Chemoradiotherapy as an Alternative to Radiotherapy Alone in Fast Proliferating Head and Neck Squamous Cell Carcinomas

Renzo Corvó, Walter Giaretti, Giuseppe Sanguinetti, Elio Gello, Almalina Bacigalupo, Roberto Orecchia, Marco Benasso, Gian Mauro Numico, Marco Merlano, Giovanni Margarino, and Vito Vitale

Oncologia Radioterapica [R. C., G. S., A. B., V. V.], Laboratorio di Biofisica-Citometria [W. G., E. G., R. O.], Oncologia Medica I [M. B., G. M. N., M. M.], and Oncologia Chirurgica [G. M.], Istituto Nazionale per la Ricerca sul Cancro di Genova, 16132 Genova, Italy

ABSTRACT

The aim of this pilot study was to explore the prognostic relevance of cell kinetics parameters on the local control of patients affected by head and neck squamous cell carcinoma (HN-SCC), randomly assigned to receive either alternating chemoradiotherapy or partly accelerated radiotherapy. Between 1992 and 1995, 40 patients with HN-SCC at stages III and IV entered the study. Multiple primary tumor biopsies were obtained 6 h after in vivo infusion of bromodeoxyuridine, an analogue of thymidine that is incorporated in DNA-synthesizing cells. In vivo S-phase fraction labeling index (LI), duration of S-phase \((T_s)\), and potential doubling time \((T_{pot})\) were obtained by analysis of the flow cytometric content of bromodeoxyuridine and DNA. Twenty patients were treated by alternating chemotherapy and conventional radiotherapy (arm A), whereas 20 other matching patients received partly accelerated radiotherapy alone (arm B). Univariate local control analysis showed that LI, \(T_s\), and \(T_{pot}\) were not prognostically significant in either arm. However, local control probability at 2 years for fast growing tumors, characterized by a LI of \(9\%\), was higher for patients treated with alternating chemoradiotherapy than it was for those treated with partly accelerated radiotherapy alone (68 versus 39%). Conversely, local control probabilities for slow proliferating tumors (LI, <9%) treated in the two arms were similar. These results suggest a potential role for alternating chemotherapy and radiotherapy in HN-SCC patients with fast growing tumors.

INTRODUCTION

HN-SCCs that are diagnosed early have a satisfactory probability of local control and cure (1). Advanced lesions, however, remain a therapeutic problem (2). The vast majority of patients with HN-SCC that is diagnosed late are considered inoperable and are treated by primary radiation therapy. Conventional schedules of radiotherapy consist of one 2-Gy dose per day for 6–8 weeks and a total dose in the range of 66–70 Gy. However, the clinical outcome obtained in advanced HN-SCC by conventional radiotherapy is poor and is characterized by a 3-year local control probability for only approximately 30% of the patients (3).

Clinical trials and laboratory data suggest that the high rate of failure observed in HN-SCC after conventional radiotherapy is partly due to the repopulation of tumor cells that occurs during the course of radiotherapy (4). Recently, accelerated radiotherapy (5) and chemoradiotherapy association (6) have been explored as ways to overcome or minimize the role of multiple biological factors (radio sensitivity, tumor oxygenation, cell proliferation, differentiation, and apoptosis) that may lead to repopulation related risk of locoregional failure.

At present, randomized trials are in progress that aim to optimize treatment for advanced inoperable HN-SCC (2, 6). In the present study, we have tested the hypothesis that treatment of fast and slow proliferating advanced HN-SCC by intensified treatments may improve local control probability. For this purpose, we have considered a preliminary group of 40 HN-SCC patients who entered a randomized study to compare partly accelerated radiotherapy and alternating chemoradiotherapy. In particular, pretreatment proliferation characteristics were obtained by in vivo BrdUrd infusion and multiparameter flow cytometric analysis, which provides LI, \(T_s\), and \(T_{pot}\) (7, 8).

PATIENTS AND METHODS

Patients and Tumor Sampling. We studied 40 patients with unresectable and nonmetastatic (M\(_0\); tumor-node-metastasis staging) HN-SCC at stages III or IV (UICC staging). They were enrolled from March 1992 to July 1995 at the National Institute for Cancer Research (Genoa, Italy). The patients were accrued in a randomized Phase III clinical trial, which compared alternating chemoradiotherapy and a partly accelerated regimen

Received 3/13/97; revised 7/9/97; accepted 7/10/97.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Applicazioni Cliniche della Ricerca Oncologica-Consiglio Nazionale delle Ricerche (Rome, Italy) Grant 96.00687.PF39.

2 To whom requests for reprints should be addressed, at Servizio di Oncologia Radioterapica, Istituto Nazionale per la Ricerca sul Cancro, Largo R. Benzi 10, 16132 Genova, Italy. Phone: 39-10-5600014; Fax: 39-10-5600039; E-mail: corvo@hp380.ist.unige.it.

