ETOPOSIDE

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Etoposide

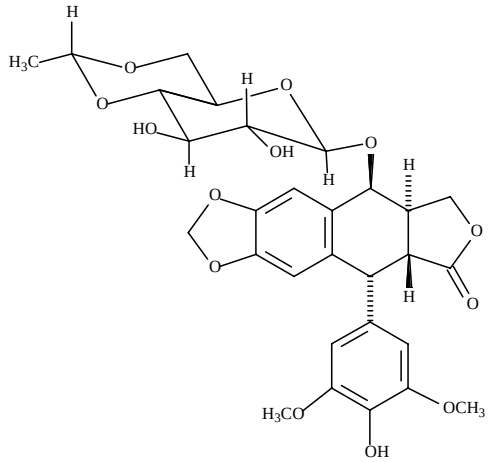
Chem. Abstr. Serv. Reg. No.: 33419-42-0
Chem. Abstr. Name: (5*R*,5a*R*,8a*R*,9*S*)-9-{[4,6-*O*-(1*R*)-Ethylidene-β-D-glucopyranosyl]oxy}-5,8,8a,9-tetrahydro-5-(4-hydroxy-3,5-dimethoxyphenyl)furo[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one
IUPAC Systematic Name: 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-(*R*)-ethylidene-β-D-glucopyranoside)
Synonyms: 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside); 4′-demethylepipodophyllotoxin ethylidene-β-D-glucoside; (–)-etoposide; *trans*-etoposide; VP 16; VP 16-123; VP 16-213

Etoposide phosphate

Chem. Abstr. Serv. Reg. No.: 117091-64-2
Chem. Abstr. Name: (5*R*,5a*R*,8a*R*,9*S*)-5-[3,5-Dimethoxy-4-(phosphonooxy)phenyl]-9-{[4,6-*O*-(1*R*)-ethylidene-β-D-glucopyranosyl]oxy}-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one
IUPAC Systematic Name: 4′-Demethylepipodophyllotoxin 9-(4,6-*O*-(*R*)-ethylidene-β-D-glucopyranoside), 4′-(dihydrogen phosphate)
Synonyms: {5*R*-[5α,5aβ,8aα,9β(*R**)]}-5-[3,5-Dimethoxy-4-(phosphonooxy)-phenyl]-9-[(4,6-*O*-ethylidene-β-D-glucopyranosyl)oxy]-5,8,8a,9-tetrahydrofuro[3′,4′:6,7]naphtho[2,3-*d*]-1,3-dioxol-6(5a*H*)-one; etoposide 4′-phosphate

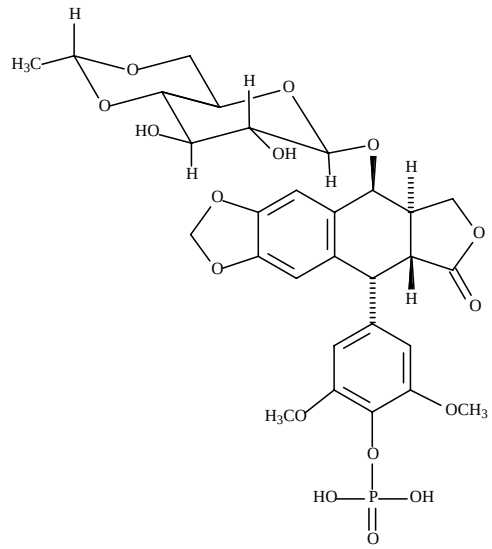
1.1.2 *Structural and molecular formulae and relative molecular mass*

Etoposide



$C_{29}H_{32}O_{13}$ Relative molecular mass: 588.57

Etoposide phosphate



$C_{29}H_{33}O_{16}P$ Relative molecular mass: 668.55

1.1.3 *Chemical and physical properties of the pure substances*

Etoposide

(*a*) *Description*: White to yellow-brown crystalline powder (Gennaro, 1995; American Hospital Formulary Service, 1997)
(*b*) *Melting-point*: 236–251 °C (Budavari, 1996)
(*c*) *Spectroscopy data*: Infrared, ultraviolet, fluorescence emission, nuclear magnetic resonance (proton and ^{13}C) and mass spectral data have been reported (Holthuis *et al*., 1989).
(*d*) *Solubility*: Sparingly soluble in water (approximately 0.03 mg/mL) and diethyl ether; slightly soluble in ethanol (approximately 0.76 mg/mL); very soluble in chloroform and methanol (Gennaro, 1995; American Hospital Formulary Service, 1997)
(*e*) *Optical rotation*: $[\alpha]_D^{20}$, –110.5° (c = 0.6 in chloroform) (Budavari, 1996)
(*f*) *Dissociation constant*: pK_a, 9.8 (Budavari, 1996)

Etoposide phosphate

(*a*) *Description*: White to off-white crystalline powder (American Hospital Formulary Service, 1997)
(*b*) *Solubility*: Very soluble in water (> 100 mg/mL); slightly soluble in ethanol (American Hospital Formulary Service, 1997)

1.1.4 *Technical products and impurities*

Etoposide is available as a 50- or 100-mg liquid-filled capsule and as a 20-mg/mL injection solution. The gelatin capsules may also contain citric acid, gelatin, glycerol, iron oxide, parabens (ethyl and propyl), polyethylene glycol 400, sorbitol and titanium dioxide. Etoposide concentrate for injection is a sterile, non-aqueous solution of the drug in a vehicle, which may be benzyl alcohol, citric acid, ethanol, polyethylene glycol 300 or polysorbate 80. The concentrate for injection is a clear, yellow solution and has a pH of 3–4.

Etoposide phosphate for injection is a sterile, non-pyrogenic, lyophilized powder containing sodium citrate and dextran 40; after reconstitution of the drug with water for injection to a concentration of 1 mg/mL, the solution has a pH of 2.9 (Gennaro, 1995; American Hospital Formulary Service, 1997; Canadian Pharmaceutical Association, 1997; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; Editions du Vidal, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998).

The following impurities are limited by the requirements of *The British Pharmacopoeia*: 4′-carbenzoxy ethylidene lignan P, picroethylidene lignan P, α-ethylidene lignan P, lignan P and 4′-demethylepipodophyllotoxin (British Pharmacopoeia Commission, 1994).

Trade names for etoposide include Abiposid, Cehaposid, Celltop, Citodox, Eposin, Etocris, Etomedac, Etopol, Etoposid Ebewe, Etoposid Pharmacia Upjohn, Etoposid Austropharm, Etoposida Filaxis, Etoposid, Etoposide Dakota, Etoposide Injection, Etoposide P&U, Etoposide Pierre Fabre, Etoposido Asofarma, Etoposido Dakota Farma, Etoposido Farmitalia, Etosid Euvaxon, Exitop, Kebedil, Labimion, Lastet, Optasid, Toposar, VePesid and Vépéside-Sandoz (Swiss Pharmaceutical Society, 1999).

Trade names for etoposide phosphate include Etopofos and Etopophos (Swiss Pharmaceutical Society, 1999).

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared absorption spectrophotometry with comparison to standards and liquid chromatography as the methods for identifying etoposide; liquid chromatography is used to assay its purity. In pharmaceutical preparations, etoposide is identified by infrared absorption spectrophotometry and thin-layer chromatography; liquid chromatography is used to assay for etoposide content (British Pharmacopoeial Commission, 1994; US Pharmacopeial Convention, 1994; Council of Europe, 1997; US Pharmacopeial Convention, 1997).

Methods for the analysis of etoposide and its metabolites in plasma, serum and urine have included reversed-phase high-performance liquid chromatography with oxidative electrochemical detection, fluorescence detection and ultraviolet detection. The limit of detection of these methods is often < 100 ng/mL (Holthuis *et al*., 1989).

1.2 Production

Etoposide is a semi-synthetic derivative of podophyllotoxin and was first synthesized in 1963. Podophyllotoxin is isolated from the dried roots and rhizomes of species of the genus *Podophyllin*, such as the may apple or American mandrake (*Podophyllin peltatum* L.) and *Podophyllin emodi* Wall (Holthuis *et al*., 1989).

Etoposide can be synthesized from naturally occurring podophyllotoxin by first treating the podophyllotoxin with hydrogen bromide to produce 1-bromo-1-deoxyepipodophyllotoxin, which is demethylated to 1-bromo-4′-demethylepipodophyllotoxin. The bromine is replaced by a hydroxy group, resulting in 4′-demethylepipodophyllotoxin. After protection of the phenolic hydroxyl, the 4-hydroxy group is coupled with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose. The protecting group at the 4′-hydroxy is removed by hydrogenolysis and the acyl groups by hydrolysis, and the cyclic *O*-4,6 acetal is formed by reaction with acetaldehyde dimethyl acetal (Holthuis *et al*., 1989).

Information available in 1999 indicated that etoposide was manufactured and/or formulated in 39 countries and etoposide phosphate in eight (CIS Information Services, 1998; Swiss Pharmaceutical Society, 1999).

1.3 Use

Podophyllotoxin is an extract of the roots and rhizomes of two plant species that have been used in folk medicine for several hundred years. It inhibits DNA topoisomerase II (Imbert, 1998). During early clinical trials for cancer chemotherapeutic use, podophyllotoxin proved to be too toxic and, in the 1960s, two epipodophyllotoxins were described, teniposide (see monograph, this volume) and etoposide (Keller-Juslén *et al*., 1971). The first clinical trial of etoposide was reported in 1971, and etoposide entered routine use after 1981 (Oliver *et al.*, 1991). The drug was approved for use in the USA in 1983 (Imbert, 1998).

Etoposide is one of the most widely used cytotoxic drugs and has strong antitumour activity in cases of small-cell lung cancer, testicular cancer, lymphomas and a variety of childhood malignancies. It is one of the most active single agents in the treatment of small-cell lung cancer (Slevin *et al.*, 1989a; Johnson *et al.*, 1991), although it is commonly used in combination with cisplatin, often as part of alternating chemotherapy with the widely used cyclophosphamide–doxorubicin–vincristine regimen (Goodman *et al.*, 1990; Roth *et al.*, 1992).

For testicular germ-cell tumours, etoposide is used in combination with bleomycin and cisplatin. Durable complete responses were achieved in about 80% of patients with disseminated germ-cell tumours; in a randomized trial, the combination resulted in longer overall survival and less toxicity than the standard cisplatin–bleomycin–vinblastine regimen (Williams *et al*., 1987). Three or four cycles of etoposide with cisplatin and bleomycin are now generally regarded as the standard treatment for this disease (Nichols, 1992).

Etoposide is active as a single agent in non-Hodgkin lymphoma, with response rates of 17–40% in previously treated patients (O'Reilly *et al.*, 1991). It has been investigated for use in combination with the widely used cyclophosphamide–doxorubicin–vincristine–prednisone regimen and in a number of new combinations.

Etoposide is less commonly used in a number of other tumour types, including non-small-cell lung cancer, breast, ovarian and gastric cancer, leukaemias, Kaposi sarcoma and in histiocytosis (Joel, 1996; Okada *et al.*, 1998), typically as part of combination chemotherapy and often in patients in whom standard first-line treatments for these malignancies have failed.

The efficacy of etoposide is clearly schedule-dependent, longer exposures of three to five days being more active than a single dose (Slevin *et al*., 1989a). A typical intravenous dose is 375–500 mg/m^2 over three to five days days (90–120 mg/m^2 per day), repeated every three weeks. More prolonged dosing with etoposide has also been described.

Etoposide is available as intravenous and oral formulations. Owing to its poor solubility, a more water-soluble pro-drug, etoposide phosphate, was developed for clinical use. Once this drug enters the systemic circulation, the phosphate is rapidly and completely cleaved by circulating phosphatases. Early clinical trials showed that equimolar

doses of etoposide and etoposide phosphate resulted in equivalent concentrations of etoposide in plasma (as measured by the area under the integrated plasma concentration–time curve) and equivalent biological effects (Schacter *et al*., 1994; Kaul *et al*., 1995).

1.4 Occurrence

Etoposide is not known to occur as a natural product. No data were available to the Working Group on occupational exposure.

1.5 Regulations and guidelines

Etoposide is listed in the British, Dutch, European, French, German, Swiss and US pharmacopoeias (Royal Pharmaceutical Society of Great Britain, 1999; Swiss Pharmaceutical Society, 1999).

2. Studies of Cancer in Humans

Several factors make it difficult to evaluate etoposide with respect to the incidence of second malignancies. First, most cancer patients are treated with combined treatment modalities (chemotherapy and radiotherapy), and multiple antineoplastic drugs are usually administered within combination chemotherapy regimens. The administration of possibly carcinogenic drugs other than etoposide was adjusted for in only a few studies. [The Working Group considered only studies in which etoposide was given to patients who did not receive treatments with alkylating agents (see IARC, 1987), with the exception of low doses of cyclophosphamide.] Secondly, first and second primary malignancies may have common risk factors. For example, there is now general agreement that the development of leukaemia in patients with mediastinal germ-cell cancer should be regarded as part of the natural history of the disease (Nichols *et al*., 1990). In studies of the risk for treatment-related leukaemia, patients with mediastinal germ-cell cancer should therefore be excluded.

In studies in which patients were treated with etoposide and/or teniposide (see monograph, this volume), the authors used various conversion factors to derive an ‘equivalent dose’ of etoposide from that of teniposide. The conversions were based, however, on the therapeutic effects with regard to the possible leukaemogenic potency at a given dose rather than on metabolic considerations.

2.1 Case reports

Since the initial reports of treatment-related acute myeloid leukaemia after treatment of cancer patients with etoposide were published in the 1980s (e.g. Ratain

et al., 1986), more than 150 such reports have appeared. In all of these, however, the development of leukaemia followed the administration of etoposide in combination with other cytostatic drugs and/or irradiation. Since several cohort studies of patients with various malignancies have been conducted to estimate the risk for second malignancies after exposure to etoposide, this section includes only case reports of the specific group of patients with Langerhans cell histiocytosis and metastatic germ-cell tumours who received etoposide. Langerhans cell histiocytosis entails proliferation of connective tissue cells which originate in the bone marrow.

An eight-year-old Peruvian girl with Langerhans cell histiocytosis of the bone who had been treated according to an Italian protocol for this disease, consisting of etoposide at a dose of 200 mg/m^2 for three consecutive days every three weeks with 15 courses administered in one year for a cumulative dose of 8400 mg/m^2, was hospitalized for acute promyelocytic leukaemia 18 months after discontinuing therapy. Etoposide was the only cytotoxic agent that had been used before the onset of acute myeloid leukaemia. This patient was one of 26 treated only with an epipodophyllotoxin for Langerhans cell histiocytosis; their follow-up periods ranged between 11 and 44 months, with a median of 29.5 months (Haupt *et al*., 1993).

In Japan, Horibe *et al*. (1993) reported two patients with secondary acute promyelocytic leukaemia after chemotherapy that included etoposide for the treatment of Langerhans cell histiocytosis. The first case was a four-year-old girl, in whom the disease was diagnosed in bone in July 1988. She initially received intravenous vinblastine and oral prednisolone, followed by etoposide injections alone at 200 mg/m^2 weekly, between June 1989 and January 1990. The total dose of etoposide administered was 6250 mg (9600 mg/m^2). In November 1990 (after 28 months), she developed acute promyelocytic leukaemia. The second case was in a five-month-old girl with Langerhans cell histiocytosis diagnosed in June 1987 who was treated with intravenous etoposide (100 mg/m^2 eight doses, two or three times a week), in addition to bolus vincristine, intravenous cyclophosphamide at doses unlikely to be leukaemogenic, intravenous methotrexate and oral prednisolone. The total doses of etoposide and cyclophosphamide administered were 1860 mg (4800 mg/m^2) and 4070 mg (10 800 mg/m^2), respectively. In June 1990 (after 36 months), acute promyelocytic leukaemia was diagnosed. Neither patient had undergone irradiation.

Oliver *et al*. (1991) reported in a letter to the editor that 115 patients in a series of 207 cases of metastatic germ-cell tumour (1978–90) in the United Kingdom were treated with low-dose etoposide combinations and that two cases of acute myeloid leukaemia occurred. The first patient, aged 34, developed acute myeloid leukaemia 63 months after treatment with bleomycin, cisplatin and etoposide. The cumulative etoposide dose was 710 mg/m^2. The other patient, aged 36, developed acute myelomonocytic leukaemia 27 months after radiotherapy and bleomycin, etoposide, vinblastine and cisplatin. The cumulative etoposide dose was 300 mg/m^2. There were also four non-haematological malignancies.

2.2 Cohort studies

In many of the cohort studies, the authors did not compare the observed number of secondary leukaemias with the number expected from rates for the general population; however, the expected number of cases of acute myeloid leukaemia in the general population can be approximated from a European standardized annual incidence rate of 3–4/100 000 (Parkin *et al*., 1997), with little variation between the countries in which the studies were carried out. Thus, the observed cumulative incidences in most studies are clearly higher than the incidence in the general population.

2.2.1 *Langerhans cell histiocytosis*

The cohort studies of patients with Langerhans cell histiocytosis are summarized in Table 1.

An Austrian, Dutch, German, Swiss cohort was formed, consisting of 363 patients (223 boys, 140 girls) who were enrolled in trials for the treatment of newly diagnosed disseminated or relapsed Langerhans cell histiocytosis between 1983 (since use of etoposide began in these countries) and 1995 (Haupt *et al*., 1997). The diagnoses were made between 1969 and 1992. The patients received various treatments, depending on the date of start. In 1983–91, the induction chemotherapy comprised prednisone, vinblastine and etoposide, and continuation treatment consisted of 46 weeks of therapy with 6-mercaptopurine and re-induction pulses of prednisone, vinblastine with/without etoposide, with/without methotrexate. The total cumulative dose of etoposide was 900 mg/m^2 for subjects who received the drug only in the induction phase and 2100 mg/m^2 for those given continuation treatment. In 1991–95, the patients were randomized into two groups, one receiving vinblastine and the other receiving etoposide (cumulative dose, 3600 mg/m^2). The median length of follow-up was 5.5 years. In this cohort, 123 patients had received etoposide alone or in combination with other drugs not known to be leukaemogenic, whereas 41 patients who had not responded adequately to these treatment protocols were subsequently given doxorubicin and/or cyclophosphamide in addition to etoposide. For subjects treated with etoposide, the total cumulative dose received ranged between 150 and 17 600 mg/m^2 (median, 2000 mg/m^2); only 14 patients were treated with doses exceeding 4000 mg/m^2. No cases of acute myeloid leukaemia were reported; however, the rate in the Saarland Cancer Registry in Germany indicated that only 0.005 cases were expected.

