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BIS-CHLOROETHYL AMINO COMPONENTS AS ALKYLATING AGENTS ON NUCLEOTIDES VIA FORMATION OF AZIRIDINIUM CATION

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ABSTRACT

Alkylating agents were one of the earliest classes of drugs used to treat cancer, beginning in the 1940's. The biggest weakness of most cancer cells is that they are very sensitive to DNA damage. Alkylating agents work by reacting with the proteins that bond together to form the very delicate double helix structure of a DNA molecule, adding an alkyl group to some or all of them. This prevents the proteins from linking up as they should, cause breakage of the DNA strands and eventually, the death of the cancer cell. This phenomenon is essentially a mutation that takes away the cancer cell's ability to multiply. While there are many different classes of alkylating agents, they all work by this same chemical mechanism. Alkylating chemotherapy drugs have this effect on a cancer cell during every phase of its life cycle, making them desirable for use on a wide range of cancers. The most benefit is seen in their use to treat cancers that grow slowly, like solid tumors and leukemia, but they are also used to treat lung cancer, ovarian cancer, breast cancer, lymphomas, sarcomas, myelomas and Hodgkin's disease. The five major categories of alkylating agents are nitrogen mustards, nitrosoureas, alkyl sulfonates, triazines and ethylenimines. The following gives a more detailed description of the most popular drugs in each category. In all cases, dosage and schedule are determined on an individual basis, considering the patient's size, overall health and the type of cancer being treated. Mechlorethamine, marketed under the trade name Mustargen®, is given by injection to treat Hodgkin's disease and non-Hodgkin's lymphoma and as a palliative therapy for breast and lung cancers and given as a topical treatment for

skin lesions of mycosis fungoides (cutaneous T-cell lymphoma). Ifosfamide, sold under the trade name Ifex®, is used to treat both Hodgkin's and non-Hodgkin's lymphoma, as well as recurrent testicular cancer and germ cell tumors, sarcomas, lung cancer, bladder cancer, head and neck cancer and cervical cancer. It is administered intravenously. Melphalan is a chemotherapy drug sold under the brand name Alkeran® and is also referred to as L-PAM or phenylalanine mustard. It is used to treat multiple myeloma, ovarian cancer, neuroblastoma, rhabdomyosarcoma and breast cancer. It comes as a 2 milligram pill to be taken daily on an empty stomach. More rarely, it can be administered by injection. Chlorambucil is sold by the trade name Leukeran® and is most widely used to treat chronic lymphocytic leukemia, malignant lymphomas including lymphosarcoma, giant follicular lymphoma and Hodgkin's disease. It has also been successfully used to treat non-Hodgkin's lymphoma, breast, ovarian and testicular cancer, Waldenstrom's macroglobulinemia, thrombocythemia and choriocarcinoma. It comes in coated tablet form. Cyclophosphamide is marketed as Cytoxan® or Neosar® and is used to treat Hodgkin's and non-Hodgkin's lymphoma, Burkitt's lymphoma, chronic lymphocytic leukemia, chronic myelocytic leukemia, acute myelocytic leukemia, acute lymphocytic leukemia, t-cell lymphoma, multiple myeloma, neuroblastoma, retinoblastoma, rhabdomyosarcoma, Ewing's sarcoma; breast, testicular, endometrial, ovarian and lung cancers. Because of the wide variety of cancers it treats, there is also a wide range of administering options. The most common methods are by intravenous injection or mouth in the form of tablets. Tablets should be taken with food and not tampered with. Less commonly, this drug is also approved to be injected directly into muscle, abdominal lining, or lung lining.

KEYWORDS: Sulfur mustard, Nitrogen mustard, DNA, Cytotoxicity, Mitosis, Aziridine, Crosslinking, Intercalation.

INTRODUCTION

Sulfur mustard, commonly known as mustard gas, is a cytotoxic and vesicant chemical warfare agent with the ability to form large blisters on the exposed skin and in the lungs. Related chemical compounds with similar chemical structure and similar properties form a class of compounds known collectively as sulfur mustards or mustard agents. Pure sulfur mustards are colorless, viscous liquids at room temperature. When used in impure form, such as warfare agents, they are usually yellow-brown in color and have an odor resembling mustard plants, garlic, or horseradish, hence the name. Sulfur mustard was originally

assigned the name LOST, after the scientists Wilhelm Lommel and Wilhelm Steinkopf, who developed a method for the large-scale production for the Imperial German Army in 1916.

