MELPHALAN

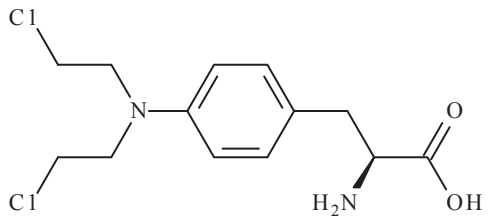
Melphalan was considered by previous IARC Working Groups in 1974 and 1987 (IARC, 1975, 1987a). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 148-82-3; 3223-07-2 [hydrochloride]
Chem. Abstr. Name: L-Phenylalanine, 4-[bis(2-chloroethyl)amino]-
IUPAC Systematic Name: (2*S*)-2-Amino-3-[4-[bis(2-chloroethyl)amino]phenyl] propanoic acid
Synonyms: Alanine nitrogen mustard; Alkeran; melphalan; L-PAM; L-phenylalanine mustard; L-phenylalanine mustard hydrochloride; phenylalanine mustard; L-sarcolysine
Description: Off-white to buff powder with a faint odour (Sweetman, 2008)

1.1.1 Structural and molecular formulae, and relative molecular mass



$C_{13}H_{18}Cl_2N_2O_2$
Relative molecular mass: 305.2

1.2 Use of the agent

Information for Section 1.2 is taken from McEvoy (2007), Royal Pharmaceutical Society of Great Britain (2007), Thomson Healthcare (2007), and Sweetman (2008).

1.2.1 Indications

Melphalan is an antineoplastic agent that acts as a bifunctional alkylating agent. It is used in the treatment of multiple myeloma, advanced ovarian adenocarcinoma, early and advanced breast cancer, childhood neuroblastoma, and *polycythaemia vera*. Melphalan is also used for regional arterial perfusion in localized malignant melanoma, and localized soft-tissue sarcoma of the extremities.

1.2.2 Dosage

Melphalan is usually given orally as a single daily dose or in divided doses; it is also given intravenously as the hydrochloride.

The oral dose administered to treat multiple myeloma may vary according to the regimen. Typical dose regimens include 150 µg/kg daily for 4–7 days; 250 µg/kg daily for 4 days; or 6 mg daily for 2–3 weeks. Melphalan is usually combined with corticosteroids. Courses are followed by a rest period of up to 6 weeks to

allow recovery of haematological function, and are then repeated, or maintenance therapy may be instituted, usually with a daily dose of 1–3 mg, or up to 50 µg/kg.

Similar oral dose regimens (150–200 µg/kg daily for 5 days, repeated every 4–8 weeks) have been used to treat ovarian adenocarcinoma and advanced breast cancer, although with the development of newer agents, melphalan is now used infrequently for the treatment of these tumours. The dose administered by mouth to treat *polycythaemia vera* is initially 6–10 mg daily, reduced after 5–7 days to 2–4 mg daily until satisfactory response, then further reduced to 2–6 mg per week for maintenance.

Melphalan has also been given intravenously; a single dose of 1 mg/kg, repeated in 4 weeks if the platelet and neutrophil counts permit, has been used to treat ovarian adenocarcinoma. In multiple myeloma, melphalan has been administered as a single agent intravenously at a dose of 400 µg/kg or 16 mg/m^2, infused over 15–20 minutes; the first four doses are given at 2-week intervals, and further doses are given at 4-week intervals depending on toxicity. High-dose melphalan has been given intravenously in some malignancies: doses of 100–240 mg/m^2 have been used for neuroblastoma, and 100–200 mg/m^2 in multiple myeloma, generally followed by autologous stem-cell transplantation. Melphalan may be given by local arterial perfusion in the management of melanoma and soft-tissue sarcoma. A typical dose range for upper extremity perfusions is 0.6–1 mg/kg, whereas for lower extremity perfusions, dose ranges of 0.8–1.5 mg/kg (in melanoma) or 1–1.4 mg/kg (in sarcoma) are typically used.