An Italian cohort (Haupt *et al*., 1994, 1997) consisted of 241 patients (132 boys and 109 girls) who were treated between 1977 and 1995 for newly diagnosed or relapsed Langerhans cell histiocytosis. The median length of follow-up was 5.8 years. The expected number of cases of leukaemia was estimated from age- and sex-specific incidence rates derived from the Varese Cancer Registry in Italy. The standardized incidence ratio (SIR) of acute myeloid leukaemia for the extended Italian cohort (Haupt *et al.*, 1997) was 520 (95% CI, 168–1213). Eighty-two patients had received etoposide

Table 1. Cohort studies of the risk for secondary acute myeloid leukaemia (AML) after treatment of Langerhans cell histiocytosis with etoposide

Study population	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed cases of secondary acute myeloid leukaemia	Median follow-up (years)	SIR for AML	Remarks
223 boys, 140 girls in Austria, Germany, Netherlands, Switzerland; cases diagnosed in 1969–92 (age not given)	150–17 600	123	None	0	Total group (n = 363; 342 alive): 5.5		Some patients received teniposide, and the cumulative dose of teniposide was transformed into equivalent etoposide dose assuming a 1:2 ratio. 14 (first cohort) patients received > 4000 mg/m^2.
	150–17 600	41	Alkylating, intercalating agents and/or radiotherapy	0			
	0	147	None	0			
	0	52	Alkylating, intercalating agents and/or radiotherapy	0			
132 boys, 109 girls in Italy; cases diagnosed in 1977–95; age, 22 days–19 years	100–30 000	82	None	4	Total group (n = 241): 5.8	1600 (440–4100)	All AML cases received cumulative etoposide doses > 4000 mg/m^2. The SIR for subjects exposed to high cumulative doses of etoposide is 1800 (570–4200). 70 patients received > 4000 mg/m^2.
	100–30 000	31	Alkylating, intercalating agents and/or radiotherapy	1			
	0	112	None	0			
	0	16	Alkylating, intercalating agents and/or radiotherapy	0			

From Haupt *et al.* (1997)
SIR, standardized incidence ratio

as a single agent or in combination with other drugs not known to be leukaemogenic, while 31 patients had received etoposide in combination with one or more agents with possible leukaemogenic activity (vincristine, prednisone, vinblastine, doxorubicin and cyclophosphamide); 128 patients were not treated with etoposide. The cumulative dose of etoposide ranged between 100 and 30 000 mg/m^2 (median, 5200 mg/m^2); 70 children received more than 4000 mg/m^2. Five cases of acute promyelocytic leukaemia were diagnosed, all in etoposide-treated patients (four girls, one boy; latency, 27–106 months). Four of the five patients had not been exposed to alkylating agents, intercalating agents or radiotherapy; the SIR for this group was 1600 (95% CI, 435–4096). The fifth case occurred in the group that had received both etoposide and alkylating agents, intercalating agents or radiotherapy (SIR, 776; 95% CI, 19–4325). All of the patients with acute promyelocytic leukaemia had received a cumulative dose of etoposide exceeding 4000 mg/m^2, and the SIR for this group was 1782 (95% CI, 574–4159). No cases of acute promyelocytic leukaemia were observed in patients who had not received etoposide.

[The Working Group noted that a possible explanation for the difference in the results of the multicentre and the Italian trials is the cumulative dose of etoposide given: The Italian patients received an average of 5200 mg/m^2 while those in the multicentre trial received 2000 mg/m^2. Moreover, when treated with etoposide, 62% of the Italian patients and 8.5% of those in the multicentre trial had received a cumulative dose of etoposide > 4000 mg/m^2. Other reasons for the difference in results cannot, however, be excluded. Both studies lacked sufficient power to detect a significant difference in leukaemia risk between patients with Langerhans cell histiocytosis treated with and without etoposide, although no cases of acute myeloid leukaemia were observed without etoposide treatment. The Working Group also noted that a small, unspecified proportion of patients in the Italian cohort were treated with teniposide (Haupt *et al.*, 1994).]

2.2.2 *Germ-cell tumours in men*

In the early years of platinum-based chemotherapy for testicular cancer, the large majority of patients received the cisplatin, vinblastine, bleomycin regimen. The absence of an increased risk for acute myeloid leukaemia after this regimen has now been documented in several large studies of the risk for second malignancies (Pedersen-Bjergaard *et al.*, 1991; Bokemeyer & Schmoll, 1993; van Leeuwen *et al.*, 1994; Bokemeyer & Schmoll, 1995; van Leeuwen, 1997). Further evidence for the absence of cases of acute myeloid leukaemia with myelodysplastic syndrome in patients treated with this regimen comes from several trials with long-term follow-up (Ozols *et al.*, 1988; Roth *et al.*, 1988).

The cohort studies of germ-cell tumours in men are summarized in Table 2.

Pedersen-Bjergaard *et al.* (1991) described four cases of acute myeloid leukaemia and one of myelodysplastic syndrome in a cohort of 212 patients in Denmark with

Table 2. Cohort studies of the risk for secondary acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) after treatment of germ-cell tumours with etoposide-containing regimens

Reference	Study population receiving etoposide	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed cases of second malignancies	Follow-up period (years)	Relative risk for AML or MDS (observed/ expected)	Cumulative risk for AML or MDS (95% CI)	Remarks
Denmark									
Pedersen-Bjergaard *et al*. (1991)	212 men Diagnosed in 1979–89	1800–3600							
		1800–2000	130	Cisplatin, bleomycin	5 0	5.7	340 (92–860)	4.7% at 5.7 years (AML + MDS)	Etoposide-treated patients
		2000–3600	82		5 (4 AML, 1 MDS)		(AML)	11% at 5.7 years	
Germany									
Bokemeyer & Schmoll (1993)	293 men Diagnosed in 1970–90	≤ 2000	221	Cisplatin, bleomycin, vinblastine, anthracyclines, dactinomycin, ifosfamide	3 (1 ALL + 2 solid)	Median, 5.1	2.3 (0.1–13)	1.0% at 5 years (0.0–2.2)	SMR for etoposide-treated patients
		> 2000	72		0				
Bokemeyer *et al.* (1995)	128 men Diagnosed in 1983–93	> 2000			1 (AML)	4.5	30–35 (NS)	0.8% (0–2.3) at 4.5 years	Etoposide-treated patients
		Median cumulative dose:							
		3750	22	Cisplatin, bleomycin, ifosfamide	1 (AML)	6.1			
		3800	50	Cisplatin, bleomycin	0	5.2			
		3800	41	Cisplatin, ifosfamide	0	3.4			
		5300	15	Carboplatin, ifosfamide, autologous stem-cell rescue	0	2.3			

Table 2 (contd)

Reference	Study population receiving etoposide	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed cases of second malignancies	Follow-up period (years)	Relative risk for AML or MDS (observed/ expected)	Cumulative risk for AML or MDS (95% CI)	Remarks
Germany + France									
Kollmannsberger *et al*. (1998)	302 men, 15–55 years old Diagnosed in 1986–96	2400–14 000			4	4.3 Median, 3.5	160 (44–411)	1.3% (0.4–3.4) at 4.3 years	SIR. Mediastinal germ-cell cancer patients were included. 161 patients were included in the trial after failing first-line therapy.
		First-line therapy 2400–6000	141	Cisplatin, ifosfamide, autologous stem-cell support	2 (AML)				
		2400–14 000	161	Cisplatin, cyclophos-phamide, ifosfamide, carboplatin, autologous stem-cell support	2 (AML) (2 MDS in mediastinal germ-cell cancer patients)	4.8–5.6			
United Kingdom									
Boshoff *et al*. (1995)	679 men Diagnosed in 1979–92	500–5000		Vincristine, methotrexate, cisplatin, bleomycin, actinomycin D, cyclophos-phamide, vinblastine, carboplatin	6 AML + 4 solid tumours	2 (n = 541) > 5 (n = 331) Median, 5.7	150 (55–326)	NR	Mediastinal germ cell-cancer patients included in patient population
		≤ 2000	636		4 (AML)				
		> 2000	25		2 (AML)				

Table 2 (contd)

Reference	Study population receiving etoposide	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed cases of second malignancies	Follow-up period (years)	Relative risk for AML or MDS (observed/ expected)	Cumulative risk for AML or MDS (95% CI)	Remarks
United States									
Bajorin *et al.* (1993) New York	340 men Diagnosed in 1982–90	800–5000		Cisplatin, cyclophos-phamide	2 (AML) (1 after cyclophos-phamide)	≥ 5	NR	< 1% at 5 years for 1 AML seen after etoposide only	Incidence
Nichols *et al.* (1993) Indiana	538 men Diagnosed in 1982–91	1500–2000		Cisplatin, bleomycin, ifosfamide	2 (AML)	Median, 4.9	66 (8–238)	NR	3 cases observed in another group (of unknown size) of patients treated with etoposide off clinical trial protocol; 2 patients received 2000 mg/m^2.

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; MDS, myelodysplastic syndrome; NR, not reported; SIR, standardized incidence ratio; SMR, standardized morbidity ratio; NS, not significant

mostly testicular germ-cell tumours who had been treated with bleomycin, etoposide and cisplatin, none of whom had mediastinal germ-cell tumours. Thirty-five patients, treated between 1979 and 1983, received cisplatin, vinblastine and bleomycin and, at relapse, bleomycin (15 mg/m^2 weekly), etoposide (120 mg/m^2 for five days) and cisplatin (20 mg/m^2 for five days). In the subgroup of 20 patients who had received a cumulative etoposide dose of > 2000 mg/m^2, two cases of acute myelomonocytic leukaemia occurred. The latent periods after etoposide treatment were 25 and 54 months, respectively. For the 177 patients treated after 1983 to 1989 with first-line bleomycin, etoposide and cisplatin, the doses were adjusted according to risk category: 115 patients received standard doses (100 mg/m^2 etopside for five days (cumulative dose, 2000 mg/m^2), 20 mg/m^2 cisplatin for five days, 15 mg/m^2 bleomycin weekly). No cases of acute myeloid leukaemia were diagnosed. In 62 patients who received high-dose treatment consisting of etoposide (200 mg/m^2 for five days; cumulative dose, 3000 mg/m^2), cisplatin (40 mg/m^2 for five days) and bleomycin (15 mg/m^2 weekly), two cases of acute myeloblastic leukaemia (one in a patient with extragonal germ-cell tumour) and one case of myelodysplastic syndrome developed. The latencies after etoposide treatment were 15 and 29 months for acute myeloblastic leukaemia and 68 months for the case of myelodysplastic syndrome. The expected number of de-novo cases of acute myeloid leukaemia was estimated from the leukaemia incidence reported in the Danish Cancer Registry for 1973–77. In comparison with the risk of the general population, the relative risk for overt leukaemia was 336 (95% CI, 92–861). The mean cumulative risk (Kaplan–Meier method) for leukaemic complications was 4.7% (SE, 2.3) 5.7 years after the start of etoposide-containing chemotherapy. No leukaemias or dysplastic syndromes were observed among the 130 patients who had received ≤ 2000 mg/m^2 etoposide, whereas five cases were seen among the 82 patients who had received > 2000 mg/m^2 (p = 0.004). The cumulative risk for leukaemia among the 82 patients receiving high-dose etoposide (> 2000 mg/m^2) was 11% (SE, 5.0) 5.7 years after the start of chemotherapy. Although five cases of leukaemia and dysplastic syndrome were found in the 212 etoposide-treated patients, none was found in a previous cohort of 127 patients with germ-cell tumour treated with vinblastine and similar doses of cisplatin and bleomycin (p = 0.08).

Bokemeyer and Schmoll (1993) assessed the risk for secondary neoplasms after therapy for germ-cell tumours in 1025 patients treated between 1970 and 1990 in Germany. Patients followed-up for longer than 12 months were eligible (1018 patients; 394 had seminomatous germ-cell tumours). The median follow-up was 61 months, and the median age of the patients at diagnosis was 28.9 years. The chemotherapy regimens consisted mainly of cisplatin, bleomycin and either vinblastine or etoposide. A total of 293 patients received etoposide during their treatment: 221 patients received cumulative doses of ≤ 2000 mg/m^2; 72 patients received > 2000 mg/m^2. The cumulative incidence of second tumours after etoposide-containing therapy was 1.0% (95% CI, 0.0–2.2), while that after chemotherapy without etoposide was 0.8% (95% CI, 0.0–2.5) (not significant). The standardized morbidity ratio of second malignancy for patients

treated with etoposide was 2.3 (95% CI, 0.1–13 (not significant)) when compared with the cancer incidence rate in the male German population (based on the Saarland Cancer Registry). Among the 221 patients who received ≤ 2000 mg/m^2 etoposide, three developed a secondary tumour: one carcinoid tumour, one rhabdomyosarcoma and one lymphoblastic leukaemia; the last patient had received four cycles of bleomycin, etoposide and cisplatin (cumulative dose of etoposide, 2000 mg/m^2), and the interval to second leukaemia was 16 months. In patients who received > 2000 mg/m^2 etoposide, no second malignancies occurred.

Bokemeyer *et al*. (1995) analysed the risk for leukaemia of long-term survivors of three treatment protocols of the German Testicular Cancer Study Group and of patients treated at Hannover University Medical School, all of whom had received cumulative doses of etoposide of > 2000 mg/m^2. All patients had non-seminomatous germ-cell tumours. The study was limited to those who had achieved complete remission or a stable partial response with no tumour markers after chemotherapy, with a minimum follow-up of 12 months. Patients with prior abdominal or mediastinal radiotherapy were excluded. The first cohort consisted of 22 patients who were treated between 1983 and 1989 with three or four cycles of bleomycin, etoposide and cisplatin as induction chemotherapy followed by cisplatin, etoposide and ifosfamide as salvage chemotherapy at relapse. The median cumulative dose of etoposide was 3750 mg/m^2. The second cohort was composed of 50 patients with metastatic testicular cancer who had been treated during 1984–88 with first-line chemotherapy consisting of a 'double-dose' of cisplatin, a 'double-dose' of etoposide and bleomycin (175 mg/m^2 cisplatin and 1000 mg/m^2 etoposide per cycle; four cycles). The median cumulative dose of etoposide was 3800 mg/m^2. The third cohort consisted of 41 patients who had been treated in a stepwise dose–escalation protocol with the cisplatin, etoposide and ifosfamide regimen as first-line therapy for 'advanced' germ-cell tumours. The patients were treated during 1989–92 with 150 mg/m^2 cisplatin and 8000 mg/m^2 ifosfamide plus either 750 mg/m^2 or 1000 g/m^2 etoposide per cycle for four consecutive cycles. The median total dose of etoposide given to these patients was 3800 mg/m^2. The fourth cohort consisted of 15 patients treated between 1990 and 1993 for relapsed testicular cancer with high doses of carboplatin, etoposide and ifosfamide followed by autologous stem-cell rescue. These patients had received primary chemotherapy that included etoposide and at least one regimen of salvage therapy with etoposide before the high-dose treatment, which resulted in a median cumulative dose of etoposide of 5300 mg/m^2. After a total median follow-up time of 4.5 years, one case of myelomonocytic leukaemia was diagnosed in the first cohort (after 6.1 years). The cumulative incidence of secondary leukaemia in the group of 128 patients after 4.5 years of median follow-up was 0.8% (95% CI, 0–2.34). When compared with the annual incidence of five cases of myeloid leukaemia per 100 000 persons in the general population, the relative risk for secondary leukaemia was increased approximately 30- to 35-fold, which is not statistically significant. [The Working Group noted that the power of this study was insufficient to detect an increased risk for leukaemia

in the individual cohorts or to detect differences between low and high doses of etoposide-containing regimens. The Working Group also noted that there may have been overlap with the previous study.]

Kollmannsberger *et al*. (1998) examined the risk for acute myeloid leukaemia after high cumulative doses of etoposide (> 2000 mg/m^2) and stem-cell transplantation in patients with advanced or relapsed germ-cell tumours. The records of 302 patients (median age, 29 years) with germ-cell tumours (241 testicular, 33 retroperitoneal and 28 mediastinal) who were treated with high-dose chemotherapy in clinical trials in Germany and France between 1986 and 1996 were reviewed. Patients had to have had a minimal follow-up of 12 months. Of the three German trials, the first included first-line therapy with one cycle of standard-dose cisplatin 20 mg/m^2, etoposide 100 mg/m^2 and ifosfamide 1200 mg/m^2 daily for five days followed by three to four cycles of of the same treatment escalated over seven doses: the highest consisted of 20 mg/m^2 cisplatin, 300 mg/m^2 etoposide and 2400 mg/m^2 ifosfamide daily for five consecutive days every three weeks. In the second German trial, patients who relapsed after receiving cisplatin and etoposide-based chemotherapy received two cycles of a standard-dose cisplatin, etoposide and ifosfamide regimen followed by two cycles of 500 mg/m^2 carboplatin, 400 mg/m^2 etoposide and 2500 mg/m^2 cyclophosphamide. In the third German trial, patients who relapsed after initial therapy with cisplatin and etoposide received two cycles of standard-dose cisplatin, etoposide and ifosfamide followed by carboplatin, 300–400 mg/m^2 etoposide and ifosfamide. All the patients in France were treated with high-dose etoposide-containing chemotherapy including cisplatin, carboplatin and cyclophosphamide or ifosfamide, either as first-line consolidation therapy (patients with poor prognostic criteria) or as treatment for relapsed germ-cell tumour. All patients received either autologous bone marrow or autologous peripheral blood stem-cell support, and most patients also received granulocyte- or granulocyte–macrophage colony-stimulating factor after high-dose chemotherapy. The median cumulative dose of etoposide was 5000 mg/m^2 (range, 2400–14 000 mg/m^2). Six patients developed a secondary haematological malignancy (four acute myeloid leukaemias and two myelodysplastic syndromes). The two cases of myelodysplastic syndrome occurred in patients with a primary mediastinal germ-cell tumour and were excluded from the analysis. For the total group of 302 patients, the cumulative incidence of acute myeloid leukaemia was 1.3% (95% CI, 0.4–3.4%) at a median follow-up time of 4.3 years. The standardized incidence ratio in comparison with data from the Saarland Cancer Registry in Germany for 1989–93 was 160 (95% CI, 44–411). The latency from start of etoposide treatment was 24–58 months. Two of the malignancies were acute monoblastic leukaemia and two were acute myelomonocytic leukaemia; three were found in patients with testicular cancer as the primary tumour.

Boshoff *et al*. (1995) reported on the incidence of second cancer in 679 male patients (634 with testicular cancer) in the United Kingdom with advanced germ-cell cancer who had been treated with one of two etoposide-containing protocols. Between 1979 and 1992, 343 patients were treated with cisplatin, vincristine, methotrexate,

bleomycin, actinomycin D, cyclophosphamide and etoposide, and 336 patients were treated with etoposide, platinum (cisplatin or carboplatin) and bleomycin with or without vinblastine. Patients who did not achieve complete remission or who died of germ-cell cancer were not excluded from the analysis. A total of 541 patients were followed-up for more than two years and 331 for more than five years. Six patients developed acute myeloid leukaemia, and four developed solid tumours. None of them had a primary mediastinal germ-cell tumour, and only one patient had received radiotherapy. The median interval between the onset of treatment and the development of leukaemia was 27 months. Four of six cases were acute myelomonocytic leukaemia, one was acute myeloid and the other acute myeloblastic leukaemia. The cumulative dose of etoposide in the cases of leukaemia ranged from 720 to 5000 mg/m^2. In the three cases treated with cisplatin, vincristine, methotrexate, bleomycin, actinomycin D, cyclophosphamide and etoposide, the relative risk for secondary leukaemia was 150 (95% CI, 55–326) ($p < 0.001$), on the basis of a comparison with data from the Office of Population Censuses and Surveys for England and Wales. Two of 25 patients who received total doses > 2000 mg/m^2 developed acute myeloid leukaemia, whereas four of 636 who received < 2000 mg/m^2 developed acute myeloid leukaemia ($p = 0.02$). Four patients developed solid tumours (excluding cancer of the contralateral testis). [The Working Group noted that the cumulative incidence in the patients given the low and high doses of etoposide was not properly compared; instead, the authors compared the frequency and also did not adjust for the doses of cyclophosphamide and actinomycin in the seven-agent regimen.]