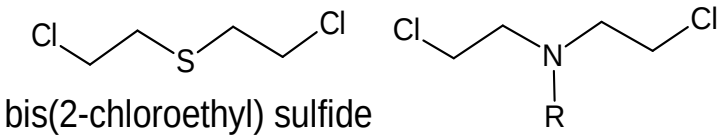


Figure-1: Sulfur mustard & Nitrogen mustard

Mustard agents are regulated under the 1993 Chemical Weapons Convention (C.W.C.). Three classes of chemicals are monitored under this Convention, with sulfur and nitrogen mustard grouped in Schedule 1, as substances with no use other than in chemical warfare. Mustard agents could be deployed on the battlefield by means of artillery shells, aerial bombs, rockets, or by spraying from warplanes.

The **nitrogen mustards** are cytotoxic chemotherapeutic agents similar to mustard gas. Although their common use is medicinal, in principle these compounds can also be deployed as chemical warfare agents. Nitrogen mustards are nonspecific DNA alkylating agents. Nitrogen mustard gas was stockpiled by several nations during the 2nd World War, but it was never used in combat. As with all types of mustard gas, nitrogen mustards are powerful and persistent blister and the main examples (**HN1, HN2 & HN3**) are therefore classified as Schedule 1 within the Chemical Weapons Convention. Production and use is therefore strongly restricted. During WWII nitrogen mustards were studied at the Yale School of Medicine by Alfred Gilman and Louis Goodman and classified human clinical trials of nitrogen mustards for the treatment of lymphoma started in December 1942. Also during WWII, an incident during the air raid on Bari, Italy, led to the release of mustard gas that affected several hundred soldiers and civilians. Medical examination of the survivors showed a decreased number of lymphocytes. After WWII was over, the Bari incident and the Yale group's studies eventually converged prompting a search for other similar compounds. Due to its use in previous studies, the **nitrogen mustard** known as HN2 became the first chemotherapy drug mustine. Nitrogen mustards are not related to the mustard plant or its pungent essence, allyl isothiocyanate: the name comes from the pungent smell of chemical weapons preparations.

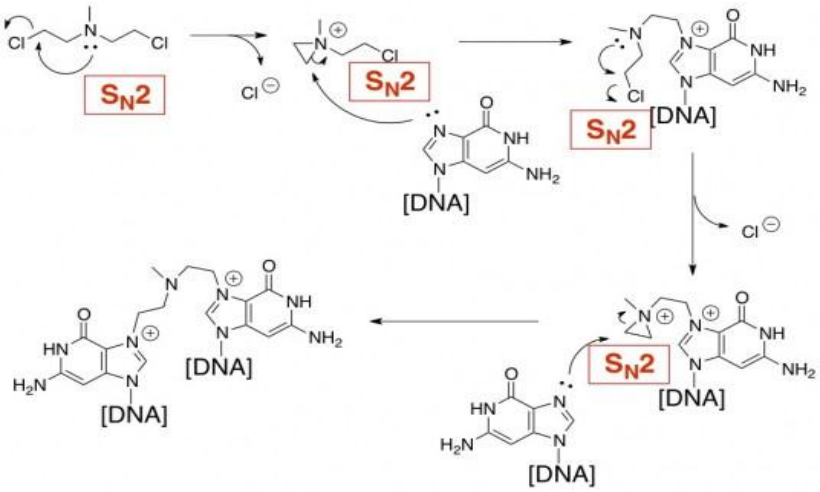
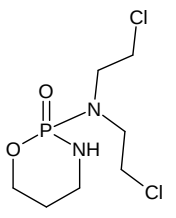


