NOVEL NEOADJUVANT IMMUNOTHERAPY REGIMEN SAFETY AND SURVIVAL IN HEAD AND NECK SQUAMOUS CELL CANCER

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Abstract: *Background.* Cellular immune suppression is observed in head and neck squamous cell cancer (HNSCC) and contributes to poor prognosis. Restoration of immune homeostasis may require primary cell-derived cytokines at physiologic doses. An immunotherapy regimen containing a biologic, with multiple-active cytokine components, and administered with cytoxan, zinc, and indomethacin was developed to modulate cellular immunity.

Methods. Study methods were designed to determine the safety and efficacy of a 21-day neoadjuvant immunotherapy regimen in a phase 2 trial that enrolled 27 therapy-naïve patients with stage II to IVa HNSCC. Methods included safety, clinical and radiologic tumor response, disease-free survival (DFS), overall survival (OS), and tumor lymphocytic infiltrate (LI) data collection.

Results. Acute toxicity was minimal. Patients completed neoadjuvant treatment without surgical delay. By independent radiographic review, 83% had stable disease during treatment. OS was 92%, 73%, and 69% at 12, 24, and 36 months, respectively. Histologic analysis suggested correlation between survival and tumor LI.

Conclusion. Immunotherapy regimen was tolerated. Survival results are encouraging. © 2011 Wiley Periodicals, Inc. *Head Neck* **33:** 1666–1674, 2011

Keywords: cytokine; head and neck squamous cell carcinoma; immune response; IRX-2; phase 2

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Head and neck squamous cell carcinoma (HNSCC) is common, representing about 6% of all cancers, with an estimated 644,000 new cases and 352,000 cancer deaths worldwide each year.[1] In the United States, it was estimated that head and neck cancers in 2008 would have accounted for 3.3% (47,560) of all new cancer diagnoses and 2% (11,260) of all cancer deaths.[2] Despite more aggressive treatments, the overall 5-year relative survival rate for HNSCC has not changed substantially, and remains at 50% to 59%.[3,4] New treatments with limited toxicity are needed that can enhance or replace current regimens, with the goal of improving the survival rate, preserving organ function, and enhancing the quality of life of patients with HNSCC.

Many patients with HNSCC are found to have defects in both innate and adaptive immunity that include dendritic cell and T cell dysfunction.[5–7] In patients with HNSCC, immunologic changes in lymph nodes have also been reported to be related to

survival,[8] whereas systemic T-cell counts are often low in HNSCC[6,9] and are negatively associated with survival.[10] Current treatments, including surgery, radiation therapy, and chemotherapy, further exacerbate cellular immune deficiencies. Attempts at modulating the immune system to treat cancer have been attempted for almost a century with only limited success. However, with the recent growth in understanding of tumor biology and immune regulatory function, new approaches have been developed over the last decade that seem to have increased potential for success. Early studies with immunomodulatory cytokines, such as interleukin 2 (IL-2), have had mixed results and have relied on traditional high-dose drug testing paradigms, which may not be the best application of these biological agents, given their adverse effects and the fact that biological systems often function within physiologic ranges where more is not necessarily better. These observations identify HNSCC as a good candidate for immunotherapy.

In an attempt to restore immune homeostasis and potentially enhance an immunologically-mediated antitumor response, we conducted a multicenter phase 2 clinical trial of a novel neoadjuvant immunologic approach based on subcutaneous injections of physiologic quantities of multiple cytokines combined with low-dose immunomodulatory cyclophosphamide, indomethacin, and zinc.

The major component of this immunotherapy regimen (IRX-2, IRX Therapeutics, New York, NY) is a primary cell-derived biologic, containing physiologic quantities of T helper type 1 (TH_1) cytokines and monokines, that has been shown in preclinical studies to enhance cell-mediated immune response.[11] The primary active components are interleukin (IL)-2, IL-1β, gamma interferon (γ-IFN), and tumor necrosis factor alpha (TNF-α).[12] These components are produced in vitro from lymphocytes and monocytes by stimulation with phytohemagglutinin. Preclinical studies in immunodeficient animal models have found an increase in both CD4+ and CD8+ T cells after treatment with such human, primary cell-derived cytokines.[11] To achieve better cytokine efficacy in patients with multiple defects in cellular immunity, the design of the immunotherapy regimen includes additional drug strategies to enhance immune responsiveness. Low-dose cyclophosphamide is used because it has been shown to enhance cell-mediated immune response by depleting and inhibiting immunosuppressive regulatory T cells.[13] The use of non-steroidal anti-inflammatory drugs such as indomethacin has also been demonstrated to activate immune responses and increase tumor infiltration in patients with cancer by reducing the immune suppressing effect of prostaglandins.[14,15] Zinc supplementation is added because the trace metal zinc plays an important role in the development and function of cellular immunity.[16] Furthermore, zinc deficiency, observed in 50% of patients with HNSCC, is associated with increased tumor size and higher overall cancer stage, and negatively affects disease-free interval.[17] Therefore, an immunotherapy regimen was designed to include zinc, indomethacin, and low-dose cyclophosphamide in addition to the primary cell-derived active cytokines. This regimen was named IRX-2 by the biotechnology company that developed it.

