

The classical anti-cancer agents comprise cytotoxic compounds. Mostly, these drugs act by exerting DNA damage. In essence, there are two major response phenotypes available to a cell upon DNA damage, such as a chemotherapeutic drug action,

- to arrest the cell cycle and repair the damage,
- to initiate a pathway to apoptosis (programmed cell death).

In either scenario, the uncontrolled growth of the tumor cells is curtailed. The major limitation of the cytotoxic anti-cancer drugs is the tumor non-specific action, and the suppression of all rapidly dividing cells¹.

DNA damaging drugs interfere with transcription and reduplication.

Affected cells respond with cell cycle arrest or programmed cell death.

DNA damaging drugs are mutagenic, teratogenic, and carcinogenic.

Adverse effects are exerted on rapidly proliferating cells (skin—hair loss, gastrointestinal tract—nausea and vomiting, bone marrow—anemia causing fatigue/leukopenia causing infections/thrombocytopenia causing bleeding).

2.1 Alkylating Agents

Alkylating agents² are electrophilic and bind covalently to electron-rich functional groups of various target molecules via first-order or second-order nucleophilic substitutions (nucleophiles are electron-rich molecules or ions, such as OH⁻, H₂O, halogenides, alcohols, thiols and amines). The first order reactants include aromatic and aliphatic nitrogen and sulfur mustards. Second order reactants include ethylene

imines and epoxides, alkylmethane sulfonates of the busulfan (Myleran) type, and α -halogenated acids, ketones, and their derivatives.

- For first-order reactions, the rate limiting step is the ionization of the alkylating agent to form a positively charged carbonium ion, which then rapidly reacts with water, or a negative center, or a nucleophilic center. Within DNA and RNA, the most reactive site is the N7 position of guanine (Fig. 2.1). In DNA, this is followed by N3 of adenine, N1 of adenine, N1 of cytosine, and N7 of adenine (Table 2.1). The rate limiting step of the reaction is the formation of positively charged cyclic immonium ions, whereas the rate of the reaction is essentially independent of the nature and concentration of the nucleophilic target being attacked.
- For second order nucleophilic substitutions, both reactants interact to form a transition complex. No carbonium ion is formed. The rate of the reaction depends on both concentrations, with bond strengths, electron affinity, and accessibility of both reagents being important (Knock 1967).

Alkylating agents exert cytotoxic effects by transferring alkyl groups to DNA, thereby damaging the DNA and interfering with DNA transcription and cell division. This class of drugs mainly works by three distinct mechanisms:

- The attachment of alkyl groups to DNA bases prevents DNA synthesis and RNA transcription, and it results in the DNA being fragmented by repair enzymes in a process to replace the altered bases.
- The two arms of mustard drugs can cross-link DNA strands. In this process, two bases are linked together. Bridges can be formed within a single molecule of DNA (intra-strand cross-links³) or a bridge may connect two DNA molecules (inter-strand cross-links). Cross-linking prevents DNA from being separated for reduplication or transcription. Although bifunctionality of alkylating

¹ Because the desired drug actions (damage to rapidly proliferating cancer cells) and the adverse effects (damage to rapidly proliferating healthy cells) are identical in targets and mechanisms, they are not separable.

² Alkyl designates a functional group (a “side chain”) that consists solely of single-bonded carbon and hydrogen atoms. Alkylating anti-cancer agents attach alkyl groups to biomolecules.

³ called limpet attachment of the drug molecule to the DNA.

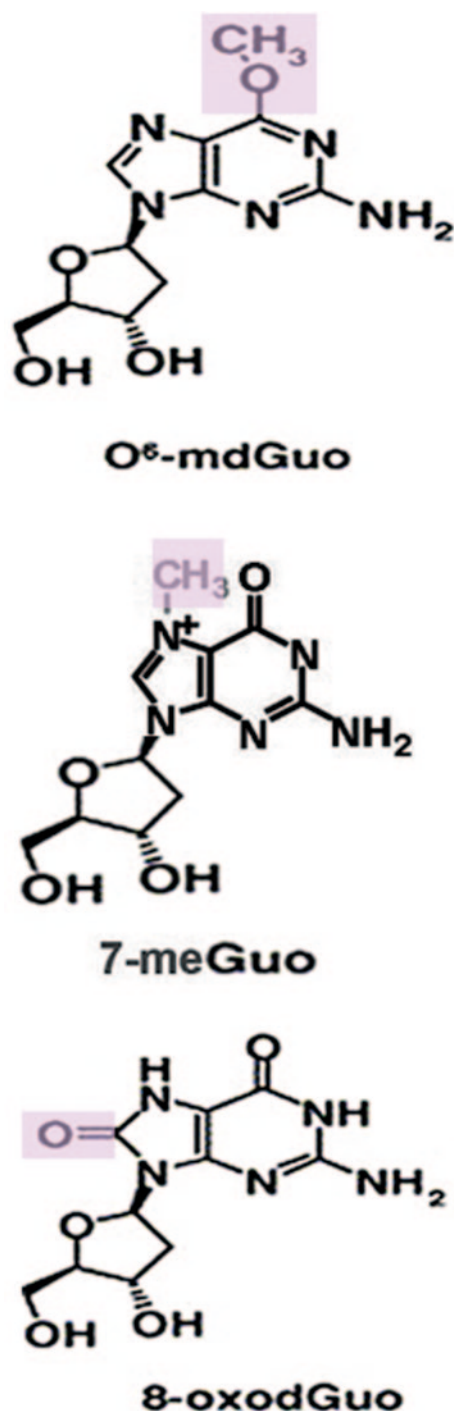


Fig. 2.1 Common DNA adducts of guanosine. DNA damaging anti-cancer drugs may alter guanine residues to mutagenize and kill proliferating cells. O⁶-methyl-2'-deoxyguanosine (*O⁶-mdGuo*), N⁷-methyl-guanosine (*7meGuo*), and 8-oxo-7,8-dihydro-2'-deoxyguanosine (*8-oxodGuo*) represent such DNA lesions. The modifications are highlighted in pink. (Adapted from Brink et al. 2006; Nay 2013)

agents is not required for their mutagenic and carcinogenic properties, it is important for high anti-cancer activity.

- Alkylating agents can induce the mispairing of nucleotides, leading to mutations. Alkylated G bases may erroneously pair with Ts. If this altered pairing is not corrected by DNA repair it can result in permanent genetic change (Fig. 2.2).