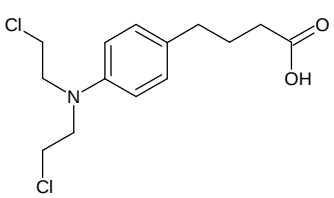
Figure-2: Alkylating action of nitrogen mustards

The original nitrogen mustard drug, mustine (HN2), is no longer commonly in use because of excessive toxicity. Other nitrogen mustards developed as treatments include cyclophosphamide, chlorambucil, uramustine, ifosfamide, melphalan, and bendamustine. Bendamustine has recently reemerged as a viable chemotherapeutic treatment. Cyclophosphamide (INN), also known as cytophosphane, is a medication mainly used in chemotherapy. It is an alkylating agent of the nitrogen mustard type (specifically, the oxazaphosphorine group). An alkylating agent adds an alkyl group to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. This interferes with DNA replication by forming intrastrand and interstrand DNA crosslinks.[1]

Cyclophosphamide is used to treat cancers, autoimmune disorders and AL amyloidosis. As a prodrug, it is converted by liver cytochrome P450 (CYP) enzymes to form the metabolite 4-hydroxy cyclophosphamide that has chemotherapeutic activity. Cyclophosphamide has severe and life-threatening adverse effects, including acute myeloid leukemia, bladder cancer, hemorrhagic cystitis and permanent infertility, especially at higher doses. For autoimmune diseases, doctors often substitute less-toxic methotrexate or azathioprine after an acute crisis.



N,*N*-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide

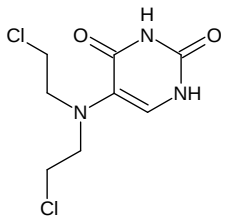


4-{4-[bis(2-chloroethyl)amino]phenyl}butanoic acid

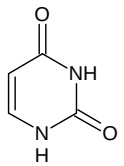
Figure-3: Cyclophosphamide and Chlorambucil as alkylating agent

It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system. The abbreviation CP is common, but abbreviating drug names is not best practice in medicine.

The main effect of cyclophosphamide is due to its metabolite phosphoramide mustard. This metabolite is only formed in cells that have low levels of ALDH (aldehyde dehydrogenase). Phosphoramide mustard forms DNA crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand cross linkages, respectively). This is irreversible and leads to cell apoptosis. Cyclophosphamide has relatively little typical chemotherapy toxicity as ALDHs are present in relatively large concentrations in bone marrow stem cells, liver and intestinal epithelium. ALDHs protect these actively proliferating tissues against toxic effects of phosphoramide mustard and acrolein by converting aldophosphamide to carboxycyclophosphamide that does not give rise to the toxic metabolites phosphoramide mustard and acrolein. This is because carboxycyclophosphamide cannot undergo β-elimination (the carboxylate acts as an electron-donating group, forbidding the transformation), preventing nitrogen mustard activation and subsequent alkylation.[2]



5-[bis(2-chloroethyl)amino]pyrimidine-2,4(1*H*,3*H*)-dione



pyrimidine-2,4(1*H*,3*H*)-dione

Figure-4: Uramustine and Uracil as alkylating agent

Cyclophosphamide induces beneficial immunomodulatory effects in adaptive immunotherapy. Suggested mechanisms include: (1) Elimination of T regulatory cells (CD4+CD25+ T cells) in naive and tumor-bearing hosts. (2) Induction of T cell growth factors, such as type I IFNs, and/or. (3) Enhanced grafting of adoptively transferred, tumor-reactive effector T cells by the creation of an immunologic space niche. Thus, cyclophosphamide preconditioning of recipient hosts (for donor T cells) has been used to enhance immunity in naïve hosts and to enhance adoptive T cell immunotherapy regimens, as well as active vaccination strategies, inducing objective antitumor immunity.

Nitrogen mustards that can be used for chemical warfare purposes are tightly regulated. Their weapon designations are:

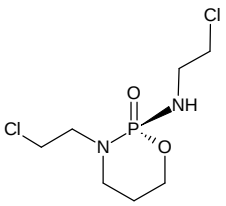
Uramustine (INN) or uracil mustard is a chemotherapy drug which belongs to the class of alkylating agents. It is used in lymphatic malignancies such as non-Hodgkin's lymphoma. It works by damaging DNA, primarily in cancer cells that preferentially take up the uracil due to their need to make nucleic acids during their rapid cycles of cell division. The DNA damage leads to apoptosis of the affected cells. Bone marrow suppression and nausea are the main side effects.

