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## TUMOR CELL DNA CONTENT AND RADIATION RESPONSE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

DNA content in tumors is reported to be a prognostic factor in many malignancies. The presence of an abnormal stem cell line, termed aneuploidy, is generally accepted as a poor prognostic factor (1). However, some studies revealed that aneuploid tumors have a better response to radiation therapy. The prognostic value of DNA content has been debatable in head and neck cancer (1). In most of the reports paraffin-embedded material is used for the measurement of DNA content and the detection of aneuploidy might have been insufficient. In the present study, we examined cellular DNA content using fresh-frozen material in patients with head and neck cancer and made a prospective investigation of the relation between DNA content and treatment results.

*Material and Methods.* From August 1991 to January 1993, fresh-frozen materials from 45 patients with head and neck squamous cell carcinomas were examined for DNA content. Out of the 45 patients, 29 who were treated with definitive irradiation were studied on the relationship between DNA content and prognosis. The commonly used radiotherapy schedule was: 2.5 Gy per fraction with a total dose of 65 Gy and with an overall treatment time of 6.5 weeks. Mean follow-up period was 20 months. Primary sites were larynx in 10 (patients), paranasal sinus in 5, oropharynx in 5, tongue in 4, nasopharynx in 2, hypopharynx in 2 and buccal mucus in 1 patient. Measurement of DNA content was carried out using a flow cytometor (FACscan, Becton-Dickinson). Lymphocytes from healthy adults were used as controls for G0/G1 peak of the sample. The coefficient variance of the G0/G1 peak of the sample was 4.4 in its mean value. A DNA index (DI) was calculated from the DNA histogram. An aneuploid tumor was defined as having a DI of more than 1.1 or less than 0.9. The initial radiation response was assessed 2 months after the completion of treatment. Complete response (CR) was defined as disappearance of the tumor. Response to irradiation was based on physical examination and/or computed tomography.

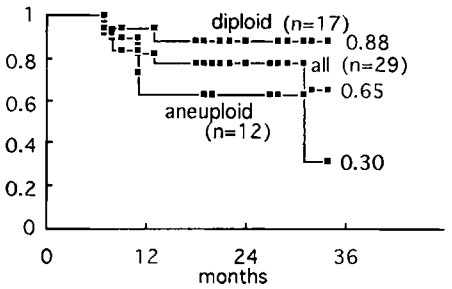


*Figure.* Survival by ploidy.

*Results.* All T1 and T2 tumors displayed a complete response regardless of the status of DNA content. Among 14 T3 or T4 tumors, CR was achieved in 8 cases. The mean value of DI in those 8 cases was higher than that of 6 residual cases, but there was no statistical significance. No difference in CR rate was found between aneuploid and dipioid tumors (63%(5/8) vs. 50%(3/6)). However, when the DI was set at 1.2 as the threshold, the CR rate was significantly higher above the threshold (100%(5/5)) than below (33%(3/9)) ($p<0.01$, $\chi^2$-test). The Figure shows survival depending on ploidy. Aneuploid tumor cases showed poor survival and disease-free survival compared with those with diploid tumor though statistical significance was not indicated. A similar pattern could be seen in T3 and T4 cases.

*Discussion.* The prognostic value of tumor DNA content in squamous cell carcinoma has been controversial. Aneuploid tumors of the uterine cervix are reported to be more radiosensitive than diploid tumors (2, 3). Franzen et al. (4) studied 24 squamous cell carcinomas treated by radiotherapy, preoperatively. They reported that 5 of 7 aneuploid tumors were eradicated histologically by preoperative irradiation. However, Muller et al. (5) have reported that aneupoloid tumors had a poorer prognosis in a study of 50 patients with maxillo-facial carcinoma, who were given radiotherapy postoperatively. In the present study, aneuploid tumor, especially that with a large DI, tended to have a better response to radiotherapy. However, this initial improved response did not lead to a better chance of survival in cases of aneuploid tumor. The reason for this was that recurrence or residual disease in aneuploid cases could not be salvaged, whereas recurrences or residual disease in diploid cases tended to stay at the primary site and did not display rapid growth. These were effectively salvaged by surgery. Recently, it was reported that aneuploid tumors have greater proliferlative activity than diploid tumors in head and neck SCC (6). Dyson et al. (2) reported that aneuploid tumors quickly spread locally and metastasize to other organs. Our findings were consistent with these studies. To conclude, aneuploid tumors seem to be radiosensitive but have a poor prognosis in head and neck squamous cell carcinoma.

