

# PHARMACEUTICALS

VOLUME 100 A  
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

LYON, FRANCE - 2012

IARC MONOGRAPHS  
ON THE EVALUATION  
OF CARCINOGENIC RISKS  
TO HUMANS

# CHLORAMBUCIL

Chlorambucil was considered by previous IARC Working Groups in 1980 and 1987 ([IARC, 1981a, 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.:* 305-03-3

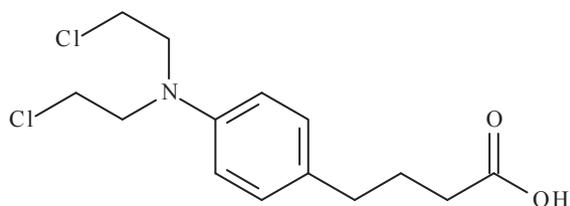
*Chem. Abstr. Name:* Benzenebutanoic acid, 4-[bis(2-chloroethyl)amino]-

*IUPAC Systematic Name:* 4-[4-[Bis(2-chloroethyl)amino]phenyl]butanoic acid

*Synonyms:* 4-[Bis(2-chloroethyl)amino]benzenebutanoic acid; 4-[*p*-[bis(2-chloroethyl)amino]phenyl]butyric acid;  $\gamma$ -[*p*-bis(2-chloroethyl)aminophenyl]butyric acid; chloraminophene;  $\gamma$ -[*p*-di(2-chloroethyl)-aminophenyl]butyric acid; Leukeran

*Description:* Flattened needles ([O'Neil, 2006](#)); white crystalline powder ([European Pharmacopoeia, 1997](#))

#### 1.1.1 Structural and molecular formulae, and relative molecular mass



$C_{14}H_{19}Cl_2NO_2$

Relative molecular mass: 304.2

### 1.2 Use of the agent

Chlorambucil is an antineoplastic agent derived from chlormethine, and has a similar mode of action. It acts on lymphocytes and to a lesser extent on neutrophils and platelets. Chlorambucil is most effective in those conditions associated with the proliferation of white blood cells, especially lymphocytes. Although formerly widely used in the management of *polycythaemia vera*, it has largely been superseded. 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

Chlorambucil is used in the treatment of chronic lymphocytic leukaemia, Waldenström macroglobulinaemia, indolent non-Hodgkin lymphoma, and in combination with other drugs in patients with Hodgkin lymphoma. Chlorambucil was previously indicated for *polycythaemia vera*.

#### 1.2.2 Dosage

When used as a single-agent antineoplastic drug for the treatment of chronic lymphocytic leukaemia, Waldenström macroglobulinaemia,

and lymphomas, chlorambucil is given orally at initial doses of 100–200 µg/kg body weight daily (usually 4–10 mg once daily), for 3–8 weeks until leukopenia occurs, then reduced to 2–8 mg daily. Lower doses may be given as part of a combination regimen. Alternatively, higher doses of chlorambucil may be given intermittently. For example, in chronic lymphocytic leukaemia, it may be given in an initial single dose of 0.6–1.0 mg/kg body weight, increased by 0.1–0.2 mg/kg body weight at 4-week intervals until control of lymphocytosis and adenopathy is achieved or toxicity occurs. When maximal response is achieved, treatment with chlorambucil is generally stopped rather than continued at lower dose for maintenance therapy. Depending on the magnitude and duration of response, chlorambucil may be restarted when progressive disease is apparent.

Chlorambucil is available as 2 mg tablets.

### 1.2.3 Trends in use

Chlorambucil is still commonly used as the initial treatment of chronic lymphocytic leukaemia, particularly in older patients. The use of chlorambucil in patients with rheumatoid arthritis has decreased substantially in favour of other immunosuppressive treatments.