In a study in New York, USA, Bajorin *et al.* (1993) investigated the risk for developing acute myeloid leukaemia of 503 patients with advanced germ-cell tumours who had been treated with etoposide-containing therapy according to a cancer centre protocol between 1982 and 1990; 340 patients with a minimum disease-free survival greater than one year were selected. Six patients with acute myeloid leukaemia were identified; however, four of them had a mediastinal germ-cell tumour. One patient aged 31 with testicular cancer had received cisplatin, etoposide (cumulative dose, 2000 mg/m^2), vinblastine, bleomycin, dactinomycin and cyclophosphamide as induction plus salvage therapy. After 56 months, he developed acute myeloblastic leukaemia. The second patient with testicular cancer, a man aged 35, had received induction therapy consisting of cisplatin, carboplatin and etoposide (cumulative dose, 1300 mg/m^2). After 26 months, he developed acute myeloblastic leukaemia. Thus, one of the 310 patients (291 treated with bleomycin, carboplastin and cisplatin) who had received only one etoposide-containing induction chemotherapy regimen subsequently developed acute myeloid leukaemia, giving a definite incidence [an approximate actuarial risk] of less than 1.0% at five years.

In a study in Indiana (USA) designed to estimate the risk for developing leukaemia of patients receiving conventional doses of etoposide, mostly with cisplatin and bleomycin, Nichols *et al.* (1993) reviewed the records of 538 previously untreated patients with (disseminated) germ-cell cancer entering clinical trials between 1982 and 1991,

who were given conventional doses of etoposide (cumulative dose, 1500–2000 mg/m^2) in combination with cisplatin and either ifosfamide (190 cases) or bleomycin, in three or four cycles administered in short intravenous infusions of 100 mg/m^2 daily for five days. Of these patients, 337 had been followed-up for longer than two years. Five of the 538 patients developed a haematological malignancy. Three of the five developed acute leukaemia associated with a primary mediastinal germ-cell tumour and were excluded from the study. Two patients (0.37%) with a primary testicular cancer developed leukaemia: after the start of bleomycin, etoposide and cisplatin therapy (cumulative dose of etoposide for both, 2000 mg/m^2), one developed acute myelomonocytic leukaemia after 2.3 years and the other developed acute undifferentiated myeloid leukaemia after 2.0 years. The number of cases of leukaemia expected was estimated from the rates for white male US Navy personnel aged 17–34, who reflect the population of patients with testicular cancer entering clinical trials at Indiana University (Garland *et al.*, 1990). The relative risk for developing leukaemia was 66 (95% CI, 8–238). [The Working Group calculated a cumulative incidence of < 1% at five years.] The authors also reported three cases of haematological abnormality (acute monoblastic leukaemia, acute myeloid leukaemia and refractory anaemia with excess blasts in transition (myelodysplastic syndrome)) in 'several hundred' patients who received a chemotherapy regimen containing etoposide, vinblastine, ifosfamide or cisplatin after failing to respond to primary chemotherapy involving treatment with total doses of etoposide of 2000 (n = 2) and 4400 (n = 1) mg/m^2.

[The Working Group calculated the relative risk for acute myeloid leukaemia or myelodysplastic syndrome in germ-cell tumour patients treated with etoposide-containing regimens with cisplatin and bleomycin and, in some cases, vinca alkaloids, from all of the studies reported above (see Table 3). Twelve cases of leukaemia or myelodysplastic syndrome (10 cases of acute myeloid leukaemia, one case of acute lymphoblastic leukaemia and one of myelodysplastic syndrome, were observed among 1720 patients with germ-cell tumours. On the basis of 8699 patient–years of follow-up and an annual incidence rate of 3–4 cases of acute myeloid leukaemia per 100 000 population (Parkin *et al.*, 1997), the relative risk for acute myeloid leukaemia or myelodysplastic syndrome in the germ-cell tumour patients was significantly elevated by a factor of 40 (95% CI, 17–81).]

Since the background incidence of acute myeloid leukaemia in the population is low, this high relative risk translates to a rather low absolute risk. According to Bokemeyer and Schmoll (1995), the cumulative risk for acute myeloid leukaemia was only 0.58% (95% CI, 0.29–0.94%) after five years in 1868 published cases of patients treated with conventional etoposide-containing regimens (cumulative dose, ≤ 2000 mg/m^2), on the basis of the six cases reported by Boshoff *et al.* (1995), two by Nichols *et al.* (1993), two by Bajorin *et al.* (1993), one by Bokemeyer and Schmoll (1993) and none by Pedersen-Bjergaard *et al.* (1991).

Cohort studies of other types of cancer are summarized in Table 4 and are described below.

Table 3. Summary of studies in which 12 cases of leukaemia or myelodysplastic syndrome were found after treatment for germ-cell tumours with etoposide-containing regimens with cisplatin and bleomycin and, in some cases, vinca alkaloids

Reference	Cumulative dose of etoposide received	Type of malignancy	Agents other than etoposide	Number treated with this regimen	
				Patients	Person–years
Pedersen-Bjergaard *et al.* (1991)	7400 mg 6075 mg 4250 mg 7500 mg 5250 mg	AML AML AML MDS AML	Cisplatin, bleomycin, vinblastine Cisplatin, bleomycin, vinblastine, vincristine Cisplatin, bleomycin (X-ray) Cisplatin, bleomycin (X-ray) Cisplatin, bleomycin	212	[848]
Bokemeyer *et al.* (1995)	3800 mg		Cisplatin, bleomycin	50	[225]
Bokemeyer & Schmoll (1993)	2000 mg/m^2	ALL	Cisplatin, bleomycin	293	[1465]
Bajorin *et al.* (1993)	1300 mg/m^2	AML	Cisplatin, carboplatin	291	[1455]
Nichols *et al.* (1993)	2000 mg/m^2 2000 mg/m^2	AML AML	Cisplatin, bleomycin Cisplatin, bleomycin	538[a]	[2690]
Boshoff *et al.* (1995)	720 mg/m^2 750 mg/m^2 1440 mg/m^2	AML AML AML	Cisplatin, bleomycin, vinblastine Cisplatin, bleomycin, vinblastine Carboplatin, bleomycin	336	[2016]
Total				1720	8699

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia

[a] Patients treated with ifosfamide included

Table 4. Cohort studies of acute myeloid leukaemia (AML) or myelodysplastic syndrome (MDS) occurring after treatment with etoposide-containing regimens of cancers other than Langerhans cell histiocytosis and germ-cell tumours

Reference	Study population	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed second malignancies	Follow-up period	Relative risk (observed/ expected)	Cumulative risk (95 % CI)	Remarks
Acute lymphoblastic leukaemia in children									
Pui *et al*. (1991) (Tennessee, USA)	Diagnosed in 1979–88 < 19 years		734	Prednisone, vincristine, asparaginase, methotrexate, mercaptopurine, cyclophos-phamide, doxorubicin, cytarabine, cranial irradiation	21 (AML)		NR	3.8% (2.3–6.1) at 6 years	Specific analysis for different schedules of drug administration
		No etoposide, no teniposide	154		1 (AML)				
		No etoposide, 600–4620 teniposide	279		7 (AML)				
		9000 etoposide, 5100 teniposide	301		9 (AML) (+ 4 AML as second adverse effect)				
Winick *et al*. (1993) (Texas, USA)	Diagnosed in 1986–91 1–18 years	9900	203	Prednisone, vincristine, daunorubicin, asparaginase, methotrexate, mercaptopurine, leucovorin, cytarabine	10 (AML)		NR	5.9 % (SE 3.2%) at 4 years	The first 33 patients received teniposide instead of etoposide at half the dose.
Other types of childhood cancer									
Smith *et al*. (1993) (USA)	**Rhabdomyo-sarcoma** diagnosed around 1984	600–900	207	Dactinomycin, cisplatin. doxorubicin, cyclophos-phamide (24 g/m^2)	4 (3 AML, 1 MDS)	Mean, 3.7 years	NR	3.2% (1.2–8.6) at 6 years	

Table 4 (contd)

Reference	Study population	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed second malignancies	Follow-up period	Relative risk (observed/ expected)	Cumulative risk (95 % CI)	Remarks
Other types of childhood cancer (contd)									
Smith *et al.* (1999) (USA)	**Various primary tumours** diagnosed in 1986–94; various ages	< 1500 (rhabdomyosarcoma, medulloblastoma)	451	Rhabdomyo-sarcoma: cyclophos-phamide (25–35 g/m^2), ifosfamide	8 (4 AML, 4 MDS)	3 years	NR	2.1% (upper 95% CI bound, 3.7%) at 4 years	A 1:2 conversion was used to equate teniposide dose to etoposide dose. Each treatment stratum consists of patients with different primary tumours, ages, treatments.
		1500–3000 (neuroblastoma, germ-cell cancer, ALL)	1270	No cyclo-phosphamide	4 (2 AML, 2 MDS)			0.4% (upper 95% CI bound, 1.0%) at 4 years	
		> 3000 (rhabdomyo-sarcoma, Ewing sarcoma)	570	Cyclophos-phamide (25–35 g/m^2), ifosfamide	5 (2 AML, 2 MDS, 1 T-cell ALL)			1.4% (upper 95% CI bound, 2.9%) at 4 years	
Heyn *et al.* (1994) (USA)	**Rhabdomyo-sarcoma** diagnosed in 1984–91; 0–21 years	643–3200	223	Cyclophos-phamide, vincristine, dactinomycin, doxorubicin, cisplatin, radiotherapy	5 (4 AML, 1 MDS) + 1 osteogenic sarcoma	Median, 3.7 years	NR	Incidence of AML: 52/10 000 person–years	Preliminary results of low-dose study of Smith *et al.* (1999)

Table 4 (contd)

Reference	Study population	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed second malignancies	Follow-up period	Relative risk (observed/ expected)	Cumulative risk (95 % CI)	Remarks
Other types of childhood cancer (contd)									
Duffner *et al.* (1998) (USA)	**Brain tumours** diagnosed in 1986–90; < 3 years	Intravenous etoposide 6.5 mg/kg bw on 2 days per cycle	198	Vincristine, cyclophos-phamide, cisplatin, irradiation		Median for 75 surviving children, 6.4 years	NR	Total group: Haematological and solid tumours: 11% (0–39%) (n = 198) at 8 years	No information on cumulative dose of etoposide
		≤ 2 years: 24 months etoposide	132		3 (2 MDS, 1 AML) + 1 sarcoma + 1 meningioma			19% (0–70%) at 8 years for children < 24 months at diagnosis; 4.8% (0–38%) at 8 years for children 24–36 months at diagnosis	
		2–3 years: 12 months etoposide	66						
Sugita *et al.* (1993) (Japan)	**Non-Hodgkin lymphoma** diagnosed in 1987–91 2–17 years; 28 boys, 10 girls	4200–5600	38	Prednisolone, vincristine, L-asparaginase, mercaptopurine, methotrexate, cranial irradiation, behenoyl cytarabine	5 AML + 3 haemato-logical relapses	Median, 19 months (6–60 months)	NR	18% at 4 years	Short follow-up

Table 4 (contd)

Reference	Study population	Cumulative dose of etoposide (mg/m^2)	No. of patients	Additional chemotherapy or radiotherapy	No. of observed second malignancies	Follow-up period	Relative risk (observed/ expected)	Cumulative risk (95 % CI)	Remarks
Lung cancer									
Ratain *et al.* (1987) (USA)	Diagnosed in 1981–84 17 men, 7 women; median age, 56 (range, 38–69)	4382–7950	24	Cisplatin, vindesine, radiotherapy	4 (AML)		NR	15% (SE, 11%) at 2 years	3 patients (no AML cases) did not receive etoposide. Patients are 1-year survivors.
Breast cancer									
Yagita *et al.* (1998) (Japan)	Diagnosed in 1985–94; 24 women	< 2000 2000–5000 > 5000	7 10 7	Doxorubicin, vindesine, cyclophos-phamide, cisplatin	2 AML + 1 MDS	1–40 months	Total group 630 (130–1800)	$p < 0.01$	Short follow-up

ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CI, confidence interval; MDS, myelodysplastic syndrome; NR, not reported; SE, standard error. The expected number of cases of acute myeloid leukaemia in the general population can be approximated from a world standardized incidence rate of 4–5 per 100 000 persons (see text).

2.2.3 *Acute lymphoblastic leukaemia in children*

Pui *et al*. (1991) reported on the risk for acute myeloid leukaemia among 734 children (< 19 years old) in Tennessee, USA, in whom acute lymphoblastic leukaemia was diagnosed between 1979 and 1988. After having achieved complete remission, the patients received maintenance treatment with epipodophyllotoxins according to seven schedules (Table 5): 580 patients received teniposide (see monograph, this volume), and a substantial proportion of these (301) also received etoposide. In addition, most patients received methotrexate, mercaptopurine, prednisone, vincristine, asparaginase and cytarabine, and some patients received cyclophosphamide, doxorubicin and cranial irradiation. Acute myeloid leukaemia developed in 21 children (as a first adverse event in 17), with an overall cumulative risk of 3.8% (2.3–6.1%) at six years; one developed in a child not receiving etoposide or teniposide. The median interval between the diagnoses of acute lymphoblastic leukaemia and acute myeloid leukaemia was 40 months. Six of the cases were acute myelomonocytic leukaemia, eight were acute monoblastic leukaemia, three were acute myeloblastic leukaemia, one was acute megakaryoblastic leukaemia, one was acute myeloid leukaemia and two were acute undifferentiated leukaemia. In four patients, acute myeloid leukaemia developed after relapse had occurred, and these were not included in the statistical analyses. In the analysis of leukaemia risk, the doses of teniposide and etoposide were weighted equally, since the potency of teniposide *in vitro*—10 times that of etoposide—is offset *in vivo* by extensive protein binding, resulting in 10 times less unbound (active) drug (see section 4). The schedule of epipodophyllotoxin treatment appeared to be a crucial factor in determining the risk for acute myeloid leukaemia, as the strongest evidence was obtained by comparing two subgroups that differed only in their schedule of epipodophyllotoxin administration. The two groups were scheduled to receive the same cumulative doses of teniposide (5100 mg/m^2) and etoposide (9000 mg/m^2); among 84 patients in the first group (XI-HR3) who received epipodophyllotoxins weekly, the risk for acute myeloid leukaemia was clearly and significantly increased (12% at six years; 95% CI, 6.1–24%) as compared with the risk of the second subgroup of 148 patients (XI-HR2) who received the agents every other week (1.6% at six years; 95% CI, 0.4–6.1% [$p = 0.01$ by log-rank test for the difference between groups]. The multivariate analysis indicated that the frequency of epipodophyllotoxin administration was a much more important determinant of risk for acute myeloid leukaemia than cumulative dose. The frequency of treatment remained significant (relative risk, 6.7; 95% CI, 1.5–31; $p < 0.01$) after adjustment in a Cox model for all competing covariates, including cranial irradiation, cyclophosphamide and several factors characteristic of a poor prognosis for acute lymphoblastic leukaemia; however, since the total dose varied only slightly among the patients, its effects could not be assessed reliable. [The Working Group noted that the carcinogenic effects of etoposide could not be evaluated because all patients were treated with teniposide and none received etoposide alone, while patients treated with and without etoposide received teniposide at different schedules.]

Table 5. Risks for secondary acute myeloid leukaemia (AML) in children with acute lymphoblastic leukaemia treated with epipodophyllotoxins, according to regimen

Regimen	Prognosis	Planned cumulative dose (mg/m^2)		Epipodophyllotoxin schedule	No. of patients treated	No. of patients with AML	Six-year cumulative risk % (95% CI)
		Teniposide	Etoposide				
X-LR1	Low risk	0	0	None	154	1	1.0 (0.6–6.3)
X-LR2	Low risk	1350	0	Every other week	155	1	1.1 (0.1–7.1)
X-HR	High risk	4620	0	Twice weekly	85	6	12 (5.7–25)
XI-LR1	Low risk	600	0	Induction only	39	0	0
XI-LR2	Low risk	5100	9000	Every other week	69	0	0
XI-HR2	High risk	5100	9000	Every other week	148	2	1.6 (0.4–6.1)
XI-HR3	High risk	5100	9000	Weekly	84	7	12 (6.1–24)

From Pui *et al.* (1991)
CI, confidence interval

Winick *et al*. (1993) studied a cohort of 203 consecutive children aged 1–18 in Texas, USA, with early B-lineage acute lymphoblastic leukaemia diagnosed in 1986 and 1991, who received induction treatment and achieved complete remission. The induction and maintenance treatment consisted of prednisone, vincristine, daunorubicin, asparaginase, methotrexate, mercaptopurine, leucovorin, intravenous etoposide (300 mg/m^2) and cytarabine. The first 33 patients received teniposide instead of etoposide at half the dose. The planned cumulative dose of etoposide was 9900 mg/m^2. Only four patients received radiation therapy; none received alkylating agents. Ten children developed secondary acute myeloid leukaemia, two of which were of the myelomonocytic type and two of the monoblastic type; one developed myelodysplastic syndrome (consistent with chronic myelomonocytic leukaemia), and one had refractory anaemia with excess blasts in transformation. The interval between the diagnosis of acute lymphoblastic and acute myeloid leukaemia ranged from 23 to 68 months. The median dose of etoposide administered was 7900 mg/m^2 (range, 5100–9900 mg/m^2). One child with acute myeloid leukaemia had received teniposide instead of etoposide. The risk for secondary acute myeloid leukaemia at four years was 5.9% (SE, 3.2%), with risks for standard- and poor-risk patients of 6.3% (SE, 4.0%) and 4.7% (SE, 5.2%) respectively. [The Working Group noted that the patients treated with etoposide and teniposide were considered together as if they had received the same treatment, assuming equivalent leukaemogenic potencies. The Working Group also noted that it was not completely clear in these two studies whether the diagnosis of acute lymphoblastic leukaemia excluded primary mixed leukaemia and thus allowed differentiation of lymphoblastic from myeloid disease.]

2.2.4 *Other types of childhood cancer*

Smith *et al*. (1993) presented the first results of a monitoring plan for secondary acute myeloid leukaemia in clinical trials of the Cancer Therapy Evaluation Program of the National Cancer Institute in the USA. A total of 465 children [ages not given] with primary rhabdomyosarcoma (diagnosis around 1984) took part in this trial. The analysis was restricted to 207 children who had survived more than 36 weeks from entry into the protocol. They had received etoposide daily in combination with two courses of dactinomycin (cumulative dose of etoposide, 600 mg/m^2) or three courses of cisplatin (cumulative dose of etoposide, 900 mg/m^2), after they had been treated with induction regimens that included cyclophosphamide and doxorubicin. The mean duration of follow up was 3.7 years. Interim analyses of the risks for acute myeloid leukaemia and myelodysplastic syndrome were carried out when four cases had been observed. Two of the four cases had received etoposide (600 mg/m^2) and dactinomycin, and two had received etoposide (900 mg/m^2) and cisplatin. The three cases of acute myeloid leukaemia were of the myelomonocytic and monoblastic types and myelodysplastic syndrome progressing to acute myeloid leukaemia; the other case was myelodysplastic syndrome. The patients in the two treatment groups in which these

four cases occurred had been treated for induction of remission with similar doses of doxorubicin (480 mg/m^2), cyclophosphamide (24 000 mg/m^2) and cisplatin (360 mg/m^2). The latency ranged from 2.0 to 3.3 years. The calculated cumulative six-year rate of development of acute myeloid leukaemia or myelodysplastic syndrome was 3.2% (95% CI, 1.2–8.6%). [The Working Group noted that the cumulative dose of cyclophosphamide was > 20 000 mg/m^2, which is known to be leukaemogenic (Curtis *et al.*, 1992).]