Chemically it is a derivative of nitrogen mustard and uracil.

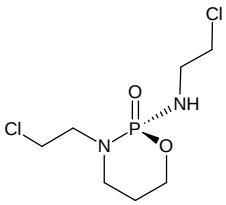
Ifosfamide (also marketed as Ifex) is a nitrogen mustard alkylating agent used in the treatment of cancer. It is sometimes abbreviated as "IFO". It is on the WHO Model List of Essential Medicines, the most important medications needed in a basic health system.

It is given as a treatment for a variety of cancers, including: (1) Testicular cancer (2) Breast cancer (3) Lymphoma (Hodgkin and non-Hodgkin) (4) Soft tissue sarcoma (5) Osteosarcoma or bone tumor (6) Lung cancer (7) Cervical cancer (8) Ovarian cancer.

Administration: It is a white powder which, when prepared for use in chemotherapy, becomes a clear, colorless fluid. The delivery is intravenous. Ifosfamide is often used in conjunction with mesna to avoid internal bleeding in the patient, in particular hemorrhagic cystitis. Ifosfamide is given quickly and in some cases can be given as quickly as an hour.



(2*R*)-*N*,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide

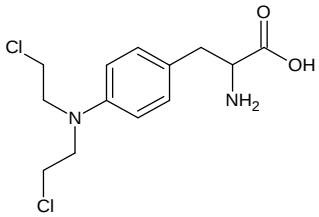


(2*S*)-*N*,3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amine 2-oxide

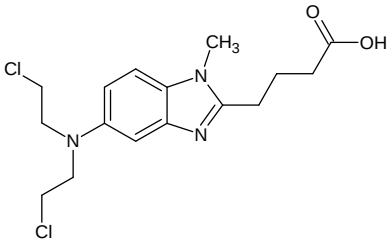
Figure-5: Ifosfamide as alkylating agent

Melphalan (trade name Alkeran, in former USSR also known as Sarcolysin) is a chemotherapy drug belonging to the class of nitrogen mustard alkylating agents. An alkylating agent adds an alkyl group (C_nH_{2n+1}) to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. Otherwise known as L-phenylalanine mustard, or L-PAM, melphalan is a phenylalanine derivative of mechlorethamine. Melphalan chemically alters through alkylation of the DNA nucleotide guanine, and causes linkages between strands of DNA. This chemical alteration inhibits DNA

synthesis and RNA synthesis, functions necessary for cells to survive. These changes cause cytotoxicity in both dividing and non-dividing tumor cells. It is used to treat multiple myeloma, ovarian cancer, AL amyloidosis and occasionally malignant melanoma. The agent was first investigated as a possible drug for use in melanoma, it was not found to be effective.[3]



2-amino-3-{4-[bis(2-chloroethyl)amino]phenyl}propanoic acid



4-{5-[bis(2-chloroethyl)amino]-1-methyl-1*H*-benzimidazol-2-yl}butanoic acid

Figure-6: Melphalan and Bendamustine as alkylating agent

On March 15, 2016 it was approved by the U.S. FDA under the trade name Evomela for use as a high-dose conditioning treatment prior to hematopoietic progenitor (stem) cell transplantation in multiple myeloma (MM) patients the palliative treatment of MM patients for whom oral therapy is not appropriate. Melphalan is currently being used to treat ocular retinoblastoma, a pediatric solid tumor. This is accomplished via transarterial catheter based slow pulsed infusion into the ophthalmic artery.

Bendamustine (INN, trade names Treakisym, Ribomustin, Levact and Treanda; also known as SDX-105) is a nitrogen mustard used in the treatment of chronic lymphocytic leukemia and lymphomas. It belongs to the family of drugs called alkylating agents. It is also being studied for the treatment of sarcoma. It is also being investigated in phase II trials for the non-cancer treatment of AL amyloidosis. It is on the World Health Organization's List of Essential Medicines, a list of the most important medications needed in a basic health system.

