first arm: TPF followed by anti-EGFR + RT versus second arm: cisplatin + RT versus third arm anti-EGFR + RT) planned by the EORTC-HNCG. This analysis will demonstrate whether the addition of docetaxel to PF provides additional benefit in the HPV+/p16+ patient subgroup. The EORTC 22071-24071 postoperative study will also undergo prospective HPV/p16 determination to demonstrate whether HPV+/p16+ patients at high risk for recurrence derive additional survival gain with the incorporation of panitumumab to standard postoperative cisplatin-containing chemoradiotherapy regimens.

signaling through the EGFR

The EGFR is a member of a receptor family known as the type I receptor tyrosine kinases or ErbB receptors. This receptor family includes the following four related receptors: EGFR (ErbB1/EGFR/HER1), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4). EGFR is a 170-kDa plasma membrane glycoprotein that consists of an extracellular ligand-binding domain, a hydrophobic transmembrane domain, and an intracellular protein kinase domain with a regulatory COOH terminal segment [28]. Ligand binding promotes receptor dimerization leading to high-affinity ligand binding, activation of the intrinsic protein kinase activity, and tyrosine autophosphorylation. These events activate a signal transduction cascade that is mitogenic and transforming.

Several lines of evidence support the conclusion that EGFR is a molecular target for therapy of HNSCC. First, overexpression of EGFR is one of the most frequent molecular alterations in HNSCC [29]. The level of EGFR expression in HNSCC is elevated compared with its expression on normal adjacent squamous mucosa in 83%–100% of cases. Secondly, increased EGFR content is often correlated with an increased production of ligands, such as transforming growth factor alpha, by the HNSCC [29]. Furthermore, treatment with EGFR-targeted therapy such cetuximab (Erbitux) inhibits EGFR signaling and sensitizes cancer cells to chemotherapy or radiation [30–32]. In an international randomized phase III trial in patients with

inoperable locally advanced disease, the addition of cetuximab to radiotherapy prolonged time to locoregional recurrence and survival compared with radiotherapy alone [30]. The overall toxicity profile was dominated by traditional known effects of curative head and neck radiation dose, although some additional, mainly skin, toxicity was attributed to cetuximab. In the recurrent/metastatic setting, a European randomized phase III trial (EXTREME study) examined the addition of cetuximab until disease progression in patients receiving six cycles of cisplatin or carboplatin/5-fluorouracil chemotherapy regimen as first-line treatment in recurrent or metastatic HNSCC and showed a significantly improved survival in cetuximab-treated patients [31]. Cetuximab alone is an acceptable second-line therapy in recurrent or metastatic HNSCC [33]. Taken together, abrogation of EGFR signaling appears to be an effective therapeutic strategy in HNSCC.

A fundamental question in EGFR-targeted therapy has been patient selection since the intensity of EGFR staining by IHC has not been closely associated with the response rate and other outcome measures. Studies evaluating EGFR expression in tumor tissues are limited by the technical difficulties inherent in assessing EGFR conventionally such as variability in immunohistochemical techniques, different methods of pathologist-based scoring, and the semiquantitative nature of the assay. To overcome this problem, a method of in situ AQUA has been developed, which allows measurements of protein expression within subcellular compartments that results in a number directly proportional to the number of molecules expressed per unit area. Thus, we avoid biases introduced from the arbitrary cut-off points used in conventional IHC. Psyrri et al. [34] analyzed a cohort of 95 patients with OPC on a tissue microarray for EGFR protein expression levels using AQUA and correlated those with clinical and pathological data. High nuclear and tumor EGFR protein levels were associated with significantly higher local recurrence rates and inferior disease-free survival and OS times. The finding that nuclear EGFR is also a significant prognostic indicator is consistent with data supporting nuclear localization and action of EGFR. Lin et al. [35] showed that EGFR may enter directly the nucleus and function as a transcription factor bypassing protein phosphorylation cascades. EGFR AQUA score may prove useful in predicting response to EGFR-targeted therapies. Assessment of EGFR gene copy number has been associated with response to EGFR-targeted therapies in other tumors. Chung et al. [36] reported that increased EGFR gene copy number by gene amplification or high polysomy using FISH was a frequent genetic alteration in a cohort of patients with HNSCC and was strongly associated with worse recurrence-free survival and OS. FISH analysis of specimens from the EXTREME study for EGFR gene amplification or polysomy failed to show an association between FISH positivity and outcomes in cetuximab-treated patients [37, 38].