The rationale for the immunotherapy regimen and trial design was supported by observations that tumor-draining neck lymph nodes often contain tumor cells in a microenvironment that is immunosuppressive. Before surgery, the multiple active cytokines are injected subcutaneously into the base of the neck and absorbed into the lymphatics, activating regional lymph node immune cells, theoretically, and allowing priming of an antitumor response. It is hypothesized that the elicited antitumor immune response could destroy any remaining microscopic or disseminated tumor cells and contribute to long-term antitumor immunity postsurgical resection and subsequently enhance long-term survival.

The same immunotherapy regimen administered in this report was previously given to 13 patients with advanced HNSCC whose surgery and/or radiation therapy was unsuccesful in a phase 1 clinical safety study.[18] The most frequent adverse events (AEs) were observed in the blood and lymphatic system and the gastrointestinal system, with lymphopenia in 4 patients; and anemia, abdominal pain, and dysphagia in 3 patients each. Anorexia, headache, and dyspnea were each reported in 3 patients. There were 3 patients (23%) who discontinued participation from the study prematurely due to an AE. Of these, 2 patients (16%) died of causes not related to the immunotherapy regimen. One patient (8%) had mental status changes. Two more patients died after the study period due to tumor progression. None of the deaths or discontinuations was considered related to the immunotherapy regimen. AEs attributed as probably drug-related included injection-site reaction in 2 patients and injection-site pain in 1 patient. Possibly related AEs were lymphopenia and pain in 2 patients each, followed by leukocytosis, neutrophilia, thrombocythemia, tinnitus, malaise, nasopharyngitis, and hypotension, each observed in 1 patient. For indomethacin, probably related AEs were abdominal pain in 2 patients, and nausea and anorexia, each observed in 1 patient. Dizziness in 1 patient was considered possibly related to cyclophosphamide, and constipation in 1 patient was reported as possibly related to zinc gluconate. The most frequent grade 3 (severe) AEs were dysphagia in 2 patients (a third patient had grade 1 dysphagia) and dehydration in 2 patients. There were no grade 4 (life-threatening) AEs. Of the 2 patients with grade 5 AEs (death), 1 died of multi-organ failure and the other died of subarachnoid hemorrhage. Neither of these grade 5 AEs (death) was considered related to the study drugs. There were 7 patients with a total of 13 serious

adverse events (SAEs). Only 1 AE, hypotension, was considered by the investigator as possibly related to the cytokine-containing biologic, whereas an acute renal failure was defined as unlikely to be related to indomethacin. Antitumor responses were noted by radiographic assessment. In the 8 patients who had antitumor data at day 21, 1 patient had complete response, 5 patients had stable disease, and 2 patients had progressive disease.

The primary goal of this phase 2 study was to further evaluate the safety and efficacy of this immunotherapy regimen in the neoadjuvant setting in previously untreated patients with advanced HNSCC undergoing surgery with curative intent.

PATIENTS AND METHODS

Patient Population. Previously untreated patients were consecutively screened at 16 academic research centers and invited to participate in the study. Eligible patients had stage II to IVa, histologically proven, squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, or larynx, were aged from 18 to 80 years ($n = 27$), and had a Karnofsky performance status $\geq$70%. Patients were excluded if they were currently under immunosuppressive therapy, used any investigational agent, had undergone surgery, radiotherapy, or chemotherapy within the previous 30 days, or had known allergies or sensitivities to study drugs. Institutional review boards at each of the 16 participating centers individually approved the study. Twelve of the 16 centers enrolled patients. All patients gave informed consent, and the experimental protocol was approved by the individual Institutional Review Board for Human Experimentation at each participating institution.

