Eur J Cancer, Vol. 27, No. 10, p. 1334, 1991.
Printed in Great Britain
0277-5379/91 $3.00 + 0.00

Ewing's Sarcoma and Epstein–Barr Virus

Søren Daugaard, Ulla Hørding and Torben Schiødt

EWING'S SARCOMA (ES) is a highly malignant tumour which occurs primarily in the bones of children and young adults. Both its aetiology and its histogenesis are unknown; immunohistochemical studies indicate that conventionally diagnosed ES is a heterogenous entity which may be related to blastomas or to peripheral neuroectodermal tumours (PNETs) [1]. The latter view is supported by the demonstration of a common chromosomal lesion, t(11;22) (q24;q12) or del(22)q(12), and expression of the MIC2 gene [2].

Epstein–Barr virus (EBV) has been demonstrated and is thought to play an aetiological role in nasopharyngeal carcinomas and various lymphoid malignancies [3, 4]. A recent case report describes a patient with persistent polyclonal B-lymphocytosis, EBV antibodies and subsequent malignant pulmonary blastoma [5].

The polymerase chain reaction (PCR) allows detection of (virus-)specific genome sequences in routinely processed, archival tissue material [6]. EBV can be detected by the amplification of a 110 bp sequence of the highly conserved *Bam* H1W region, which is reiterated 10–11 times [6].

We examined pathological material from 7 patients with ES (aged 3, 5, 13, 15, 16 (extraskeletal), 19 and 61 years, respectively) and 2 with rhabdomyosarcomas (4 and 18 years old), all immunohistochemically confirmed. Diagnostic paraffin blocks were chosen and 7 μm sections were cut from each, placed in a 0.5 ml tube and deparaffinised by boiling. The supernatant was transferred to another tube, and PCR buffer including *Taq* polymerase, the four deoxynucleotide triphosphates and oligonucleotide primers specific for EBV was added to a total of 0.1 ml. The reaction mixture was overlaid with mineral oil to prevent vaporisation and subjected to 30 cycles of amplification, followed by dot blotting and hybridisation with a ^{32}P-endlabelled oligonucleotide probe specific to the internal part of the amplified sequence of EBV. Negative controls consisted of heart tissue, and paraffin-embedded pellets of Raji cells (which contain about 50 EBV genome copies each) were used as positive controls. Sections of paraffin-embedded tissue from nasopharyngeal carcinomas in two Greenlandic patients were also included.

As would be expected, EBV was present in both nasopharyngeal carcinomas, but we could not demonstrate its presence in the nine sarcomas.

Considering the extreme sensitivity of the PCR method, it is thus highly unlikely that EBV plays a role in the evolution of these sarcomas.

1. Daugaard S, Kamby C, Sunde LM, Myhre-Jensen O, Schiødt T. Ewing's sarcoma. A retrospective study of histological and immunohistochemical factors and their relation to prognosis. *Virchows Archiv A Pathol Anat* 1989, **414**, 243–251.
2. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H, Salzer-Kuntschik M. MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors. *Cancer* 1991, **67**, 1886–1893.
3. Chang YS, Tyan YS, Liu ST, Tsai MS, Pao CC. Detection of Epstein-Barr virus DNA sequences in nasopharyngeal carcinoma cells by enzymatic DNA amplification. *J Clin Microbiol* 1990, **28**, 2398–2402.
4. Ohshima K, Kikuchi M, Eguchi F, *et al.* Analysis of Epstein-Barr viral genomes in lymphoid malignancy using Southern blotting, polymerase chain reaction and *in situ* hybridisation. *Virchows Archiv B Cell Pathol* 1990, **59**, 383–390.
5. Lawlor E, Murray M, O'Briain DS, *et al.* Persistent polyclonal B lymphocytosis with Epstein-Barr antibodies and subsequent malignant pulmonary blastoma. *J Clin Pathol* 1991, **44**, 341–342.
6. Peiper SC, Myers JL, Broussard EE, Sixbey JW. Detection of Epstein-Barr virus genomes in archival tissues by polymerase chain reaction. *Arch Pathol Lab Med* 1990, **114**, 711–714.