Mechanisms of resistance to EGFR-targeted therapies have extensively been studied in other tumor types. In lung cancer for instance, catalytic domain EGFR mutations predict for sensitivity to small-molecule tyrosine kinase inhibitors. In colon cancer, KRAS and BRAF mutations predict for resistance to cetuximab. These mutations are rare in patients with head and neck cancer. At this point, the mechanisms of resistance

to EGFR-targeted therapies in patients with head and neck cancer are largely unknown. Potential mechanisms of resistance include the following: (i) constitutive up-regulation of downstream targets of EGFR (i.e. the downstream target is no longer regulated by EGFR), (ii) compensatory up-regulation of redundant receptor tyrosine kinases (RTKs) that signal through common effectors (pAKT is one such effector). Benavente et al. [39] have shown constitutive activation of MET and ErbB3 RTKs in cetuximab- or erlotinib-resistant head and neck and lung cancer cell lines. Seiwert et al. [40] demonstrated a greater-than-additive inhibition of cell growth by combining a MET inhibitor with erlotinib (small-molecule tyrosine kinase EGFR inhibitor) and synergy was mediated via ErbB3/AKT signaling. (iii) Ligand-independent signaling (i.e. EGFRvIII): EGFRvIII is a mutant receptor with an in-frame deletion of the extracellular domain that renders the receptor constitutively active despite its inability to bind EGF. EGFRvIII expression was detected in 42% of HNSCC where EGFRvIII was always found in conjunction with wild-type EGFR [41]. HNSCC cells expressing EGFRvIII showed increased proliferation in vitro and increased tumor volumes in vivo compared with vector-transfected controls. EGFRvIII-transfected HNSCC cells showed decreased apoptosis in response to cisplatin and decreased growth inhibition following treatment with cetuximab compared with control cells. (iv) Transcriptional regulatory mechanisms that control EGFR expression: single-nucleotide polymorphisms (SNPs) in EGFR promoter region [i.e. GC (EGFR*1)] or common CA dinucleotide repeat in intron 1 of EGFR affects EGFR mRNA levels. Higher promoter activity and stronger mRNA expression have been observed in the non-GC-containing haplotype and this haplotype may be associated with greater sensitivity to gefitinib. Shorter number of CA dinucleotide repeats in intron 1 of EGFR was associated with greater in vitro sensitivity to erlotinib in 14 head and neck cancer cell lines [42]. (v) Inhibition of the ubiquitin-mediated degradation of EGFR: the chromosome 11q13 region is frequently amplified in HNSCC (36%) and results in an increased expression of cortactin [43, 44]. Cortactin acts as an important regulator of the actin cytoskeleton and mediates the invasive potential of tumor cells [45]. Cortactin also participates in receptor-mediated endocytosis and its overexpression inhibits the ubiquitin-mediated degradation of EGFR, resulting in a sustained ligand-induced EGFR activity [45].

Chung et al. [46] have shown that a matrix-assisted laser desorption/ionization mass spectrometry (MS) profile in serum or plasma can predict HNSCC survival after treatment with EGFR inhibitors (EGFRI).