The Immunotherapy Regimen and Treatment Schedule. The immunotherapy regimen is a 21-day neoadjuvant immune restorative treatment regimen to be followed by surgery with curative intent. The cytokine biologic, also referred to as IRX-2, contains physiologic quantities of cytokines, which are measured by quantitative enzyme-linked immunosorbent assay and serve as product release criteria.[21] In addition to the cytokines that define product release, IRX-2 also contains IL-6, IL-8, granulocyte colony-stimulating factor, and granulocyte macrophage colony-stimulating factor. Drug product dosing is based on 4 primary cytokine components that are listed in Table 1. The cytokine biologic is produced from healthy blood donors' leukocytes obtained from United States Food & Drug Administration (FDA) licensed blood centers. Purified mononuclear cells were prepared and stimulated with phytohemagglutinin to induce cytokine production. The good manufacturing process ensures consistency, safety from bloodborne pathogens, and adherence to FDA guidelines for good manufacturing process biologics production under an approved United States Investigational New Drug application. Each biologic lot was tested for adherence to FDA-approved specifications, for content of IL-2, IL-1β, γ-IFN, TNF-α, for protein, for sterility, and for the absence of various viruses and endotoxin.

Table 1. Phase 2 lots cytokine unit dosing.

Lot no.	IL-1β (ng/mL)	IL-2 (ng/mL)	γ-IFN (ng/mL)	TNF-α (ng/mL)
2050426	0.6	5.8	1.5	1.8
2007001	0.7	6.4	2.6	2.3
2007002	0.7	5.9	2.1	1.6

Abbreviations: IL-1β, interleukin 1 beta; IL-2, interleukin 2; γ-IFN, gamma interferon; TNF-α, tumor necrosis factor alpha.

The subcutaneous immunotherapy injections were administered over 10 days (Monday through Friday over a 2-week period) as 2 bilateral injections of 115 U each in the mastoid region of the left and right necks in close proximity to the regional nodal basins. The regimen also included a 1-time infusion of a noncytotoxic dose of cyclophosphamide (300 mg/m^2) on day 1, and daily oral indomethacin (25 mg; 3 times daily) and zinc gluconate (24 mg; 1 daily) for 21 days. Although not considered part of the regimen, daily omeprazole (20 mg; 1 daily) for 21 days was recommended as supportive care to decrease potential gastric symptoms related to the indomethacin. Posttreatment patient and tumor assessments were carried out on day 21. Patients were assessed for AEs and toxicity during treatment and for 30 days after surgery. The protocol recommended that investigators give postoperative radiation therapy, especially in patients with suspected microscopic residual disease. In addition, postoperative chemotherapy concurrent with radiation therapy was advised for candidates where appropriate and in accordance with recommendations of recent cooperative study findings. Study design included periodic patient follow-up for 5 years after surgery.

Safety and Tumor Assessment. The primary study objective was to demonstrate the safety of this immunotherapy regimen based on AEs, changes in clinical laboratory measures (hematology, chemistry, and urinalysis were determined at baseline, day 1, day 8, day 15, day 21; and 1, 3, 6, 9, 12, 18, 24, 30, 36, 48, and 60 months postsurgery), vital signs, and physical examinations. Stools for guaiac were collected at baseline and as clinically indicated through day 21. SAEs were collected throughout the treatment and 30 days postoperatively. AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). All serious SAEs were followed to resolution. A sample size of 25 patients was determined based on the assumption that, for any given category of safety risk, if no event

of that type occurs, one can rule out that the true rate of occurrence of that type of risk is at least 12%. If the true frequency of a given adverse event is 5%, a study of 25 patients would provide 87% power to exclude a likelihood of that event of greater than 25%.

Secondary objectives were clinical, pathologic, and radiographic tumor response; and patient disease-free survival (DFS) and overall survival (OS). Tumor response data were prospectively evaluated using modified Response Evaluation Criteria in Solid Tumors (RECIST) based on central independent radiologic evaluation at baseline and day 21 (after completion of immunotherapy but before surgery). Data collected included a description of the change in the sum of the longest diameters of all target lesions. A tumor response was categorized as "progression" if there was an increase of single longest diameter of >20%, and as a "partial response" if there was a decrease in the single longest diameter of >30%. Pretreatment and posttreatment CT or MRI scans were compared, with no clinical information provided, by centralized independent reviewers (Perceptive Informatics, Waltham, MA). The recording of baseline data was done without seeing the day-21 scan.

Tumor pathology was evaluated from tissue specimens obtained at tumor resection. Formalin-fixed, paraffin-embedded blocks, or unstained slides from the primary tumor were submitted to an independent pathology laboratory (PhenoPath Laboratories, Seattle, WA) for hematoxylin and eosin staining, and evaluation of lymphocyte infiltration. It was estimated that up to 70% of patients would demonstrate substantial biologic effect as defined by a 20% or greater increase in tumor lymphocyte infiltration. If at least 20 patients were included in the sample size, the 95% 2-sided confidence intervals for 20% response would range from 0.03 to 0.37. Changes in laboratory values and clinical symptoms related to treatment were also recorded during treatment and on day 21 posttreatment.