Melphalan is available as 2 mg tablets for oral administration, and as a 50 mg (melphalan hydrochloride) solution for injection for parenteral administration.

Melphalan is also used intravenously as part of isolated hyperthermic limb perfusions for patients with malignant melanoma in transit and limb sarcomas.

1.2.3 Trends in use

The availability of several newer chemotherapeutic and biological therapies has substantially reduced the use of melphalan in patients with solid tumours, whereas the use of high-dose intravenous melphalan followed by autologous stem-cell infusion has increased following randomized studies that demonstrated a disease-free, and in some studies, an overall survival advantage in patients with multiple myeloma.

2. Cancer in Humans

Epidemiological studies of patients with ovarian carcinoma (Reimer *et al.*, 1977; Einhorn *et al.*, 1982; Greene *et al.*, 1982, 1986; Kaldor *et al.*, 1990), multiple myeloma (Gonzalez *et al.*, 1977; Law & Blom, 1977; Bergsagel *et al.*, 1979; Dent *et al.*, 2000) or breast cancer (Fisher *et al.*, 1985; Curtis *et al.*, 1992) have consistently shown very large excesses of acute myeloid leukaemia in the decade following therapy with melphalan.

Since then, a large number of epidemiological studies have contributed to the weight of evidence for the carcinogenicity of melphalan, in particular, two large case–control studies detailed below.

A case–control study conducted in a cohort of 82700 women who received adjuvant chemotherapy for breast cancer, and who were followed up for at least 18 months after completion of treatment, identified 90 cases of leukaemia, of which 80 were acute myeloid leukaemia/myelodysplatic syndromes. The relative risk (RR) of developing acute myeloid leukaemia was highest in patients who received alkylating agents with radiation treatment (RR, 17.4) compared to those who were treated with alkylating agents (RR, 10) or radiation treatment alone (RR, 2.4). The relative risk was 10-fold greater for patients who received

melphalan (RR, 31.4) compared to those who received cyclophosphamide (Curtis *et al.*, 1992).

A case–control study compared the relative risk of leukaemia in patients treated with chemotherapy or radiation to patients who only underwent surgery (Kaldor *et al.*, 1990). Approximately 90 cases [The Working Group noted that older nomenclature was used, and it was difficult to precisely assess the diagnosis in some patients] of acute myeloid leukaemia or myelodysplatic syndromes were identified among ~99000 patients with ovarian cancer. All of the alkylating agents (including chlorambucil, cyclophosphamide, thiotepa, treosulfan, and melphalan) increased the risk of developing leukaemia in a dose-dependent manner. Relative risks for melphalan ranged from 12–23 depending on the total dose used.

3. Cancer in Experimental Animals

Melphalan has been tested in mice by oral, intraperitoneal, and dermal application; in rats by intraperitoneal injection, and in monkeys by oral administration (Table 3.1).

In mice, the administration of melphalan produced forestomach papillomas, lymphosarcomas, and skin and lung tumours (IARC, 1975, 1987a; Satoh *et al.*, 1993; Eastin *et al.*, 2001). In rats, melphalan caused mammary gland tumours, and peritoneal sarcomas (IARC, 1975, 1987a). Results in monkeys were inconclusive (Thorgeirsson *et al.*, 1994; Schoeffner & Thorgeirsson, 2000).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans, following oral administration, melphalan is absorbed from the gastrointestinal tract with a wide range in bioavailability (range 25–89%, mean 56%) (Sweetman, 2008). It also exhibits considerable variability with respect to the time of its appearance in the plasma (range ~0–6 hours), and in the peak plasma concentration achieved (range 70–4000 ng/mL, depending upon the dose) (GlaxoSmithKline, 2007). This variability may be due to incomplete intestinal absorption, variable first-pass metabolism, and/or rapid hydrolysis. Upon absorption, approximately 60–90% of plasma melphalan is bound to plasma proteins such as albumin, and to a lesser degree, α_1-acid glycoprotein, with approximately 30% being bound irreversibly (GlaxoSmithKline, 2007). Melphalan does not undergo metabolic activation and is inactivated in the plasma, primarily by non-enzymatic hydrolysis to monohydroxymelphalan and dihydroxymelphalan. Apart from these hydrolysis products, no other melphalan metabolites have been detected in humans (GlaxoSmithKline, 2007). Melphalan enters cells through active transport, mostly by the high-affinity L-amino acid transport system, which carries glutamine and leucine (Nieto & Vaughan, 2004).