Using both the tumor and the skin specimens from EORTC 24061, the predictive value of ErbB signaling on the clinical outcome of patients with HNSCC treated with a TPF induction regimen combined with cetuximab in one of the treatment arms will be tested. The tumor specimens from EORTC 22071-24071 offer a valuable opportunity to probe the molecular profile of patients with HNSCC who have all received panitumumab in their treatment. A gene expression classifier before and after administration of a single panitumumab dose will be correlated with PET response. These studies offer a unique opportunity to identify gene expression changes predictive of response to

EGFR-targeted therapy. If activation of specific pathways is associated with resistance, then targeting these pathways will reverse resistance to EGFR-targeted therapies.

prognostic molecular signatures using genomic and proteomic profiling

Several research groups have attempted to classify patients with HNSCC in terms of prognosis using genomic and proteomic approaches. The most commonly used genomic platforms are DNA microarrays. This technology has been very effective in defining prognostic tumor subsets in breast cancer and lymphomas. A major limitation of its applicability in clinical trial specimens had been the requirement for frozen tissue material. Recently, a successful technology for extraction of RNA from FFPE tissues has been developed. Chung et al. [4], using complementary DNA (cDNA) microarray technology in 60 fresh frozen HNSCC samples, categorized these tumors into the following four distinct subtypes with statistically significant differences in recurrence-free survival: a subtype with a possible EGFR pathway signature, a mesenchymal-enriched subtype, a normal-epithelium-like subtype, and a subtype with high levels of antioxidant enzymes. This signature was validated in an independent cohort of 40 patients with HNSCC and the RNA used was extracted from FFPE tumors [47]. Therefore, global gene expression analysis is feasible using formalin-fixed tissue. As discussed previously, cDNA microarray technology will be used to determine the gene expression signature predictive for response to panitumumab in the postoperative EORTC 22071-24071 clinical trial.

To carry out comprehensive analysis of genomic abnormalities in HNSCC, various platforms are available such as whole-genome array comparative genomic hybridization (CGH) analysis, structural variation analysis/SNP array screening, and chromosome- or gene-specific CGH arrays. Gibcus et al. [43, 48] reported on the identification of several HNSCC-specific abnormalities, with chromosome 11q13 amplification as the most common. Structural analysis of the 11q13 amplicon combined with expression analysis of the genes located in the amplicon revealed that cyclin D1, cortactin, and fas-associated protein death domain are interesting candidate genes to mediate resistance to chemoradiation treatment in HNSCC [49]. Therefore, in parallel, gene expression signature confined to regions with chromosomal abnormalities will be tested as predictors for response to panitumumab in the postoperative EORTC 22071-24071 clinical trial.

The field of biomarker discovery has been enriched with the microRNA microarray technology. The widespread use of microRNA microarrays has enabled the identification of a number of microRNAs as potential biomarkers for cancer in both formalin-fixed tissue and blood [50]. Many microRNAs function as oncogenes, tumor suppressors, or modulators of cancer stem cells and metastasis. Studies have not only identified microRNA biomarkers but also found their target genes. MicroRNAs have also been implicated in resistance to EGFR-targeted therapies [51].

In terms of proteomics, the most commonly used platforms include tissue microarrays (TMAs), MS-based assay, and protein arrays. TMA technology has emerged as a very useful tool in facilitating biomarker validation; it is a method used to analyze hundreds (or even thousands) of tissue specimens on a single TMA slide. The most widely used end application for TMA is IHC, although the technique can be coupled with other molecular biology methods including ISH or *in situ* PCR. Essentially, a TMA is made up of individual cylindrical 'cores' taken from representative areas of each sample in a cohort. These cores are then arrayed in an orderly grid into a recipient paraffin block. Subsequent tissue sections are then made of this recipient block and processed identically to traditional whole-section slides. The advantages of TMA over individual whole-slide sections are several. First, all specimens are treated to identical conditions throughout the IHC process. In a TMA, all spots are being incubated simultaneously, thus greatly reducing variability that might obscure true findings. The second advantage is the practicality of this approach due to the utilization of FFPE tissues. Last but not least, another advantage of TMA is cost and time savings. TMAs have been used to validate immunohistochemical expression profiles of proteins that have been indicated as predictors for laryngeal carcinoma [52, 53]. Using AQUA protein analysis on TMAs composed of annotated HNSCC cohorts, Psyrri et al. have identified several prognostic biomarkers as well as biomarkers that distinguish HPV-positive versus HPV-negative HNSCCs [34, 54–57]. TMAs will be constructed from tissue specimens of ongoing EORTC clinical trials and will be used for biomarker validation. MS and protein arrays require frozen tissues and will be more widely used in the future.