## 2. Cancer in Humans

Many case reports and a few earlier small epidemiological studies of malignancy after therapy with chlorambucil have been described among patients treated for breast cancer, juvenile arthritis, glomerulonephritis, and ovarian cancer (IARC, 1981a, 1987a; Greene *et al.*, 1982; Patapanian *et al.*, 1988; Jones *et al.*, 1996; Asten *et al.*, 1999). [The Working Group noted that though in each study an excess of subsequent malignancy, especially acute myeloid leukaemia is suggested, these reports are difficult to interpret because the cases are few or because they also received radiation or other putative carcinogens.]

More recent studies are presented below.

### 2.1 Cancers following treatment for various diseases

In a randomized therapy trial of 431 patients with *polycythaemia vera* (Berk *et al.*, 1981), a significant increase in the incidence of acute myeloid leukaemia occurred in patients treated with chlorambucil when compared to phlebotomy or radiotherapy. The excess of acute myeloid leukaemia incidence declined after the first decade after treatment (Najean *et al.*, 1994).

A case–control study compared the relative risk of leukaemia in patients treated with chemotherapy or radiation with patients who only underwent surgery (Kaldor *et al.*, 1990). Approximately 114 cases of leukaemia were identified among 99113 patients with ovarian cancer. All of the alkylating agents assessed, including chlorambucil, cyclophosphamide, thiotepa, treosulfan and melphalan, increased the risk of developing leukaemia. The relative risks attributed to chlorambucil monotherapy were 14 and 23 in the lower and higher dose groups, respectively.

A retrospective analysis compared patients with advanced rheumatoid arthritis treated for a median time of 2 years with either chlorambucil ( $n = 39$ ) or the antimetabolite 6-mercaptopurine ( $n = 30$ ) (Patapanian *et al.*, 1988). An increase in the number of skin cancers was observed in the chlorambucil recipients compared to the 6-mercaptopurine recipients that included melanoma and squamous cell carcinoma (8 versus 1, respectively), as well as an increase in acute myeloid leukaemia/myelodysplastic syndromes (AML/MDS) (3 versus 0, respectively).

Some reports have suggested an increased rate of AML/MDS and other cancers in patients with Hodgkin lymphoma treated with variants of the MOPP regimen that include chlorambucil (chlorambucil, vincristine, procarbazine, prednisone [ChlVPP]) (Selby *et al.*, 1990; Swerdlow *et al.*, 1992). However, it is difficult to determine the unique contribution of chlorambucil compared to the effects of the other drugs and the

radiation therapy, which many of these patients also received.

## 2.2 Cancers following treatment for chronic lymphocytic leukaemia

### 2.2.1 AML/MDS

The French Cooperative Group on Chronic Lymphocytic Leukaemia conducted two large successive trials that randomized 1535 patients with early-stage chronic lymphocytic leukaemia to observation until disease progression or initial treatment with chlorambucil ([Dighiero et al., 1998](#)). Four cases of AML/MDS were reported in the treatment group, and two in the observation group. No information was provided about the nature of treatment these two patients might have received before the onset of acute myeloid leukaemia (no relative risk available). Another retrospective analysis included 389 patients, approximately half of whom were observed, and the others treated with prolonged courses of chlorambucil including maintenance treatment. Four cases of AML/MDS were noted, all in the chlorambucil-treated patients ([Callea et al., 2006](#)). [The Working Group noted that all of these patients had also received fludarabine in combination with cyclophosphamide for progressive disease as well.]

In another study assessing initial treatment with chlorambucil compared with fludarabine or the combination of the two drugs, AML/MDS was seen in none of 191 patients treated with chlorambucil alone compared to 1/188 of fludarabine recipients, and 5/142 patients receiving the combination ([Morrison et al., 2002](#)). Overall, there does not appear to be a significant increase in AML/MDS in patients with chronic lymphocytic leukaemia treated with chlorambucil alone despite prolonged exposure, older patient age, and long-term patient survival permitting adequate patient follow-up. [The Working Group noted however that because of the number of patients

in these studies, it is not possible to exclude a small effect on the relative risk of AML/MDS.]

