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Controlled Trial of Methotrexate and *Bacillus Calmette-Guérin* Therapy for Advanced Head and Neck Cancer[1]

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ABSTRACT

Thirty-eight patients with advanced, inoperable squamous cell carcinoma of the head and neck were randomized to receive methotrexate alone or methotrexate with *Bacillus Calmette-Guérin*. The response rates with methotrexate (3 of 19) and methotrexate plus *B. Calmette-Guérin* (4 of 16) were similar, as was the duration of response and survival of the two groups. The results of *in vitro* immunological studies of lymphocytes were assessed. Marked weight loss, poor performance status, and distant metastases were the most important prognostic factors. The presence of anergy was significantly correlated with weight loss. This study also indicated that a large tumor burden is a frequent occurrence in advanced head and neck cancer and may account for the lack of efficacy of *B. Calmette-Guérin*.

INTRODUCTION

The treatment of recurrent or advanced epidermoid carcinoma arising in the head and neck is a continuing challenge to the medical oncologist. Methotrexate, the most widely used antineoplastic agent, generally provides partial regression of tumor for brief periods. Despite alterations of dosage and schedules, complete and prolonged responses are rare. Indeed, chemotherapy has had little impact on the course of the disease.

Efforts to obtain more substantial benefit from chemotherapy have recently involved the use of adjunctive immunotherapy. Interest in the use of adjunctive immune stimulation for patients with carcinomas arising in the head and neck is based upon several observations relating to immunological status and function in this disease (8, 14). Impairment of cell-mediated immunity is observed in patients with carcinomas arising in the head and neck (17). Since immune compromise may lead to the development of neoplasia, it is interesting that epidermoid carcinoma of the head and neck has recently been documented to occur in a patient receiving long-term immunosuppression for transplantation (12).

Evidence exists that suggests that the immune status of patients with head and neck cancers has prognostic implications. The induction of response to skin sensitization with dinitrochlorobenzene is highest in patients with early disease and decreased in patients with advanced disease. In early disease, lack of response to dinitrochlorobenzene, even with limited extent of disease, may predict a poor survival (9).

Immunotherapy is felt to be useful when the tumor cell burden is small. In head and neck carcinomas, there is a general impression that distant metastases are uncommon, so that tumor cell burden may be small. Theoretically, head and neck carcinomas should respond to immunological manipulations.

Interest in the use of immunotherapy was also generated by the reports of the striking success of methotrexate with BCG[5] in uncontrolled trials (4, 5). For these reasons we initiated a prospective randomized trial of methotrexate with or without BCG in patients with advanced epidermoid carcinomas of the head and neck (15). In addition to analyzing the rate and degree of response, immunological function and prognostic factors were evaluated.

METHODS

Patient Selection and Evaluation

Thirty-eight patients with advanced, inoperable, measurable squamous cell carcinoma of the head and neck area were randomized to receive methotrexate with BCG or methotrexate without BCG. Of these 38 patients, 3 were non-evaluable, 2 received concomitant radiation therapy to only sites of known disease, and 1 patient died from tumor 7 days after receiving BCG, without receiving any methotrexate. All patients had biopsy-proven squamous cell carcinoma, except for 1 who had anaplastic carcinoma of the nasopharynx.

Pretreatment studies included routine blood counts and chemistries, creatinine clearance, bone marrow aspirate, and the immunological studies described below. Radioisotope scans were performed only when clinically indicated by symptoms or laboratory evidence of metastases. Eight of our 35 patients had distant metastases clinically evident when therapy was begun. These distant metastases were found in the following areas: bone, 2 cases; lung, 3 cases; liver, chest wall, and axillary node, 1 case each. Of our 35 patients, 1 had received no previous treatment, 1 had received surgery, 13 had received radiotherapy, and 20 had received both surgery and radiotherapy. No patient had been treated with chemotherapy.

Treatment Schedule

Methotrexate, 0.8 mg/kg i.v. or i.m. biweekly, was given

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[5] The abbreviations used are: BCG, *Bacillus Calmette-Guérin* (Tice strain); i.d., intradermal(ly).

until mild toxicity, stomatitis or leukopenia, appeared and then resumed weekly after toxicity abated. Previous results with this dosage and schedule of methotrexate at this institution have been reported (16).