molecular signatures: predictors of toxicities

In recent years, the data imply a genetic basis for a susceptibility to the development of radiation injury after (chemo)radiotherapy [52, 53, 58]. A recent study [59] examined whether patients with severe radiation-induced sequelae (RIS) both show a low capacity of radiation-induced CD8 lymphocyte apoptosis (RILA) *in vitro* and bear certain SNPs located in candidate genes associated with the response of cells to radiation. DNA was extracted from blood samples obtained from 399 patients enrolled in the Swiss prospective study evaluating the predictive effect of *in vitro* RILA and RIS. SNPs in the *ATM, SOD2, XRCC1, XRCC3, TGFB1,* and *RAD21* genes were screened in patients who experienced severe RIS (group A, $n = 16$) and control subjects who did not manifest RIS (group B, $n = 18$). Overall, 13 and 21 patients were found to possess a total of less then four and four or more SNPs, respectively, in the candidate genes. The median (range) RILA in group A was 9.4% and 94% of the patients (15 of 16) had four or more SNPs. In group B, median (range) RILA was 25.7% and 33% of patients (6 of 18) had four or more SNPs ($P < 0.001$). These findings imply that patients with severe RIS bear four or more SNPs in candidate genes and display low RILA *in vitro*. Mahmut Ozsahin will attempt to validate these findings in the EORTC 22071-24071 study. This study has been selected to receive financial support from the EORTC. In the same study, SNPs in candidate genes will also be screened, and this study will also be financed by the Swiss National Funds.

strategies within EORTC to facilitate TR

The EORTC Translational Research Advisory Committee (TRAC) will promote TR with the following mechanisms: (i) availability of large numbers of clinical specimens from clinical trials, (ii) team expertise, and (iii) funding through EORTC.

tissue banking

EORTC is developing adopted standardized protocols for specimen collection, storage, and processing. Kits used with that contain the required materials and detailed instructions. The aim of the EORTC tissue bank is to provide qualified researchers with clinically annotated tissue specimens.

The specimens are sent to the central repository, de-identified of personal information coded with the study and case number, and stored. Samples are reviewed by the pathologist to ensure that tumor tissue is included in the specimen. Collected material includes unstained slides, tissue blocks, frozen tissue, and fluids such as plasma. These specimens become available to the researchers after submission and endorsement of proposals to the EORTC TRAC. These proposals are reviewed by the EORTC for scientific merit, sample availability, and statistical validity.

EORTC NOCI group

EORTC NOCI consists of 23 high-level cancer centers highly involved in EORTC activities, which have signed a consortium agreement to conduct TR. NOCI institutions have been selected for their earlier accomplishments and successful work in various EORTC groups. The principal goal of NOCI is contacting the most challenging research studies in the field of phase II trials of novel compounds and bridging the gap from laboratory to the clinic by carrying out TR studies. The work within NOCI group involves also mutual collaboration of all EORTC groups covering cancer sites and oncology branches. Having organized the network NOCI, EORTC proved once more the organization's efforts to carry out top-quality clinical research and its commitment to translational clinical research in oncology.

The NOCI call at EORTC Group Annual Meetings (EGAM) was conceived to promote EORTC scientific strategy and NOCI projects. The EORTC Board has allocated support for this initiative to promote clinico-genomic trials that cannot be adequately supported by the pharmaceutical industry. In 2009, eight proposals representing nine EORTC groups were presented at a session dedicated to this call for NOCI projects at the 2009 EGAM. The criteria used to evaluate the proposals were as follows: originality, innovation and adherence to EORTC scientific strategy, the potential impact of the study on clinical practice, the strength of the TR component, the suitability of the methodology, the feasibility, and statistical robustness. Three proposals were selected to receive grants including one proposal from the EORTC-ROG and the EORTC-HNCG: "Radiation-induced normal tissue toxicities in patients to be included in the randomized phase III trial on postoperative chemoradiation in combination with anti-EGFR antibody versus postoperative chemoradiation alone in head and neck squamous-cell carcinomas with high risk of locoregional recurrence (Protocol 22071-24071)" presented by Mahmut Ozsahin.