### 2.2.2 Epithelial cancers

The initial report of the first large aforementioned observation study suggested an increase in the incidence of epithelial cancers [not further defined] in the chlorambucil recipients ([The French Cooperative Group on Chronic Lymphocytic Leukemia, 1990](#)). In contrast, the aggregate data from the two trials mentioned above ([Dighiero et al., 1998](#)) and from another observational trial ([Cellai et al., 2001](#)) showed no difference between the treated and observation groups, both in the total number of cancers, and in the incidence of skin and lung cancers.

## 3. Cancer in Experimental Animals

Chlorambucil has been tested for carcinogenicity in mice and rats by intraperitoneal injection, and in male and female mice and female rats by gavage.

Chlorambucil increased the incidence and multiplicity of tumours of the lung and the incidence of tumours of the haematopoietic system in mice ([Shimkin et al., 1966](#); [Weisburger et al., 1975](#); [IARC, 1981b](#)), haematopoietic tumours in male rats, and haematopoietic tumours and lymphomas in female rats and mice ([Weisburger et al., 1975](#); [IARC, 1981b](#); [Berger et al., 1985](#); [Cavaliere et al., 1990](#)). It induced lung tumours in male and female mice, and mammary gland tumours in female rats and mice ([Berger et al., 1985](#); [Cavaliere et al., 1990](#)). It also produced nervous system tumours in rats ([Berger et al., 1985](#)).

It had an initiating effect in a two-stage skin carcinogenesis experiment in mice ([Salaman & Roe, 1956](#); [IARC, 1981b](#)).

See [Table 3.1](#).

**Table 3.1 Studies of cancer in experimental animals exposed to chlorambucil**

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, S (NR) 22 wk <a href="#">Salamán &amp; Roe (1956)</a>	Skin 0, 2.7 mg total dose in methanol, administered by skin painting once weekly for 10 wk; croton oil used as promoter (from Week 5 to Week 22) 20 (control), 25	Skin (papillomas): 0/17, 11/19 (58%)	$P < 0.01$	Purity NR
Mouse, A/J (M, F) 39 wk <a href="#">Shimkin et al. (1966)</a>	i.p. 0, 9.6, 37, 150, 420 mg/kg bw (total dose), 3 ×/wk for 4 wk 45, 60, 60, 60, 173 (male control), 157 (female control)	Lung (adenomas and adenocarcinomas): 43% (0.53 tumours/mouse); 32% (0.42 tumours/mouse); 18/38 (47%, 0.6 tumours/mouse); 48/56 (86%, 1.6 tumours/mouse); 45/47 (96%, 5.1 tumours/mouse); 30/30 (100%, 8.9 tumours/mouse)	[ $P < 0.001$ ] [ $P < 0.001$ ] [ $P < 0.001$ ]	Purity NR
Mouse, Swiss-Webster (M, F) 15 mo <a href="#">Weisburger et al. (1975)</a>	i.p. 3 mg/kg bw (MTD) or 1.5 mg/kg bw, 3 ×/wk for 6 mo 25/sex/group	Lung: M–22/35 F–20/28 (controls: 10/101 M, 21/153 F) Lymphoma-myeloid leukaemia: M–6/35 F–4/28 (controls: 3/101 M, 3/153 F) Ovary: F–10/28 (controls: 6/153 F)	$P < 0.001$ , $P < 0.001$	Results reported had been combined for the two doses
Rat, Sprague-Dawley (M, F) 15 mo <a href="#">Weisburger et al. (1975)</a>	i.p. 4.5 mg/kg bw (MTD) or 2.2 mg/ kg bw, 3 ×/wk for 6 mo 25/sex/group	Haematopoietic/lymphatic system: M–8/33 (control: 2/179 M, 1/181 F)	$P < 0.001$	Results reported had been combined for the two doses