As a consequence of its inconsistent absorption, melphalan can also exhibit considerable variability in its elimination. For example, when given intravenously, melphalan exhibited a fairly consistent half-life (14–40 minutes in a study of ten patients given a 20 mg/m^2 dose) but was absorbed variably, and found to have a half-life of 36–552 minutes in a study of 13 patients administered a 0.6 mg/m^2 oral dose (Hall & Tilby, 1992). About 10% of the drug is excreted unchanged in the urine within 24 hours, and about 30% of administered melphalan (including metabolites) is excreted in the urine within 9 days of oral administration (McEvoy, 2007; Sweetman, 2008). Approximately 20–50% of the dose is eliminated via faeces (McEvoy, 2007).

Table 3.1 Studies of cancer in experimental animals exposed to melphalan

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, S (NR) 22 wk Roe & Salaman (1956)	Skin 0, 1.44, 3 mg total dose administered by skin painting once weekly for 10 wk 60, 25, 25	Skin (papillomas): 5/53, 2/19, 4/22 Lung (adenomas): 10/17 (1.7/mouse), 13/18 (3.6/mouse), 11/13 (5.1/mouse)	[$P < 0.05$] for 3 mg	Croton oil used as promoter (experimental Weeks 5–22)
Mouse, A/J (M, F) 39 wk Shimkin et al. (1966)	i.p. 0, 0.27, 1.07, 4.27, 17.1 mg/kg bw total dose 3 times/wk for 4 wk 1400, 60, 60, 60, 60	Lung: 307/777, 23/58, 37/56, 43/56, 40/41 Tumours/mouse : 0.5, 0.6, 1.0, 2.1, 4.0	[$P < 0.0001$] for 1.07, 4.27 and 17.1 mg/kg bw	
Mouse, Swiss (M, F) 18 mo Weisburger et al. (1975)	i.p. 0.75, 1.5 mg/kg bw 3 times/wk for 6 mo 25 (M) + 25 (F)	Lung: M–11/44 F–10/23 Lymphosarcomas: M–13/44	 $P = 0.012$ $P = 0.001$ $P < 0.001$	Dosage groups were combined for each sex; 254 mice served as untreated controls
Mouse, Tg.AC (M, F) 27 wk Eastin et al. (2001)	Oral gavage 0, 0.25, 1.0 & 4.0 mg/kg bw/wk for 26 wk 15/sex/group	Forestomach (squamous cell papillomas): M–8/14, 14/15, 15/15, 14/15; F–12/15, 14/14, 13/14, 13/14	[$P \leq 0.03$] for 0.25, 1.0, 4.0 mg/kg bw doses in male mice	
Mouse, Tg.AC (M, F) 27 wk Eastin et al. (2001)	Topical application (in methanol, 3.3 mL/kg bw), 0, 0.25, 1.0, 4.0 mg/kg bw/wk for 26 wk 15/sex/group	Skin tumours (in area of the vulva): F–1/15, 3/15, 7/15, 12/15	[$P \leq 0.02$] for 1.0 & 4.0 mg/kg bw in female mice	
Mouse, A/J (F) 24 wk Satoh et al. (1993)	i.p. 0, 4.0 µmol/kg bw/d, 3 d/wk 12, 13	Lung (adenomas): 4/12 & 13/13 (incidence) Tumours per mouse: 0.42 ± 0.67, 12.15 ± 1.68	[$P = 0.0005$] (incidence) [$P < 0.001$] for multiplicity	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, (F) 17 mo Presnov & Iushkov (1964)	i.p 0, 10 mg/kg bw, single application 40, 60	Mammary gland (fibroadenomas): 0/30, 9/33		A mixture of melphalan and medpalan (D isomer of melphalan) was used
Rat, Sprague-Dawley CD (M, F) 18 mo Weisburger et al. (1975)	i.p. 0.9, 1.8 mg/kg bw 3 times/wk for 6 mo 25/sex	Peritoneum (sarcomas): M–11/20 F–10/23	 $P < 0.001$ $P < 0.001$	Dosage groups were combined for each sex; 360 rats served as untreated controls
Monkey, (NR) 16 yr Thorgeirsson et al. (1994), Schoeffner & Thorgeirsson (2000)	Nasogastric tube (in DMSO, volume NR) 0.1 mg/kg bw/5 d/wk	Malignant tumours: 5/12		Incidence in control animals could not be determined

