Combination Immunotherapy of Squamous Cell Carcinoma of the Head and Neck

A Phase 2 Trial

Jose Luis Barrera, MD; Emma Verastegui, MD; Abelardo Meneses, MD; Juan Zinser, MD; Jaime de la Garza, MD; John W. Hadden, MD

Objectives: To test the efficacy of a natural cytokine mixture (IRX-2), cyclophosphamide, indomethacin, and zinc to induce immune regression of squamous cell carcinoma (SCC) of the head and neck (H&N) prior to conventional therapy and to characterize the responses.

Patients and Design: A phase 2 trial was performed in 15 adults with recently diagnosed, biopsy-confirmed H&N SCC (3 with stage II disease, 6 with stage III disease, and 6 with stage IV disease). The patients were treated with 20 days of perilymphatic injections of IRX-2 (administered subcutaneously at the base of the skull) in combination with contrasuppression consisting of a low-dose infusion of cyclophosphamide (300 mg/m²), and daily oral indomethacin and zinc (StressTabs) in a 21-day cycle before surgery and/or radiotherapy. Tumor dimensions, toxic effects, and disease-free survival were monitored. The tumor sections were histologically examined after surgery, and tumor reduction, fragmentation, and lymphoid infiltration were assessed.

Results: All 15 patients responded clinically to the 21-day IRX-2 protocol: 1 with a complete response, 7 with a partial response, and 7 with a minor response. All 15 patients responded pathologically with tumor reduction (mean, 42%) and fragmentation (mean, 50%) in the histological section and increased lymphoid infiltration. The adverse effects of the IRX-2 protocol were negligible except for an allergic skin rash (n = 1) and parotiditis (n = 1). Indomethacin caused gastritis in 1 patient. Reduction of pain and ulceration and bleeding were observed in 8 and 4 patients, respectively. Four of 5 patients with lymphopenia showed increased CD3, CD4, and CD8 cell counts. After surgery (n = 13) and/or radiotherapy (n = 10) and with a mean follow-up of 17 months, 3 patients have had recurrences, 1 patient has died of disease, 1 patient has been re-treated with immunotherapy and has no evidence of disease, and 1 patient is alive with disease. Two patients died of other causes with no evidence of disease.

Conclusions: The IRX-2 immunotherapy induced lymphocyte mobilization and infiltration in H&N SCC associated with clinical and histological tumor responses indicative of immune regression in all 15 patients. Minimal toxic effects were observed, and overall survival may have been improved. A phase 3 trial seems warranted.
PATIENTS AND METHODS

PATIENTS

Fifteen patients with biopsy-documented H&N SCC were included in the study, which was approved by the INCAN institutional review board and research committee and by the Mexican Drug Authority (equivalent to the Food and Drug Administration in the United States). The patients participated in informed consent as outlined in the declaration of Helsinki (Finland). All patients were staged by standard TNM criteria, determined to be surgical candidates, and demonstrated a positive intradermal skin test reaction to 0.1 mL of IRX-2 (>3 mm of erythema and induration at 24 hours). None of the patients had other debilitating diseases. Two had previously received chemotherapy (>3 months).

NATURAL CYTOKINE MIXTURE (IRX-2)

The natural cytokine mixture is produced from mononuclear cells from human blood. Theuffy coats are prepared with blood from the INCAN Blood Bank that has been fully tested for transfusion and determined negative for syphilis, hepatitis core antigen, hepatitis B and C viruses, alanine aminotransferase, human immunodeficiency virus, and human T-lymphotrophic viruses 1 and 2. The heterologousuffy coat cells are further separated by gradient centrifugation to exclude red blood cells, polymorphonuclear leukocytes, and platelets. The mononuclear cells obtained are exposed to a mitogen phytohemagglutinin (PHA) (Murex, Atlanta, Ga) to stimulate cytokine biosynthesis in vitro serum-free medium (X-VIVO; BioWhitaker Inc, Walkersville, Md). This medium was originally designed for experimental use in IL-2–lymphokine-activated killer protocols in humans and is approved by the Food and Drug Administration. The cells are cultured for a short time in the presence of an antibiotic, and the supernatants are collected and passed through a 0.22-µm filter (Millipore-Millipak Gold; Millipore Corp, Bedford, Mass) for sterilization. After release of the bulk product, the final product is packaged in 10-mL sterile glass bottles, which are equipped with flanged, airtight rubber stoppers and secured with crimped aluminum covers, and then sampled for quality control testing. Final product specifications include standard tests for potency (IL-1, IL-2, and interferon gamma), bacterial endotoxin, sterility, and pyrogens. The product is controlled both at the bulk product stage and at final bottling. The bottle is properly labeled and kept frozen until use.