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague-Dawley (F) Lifetime (3 yr) <a href="#">Berger et al. (1985)</a>	Oral 0, 3, 6, 13.5, 27 mg/kg bw per mo by gavage for 18 mo 30/group, 120 controls	Mammary gland (malignant): 8/120, 2/30, 4/30, 10/30, 5/30  Central & peripheral nervous tissue (malignant): 2/120, 2/30, 1/30, 3/30, 3/30  Haematopoietic & lymphatic tissue: 1/120, 0/30, 4/30, 0/30, 0/30  External auditory canal (malignant): 0/120, 2/30, 0/30, 0/30, 3/30	[ <i>P</i> < 0.001] (27 mg/kg)  [ <i>P</i> < 0.05] (13.5 and 27 mg/kg)	Purity > 99% The dose of 3 mg/kg bw was kept constant over all treatment groups. To increase the dose, the frequency of administrations was increased
Mouse, BALB/c (M, F) Lifetime (2 yr) <a href="#">Cavaliere et al. (1990)</a>	Oral 0 or 1 mg/kg bw by gavage 5 ×/wk for 12 wk 53 males, 54 females, 50 (male control), 50 (female control)	Lymphoreticular system: 5/50, 4/50, 7/53, 24/53  Lung (adenomas): 19/50, 7/50, 47/53, 46/54  Mammary gland: 0/50, 2/50, 0/53, 4/54	<i>P</i> < 0.01 (F)  <i>P</i> < 0.001 (M, F)  <i>P</i> < 0.05 (F)	Purity > 99% Survival was reduced in treated animals of both sexes ( <i>P</i> < 0.001)

bw, body weight; d, day or days; F, female; i.p., intraperitoneal; M, male; mo, month or months; MTD, maximum tolerated dose; NR, not reported; wk, week or weeks; yr, year or years

## 4. Other Relevant Data

### 4.1 Absorption, distribution, metabolism, and excretion

Chlorambucil is rapidly absorbed following administration to animals as a solution or as an emulsion ([Newell et al., 1981](#); [Ganta et al., 2008](#)). It is a highly lipophilic drug but is also a weak acid and can therefore be taken up into cells by passive diffusion. The weak acidic function is ionized to a lesser extent at acidic pH, and therefore favours drug uptake into the relatively neutral intracellular compartment ([Parkins et al., 1996](#); [Kozin et al., 2001](#)). Drug accumulation by chronic lymphocytic leukaemia lymphocytes peaks at 30 seconds, while efflux from cells loaded with chlorambucil is almost complete within 30 seconds ([Bank et al., 1989](#)). The extracellular pH of tumour tissue is significantly lower than the extracellular pH of normal tissue, and it is expected that this extracellular acidity may enhance the intracellular uptake of chlorambucil by increasing the amount of the free acid ([Kozin et al., 2001](#); [Gerweck et al., 2006](#)).

Once inside the cell, chlorambucil reacts as a bifunctional alkylating agent, with common reaction sites including the  $N^7$  position of guanine or adenine, the  $N^3$  position of adenine (the predominant binding site being the  $N^7$  of guanine), and thiol groups of proteins and peptides ([Bank, 1992](#); [Barnouin et al., 1998](#)). Reaction with thiol groups of glutathione may lead to export of the conjugate by multidrug resistance proteins, potentially reducing cellular effects ([Barnouin et al., 1998](#)). Overexpression of cytosolic glutathione S-transferase in some cells leads to increased conjugation to glutathione and consequent removal from the cell, contributing to the resistance of these cells to the effects of chlorambucil ([Zhang & Lou, 2003](#); [Zhang et al., 2004](#)). Some cells are also able to cause extensive metabolism of chlorambucil to phenylacetic acid

mustard, which is also excreted, again contributing to resistance ([Alberts et al., 1980](#)).