Adverse Effects Due to the genetic damage exerted by the drug class, the treatment of cancer patients with alkylating agents is linked to an increased risk for secondary cancers. Alkylating agents can cause mutations not unlike those produced by radiation. These mutations occasionally lead to transformation. One form of cancer that may arise is a relatively rare type of acute non-lymphocytic leukemia (ANL). Highly treatment resistant cases appear as early as 2 years following initial therapy and peak around 5 years after exposure to alkylating agents. The risk of osteosarcomata may be elevated after the treatment of childhood cancers with alkylating agents.

Alkylating agents attach alkyl groups to DNA bases, leading to DNA fragmentation.

Alkylating agents cause intra- and inter-strand DNA cross-links.

Alkylating agents can induce mispairing of nucleotides, leading to mutations.

Alkylating agents exert cytotoxicity in all phases of the cell cycle.

Nitrogen mustards and chlorethyl nitrosoureas have a preference for guanine-N7 alkylation.

2.1.1 Nitrogen Mustards

The beginning of the modern era of cancer chemotherapy is rooted directly in the discovery of nitrogen mustard. Mustard gas (sulfur mustard, H-gas) had been used as a chemical warfare agent during World War I, the first documented battlefield use being in Ypres, Belgium in 1917. The name assigned to the gas by the German military was Lost (referring to Lommel and Steinkopf, who in 1916 proposed the military use to the German Imperial General Staff). Exposure to mustard gas induces severe injuries to the eyes, skin, and respiratory tract. In 1917, Krumbhaar, a Captain in the U.S. Medical Corps, noted the development of profound leukopenia in individuals who survived a gas attack for several days (Krumbhaar 1919; Krumbhaar and Krumbhaar 1919). Following up on this observation, a group from the U.S. Office of Scientific Research and Development (OSRD) at Yale Medical School secretly studied the effects of nitrogen mustard on lymphomata. Milton Winternitz, who had worked on sulfur mustards in World War I, obtained the OSRD contract to study the chemistry of mustard compounds. He recruited the pharmacologists Louis S. Goodman and Alfred Gilman

Table 2.1 DNA target sites for alkylating anti-cancer agents

Base	Target	Drug	Class	Recognition sequence
Guanine	N7	Melphalan	Nitrogen mustard	
		Cyclophosphamide	Phosphoramidate mustard	
		Temozolomide	Triazene	
		Cisplatin	Platinum drug	
		Fotemustine	Nitrosourea	3' end of guanine tracts
Guanine	N1	Fotemustine	Nitrosourea	
Guanine	N2	Ecteinscidin-743	Minor groove binding antibiotic (G/C preference)	AGC, CGC, TGG
Guanine	N3	Duocarmycin A	Cyclopropylpyrroloindole antibiotic	5'-GCAATTGCG-CAATTGC-3'
Guanine	O6	Temozolomide	Triazene	
		Dacarbazine	Triazene	
		Laromustine	Hydrazine	
Guanine		Duocarmycin A	Cyclopropylpyrroloindole antibiotic	5'-CGCGTTGGGAG-3'
		Mithramycin A	Aureolic acid minor groove binder	
Adenine	N3	Duocarmycin A	Cyclopropylpyrroloindole antibiotic	
		CC-1065	Cyclopropylpyrroloindole antibiotic	5'-d(A/G)NTTA-3'
				5'-dAAAAA-3'
		Adozelesin, carzelesin, bizelesin	Minor groove binding antibiotic (A/T preference)	5'-(A/T)(A/T)A-3'
		Tallimustine	Minor groove binding antibiotic (A/T preference)	5'-TTTTGA-3'
Adenine	N7	Cisplatin	Platinum drug	
Adenine	N1	(Uncommon)		
Cytosine	N3	Fotemustine	Nitrosourea	
Cytosine	N1	(Uncommon)		

to perform the necessary animal experiments. Based on their successful research, Gustav Lindskog successfully treated a radio-resistant lymphosarcoma that compressed a patient's trachea with the injection of nitrogen mustard in December 1942. In 1943, Goodman and Gilman initiated the experimental treatment of Hodgkin disease and lymphosarcoma with nitrogen mustard. None of this was made public until 1946, when Goodman and Gilman reported their observation that exposure to mustard gas caused profound lymphoid and myeloid suppression, suggesting its utility for the treatment of lymphomata (Goodman et al. 1946; Gilman and Philips 1946). There was a parallel development: In World War II, General Eisenhower had ordered that a stockpile of mustard gas be kept near the front for possible use in a reprisal if the Nazis resorted to chemical warfare. During a military operation, allied ships in Bari Harbor, Italy⁴, were sunk in an air assault by the German Luftwaffe on 2nd December 1943. At the center of the destruction was the vessel Liberty Ship S.S. John Harvey, laden with ammunition, supplies, and 2000 mustard gas bombs. A large number of military personnel were accidentally exposed to mustard gas and were later found by U.S. medical officer Lieutenant Colonel Stewart F. Alexander to have abnormally low white blood cell counts as a consequence of poisoning. It was implied that an agent, which damaged the rapidly growing white blood cells, might have a similar effect on cancer cells. Cornelius P. Rhoads

served as chief of the medical division of the U.S. Army's chemical warfare unit during World War II. Based on his experience in the Bari incident, he investigated mustard gas as a tumor killing agent (Rhoads 1946). The research presaged classical chemotherapy⁵. Rhoads moved on to head one of the largest drug development programs at the Sloan-Kettering Institute in New York and pioneered the practice of contract research for pharmaceutical companies under confidentiality agreements.

Mustard gas is not derived from the mustard plant, it gets its name from its impurities. Impure mustard gas is yellow-brown and has an odor resembling mustard plants. Upon contact with the skin, it causes a burning sensation, which is similar to that caused by the oil from black mustard seeds. Sulfur mustards (bis(2-chloroethyl)-sulfide, 1,5-dichloro-3-thiapentane), which are related to mustard gas, are vesicants that have the ability to form large blisters on exposed skin. While sulfur mustards are too toxic for medical applications, nitrogen mustards⁶ have found use as therapeutics.

⁴ Allied ships were stationed in Bari under the assumption that the harbor was too far south to be reached by the Luftwaffe (the German air-force). The air raid was later called The Little Pearl Harbor.

