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Strategic plans to promote head and neck cancer translational research within Radiation Therapy Oncology Group: A report from the Translational Research Program

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Abstract

Head and neck cancer is the fifth most common cancer in the U.S. with an overall survival rate of approximately 40–50%. In an effort to improve patient outcomes, research efforts designed to maximize benefit and reduce toxicities of therapy are in progress. Basic research in cancer biology has accelerated this endeavor and provided preclinical data and technology to support clinically relevant advances in early detection, prognostic and predictive biomarkers. Recent completion of the Human Genome Project has promoted the rapid development of novel “omics” technologies that allow more broad based study from a systems biology perspective. However, clinically relevant application of resultant gene signatures to clinical trials within cooperative groups has advanced slowly. In light of the large numbers of variables intrinsic to biomarker studies, validation of preliminary data for clinical implementation presents a significant challenge and may only be realized with large trials that involve a significant patient numbers. The Radiation Therapy Oncology Group (RTOG) Head and Neck Cancer Translational Research Program recognizes this problem and brings together three unique features to facilitate this research; 1) availability of large numbers of clinical specimens from homogeneously treated patients through multi-institutional clinical trials, 2) a team

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of physicians, scientists and staff focused on patient-oriented head and neck cancer research with the common goal of improving cancer care, and 3) a funding mechanism through the RTOG Seed Grant Program. In this position paper we outline strategic plans to further promote translational research within the framework of the RTOG.

Keywords

head and neck cancer; RTOG; biomarkers; translational research; funding

INTRODUCTION

Clinical trials of combined modality therapy or radiation dose intensification have led to incremental improvement in head and neck cancer outcomes, resulting in improved local control, organ preservation, and/or survival. Radiation therapy (RT) remains a critical component of this success. Large cooperative oncology research groups like the Radiation Therapy Oncology Group (RTOG) have played a key role in the success of dose escalation and combined modality treatment approaches that involve RT. Despite these gains in optimizing cancer care, over 50% of head and neck cancer patients die of their disease in the U.S. (1). Furthermore, these successes come at a price of increased acute and/or late treatment-related toxicities, including treatment-related deaths in some cases. Thus, the risks and benefits of aggressive treatment should be assessed using a therapeutic ratio that balances the likelihood of treatment benefit or toxicity versus measured treatment intensity. The conceptual curves that estimate therapeutic ratio are based on the population averages of treatment benefit and the likelihood of side effects. These curves estimate the ratios by using traditional clinical parameters such as primary tumor site, histological features, tumor size, nodal status, patient's age, performance status and/or other co-morbidities. However, the biological composition of the tumors and the patients being treated are heterogeneous. Thus, relying on population averages is an imprecise method to predict for therapeutic success. Therefore, improved methods of predicting clinical outcomes and toxicity will ultimately maximize the therapeutic ratio for individual patients.

Biomarkers have been evaluated as surrogate endpoints to better understand and predict treatment effects as seen in the analyses of epidermal growth factor receptor (EGFR) (2). Furthermore, biomarker studies have usually been performed focusing on a handful of markers at any one time. However, this approach remains limited in its ability to represent the relevant molecular heterogeneity of a particular type of cancer. Recently, there has been rapid development of novel "omics" technologies that allow us to examine cancer as a whole in the view of systems biology. The "omics" technologies include various large-scale methods to profile each cellular component and their interactions including the genome, transcriptome, proteome, lipidome and/or metabolome (reviewed by Joyce, *et al.*; (3)). Combination of mechanism-driven biomarkers with well-established methods and innovative molecular signatures with still evolving "omics" technologies will accelerate understanding of cancer biology and provide clinically applicable biomarkers for early disease detection, prognostication, and treatment response assessment.