Smith *et al*. (1999) described the results of the second analysis of the monitoring plan, with results for the groups receiving low, moderate and higher cumulative doses of epipodophyllotoxins. Twelve trials were selected from a pool of approximately 100 in which etoposide or teniposide had been used. [The data from these trials do not appear to have been published elsewhere.] The 12 trials were selected on the basis of the length of accrual and the treatment of patient populations with significant numbers of survivors two to three years after treatment. Selection was made without knowledge of the number of secondary leukaemias that had occurred to date in the trials. The 12 trials (11 for patients with solid tumours and one for patients with acute lymphoblastic leukaemia) were divided into three strata according to the cumulative dose of etoposide: low (< 1500 mg/m^2), moderate (1500–3000 mg/m^2) and high (> 3000 mg/m^2). For trials in which teniposide was used, a 1:2 ratio was used to convert the dose of teniposide to that of etoposide. Patients treated with the low dose had primary rhabdomyosarcoma (n = 222) or medulloblastoma (advanced stage) (n = 229). The patients with rhabdomyosarcoma had also received cyclophosphamide or equivalent doses of ifosfamide (25 000–35 000 mg/m^2). Patients treated with the moderate dose had primary neuroblastoma (n = 319), germ-cell tumour (adult and paediatric) (n = 700) or acute lymphoblastic leukaemia (high risk) (n = 251). Patients given the higher dose had primary rhabdomyosarcoma (n = 313) or Ewing sarcoma (n = 257). They also received cyclophosphamide or equivalent doses of ifosfamide (25 000–35 000 mg/m^2). For each interim analysis (see Smith *et al.*, 1993), the total patient follow-up was calculated for all protocols within the treatment group, excluding the first 36 weeks of follow-up, since the incidence of leukaemia development during this period is extremely low, and the four-year and six-year cumulative incidence rates were estimated. The six-year actuarial risks for acute myeloid leukaemia or myelodysplastic syndrome were 3.3% (upper 95% CI bound, 5.9%) with the low cumulative dose of epipodophyllotoxin, 0.7% (upper 95% CI bound, 1.6%) with the moderate cumulative dose and 2.2% (upper 95% CI bound, 4.6%) with the high cumulative dose. The p values for homogeneity of the risks for secondary leukaemia across the cumulative dose strata were 0.012 (parametric test) and 0.011 (non-parametric test). Thus, the data provide no support for an effect of the cumulative dose of epipodophyllotoxins on leukaemogenic activity, at least not within the cumulative dose range encompassed by the monitoring plan. [The Working Group noted that the three treatment strata were compared as if the cumulative dose of epipodophyllotoxin were the only difference between them; however, the strata also differed with respect to the

primary tumour (stratum with solid tumours versus stratum with solid and lymphoid tumours) and treatment (one stratum with high-dose epipodophyllotoxin and high-dose cyclophosphamide versus a stratum with no cyclophosphamide and a moderate dose of epipodophyllotoxin and a stratum with high-dose cyclophosphamide given to part (one trial) of the stratum with low dose epipodophyllotoxin). It is also not clear which patients received teniposide and which received etoposide.]

Between 1984 and 1991, 1062 patients with rhabdomyosarcoma (age, 0–21 years) entered a trial in the USA (Heyn *et al*., 1994). Of these, 223 patients received etoposide in combination with cyclophosphamide, vincristine, dactinomycin, doxorubicin and cisplatin, with a total dose of etoposide of 600–900 mg/m^2. All patients also received radiotherapy. The median follow-up time was 3.7 years. Four cases of acute myeloid leukaemia, one of myelodysplastic syndrome and one of osteogenic sarcoma were reported. The median time from the initiation of primary treatment to the diagnosis of leukaemia was 39 months. Three of four leukaemia patients had received etoposide in combination with doxorubicin, cyclophosphamide (13 000–21 900 mg/m^2), cisplatin and other agents and radiotherapy during their treatment. The cumulative doses of etoposide were 643, 765, 911 and 3197 mg/m^2. Two cases were myelomonocytic and two were monoblastic leukaemia. The incidence of acute myeloid leukaemia among patients who had received etoposide in combination with doxorubicin, cyclophosphamide, cisplatin and other agents and radiotherapy during their treatment was 52 per 10 000 person–years. When cyclophosphamide alone or cyclophosphamide plus doxorubicin but no etoposide was part of the regimen, the incidence was 7.6 per 10 000 person-years. The relative risk for acute myeloid leukaemia in a comparison of the etoposide-containing regimen and that without etoposide was thus 7.2 (95% CI, 0.8–65; $p = 0.06$). The patient who developed myelodysplastic syndrome after five years and seven months had received etoposide (840 mg/m^2) in combination with doxorubicin, cyclophosphamide (18 500 mg/m^2), cisplatin and other agents and radiotherapy during his treatment. [The Working Group noted that it is not clear whether the two treatment groups differed with respect to the doses of drugs other than etoposide or of radiation.]

Between 1986 and 1990, 198 children under three years of age with primary brain tumours were included in the study of Duffner *et al*. (1998) in the USA. Chemotherapy was started two to four weeks after surgery and consisted of vincristine, cyclophosphamide, cisplatinum and intravenous etoposide (6.5 mg/kg bw on days 3 and 4, every three months). Chemotherapy was planned for 24 months for children 0–23 months of age at diagnosis, and for 12 months for those 24–36 months of age. Irradiation therapy was started three to four weeks after the last cycle of chemotherapy. The median duration of follow-up for the 75 surviving children was 6.4 years. Five children developed a secondary malignancy. One developed a sarcoma, one a meningioma, and three developed haematological malignancies: two myelodysplastic syndromes with latencies of 7.7 and 4.8 years and an acute myeloid leukaemia with a latency of 2.8 years. The child with acute myeloid leukaemia had received a cumulative dose of etoposide of approximately 2400 mg/m^2. The actuarial risk for developing a second

malignancy (solid or haematological) eight years after diagnosis was 11% (95% CI, 0–39). [The Working Group noted that the risk for secondary haematological malignancy was not analysed separately. The possibility that cyclophosphamide contributed to the risk for leukaemia could not be excluded, but the dose was lower than that considered to be leukaemogenic (Curtis *et al.*, 1992).]

Sugita *et al.* (1993) reported on 38 patients, 2–17 years old, in Japan who were treated for non-Hodgkin lymphoma diagnosed between 1987 and 1991 with a protocol that included etoposide (cumulative dose, 5600 mg/m^2). Etoposide (200 mg/m^2) and behenoyl cytarabine were given twice weekly before and after a conventional four-week induction course of prednisolone, vincristine and L-asparaginase. Maintenance therapy consisted of 6-mercaptopurine and methotrexate, administered for 2.5 years, with five two-week pulses of etoposide (200 mg/m^2) and behenoyl cytarabine given every 10 weeks during the first year. All received periodic infusions of methotrexate and prophylactic cranial irradiation. The median follow-up was 19 months. Five of eight patients with haematological relapses developed secondary acute myeloid leukaemia, with a cumulative risk at four years of 18.4% (Kaplan-Meier estimate). [Insufficient information was available to calculate the confidence interval.] Two cases occurred in patients while they were being treated with etoposide. One had been treated for relapse of non-Hodgkin lymphoma with higher doses of etoposide, cyclophosphamide, doxorubicin and also ifosfamide, vincristine, pirarubicin and mitoxantrone. The five patients with acute myeloid leukaemia had received a cumulative dose of etoposide of 4200–5600 mg/m^2; the latent period was 13–30 months. Four of the cases were acute monoblastic leukaemia and the other was acute myeloblastic leukaemia.

2.2.5 *Lung cancer*

Ratain *et al.* (1987) entered 119 patients with unresectable non-metastic lung cancer (histological type, other than small-cell) between 1981 and 1984 into a phase II trial of vindesine, etoposide (300 mg/m^2 intravenously) and cisplatin; etoposide (300 mg/m^2 intravenously) and cisplatin; or vindesine and cisplatin. The patients had had no prior chemotherapy. Twenty-four patients survived more than one year after initiation of therapy. Three of these had received vindesine and cisplatin, nine had received etoposide and cisplatin, and 12 had received vindesine, etoposide and cisplatin; 19 had received palliative radiotherapy (usually in the thorax). Four cases of acute myeloid leukaemia occurred (two acute monoblastic leukaemia, one acute myelomonocytic leukaemia). The rate of acute myeloid leukaemia was 0.30 per person–year (95% CI, 0.11–0.80), and the cumulative risk was 15% [95% CI, 2–45%] at two years. Two patients had received etoposide (7350 and 6240 mg/m^2) and cisplatin, and developed acute leukaemia 28 and 35 months after the start of therapy. The two others had received vindesine, etoposide (7950 and 4382 mg/m^2) and cisplatin, and developed acute myeloid leukaemia 19 and 13 months after the start of therapy, respectively. A comparison of the median cumulative dose of etoposide in the

four patients with leukaemia (6795 mg/m^2) and the 20 patients without leukaemia (3025 mg/m^2) showed that those who eventually developed acute myeloid leukaemia had received significantly more etoposide than those who did not ($p < 0.01$). [The Working Group noted that three of the 24 one-year survivors who did not develop acute myeloid leukaemia had not received etoposide but were included in the calculation of cumulative risk.]

2.2.6 *Breast cancer*

A study in Japan (Yagita *et al*., 1998) included 119 women who were treated for recurrent breast cancer between 1985 and 1994. Before recurrence, the patients had been treated with 5-fluorouracil, cyclophosphamide, doxorubicin, tamoxifen or radiation. All of the patients with recurrences were first treated with doxorubicin (or pirarubicin), vindesine and cyclophosphamide or cisplatin (or carboplatin). Twenty-four patients received etoposide (orally at 50 or 100 mg per day for five to seven days at four-week intervals); the cumulative doses were < 2000 mg for seven patients, 2000–5000 mg for 10 and > 5000 mg for seven. The length of follow-up from the start of etoposide treatment ranged from 1 to 40 months. The cumulative risk for acute myeloid leukaemia and myelodysplastic syndrome on the basis of three cases among the 119 patients was 9.1% (SE, 5.6%) 91–120 months after the operation. Two cases of acute myeloid leukaemia and one of myelodysplastic syndrome developed in the subgroup of 24 patients who had received etoposide orally, and no cases occurred in the group that did not receive etoposide ($p < 0.01$; Fisher's exact test). The latency from start of etoposide treatment was 31 months, 25 months and seven months, and the cumulative doses of etoposide were 1750 mg, 11 900 mg and 4550 mg, respectively. [The Working Group noted that one case of acute myeloid leukaemia occurred shortly (seven months) after the start of etoposide treatment. The comparison of etoposide-exposed patients with patients not treated with etoposide may not be valid, since the two groups were treated with different agents both initially and for recurrent breast cancer.]

3. Studies of Cancer in Experimental Animals

Oral administration

Mouse: Etoposide was tested in a neurofibromatosis type 1 (*Nf1*) transgenic knockout mouse model of myeloid leukaemia. Approximately 10% of heterozygous *Nf1* mice (*Nf1*$^{+/-}$) spontaneously develop myeloid leukaemia at around 15 months of age. Groups of 31–46 *Nf1* wild-type (+/+) or *Nf1* heterozygous (+/–) mice, 6–10 weeks of age [sex unspecified], were treated with 0 or 100 mg/kg bw etoposide weekly for six weeks by gastric intubation and were observed for up to 18 months. Histological examination

was limited to smears of peripheral blood and, in some cases, bone marrow and spleen. The incidences of leukaemia were 2/31 in controls and 8/46 in *Nf1*$^{+/+}$ and *Nf1*$^{+/-}$ mice compared with 0/26 in etoposide-treated *Nf1*$^{+/+}$ and 8/32 in *Nf1*$^{+/-}$ mice ($p = 0.20$). In contrast, the alkylating agent, cyclophosphamide, induced myeloid leukaemias in 0/5 *Nf1*$^{+/+}$ and 7/12 *Nf1*$^{+/-}$ treated mice (Mahgoub *et al*. (1999). [The Working Group noted that the model was applicable for alkylating agents.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

Numerous studies and several reviews (Clark & Slevin, 1987; Fleming *et al*., 1989; Slevin, 1991) have reported the pharmacokinetics of etoposide after intravenous administration in humans. The pharmacokinetics of intravenously administered etoposide in children is similar to that in adults, with a total plasma clearance of 20–40 mL/min per m^2 in children and 15–35 mL/min per m^2 in adults, a distribution volume of 5–10 L/m^2 in children and 7–17 L/m^2 in adults and an elimination half-life of 3–7 h in children and 4–8 h in adults (Slevin, 1991). In most studies, a bi-exponential elimination is described, with a distribution half-life of about 1 h (Hande *et al*., 1984). The proportion of unchanged etoposide recovered in urine represented 20–40% of the dose, but more radiolabel was generally recovered in earlier studies with [^{3}H]etoposide (Allen & Creaven, 1975) than with the more specific high-performance liquid chromatography or radioimmunoassay methods. Studies with high doses of etoposide (up to 3.5 g/m^2) in which blood samples were collected longer than after standard dosing have shown tri-exponential elimination, with a terminal half-time of 18 h or longer, possibly reflecting release of etoposide from tissues (Holthuis *et al*., 1986; Mross *et al*., 1994). The area under the integrated time–concentration curve (AUC) was linear up to doses of 3 g/m^2 in one study (Holthuis *et al*., 1986). With standard doses of 100 mg/m^2 delivered over 1–2 h, the peak concentrations are 10–20 μg/mL (Clark *et al.*, 1994).

The pharmacokinetics of orally administered etoposide has been summarized (Clark & Slevin, 1987; Fleming *et al*., 1989; Slevin, 1991). The bioavailability from an oral capsule is about 50%, but there is evidence that the bioavailability is dose-dependent, with decreasing absorption of doses > 200 mg (Harvey *et al*., 1986; Slevin *et al*., 1989b; Hande *et al*., 1993). In one study, the bioavailability of a 100-mg dose was 76%, while that of a 400-mg dose was 48% ($p < 0.01$) (Hande *et al*., 1993). This effect might be related to a concentration-dependent reduction in the solubility of etoposide in the stomach and small intestine (Joel *et al*., 1995a). The bioavailability of etoposide varies widely among and within patients (Harvey *et al*., 1985; Hande *et al*., 1993).

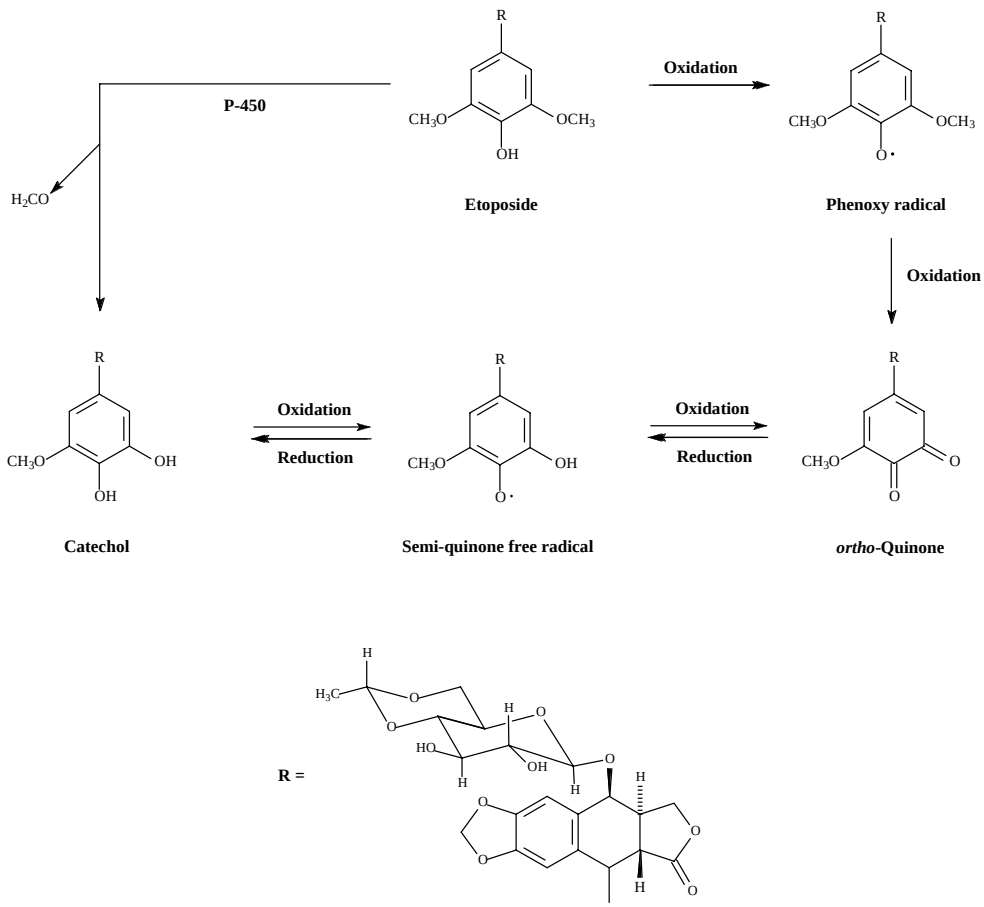
About 94% of a dose of etoposide is bound to protein in adult cancer patients with normal hepatic function (Liu *et al*., 1995; Joel *et al*., 1996; Liliemark *et al*., 1996; Nguyen *et al*., 1998) and 97.5% in children (Liliemark *et al*., 1996). The haematological toxicity of etoposide correlated better with the AUC for free compound than with that for total etoposide (Stewart *et al*., 1991; Joel *et al*., 1996). The AUC for intracellular etoposide in leukaemic cells from patients with acute myeloid leukaemia was ~10% that of the plasma AUC (Liliemark *et al*., 1993).

Little etoposide penetrates into other fluid spaces, almost certainly because of its extensive protein binding. The concentrations of etoposide in cerebrospinal fluid were only 1–2% of the plasma concentration after high doses (Hande *et al*., 1984; Postmus *et al*., 1984a; Holthuis *et al*., 1986), and none was detectable after a standard dose of etoposide (D'Incalci *et al*., 1982). Etoposide was detectable in brain tumours after standard doses (Stewart *et al*., 1984), at concentrations higher than in the cerebrospinal fluid immediately after administration of a high dose (400–800 mg/m^2 by infusion) (Hande *et al*., 1984), but the concentrations in tumours were generally lower in primary or metastasized intracerebral than in extracerebral tumours (Stewart *et al*., 1984). The concentration in saliva after a high dose was only 1.5% of the concurrent plasma concentration at several intervals (Holthuis *et al*., 1986). After administration of a high dose, the peak concentrations in ascites and pleural fluid were considerably lower than the peak plasma concentration, but at later times (> 10 h) the concentrations were higher than in plasma, suggesting slow clearance from these fluid compartments (Hande *et al*., 1984; Holthuis *et al*., 1986).

Because etoposide is excreted renally, clearance is reduced in patients with impaired renal function (Arbuck *et al*., 1986; D'Incalci *et al*., 1986; Pflüger *et al*., 1993; Joel *et al*., 1996). Changes in the pharmacokinetics of etoposide are more subtle in patients with impaired liver function. While the pharmacokinetics of total plasma etoposide may be unchanged, a reduction in protein binding has been reported in these patients, which is associated with decreased serum albumin and/or increased serum bilirubin (Stewart *et al*., 1989; Liu *et al*., 1995; Joel *et al*., 1996). This increase in free etoposide is associated with greater toxicity in this group of patients (Joel *et al*., 1996).

The fate of an intravenous dose of etoposide is still not clear. Generally, few or no etoposide metabolites have been detected in plasma. Etoposide is administered as the *trans*-lactone (ring furthest to the right, Figure 1), but *cis*-etoposide can also be detected in human urine (Holthuis *et al*., 1986). This might be a storage phenomenon, since isomerization sometimes occurs during freezing of plasma samples under slightly basic conditions (Rideout *et al*., 1984). The *cis* isomer accounts for < 1% of the dose (Holthuis *et al*., 1986; Holthuis, 1988). The catechol metabolite has also been reported in patients receiving 600 mg/m^2 etoposide, with an AUC of around 2.5% that of etoposide (Stremetzne *et al*., 1997). In patients given 90 mg/m^2 etoposide, the catechol metabolite represented 1.4–7.1% of the urinary etoposide and < 2% of the administered dose (Relling *et al*., 1994).