⁵ The recognition would have assured Rhoads' place in medical history had it not been revealed that he had likely committed serious ethics violations in unrelated assignments. In Puerto Rico, 1931, after Rhoads' car had been vandalized he wrote generally hateful comments on Puerto Ricans in a letter. He claimed that he had deliberately injected several Puerto Rican citizens with cancer cells.

⁶ The characteristic bis(2-chloroethyl)amine- domain, which generates DNA cross-links, is contained in nitrogen mustards, phosphoramidate mustards (ifosfamide is unique in its chain length that generates 7-membered cross-links), and selective minor groove DNA binding antibiotics (tallimustine and MEN 10710).

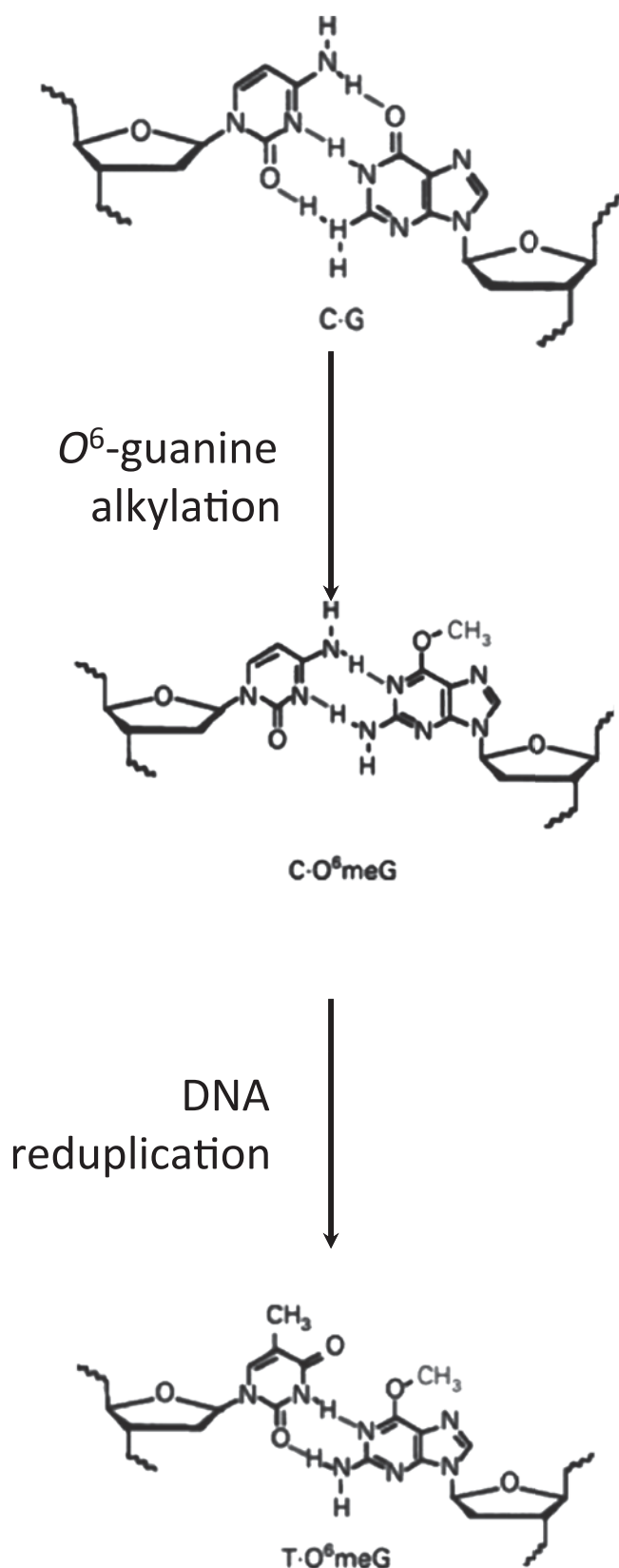


Fig. 2.2 DNA adducts caused by alkylating agents. The reaction scheme depicts the O⁶ alkylation of guanine and the ensuing mispairing during DNA reduplication. (Adapted from Marra and Schär 1999)

After uptake, nitrogen mustard is metabolized to a highly reactive ethylene immonium derivative, which alkylates DNA (Papirmeister et al. 1985) and inhibits DNA reduplication (Fig. 2.3). In addition, increasing nitric oxide produced by Nitric Oxide Synthase may be responsible for some of the damage exerted by mustard drugs (Sawyer 1998). This toxicity probably comes from an over-production of reactive nitrogen species, in particular peroxynitrite (ONOO⁻), by the reaction of nitric oxide and superoxide. There are several generations of nitrogen mustard drugs.

- In first generation nitrogen mustards, aliphatic radicals were attached to the mustard pharmacophore -N(CH₂CH₂Cl)₂.
- In second generation nitrogen mustards, electron withdrawing aromatic radicals were attached to reduce the reactivity and permit oral use.
- In third generation nitrogen mustards, the pyrimidine nucleus was chosen as a carrier for the mustard pharmacophore, which permits oral administration.
- More recent modifications include steroid-coupled nitrogen mustards and phosphoramidate mustards

First generation nitrogen mustards The aliphatic alkylating agent mechlorethamine hydrochloride (2-chloro-*N*-(2-chloroethyl)-*N*-methyl-ethanamine, nitrogen mustard, chlormethine hydrochloride, mustine, chlorethazine hydrochloride, HN2 hydrochloride, N-Lost) (NSC-762) <Mustargen, Caryolysine, Cloramin, Erasol, Onco-Cloramin> is the salt of a synthetic nitrogen-containing sulfur mustard derivative (Fig. 2.4) with anti-neoplastic and lympholytic properties. Mechlorethamine hydrochloride is used because it induces a rapid response. It is primarily administered as part of the MOPP regimen. The agent may be indicated in treating Hodgkin disease (stages III and IV), lymphosarcoma, chronic myelocytic or chronic lymphocytic leukemia, polycythemia vera⁷, mycosis fungoides⁸, and bronchogenic carcinoma. It may also be included in the treatment of small cell lung cancer or medulloblastoma. The drug is not active in acute leukemias or chronic granulocytic leukemias (Knock 1967).