The natural cytokine mixture is a collection of natural human cytokines induced from human peripheral blood mononuclear cells. It contains IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, interferon gamma, tumor necrosis factor alpha, and granulocyte-macrophage and granulocyte colony-stimulating factor in nanogram quantities. It lacks IL-3, IL-4, IL-5, and IL-7. It has undetectable PHA, endotoxin, bacteria, mycobacteria, fungi, hepatitis C, and human immunodeficiency virus. It is administered in 1.0-mL injections that contain approximately 250 U of IL-2 on enzyme-linked immunosorbent assay and 640 U of IL-2 on bioassay. Perilymphatic subcutaneous injections are administered bilaterally at the insertion of the sternocleidomastoid muscle at the base of the skull for 20 consecutive days (Figure 1). Also, in the 8 patients with tumors in the oral cavity, a daily injection was administered in the oral cavity to feed into the surrounding mucosa-associated lymphoid tissue.

TREATMENT PLAN

Fifteen patients with biopsy-proven H&N SCC met the study criteria. History and physical examination with tumor staging were performed using standard TNM classification. Tumor diameter was measured in centimeters in 2 dimensions for each lesion. Initial laboratory tests included a complete blood cell count, differential cell count, sequential multiple automated chemistry system (SMAC), T-cell subsets, and chest radiography. A skin test was performed with 0.1 mL of IRX-2 on the palmar surface of a forearm. The skin test, which uses preformed cytokines and is read at 24 hours for millimeters of erythema and induration, is an assessment of the efferent limb of the cellular immune response and is a sensitive indicator of immune response. Subjects with negative skin test results were not included in the study.

The patients also underwent skin tests with PHA (0.5 and 1.0 µg) and purified protein derivative (tine test; LEDere Laboratories, Pearl River, NY). They were introduced to the protocol with an infusion of low-dose cyclophosphamide (300 mg/m²) and a daily oral regimen consisting of indomethacin (25 mg 3 times daily) and zinc (StressTabs), followed after 3 days with 20 days of outpatient treatments with IRX-2 (2-3 mL) administered bilaterally in the neck and, in the case of the patients with oral cancer, in mucosa-associated lymphoid tissue (Figure 1). After the last injection, the indomethacin treatment was discontinued, and the patients were reevaluated and given standard surgical treatment and/or radiotherapy.

Study parameters and criteria of evaluation included (1) clinical tumor assessment; (2) pathological tumor assessment on the surgical specimen according to the criteria described by Meneses et al10; (3) grading of adverse reactions according to standard criteria; and (4) evaluation of subjective responses concerning tumor changes, pain, voice, bleeding, swallowing, nutrition, and well-being.
RESULTS

The H&N SCC study population (13 men and 2 women; average age, 63 years; age range, 34-68 years) consisted of 9 patients with cancers of the oral cavity (n = 8) or oropharynx (n = 1), 5 patients with laryngeal cancer (T3 or T4), and 1 patient with neck metastases from an unknown primary site, which was later found to be the larynx (Table). Nine patients had neck metastases (N1-N3) at the time of diagnosis. None had known distant metastases (M0).

Eleven patients were smokers, 12 used alcohol regularly, and 3 were clinically malnourished. All 15 patients had a positive IRX-2 skin test result averaging 1 cm. Eleven skin tests were positive for intradermal PHA (mean, 1.4 cm), and 7 were positive for purified protein derivative. Five patients had lymphopenia (lymphocyte count, <1.5 x 10^9/L). Approximately 80% to 90% of INCAN patients with H&N SCC who have been tested to date have been positive for IRX-2 and PHA, but most have been negative for purified protein derivative. These patients are, by nature of their positive IRX-2 skin test results, thereby better-off immunologically than the average INCAN patients with H&N SCC.