The abbreviations used are: HN-SCC, head and neck squamous cell carcinoma; LI, S-phase fraction labeling index; \(T_s\), S-phase fraction duration; \(T_{pot}\), potential doubling time; UICC, Union International Contra Cancer; BrdUrd, bromodeoxyuridine; TR, tumor regression; PCNA, proliferating cell nuclear antigen.

3 The abbreviations used are: HN-SCC, head and neck squamous cell carcinoma; LI, S-phase fraction labeling index; \(T_s\), S-phase fraction duration; \(T_{pot}\), potential doubling time; UICC, Union International Contra Cancer; BrdUrd, bromodeoxyuridine; TR, tumor regression; PCNA, proliferating cell nuclear antigen.
of radiotherapy alone. All patients gave written consent according to the recommendation of the Ethical Committee at the National Institute for Cancer Research.

The present pilot clinical trial is part of a larger project that aims to optimize clinical outcome in individual HN-SCC patients based on flow cytometric tumor cell kinetics characteristics evaluated in vivo. As previously reported (8), the patients were given a 20–30-min infusion of BrdUrd (Sigma Chemical Co., St. Louis, MO) dissolved at the concentration of 250 mg in 100 ml of sodium chloride physiological solution. Multiple tumor biopsies were obtained 4–6 h after BrdUrd infusion.

**Flow Cytometry.** Samples were processed according to the BrdUrd/DNA content method of Dolbeare et al. (9). Briefly, the main steps included mechanical disaggregation of tumor fragments and incubation in 0.4 mg/ml pepsin, diluted in 0.1 N HCl, for 30 min at 37°C, followed by resuspension in 1 N HCl for an additional 30 min at 37°C. Nuclei suspensions were then incubated with anti-BrdUrd Mabs at a concentration of 1:100 (Bio Cell Consulting, Grellingen, Switzerland), indirectly conjugated with FITC, and counterstained with the DNA-intercalating dye, propidium iodide.

Measurements and analysis were performed using a flow cytometer/cell sorter (FACS440; Becton Dickinson, Mountain View, CA), equipped with a laser (Spectra Physics, Mountain View, CA) for excitation at 488 nm of FITC and propidium iodide. Green and red fluorescence detection was obtained with a 520/30-nm band filter and a 610 long-pass filter. The flow cytometer was online to a 486 PC, equipped with dedicated software (Phoenix Flow Systems, San Diego, CA). The number of nuclei analyzed for each specimen ranged from 10,000 to 30,000.

DNA aneuploidy was considered to be present when DNA histograms had at least two clearly separated G0-G1 peaks, according to internationally agreed upon criteria (10). The degree of DNA aneuploidy (DNA index) was expressed as the ratio of the mean channel number of the DNA aneuploid G0-G1 peak and the mean channel number of the DNA diploid G0-G1 peak. DNA ploidy corresponds to DNA index = 1. The coefficient of variation of the diploid G0-G1 peaks was evaluated for all samples as an indication of measurement accuracy after DNA histogram fitting by means of commercially available software (Phoenix Flow Systems). The mean coefficient of variation evaluated from the diploid G0-G1 peak was 6%. The calculation of T5 and Tpot values was done as described previously (8), according to the method reported by Begg et al. (11).

**Treatment.** Combined chemoradiotherapy consisted of chemotherapy (four courses during weeks 1, 4, 7, and 10) and conventional radiotherapy (three courses during weeks 2–3, 5–6, and 8–9; arm A). Chemotherapy consisted of cisplatinum (Platinex; Bristol Myers, Rome, Italy) at a dose of 20 mg/m² per day and fluorouracil (Fluorouracile; Roche, Milan, Italy) at a dose of 200 mg/m² per day, both given i.v. for 5 consecutive days (12). Cisplatinum was given during a 2-h period, with forced hydration with 2 liters of saline containing 6 mmol of potassium chloride per liter and 2 g of magnesium sulfate. Fluorouracil was administered in a i.v. bolus dose at the end of hydration. Radiotherapy was delivered for a total dose of 60 Gy, split into three courses of 20 Gy each and given in fractions of 2 Gy once a day, 5 times a week.

Patients assigned to receive partly accelerated radiotherapy alone were treated with a conventional radiotherapy course of 6 weeks, superimposed with a second course of 1.5 Gy once a day/5 times a week during the last 2 weeks, which encompassed only the macroscopic disease (Ref. 8; arm B).

**Clinical Evaluation Criteria.** Two clinical intermediate end points were considered. First, reduction of the original tumor areas (< and ≥ 50%) was evaluated after 40 Gy and subdivided into two classes, corresponding to minor and major 40 Gy-TR (13). Evaluation of 40 Gy-TR in arm A was performed at the end of the first 6 weeks of treatment (two cycles of chemoradiotherapy alternating with two cycle of conventional radiotherapy), and evaluation of 40 Gy-TR in arm B was performed at the end of 4 weeks of conventional radiotherapy.