Mechlorethamine cannot be taken orally. It is given as an intravenous infusion over 20 min. The dosage varies with the clinical situation, the initial therapeutic response, and the magnitude of hematologic depression. Usually, a total dose of 0.4 mg/kg of body weight for each course is given either as a single dose or in divided doses of 0.1–0.2 mg/kg per day. Subsequent courses should not be given until the patient has recovered hematologically from the previous course. The drug administered intrapleurally, intraperitoneally, or

⁷ A myeloproliferative disorder that results in the over-production of red blood cells.

⁸ The most common form of cutaneous T-cell lymphoma.

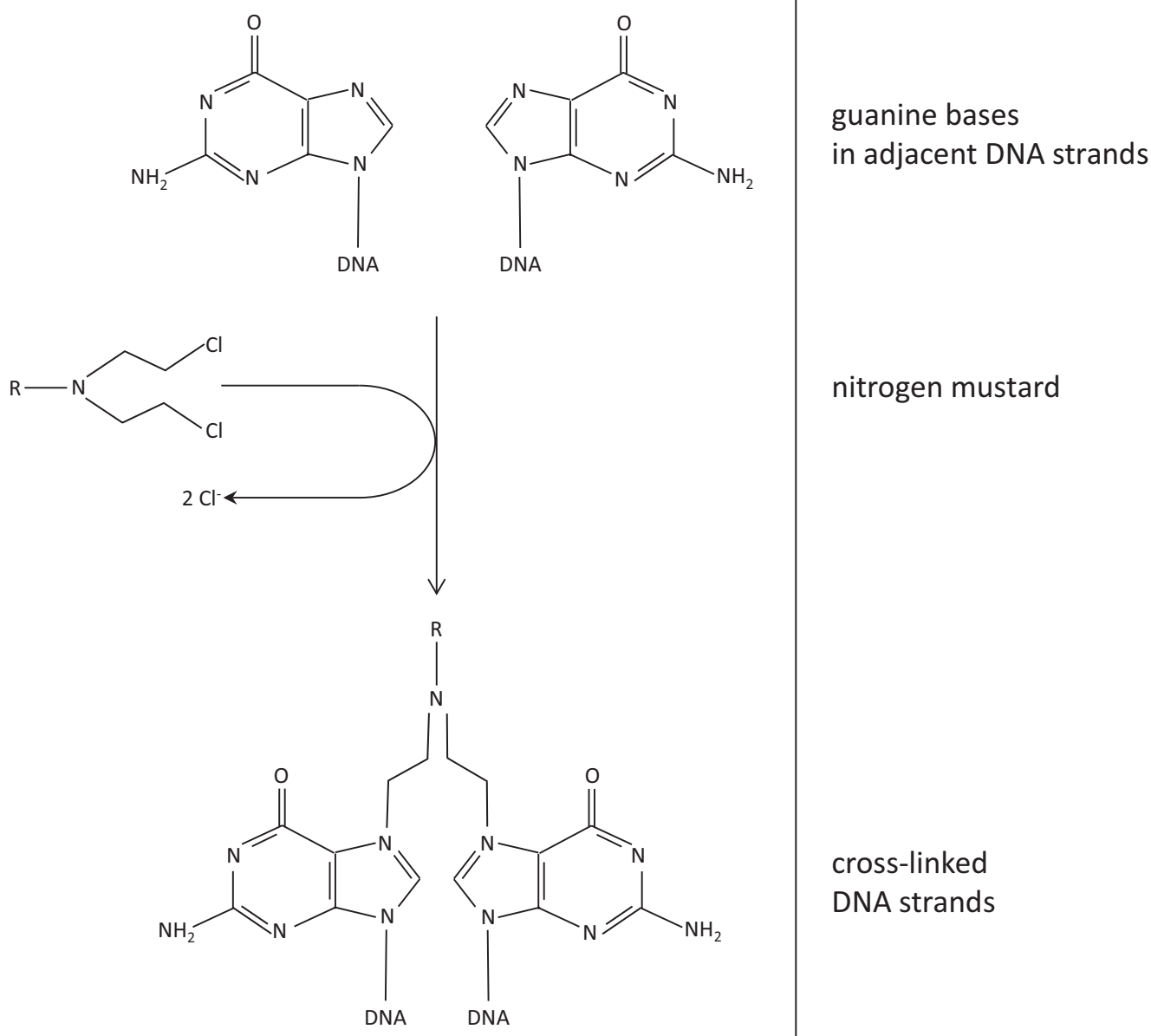


Fig. 2.3 Mechanism of action for nitrogen mustard. Alkylation of guanine bases in the DNA is partially responsible for the cytotoxic effect of nitrogen mustards. The alkylation of two guanines by the arms of the mustard leads to DNA cross-linking

intrapericardially is indicated for the palliative treatment of metastatic carcinoma resulting in effusion. Local therapy with nitrogen mustard is used only when malignant cells are present in the effusion. Intracavitary injection is not recommended when the accumulated fluid is chylous, because the results are likely to be poor. Paracentesis is first performed with most of the fluid being removed from the pleural or peritoneal cavity. The position of the patient should be changed every 5–10 min for an hour after injection to obtain more uniform distribution of the drug throughout the serous cavity.

- Mechlorethamine gel <Valchlor> is a topical gel for the second-line treatment of stage IA and IB mycosis fungoides cutaneous T-cell lymphoma

Pharmacokinetics Because of its extreme reactivity with water, nitrogen mustard is reconstituted immediately before use. In neutral or alkaline aqueous solution the drug is highly unstable and undergoes rapid chemical transformation. In body fluids, mechlorethamine combines with water or reactive compounds of cells within a few minutes after administration, so that the drug is no longer present in its active form.