Correspondence to S. Daugaard.
S. Daugaard is at the Department of Pathology, Frederiksberg Hospital, DK-2000 Frederiksberg; U. Hørding is at the Department of Gynaecology; and T. Schiødt at the Department of Pathology, Rigshospitalet, Copenhagen, Denmark.
Received 4 July 1991; accepted 18 July 1991.

Eur J Cancer, Vol. 27, No. 10, pp. 1334–1335, 1991.
Printed in Great Britain
0277-5379/91 $3.00 + 0.00

Excessive Toxicity of Mitoxantrone Combined with Etoposide in Advanced Non-small Cell Lung Cancer

Brian M. O'Connor, Patricia Ziegler and Monica B. Spaulding

NON-SMALL CELL LUNG CANCER (NSCLC) is responsive to combination chemotherapy and a short but significant prolongation of survival has been achieved in advanced disease with treatment [1]. At present, however, there is no standard chemotherapeutic regimen for NSCLC and existing protocols still lack the efficacy required to produce an acceptable number of complete remissions. Etoposide has an objective response rate of 11% in NSCLC as a single agent [2]. Mitoxantrone alone has also shown modest activity in NSCLC, with response rates ranging from 10% to

Correspondence to B. M. O'Connor, Frederick Memorial Hospital, Regional Cancer Therapy Center, 501 West Seventh St, Frederick, Maryland 21701, U.S.A.
The authors are at the Oncology Section, Department of Medicine, State University of New York at Buffalo, Department of Veterans' Administration Medical Center, New York, Buffalo, U.S.A.
Received 27 May 1991; accepted 13 June 1991.

12% [3, 4]. The two agents were thus combined in a non-randomised phase II study to determine joint activity in NSCLC.

All patients had to have histologically confirmed inoperable stage IIIB or IV NSCLC with measurable or evaluable disease, an ECOG performance status ≤ 2, absence of weight loss > 10% in the preceding 3 months, no history of prior chemotherapy or concurrent malignancy, no major organ dysfunction and a projected life expectancy of 6 months. Signed informed consent was obtained from each patient according to institutional policy. Based on prior trials, mitoxantrone was administered as a bolus injection at a dosage of 10 mg/m^2 on day 1 [4–7]. This dosage was to be escalated to a maximum of 14 mg/m^2 depending on the degree of myelosuppression. The mitoxantrone dose was reduced by 1 mg/m^2 for nadir neutrophil counts < 1000/μl associated with suspected or documented infection or nadir neutrophil counts alone < 500 μl. Patients with prior radiation were started at 9 mg/m^2. Etoposide was given as a 2-hour infusion on 3 consecutive days at a dosage of 100 mg/m^2/day. Chemotherapy was to be repeated every 3 weeks. Tumour measurements were recorded at the initiation of therapy and after every two cycles. Responses were assessed using standard criteria. Toxicities were graded according to WHO criteria [8].

9 patients with NSCLC were entered into the study and received a total of 22 courses of mitoxantrone and etoposide. All were men with a mean age of 59 years (range 44–74). No patient had received prior chemotherapy, but 3 (33%) had received prior radiation therapy with curative intent and had relapsed outside the radiation port. 1 patient had relapsed postsurgery. More than half were squamous cell type (55.6%) and most were poorly differentiated (55.6%). There were no partial or complete responders; 7 patients had stable disease for more than two cycles. The patients with stable disease eventually were taken off study for progression (3), toxicity (1), and increasingly severe pain without measurable progression (3; 1 of these also had a haemoptysis and another had hypercalcaemia). Time to progression of the 3 patients with stable disease was a mean of 74 (S.D. 33) days. All patients died after a mean survival of 150 days (5 months), including 1 early death.

The primary toxicity was haematological. 4 patients experienced neutropenic fever; only one episode was grade 3 with a *Klebsiella pneumoniae* bacteraemia, and another was grade 5 with death secondary to septic shock (see Table 1). Nine of the 22 courses (41%) were followed by grade 3 (1000–2000/μl) or 4 (< 1000/μl) leukopenia, while 15 of 22 (68%) courses had neutropenia of equal grade (500–1000/μl and < 500/μl, respectively) in spite of a total of five dose reductions. Only 1 patient developed thrombocytopenia in relation to septic shock. 2 patients with clinically stable pulmonary disease experienced a severe wasting syndrome accompanied by grade 3 anorexia and weight loss of 12 and 28 pounds, respectively. Other toxicities were infrequent and of no major consequence. 2 patients tolerated the regimen as outpatients.