Figure 1. Possible metabolic conversions of etoposide



From Mans *et al.* (1990)
P-450, cytochrome P450 mixed-function oxidases

The major urinary metabolite of etoposide in humans is reported to be the glucuronide conjugate. Although urinary glucuronide and/or sulfate conjugates were reported to account for 5–22% of an intravenous dose of etoposide (D'Incalci *et al.*, 1985), other studies suggest that the glucuronide predominates (Holthuis *et al.*, 1986). Etoposide glucuronide in the urine of treated patients accounted for 8–17% of a dose of 0.5–3.5 g/m^2 etoposide (Holthuis *et al.*, 1986) and 29% of a dose of 100–800 mg/m^2 etoposide (Hande *et al.*, 1990), with no other metabolites other than etoposide glucuronide detected in the latter study. In patients with renal or liver impairment given somewhat lower doses of 70–150 mg/m^2, 3–17% of the dose was excreted in the urine within 72 h as etoposide glucuronide (D'Incalci *et al.*, 1986).

The proposed hydroxy acid metabolite of etoposide, formed by opening of the lactone ring, has been detected in human urine, but only at low concentrations, accounting for 0.2–2.2% of the administered dose (Hande *et al*., 1984; Holthuis *et al*., 1986).

These findings are in broad agreement with those of early studies in which [^{3}H]etoposide was used, which indicated that 35–66% of the administered dose of radiolabel was recovered in the urine (Allen & Creaven, 1975).

Less than 4% of a dose was recovered in the bile after 48 h in patients with biliary drainage tubes (Arbuck *et al*., 1986; D'Incalci *et al*., 1986; Hande *et al*., 1990). The faecal recovery of radiolabel after intravenous administration of [^{3}H]etoposide (130–290 mg/m^{2}) was variable, representing 0–16% of dose, but the collections were known to be incomplete because of faecal retention and other difficulties associated with the poor general condition of many of the patients (Creaven & Allen, 1975). In a study reported as an abstract in four patients with small-cell lung cancer given [^{14}C-glucopyranoside]etoposide, 56% of the radiolabel was recovered in urine and 44% in faeces over five days, for a total recovery of 100 ± 6% (Joel *et al*., 1995b).

Studies in lung cancer patients have shown that the plasma concentrations associated with haematological toxicity are higher than those required for antitumour activity. The plasma concentration associated with antitumour activity may be different for different tumour types (Minami *et al*., 1993; Clark *et al*., 1994; Minami *et al*., 1995; Joel *et al*., 1998).

No differences in the pharmacokinetics of etoposide or in the AUC of etoposide catechol were seen in eight children who developed acute myeloid leukaemia after receiving etoposide as part of combination chemotherapy for acute lymphoblastic leukaemia, when compared with 23 children who did not develop secondary acute myeloid leukaemia (Relling *et al*., 1998). These children were included in the study of Pui *et al*. (1991), described in section 2.2.3.

Felix *et al*. (1998) investigated the frequency of a cytochrome P450 CYP3A4 variant with an alteration in the 5′ promoter region of the gene, in leukaemic cells with *MLL* translocations from 42 de-novo cases and 19 that followed epipodophyllotoxin treatment. A significantly decreased frequency of the CYP3A4 variant genotype was found in patients with leukaemia that occurred after treatment with epipodophyllotoxins. Possible changes in the pharmacokinetics related to the CYP3A4 variant were not investigated, but the wild type metabolizes epipodophyllotoxins to the catechol and quinone metabolites.

The pharmacokinetics of etoposide is influenced by concurrent administration of a number of other drugs: clearance may be increased by phenytoin (Mross *et al*., 1994), strongly decreased by cisplatin, which is often given with etoposide, resulting in a 30% increase in the AUC for etoposide (Relling *et al*., 1994), and decreased by up to 40% by ciclosporin, resulting in an increase in the AUC for etoposide of up to 80% (Lum *et al*., 1992).

4.1.2 *Experimental systems*

Few published data on the pharmacokinetics of etoposide in non-human species are available, and many of the preliminary studies conducted before the early clinical trials have not been published in full.

Rhesus monkeys given [^{3}H]etoposide showed biphasic elimination, with a distribution phase half-time of about 1.3 h and a terminal elimination phase of 43 h, as reported in a review by Achterrath *et al*. (1982) that included unpublished reports. Sixty per cent of the dose was excreted renally within 60 h, and 30% faecally. Biphasic elimination was also observed in mice, with a distribution half-time of 1.5 min and an elimination half-time of 33 min. The clearance rate was 17 mL/kg bw per min, and the distribution volume was 820 mL/kg bw (Colombo *et al*., 1986).

After intravenous infusion (5 min) of etoposide phosphate to beagle dogs at doses of 57–461 mg/m^2, a dose-proportional increase was seen in the maximal plasma concentration and AUC for etoposide. The total plasma clearance rate (342–435 mL/min per m^2) and the distribution volume (22–27 L/m^2) were not dose-dependent. The peak plasma concentration occurred at the end of the infusion of etoposide phosphate, indicating rapid conversion of the pro-drug to etoposide (Igwemezie *et al*., 1995).

Thirty minutes after intravenous administration of etoposide to rats, the highest concentrations were found in the liver, kidneys and small intestine. By 24 h after the dose, the tissue concentrations were negligible (Achterrath *et al*., 1982).

In leukaemic cells, the uptake appeared to be linear up to 5 min and reached a steady state by 20–30 min (Allen, 1978; Colombo *et al*., 1986), with intracellular concentrations about twice those of the extracellular medium (Allen, 1978). After removal of the drug, an exponential efflux with a half-time of just 3 min was observed (Allen, 1978). At the same extracellular concentration, the intracellular concentrations of etoposide were 15–20 times lower than those of the closely related drug teniposide (Allen, 1978; Colombo *et al*., 1986).

In rat liver homogenates, liver microsomes and in rats *in vivo*, etoposide was extensively metabolized to only one major metabolite, which was not formally identified (van Maanen *et al*., 1982). In perfused isolated rat liver incubated with etoposide, the total recovery in bile was 60–85%, with roughly equal amounts of etoposide and two glucuronide metabolites (Colombo *et al*., 1985; Hande *et al*., 1988), confirmed as glucuronide species by liquid chromatography and mass spectrometry (Hande *et al*., 1988). After intravenous injection of [^{3}H]etoposide to rabbits, the total urinary excretion of radiolabel was 30% after five days, with very little thereafter. A single glucuronide metabolite was identified in rabbit urine, which was present in larger amounts than etoposide. No hydroxy acid was identified in either species (Hande *et al*., 1988).

A number of authors have reported the peroxidase-mediated oxidation of etoposide to a phenoxy radical, with further oxidation to the *ortho*-quinone, semi-quinone and catechol derivatives (Broggini *et al*., 1985; van Maanen *et al*., 1986; Haim *et al*., 1987;

Kalyanaraman *et al*., 1989). Cytochrome P450-mediated demethylation directly to the catechol has also been reported (van Maanen *et al*., 1987; Relling *et al*., 1992), which is catalysed mainly by the CYP3A4 isoform (Relling *et al*., 1994). These reactive species bind to intracellular macromolecules, including DNA (Haim *et al*., 1987). The *ortho*-quinone and catechol, but not etoposide itself, induced direct DNA damage, as measured by inactivation of single-stranded and double-stranded biologically active $\Phi \times 174$ [bacteriophage] DNA (van Maanen *et al*., 1988). The *ortho*-quinone retains the DNA topoisomerase II inhibitory activity of etoposide (Gantchev & Hunting, 1998). It remains unclear how much these reactive metabolites contribute to the cytotoxic or mutagenic activity of etoposide.

4.2 Toxic effects

4.2.1 *Humans*

The toxicity of etoposide has been summarized (Fleming *et al.*, 1989; Hainsworth & Greco, 1995). The main, dose-limiting toxic effect is myelosuppression, manifest principally as leukopenia. After standard intravenous doses (375–500 mg/m^2 total dose) of etoposide administered alone over three to five days, 20–50% of previously untreated patients experienced moderate to severe leukopenia or neutropenia, typically occurring around day 10–12, with recovery by day 21. Nausea and vomiting are generally mild but may be more common after oral administration. Alopecia occurs in most patients. Mucositis can occur at standard doses, when it is generally mild, but at high doses (< 3500 mg/m^2), mucositis can become dose-limiting (Postmus *et al*., 1984b). Hypotension has been reported, which may be related to the duration of infusion.

Hypersensitivity reactions to etoposide have been reported but are uncommon (O' Dwyer & Weiss, 1984). In eight patients reported to the Investigational Drug Branch of the National Cancer Institute between January 1982 and May 1983, these reactions included flushing, respiratory problems, changes in blood pressure and abdominal pain, often occurring soon after the start of drug administration and generally resolving rapidly when the infusion was stopped. These reactions are less common with etoposide than with the related drug teniposide and have not been reported after oral administration, suggesting that other agents in the formulation may be at least partly responsible. The very low incidence of reported cases may reflect only serious hypersensitivity reactions (Weiss, 1992), as mild reactions were found in 51% of patients receiving etoposide as part of combination chemotherapy for Hodgkin disease (Hudson *et al*., 1993) and 34% of children receiving etoposide as part of a multi-agent induction regime for leukaemia (Kellie *et al*., 1991). Most patients can be successfully re-treated with etoposide after a premedication comprising antihistamine and/or corticosteroids (Hudson *et al*., 1993).

Cardiotoxicity was reported in three of eight patients with pre-existing cardiac disease who received etoposide by infusion (Aisner *et al*., 1982). Dose-related

cutaneous toxicity has been reported, more commonly at doses > 2000 mg/m^2, but the symptoms can be controlled by corticosteroids (Murphy *et al*., 1993).

4.2.2 *Experimental systems*

Much of the pre-clinical toxicology of etoposide has not been published in full, but summary data have been reported in reviews. The acute toxicity of the drug after intravenous dosing was investigated, with LD_{50} values of 118 mg/kg bw in mice, 68 mg/kg bw in rats and > 80 mg/kg bw in rabbits (Achterrath *et al*., 1982). In a later study, the LD_{50} in mice after intraperitoneal administration was reported to be 108 mg/kg bw (Lee *et al*., 1995).

Four-week studies of toxicity were conducted in rats treated intraperitoneally at 0.6–6.0 mg/kg bw per day and in monkeys treated intravenously at 0.4–3.6 mg/kg bw per day. At the highest doses, the main toxic effect was myelosuppression, with anaemia, leukopenia and thrombocytopenia, and some hepatotoxicity. Pathological changes were noted in the lung in rats, and mild enteritis was seen in dogs. After oral and intravenous administration at the same doses as in the previous studies, no additional toxicity was observed up to nine weeks (review of unpublished studies by Achterrath *et al*., 1982).

Oral administration to rats and dogs at a dose of 0.5–5 mg/kg bw per day for five days a week for 26 weeks also resulted in myelosuppression as the major toxic effect in both species. No other effects were seen in the rats, while those in dogs included renal and hepatic impairment, electrocardiographic changes, decreased testis weight and disorders of spermatogenesis (review of unpublished studies by Achterrath *et al*., 1982). These changes were largely reversible after four weeks without treatment.

After intraperitoneal administration of a clinical formulation or intrapleural administration of etoposide dissolved in dimethyl sulfoxide and Tween 80 diluted in Hank's buffer to rats and mice, delayed chronic pleuritis and peritonitis, with liver and spleen inflammation were reported. The vehicle had no effect when given alone (Stähelin, 1976).

After intravenous infusion of a single dose of 461 mg/m^2 etoposide phosphate to dogs over 5 min, all animals vomited, and leukopenia and thrombocytopenia were seen at this and lower doses (Igwemezie *et al*., 1995).

Etoposide- and etoposide phosphate-induced sensory neuropathy has been reported in mice after single doses of 88 mg/kg bw and 100–150 mg/kg bw, respectively (Bregman *et al*., 1994).

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Five case reports of treatment with etoposide during pregnancy were located. A woman was treated at 26 weeks of gestation with a combination of bleomycin, etoposide

(165 mg/day) and cisplatin on three consecutive days for an unknown primary cancer. Six days later, she developed neutropenia and septicaemia and had a spontaneous vaginal delivery. The female infant developed profound leukopenia with neutropenia three days later (10 days after in-utero exposure), which had resolved by day 13. At 10 days of age, the infant started to lose her hair, which was growing again when she was discharged at 12 weeks. At follow-up at one year, she was essentially normal (Raffles *et al*., 1989). A woman was treated for acute leukaemia at 25 and 30 weeks of gestation with cytarabine, daunorubicin and etoposide (400 mg/m^2 per day for three days). Her infant, delivered by caesarean section at 32 weeks because of fetal distress, had leukopenia with profound neutropenia, which was confirmed to be due to bone-marrow suppression by measurement of circulating haemopoietic progenitor cells. This condition responded to transfusion of packed cells and subcutaneous injections of granulocyte colony-stimulating factor, and the infant was well at follow-up at one year (Murray *et al*., 1994). Three women treated for acute leukaemia, ovarian cancer and non-Hodgkin lymphoma with multiple drug cycles including etoposide (100–125 mg/m^2 per day) in the third trimester had normal, healthy infants (Buller *et al*., 1992; Brunet *et al*., 1993; Rodriguez & Haggag, 1995). Etoposide was used to induce abortion in two cases of ectopic pregnancy. In one case, a woman with a cervical ectopic pregnancy of six weeks was given oral doses of etoposide of 200 mg/m^2 for five days. The pregnancy was terminated, but there was evidence of bone-marrow suppression in the mother and almost complete loss of hair (Segna *et al*., 1990). The second case, a tubal pregnancy of five weeks, was successfully terminated by two injections of 50 mg etoposide locally into the gestational sac, with no side-effects (Kusaka *et al*., 1994).

Ovarian function may be impaired by etoposide. In a study of 20 young and two older (> 50 years) women with gestational trophoblastic disease treated orally with etoposide and who had serial hormone assays, transient ovarian failure lasting two to four months was observed in five of the young women, and the two older women both had permanent ovarian failure. In the younger women, fertility was unaffected and six became pregnant within one year of therapy (Choo *et al*., 1985). In a similar study on 47 women treated with etoposide, ovulation ceased in about half of the patients but returned within four months after treatment in all of the patients under 40 years of age. In nine patients over 40 years of age, ovulation did not return within the follow-up period of 12 months. The effects on the ovary were not related to the dose of etoposide but were related to the age of the patient (Matsui *et al*., 1997). Etoposide was not found to have any long-term effect on fertility in 77 women treated for gestational trophoblastic tumours (Adewole *et al*., 1986).

Excretion of etoposide in breast milk was demonstrated in a woman with acute promyelocytic leukaemia receiving daily doses of 80 mg/m^2 [route not stated]. Peak concentrations of 0.6–0.8 μg/mL were measured immediately after dosing but had decreased to undetectable levels by 24 h (Azuno *et al*., 1995).

Reproductive capacity was assessed in 30 men with germ-cell tumours after treatment with cisplatin, etoposide and bleomycin. A single semen sample was obtained

for analysis 24–78 months after initiation of therapy. The results are difficult to interpret, since most men with testis tumours are oligospermic before chemotherapy. Oligospermia ($< 40 \times 10^6$ total sperm) was diagnosed in 13 of the men, including six with azoospermia. Morphological abnormalities were common, and only one man had more than 50% normal sperm. Eight of the men subsequently fathered children, none of whom had birth defects (Stephenson *et al*., 1995).

Etoposide did not cause permanent damage to the germinal epithelium in 47 young men receiving it for Hodgkin disease (Gerres *et al*., 1998).

4.3.2 *Experimental systems*

Groups of 4–10 pregnant Swiss albino mice were given a single dose of etoposide at 1.0, 1.5 or 2.0 mg/kg bw intraperitoneally on day 6, 7 or 8 of gestation (vaginal plug, day 0), and the fetuses were removed and examined on day 17. No effect on maternal body-weight gain was seen in any group. In animals injected on day 6, no embryotoxicity was seen with 1.0 or 1.5 mg/kg bw, but 2.0 mg/kg bw increased the frequencies of intrauterine death, fetal malformations and reduced fetal body weight. Injection on day 7 caused dose-related embryolethality, fetal malformations and reduced fetal weight. Injection on day 8 caused no embryolethality and no effect on fetal body weight, but the frequency of fetal malformations was increased at doses of 1.0 and 2.0 mg/kg bw. The commonest malformations observed at the highest dose were hydrocephalus (12.2%) and open eyelids (16.7%) after injection on day 6, exencephaly and encephalocoele at frequencies of 13% and 10% on days 7 and 8 and axial skeletal defects at frequencies of 28% and 7.7% on days 7 and 8, respectively (Sieber *et al*., 1978).

The results of standard studies of reproductive toxicity with etoposide have been published. Groups of 30 male Crj:CD Sprague-Dawley rats were given etoposide at a dose of 1, 3 or 10 mg/kg bw orally by gavage for 64 days and 30 females for 15 days before mating, and treatment was continued during mating and, for females, until day 7 of gestation. The high dose suppressed body-weight gain during the first two weeks of treatment in the females only. In males at the highest dose, the weights of the testis, epididymides and thymus were reduced, and the organs appeared atrophic macroscopically; however, reproductive function was not significantly affected. Females at the high dose had decreased numbers of corpora lutea and implants and reduced litter size and an increased frequency of resorptions. Fetal body weight was significantly reduced and the number of malformed fetuses greater than in controls. The malformations observed included exencephaly, anury, cerebral atrophy, cerebral ventricular dilatation, anophthalmia and microphthalmia (Takahashi *et al*., 1986a).

Groups of 22–24 pregnant Crj:CD Sprague-Dawley rats were given etoposide at a dose of 1, 3 or 10 mg/kg bw orally by gavage from day 17 of gestation until day 20 *post-partum*. The high dose produced thymic atrophy in the dams but did not affect the duration of gestation or parturition. The mortality rate of pups was slightly

increased during the first three days after birth, and their body-weight gain was transiently suppressed. Other aspects of postnatal physical, functional and behavioural development were unaffected. The reproductive function of the F_1 generation was normal, and the growth and development of the F_2 generation were normal. Long-term observation of the F_1 animals showed no delayed toxicity or carcinogenesis [details not given] (Takahashi *et al*., 1986b).

Groups of 20–22 male Crj:CD Sprague-Dawley rats were treated with etoposide at a dose of 0.05, 0.2 or 0.8 mg/kg bw intravenously for 61 days and females for 14 days before mating, and treatment was continued during mating and, for females, until day 7 of gestation. The high dose suppressed body-weight gain in animals of each sex. In males at the high dose, the weights of the testis and epididymides were reduced, and the organs appeared atrophic macroscopically; however, reproductive function was not significantly affected. The weight of the thymus was reduced at the intermediate and high doses. Females at the high dose had smaller litters and more resorptions than controls. Fetal body weight was significantly reduced, and the number of malformed fetuses was increased when compared with controls. The malformations observed included cerebral ventricular dilatation, anophthalmia and microphthalmia (Takahashi *et al*., 1986c).