Survival Assessments. The intent-to-treat population included all patients who entered the study and received any amount of any component of the immunotherapy regimen. Analyses and evaluations of DFS and OS outcomes were determined.

Measure of Lymphocytic Infiltrate. An independent review of tumor-infiltrating lymphocytes was performed on representative tumor sections from samples obtained at surgery for analysis using a 100-mm visual analog scale (VAS) as an assessment of lymphocytic infiltrate (LI). Tumor specimens were evaluable in 24 of the 27 patients; 1 patient refused surgery, there was not enough tumor to analyze in 1 patient, and in 1 patient no tumor was visible in the resected specimen. A blinded VAS reading of hematoxylin-eosin-stained sections was performed for LI, with 100 mm signifying LI encompassing the entire primary tumor section or maximum LI, and 0 mm signifying no LI in the tumor specimen. The patients were then grouped for analysis into high-LI and low-LI cohorts based on a mean VAS cutpoint of 23 mm.

Quality Control. A surgical quality control committee (authors G.T.W., J.S.M., and principal investigator W.K.) was used to prospectively assure proper tumor staging, patient eligibility, adherence to protocol surgical guidelines, and to review all operative and pathology reports. As described above, independent central laboratories were used to evaluate imaging quality, peripheral blood, serum, and histopathology results.

RESULTS

Patient Characteristics. Mean patient age was 57 years (range, 36–79 years). Twenty patients (74%) were men. Fifteen patients had oral cavity cancer, 8 patients had oropharyngeal cancer, 1 patient had hypopharyngeal cancer, and 3 patients had laryngeal cancer. Most patients (89%) had Karnofsky performance status >80. Three patients had stage II disease, 8 patients had stage III, and 16 patients had stage IVa (60%). Nodal class was N0 in 5 patients, N1 in 8 patients, and N2 in 14 patients; no patient had N3 disease.

Toxicity. The 21-day neoadjuvant immunotherapy regimen was well tolerated with minimal toxicity. Compliance was excellent; all patients completed the regimen. Of the 26 patients completing surgery, 19 received surgery and postsurgical radiation therapy and 10 of the 19 patients also received chemotherapy. One patient died from aspiration pneumonia before starting postsurgical adjuvant therapy. There are 17 patients still alive in follow-up, with all being followed for at least 36 months postsurgery.

Eight SAEs were reported during treatment and the 30-day postoperative period in 7 patients, including 3 patients with aspiration pneumonia, 1 patient with asthma exacerbation secondary to upper respiratory infection, 1 patient with a postoperative wound infection, 1 patient with a neck abscess, and 1 patient with an episode of alcohol withdrawal. Only 1 case of aspiration pneumonia was deemed life threatening (grade 4). None of the SAEs was considered related to treatment except for the postoperative wound infection, which was considered possibly related. All AEs resolved without sequelae. Other minor (grade 1 or 2) AEs included headache (30%), injection-site pain (22%), nausea (22%), constipation (15%), dizziness (15%), fatigue (11%), and myalgia (7%). There were minor (grade 1) alterations in posttreatment lab values. The most common (>5%) shifts from normal at

baseline to a toxicity at day 21 were observed for decreased hemoglobin (18.5%), decreased leukocytes (7.4%), high non-fasting glucose (48.1%), and decreased albumin (18.5%). At day 21, the only laboratory parameter that worsened more than 1 grade from baseline was phosphate (3-grade worsening in 1 patient, 3.7%). No important mean changes in vital sign assessments were detected at any time point through day 21.

There were no unplanned delays in surgery as a result of the immunotherapy regimen. Mean time from date of informed consent to surgery was 31 days (range, 22–47 days). Mean time from the last dose of the regimen to surgery was 5 days (range, 1–11 days). All patients underwent extirpative surgery as planned before treatment, except for 1 patient who had more extensive tumor than expected, which would have required a total glossectomy. This patient refused surgery. Surgery and wound healing were uneventful in all patients but 1; this patient developed an early wound infection. There were no fistulae. A total of 9 patients (35%) had close margins on final pathology, and 1 (4%) had a microscopically positive margin. A total of 17 patients (65%) had histologically positive nodes, of whom 13 patients (50%) had multiple positive nodes and 6 patients had extracapsular extension.