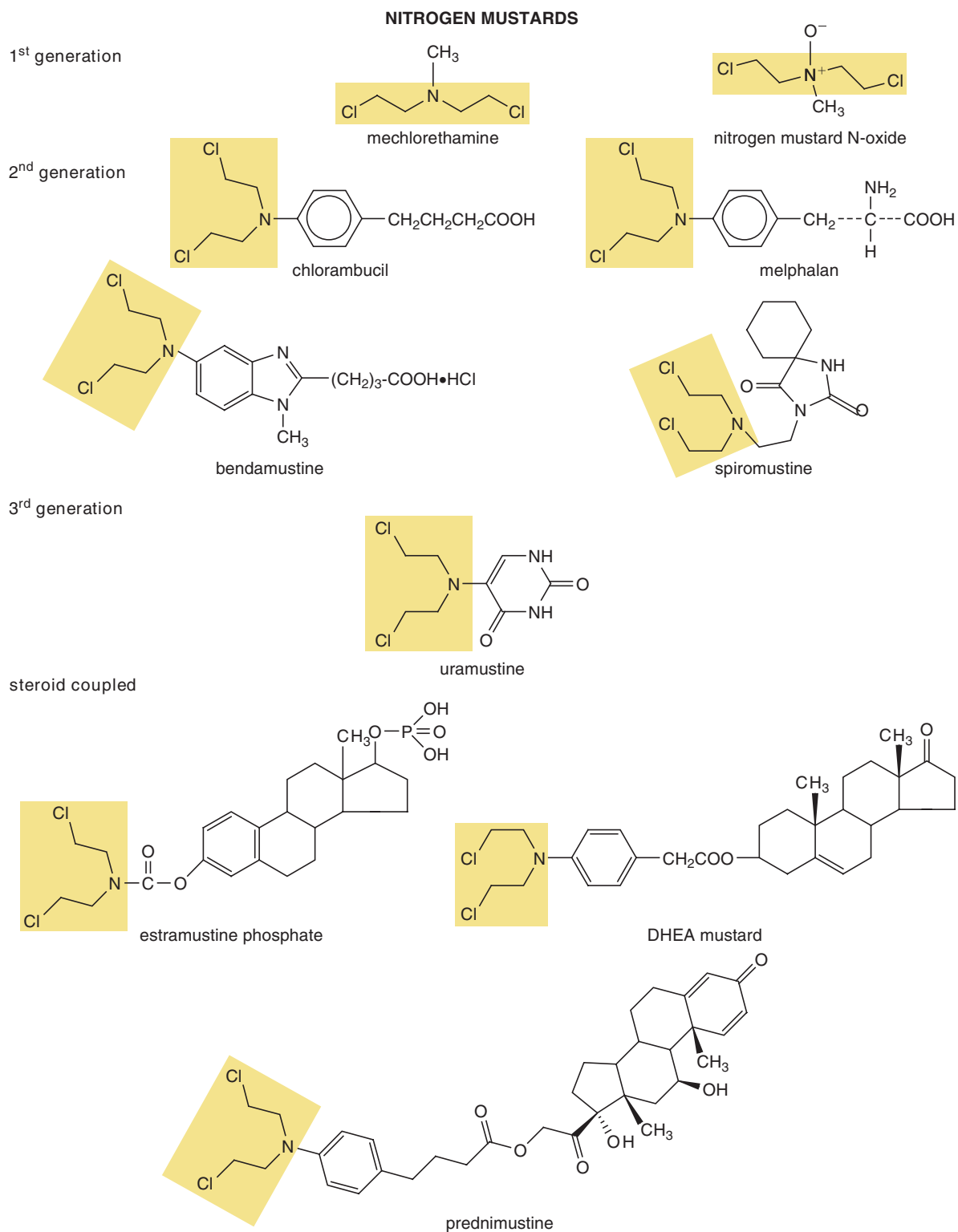


Fig. 2.4 Structures of nitrogen mustards. Three consecutive generations in this class of drugs have acquired increasing oral bioavailability. A 4th generation is coupled to steroids for bifunctionality. The common functional mustard moiety is highlighted in yellow

Adverse Effects For the comfort of the patient, nitrogen mustard may be given at bedtime, following administration of a barbiturate and an anti-emetic to minimize the adverse effects of nausea and vomiting, which usually occurs after

1–3 h. Emesis may disappear in the first 8 h, but nausea may persist for 24 h. Depression of the hematopoietic system may be present for up to 50 days (or more) after starting therapy. Mustard treatment generally produces lymphocytopenia

within 24 h after the first injection. Substantial granulocytopenia occurs within 6–8 days and lasts for 10–21 days. Thrombocytopenia is variable but the time course of the appearance and recovery from reduced platelet counts generally parallels the granulocyte levels. Rarely, hemolytic anemia associated with such diseases as lymphomata and chronic lymphocytic leukemia may be precipitated by treatment with alkylating agents including mechlorethamine. Serious adverse effects can include anaphylactic reactions or bleeding (bloody urine, tar stools, bleeding gums). Other common adverse effects include hyperuricemia, fatigue, hair loss, maculopapular skin eruptions, and herpes zoster. Jaundice, vertigo, tinnitus and hearing loss occur infrequently. As nitrogen mustard therapy can contribute to the extensive and rapid development of amyloidosis, it should be used only if foci of acute or chronic suppurative inflammation are absent.

Alkylating agents are carcinogenic, mutagenic and teratogenic. Oligomenorrhea or azoospermia can be induced by mechlorethamine and may not recover for years after termination of therapy. During pregnancy, the agent can cause damage to the fetus. It is in the U.S. FDA Pregnancy Category D.

After intravenous injection, a local rash, pain, or burning may occur. Extravasation into the tissue surrounding the injection site is a potentially serious problem. It causes severe painful induration. The area can be infiltrated with an isotonic solution of 0.11 M sodium thiosulfate as an antidote. The mustard also poses a risk for venous thromboses at the site of injection from direct sclerosing action. Transient cardiac irregularities may occur with intrapericardial injection.

A contraindication is hypersensitivity to mechlorethamine or any component of the formulation. The presence of known infectious diseases may be an indication against immunosuppressive agents, such as mechlorethamine. Because of the bone marrow suppression, vaccinations during or shortly before or shortly after chemotherapy with mechlorethamine should be avoided.

Drug Interactions Turmeric may decrease the effect of mechlorethamine. The use of this spice in the diet should be avoided while receiving treatment. Precautions must be observed with the use of mechlorethamine and radiation therapy in alternating courses. Both depress hematopoietic function and neither regimen should follow the other until bone marrow function has recovered. In particular, irradiation of such areas as sternum, ribs, and vertebrae shortly after a course of nitrogen mustard may lead to hematologic complications. The decrease in platelet count can increase the risk of bleeding. Therefore, any aspirin or salicylate containing medicines are to be avoided.

Nitrogen mustard *N*-oxide hydrochloride (2-chloro-*N*-(2-chloroethyl)-*N*-methyl-ethanamine-*N*-oxide hydrochloride, NMNO) (NSC-10107, SK-598) <Nitromin> is the chloride salt of the oxide of nitrogen mustard. It is closely related to mechlorethamine. Nitrogen mustard *N*-oxide has been used

especially in Japan⁹. Nitrogen mustard *N*-oxide is usually administered intravenously at 1 mg/kg/day for 10 days. Its therapeutic spectrum resembles that of nitrogen mustard.