Bendamustine is a white, water-soluble microcrystalline powder with amphoteric properties. It acts as an alkylating agent causing intra-strand and inter-strand cross-links between DNA bases. After intravenous infusion it is extensively metabolised in the liver by cytochrome p450. More than 95% of the drug is bound to protein - primarily albumin. Only free bendamustine is active. Elimination is biphasic with a half-life of 6–10 minutes and a terminal half-life of approximately 30 minutes. It is eliminated primarily through the kidneys.

Cisplatin is a chemotherapy agent. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum

complexes react in the body, binding to DNA and causing the DNA strands to crosslink, which ultimately triggers cells to die in a programmed way. Cisplatin was discovered in 1972. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system. Cisplatin interferes with DNA replication, which kills the fastest proliferating cells, which in theory are carcinogenic. Following administration, one of the two chloride ligands is slowly displaced by water to give the aquo complex *cis*-$[PtCl(NH_3)_2(H_2O)]^+$, in a process termed aquation.

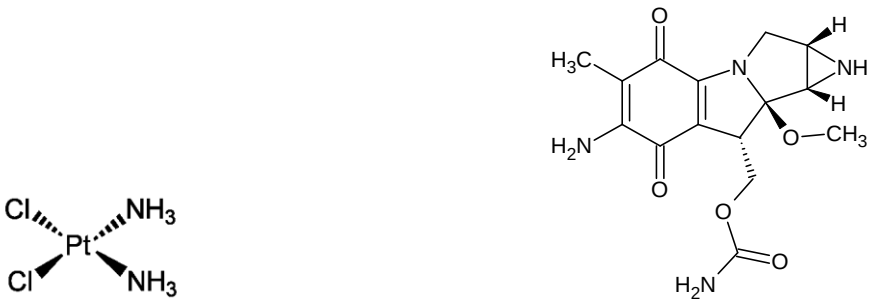


Figure-7: Cisplatin and Mitomycin C as alkylating agent

Dissociation of the chloride ligand is favored inside the cell because where the background chloride concentration is 3–20% of the approximately 100 mM chloride concentration in the extracellular fluid. The aqua ligand in *cis*-$[PtCl(NH_3)_2(H_2O)]^+$is itself easily displaced by the N-heterocyclic bases on DNA. Guanine preferentially binds. Subsequent to formation of $[PtCl(guanine\text{-}DNA)(NH_3)_2]^+$, crosslinking can occur via displacement of the other chloride ligand, typically by another guanine. Cisplatin crosslinks DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible. In 2008, researchers were able to show that the apoptosis induced by cisplatin on human colon cancer cells depends on the mitochondrial serine-protease Omi/Htra2. Since this was only demonstrated for colon carcinoma cells, it remains an open question if the Omi/Htra2 protein participates in the cisplatin-induced apoptosis in carcinomas from other tissues.[4]