Overall, the pattern of SAEs with the immunotherapy regimen was consistent with that expected in this patient population and did not suggest that the regimen was associated with the occurrence of any unique SAEs. Patients' symptoms and signs were subjectively assessed by clinical investigators at both baseline and at day 21 after the start of immunotherapy treatment. Clinical investigators described post-immunotherapy regimen tumor "softening" in 4 patients. Decreased pain was reported by 5 patients, and improved swallowing was reported by 4 patients. There were no significant progressive symptoms in either breathing or tumor bleeding among the patients.

Tumor Response. Clinical response of the tumor after the 21-day regimen before surgery was assessed by clinical examination and radiographic imaging (CT or MRI). All images were reviewed by an independent central imaging group (Perceptive Informatics, Waltham, MA) and graded using modified RECIST. A total of 25 patients had images that were satisfactory for review. Two more patients were non-evaluable because of imaging artifact from extensive dental restorations, or because the tumor could not be definitely identified at baseline. Thus, in 23 patients, the primary tumor was evaluable, and in 16 patients, at least 1 lymph node was evaluable for imaging response. Overall, there was modest tumor shrinkage as measured by RECIST criteria at the end of the 21-day treatment period just before surgery (Figure 1). Based on target lesion (sum of longest diameter), evaluation of the 23 evaluable patients, 4 patients had −20% to <−10% change, 7 patients had

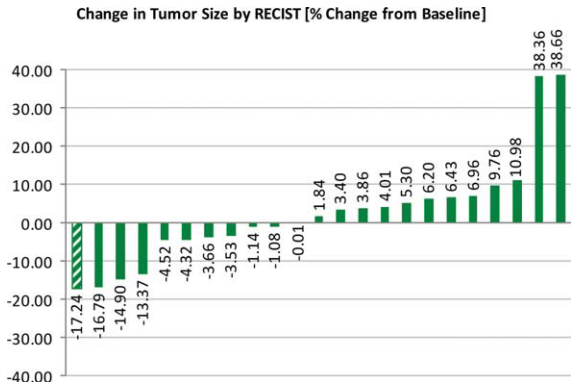

FIGURE 1. Central diagnostic radiology assessment of target lesions using modified Response Evaluation Criteria in Solid Tumors (RECIST) at 3 weeks (percent change from baseline). One patient was adjudged by the central reviewer to be a complete response. CT/MRI of this site showed a −17.2% change (bar with diagonal stripes), which was in concurrence with the pathology report.

−10% to <0% change, 9 patients had 0% to <10% change, 1 patient had 10% to <20% change, no patients had 20% to <30% change, and 2 patients had ≥30% change in tumor (target lesion) size. Based on the overall assessment, 19 patients (83%) had stable disease and 4 patients (17%) had some tumor growth at day 21 (completion of immunotherapy before surgery). Two of the 4 patients had some tumor growth at day 21, as measured by the target lesions. One of the 4 patients had a −13.37% change in the target lesions, but was classified as progressive disease because of a new small non-target focus node; and 1 patient with a −3.66% change in the target lesion longest diameter also had a new left side node (non-target lesion) plus an enlarged jugulodigastric node and was classified as having progressive disease.

There were no obvious deleterious effects in the 2 patients with tumor progression by RECIST on day 21. In the event that a tumor progressed during the 21-day regimen, the surgeon had the option of withdrawing the patient from the immunotherapy regimen and proceeding directly to surgery. No surgeon opted to withdraw a patient. One patient had surgery earlier than planned due to clinical enlargement of the primary tumor. For most patients, there was little apparent change in tumor/node size. Review of the radiologic findings in relation to the pathology nodal status made it apparent that lymph node enlargement due to reactive hyperplasia could not be distinguished from that due to tumor involvement based on a CT or MRI scan.

It is of interest that 1 patient with tongue base cancer underwent a fluorodeoxyglucose-PET CT scan at baseline and at completion of the immunotherapy regimen. In 2 lymph nodes and the tumor, this patient showed elevated pretreatment glycolytic activity; the mean decrease in glycolytic activity in these tumors was 75% after therapy (Figure 2).