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## SYSTEMIC MITOMYCIN C AS SECOND-LINE TREATMENT FOR METASTATIC UROTHELIAL CANCER

Mitomycin C has shown effect primarily as a drug for local treatment of non-invasive bladder tumors. As single agent it has been reported to cause tumor reduction both in patients with invasive bladder tumors and in those with metastatic disease (1, 2). These studies, however, have not well-defined response criteria, and the number of patients included is small. Few studies have reported results of treatment with mitomycin C in combination with other drugs, achieving a response rate of 31-48% (3, 4). The drug induces myelosuppression, but has limited renal toxicity which makes it feasible as second-line treatment after cisplatin-based chemotherapy.

This report summarizes the results of a phase II study with systemic mitomycin C as single drug therapy in patients with locally advanced or metastatic urothelial cancer with progression after first-line chemotherapy.

*Material and methods.* Two institutions entered 23 patients with histologically proven measurable advanced transitional cell carcinoma of the urinary tract.

The criteria of eligibility were: 1) Progression of disease after or during first-line chemotherapy with cisplatin and methotrexate, or cisplatin, methotrexate plus carboplatin in our previous phase II trials (5, 6). Also patients who where non-eligible for first-line treatment because of reduced renal function or other medical reasons were included. 2) No prior local or systemic treatment with mitomycin C. 3) Performance status score (WHO) $\leq 2$. 4) Blood tests: White blood cell count $\geq 3 * 10^9/l$, platelet count $\geq 150 * 10^9/l$, prothrombin level $> 70\%$, and bilirubin 4-22 micromol/l. 5) No other malignancy. 6) Informed consent.

All patients were assessed initially by clinical examinations and laboratory investigations including complete blood count, serum creatinine, electrolytes, liver function tests, $^{51}$Cr-EDTA clearance, and chest x-ray. CT-and ultrasound scans were performed when necessary for evaluation of measurable disease.

Mitomycin C, 20 mg/m$^2$, was administered in 500 ml 0.9% saline solution, given intravenously in 30 min and repeated every sixth week. Antiemetic treatment was given prophylactically, in most cases as metopimazin 30 mg, 4 times daily for 3-4 days. The dose of mitomycin C was reduced if the nadir white blood cell or platelet counts were below WHO grade 3 or more, and the treatment was delayed if the blood counts were below normal at the day of treatment. Treatment was discontinued in case of severe lung, renal or prolonged hematological toxicity (more than 3 weeks). Full reassessment of evaluable disease was performed every third course. Response and toxicity were defined according to the WHO criteria. Early deaths, i.e. before the first evaluation, were classified as progressive disease (PD), except when due to toxicity. The study was approved by the local ethics committee.

*Results.* A total of 23 patients were included in the study. Two patients were non-eligible; one patient did not have progressive disease after first-line chemotherapy and one had elevated bilirubin. Nineteen of 21 patients were evaluable for response. In the two remaining patients treatment had to be discontinued after two courses because of toxicity. The male/female ratio was 6/15 and the median age was 61 years (range 51-71). One patient had a large adherent tumor in the pelvis. Fifteen patients had an advanced local recurrent tumor in the bladder and 14 patients had distant metastases as well. Six patients had metastatic disease only. Liver metastases were recorded in 12 patients, metastases in distant lymph nodes in 15 patients, lung metastases in 5, and brain metastases in one patient.

First-line chemotherapy was cisplatin, methotrexate and carboplatin in 18 patients (6). Three patients did not receive first-line chemotherapy for metastatic disease because of impaired renal function, congestive heart failure and neoadjuvant treatment with cisplatin and methotrexate respectively. Performance status score was 0 in 4 patients, 1 in 11 patients and 2 in 6 patients. Treatment was discontinued in 14 patients after one or two courses because of progressive disease. Three patients received 3 courses, but had progressive disease at the first evaluation. Two patients had no change (NC), but treatment had to be stopped after 4 courses because of prolonged hematological toxicity. Two patients were eliminated from the study after two courses because of lung and prolonged hematological toxicity respectively. The first patient had no change when treatment was discontinued and the second one achieved minor response (less than 50% tumor reduction).

The median survival of all patients was 4 months (range 1.3-15.9). Patients with progressive disease had a median survival of 2.9 months and the two patients with NC survived for 8.6 and 15.9 months respectively. Two patients are still alive; one patient with progressive disease and one patient who was eliminated from the study because of lung toxicity.

The median dose was 36 mg per course; no patient had dose reduction. Hematological toxicity was severe, especially thrombocytopenia, with half of the patients having grade 3 or 4 toxicity, and two of these patients had severe bleeding episodes. Nine patients had leukopenia, but mostly grade 1 or 2. Five patients had infections (mostly from urinary tract) but none in relation to leukopenia. Nineteen patients developed anemia and most patients needed transfusions. Emesis and vomiting were moderate. Four patients had transient grade 1 nephrotoxicity. One patient developed lung toxicity with pulmonary infiltrations and severely reduced diffusion capacity and was excluded from study with no further examinations. One patient had a cerebral infarction two