The application of these promising biomarkers to clinical trials within cooperative groups has been slow due to the fact that the validation studies require sufficient numbers of specimens with detailed clinical annotation from large population-based studies. Also, for "omics" studies, the bioinformatics methods for molecular signature analyses need non-traditional statistical approaches. In this position paper we will summarize ongoing and planned clinical trials, highlight correlative studies performed using specimens collected through the RTOG,

describe planned translational projects, and outline strategic plans to further promote future translational research within the framework of the RTOG.

CLINICAL TRIALS IN HEAD AND NECK CANCER AT THE RTOG

As the detailed description of each clinical trial is beyond the scope of this paper, we will highlight a few examples. The comprehensive information can be found at the RTOG web site (<http://www.rtog.org>). Head and neck cancer clinical trials at the RTOG provide unique opportunities to test hypothesis-driven translational research questions. RTOG 9003 provides an example of this paradigm. RTOG 9003 was a randomized phase III study comparing 4 different radiation fractionation regimens that completed accrual of 1113 patients with head and neck squamous cell carcinoma (HNSCC) in 1997 (4). To date six translational research studies have been published utilizing tissue from this study (2,5–7). Other studies utilizing tissue specimens from the RTOG 9003 are in progress, including studies examining radiation repair gene polymorphisms and the effects of Human Papilloma Virus (HPV).

Current RTOG clinical trials that incorporate chemotherapy and targeted agents further enhance opportunities in translational research. RTOG 0234 is a recently completed post-operative clinical trial in locally advanced HNSCC that examined different drug combinations of cetuximab/chemotherapy and concurrent RT. A translational component of this study will correlate clinical outcomes with protein expression of EGFR, phosphorylated mitogen-activated protein kinase (MAPK), phosphorylated protein kinase AKT, signal transducer and activator (STAT)-3, Ki67, COX-2 and Cyclin B1. An ongoing study, RTOG 0522, is a clinical trial for locally advanced stage HNSCC that examines the efficacy of concurrent cisplatin and cetuximab versus cisplatin alone with the radiation therapy. This study will examine the protein expressions of MAPK, AKT, STAT-3 and protein kinase C (PKC) as well as pretreatment PET imaging. Their results will be correlated with clinical parameters for validation as predictive biomarkers of outcomes.

Among the RTOG head and neck clinical trials that will soon be activated, RTOG 0615 and 0619 are highly interesting as they will permit investigation of biomarkers for angiogenesis as well as for normal tissue toxicity. RTOG 0615 will examine concurrent chemoradiation with bevacizumab, an anti-VEGF antibody, for nasopharyngeal carcinoma and RTOG 0619 will examine concurrent chemoradiation with or without vanitinib (ZD 6474, a dual EGFR and VEGFR tyrosine kinase inhibitor) for high-risk post-operative HNSCC.

DEVELOPMENT OF TISSUE-BASED MOLECULAR BIOMARKERS

In recent years there has been a tremendous effort to develop biomarkers for prognosis and prediction of clinical response to a given treatment by studying the difference between the normal tissue and tumors as well as the differences in the tumors across a cohort of patients. We have gained valuable insights in the development and validation of biomarkers and their application to subsequent clinical trials. We will describe a few examples of how biomarker studies can be further developed through the resources at the RTOG.

Epidermal Growth Factor Receptor (EGFR) as a prognostic biomarker

Epidermal Growth Factor Receptor (EGFR) is expressed in greater than 90% of HNSCC and has been shown to associate with poor prognosis (5,8). The advent of EGFR inhibitors including monoclonal antibodies (i.e. cetuximab and panitumumab) or tyrosine kinase inhibitors (i.e. gefitinib and erlotinib) has enabled therapeutic inhibition of EGFR activation. In a recent international randomized trial comparing radiation versus radiation plus cetuximab in HNSCC patients with locally advanced disease, a combination of radiation and cetuximab increased local-regional control and survival compared to radiation alone (9). Unlike the

addition of chemotherapy, only mild toxicities were observed with the addition of cetuximab to radiation therapy (9). However, the current standard of care for advanced stage HNSCC is concurrent chemoradiation rather than radiation therapy alone. Without a randomized trial comparing concurrent cetuximab/radiation versus chemoradiation, the benefit of the addition of cetuximab in comparison to chemoradiation in this patient population remains unclear.