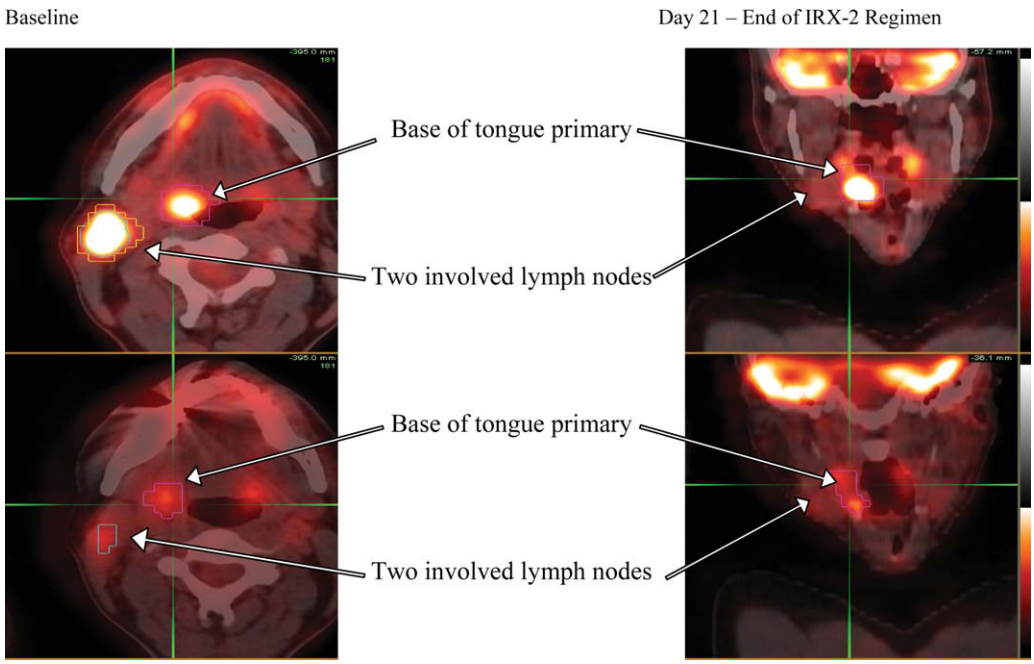

FIGURE 2. Fluorodeoxyglucose-positron emission tomography (FDG-PET) CT scan at baseline and before surgery at the completion of immunotherapy regimen (IRX-2) therapy.

Survival. After over more than 36 months of follow-up, 11 of the 27 patients enrolled in this study have experienced tumor relapse ($n = 1$) or death ($n = 10$). Figures 3 and 4, show DFS and OS from the date of surgery in the 26 patients completing surgery, respectively. The pattern of first HNSCC relapse included 3 patients with primary site recurrence, 2 with recurrences in the neck, and 2 with distant metastases. Of the 10 patients who died, 6 died of cancer (1 from a new primary) and 4 died of other causes. The 1-year, 2-year, and 3-year DFS probabilities after surgery were 72%, 64%, and 62%, respectively. Of the 26 patients whose primary tumor was resected surgically, 2 patients died during the first year and 5 patients died during the second year after surgery. The probability of surviving after surgery was 92% the first year, 73% the second year, and 69% the third year. Median DFS or OS were not reached after all patients were followed for at least 3 years.

Histologic Results and Survival. Representative posttreatment tumor samples were available from 24 patients and submitted for central independent pathology review (PhenoPath Labs, Seattle, WA). Three patients had no grading for tumor lymphocytic infiltration because the tumor was not surgically resected, the tumor specimen was too small, or no tumor was evident in the tumor resection. A lymphocyte infiltration 100-mm VAS score was used for analysis, in which 100 mm signified lymphocyte infiltration of the entire primary tumor section and 0 mm signified no lymphocyte infiltration in the tumor specimen. The mean VAS score for all patients was 22.6 mm on the samples obtained at surgery. Patients were grouped into a low VAS score (below the overall mean) and high VAS score (above the overall mean) cohort. There were 14 patients in the low VAS score cohort with scores between 2 and 21 (median of 9.5), and there were 10 patients in the high VAS score cohort

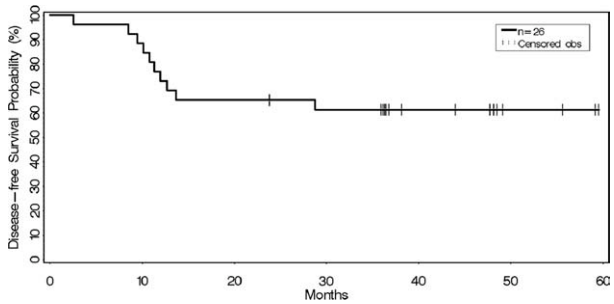

FIGURE 3. Disease-free survival from date of surgery.

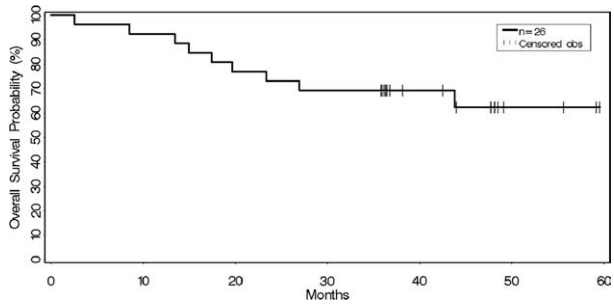

FIGURE 4. Overall survival from date of surgery.