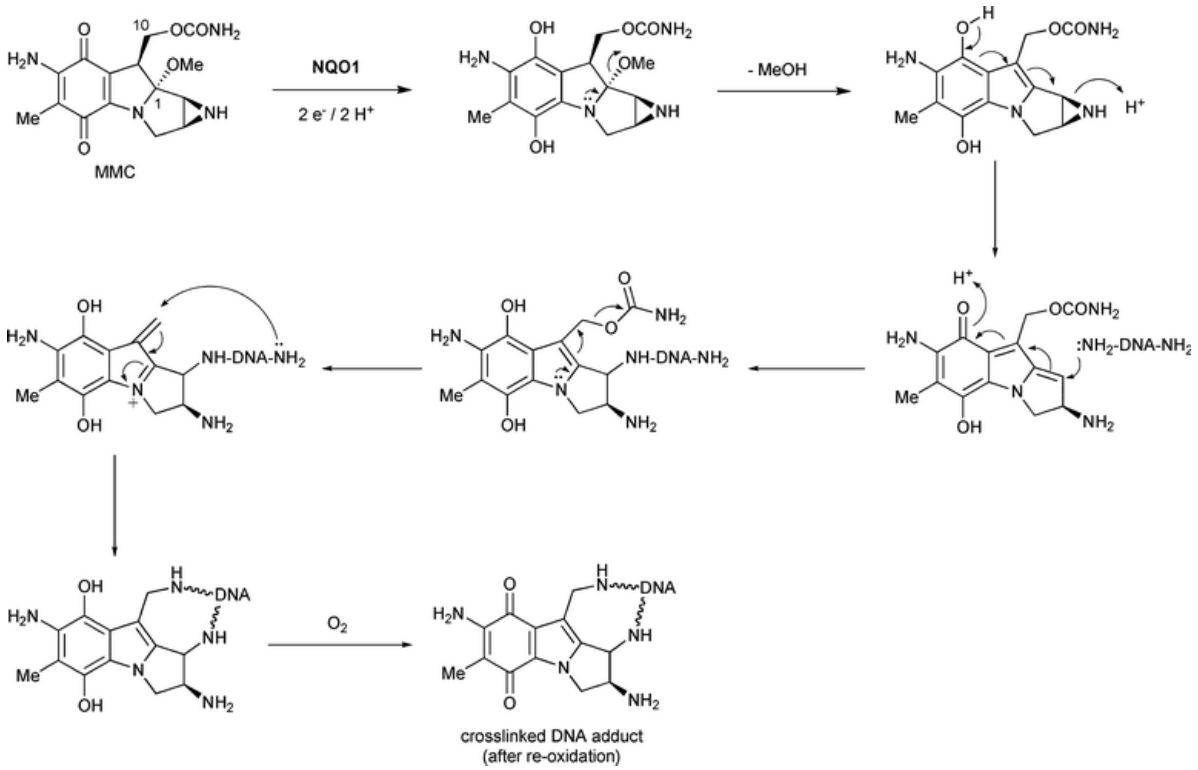


Figure-8: Mitomycin C as alkylating agent

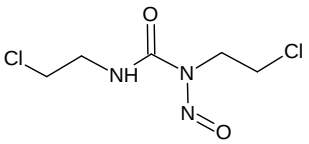
Most notable among the changes in DNA are the 1,2-intrastrand cross-links with purine bases. These include 1,2-intrastrand d(GpG) adducts which form nearly 90% of the adducts and the less common 1,2-intrastrand d(ApG) adducts. 1,3-intrastrand d(GpXpG) adducts occur but are readily excised by the nucleotide excision repair (NER). Other adducts include inter-strand crosslinks and nonfunctional adducts that have been postulated to contribute to cisplatin's activity. Interaction with cellular proteins, particularly HMG domain proteins, has also been advanced as a mechanism of interfering with mitosis, although this is probably not its primary method of action. Although cisplatin is frequently designated as an alkylating agent, it has no alkyl group and it therefore cannot carry out alkylating reactions. It is correctly classified as alkylating-like.

Mitomycin C is a mitomycin that is used as a chemotherapeutic agent by virtue of its anti-tumour activity. It is given intravenously to treat upper gastro-intestinal cancers (e.g. esophageal carcinoma), anal cancers and breast cancers, as well as by bladder instillation for superficial bladder tumours. It causes delayed bone marrow toxicity and therefore it is usually administered at 6-weekly intervals. Prolonged use may result in permanent bone-marrow damage. It may also cause lung fibrosis and renal damage. Mitomycin C has also been used topically rather than intravenously in several areas. The first is cancers, particularly bladder cancers and intraperitoneal tumours. It is now well known that a single instillation of this

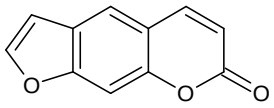
agent within 6 hours of bladder tumor resection can prevent recurrence. The second is in eye surgery where mitomycin C 0.02% is applied topically to prevent scarring during glaucoma filtering surgery and to prevent haze after PRK or LASIK; mitomycin C has also been shown to reduce fibrosis in strabismus surgery. The third is in esophageal and tracheal stenosis where application of mitomycin C onto the mucosa immediately following dilatation will decrease re-stenosis by decreasing the production of fibroblasts and scar tissue. Mitomycin C is a potent DNA crosslinker. A single crosslink per genome has shown to be effective in killing bacteria. This is accomplished by reductive activation of mitomycin to form a mitosene, which reacts successively via N-alkylation of two DNA bases. Both alkylations are sequence specific for a guanine nucleoside in the sequence 5'-CpG-3'. Potential bis-alkylating heterocylic quinones were synthetised in order to explore their antitumoral activities by bioreductive alkylation. Mitomycin is also used as a chemotherapeutic agent in glaucoma surgery.[5]