Cetuximab was also studied in combination with either cisplatin or carboplatin in HNSCC patients with recurrent and/or metastatic disease, and the response rates were in the range of 10–13% in platinum-refractory patients (10–12). Gefitinib and erlotinib were also found to produce modest response rates as monotherapy in this patient population, in the range of 4.3–10% (13–15), although the disease control rate was much higher at 38–53% with minimal toxicities (13,14). Therefore, there is a clear clinical benefit of EGFR inhibition for a subset of HNSCC patients as monotherapy and in combination with radiation or conventional chemotherapies. Consequently, it is critical to identify biomarkers to help predict the likelihood of treatment benefit with anti-EGFR therapies. Such biomarkers would facilitate the selection of optimal patients and treatment regimens to maximize clinical benefit and lower toxicity.

The biomarker discovery process for EGFR has been challenging due to technical limitations of reagents and laboratory techniques. The data published on the correlation between the clinical outcomes of EGFR inhibitors and EGFR protein expression levels measured by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tumor sections have shown mixed results and have been difficult to interpret. In the samples from RTOG study 9003, Ang, *et al.* examined 155 tumors for EGFR expression levels using IHC. While there was no correlation with TNM staging and expression of EGFR, high EGFR expression was associated with lower overall and disease-free survival and a higher rate of locoregional recurrence (5). The strength of the study was the large sample size, a well-defined patient population, quantitative EGFR IHC and objective scoring of the stains using an automated system without the knowledge of the clinical data. Further, Chung, *et al.* examined increased *EGFR* gene copy number by gene amplification or high polysomy using Fluorescent *In Situ* Hybridization (FISH) and reported that 58% (43 of 75 tumors) of HNSCC tumors had FISH positivity (16). The FISH positivity was strongly associated with worse recurrence-free survival and overall survival. Although the patients in this study were not treated with EGFR inhibitors, it suggests that FISH may be one of the molecular techniques beneficial in patient selection.

The recent identification of catalytic domain EGFR mutations that predict sensitivity to small molecule tyrosine kinase inhibitors in a cohort of lung cancer patients represents a landmark development in the EGFR cancer therapeutic field (17,18). The infrequency of such mutations in head and neck cancer patients and the low relevance of these mutations for patients receiving anti-EGFR monoclonal antibody therapies indicate that other mechanisms must govern response and resistance to EGFR inhibition (19,20). Investigators have undertaken several innovative approaches to help identify biologic factors that may predict for response and resistance to anti-EGFR therapies. One experimental approach involves the establishment of resistant tumor cell lines to EGFR inhibitors following long-term exposure to EGFR inhibitors in culture and/or in animal model systems (21,22). Through rigorous comparative analysis of EGFR inhibitor-resistant versus sensitive tumors using high-throughput screening, specific molecular targets that may play a role in regulating response and resistance can be identified. Using an antibody based array to screen a panel of receptor tyrosine kinases (RTK), Harari, *et al.* (23) have identified constitutive activation of alternative RTKs including ErbB3 and c-Met in cetuximab- or erlotinib-resistant head and neck and lung cancer cells (Figure 1). Consistent with this finding, several recent reports show that constitutively active ErbB3 may contribute to resistance to EGFR inhibitors (24–26). These results suggest that activation of

alternative RTKs that bypass the EGFR pathway and/or activate signaling pathways downstream of EGFR may induce resistance to anti-EGFR therapies (Figure 2).