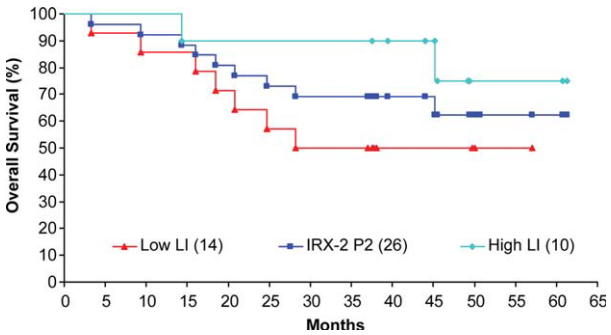

FIGURE 5. Overall survival – high lymphocytic infiltrate (LI) versus low LI. Immunotherapy regimen (IRX-2).

with scores between 27 and 66 (median of 37.0). Patients in the high-LI group included fewer oral cavity patients (50% in high LI vs 60% in low LI) but were similar with respect to tumor sites. Seventy percent of high-LI patients were stage IV, whereas only 60% of low LI were stage IV. The LI score was used to determine whether the degree of LI correlated with survival. Patients with a high-LI score had an improved survival trend compared to those with low LI, and superior to the survival rate for the combined overall group (Figure 5).

DISCUSSION

This phase 2, multi-institutional, clinical trial was conducted to determine the safety and efficacy of a regimen of neoadjuvant immune reconstitution, in newly diagnosed patients with HNSCC who were to undergo surgery with curative intent. The results confirm the feasibility and modest toxicity with this low-dose, physiologic cytokine regimen. The regimen has been shown to have a favorable safety profile in this study and in a prior phase 1 trial.[18] Most AEs were grade 1 or 2, with the predominant ones being headache, injection-site pain, nausea, constipation, dizziness, anemia, and fatigue. All patients completed the immunotherapy regimen, and delays in surgery beyond the 3-week treatment period were rare. Only 1 SAE (postoperative wound infection) was felt to be possibly related to the immunotherapy and this event only resulted in slight prolongation of hospitalization after surgery. All participating investigators were skilled head and neck surgeons with extensive clinical experience. There was no increase in surgical complications, rate of positive surgical margins, or compromise of surgical resection reported by any investigator compared to what is usually expected in this patient population. Although the trial was not randomized and the treatment period was brief, it was encouraging to see symptomatic improvement and clinical decrease in tumor size among some individual patients. An improvement in tumor status over this short neoadjuvant period of 3 to 4 weeks in this trial was not expected.

The immune regimen seemed less toxic than typically experienced with high-dose cytokine therapy or neoadjuvant chemotherapy. Locoregional delivery of physiologic doses of cytokines may reduce systemic toxicity. Most recombinant cytokines, such as IL-2, are tested in the same manner as traditional oncology drugs, where the maximum tolerated dose is sought. Typical cytokine therapies in cancer treatment use extremely high doses, in the millions of units per administration.[19–22] Thus, AEs such as fever, hypotension, malaise, anemia, leukopenia, and hepatic and renal dysfunction are commonly reported, and often lead to discontinuation of the treatment.[20,22] In addition, it is theoretically possible that such high-dose immune modulation will lead to feedback suppressive effects. IV administration of cytokines is frequently associated with an acute phase reaction characterized by rigors, fever, an increase in neutrophils, a decrease in lymphocytes, and changes in hormone levels.[19] The current regimen, which contains physiologic quantities of ILs, showed greatly improved tolerability over typical recombinant cytokine therapies. Regional administration was expected to reduce systemic toxicity by lowering circulating concentration in the normal tissues, and also provide higher concentrations to the tumor-draining lymph nodes.

In this trial, a low-dose cytokine-containing biologic was delivered subcutaneously near the tumor-draining lymph nodes where the priming of an antitumor response is thought to begin. Indirect evidence that an immunologically mediated antitumor effect may be occurring is suggested by pronounced lymphocytic infiltration seen in some tumors and by the tumor reductions observed at the end of the 21-day regimen in 11 patients, and by a mean 75% reduction of glycolytic activity in the tumor and lymph nodes on posttreatment PET scan in 1 patient. Enhanced immune response has been shown to correlate with positive outcomes for patients with cancer. For example, the presence of tumor-infiltrating T cells has been correlated with improved progression-free survival and/or OS in various cancers, including advanced ovarian cancer,[23] advanced melanoma,[24] and HNSCC.[25] Because the immunobiology of HNSCC is so intimately associated with the host immune system, the reversal of immunosuppression is a particularly attractive therapeutic goal for patients with this tumor type.