BCG (Research Foundation, Chicago, Ill.; total dose, 2.2 × 10^8 organisms), 0.1 ml was given i.d. in 8 sites, bilaterally in the infraclavicular, axillary, subcostal, and inguinal regions. The method of Donaldson (5) was followed, except that a groin site was deleted and an infraclavicular site was utilized to provide more immunostimulation in anatomic proximity to the tumor. Fourteen patients received BCG 1 week before methotrexate, and 2 patients were begun on BCG and methotrexate simulataneously. BCG injections were repeated at 3-month intervals. A protocol violation occurred when 2 patients randomized to methotrexate and BCG received methotrexate alone. Neither responded and a decision was made prior to data review to include them in the methotrexate alone group. Isoniazid, 300 mg daily, was given to the 11 patients who had a positive purified protein derivative of tuberculin.

Statistical Methods and Evaluation of Response

Statistical methods used for analysis were Student's *t* test for comparison of means and Yates' corrected χ^2. Survival tables for treatment groups were constructed by the life table (actuarial) method for comparing methotrexate *versus* methotrexate plus BCG. A computer-based DATA TEXT program was used to compute cross-tabulations and calculate χ^2 for response and survival with each prognostic factor. In this analysis of prognostic factors, the 7 patients still alive were excluded.

Partial response was defined as >50% reduction of the product of 2 diameters of all measurable disease lasting at least 4 weeks. Responses >50% lasting <4 weeks and responses 25 to 50% lasting >4 weeks were defined as disease stabilization. Duration of response was measured from the date of first treatment to the date of disease progression. No patients were lost to follow-up.

Immunological Studies

Immune status was evaluated by the following tests.

Skin Tests. Purified protein derivative of tuberculin, *Candida, Trichophyton,* and mumps antigen were applied to the volar surface of the forearm and observed at 24 and 48 hr. If the area of induration exceeded 10 mm, the test was considered to be positive.

Lymphocyte Subpopulations. Thymic-derived lymphocytes (T-cells) were assessed by the ability to form rosettes with sheep erythrocytes. Bone marrow-derived B-cells were enumerated by quantitating immunofluorescence of separated peripheral blood lymphocytes.

Peripheral blood lymphocytes were separated on Ficoll-Hypaque gradients, and an aliquot of 2 × 10^6 suspension in 0.25 ml Medium 199 was added to a 0.25-ml suspension of 0.5% washed sheep RBC in 0.9% NaCl solution. Trypan blue was added to a drop of the final mixture, and rosettes were enumerated in a hemocytometer, counting 200 viable cells. Rosettes were defined by the presence of 3 or more adherent cells.

Bone marrow-derived lymphocytes were studied by mixing blood lymphocytes in Hanks' balanced salt solution with fluorescein-conjugated polyvalent immunoglobulin antiserum in the presence of sodium azide. The cell suspension was examined under fluorescent microscopy, and a minimum of 200 cells were counted.

RESULTS

Of the 35 evaluable patients, there were 7 partial responses and no complete responses. The 95% confidence limits for this overall partial response rate of 20% are 7 to 33%. The median duration of response was 15.8 weeks. Of the 16 patients receiving methotrexate plus BCG, 4 responded, and of 19 patients receiving methotrexate alone, 3 responded. The difference is not statistically significant ($p_a > 0.05$; $p_b = 0.337$; $\Delta = 0.20$). Four patients had disease stabilization, 3 of whom received BCG (see Table 1). No significant difference was observed in response duration between patients receiving methotrexate plus BCG or methotrexate alone. Chart 1 presents life table survival curves for the 2 groups. The differences are not statistically significant. The trend toward higher early mortality in the methotrexate alone group is due to 2 drug-related deaths and to 1 patient who, although randomized to BCG, received methotrexate alone because he was deemed to be too sick to benefit from BCG; he was analyzed with the methotrexate alone group. No difference was observed between the 2 treatment groups in age, primary site, presence of distant metastases, skin test reactivity, performance status, or pretreatment weight loss. Median survival for the responders was 40 weeks and for the nonresponders was 12 weeks.

Table 1

Responses to methotrexate with or without BCG

	Partial response	Disease stable
Methotrexate alone	3/19[a]	1/19
Methotrexate + BCG	4/16	3/16

[a] Patients responding/total patients.