The tumor specimens from RTOG 0234 afford a valuable opportunity to probe the molecular profile of 230 HNSCC patients who have all received the EGFR inhibitor cetuximab in their treatment. Although specimens will not be available from all patients, it will still be a valuable sample set. This specimen cohort will enable screening for correlation between potential EGFR inhibitor-resistance markers and ultimate clinical outcome. Furthermore, approximately 35–50% of patients in this trial are expected to manifest eventual disease recurrence, thereby affording additional tumor specimens for repeat molecular analysis following EGFR inhibitor-based therapy with each patient effectively serving as his own control. This affords a unique clinical resource to compare underlying molecular alterations identified in the preclinical acquired resistance models with those occurring in patients who demonstrate tumor recurrence following EGFR inhibitor therapy. If the presence of activated RTKs such as ErbB3 or c-Met (23–26) are identified in tumors that manifest recurrence following treatment, for example, this may facilitate the design of new treatment strategies that incorporate ErbB3 and/or c-Met inhibitors in future trial designs for selected patients.

Human Papilloma Virus (HPV) as a prognostic biomarker

Reports of the prevalence of HPV infection indicate that 15–35% of HNSCC may harbor HPV DNA, depending on the detection method used (27–30). HPV is most commonly found in tonsillar and base-of-tongue tumors (approximately 50%) with HPV type 16 being found in the vast majority (>90%) (27,31). Evidence for the causal relationship between the presence of HPV and HNSCC incidence comes from prospective studies indicating increased risk for developing HNSCC in patients who are seropositive for anti-HPV antibodies. In a large, nested case-control study from a Scandinavian cohort of almost 900,000 individuals, HPV-16 seropositivity was observed on average 9.4 years prior to the onset of disease and was associated with a 14-fold increased risk of oropharyngeal HNSCC and an overall 2.2-fold increased risk for any HNSCC (29). Some studies have suggested that HPV not only represents a molecularly distinct disease but that it is also associated with a better prognosis (reviewed in (32)). This would have significant implications for treatment options for patients with HPV(+) tumors.

Differences in the molecular pathways that are altered between HPV(+) and HPV(–) tumors based on their gene expression analyses suggest that there may be differences in the response to RT and/or chemotherapy treatment in HNSCC (33). The evidence for an interaction between HPV and response to RT is limited. Data from a clinical trial suggested that 4 patients with HPV(+) tumors who did not receive RT had comparatively worse survival than the 39 patients with HPV(+) tumors who did receive RT, although this interaction did not reach statistical significance (34). Other studies support a beneficial effect of HPV(+) status on clinical outcomes. That is, HPV(+) HNSCCs appear to be more responsive to RT despite having higher nodal stage and other factors related to worse disease outcome (35,36). None of these studies, however, directly tested the possible interaction between the presence of HPV and radiation sensitivity. The availability of specimens from the RTOG trial 9003 will allow us to study this question in a large, well-controlled randomized study.

Hypoxic response as a biomarker of distant metastasis

The integration of modern radiation delivery, concurrent chemotherapy and altered fractionation has resulted in improved locoregional control in head and neck cancers. However, the incidence of distant metastasis has not changed over the years. Identification of molecular predictors for metastatic disease is of special interest to RTOG as it would help to define the role of induction or adjuvant chemotherapy in addition to current therapeutic approaches. Lysyl

Oxidase (LOX), an enzyme essential for the formation of the extracellular matrix, has been recently identified as a hypoxia and HIF-1 α (hypoxia inducible factor-1 α) regulated gene (37). LOX is mechanistically essential for hypoxia induced metastasis by affecting the invasive properties of hypoxic human adenocarcinoma and squamous cell carcinomas (37). Importantly, in a group of 101 patients with locally advanced HNSCC, those with high LOX expressing tumors as assessed by IHC had shorter Time-to-Metastasis and inferior overall survival compared to those with low or no expression (Figure 3). A proposal for confirming the relationship between LOX expression and distant relapse is currently under review by the RTOG Translational Group.

Global molecular signatures using genomic and proteomic profiling as biomarkers of prognosis in HNSCC

Despite the relative novelty of this technology, the ability to examine genomics using DNA microarrays and quantitative RT-PCR for generation of clinically relevant molecular signatures is now widely accepted in the scientific literature (38–53). While a detailed discussion of each study is beyond the scope of this paper, a comprehensive review regarding the clinical application of genomics and proteomics can be found in an article by Chung, *et al.* (54). However, we will present a few examples to support the validity and applicability of studying molecular signatures as clinical outcome predictors using samples from the RTOG clinical trials.