### 4.2 Genotoxic effects

#### 4.2.1 Induction of DNA damage

Reaction of one of the two chloroethyl groups of chlorambucil with the  $N^7$  position of guanine or adenine of double-stranded DNA leads to the formation of mono-adducts. These are repaired rapidly in an error-free fashion by methylguanine methyltransferase (sometimes called alkylguanine alkyltransferase). However, some cells lack this repair activity, usually because of silencing of the corresponding gene, and the unrepaired DNA mono-adduct then forms a complex with mismatch-repair enzymes. The subsequent inhibition of DNA replication can eventually induce DNA breakage ([Caporali et al., 2004](#)). The second chloroethyl group of the DNA mono-adduct with chlorambucil can interact with proteins ([Loeber et al., 2008](#)) but more importantly, because of its juxtaposition to other bases in the major groove of DNA, it can react with a DNA base to form an interstrand DNA cross-link. This DNA cross-link complex is quite stable ([Jiang et al., 1989](#); [Loeber et al., 2008](#)), and its repair requires nucleotide excision repair factors (such as xeroderma pigmentosum complementation group F-excision repair cross-complementing rodent repair deficiency, complementation group, 1-XPF-ERCC1) that act slowly by homologous recombination ([Drabløs et al., 2004](#)). The DNA cross-link attracts several binding proteins, probably the BRCA1 and BRCA2 proteins, Fanconi anaemia gene product, and Nijmegen breakage syndrome gene product to form a complex ([Wilson et al., 2001](#)). As shown in cultured HeLa cells, addition of chlorambucil prolongs S-phase and induces a corresponding mitotic delay. The magnitude of these effects correlates with the level of DNA cross-links. Treatment of cells in the  $G_2$ -phase of the cell cycle does not induce mitotic delay but

does inhibit DNA synthesis in the subsequent cell cycle, and causes a delay in the next mitosis, suggesting that at least some lesions induced by chlorambucil are long-lasting ([Roberts, 1975](#)).

#### 4.2.2 Mutational consequences of DNA damage

Chlorambucil has been tested for genotoxicity in several short-term assays *in vitro* and *in vivo*. It has been shown to be mutagenic in bacteria after metabolic activation, to cause gene conversion in yeast, sex-linked recessive mutations in *Drosophila*, mutations in Chinese hamster ovary cells, and clastogenic effects in human lymphocytes *in vitro*, and in animals *in vivo* ([IARC, 1987b](#)).

The mutagenicity of chlorambucil has been reported to be related to its ability to form DNA cross-links as well as to transfer an alkyl group to form DNA mono-adducts ([Sanderson & Shield, 1996](#)), suggesting that lesions responsible for S-phase and mitotic delays are also responsible for mutagenicity, probably as a consequence of unrepaired DNA damage persisting after DNA replication ([Shi & King, 2005](#)). Such mechanisms may involve changes to chromosomes, consistent with observations that chlorambucil can induce sister chromatid exchange, chromosomal aberrations ([Speit et al., 1992](#)), and micronuclei ([Ashby & Tinwell, 1993](#); [Yaghi et al., 1998](#)). The ability of chlorambucil to induce aneuploidy ([Efthimiou et al., 2007](#)) may contribute to its carcinogenicity.

Exposure to chlorambucil increases the frequency of micronucleus induction and chromosomal aberrations in rat bone marrow and spleen *in vivo* ([Moore et al., 1995](#)), of mutations at the hypoxanthine-(guanine) phosphoribosyl transferase (*Hprt*) locus in Chinese hamster V79 cells ([Speit et al., 1992](#)), of deletions in Chinese hamster (CHO)-AS52 cells ([Yaghi et al., 1998](#)), and of gene deletions and translocations in mouse spermatids *in vivo* ([Russell et al., 1989](#); [Rinchik et al., 1990](#)).

### 4.3 Synthesis

Chlorambucil 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 chlorambucil. Chlorambucil causes acute myeloid leukaemia.

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

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

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