All 15 patients responded clinically to the IRX-2 protocol with a tumor reduction of more than 25%. One patient had a complete response, 7 patients had a partial response (>50%), and 7 patients had a minor response (<50% but >25%). Eight patients with oral cancer and significant pain had relief of pain. Seven who reported bleeding had a marked reduction of bleeding, and 4 with gross ulceration showed healing. As a result, their ability to swallow and eat improved, allowing better nutrition.

Four of the 5 patients with lymphopenia showed increases in total lymphocyte counts (from 0.9 x 10^9/L to 1.5 x 10^9/L) and in CD3+ (from 0.61 x 10^9/L [611/µL] to 0.91 x 10^9/L [906/µL]), CD4+ (0.36 x 10^9/L to 0.62 x 10^9/L), and CD8+ (0.21 x 10^9/L to 0.47 x 10^9/L) cell counts. The pooled increases in lymphocyte counts were significant (P<.01, paired t test). These changes reflect evidence of lymphocyte mobilization resulting from the IRX-2 therapy.

The adverse effects of the IRX-2 protocol were negligible; however, 1 patient developed grade 2 parotiditis that was caused by injection of the IRX-2 into the parotid gland. One patient developed indomethacin-associated grade 1 gastritis, and 1 patient developed a grade 2 allergic skin rash, which, because of tumor eosinophilia (20%), may have represented a reaction to the tumor.

Fourteen patients underwent wide local resections with (radical) neck dissections (9 patients) and postoperative radiotherapy (9 patients). Patient 4 underwent a biopsy and was referred to radiotherapy when her T2 floor of the mouth lesion showed an approximately 90% regression. Patient 13 did not undergo radiotherapy because he had previously undergone irradiation for an earlier SCC. All patients had no evidence of disease after surgery and/or radiotherapy.

All the surgical specimens, as well as the post-IRX-2 treatment biopsy specimen from patient 4, showed histological changes indicating tumor reduction and fragmentation and infiltration with leukocytes (Figure 2). The typical pretreatment biopsy specimen averaged 76% solid tumor with 24% stroma, of which almost one half contained a light infiltration of leukocytes (10%). After the IRX-2 treatment, the typical tumor section con-
cells (≥70%), the tumor infiltrate could be classified as T-cell dominant in 5 patients, B-cell dominant in 4 patients, and mixed in 6 patients. Where examined, regional nodes reflected the situation in the tumor, all nodes were hyperplastic, with sinusoidal histiocytosis in T-cell dominant nodes and follicular hyperplasia in B-cell dominant nodes. After a mean follow-up of 17 months, 10 patients were alive with no evidence of disease. To date, only 1 death has been attributed to cancer.

This IRX-2 strategy uses perilymphatic local administration along with contrasuppression with low-dose cyclophosphamide and indomethacin and with zinc replacement therapy (as an immunorestorative). The data presented herein demonstrate that H&N SCC can respond very well to immunotherapy: there was a response rate of 100% in this series of 15 patients, with clinical reduction in tumor (1 complete response, 7 partial responses, and 7 minor responses) and histological evidence of tumor regression of 42%. Overall, the average combined estimated tumor reduction exceeded 70%. Also, patients with oral cancer noted marked analgesic and hemostatic effects from this therapy, with healing of oral lesions.

The mechanism of this tumor reduction involved a heavy lymphoid infiltration with both T and B lymphocytes and a lesser infiltration with granulocytes (polymorphonuclear and eosinophilic leukocytes), macrophages, and giant cells. Since the tumors themselves were not injected and the findings of histological examination of local lymph nodes reflected corresponding hyperplastic changes (unpublished data), the appearance of these cells in the tumors with fragmentation and reduction represented cellular and humoral immune responses, which were probably elicited at the level of regional lymph nodes that contained tumor antigen. The observation that T-dominant reactions and B (plasma cell)-dominant tumor and node reactions are correlated with major tumor reduction indicates that both are effective antitumor mechanisms. It is known that many human tumors express both T and B epitopes as tumor-associated antigens.1,12 The extent to which these antigens might be mobilized to induce immune regression has heretofore been underestimated.