Adverse Effects As a DNA damaging agent, nitrogen mustard *N*-oxide hydrochloride is potentially carcinogenic.

Second generation nitrogen mustards Chlorambucil (4-[bis(2-chlorethyl)amino]benzenebutanoic acid) <Leukeran> was developed in 1953 at the Chester Beatty Research Institute, England. It is an orally active, bifunctional, aromatic nitrogen mustard that alkylates and cross-links DNA during all phases of the cell cycle, resulting in a disruption of DNA function, cell cycle arrest, and apoptosis. The electron withdrawing properties of the aromatic ring lead to slower reactions with serum and cellular constituents compared to first generation nitrogen mustards. Therefore, chlorambucil can be given orally in tablet form. Chlorambucil is indicated in the palliative treatment of chronic lymphocytic leukemia, malignant lymphoma, lymphosarcoma, giant follicular lymphoma, and Hodgkin disease. It has been used in the treatment of Waldenström macroglobulinemia¹⁰, polycythemia vera, oat cell and undifferentiated carcinomata of the lungs, trophoblastic neoplasms, and ovarian carcinoma.

Chlorambucil is well tolerated by most patients, although for initial treatment it has been largely replaced by fludarabine (Rai 2000).

- Chlorambucil is usually given at 0.1–0.2 mg/kg/day for 3–6 weeks. The entire daily dose may be administered at one time. The dose is reduced when leukocyte counts drop or there are signs of clinical improvement. When lymphocytic infiltration of the bone marrow is present, or when the bone marrow is hypoplastic, the dose should not exceed 0.1 mg/kg/day. The maintenance dose is usually about 2 mg/day and can be extended over months or years.
- Alternate schedules for the treatment of chronic lymphocytic leukemia employ intermittent, bi-weekly, or once-monthly pulse doses of chlorambucil. Intermittent schedules begin with an initial single dose of 0.4 mg/kg, which is increased by 0.1 mg/kg until control of lymphocytosis or toxicity is observed. Subsequent doses are modified to produce mild hematologic toxicity.
- Continuous maintenance therapy is considered less safe than short courses of treatment. If a maintenance dosage is used, it should not exceed 0.1 mg/kg/day and may be as low as 0.03 mg/kg/day. It may be desirable to withdraw the drug after maximal control has been achieved, since intermittent therapy—reinstated at the time of relapse—may be as effective as continuous treatment.

⁹ Of note, in the U.K. Nitromin is a brand name for the unrelated agent glyceryl trinitrate.

¹⁰ Waldenström macroglobulinemia (lymphoplasmacytic lymphoma) is a lymphoproliferative disease of IgM secreting B-lymphocytes.

Pharmacokinetics Oral chlorambucil undergoes rapid and complete gastrointestinal absorption and blood clearance. After single oral doses of 0.6–1.2 mg/kg, peak chlorambucil levels in the blood are reached within 1 h and the terminal elimination half-life of the parent drug is roughly 1.5 h. The agent is extensively metabolized in the liver, primarily to phenylacetic acid mustard, which has anti-neoplastic activity. Chlorambucil and its major metabolite spontaneously degrade, forming monohydroxyl and dihydroxyl derivatives. Both chlorambucil and its metabolites are extensively bound to plasma and tissue proteins (99%), specifically to Albumin. Urinary excretion is below 1% in 24 h.

Adverse Effects A prominent adverse effect is myelosuppression (anemia, neutropenia, thrombocytopenia). Severe neutropenia usually occurs only in patients who have received a total dosage of 6.5 mg/kg or more in one course of therapy with continuous dosing. Upon withdrawal from the drug, this effect may be reversible after about 10 days from the last dose, but bone marrow failure can arise in rare cases. Less commonly occurring adverse effects are gastrointestinal distress (nausea, vomiting, diarrhea, oral ulcerations) and central nervous system damage (tremors, muscular twitching, confusion, agitation, ataxia, hallucinations; increased risk of seizures in children with nephrotic syndrome and patients receiving high pulse doses of chlorambucil) that often resolves upon discontinuation of the drug, skin reactions (urticaria, angioneurotic edema, rarely skin rash progressing to erythema multiforme, toxic epidermal necrolysis, and Stevens-Johnson syndrome), and infertility (sterility when administered to prepubertal and pubertal males, induction of amenorrhea in females). Uncommon adverse reactions include pulmonary fibrosis, hepatotoxicity and jaundice, drug fever, peripheral neuropathy, interstitial pneumonia, and sterile cystitis. As all DNA damaging agents, chlorambucil is itself carcinogenic and bears a risk for secondary malignancies (specifically acute leukemia) with increasing cumulative dose.

Radiation and cytotoxic drugs render the bone marrow more vulnerable to damage, and chlorambucil should be used with particular caution within 4 weeks of a full course of radiation therapy or chemotherapy. If bone marrow is infiltrated by the tumor, the daily dosage of chlorambucil should not exceed 0.1 mg/kg. The drug crosses the placenta and is contraindicated during pregnancy (Category D), but it is not known whether it is excreted into breast milk.

Chlorambucil should not be used in patients whose disease has shown a prior resistance or known hypersensitivity to the agent. There may be cross-hypersensitivity between chlorambucil and other alkylating agents.

Melphalan A (2-amino-3-[4-[bis(2-chloroethyl)amino]phenyl]-propanoic acid, L-phenylalanine mustard, L-PAM,

L-sarcosylsin) <Alkeran> is a bifunctional phenylalanine derivative of nitrogen mustard¹¹. Melphalan alkylates DNA at the N7 position of guanine and induces DNA inter-strand cross-linkages. This results in the inhibition of DNA and RNA synthesis and cytotoxicity against both dividing and non-dividing tumor cells.

Melphalan is indicated for the palliative treatment of multiple myeloma and for the palliation of non-resectable epithelial ovarian carcinoma. It is also used to treat breast cancer, neuroblastoma, and rhabdomyosarcoma. While it is occasionally applied to malignant melanoma, melphalan is not enriched in melanocytes, despite the role of phenylalanine as a precursor in the synthesis of Melanin. Administration is oral or intravenous. Dosing varies by purpose, route of administration, and patient weight. The entire daily dose may be given at one time.