Many research groups have described the heterogeneous nature of HNSCC tumors at the molecular level and their characteristics using DNA microarray (39,55–59). They also have shown that different molecular characteristics associate with prognosis and metastasis, and identified molecular signatures to predict outcomes. A major limitation to implement these approaches to retrospective analyses of RTOG trials has been the requirement for fresh frozen tumors. The attempt to collect frozen tumors routinely on all large trials within RTOG is a relatively recent effort. However, the ability to utilize RNA isolated from formalin-fixed paraffin-embedded (FFPE) tissue represents a breakthrough in genomics. Recently Chung, *et al.* identified a gene expression signature that identifies head and neck cancer patients with a high-risk of recurrence using RNA isolated from FFPE tissues analyzed on DNA microarray, and the signature was validated in an independent data set (Figure 4 and Figure 5) (60). Therefore, using this approach, the implementation of genomic molecular signatures as a prognostic biomarker within the RTOG clinical trials is immediately applicable.

The most commonly used proteomic platforms are tissue microarray, mass spectrometry-based assay and protein arrays. Using a tissue microarray generated from 552 breast tumors and immunohistochemistry, a set of 21 proteins were identified as a prognostic profile (61). When the patients with poor- versus good-prognosis profiles were compared, the two groups were significantly different (37% vs. 9% metastatic relapse, respectively, $p < 0.0001$) in terms of 5-year metastasis-free survival. Similar studies in head and neck cancer can be done using tissue microarrays from samples collected during trials that are already available through the RTOG Translational Research Program and being used in several ongoing studies. The application of mass spectrometry-based assays and protein arrays requires frozen tissue specimens and should be increasingly possible in the future as frozen tissue collection effort gain momentum in the RTOG.

A molecular signature that can predict metastatic disease versus local recurrent disease would be of special interest to RTOG. For example, if molecular predictors demonstrate a high-risk of metastatic disease, chemotherapy in adequate doses would be a critical part of the regimen. On the other hand, patients with a high-risk of local failure may need an intensified RT regimen. Also, the ability to predict radiation resistance either alone or in combination with chemotherapy remains an important clinical goal. Radiation response in tumor cell lines can

be predicted based on gene expression profiles (62), and thus it is reasonable to pursue research strategies aimed at determining whether this observation applies to patients treated with RT. A molecular signature that predicts radiation response and toxicity could improve our ability to identify patients who will have the greatest therapeutic ratio for RT.

DEVELOPMENT OF BIOFLUID-BASED BIOMARKERS

Biomarkers in biofluids such as blood, saliva or urine hold great promise as both diagnostic and prognostic markers for head and neck cancers because of relative ease of obtaining the clinical samples. Therefore, biomarker studies using biofluid-based assays have an advantage in the ability obtain clinical samples through multi-institutional clinical trials. Several ongoing RTOG trials include collection procedures for saliva, serum and plasma, with kits provided to enhance collection feasibility. Circulating biomarkers may be nucleic acid- or protein-based. Below we described a few examples of such markers.

Epstein-Barr Virus (EBV) for nasopharyngeal carcinomas (NPC)

In NPC, circulating pre- and post-treatment EBV DNA has been shown to be a prognostic factor for patients treated with RT alone, induction chemotherapy followed by RT or concurrent chemoRT (63–65). Most of the EBV data were generated from Asia where NPC is endemic and these findings need to be replicated in western countries where the association between EBV and NPC is less clear, especially in patients with WHO grade I tumors. Thus, the relationship between circulating EBV DNA levels pre- and post-treatment to treatment response and outcome will be investigated in the upcoming Phase II trial, RTOG 0615, which will address the role of bevacizumab with concurrent chemoradiation in patient with locally advanced NPC.