The immunology of H&N SCC has been reviewed in detail elsewhere.13 Squamous cell carcinoma of the head and neck is associated with more profound defects of cellular immunity than any other cancer except, perhaps, SCC of the cervix. The cellular immune defects in patients with H&N SCC are present at the time of diagnosis and likely predate the cancer, at least in part. The immunodeficiency reflected in the patients in this study, including lymphopenia (n = 5) and anergy to intradermal PHA (n = 5) and purified protein derivative (n = 8), supports this observation; however, the presence of a positive IRX-2 skin test result as an inclusion criterion indicates that they were not the most severely immunosuppressed 10% to 20% of patients with H&N SCC.

Four of the 5 patients with lymphopenia showed correction of total lymphocytes, including CD3+/CD4+ and
CD3+/CD8+ subsets of T cells, which reflects the ability of this protocol to mobilize T cells from the periphery and perhaps those from the thymus (since the CD45RA/CD45RO ratio was increased in several patients [E.V. and J.W.H., unpublished data]). The findings of this study confirm the results of our previous phase 2 trial using a 10-day injection protocol with IRX-2.9,10

The marked influx of leukocytes, particularly lymphocytes, into these tumors was notable; yet compared with the 10-day injection protocol,10 the influx was less, 28%

Figure 3. Representative histological sections of head and neck squamous cell carcinoma biopsy specimen (A) and of surgical specimen undergoing immune rejection induced by the immunotherapy protocol (B-D).
and granulocyte-macrophage and colony-stimulating factor; it does not contain IL-3, IL-4, IL-5, or IL-7. It is therefore a mixture of monokines and T-helper 1 cytokines, deficient in T-helper 2 cytokines. As such, it would be theoretically more active to induce cell-mediated T-cell-dependent immunity as an immune adjuvant. The lack of activity of rIL-2 in this adjuvant setting, even when its gene is transfected into H&N SCC, underscores the importance of the natural synergy that cytokine action uses.

The findings of animal studies21 convinced us that IRX-2 has activity that is not shared by recombinant cytokines, even the mixture of rIL-1 and rIL-2. We therefore consider the natural mixture critical to the success of this protocol. We use it not because we like partially uncharacterized mixtures; we use it because it works. Some investigators suggest that the Food and Drug Administration, or an equivalent authority, would not approve IRX-2, yet we should remember that licensed γ-globulin preparations, such as intravenous immunoglobulin, are uncharacterized antibody mixtures of IgG, IgM, and IgA isotypes, and that 2 licensed interferon preparations, interferon alfa-n3 (Alferon) and interferon alfa-n1 (Wellferon), are natural mixtures.

The possible impact of the current protocol on survival is of interest. At the last point of mean follow-up (16 months), we had 3 recurrences and 1 death from disease. At INCAN, concomitant but nonrandomized historical controls with an equivalent stage of H&N SCC have had a mean survival rate of 40% at 2 years. This figure is similar to those from a comparable institution.22

It is important to note that adjuvant chemotherapy is not used at INCAN. Many studies23 indicate that treatment with fluorouracil and cisplatin, the combination most in use, is effective for reducing tumors in the majority of patients; however, with no meaningful impact on survival, their routine use in the United States has recently been questioned.24,25 The expense, toxic effects, and lack of effectiveness of both drugs has made their use in other less affluent countries unwarranted. The current data on the use of IRX-2 in this and other protocols8-10 hint at improved survival, and a phase 3 randomized controlled study comparing this protocol with chemotherapy arm is to be initiated.

Accepted for publication May 6, 1999.

This study was sponsored by Immuno-Rx Inc, Bradenton, Fla.

Presented in part at the First World Congress on Head and Neck Cancer, Madrid, Spain, December 2, 1998; the Third International Congress on Immunorehabilitation, Canary Islands, May 3 1999; and the annual meeting of the American Head and Neck Society, Palm Desert, Calif, April 25, 1999.

Reprints: John W. Hadden, MD, 428 Harbor Rd, Cold Spring Harbor, NY 11724.

REFERENCES


**Correction**

Error in Table Row Heading. In the Original Article titled “A New Classification for Malignant Tumors Involving the Anterior Skull Base,” published in the November issue of the ARCHIVES (1999;125:1252-1257), a row heading under “Histological characteristics” in Table 2 (page 1254) was incorrect. “Squamous odontogenic tumor” should have appeared as “Squamous cell tumor.”