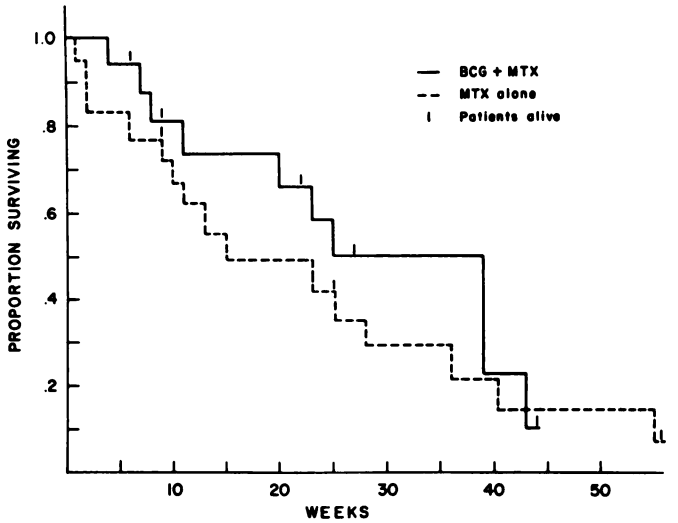

Chart 1. Life table survival curves for patients receiving BCG and methotrexate (*MTX*) or methotrexate alone. The differences are not significant (see "Results").

Toxicity from the methotrexate and BCG was appreciable. Two patients died of methotrexate-induced pancytopenia with fever and gastrointestinal bleeding; each had received 4 doses of biweekly methotrexate. Four other patients also had methotrexate-induced pancytopenia and bleeding requiring transfusions and/or antibiotics. One patient had transient asymptomatic thrombocytopenia (platelet count, 34,000). All of the other 28 patients maintained WBC > 1000 and platelets > 150,000, although most had mild stomatitis as the protocol was designed to produce mild toxicity. At autopsy, 1 patient had findings consistent with methotrexate hepatitis.

BCG produced fever the night of the injection in most patients and brisk local reactions in all patients. In 1 patient the BCG injection sites failed to heal, and viable mycobacteria were cultured from the injection sites for several months. No instances of systemic infection with BCG were documented.

The results of the *in vitro* immunological studies are presented in Table 2. Although the percentage of T-cells in the peripheral blood was higher in responding patients compared to nonresponders, the results were not statistically significant. In 2 patients the *in vitro* immunological studies were repeated after these patients had responded to methotrexate and BCG and shown an increase in the number of T-cells.

Various parameters were examined for their influence on response and survival in these patients with advanced disease. Marked weight loss, poor performance status, and distant metastases seemed to be the most important prognostic factors. None of the 4 patients with weight loss >20% in the 2 months preceding chemotherapy responded to the chemotherapy, and their median survival of 8.7 weeks was borderline, significantly shorter than those of patients with <10% weight loss who had a median survival of 24.2+ weeks ($p < 0.05$). Weight loss also correlated with anergy as defined by absence of response to any of the i.d. skin tests. Six of the 7 anergic patients had weight loss >10% in the previous 2 months, whereas only 9 of 21 patients with positive skin tests had experienced that degree of weight loss ($\chi^2 = 2.34$; $0.05 < p < 0.10$). Weight loss did not seem to affect absolute lymphocyte counts, T-cell rosettes, or B-cells. Anergy had a weak effect on response and survival that may have been due to the associated and more prognostically significant weight loss.

Eight patients were totally bedridden (Eastern Cooperative Oncology Group Performance Status 4); none of them responded to therapy. Their survival was significantly shorter than partially ambulatory patients (8.6 *versus* 24+ weeks; $p < 0.02$). In addition, patients who did not require narcotic analgesics at the time of entry to the study lived somewhat longer, although the difference was not statistically significant (32.5+ *versus* 17.6+ weeks).

The primary sites of disease appeared to influence response, although the differences were statistically insignificant because of the small sample size. Six of 23 patients whose primary site was in the oral cavity or oropharynx responded, whereas only 1 of 12 patients with other primary sites responded. This is consistent with the findings of others (1) who noted higher responses with primary carcinoma of the oral cavity. None of the 8 patients with distant metastases had a partial response, although 1 had stable disease.