Hypoxia markers

The tumor microenvironment plays an important role facilitating tumor cell growth in the progression of cancer. The microenvironment is complex and consists of stromal fibroblasts, inflammatory cells, components of the vasculature, normal epithelia, and extracellular matrix. It works in concert with tumor cells to promote tumor progression through the release of growth factors, cytokines, proteases and other bioactive molecules. These proteins can be shed and detected in the blood. Several hypoxia induced proteins have been found in patients' plasma and sera and pre-treatment protein levels were shown to correlate with prognosis in head and neck cancer patients. A few examples are vascular endothelial growth factor (VEGF), Osteopontin, Interleukin-6, Interleukin-8, Connective Tissue Growth Factor (CTGF), Transforming Growth Factor- α (TGF- α) and others (66–73).

However, the results of serum/plasma biomarker studies have often been mixed because of technical or biological noise and limitations in the detection methods. In order to validate their roles as prognostic markers in large sets of biomarker candidates, multiplexed and sensitive detection technologies with low sample consumption are required. Most conventional immunoassays rely on a solid support for capturing the target protein and for the removal of excess secondary reporter antibody by washing. These sandwich assays therefore have low sensitivity and specificity. This is especially challenging when performing multiplexed reactions with many detection antibodies, which requires extensive optimization through the careful selection of antibody combinations in order to minimize cross-reactivity. Proximity ligation is a novel method that was developed as a potential diagnostic tool to bridge the fields of protein and nucleic acid chemistry with the goal of improving sensitivity, specificity, dynamic range, and scalability into multiplex assays (74,75). The technology employs a pair of proximity probes each composed of an antibody linked to an oligonucleotide. As these two probes bind to the protein in solution, their proximity allows the oligonucleotides to hybridize

to each other. This is then followed by an enzymatic ligation uniting the 3'-end of the first probe with the 5'-end of the second probe, resulting to the formation of a target-specific reporter amplicon that contains a unique molecular barcodes (76). These molecular barcodes then serve as primer sites for quantitation by real-time PCR. This novel approach has been used to measure circulating cytokines with femtomolar detection sensitivities in low volume samples in the range of microliters (75). It can be used to study the expression of multiple markers in large numbers of RTOG plasma and serum samples to identify the most optimal cytokine and circulating protein profiles for prognostication and targeting.

Predictive markers for anti-angiogenic therapies

Vascular endothelial growth factors (VEGFs) are secreted molecules that can stimulate tumoral angiogenesis. A comprehensive review of VEGF family members and their receptors can be found in an article by Ferrara, *et al.* (77). High levels of circulating VEGF correlated strongly with high VEGFR-2 levels in patients with HNSCC and the expression of both ligand and receptor was associated with enhanced tumor proliferation and worse survival (78). In addition, enhanced VEGF expression was correlated with resistance to anti-EGFR inhibitors (79). These data provided mechanistic support for targeting the VEGF pathway in combination with either conventional therapy or anti-EGFR therapy. The development of a monoclonal antibody targeting VEGF, bevacizumab, has attracted considerable interest in cancer therapy due to its increased tumor specificity and potential for less treatment-related toxicity.

Positive phase III clinical trials with bevacizumab in colorectal, non-small cell lung cancers and renal cell carcinomas (80–82) have also spurred interests in its application to head and neck cancers. A phase I clinical trial of bevacizumab, 5-Fluorouracil, hydroxyurea and concomitant hyperfractionated RT was performed in 43 patients with locally advanced or recurrent HNSCC (83). A dose level of 10 mg/m² every 2 weeks was found to be tolerable for integration into this aggressive chemoradiation regimen and a randomized phase II study is ongoing to evaluate the efficacy of bevacizumab in a lower risk HNSCC patient population. Also, the combination of bevacizumab and erlotinib has been tested in patients with recurrent/metastatic HNSCC in phase I and II studies (84). Since no dose limiting toxicity was noted, a phase II randomized study was conducted using the highest dose level of 15 mg/kg every 3 weeks concurrently with oral daily erlotinib (150 mg/day); patients were randomized to receive bevacizumab on either day 1 or day 15 of the daily scheduled erlotinib dose. Although 3 serious hemorrhages were noted, only one was fatal and not related to the study drug. The median progression-free survival was 3.8 months and overall survival was 6.8 months. These studies suggest that combinations of anti-VEGF therapy with anti-EGFR or chemoradiation are reasonably safe. These trials formed the basis for a future RTOG phase II study, RTOG 0615, where bevacizumab will be added to cisplatin and RT in patients with locally advanced NPC.