collaboration between individual groups and the Translational Research Division

The Translational Research Division (TRD) consists of basic scientists focusing on cancer research. Personalized cancer care requires treatment tailoring depending on the molecular profile of the individual tumor and a close collaboration between scientists and clinicians becomes more urgent. The division includes the EORTC Pharmacology and Molecular Mechanisms, the PathoBiology Group, and the functional imaging group during the EGAM. This collaboration aims to identify basic science discoveries 'ripe' for translation and accelerate prioritized opportunities.

funding mechanism

The scientific merits of proposals are evaluated based on the scientific, statistical, and financial information provided in the TR project application form to justify the requested use of tissue bank specimens and funding (Figure 1). Proposals that seek to validate biomarkers and have applicability to future clinical trials are deemed as high priority. Hypothesis generating projects that bring innovative research and appear promising are also considered.
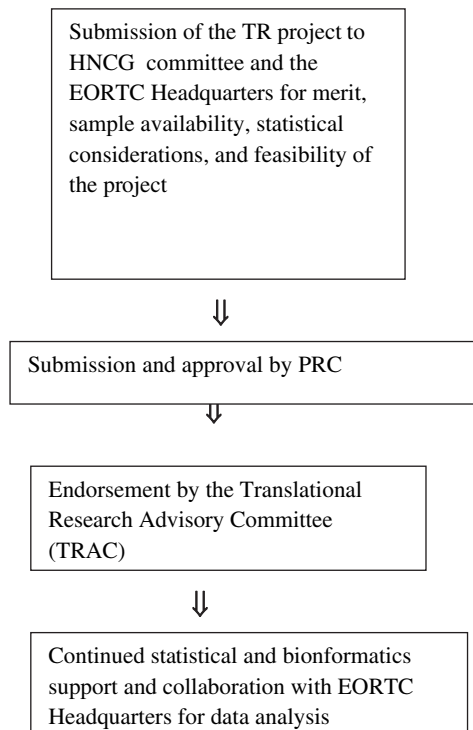

Submission of the TR project to HNCG committee and the EORTC Headquarters for merit, sample availability, statistical considerations, and feasibility of the project

⇓

Submission and approval by PRC

⇓

Endorsement by the Translational Research Advisory Committee (TRAC)

⇓

Continued statistical and bioinformatics support and collaboration with EORTC Headquarters for data analysis

Figure 1. Schema for the translational research program application review. EORTC, European Organisation for Research and Treatment of Cancer; HNCG, Head and Neck Cancer Group; PRC, project review committee; TR, translational research.

review

bioinformatics and statistical support

EORTC will provide research support in experimental design, bioinformatics, and statistics through the statistics group at EORTC Headquarters. Projects using genomic and proteomic platforms require strong support in bioinformatics.

conclusion/future perspectives

The substantial progress in head and neck cancer research in research laboratories has not yet resulted in the translation of the findings to the clinic. Advances in molecular biology such as the 'omic' approaches have demonstrated distinct prognostic head and neck cancer patient subsets beyond those defined by traditional clinical–pathological factors. Validation of these biomarkers in large prospective clinical trials is required before their clinical implementation. The strategy of translating these research findings into population-based, multi-institutional EORTC clinical trials for subsequent clinical application remains a challenge. The head and neck cancer TR subcommittee at the EORTC is committed to accelerate the translation of prioritized basic science discoveries. The construction of a tissue/serum bank of clinically annotated samples combined with the coordinated efforts of the EORTC-HNCG and the TRD aims to accelerate TR and personalize head and neck cancer management.

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