The second intermediate end point was the clinical local response 3 months after the end of treatments (14). Local control failure was defined as either recurrence in the treatment field after complete tumor clearance or presence of residual disease when tumor response was incomplete.

**Statistical Analysis.** Prognostic significance tests were done for type of treatment (chemoradiotherapy versus partly accelerated radiotherapy), clinical factors (sex, age, primary site, tumor and nodal classification, UICC stage, degree of differentiation, and 40 Gy-TR), and cell kinetics factors (LI, T5, Tpot, and DNA ploidy). Univariate analyses were performed against local control data according to Kaplan and Meyer (15). For the continuous variables (LI, T5, and Tpot), a median value was taken to separate between two subgroups of patients. Differences between Kaplan-Meyer univariate curves were assessed by the Mantel-Cox test (16). Statistical significance was set at P < 0.05.

**RESULTS**

**Tumor Cell Kinetics by Flow Cytometry.** Cell kinetics data were available for 40 HN-SCC patients, of which 20 were assigned to chemoradiotherapy (arm A) and 20 were assigned to partly accelerated radiotherapy alone (arm B). Clinical and histological characteristics of the two patient groups and cell kinetics data (LI, T5, Tpot, and DNA ploidy) are reported in Tables 1 and 2. The patients of the two arms had comparable values for age, sex, and TNM and UICC stage and grading. Also, note that the median values of LI, T5, and Tpot, despite broad ranges of values, were similar.

**Treatment and Clinical Tumor Response.** The patients of arm A were treated with a total dose of 60 Gy of conventional radiation (range, 58–60 Gy) and four chemotherapy cycles (except for two patients who received only one cycle) over a median treatment time of 11 weeks (range, 8–14 weeks).

The patients of arm B received an average dose of partly accelerated radiotherapy of 73 Gy (range, 72–75 Gy) over a median treatment time of 6.5 weeks (range, 6–7 weeks).

Major 40 Gy-TR occurred in arm A for 14 of 20 (79%) patients, compared to 8 of 20 (40%) patients in arm B (P = 0.05).

Three months after the end of treatments, clinical local control was evident in 27 (67%) patients overall, 15 of 20 (75%) patients in arm A and 12 of 20 (60%) patients in arm B; i.e., 13 patients had local failure, 5 in arm A and 8 in arm B (P, not
respectively (P = 0.01; data not shown). Among patients treated by partly accelerated radiotherapy (arm B), significant differences in local control were found for T4 stage versus T3 stage (17 versus 56%, respectively, P = 0.05) and primary tumor sites (oral cavity versus other sites; 33 versus 50%, respectively, P = 0.01; data not shown).

DNA ploidy, LI, T50, and Tpost values had no statistically significant effect on local control probability in either arm, as shown in Table 3.

Table 3 shows the local control probabilities after subgrouping the patients of both A and B arms according to slow and high proliferation characteristics, using median values as thresholds. We found significantly better local control probabilities when the therapy modality was chemoradiotherapy, compared to when the modality was partly accelerated radiotherapy alone for the fast growing tumors, characterized by LI values of ≥9% (Table 3).

Fig. 1 shows that local control probability at 2 years for tumors with LI of ≥9% was 68% for patients treated by chemoradiotherapy versus 39% for patients treated by radiotherapy alone (P = 0.03). In other words, we obtained an higher complete tumor response rate in arm A with respect to arm B [11 of 13 (84%) versus 8 of 12 (66%), respectively] and a minor relapse rate occurring in complete responders in arm A with respect to arm B [2 of 11 (18%) versus 4 of 8 (50%), respectively].

A trend for a better local control rate was observed with alternating chemoradiotherapy than that with partly accelerated radiotherapy in patients with a $T_{post}$ of ≤5 days (P = 0.1).

Similar local control rates (48% at 2 years) were obtained for slowly proliferating tumors (LI, <9%) independently from treatment type.

A trend for a better local control rate with chemoradiotherapy than that with radiotherapy alone was also observed for patients with tumors with T4 stage (P = 0.07) and G3 differentiation (P = 0.08).

DISCUSSION

Due to the still very high relapse and death rate of patients with advanced inoperable squamous cell carcinoma of the HN-SCCs, several Phase III clinical trials are presently in progress, with the aim of improving treatment (17). One basic difference among the different clinical trials was the evaluation of parameters related to biological properties, including tumor growth rate, for the individual patients. It is conceivable that a clinical study based on biological parameters should allow the gain of supplementary information for a better interpretation of the clinical outcome. Proliferation characteristics are of particular interest in this context because they are associated with individual tumor’s biological aggressiveness (4, 18). In a recent study conducted with use of flow cytometry and in vivo BrdUrd infusion to quantitatively evaluate cell kinetics parameters, we suggested that partly accelerated radiotherapy may be superior to conventional radiotherapy in the treatment of fast growing HN-SCCs (8).