Groups of 23–24 pregnant Crj:CD Sprague-Dawley rats were given etoposide intravenously at a dose of 0.05, 0.2 or 0.8 mg/kg bw from day 17 of gestation until day 20 *post-partum*. Animals at the intermediate and high doses had reduced body-weight gain and thymic atrophy, but the duration of gestation and parturition was not affected. Treatment had no effect on pup survival, but their body-weight gain was transiently suppressed. Other aspects of postnatal, physical, functional and behavioural development were unaffected, except for a slight delay in vaginal opening by 1.4 days in the group at the high dose. The reproductive function of the F_1 generation was normal, and the growth and development of the F_2 generation were normal (Takahashi *et al*., 1986d).

Day-10 rat embryos [strain not specified] cultured for 24 h *in vitro* were exposed for the first 3 h to etoposide at concentrations of 1.0–10 μmol/L. A dose-related increase in the incdence of malformations was observed at doses of 2.0 and 5.0 μmol/L, and at the latter concentration 100% of the embryos were malformed. The dose of 10 μmol/L was lethal to all embryos. The malformations observed consisted mainly of hypoplasia of the prosencephalon, microphthalmia and oedema of the rhombencephalon. Comparison of the concentrations necessary to produce 50% lethality and 50% malformations showed that amsacrine (see monograph, this volume) was 10 times and 20 times more potent, respectively, than etoposide. The authors suggested that the malformations were related to the inhibition of DNA topoisomerase II activity in the embryo, but presented no data to support the proposal (Mirkes & Zwelling, 1990).

Etoposide has been reported to cause degeneration of rat spermatogonia and early spermatocytes, the appearance of large multinucleated spermatids and nuclear and cytoplasmic changes in Sertoli cells. The stage-specific changes produced by etoposide were studied by following effects on DNA synthesis as measured by incorporation of

[^{3}H]thymidine. Groups of three Sprague-Dawley rats, two to three months of age, were injected intraperitoneally with 5 or 10 mg/kg bw etoposide and killed 1, 3 or 18 days later. Premitotic DNA synthesis was inhibited by about 40–70% in spermatogonial stages II–V at both doses, when compared with controls. The effects on premeiotic DNA synthesis were less marked, with a maximum inhibition of about 40%. Markedly increased incorporation of thymidine was also observed at stage VII, at which no DNA synthesis normally occurs. The effects of etoposide were most marked after one and three days, but some effects were still present 18 days after treatment (Hakovirta *et al*., 1993).

In adult Sprague-Dawley rats injected intraperitoneally with a single dose of 5 or 10 mg/kg bw etoposide, it was a powerful inducer of micronuclei in early spermatids, whereas the major cytotoxic action is on the early spermatogonial stages. Thus, the cytotoxicity is separate from genotoxicity (Lähdetie *et al*., 1994).

In a study of teratogenicity, groups of 11–21 JW-NIBS rabbits were given 0.3, 1, 3 or 10 mg/kg bw etoposide orally by gavage on days 6–18 of gestation. The highest dose caused marked depression of body-weight gain and food intake throughout gestation, and only two animals were alive at the end of the study. Deaths were observed at 3 mg/kg bw per day, and only 16/21 animals in this group were alive at termination. Doses up to and including 3 mg/kg bw had no adverse effect on embryo or fetal development, fetal weight, ossification or the incidence of malformations (Takahashi *et al*., 1986e).

Five groups of eight pregnant Japanese white rabbits (Kbl:JW) were given etoposide at a dose of 0, 0.25, 0.5, 1.0 or 2.0 mg/kg bw per day intravenously on days 7, 8 and 9 of gestation. The fetuses were removed for visceral and skeletal examination on day 28 of gestation. The body-weight gain of dams was depressed and liver damage was observed at the highest dose, but fetal survival and weight were unaffected and no gross malformations were observed. A low incidence (4/64, $p < 0.05$) of fetuses with rib and vertebral abnormalities was noted. Histological examination of the fetal telencephalon showed no increase in cell death (Nagao *et al*., 1999).

4.4 Genetic and related effects

4.4.1 *Humans*

Evidence that etoposide causes genetic changes in humans derives from three lines of investigation. The first involves rather limited studies of DNA damage and mutations in patients undergoing treatment. The second involves cytogenetic analyses of cells from patients with leukaemias associated with this treatment. The third involves molecular characterization of the translocation break-points.

(*a*) *DNA damage and mutations in patients undergoing treatment with etoposide*

Osanto *et al.* (1991) examined the presence of micronuclei in binucleated peripheral blood lymphocytes of patients treated with etoposide-containing combination chemotherapy for testicular carcinoma. An increased frequency of micronuclei was found in these samples compared with those obtained from untreated cancer patients or healthy, age-matched controls at a mean interval of 4.6 years after cessation of chemotherapy. This indicates that DNA damage persists for a long time after treatment.

The analysis by Karnaoukhova *et al.* (1997) of *HPRT* gene mutation frequency and mutation spectra by a T-cell cloning assay in lymphocytes from 12 patients with small-cell lung cancer receiving chemotherapy with etoposide provides additional evidence that it is associated with genetic changes. The regimens included up to six cycles of two daily oral doses of 50 mg/kg bw over 10–14 days separated by two weeks of rest. The total doses ranged from 1.4 to 8.4 g, and follow-up was for 0.7–5.3 months from the start of treatment. There was considerable variation between patients, but no significant increase in *HPRT* gene mutation frequency was seen after treatment when compared with pre-treatment control values. The post-treatment mutation spectrum showed an increase in AT→TA transversions and a decrease in GC→TA transversions when compared with the pre-treatment spectrum. No gross rearrangements or deletions were detected.

(*b*) *Cytogenetic analyses of cells from patients with leukaemias associated with etoposide treatment*

The most direct evidence that etoposide causes genetic changes in humans *in vivo* derives from the finding in the 1980s of a distinct form of leukaemia characterized by chromosomal translocations at the time this agent was introduced into clinical usage. Taken together, these analyses suggest that etoposide is responsible for several non-random chromosomal translocations that are central to leukaemogenesis. It is also noteworthy that etoposide treatment of phytohaemagglutinin-stimulated human lymphocytes in culture is associated with an excess frequency of reciprocal translocations in addition to other abnormalities, which include dicentrics and, less often, unbalanced or complex rearrangements, deletions and inversions. Chromosomes 1, 11 and 17 are frequent targets of these abnormalities (Maraschin *et al.*, 1990; Pedersen-Bjergaard & Rowley, 1994).

Pedersen-Bjergaard *et al.* (1995) evaluated the cytogenetic characteristics in 137 cases of leukaemia and myelodysplastic syndrome after primary cancer treatment. The results of this analysis provide conclusive evidence that the kinds of aberrations associated with DNA topoisomerase II inhibitors are different from those observed with alkylating agents. Deletions or loss of chromosomes 5 and 7 were significantly associated with exposure to alkylating agents ($p = 0.002$), and balanced translocations to bands 11q23, 21q22 and 3q23 were seen with DNA topoisomerase II inhibitors ($p = 0.00005$).

Most of the translocations with which etoposide is associated in treated patients involve chromosome band 11q23, but this and other DNA topisomerase II inhibitors have also been associated with leukaemias with t(8;21), t(3;21), inv(16), t(15;17) or t(9;22) translocations (Ratain *et al*., 1987; Pui *et al*., 1990; Pedersen-Bjergaard & Philip, 1991; Pui *et al*., 1991; Detourmignies *et al*., 1992; Pedersen-Bjergaard, 1992; Hunger *et al*., 1993; Quesnel *et al*., 1993; Winick *et al*., 1993; Felix *et al*., 1995a; Nucifora & Rowley, 1995; Strissel Broeker *et al*., 1996; Pedersen-Bjergaard *et al*., 1997). These are also the common translocations in de-novo cases of leukaemia; however, while translocations of chromosome band 11q23 are present in most cases of acute lymphoblastic leukaemia in infants and cases of monoblastic leukaemia in infants and young children, they are found in only about 5% of acute leukaemias in adults. More recently, chromosome band 11p15 was recognized as a site of recurrent translocations in leukaemias that follow etoposide-containing therapy (Stark *et al*., 1994; Kobayashi *et al*., 1997).

(i) *Translocations involving band 11q23*

Etoposide has most often been used in combination chemotherapy. Ratain *et al.* (1987) described the occurrence of leukaemia, primarily with monoblastic features and translocations of chromosome band 11q23, in patients treated with etoposide in combination chemotherapy for non-small-cell carcinoma of the lung. A group of 119 patients with advanced non-small-cell lung cancer were treated with four different cisplatin-based regimens, one of which contained etoposide; four patients developed acute myeloid leukaemia 13, 19, 28 and 35 months after the start of treatment, respectively. All four had received etoposide and cisplatin with or without vindesine. Three of the leukaemias had monoblastic features; in one case there was a t(9;11)(p22;q23) translocation; in another there was t(9;11;18)(p22;q23;q12). The fourth case had a complex karyotype, with -5 and -7 abnormalities typical of alkylating agent-induced cases.

Several additional case reports and cohort studies have indicated that, while the 9p22 locus is commonly involved in translocations with band 11q23 (Pedersen-Bjergaard *et al*., 1991), chromosome band 11q23 can fuse with numerous other chromosomal loci. This was confirmed in studies of de-novo cases (Felix, 1998). For example, DeVore *et al.* (1989) and Nichols *et al.* (1993) reported cases of acute myeloid leukaemia with t(11;19)(q23;p13) after etoposide-containing chemotherapy of germ-cell tumours. Observations of cases of undifferentiated leukaemia and acute lymphoblastic leukaemia with t(4;11)(q21;q23) suggested additional heterogeneity in the partner chromosomes involved in translocations with band 11q23 (Hunger *et al*., 1992; Nichols *et al*., 1993).

When Pui *et al.* (1991) examined 21 cases of acute myeloid leukaemia that occurred as a second cancer in 734 children with acute lymphoblastic leukaemia who received maintenance therapy including teniposide with or without etoposide, a translocation of chromosome band 11q23 was found in 15 cases. The karyotype revealed t(9;11)(p21–p22;q23) in seven cases, but other chromosomal regions involved in

translocations with band 11q23 in one case each were 21q22, Xq13, 12p13, 16p13, 19q13, 19p13, and 17q21. The translocation at band 11q23 was usually the only abnormality, but in five cases additional structural or numerical changes were detected. In one case the karyotype showed inv(11)(p15q23) and t(10;11)(p13;q13). Similarly, Winick *et al.* (1993) examined the occurrence of acute myeloid leukaemia as a second cancer in 205 children with B-lineage acute lymphoblastic leukaemia treated according to a protocol that included etoposide during central nervous system consolidation and continuation phases. Ten patients developed acute myeloid leukaemia. Translocation of chromosome band 11q23 occurred in eight of the 10 cases, with fusions to band 9p21 or 9p22 in three cases and fusions to band 17q25, 19p13.1, 16p13.3, 2q37 or 1q32 in one case each.

Heterogeneous translocations involving band 11q23 were also observed in cases of leukaemia after epipodophyllotoxin-containing treatment for paediatric solid tumours. In the series reported by Pui *et al.* (1990), 12 cases of leukaemia occurred in 3365 children, and cytogenetic analysis was available for eight cases. A translocation t(9;11)(p21;q23) was seen in two cases, one of which also contained t(4;11)(q26;p13). One case showed t(1;11)(p32;q23) and t(11;?)(p15;?). The treatment regimens were not described completely, but epipodophyllotoxins were used in two patients with translocations involving band 11q23. Laver *et al.* (1997) reported a case of myelodysplastic syndrome without excess blasts which showed t(11;16)(q23;p13.3) in a paediatric patient given etoposide-containing therapy for Ewing sarcoma. Karyotypic features in leukaemias were evaluated after more dose-intensive, repetitive administration of high-dose alkylating agents, anthracycline and etoposide for paediatric non-lymphoid solid tumours (Kushner *et al*., 1998a) and neuroblastoma (Kushner *et al*., 1998b), in which the incidence of leukaemia is high. Here, cytogenetic analysis revealed 5q and 7q deletions, consistent with alkylating agent-related leukaemia and myelodysplastic syndrome in some cases, and t(9;11)(p22;q23) translocation, more typical of leukaemias associated with DNA topoisomerase II inhibitors in others. One case that occurred 24 months after the start of neuroblastoma treatment showed del(7)(q22) and del(11)(q13q23), possibly reflecting the combined effects of an alkylating agent and an epipodophyllotoxin (Kushner *et al*., 1998b).

(ii) *Translocations t(8;21)(q22;q22) and t(3;21)(q26;q22)*

The t(8;21)(q22;q22) translocation is the hallmark of de-novo acute myeloid leukaemias of myeloblastic morphology, but this translocation is also observed after etoposide-containing therapy (Felix *et al*., 1995b). Two cases of acute myeloid leukaemia with t(8;21) were observed among 212 patients who received etoposide, cisplatin and bleomycin for germ-cell tumours (Pedersen-Bjergaard *et al*., 1991). Quesnel *et al.* (1993) identified the t(8;21) translocation in three cases of treatment-related leukaemia in which prior treatment had included an epipodophyllotoxin in combination with either an anthracycline or an alkylating agent, but it was not specified whether etoposide or teniposide was used. Translocation t(8;21) has been observed in cases of epipodo-

phyllotoxin-related leukaemia occurring after paediatric solid tumours. In the paediatric patients with primary solid tumours reported by Pui *et al.* (1990), one leukaemia showed t(8;21)(q22;q22), with additional abnormalities of chromosome 17. One case showed t(8;21)(q22;q22) and del(16)(q22). Translocations t(3;21)(q26;q22) are uncommon variants in treatment-related acute myeloid leukaemia and treatment-related myelodysplastic syndrome (Nucifora & Rowley, 1995). Of five cases of leukaemia with t(3;21) after heterogeneous chemotherapy, one had been treated with etoposide (Rubin *et al.*, 1990).

(iii) *Abnormality inv(16)(p13q23)*

The chromosomal abnormality inv(16) is infrequent in treatment-related acute myeloid leukaemia associated with the monocytic subtype with eosinophilia (Quesnel *et al.*, 1993; Pedersen-Bjergaard & Rowley, 1994). Fenaux *et al.* (1989) described a case of acute monocytic leukaemia with eosinophilia in which the karyotype showed del(3)(q26), del(7)(q31), inv(16)(p13q23); the leukaemia had occurred after chemotherapy for Hodgkin disease with procarbazine, vincristine, prednisone and nitrogen mustard (MOPP) and for a germ-cell tumour with cisplatin, doxorubicin and etoposide in the same patient. Quesnel *et al.* (1993) identified the inv(16) in one case of treatment-related leukaemia in which prior treatment had included an epipodophyllotoxin in combination with an alkylating agent, but it was not specified whether etoposide or teniposide was used.

(iv) *Translocation t(15;17)*

Acute promyelocytic leukaemia with translocation t(15;17) occurs infrequently after treatment with etoposide and other DNA topoisomerase II-targeted anticancer drugs. Individual case reports indicate that t(15;17) is also a recurrent chromosomal alteration after etoposide-containing treatment for a variety of tumours (Raiker *et al.*, 1989; Detourmignies *et al.*, 1992; Lopez-Andreu *et al.*, 1994; Smith *et al.*, 1999). Etoposide has rarely been used as a single agent, except in some cases for the treatment of Langerhans cell histiocytosis (Haupt *et al.*, 1997). Haupt *et al.* (1997) observed five cases of leukaemia among 241 patients, 113 of whom had received etoposide as part of their treatment. Morphologically, the leukaemias were acute promyelocytic. Although t(15;17) is present in the vast majority of cases of acute promyelocytic leukaemia, the karyotypes in cases where etoposide was used a single agent revealed -6, -10, +mar, +ring in one case and del(20)(q11q13) in another. Cytogenetic analysis in one case in which etoposide was used with vinblastine and high-dose methylprednisolone did reveal the t(15;17), but in another case in which etoposide was used the t(15;17) was detectable only by molecular analysis.

(v) *Translocation t(9;22)*

A case of chronic myeloid leukaemia with t(9;22) and the *BCR/ABL* fusion gene was observed in an adult male 5.5 years after treatment of testicular cancer with a regimen that contained etoposide. Acute lymphoblastic leukaemia and acute myeloid leukaemia

with t(9;22) may also occur after treatment with etoposide (Pedersen-Bjergaard *et al.*, 1997).

(vi) *Translocations involving chromosome band 11p15*

Chromosome band 11p15 has emerged as another site of chromosomal abnormalities in leukaemias occurring after etoposide-containing treatment. At the cytogenetic level, abnormalities including add(11)(p15), inv(11)(p15q22), t(11;20)(p15;q11.2) and t(2;11)(q31;p15) have been reported (Stark *et al.*, 1994; Felix *et al.*, 1995b; Kobayashi *et al.*, 1997; Raza-Egilmez *et al.*, 1998), while additional cases with inv(11)(p15q22) and t(1;11)(q23;p15) after unspecified DNA topoisomerase II inhibitors have been observed (Arai *et al.*, 1997; Nakamura *et al.*, 1999).

(*c*) *Molecular characterization of the etoposide-related translocation break-points*

Molecular characterization of the translocation break-points in the leukaemias provides detailed insight about the genetic changes associated with etoposide treatment and clues to the mechanism whereby they occur. Cytogenetic studies revealed that translocations, especially those involving chromosome band 11q23, are hallmark features of leukaemias related to etoposide. In the early 1990s, several laboratories isolated the break-point region of the relevant gene at chromosome band 11q23 and named the gene *ALL-1*, *MLL*, *HTRX1* and *HRX* (Djabali *et al.*, 1992; Gu *et al.*, 1992; McCabe *et al.*, 1992; Tkachuk *et al.*, 1992), the last two designations for its homology to *Drosophila trithorax*. After the cloning of the *MLL* gene, several laboratories used Southern blot analysis to study leukaemias that had occurred in etoposide-treated patients and isolated the genomic break-points and the fusion transcripts.

Southern blot analyses of leukaemias with cytogenetic evidence of translocations involving band 11q23 and chromosomal loci, such as 9p22, 19p13, 3q25, 1p32, 4q21, 6q27, 5q13, 9q27, 16p13 or del(11)(q23), revealed that virtually all disrupted the same break-point cluster region between exons 5 and 11 of the *MLL* gene, which is also involved in de-novo leukaemias with translocations of chromosome band 11q23 (Felix *et al.*, 1993; Gill Super *et al.*, 1993; Domer *et al.*, 1995; Felix *et al.*, 1995b; Strissel Broeker *et al.*, 1996). In addition, Southern blot analysis indicated that *MLL* gene rearrangements could be present even when the karyotype did not reveal the translocation (Felix *et al.*, 1995b). Later, detailed mapping by Southern blot analysis suggested a biased distribution of the translocation break-points in treatment-related leukaemias in the 3′ break-point cluster region (Strissel Broeker *et al.*, 1996). Low-affinity and high-affinity scaffold attachment regions were identified centromeric to and within the telomeric break-point cluster region, respectively, and sequence homologies to a putative in-vitro DNA topoisomerase II recognition sequence were observed proximal to and within the telomeric scaffold attachment region, leading Strissel Broeker *et al.* (1996) to suggest that chromatin structure may be important in determining the distribution of the break-points.

Domer *et al.* (1995) were the first to clone an *MLL* genomic translocation break-point in a case of leukaemia after etoposide-containing therapy when they isolated the der(11) breakpoint junction in a case of acute lymphoblastic leukaemia with a t(4;11). The *MLL* break-point was 3′ in intron 8. The partner gene was *AF-4* at band 4q21. The *MLL* and *AF-4* break-points were both proximal to regions of homology to a putative in-vitro DNA topoisomerase II recognition site. The authors suggested a role for DNA topoisomerase II in the translocation process. Cloning of additional *MLL* genomic break-points and attempts to understand the translocation mechanism followed.