bw, body weight; d, day or days; DMSO, dimethylsulfoxide; F, female; i.p., intraperitoneal; M, male; mo, month or months; NR, not reported; w, week or weeks; yr, year or years

4.2 Mechanisms of carcinogenesis

4.2.1 Induction of DNA damage

Melphalan is a direct-acting, bifunctional alkylating agent that binds to cellular macromolecules (Osborne *et al.*, 1995). As a phenylalanine derivative of nitrogen mustard, it is capable of producing a variety of DNA adducts including mono-adducts at the N^7 of guanine and the N^3 of adenine as well as interstrand cross-links, and pre-mutagenic lesions that are believed to play a critical role in its toxic and carcinogenic effects (Povirk & Shuker, 1994; Lawley & Phillips, 1996; GlaxoSmithKline, 2007).

Melphalan has been shown to bind to DNA, RNA, and protein in cells *in vitro* (Tilby *et al.*, 1987, 1995; Povirk & Shuker, 1994; Osborne *et al.*, 1995), and DNA-binding has been seen in rats treated *in vivo* (Van den Driessche *et al.*, 2004a, b). The formation of DNA cross-links *in vitro* and *in vivo* has also been reported based on the measurement of specific adducts (Osborne & Lawley, 1993) or by changes in DNA migration in DNA strand breakage or electrophoretic assays (Ringborg *et al.*, 1990; Popp *et al.*, 1992; Souliotis *et al.*, 2003, 2006; Cordelli *et al.*, 2004; Dimopoulos *et al.*, 2005).

4.2.2 Mutational consequences of DNA damage

Melphalan has been tested for genotoxicity in an assortment of short-term assays, both *in vitro* and *in vivo*. Increased frequencies of dominant lethal mutations, chromosomal aberrations, micronuclei, and DNA strand breaks have been observed in several studies following treatment of rodents with melphalan (IARC, 1987b; Shelby *et al.*, 1989; Generoso *et al.*, 1995; Morita *et al.*, 1997; Tsuda *et al.*, 2000; Sgura *et al.*, 2005, 2008; Ranaldi *et al.*, 2007). In the mouse-specific locus mutation and heritable translocation tests, increases in mutations were seen in both spermatagonial as well as postspermatogonial germ cells (Russell *et al.*, 1992a, b; Generoso *et al.*, 1995; Witt & Bishop, 1996). The observed mutations originated primarily from large deletions in the postspermatogonial cells whereas other types of mutagenic mechanisms predominated in the spermatogonial cells (Witt & Bishop, 1996).

Melphalan also induced chromosomal aberrations, sister chromatid exchange, micronuclei, mutations at the *HPRT* gene, and DNA damage in human cells *in vitro* (IARC, 1987b; Mamuris *et al.*, 1989a, b; Sanderson *et al.*, 1991; Routledge *et al.*, 1992; Sorsa *et al.*, 1992; Vock *et al.*, 1999; Efthimiou *et al.*, 2007; Escobar *et al.*, 2007). It also induced transformation of C3H 10T1/2 and other cells (IARC, 1987b; Miller *et al.*, 1994; Kowalski *et al.*, 2001). In cultured rodent cells, it induced chromosomal aberrations, sister chromatid exchange, gene mutations, and DNA damage (IARC, 1987b; Austin *et al.*, 1992; Preuss *et al.*, 1996; Allan *et al.*, 1998). In addition, it induced aneuploidy and sex-linked recessive lethal mutations in *Drosophila*, and mutation in bacteria (IARC, 1987b).