In an effort to determine the biomarkers of treatment response in these trials, correlative studies were performed. In the phase I study by Seiwert, *et al.*, baseline plasma VEGF levels did not correlate with either survival or treatment response; however, measurement of VEGF levels during therapy was hampered by the presence of bevacizumab (83). Preliminary correlative studies from Vokes, *et al.* showed a decrease in circulating VEGF levels but increased TGF- α levels in the post-treatment sera. Unfortunately the baseline levels of these two markers did not correlate with either response or survival. Studies to detect circulating biomarkers to evaluate response to anti-angiogenic therapy are an active area of investigation in the RTOG TRP.

Molecular signatures as a predictive biomarker of toxicities

One of the promising applications of pharmacogenomic signatures generated from peripheral blood mononuclear cells is the ability to predict toxicity to a given treatment rather than using

a population-based clinical estimate. Polygenic pharmacogenomics, which studies the variations in DNA sequences as single nucleotide polymorphisms in a set of genes within common biological or metabolic pathways, represents another approach to predict these outcomes. In fact, these DNA sequence variations may be able to predict different clearance rates of certain drugs or differences in gene expression of drug targets (85). Similar techniques may also be applied to the field of metabolomics, which comprehensively studies the biochemical properties of metabolites in biofluids and can be applied to response and toxicity assessments (86). Again, these methods can be applied to currently available clinical specimens in RTOG. The large amount of clinical information that RTOG has already collected can be utilized to further mine the “omics” data and/or applied as validation sets for already identified molecular signatures.

STRATEGIES WITHIN RTOG TO PROMOTE TRANSLATIONAL RESEARCH

The head and neck cancer TRP committee at the RTOG will promote and facilitate comprehensive translation research by providing large numbers of clinical specimens from multi-institutional clinical trials, expertise within the TRP committee and funding through the RTOG Seed Grant. Detailed information is provided at the RTOG web site (<http://www.rtog.org>).

Specimen collection and allocation

One of the major obstacles to generate highly predictive and prognostic biomarkers is biological and technical “noise”. The biological noise is inherent and unavoidable from population-based studies such as RTOG clinical trials. However, studies of homogeneous patient populations with well-defined treatment regimens can mitigate the problem. The technical noise can be minimized by using clinical specimens that are properly collected and stored using standard operating protocols with accurate clinical annotation. The RTOG Tissue Bank was established with these concerns in mind. All specimens are collected according to standardized collection methods. Kits are provided that contain all the necessary materials and detailed instructions. Storage and processing procedures occur in a laboratory environment compliant with standard laboratory tissue handling practices that are CAP, JCAHO and HIPAA compliant.

The goal of the RTOG tissue bank is to provide clinically annotated specimens to qualified researchers. The specimens from cancer patients enrolled in the RTOG clinical trials are sent to the central repository, coded with the study and case numbers, de-identified of personal information and stored in a manner that can be available to the institution within 24 hours if necessary. Samples are reviewed by pathologists within 1 week of receipt to ensure that tumor tissue is included in the specimen. The collected specimens include unstained slides, tissue blocks, frozen tissue and fluids such as plasma or serum. The funding for the tissue collection effort is supported by the NCI RTOG contract. Specimen availability, identified by trial numbers, can be reviewed online. Proposals to utilize the resource are reviewed for scientific merit, tissue availability and statistical validity by various RTOG committees (Figure 6). The investigators must have funds to complete the research project. Once the merit of the project is determined, the samples are allocated using a defined process so that samples may be tracked.