In preclinical studies, the cytokine-containing biologic used in this trial has been shown to be a potent activator of dendritic cells (DCs).[12] Low-dose cytokine treatment of DCs has resulted in the upregulation of major histocompatibility-II and intercellular adhesion molecule-1 expression, markers associated with antigen presentation. Furthermore, it caused an increase in CD86 and CD40 protein expression, both of which

are directly involved in T cell stimulation. It also caused an increase in both CD83 protein expression and the percentage of cells expressing CD83, a marker for DC maturation. Treated DCs were able to effectively stimulate and cause proliferation of allogeneic T cells as compared to untreated DCs.

The cytokine-containing biologic was recently shown to protect human T cells in vitro from apoptosis in response to several stimuli including tumor-derived FasL+ microvesicles.[26] Inhibition of apoptosis was both dose-dependent and time-dependent and was equivalent or better than that provided by higher concentrations of IL-2, IL-7, and/or IL-15. These results further bolster the argument that the multiple cytokines act synergistically. A detailed evaluation of the molecular pathways involved in tumor-mediated apoptosis revealed that this biologic targets Akt via Jak3/STAT 5 signaling and thereby restores the balance of pro-apoptotic versus anti-apoptotic proteins in the direction of survival. Collectively, these data indicate that low-dose cytokines are a potent activator of human DCs, protects T cells from tumor-induced apoptosis, and thus may be able to overcome tumor-mediated suppression of cellular immunity.

IL-2 has been administered to patients with HNSCC using a variety of delivery methods, including intralesional injection (recombinant IL-2), synthetic gene delivery systems, and primary cell-derived cytokines with or without cyclophosphamide and indomethacin. Clinical responses and tumor histologic changes have been reported that have been interpreted as derived from immune-rejection phenomena.[27] A major advantage of the current regimen is use of physiologic quantities of IL-2 administered with a variety of other low-dose cytokines that may be important in restoring immune homeostasis and modulating the overall response. A concomitant noncytotoxic dose of cyclophosphamide was used to reduce the negative effects of regulatory T cells combined with indomethacin-induced prostaglandin inhibition to reduce immunosuppressive chronic inflammation in the tumor microenvironment.

A second finding of the current study was that some tumors showed some decrease in overall size after the immunotherapy regimen. Overall tumor shrinkage was modest, although in 4 patients, independent, objective imaging documented a >10% decrease in tumor size. This was unexpected and encouraging after only 3 weeks of presurgical neoadjuvant immunotherapy. No patient achieved a true partial response by RECIST criteria. Increases in tumor measurements were also seen in some patients, but most patients showed negligible change in tumor dimensions. These findings support the safety of the neoadjuvant regimen.

This phase 2 trial did not include a randomized control cohort. However, the disease-free and OS rates with reasonably long follow-up were very good. Median DFS or OS were not reached after all patients were followed for at least 3 years. We believe the safety results and feasibility of this immunotherapy regimen are intriguing enough to warrant further study and appropriate comparison in a randomized trial. New therapies are needed for patients with HNSCC. Patients with advanced, surgically resectable cancers typically receive adjuvant radiation and/or chemotherapy, but maximum toxicity has been reached without significant improvement in survival, and new therapies capable of combination with current chemotherapy/radiation treatments and with acceptable safety profiles are needed.

Interestingly, LI in resected tumor specimens was considered high in 40% of the patients. The 10 patients with a high-LI score showed an improved survival trend in comparison to the low-LI group ($n = 15$) and to the entire study population ($n = 26$). It is difficult to directly compare these subgroups, because there was some imbalance, with a slightly higher percentage of oral cavity patients in the low-LI group. Detailed immunologic studies of peripheral blood and tumor infiltrates from tissue specimens from this clinical trial are underway and will be reported separately. In the absence of a randomized control, it is impossible to directly attribute the LI to the immunotherapy regimen. In addition, further studies are needed to determine if increases in lymphocyte subpopulation infiltrates in the tumor are associated with functional changes in tumor immunity or with tumor regressions, which could lead to more specific patient selection and further improvements in survival.

In conclusion, the results of this small, phase 2 clinical trial indicate that this neoadjuvant immunotherapy regimen is safe and well tolerated in patients with newly diagnosed HNSCC who are undergoing surgery for curative intent. The safety of the treatment makes it feasible for a patient population that is going to receive maximally tolerable doses of radiation and chemotherapy. OS results are encouraging.

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