Table 1. Toxicity

	WHO grade*					
	1	2	3	4	5	Total ≥ 3 (%)
Infection	1	1	1	0	1	2 (9)
Leukopenia	0	9	6	3	0	9 (41)
Neutropenia	1	1	7	8	0	15 (68)
Thrombocytopenia	0	0	0	1	0	1 (5)
Anaemia	9	6	1	1	0	2 (9)
Alopecia	0	9	0	0	0	0
Anorexia	1	1	3	0	0	3 (14)
Nausea	4	1	2	0	0	2 (9)
Emesis	0	1	2	0	0	2 (9)
Fatigue	0	2	0	2	0	2 (9)

*Grade 5 is toxicity causing death.

The present study was an attempt to use a new drug combination in NSCLC: mitoxantrone with etoposide. These two drugs may be synergistic since both cause DNA breaks [9, 10]. This combination has shown activity in haematological malignancies without renal, hepatic or cardiac toxicity [11]. Patient accrual was terminated early due to excessive haematological toxicity. The frequency of leukopenia in this study was similar to that seen with mitoxantrone alone, 38% [6]; however, the occurence of neutropenia was more than doubled by adding etoposide [4]. The degree of leukopenia and neutropenia obtained represented necessary endpoints as previous phase II studies with mitoxantrone have shown that dose intensity is important [12]. In spite of this, there were no objective responses in the present study. The haematological toxicities with the described dosing schedule were prohibitive and precluded the continued use of the combination mitoxantrone and etoposide for the treatment of advanced NSCLC.

1. Rapp E, Pater JL, Willan A, *et al.* Chemotherapy can prolong survival in patients with advanced non-small cell lung cancer—report of a Canadian multicenter randomized trial. *J Clin Oncol* 1988, **6**, 633–641.
2. Bakowski MT, Crouch JC. Chemotherapy of non-small cell lung cancer: a reappraisal and a look to the future. *Cancer Treat Rev* 1983, **10**, 159–172.
3. Valdivieso M, Umsawasdi T, Spitzer G, *et al.* Phase II clinical evaluation of dihydroxyanthracenedione in patients with advanced lung cancer. *Am J Clin Oncol* 1984, **7**, 241–244.
4. Anderson KC, Cohen GI, Garnick MB. Phase II trial of mitoxantrone. *Cancer Treat Rep* 1982, **66**, 1929–1931.
5. Ettinger DS, Finkelstein DM, Harper GR, *et al.* Phase II study of mitoxantrone, aclarubicin, and diaziquone in the treatment of non-small cell lung carcinoma: an eastern cooperative oncology group study. *Cancer Treat Rep* 1985, **69**, 1033–1034.
6. Suga J, Saijo N, Shinkai T, *et al.* Phase II study of mitoxantrone in patients with non-small cell lung cancer. *Jpn J Clin Oncol* 1986, **16**, 147–151.
7. De Jager R, Cappelaere P, Armand JP, *et al.* An EORTC phase II study of mitoxantrone in solid tumours and lymphomas. *Eur J Cancer Clin Oncol* 1984, **20**, 1369–1375.
8. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981, **47**, 207–214.
9. O'Dwyer PJ, Leyland-Jones B, Alonso MT, Marsoni S, Wittes RE. Etoposide (VP-16-213), current status of an active anticancer drug. *N Engl J Med* 1985, **312**, 692–700.
10. Tewey KM, Rowe TC, Yang L, *et al.* Adriamycin induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* 1984, **226**, 446–448.
11. Ho AD, Lipp T, Ehninger G, *et al.* Combination of mitoxantrone and etoposide in refractory acute myelogenous leukemia—an active and well-tolerated regimen. *J Clin Oncol* 1988, **6**, 213–217.
12. Kramer BS, Gams R, Birch R, Einhorn L, Buchanan R. Phase II evaluation of mitoxantrone in patients with bronchogenic carcinoma: a southeastern cancer study group trial. *Cancer Treat Rep* 1984, **68**, 1295–1296.

Acknowledgement—This work was supported in part by a grant from Lederle.