The cloning of *MLL* translocation break-points in leukaemias in etoposide-treated patients has led to further characterization of intronic regions of known genes fused with *MLL* (typically called 'partner genes'), because the break-points are in introns. It also led to the discovery of new partner genes. This line of investigation yields insights about regions of the genome affected by the drugs and provides some clues about the mechanism. Megonigal *et al.* (1997) cloned the *MLL* genomic break-point in a case of acute lymphoblastic leukaemia with t(4;11)(q21;q23) in a patient treated with etoposide. The der(11) *MLL* translocation break-point was in intron 6 in the 5′ break-point cluster region. The sequence of the partner DNA was not homologous to known cDNA or genomic sequences of the *AF-4* gene at chromosome band 4q21, but reverse transcriptase polymerase chain reaction analysis showed that the t(4;11) was an *MLL-AF-4* fusion. The break-point in the *MLL* break-point cluster region was in an *Alu* repeat and there was an *Alu* repeat near the break-point in the partner DNA, suggesting that the repetitive sequences are important for this type of rearrangements. The break-point deviated from the predilection for 3′ distribution in the break-point cluster region that was suggested in the adult cases (Strissel Broeker *et al*., 1996).

While the karyotypes imply considerable overlap in partner genes in treatment-related and de-novo leukaemias with *MLL* gene translocations, some partner genes were discovered in etoposide-related acute myeloid leukaemia or myelodysplastic syndrome. Examples include the cAMP response element-binding (CREB) protein gene (*CBP*) at chromosome band 16p13.3 (Rowley *et al*., 1997; Sobulo *et al*., 1997; Taki *et al*., 1997) and the gene encoding p300 at chromosome band 22q13 (Ida *et al*., 1997). Sobulo *et al.* (1997) isolated the der (16) genomic break-point of a t(11;16) translocation and localized the *MLL* break-point to position 1502 in intron 6, also in the 5′ break-point cluster region. The *MLL* genomic break-point in the der(11) chromosome of the t(11;22) translocation involving p300 was at position 7206 in intron 9 in the 3′ break-point cluster region (Ida *et al*., 1997), confirming heterogeneity in *MLL* genomic break-point distribution. The *CBP* gene also contains mutations in patients with Rubenstein-Taybi syndrome, indicating involvement of a common region of the genome in leukaemia and a constitutional disorder (Taki *et al*., 1997). Since the *CBP* gene product is a histone acetyltransferase, the t(11;16) potentially could lead to histone acetylation of genomic regions targeted by *MLL* AT hooks and transcriptional deregulation (Sobulo *et al*., 1997). p300 is a transcriptional co-activator with *CBP* (Ida *et al*., 1997).

Thus, like *MLL* break-points in leukaemia in infants, *MLL* translocation break-points in etoposide-related leukaemias are distributed in introns within the 8.3-kilobase break-point cluster region between *MLL* exons 5–11. Heterogeneous partner genes have been reported to be involved in the translocation. The *MLL* genomic break-point region involved in translocations has been studied in DNA topoisomerase II cleavage assays of naked DNA and after exposing human haematopoietic cells to etoposide in tissue culture. In an assay for DNA topoisomerase II cleavage *in vitro*, Lovett *et al.* (1999) reported (in an abstract) that multiple DNA topoisomerase II cleavage sites within the *MLL* break-point cluster region are enhanced not only by etoposide but also by its catechol and quinone metabolites. Aplan *et al.* (1996) and Stanulla *et al.* (1997a) observed site-specific cleavage *in vitro* within the 3′ *MLL* break-point cluster region by Southern blot analysis after exposing human peripheral blood mononuclear cells to etoposide as well as to doxorubicin, catalytic DNA topoisomerase II inhibitors and other genotoxic and non-genotoxic stimuli of apoptosis. The site-specific cleavage was attributed to the higher-order chromatin fragmentation which occurs during apoptosis. Similar site-specific cleavage was identified within *AML1*, the gene at band 21q22 in the t(8;21) and t(3;21) translocations (Stanulla *et al*., 1997b). Indeed, it later was proposed that DNA cleavage induced directly by DNA topoisomerase II or by the drug-induced apoptotic cellular response is responsible for the non-random chromosomal translocations leading to leukaemogenesis (Dassonneville & Bailly, 1998).

The *NUP98* gene at chromosome 11p15 is involved in at least six different chromosomal aberrations and, like *MLL*, appears to be a target gene in treatment-related myelodysplastic syndrome and acute myeloid leukaemia, with multiple translocation partners. The NUP98 gene product is a 98-kDa component of the nuclear pore complex which functions as a docking protein in nucleocytoplasmic transport (Radu *et al*., 1995). The multiple FXFG repeats in the N-terminal portion of the protein are required for its docking function. The translocations t(7;11)(p15;p15), t(2;11)(q31;p15), t(1;11)(q23;p15) and t(4;11)(q21;p15) and the inversion inv (11)(p15q22) result in fusion transcripts that encode chimaeric oncoproteins fusing the FXFG region of NUP98 with HOXA9, HOXD13, PMX-1, all homeodomain-containing proteins, or with RAP1GDS1 or DDX-10, a putative RNA helicase, respectively (Nakamura *et al*., 1996; Arai *et al*., 1997; Raza-Egilmez *et al*., 1998; Hussey *et al*., 1999; Nakamura *et al*., 1999). Most recently, the t(11;20)(p15;q11) was identified in two paediatric cases of treatment-related myelodysplastic syndrome after exposure to multi-agent chemotherapy in which etoposide was included. The t(11;20) translocation fuses *NUP98* with the *TOP1* gene (Ahuja *et al*., 1999).

4.4.2 *Experimental systems*

General reviews on the mutagenicity of inhibitors of DNA topoisomerase II enzymes, including etoposide, have been published (Anderson & Berger, 1994; Ferguson & Baguley, 1994, 1996; Baguley & Ferguson, 1998; Ferguson, 1998).

Jackson *et al.* (1996) collated a genetic activity profile for this drug. The results are summarized in Table 6.

Etoposide gave mainly negative responses in a range of assays in prokaryotes and lower eukaryotes. Thus, in most studies, it did not cause significant increases in reverse mutation frequency as measured in *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100, *Escherichia coli* WP2 *uvrA*, *E. coli* K12 (forward and reverse mutation) and in other *E. coli* assays in the presence or absence of exogenous metabolic activation. Etoposide caused a slight (about twofold) increase in the frequency of revertant colonies in *S. typhimurium* TA102 and a clearly positive response in strain TA1978. It caused differential toxicity in *Bacillus subtilis* H17 *rec*$^{+}$ and M45 *rec*$^{-}$. Toxicity but not mutagenicity occurred at a dose of about 800 μg/plate, which is higher than those studied in mammalian cells. In *Saccharomyces cerevisiae* strain D5, etoposide did not induce either mitochondrial 'petite' mutations or mitotic recombination. It did not induce forward or reverse mutations in *Neurospora crassa*.

Etoposide is a potent inducer of DNA breakage in mammalian cells, both *in vitro* and *in vivo*. It caused protein-masked DNA double-strand breaks, DNA–protein cross-links and a small proportion of DNA single-strand breaks in various animal cells as well as in human cell lines at a concentration of about 1 μmol/L. At lower concentrations, the DNA strand breakage caused by etoposide was enhanced by various metal ions, but this may occur through a mechanism involving free-radical formation rather than DNA topoisomerase II. Etoposide induced highly specific DNA double-strand cleavage in a range of human leukaemic cell lines. In particular, breaks were seen at *MLL* sites that have been associated with translocations in human leukaemia. Single-strand breaks typically occur rapidly during exposure to the drug, reaching a maximum by 15 min, whereas double-strand breaks accumulate more slowly, reaching a plateau between 1–2 h after the start of exposure (Long *et al.*, 1985). These DNA strand breaks are rapidly repaired when cells are placed in a drug-free medium, and 50% of the strand breaks are repaired within 60 min. All of the DNA strand breaks induced by etoposide were protein-associated (Kerrigan *et al.*, 1987), suggesting that its cytotoxicity is dependent on DNA topoisomerase II inhibition. As with the related drug teniposide, single-strand DNA breaks are more common at low concentrations of etoposide, the number of double-strand breaks increasing with increasing concentration (Long *et al.*, 1985). Cells from patients with ataxia telangiectasia show increased sensitivity to etoposide, accompanied by an increased frequency of chromosomal aberrations (Pandita & Hittelman, 1992). Treatment of human leukaemic T lymphoblasts with etoposide led to DNA breakage and also caused a nadir in cellular nucleotide pools 2–6 h after treatment. Etoposide has been suggested to inactivate DNA synthesis by inhibiting replicon cluster initiation (Suciu, 1990). In DNA from HeLa cells, DNA topoisomerase II enzymes were active in cleaving the telomere DNA repeat at a 5′-TTAGG*G3′ site, and this reaction was strongly stimulated by etoposide but not by other DNA topoisomerase II poisons (Yoon *et al.*, 1998).

Table 6. Genetic and related effects of etoposide

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Bacillus subtilis H17 and M45 *rec* strains, differential toxicity	+	NT	50 µg/disc	Nakanomyo *et al.* (1986)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	1000 µg/plate	Nakanomyo *et al.* (1986)
Salmonella typhimurium TA100, reverse mutation	–	–	500 µg/plate	Gupta *et al.* (1987)
Salmonella typhimurium TA102, reverse mutation	(+)	(+)	600 µg/plate	Gupta *et al.* (1987)
Salmonella typhimurium TA1537, TA1538, reverse mutation	–	–	1000 µg/plate	Ashby *et al.* (1994)
Salmonella typhimurium TA98, TA1537, TA1538, reverse mutation	(+)	(+)	1000 µg/plate	Nakanomyo *et al.* (1986)
Salmonella typhimurium TA98, TA1538, reverse mutation	–	NT	2000 µg/plate	Matney *et al.* (1985)
Salmonella typhimurium TA98, reverse mutation	–	–	800 µg/plate	Gupta *et al.* (1987)
Salmonella typhimurium TA98, reverse mutation	(+)	(+)	50 µg/plate	Ashby *et al.* (1994)
Salmonella typhimuriumi TA1978, reverse mutation	+	NT	200 µg/plate	Matney *et al.* (1985)
Escherichia coli 343/113, forward mutation	–	NT	500 µg/plate	Gupta *et al.* (1987)
Escherichia coli WP2 *uvrA*, reverse mutation	–	–	4000 µg/plate	Nakanomyo *et al.* (1986)
Escherichia coli WP2S, WP44SNF, reverse mutation	–	NT	400 µg/plate	Gupta *et al.* (1987)
Saccharomyces cerevisiae D5, mitochondrial 'petite' mutation	–	NT	3000	Ferguson & Turner (1988a)
Saccharomyces cerevisiae D5, mitotic recombination	–	NT	900	Ferguson & Turner (1988b)
Neurospora crassa, forward mutation	–	NT	100	Gupta (1990)
Neurospora crassa, reverse mutation	–	NT	470 µg/plate	Gupta (1990)
Drosophila melanogaster, genetic crossing-over or recombination (*white–ivory* assay)	+		25 in feed	Ferreiro *et al.* (1997)
Drosophila melanogaster, somatic mutation (and recombination)	+		300 in feed	Frei & Würgler (1996)
Drosophila melanogaster, somatic mutation (and recombination)	+		600 in feed	Torres *et al.* (1998)
DNA single- and double-strand breaks, mouse leukaemia L1210 cells *in vitro*	+	NT	1.8	Wozniak & Ross (1983)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA double-strand breaks, L1210 mouse leukaemia cells *in vitro*	+	NT	3	Ross *et al.* (1984)
DNA single-strand breaks (protein-associated) and DNA–protein cross-links, mouse leukaemia L1210 cells *in vitro*	+[c]	NT	9	Kerrigan *et al*. (1987)
DNA single- and double-strand breaks, mouse embryo fibroblast 3T3 cells *in vitro*	+	NT	12	Markovits *et al*. (1987)
DNA–protein cross-links, Chinese hamster ovary CHO-K1 cells and *xrs-1* cells *in vitro*	+	NT	3	Jeggo *et al*. (1989)
DNA–protein cross-links, MCF-7 cells *in vitro*	+	NT	12	Nutter *et al.* (1991)
DNA strand breaks (single-cell gel electrophoresis assay), Chinese hamster lung V79-171b cells *in vitro*	+	NT	2	Olive & Banáth (1993)
DNA double-strand breaks (contour clamped, homogeneous electric field assay), Chinese hamster ovary AA8 cells *in vitro*	+	NT	3	Sestili *et al.* (1995)
DNA strand breaks (single-cell gel electrophoresis assay), Chinese hamster CHO-K1 cells *in vitro*	+	NT	0.12	Vigreux *et al*. (1998)
Recombination, Chinese hamster lung V79-SP5 cells *in vitro*	–	NT	0.6	Zhang & Jenssen (1994)
Mutation, Chinese hamster ovary cells, *Hprt* locus *in vitro*	+	NT	0.8	Singh & Gupta (1983a)
Mutation, Chinese hamster ovary CHO W-14 cells, *Hprt* locus *in vitro*	+	NT	0.4	Singh & Gupta (1983b)
Mutation, mouse lymphoma L5178Y cells, *Tk* locus *in vitro*	+	NT	0.005	Ashby *et al*. (1994)
Mutation, mouse L cells, *Hprt* locus *in vitro*	+	NT	0.4	Gupta *et al*. (1987)
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	+	NT	0.1	Singh & Gupta (1983a)
Sister chromatid exchange**,** Chinese hamster lung DC3F cells *in vitro*	+[d]	NT	12	Pommier *et al*. (1988)
Micronucleus formation, spermatids of Sprague-Dawley rats *in vitro*	+	NT	0.3	Sjöblom *et al*. (1994)
Micronucleus formation, mouse splenocytes *in vitro*	+	NT	0.06	Record *et al*. (1995)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	1.2[e]	Johnston *et al*. (1997)
Chromosomal aberrations, Chinese hamster lung DC3F cells *in vitro*	+[d]	NT	12	Pommier *et al*. (1988)
Chromosomal aberrations, Chinese hamster lung CHL cells *in vitro*	+	NT	0.15	Suzuki & Nakane (1994)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	0.12	Vigreux *et al*. (1998)
Chromosomal aberrations, mouse lymphoma L5178Y cells *in vitro*	+	NT	0.02	Ashby *et al*. (1994)
Polyploidy, Chinese hamster ovary CHO cells *in vitro*	+	NT	24	Sumner (1995)
DNA single-strand breaks, human carcinoma A549 cell line *in vitro*	+	NT	0.12	Long *et al*. (1985)
DNA single- and double-strand breaks, SW1272 human lung carcinoma cells *in vitro*	+	NT	0.06	Long *et al.* (1986)
DNA single-strand breaks (protein-associated) and DNA–protein cross-links, human embryo fibroblast VA-13 and colon carcinoma HT-29 cells *in vitro*	+[c]	NT	15	Kerrigan *et al*. (1987)
DNA double-strand breaks, T-47D human breast cancer cells *in vitro*	+	NT	1.5	Epstein & Smith (1988)
DNA single-strand breaks, MCF-7 human breast cancer cells *in vitro*	+	NT	3	Sinha *et al.* (1988)
DNA strand breaks, human leukaemic T lymphoblasts *in vitro*	+	NT	12	Marks & Fox (1991)
DNA double-strand breaks (DNA unwinding assay), human promyelocytic leukaemia HL60 WT cells *in vitro*	+	NT	1.5	Sinha & Eliot (1991)
DNA single-strand breaks, primary acute myeloid leukaemia cells *in vitro*	+	NT	5	Chiron *et al*. (1992)
DNA strand breaks, Molt 4 human T lymphoblastoid cells and HL-60 promyelocytic leukaemia cells *in vitro*	+	NT	1	Shimizu *et al.* (1992)
DNA double-strand breaks, human ovarian A2780 cells *in vitro*	+[f]	NT	3	Noviello *et al*. (1994)
DNA double-strand breaks, human T lymphocytes *in vitro*	+	NT	0.6	Russo *et al*. (1994)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA double-strand breaks, human lung carcinoma A549 cells *in vitro*	+	NT	1.2	Long *et al*. (1985)
DNA double-strand breaks (*MLL BCR* site-specific), human leukaemia cell lines *in vitro*	+	NT	0.6	Aplan *et al*. (1996)
DNA strand breaks (single-cell electrophoresis assay), human lymphocytes *in vitro*	+	NT	12	Lebailly *et al*. (1997)
DNA double-strand breaks within the *AML-1* locus, various human leukaemia cell lines *in vitro*	+	NT	6	Stanulla *et al*. (1997b)
DNA double-strand breaks, human lung carcinoma A549 cell line *in vitro*	+	NT	60	Vock *et al*. (1998)
Mutation, human lymphoid CCRF-CEM, *HPRT* locus (deletion exons 2 and 3) *in vitro*	+	NT	0.15	Chen *et al*. (1996a)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.025	Tominaga *et al*. (1986)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	0.06	Ribas *et al*. (1996)
Sister chromatid exchange, human ovarian A2780 cells *in vitro*	+[f]	NT	12	Noviello *et al*. (1994)
Sister chromatid exchange, human lymphoblastoid cell lines derived from patients with ataxia telangiectasia, *in vitro*	+	NT	0.002	Fantini *et al*. (1998)
Micronucleus formation, neonatal human lymphocytes *in vitro*	+	NT	0.03	Slavotinek *et al*. (1993)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	0.025	Tominaga *et al*. (1986)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	50	Maraschin *et al*. (1990)
Chromosomal aberrations at the 1cen-1q12 region, human lymphocytes *in vitro*	+	NT	0.12	Rupa *et al*. (1997)
Chromosomal aberrations, human lymphoblastoid cell lines derived from patients with ataxia telangiectasia, *in vitro*	+	NT	0.18	Caporossi *et al*. (1993)
Chromosomal aberrations, human ovarian A2780 cells *in vitro*	+[f]	NT	12	Noviello *et al*. (1994)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, human lymphoblastoid cell lines derived from patients with ataxia telangiectasia, *in vitro*	+	NT	0.02	Fantini *et al.* (1998)
Chromosomal aberrations, TK6 and WI-L2-NS human B-lymphoblast cells *in vitro*	+	NT	1	Greenwood *et al.* (1998)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	30	Mosesso *et al.* (1998)
Fragmentation of centromeric DNA, spermatids of BALB/c mice *in vivo*	+		10 ip × 1	Kallio & Lähdetie (1996)
Mutation, primary spermatocytes in (101/R1 × C3H/R1)F_1 mice *in vivo*	+		75 ip × 1	Russell *et al.* (1998)
Sister chromatid exchange, bone-marrow cells of male Swiss mice *in vivo*	+		0.5 ip × 1	Agarwal *et al.* (1994)
Micronucleus formation, bone-marrow cells of CD-1 mice *in vivo*	+		0.75 ip × 1	Nakanomyo *et al.* (1986)
Micronucleus formation, spermatids of Han:NMRI mice *in vivo*	+		25 ip × 1	Kallio & Lähdetie (1993)
Micronucleus formation, bone-marrow cells of mice *in vivo*	+		0.1 po × 1	Ashby *et al.* (1994)
Micronucleus formation, bone-marrow cells of male CBA mice *in vivo*	+		1 po or ip × 1	Ashby & Tinwell (1995)
Micronucleus formation, spermatids of BALB/c mice *in vivo* (kinetochore-positive, indicative of aneuploidy)	+		20 ip × 1	Kallio & Lähdetie (1997)
Micronucleus formation, spermatids of Sprague-Dawley rats *in vivo*	+		5 ip × 1	Lähdetie *et al.* (1994)
Micronucleus formation, bone-marrow cells of male and female Fischer 344 rats *in vivo*	+		57 po × 14	Garriot *et al.* (1995)

Table 6 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, bone-marrow cells of pregnant Swiss mice and embryonic tissue cells *in vivo*	+		1.5 ip × 1, day 6 of gestation	Sieber *et al.* (1978)
Chromosomal aberrations, bone-marrow cells of male Swiss mice *in vivo*	+		5 ip × 1	Agarwal *et al.* (1994)
Chromosomal aberrations, metaphase II oocytes, ICR mice *in vivo*	+		40 ip × 1	Mailhes *et al.* (1994)
Aneuploidy, metaphase II oocytes, ICR mice *in vivo*	+		40 ip × 1	Mailhes *et al.* (1994)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitoneal; po, oral
[c] Protein-free single-strand breaks were not induced.
[d] Negative in topoisomerase II inhibitor-resistant DC3F/9-OHE cells
[e] CHO-K1 repair-deficient cells (*xrs* 5) were more sensitive (LED, 0.12 µg/mL) than CHO-K1 cells.
[f] Negative in multi-drug resistant, A2780-DX3 cells

Etoposide was highly effective in causing chromosomal aberrations in cultured Chinese hamster cells, in other rodent cell lines and in human peripheral blood lymphocytes in tissue culture. It also induced micronucleus formation in neonatal lymphocytes grown *in vitro* and in mouse splenocytes in culture. Various human lymphoblastoid cell lines derived from patients with ataxia telangiectasia were hyperensitive to the induction of chromosomal aberrations by etoposide. The Chinese hamster cell line *xrs-1* was also hypersensitive to etoposide, with an elevated frequency of micronucleus induction; however, the two ionizing radiation-sensitive cell lines, *irs1* and *irs3*, appeared to be similar to the parental V79-4 cell line in terms of micronucleus induction. Etoposide caused both clastogenic and aneuploidogenic effects in all these cell lines (Hermine *et al.*, 1997). Fluorescence in-situ hybridization techniques revealed that etoposide caused almost equal numbers of dicentric and stable translocations in human peripheral blood lymphocytes in culture (Mosesso *et al.*, 1998). It induced micronuclei and/or chromosomal aberrations in the bone marrow of mice and rats.