- Orally, melphalan is usually given at 6–10 mg per day for 2–3 weeks, followed by a rest period of several weeks before initiation of the next cycle. When the white blood cell and platelet counts are rising, a maintenance dose of 2 mg daily may be instituted. In multiple myeloma, melphalan shows clinical effectiveness in 50% of cases at 0.2 mg/kg/day orally.
- Alternatively, an initial course of 10 mg/day for 7–10 days is administered. The maximal suppression of the leukocyte and platelet counts occurs within 3–5 weeks and recovery within 4–8 weeks. Continuous maintenance therapy with 2 mg/day is instituted when the white blood cell count is greater than 4000 cells/ μ L and the platelet count is greater than 100,000 cells/ μ L. Dosage is adjusted to 1–3 mg/day depending upon the hematologic response. It is desirable to try to maintain a significant degree of bone marrow depression so as to keep the leukocyte count in the range of 3000–3500 cells/ μ L.
- A commonly employed regimen for the treatment of ovarian carcinoma is the administration of melphalan at a dose of 0.2 mg/kg daily for 5 days as a single course. Courses are repeated every 4–5 weeks, depending upon hematologic tolerance.

Melphalan has been used in combination with prednisone to boost effectiveness in the treatment of multiple myeloma. Early efforts to enhance chemotherapy with autologous bone marrow transplantation were performed with melphalan. For disseminated melanoma, bone marrow withdrawn from the sternum and stored cooled in citrate buffer plus dextrose was reinfused intravenously 6–8 h after administration of the chemotherapeutic (Ariel and Pack 1967).

¹¹ Melphalan is the active L-isomer of the D-isomer compound melphalan that was first synthesized in 1953 by Bergel and Stock. The L form is superior to the D form in anti-tumor activity.

Pharmacokinetics Blood melphalan levels are highly variable after oral dosing, possibly due to incomplete intestinal absorption, variable first-pass hepatic metabolism, or rapid hydrolysis. The time of the first appearance in the blood stream ranges approximately 0–6 h. The peak plasma concentration range is 70–4000 ng/mL, depending upon the dose. The extent of melphalan binding to plasma proteins ranges 60–90%. Serum Albumin is the major binding protein, while α_1 -Acid Glycoprotein may account for about 20% of the plasma protein binding. Approximately 30% of melphalan is irreversibly bound to plasma proteins. Interactions with Immunoglobulins are negligible. Melphalan is eliminated from the circulation primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. The terminal elimination half-life of the parent drug in the blood is about 1.5 h; its 24-h urinary excretion is about 10%, indicating that renal clearance is not a major route of elimination. Penetration into cerebrospinal fluid is low.

Adverse Effects Myelosuppression with reduced white blood cell count, increased risk of infection, and decreased platelet count (causing an elevated risk of bleeding) is common. Although bone marrow suppression frequently occurs, it is usually reversible if melphalan is withdrawn early enough. However, irreversible bone marrow failure has occurred. Common adverse effects include nausea and vomiting. Less frequent adverse effects comprise pulmonary fibrosis after prolonged use, interstitial pneumonitis, compromised ovarian or testicular functions, and hair loss. Allergic reactions, including urticaria, edema, skin rashes, and rare anaphylaxis, have arisen after multiple courses of treatment. Cardiac arrest can rarely result in association with such events.

Secondary malignancies, including acute non-lymphocytic leukemia, myeloproliferative syndrome, and carcinoma can occur in patients treated with alkylating agents. At cumulative doses above 700 mg, the 10-year cumulative risk increases from about 2% to about 20%.

Melphalan should be used with extreme caution in patients whose bone marrow reserve may have been compromised by prior irradiation or chemotherapy, or whose marrow function is recovering from previous cytotoxic therapy. During treatment with melphalan A, the intake of salicylic acid <Aspirin> should be avoided as it could intensify any bleeding problems. Interactions with cimetidine, steroids, and cyclosporine are possible. The drug is Pregnancy Category D; it is not known whether this agent is excreted into breast milk.

In the 1960s, bendamustine hydrochloride (4-[5-[bis(2-chloroethyl)amino]-1-methylbenzimidazol-2-yl]butanoic acid) (SDX-105) <Treanda, Ribomustin, Treakisym, Levact> was designed with the aim of creating a bifunctional anti-cancer agent that possesses DNA damaging properties (by virtue of an alkylating group) and also potential anti-metabolite

properties (associated with a purine-like benzimidazole ring). It was first marketed in Germany in the early 1970s. In 2008, bendamustine was approved by the U.S. Food and Drug Administration (FDA) for the treatment of chronic lymphocytic leukemia (CLL). The drug also has therapeutic activity against multiple myeloma, and indolent B-cell non-Hodgkin lymphoma that has progressed within 6 months of treatment with a rituximab containing regimen. For these conditions, bendamustine has strong efficacy as well as low cross-resistance with other alkylating agents and fludarabine. The agent causes DNA damage that leads to cell death via several pathways, including apoptosis and mitotic catastrophe. A standard dose is 100–120 mg/m² body surface area, administered intravenously over a period of 30–60 min, for chronic lymphocytic leukemia on days 1 and 2 of a 28-day cycle up to six cycles, for non-Hodgkin lymphoma on days 1 and 2 of a 21-day cycle up to eight cycles.

Pharmacokinetics The binding of bendamustine to plasma proteins is largely concentration independent and ranges 94–96%. The drug distributes freely in red blood cells, rendering the mean steady state volume of distribution approximately 25 L. Bendamustine is primarily hydrolyzed to metabolites with low cytotoxic activity. Two active minor metabolites, γ -hydroxy bendamustine (M3) and N-desmethyl-bendamustine (M4), are primarily formed via CYP1A2. The parent drug does not induce or inhibit Cytochrome P450 enzymes. While the intermediate half-life of the parent compound is approximately 40 min, the mean apparent terminal elimination of the metabolites γ -hydroxy bendamustine and N-desmethyl-bendamustine are approximately 3 h and 30 min, respectively. 90% of the drug is eliminated unmetabolized, mostly in the feces. Due to pharmacogenetic predisposition, Japanese patients may have higher exposure than non-Japanese subjects to identical doses.