Funding mechanism through RTOG seed grants

In an effort to promote translational research, funding opportunities are provided through the RTOG seed grant mechanism (Figure 6). The scientific merits of the proposals are evaluated based on the information provided in the RTOG Translational Research Program (TRP) Project Application Form. This includes scientific, financial and statistical information that is needed to justify the requested use of RTOG Tissue Bank specimens and funding. Research projects

regarding generation and application of molecular signatures will be reviewed by the Molecular Signatures Subcommittee within RTOG TRP. Projects that involve validation of molecular signatures for immediate clinical implementation and that demonstrate applicability to future RTOG clinical trials will be given the highest priority. However, hypothesis generating and innovative research that does not involve the clinically annotated samples in the RTOG tissue bank will also be evaluated for funding.

Bioinformatics and statistical support

Identification of informative molecular signatures requires strong support in informatics and statistics. RTOG will provide research support in experimental design, bioinformatics and statistics through the TRP Molecular Signatures Subcommittee and the statistics group at RTOG Headquarters. Because most of the primary research data to generate molecular signatures can be mined for numerous clinical parameters, the researchers will be required to deposit the primary molecular data generated from RTOG clinical specimens into the RTOG database once the proposed project is completed. For example, a primary gene expression data set used to validate a molecular signature that predicts recurrence after a given therapy can be mined as a discovery project to generate a new signature using a different supervising parameter such as mucositis as a discovery project. The database will be maintained at the RTOG headquarters and will be compatible with the NCI initiative, Cancer Biomedical Informatics Grid™ (caBIG™). This allows the cancer research community to share clinical data elements and primary research data for mining and to facilitate information exchange and applications using a common standard.

SUMMARY

Significant progress has been made in head and neck cancer research in a large number of research laboratories; however, difficulties persist in the translation of the data to the clinics. The “omic” technologies, in particular, are among the most powerful hypothesis-generating tools available today and have provided valuable new information in both basic and clinical research. Numerous biomarker studies generated from the comprehensive analyses of genetic information and their expression as DNA, RNA, proteins and metabolites can distinguish clinically relevant differences within histologically identical tumors and provide information about factors influencing treatment response, recurrence and survival for patients. The issue of how to translate these research findings into population-based, multi-institutional RTOG clinical trials for subsequent routine clinical use remains a challenge. The head and neck cancer committee at the RTOG is committed to contribute to the rapid progress through the promotion of translational research, effective collaboration and validation of biomarkers within the RTOG clinical trials and Translational Research Program.

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Figure 1. (A) Images from the phospho-Receptor Tyrosine Kinase (RTK) array depicting increased expression of p-ErbB3 and p-cMet in cetuximab-resistant (Cet-R) and erlotinib-resistant (Erl-R) cells. (B) Relative expression changes of p-RTKs in Cet-R and Erl-R cells as compared to corresponding parental cells (P and PD) following quantification of scanned images in (A).

Figure 2.

Schematic illustration depicts the activation of alternative receptor tyrosine kinases that bypass the EGFR pathway and/or activate signaling pathways downstream of EGFR that may induce resistance to anti-EGFR therapies.

Figure 3.

Time to metastatic progression and overall survival in 101 patients with locally advanced HNSCC by LOX protein expression (adapted from (37)).

Figure 4.

Comparison of DNA microarray signatures generated from 29 formalin-fixed and 6 matched-frozen head and neck squamous cell carcinomas. The matched samples are denoted with blue bars (adapted from (60)).

Figure 5.

Kaplan-Meier plot of recurrence-free survival based on the high and low-risk determination based on the molecular signature. A) 29 patients with the gene expression data from formalin fixed tissue. B) 60 patients with the gene expression data from frozen tissue (adapted from (60)).

Figure 6.
Flow chart for the translational research program application review.