Etoposide induced sister chromatid exchange in Chinese hamster lung cells and in human lymphocytes and other human cell lines *in vitro*. Sister chromatid exchange induction has also been seen in mouse bone-marrow cells *in vivo*. Etoposide induced mutation and somatic recombination in *Drosophila melanogaster* in the wing spot test. It also induced a positive response in the *Drosophila white–ivory* assay, probably again through recombinogenic events.

Somatic intrachromosomal recombination can result in inversions and deletions in DNA. pKZ1 mice have an *E. coli lac*Z transgene that is expressed only after a DNA inversion involving the transgene; the *E. coli* β-galactosidase protein, which is encoded by the *lac*Z gene, can then be detected in frozen tissue sections with a chromogenic substrate. These mice can therefore be used to detect somatic intrachromosomal recombination inversion events *in vivo* in various tissues. When these mice were given a single intraperitoneal injection of etoposide and spleen cells were examined three days later, significant induction of inversion events was found by histochemical staining of tissue sections (Sykes *et al.*, 1999).

Etoposide induced mutations at the *HPRT* locus in human leukaemic CCRF-CEM cells and at the *Hprt* locus in Chinese hamster ovary and mouse L cells. It induced primarily small colony mutants at the *Tk* locus in mouse lymphoma L5178Y cells. Small colony mutants in L5178Y cells are usually caused by chromosomal mutations (DeMarini *et al.*, 1987). Di Leonardo *et al.* (1993) showed an etoposide-induced increase in resistance to *N*-phosphonoacetyl-L-aspartate, an event that has been shown to result from gene amplification.

Whether cellular damage results in mutation or apoptosis depends on a number of factors (Ferguson & Baguley, 1994). Etoposide-induced apoptosis has been demonstrated in cultured retinoblastoma Y79 cells (Lauricella *et al.*, 1998), in mouse fibroblasts (Mizumoto *et al.*, 1994), in human leukaemia HL-60 and K562 cells (Ritke *et al.*, 1994) and in neurons cultured from the fetal rat central nervous system (Nakajima *et al.*, 1994). It caused a concentration-dependent induction of apoptosis in immature thymocytes

from male Fischer 344 rats (Sun *et al.*, 1994a,b). Fritsche *et al.* (1993) showed that etoposide caused accumulation of p53 protein in a range of murine, simian and human cell lines. In mice, etoposide caused apoptosis through a *p53*-dependent pathway in immature thymocytes and also through a *p53*-independent pathway in a particular sub-population of these cells. The drug induced apoptosis at significantly lower levels and at later times in *p53* null as compared with *p53* wild-type mice (MacFarlane *et al.*, 1996).

Chen *et al.* (1996a) provided evidence that etoposide-induced deletions in the *HPRT* gene of human lymphoid CCRF-CEM cells occur through illegitimate V(D)J recombination. In human leukaemic CCRF-CEM cells, etoposide concentrations resulting in equal cytotoxicity (95%) after a 4-h exposure (2.5 μmol/L) and a 24-h exposure (0.5 μmol/L) caused significantly fewer recombinogenic events (as measured by VDJ recombinase-mediated deletions in exons 2 and 3 of the *HPRT* gene at day 6) with the more prolonged schedule (4.1×10^{-7} after 24 h versus 14.2×10^{-7} after 4 h) (Chen *et al.*, 1996b). These results indicate an improved therapeutic index with the prolonged schedule. Similar results were not seen in the myeloid cell lines KG-1A or K562, but Edwards *et al*. (1987) observed that CCRF-CEM cells are especially sensitive to etoposide, probably because of their high content of DNA topoisomerase II enzymes. Aratani *et al*. (1996) used gene transfer assays to study whether etoposide affects non-homologous (illegitimate) recombination and found that it stimulated integration of closed circular or linearized plasmids carrying the wild-type *Aprt* gene into *Aprt*-deficient Chinese hamster cells by non-homologous (illegitimate) recombination. It did not, however, significantly influence intrachromosomal recombination in SP5/V79 Chinese hamster cells (Zhang & Jenssen, 1994). Given their size, it is probable that etoposide-induced deletions and recombination events are mediated by a series of subunit exchanges between overlapping DNA topoisomerase II dimers at the bases of replicons or larger chromosomal structures such as replicon clusters or chromosome minibands.

A fluorescence in-situ hybridization procedure involving tandem DNA probes was used to show that etoposide caused hyperdiploidy of chromosome 1 and stimulated DNA breakage in the centromeric region of this chromosome. Polyploidy was also demonstrated by cytogenetic techniques in Chinese hamster ovary cells. Etoposide inhibited accurate chromosomal segregation in both HeLa and PtK2 cells (Downes *et al.*, 1991). It also retarded chromatid separation *in vitro* in a system derived from sperm nuclei in an extract of *Xenopus laevis* eggs. Etoposide induced differentiation of human HL-60 leukaemia cells (Gieseler *et al.*, 1993). It caused hypermethylation of DNA at CpG sites, resulting in altered patterns of distribution of 5-methylcytosine residues at these sites, thereby modifying gene expression (Nyce, 1989; Wachsman, 1997).

In general, events in mammalian cells *in vitro* occurred in the absence of exogenous metabolic activation. Nevertheless, etoposide is metabolized by human liver microsomes (Kawashiro *et al*., 1998). Various metabolic species have been identified, but their mutagenic properties have not been studied.

Etoposide mutates not only somatic cells but also germ cells. It readily induced micronuclei associated with aneuploidy in stage 1 spermatids. Sjöblom *et al*. (1994)

showed that the drug increased the frequency of meiotic micronuclei in cultured rat seminiferous tubules. Hakovirta *et al*. (1993) found that it affected stage-specific DNA synthesis during rat spermatogenesis, inhibiting specific stages of premitotic DNA synthesis more effectively than premeiotic DNA synthesis. Russell *et al*. (1998) commented that etoposide is almost unique in causing peak mutagenicity in primary spermatocytes of mice. These effects are manifest as recessive mutations at specific loci and dominants at other loci. Deletion mutations occurred commonly, and these authors suggested that they had a recombinational origin.

Treatment of germ cells of male BALB/c mice with etoposide led to fragmentation of centromeric DNA. Lähdetie *et al.* (1994) found that the sensitivity of rats to etoposide was greatest in diplotene-diakinesis of primary spermatocytes, reduced in late pachytene and low in preleptotene stages, a very different pattern from that induced by DNA alkylating chemicals. These authors suggested that etoposide caused a failure of resolution of recombined chromosome arms, probably associated with cell cycle arrest and triggering of the apoptotic pathway. Etoposide also induced aneuploidy, polyploidy and M-phase cycle arrest when introduced during the meiotic M phase. Kallio and Lähdetie (1996) reported etoposide-induced DNA breakage at both the centromeric regions and chromatid arms of dyads. Additionally, many cells were arrested at late anaphase I, and the frequency of second divisions with a diploid chromosome number was significantly elevated. These authors also noted some unique effects in etoposide-treated germ cells, including minute micronuclei most of which contained only centromeric DNA. Chromosomal aberrations and aneuploidy were induced in metaphase II oocytes of ICR mice treated *in vivo* with etoposide.

Cytogenetic changes were measured in pregnant mice given a single intraperitoneal injection of 1.5 mg/kg bw etoposide on day 6, 7 or 8 of gestation and killed 48 h later. Injection on day 7 increased the frequency of embryonic cells with structural aberrations, one-third of which were stable, consisting of chromosomes with metacentric or submetacentric markers. Injection on day 6 or 8 increased the percentage of embryonic cells with numerical aberrations, most of which were hypoploidy (monosomy) (Sieber *et al*., 1978).

4.5 Mechanistic considerations

Etoposide has two properties that are likely to lead to mutation.

1. It is an inhibitor of DNA topoisomerase II enzymes: Etoposide is a eukaryotic DNA topoisomerase II poison that has been shown to promote DNA cleavage, with a strong preference for C and to a lesser extent T at the –1 position (Capranico & Binashi, 1998). It does not inhibit bacterial topoisomerases and may not mutate bacterial cells by the same mechanism as mammalian cells. Unlike many other DNA topoisomerase II poisons, etoposide does not bind to DNA, either covalently or by intercalation. Instead, it appears to interact directly with the DNA topoisomerase II enzyme (Burden & Osheroff, 1998). Most of the mutational events found in mammalian cells, including

point mutations, chromosomal deletions and exchanges, as well as aneuploidy, can be explained by this activity.

2. *It possesses readily oxidizable functions*: Some of the etoposide-induced effects have been ascribed to the formation of free radicals by oxidation of its 4′-phenolic hydroxy group to a semiquinone free radical (Sakurai *et al*., 1991). The hydroxy radical •OH may be responsible for the metal- and photo-induced DNA breakage produced by this compound; however, none of the mutations seen with etoposide is of the type usually associated with oxygen radicals.

The role of etoposide in the translocations associated with leukaemia is unknown. Two possibilities are plausible. The first is that etoposide itself causes the translocations, perhaps through a cytotoxic action. The planar ring structures of epipodophyllotoxins confer an ability to form stable, stacked complexes with DNA and DNA topoisomerase II. DNA topoisomerase II changes the topology of DNA by transiently cleaving and re-ligating both strands of the double helix (Ross *et al*., 1988; Liu & Wang, 1991; Pommier *et al*., 1991; Pommier, 1993). In the presence of epipodophyllotoxin, the rate of re-ligation is decreased, causing double-stranded breaks in DNA that are ultimately cytotoxic (Chen *et al*., 1984; Long *et al*., 1985; Epstein, 1988; Osheroff, 1989; Wang *et al*., 1990; Osheroff *et al*., 1991; Chen & Liu, 1994). In one model in which the drugs cause the translocations, the process involves drug-induced DNA topoisomerase II-mediated chromosomal breakage and formation of the translocations by further processing and resolution of the breakage through cellular mechanisms of DNA repair (Felix, 1998).

The second possibility for the role of etoposide in causing translocations is that it selects for cells that already have translocations. Indeed, *MLL* tandem duplications, a form of translocation, have been identified in peripheral blood and bone marrow of healthy adults (Schnittger *et al*., 1998). Chemotherapy has profound effects on the kinetics of the marrow: it causes cell death, forcing many marrow stem cells to divide, which might select for the rare stem cells with a translocation (Knudson, 1992).

In favour of the first possibility is the specificity of the association between DNA topoisomerase II inhibitors, but not other forms of chemotherapy that cause cell death in the bone marrow, and leukaemias characterized by translocations.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Etoposide is a semi-synthetic podophyllotoxin derivative that has been used in cancer treatment since the early 1970s. This DNA topoisomerase II inhibitor is one of the most widely used and effective cytotoxic drugs in combination therapy, particularly

in the treatment of lymphoma, small-cell lung cancer, testicular cancer, childhood malignancies and, to a lesser extent, a number of other cancers.

5.2 Human carcinogenicity data

One cohort study of patients with Langerhans cell histiocytosis and several cohort studies of patients with germ-cell tumours or lung cancer treated with etoposide-containing chemotherapy showed increased risks for acute myeloid leukaemia.

In the patients with Langerhans cell histiocytosis, a strongly increased risk for acute myeloid leukaemia of the promyelocytic type was found after treatment with etoposide alone; however, the possibility could not be ruled out that such patients have an inherently increased risk for acute promyelocytic leukaemia.

In several cohort studies of germ-cell tumours in men, treatment with etoposide, cisplatin and bleomycin was associated with an increased risk for acute myeloid leukaemia. On the basis of the combined data from six studies, the relative risk for acute myeloid leukaemia was 40 times greater than that of the general population; substantially higher relative risks have been found with high cumulative doses of etoposide. Although the other two agents (cisplatin and bleomycin) in etoposide-containing chemotherapy regimens for germ-cell tumours may have contributed to the positive association seen in the cohort studies, use of these agents in a similar regimen without etoposide has not been associated with acute myeloid leukaemia. As the background risk for acute myeloid leukaemia is low, the absolute risk for this disease in men treated for germ-cell tumours with etoposide-containing regimens is low. A strongly increased risk for acute myeloid leukaemia was also found in one cohort study of lung cancer patients treated with etoposide, cisplatin and vindesine. The possibility cannot be excluded that etoposide exerts its effects only in the presence of other cytotoxic agents.

Several other cohort studies reported strongly increased risks for acute myeloid leukaemia following treatment of various primary malignancies with etoposide-containing regimens that also included alkylating agents, or etoposide-containing regimens in combination with teniposide. In these studies, the possibility cannot be excluded that the excess leukaemia risk was partly or wholly due to the other agents.

5.3 Animal carcinogenicity data

Etoposide was tested in one experiment in wild-type and heterozygous neurofibromatosis type 1 gene (*Nf1*) knock-out mice. No increase in the incidence of leukaemia was observed.

5.4 Other relevant data

In humans, etoposide is eliminated biphasically, with an elimination half-time of 3–9 h. The pharmacokinetics of this compound is linear up to 3.5 mg/m^2 (typical single

dose, 100 mg/m^2). Its bioavailability is around 50%, but this decreases with oral doses of > 200 mg. Etoposide is about 95% protein-bound in plasma. About 50% of an intravenous dose of etoposide is recovered in urine; up to 17% is excreted as a glucuronide metabolite and less than 2% as a catechol metabolite. Preliminary studies suggest that the remainder of the dose is excreted in the faeces. The catechol metabolite has also been detected in plasma at concentrations around 2.5% that of etoposide.

Biphasic elimination is seen in a number of animal species. In rhesus monkeys, 60% of a radiolabelled dose of etoposide was excreted in urine and 30% in faeces. Glucuronide metabolites have been reported in the urine of rabbits and rats. Oxidation of etoposide to quinone species and a catechol metabolite have been reported in cell systems, occurring either by peroxidase oxidation or cytochrome P450-mediated demethylation involving CYP3A4. These oxidation products have cytotoxic activity, but it is unclear how much they contribute to the activity of etoposide.

The major dose-limiting toxic effect of etoposide in humans is myelosuppression, manifest principally as leukopenia. Other toxic effects include nausea and vomiting, mucositis and alopecia. Cases of hypotension were reported in early trials in which short infusions were given, but this effect is rarely seen with infusions of longer than 30 min. Hypersensitivity reactions have been reported but are seen much less frequently than with teniposide. Cardiotoxicity and cutaneous toxicity have been reported but are rare.

Myelosuppression was the main toxic effect of intravenously administered etoposide in a number of the animal species studied. Other effects included changes in the lung in rats and renal and hepatic toxicity, electrocardiographic changes, decreased testis weight and disorders of spermatogenesis in rats and dogs. After intrapleural and intraperitoneal administration to mice and rats, delayed chronic pleuritis and peritonitis, with liver and spleen inflammation, were reported. Teratogenic effects especially on the central nervous system have been observed.

Etoposide does not bind to DNA by forming covalent bonds or through intercalation. The drug is orders of magnitude more toxic in mammalian than in microbial cells. The effects in mammals arise primarily because etoposide is a poison of DNA topoisomerase II enzymes. Etoposide also induces both aneuploidy and polyploidy. It enhances gene amplification and affects gene expression through hypermethylation of DNA. Treatment of cells with etoposide leads to an accumulation of protein-masked double-stranded DNA breaks and, with time, a variety of chromosomal aberrations. The predominant mutagenic effects detected involve the deletion and/or interchange of large DNA segments, especially balanced translocations. *In vitro*, etoposide and its catechol and quinone metabolites enhanced DNA topoisomerase II-mediated DNA strand breaks within the *MLL* gene which is implicated in leukaemia.

Etoposide-containing regimens have been associated with the development, after a short latency, of leukaemia which is characterized by chromosomal translocations. The translocations that are observed are the same as those found in de-novo cases of acute leukaemia; however, while translocations of the *MLL* gene at chromosome band 11q23 occur in only about 5% of cases of leukaemia in adults and are seen primarily in de-novo

leukaemia in infants and young children, translocations of chromosome band 11q23 comprise the majority of the aberrations that follow leukaemias associated with administration of DNA topoisomerase II inhibitors. The translocations are considered to be primary events in leukaemogenesis. Etoposide is often used in combination chemotherapy with alkylating agents, which are themselves associated with leukaemia with specific chromosomal aberrations after a longer latency. These chromosomal aberrations are unbalanced chromosomal losses and deletions, especially monosomy 7, 7q and 5q deletions. Since the primary aberrations associated with alkylating agents are distinct from the balanced translocations with which DNA topoisomerase II inhibitors are associated, balanced translocations are specific events of epipodophyllotoxins that can be distinguished even when DNA topoisomerase II inhibitors are used in combination chemotherapy.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of etoposide.

There is *sufficient evidence* in humans for the carcinogenicity of etoposide given in combination with cisplatin and bleomycin.

There is *inadequate evidence* in experimental animals for the carcinogenicity of etoposide.

Overall evaluation

Etoposide is *probably carcinogenic to humans (Group 2A)*.

In reaching this conclusion, the Working Group noted that etoposide causes distinctive cytogenetic lesions in leukaemic cells that can be readily distinguished from those induced by alkylating agents. The short latency of these leukaemias contrasts with that of leukaemia induced by alkylating agents. Potent protein-masked DNA breakage and clastogenic effects occur in human cells *in vitro* and in animal cells *in vivo*.

Etoposide in combination with cisplatin and bleomycin is *carcinogenic to humans (Group 1)*.

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