Adverse Effects The most common hematologic abnormalities for both indications (indolent B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia) are lymphopenia, anemia, leukopenia, thrombocytopenia, and neutropenia. Most common non-hematologic adverse reactions in chronic lymphocytic leukemia are pyrexia, nausea and vomiting. Most common non-hematologic adverse reactions for non-Hodgkin lymphoma are nausea and vomiting, diarrhea or constipation, anorexia, headache, fatigue, pyrexia, cough, dyspnea, rash, and stomatitis. Dose reduction or delayed administration is required if grade 3 or grade 4 toxicities occur. Dose re-escalation in subsequent cycles may be considered. Tumor lysis syndrome can arise within the first treatment cycle. Without intervention it may lead to acute renal failure and death. Preventive measures include maintaining adequate volume status, and close monitoring of blood chemistry, particularly potassium and uric acid levels. Allopurinol may be used during the beginning of bendamustine therapy, however, it poses an increased risk of severe

skin toxicity (Stevens-Johnson syndrome or the more severe manifestation of toxic epidermal necrolysis).

Contraindications are hypersensitivities to bendamustine or mannitol (in rare instances severe anaphylactic and anaphylactoid reactions can occur, particularly in the second and subsequent cycles of therapy), renal impairment with creatinine clearance below 40 mL/min, or moderate to severe hepatic impairment. The agent is Pregnancy Category D.

Drug Interactions Because the active bendamustine metabolites, γ -hydroxy bendamustine and N-desmethyl-bendamustine, are formed via Cytochrome P450 CYP1A2, the concomitant intake of CYP1A2 inhibitors (such as fluvoxamine <Luvox> or ciprofloxacin <Cipro>) has the potential to increase the exposure to bendamustine and decrease the exposure to the active metabolites. Conversely, inducers of CYP1A2 (including cigarette smoke and omeprazole <Losec, Antra, Gastroloc, Mopral, Omepral, Prilosec>) have the potential to decrease the concentration of bendamustine and increase the concentrations of its active metabolites in the body.

Spiromustine (spirohydantoin mustard) (NSC 172112) is a nitrogen alkylating agent that contains a lipophilic hydantoin group, which serves as a carrier to cross the blood-brain barrier. This lipophilicity may also enhance alkylating activity against tumors outside the brain. Spiromustine forms covalent linkages with nucleophilic centers in DNA, causing depurination, base pair miscoding, strand scission, and DNA cross-linking, which may result in cytotoxicity. The agent acts in a cell cycle non-specific manner.

Pharmacokinetics In an aqueous environment, spiromustine is rapidly hydrolyzed. The drug has a biphasic plasma decay curve, with hepatic metabolism and excretion, enterohepatic circulation of metabolites, and approximately 50% renal excretion of the unmetabolized drug.

Third generation nitrogen mustards Uracil mustard (uramustine, 5-[bis(2-chloroethyl)amino]-1*H*-pyrimidine-2,4-dione) <Dopan> is a third generation nitrogen mustard that can be absorbed from the gastrointestinal tract. The drug is a bifunctional alkylating agent that acts in a cell cycle phase non-specific manner. Its activity is a result of the formation of an unstable ethylenimmonium ion. Uracil mustard has been administered to treat chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), Hodgkin lymphoma, non-Hodgkin lymphomata of the histiocytic or lymphocytic type, lymphosarcoma, breast cancer, and ovarian cancer. It has generated response rates of 65–75% in hematologic malignancies (Kennedy 1999), although its use has generally been replaced by that of other agents. The drug can be taken orally and is available as capsules. It is usually taken once a week for at least 4 weeks. The typical oral adult dose is 150 μ g/kg; the typical oral pediatric dose is 300 μ g/kg of body weight.

Pharmacokinetics The blood levels of uracil mustard drop to essentially undetectable levels within 2 h of administration. Less than 1 % of the dose is excreted unchanged in the urine.

Adverse Effects Uracil mustard is relatively well tolerated and does not cause alopecia. Adverse effects comprise nausea and vomiting, diarrhea, and dermatitis. They can also include nervousness, irritability, and depression. The bone marrow depressant effects of uracil mustard may result in an increased incidence of microbial infections, delayed healing, and bleeding. Dental work should be completed prior to initiation of therapy or deferred until the blood counts have returned to normal. Uracil mustard may rarely cause stomatitis, associated with considerable discomfort.

Drug Interactions Uracil mustard can raise the concentration of blood uric acid. Drug interactions may arise with anti-gout agents, such as allopurinol, colchicines, probenecid, or sulfipyrazone. Dosage adjustment may be necessary. Allopurinol may be preferred to prevent or reverse uracil mustard induced hyperuricemia and the risk of uric acid nephropathy.

Steroid-coupled nitrogen mustards Estramustine phosphate sodium (estradiol 3-[bis(2-chloroethyl)carbamate] 17-(dihydrogen phosphate), disodium salt, monohydrate) <Emcyt> is an orally available synthetic drug that combines estradiol and mechlorethamine through a carbamate link. The molecule was designed with the intent that its estradiol portion would facilitate uptake of the alkylating agent into hormone sensitive prostate cancer cells. It is phosphorylated for better water solubility. This agent exhibits anti-androgenic effects and is used for the palliative treatment of metastatic or progressive prostate cancer. In the mid-1980s, the classification of estramustine as an alkylating agent was called into doubt as it may act as an anti-microtubule agent. Estramustine and its major metabolite bind covalently to microtubule-associated proteins (MAPs) and Tubulin, thereby causing their separation from the microtubules, inhibiting microtubule assembly, and eventually causing their disassembly.

Estramustine is taken orally, at least 1 h before or 2 h after meals. The recommended daily dose is 14 mg/kg body weight, given in three or four doses. Patients should be treated for 30–90 days before a determination is made of the possible benefits of continued therapy. Therapy should be continued as long as the favorable response lasts, and may extend for years.

Pharmacokinetics Estramustine phosphate taken orally is readily dephosphorylated during absorption, and the major metabolites in the blood are estramustine, its estrone analog, and estradiol. Prolonged treatment produces elevated total blood concentrations of estradiol. The metabolic urinary patterns of the estradiol moiety of estramustine phosphate and estradiol itself are very similar, although the metabolites derived from estramustine phosphate are excreted at a slower rate. Estramustine may be poorly metabolized in patients with impaired liver function.

Molecular Therapies of Cancer

Weber, G.F.

2015, XV, 488 p. 120 illus., 86 illus. in color., Hardcover

ISBN: 978-3-319-13277-8