In the present pilot study, we have found that local control probability at 2 years for fast growing HN-SCCs, characterized by high labeling of BrdUrd for cells actively synthesizing DNA
Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Alternating chemoradiotherapy, arm A</th>
<th>Partly accelerated radiotherapy, arm B</th>
<th>Arm A vs. arm B</th>
</tr>
</thead>
<tbody>
<tr>
<td>LI</td>
<td>n</td>
<td>LCa (%)</td>
<td>P</td>
</tr>
<tr>
<td>≥9%</td>
<td>13</td>
<td>68</td>
<td>0.1</td>
</tr>
<tr>
<td>&lt;9%</td>
<td>7</td>
<td>48</td>
<td>0.8</td>
</tr>
<tr>
<td>10h</td>
<td>&gt;10 h</td>
<td>7</td>
<td>85</td>
</tr>
<tr>
<td>10 ≤h</td>
<td>13</td>
<td>39</td>
<td>0.4</td>
</tr>
<tr>
<td>≥5 days</td>
<td>&gt;5 days</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>5 ≤days</td>
<td>13</td>
<td>62</td>
<td>0.3</td>
</tr>
<tr>
<td>DNA</td>
<td>Diploidy</td>
<td>9</td>
<td>66</td>
</tr>
<tr>
<td>Aneuploidy</td>
<td>11</td>
<td>54</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a LC, local control.

Fig. 1 Local control probabilities for fast growing (BrdUrd-LI, ≥9%) HN-SCC, treated with alternating chemoradiotherapy or partly accelerated radiotherapy.

(indicated by LI of ≥9%), was higher for patients treated by alternating chemoradiotherapy in comparison with those treated by partly accelerated radiotherapy alone (68 versus 39%). Conversely, local control probabilities for slow proliferating tumors (LI, <9%) treated in the two arms were similar. These results suggest that a bimodal treatment of chemotherapy alternated to radiotherapy in HN-SCC patients with fast growing tumors may be more effective than radiotherapy alone.

Thus, if this trend is confirmed with a larger number of patients, fast growing HN-SCCs may still benefit from alternating chemoradiotherapy rather than merely changing the modality of radiotherapy from conventional to partly accelerated.

A recent study investigating HN-SCC patients treated with chemoradiotherapy, in which the protein PCNA amount was evaluated by immunocytochemistry, indicated a better local control for patients with higher PCNA values (19). Because both PCNA amount and LIs are proliferation-associated parameters, it appears that both studies reinforce our present interpretation. That local control probabilities of slowly proliferating tumors treated with either therapy modality were similar suggests the relevance of subdividing the patients according to fast and slow proliferation.

Different hypotheses regarding the potential efficacy of combined chemoradiotherapy for treating fast growing HN-SCCs have been considered.

Cell proliferation rate during cytotoxic treatment of HN-SCCs may not be interrupted for long time by the treatment, as has been recently reported (18), because a cell cycle block may be reversed. Fast growing HN-SCC cell repopulation would be expected to be massive in these individual patients. One possibility is that the longer treatment time of the combined chemoradiotherapy schedule for fast growing HN-SCCs with respect to radiotherapy alone (10 versus 6 weeks respectively) has the potential to increase killing of cells reversing from cell cycle blocks. Moreover, it is conceivable, at least in fast growing HN-SCCs, that the administration of different antineoplastic agents in strict temporal association with radiotherapy may reduce the probability of developing chemoresistant proliferating subclones (19, 20, 21). An additional possibility is that the reentry of kinetically quiescent tumor cells into the cell cycle during treatment, as has been documented for HN-SCCs (18), is also better controlled by a combined therapy than by radiotherapy alone.

The reason why slow proliferating HN-SCCs appear to respond similarly to the two different treatment modalities is not easy to understand. We speculate that a cell repopulation factor is less prominent in these individual patients and that hematological side-effects induced by chemotherapy administration (12) may be avoided by using only the radiotherapy modality.

In conclusion, our preliminary data suggest that the type of treatment influences local failure rate in advanced HN-SCCs characterized by fast growth and that the tumor response rate obtained in arm A by alternating chemotherapy and radiotherapy was statistically significantly better than treatment in arm B with radiotherapy alone. Although the follow-up time was long enough to suggest this interpretation, the results of this study need to be confirmed with a larger number of patients.

REFERENCES

Chemoradiotherapy as an alternative to radiotherapy alone in fast proliferating head and neck squamous cell carcinomas.

R Corvó, W Giaretti, G Sanguineti, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/3/11/1993

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.