In this small series we were unable to demonstrate any significant effect upon response or survival due to age, sex, tumor differentiation, or isoniazid administration.

Table 2

Immunological Studies

	Absolute lymphocyte count	% of T-cell rosettes	% of B-cells
All patients	939 ± 479[a]	57.9 ± 13.7	16.3 ± 10
Partial responders	878 ± 456	69.0 ± 6.1[b]	19.0 ± 9
Stable + non-responders	950 ± 480	55.6 ± 11.0	15.9 ± 10.3

[a] Mean ± S.D.

[b] For partial responders *versus* stable + nonresponders, $0.10 > p > 0.05$.

DISCUSSION

In this randomized prospective study, BCG as an adjunct to chemotherapy with methotrexate in advanced epidermoid carcinomas of the head and neck did not improve results achieved with methotrexate used singly. The duration and degree of response are unimpressive in contrast with those reported by Donaldson (5) who reported responses in 20 of 32 cases given methotrexate, BCG, and isoniazid. The median duration of response in his study is 322 days. However, 3 separate recently concluded randomized studies by Woods *et al.* (23). Buechler *et al.* (2), and Richman *et al.* (19) also showed no increase in response rate when BCG was added to chemotherapy in advanced head and neck cancer. Our results support a lack of efficacy of BCG in this situation. The study by Richman *et al.* (19) showed slightly prolonged survival in the group receiving BCG, but this was probably due to the 4 drug-related deaths in this group, which received chemotherapy only.

Our response rate, although low, was comparable to that reported by Woods *et al.*, (23) (6 of 59 cases). Almost all of our patients had received prior radiation therapy; other investigators (1) have also found a poor response to chemotherapy when it follows radiation in this disease. The toxicity that we encountered with chemotherapy was similar to that seen by other investigators (7, 18, 19).

In view of the failure to obtain benefit from the use of BCG as an adjunct, it seems important to examine the theoretical concepts that provide a rationale for its use in advanced head and neck carcinomas. With regard to immune defects in patients with head and neck carcinoma, our study showed cutaneous anergy in 25%. The occurrence of cutaneous anergy correlated with profound weight loss. Possibly cachexia, rather than head and neck carcinoma *per se*, is the basis for the observed immune defect in such patients (11). Future immunological studies of patients with head and neck cancer should consider the effect that malnutrition has on immune function.

Lymphopenia was frequent at the onset of inclusion into this trial, but most patients had had prior radiotherapy. Decreased T-cell numbers occurred in some cases, but the median number of T-cells (defined as E-rosette-forming cells) was in the low normal range. Wanebo *et al.* (22),

Eastham *et al.* (6), and Deegan and Coulthard (3) reported decreased T-cell numbers in patients with head and neck cancer. In this study a rise in T-cell numbers was observed in patients who experienced a response to methotrexate and BCG. These patients also experienced marked reactions to the BCG, an observation noted by others (21).

Another theoretical argument for the use of BCG is derived from the concept that tumor cell burden is small in advanced head and neck carcinoma because distant metastases are infrequently noted. A recent report (20) states that distant metastases are uncommon in head and neck carcinoma, although a larger series reported in 1971 cites an incidence of 46.7% distant metastases (13). In the present study, 8 of 35 patients had clinically apparent distant metastases and, of the 13 patients who had autopsy examination, 6 had remote metastatic deposits. Therefore it seems likely that the tumor burden is not small in advanced head and neck carcinomas.

Despite the lack of benefit from BCG in this trial, it is possible that immunotherapy will prove useful in patients with early stages of the disease. Experiments involving animal models of squamous cell cancer do substantiate the concept that limited tumor burden is conducive to benefit from use of BCG (10). Also, it is possible that more effective forms of immunotherapy will have an impact upon advanced head and neck carcinoma. Similarly effective chemotherapy resulting in a greater degree of tumor regression will provide an opportunity for immunotherapy to be of greater benefit.

This study includes a diversity of carcinomas of the head and neck. In selected tumor types, greater numbers of cases might reveal some advantage of adjunctive immunotherapy. In conclusion we reaffirm the necessity for controlled prospective trials in the use of adjunctive immunostimulating agents for advanced carcinomas of the head and neck.

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