Increased frequencies of chromosomal aberrations and sister chromatid exchange occurring in the peripheral blood lymphocytes have been reported in multiple studies of patients treated therapeutically with melphalan (IARC, 1987b; Raposa & Várkonyi, 1987; Mamuris *et al.*, 1989b, 1990; Popp *et al.*, 1992; Amiel *et al.*, 2004). In addition, DNA-binding (DNA mono-adducts and interstrand cross-links) to *TP53* and *N-RAS*, two important cancer-related genes, has been seen in the peripheral blood cells of patients administered melphalan (Souliotis *et al.*, 2003, 2006; Dimopoulos *et al.*, 2005, 2007). Decreased migration of DNA in strand breakage assays indicative of DNA cross-link formation has been observed in melphalan-treated cancer patients (Popp *et al.*, 1992; Spanswick *et al.*, 2002). Haematotoxicity and immunosuppression have also been reported in patients treated with this anticancer agent (Goldfrank *et al.*, 2002; GlaxoSmithKline, 2007).

Acute myeloid leukaemia that develops in patients previously treated with alkylating agents such as melphalan frequently exhibits distinctive characteristics that allow it to be distinguished from acute myeloid leukaemia induced by other agents (such as topoisomerase II inhibitors) or acute myeloid leukaemia that occurs spontaneously (Rödjer *et al.*, 1990; Pedersen-Bjergaard & Rowley, 1994; Jaffe et al, 2001; Pedersen-Bjergaard *et al.*, 2006). One of the hallmarks of leukaemias induced by alkylating agents is that they frequently exhibit a clonal loss of either chromosome 5 or 7 (–5, –7) or a loss of part of the long arm of one of these chromosomes (5q–, 7q–). For example, a deletion within the long arm of chromosome 5 involving the bands q23 to q32 is often seen (Jaffe *et al.*, 2001). Leukaemias developed in patients treated with melphalan (often in combination with other agents) frequently exhibit these clonal chromosomal changes (Rödjer *et al.*, 1990).

In addition, mutations in *TP53* are frequently seen in leukaemias with the –5/5q– karyotype, and mutations involving the *AML1* gene as well as mutations in *TP53* and *RAS* are often seen in a subset of leukaemias that exhibit the –7/7q– karyotype (Christiansen *et al.*, 2001, 2005; Pedersen-Bjergaard *et al.*, 2006). These treatment-related acute myeloid leukaemias also frequently exhibit increased methylation of the *p15* promoter (Pedersen-Bjergaard *et al.*, 2006). While there is some evidence that melphalan may directly induce damage targeting chromosomes 5 or 7 (Mamuris *et al.*, 1989a, b, 1990; Amiel *et al.*, 2004), this drug has also been reported to induce nonspecific chromosomal alterations in a variety of experimental models, and in the lymphocytes of treated patients (described above). The detection of elevated levels of chromosomal aberrations in the peripheral blood lymphocytes of melphalan-treated patients is of particular note, as multiple prospective studies have now shown that individuals with increased levels of chromosomal aberrations in these cells are at increased risk of developing cancer later in life (Hagmar *et al.*, 1998, 2004; Liou *et al.*, 1999; Smerhovsky *et al.*, 2001).

4.3 Synthesis

Melphalan is a direct-acting alkylating agent that is carcinogenic via a genotoxic mechanism.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of melphalan. Melphalan causes acute myeloid leukaemia.

There is *sufficient evidence* in experimental animals for the carcinogenicity of melphalan.

Melphalan is *carcinogenic to humans (Group 1).*

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