Carmustine (bis-chloroethylnitrosourea, BCNU, BiCNU) is a medication used mainly for chemotherapy and sometimes for immunosuppression before organ transplantation. It is a nitrogen mustard β-chloro-nitrosourea compound used as an alkylating agent. As a dialkylating agent, BCNU is able to form interstrand crosslinks in DNA, which prevents DNA replication and DNA transcription. It has the appearance of an orange-yellow solid.

Carmustine for injection was earlier marketed under the name BiCNU by Bristol-Myers Squibb and now by Emcure Pharmaceuticals. In India it is sold under various brand names, including Consium. It is used in the treatment of several types of brain cancer (including glioma, glioblastoma multiforme, medulloblastoma and astrocytoma), multiple myeloma and lymphoma (Hodgkin's and non-Hodgkin). BCNU is sometimes used in conjunction with alkyl guanine transferase (AGT) inhibitors, such as O6-benzylguanine. The AGT-inhibitors increase the efficacy of BCNU by inhibiting the direct reversal pathway of DNA repair, which will prevent formation of the interstrand crosslink between the N1 of guanine and the N3 of cytosine.



1,3-bis(2-chloroethyl)-1-nitrosourea



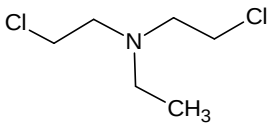
7*H*-furo[3,2-*g*]chromen-7-one

Figure-9: Carmustine and Psoralen as alkylating agent

It is also used as part of a chemotherapeutic protocol in preparation for hematological stem cell transplantation, a type of bone marrow transplant, in order to reduce the white blood cell count in the recipient (patient). Use under this protocol, usually with Fludarabine and Melphalan, was coined by oncologists at the University of Texas MD Anderson Cancer Center.

Psoralen (also called psoralene) is the parent compound in a family of natural products known as furo-coumarins. It is structurally related to coumarin by the addition of a fused furan ring and may be considered as a derivative of umbelliferone. Psoralen occurs naturally in the seeds of *Psoralea corylifolia*, as well as in the common fig, celery, parsley, West Indian satinwood and in all citrus fruits. It is widely used in PUVA (psoralen + UVA) treatment for psoriasis, eczema, vitiligo, and cutaneous T-cell lymphoma. Many furocoumarins are extremely toxic to fish, and some are deposited in streams in Indonesia to catch fish. Psoralen intercalates into the DNA double helix where it is ideally positioned to form adduct(s) with adjacent pyrimidine bases, preferentially thymine, upon excitation by an ultraviolet photon. Several physicochemical methods have been employed to derive binding constants for psoralen-DNA interactions. Classically, two chambers of psoralen and buffered DNA solution are partitioned by a semi-permeable membrane; the affinity of the psoralen for DNA is directly related to the concentration of the psoralen in the DNA chamber after equilibrium. Water solubility is important for two reasons: pharmokinetics relating to drug solubility in blood and necessitating the use of organic solvents (e.g. DMSO). Psoralens can also be activated by irradiation with long wavelength UV light. While UVA range light is the clinical standard, research that UVB is more efficient at forming photoadducts suggests that its use may lead to higher efficacy and lower treatment times. The photochemically reactive sites in psoralens are located at each of the carbon-carbon double bonds in the furan ring (the five-member ring) and the pyrone ring (the six-member ring). When appropriately intercalated adjacent to a pyrimidine base, a four-center photocycloaddition reaction can lead to the formation of either of two cyclobutyl-type monoadducts. Ordinarily, furan-side monoadducts form in a higher proportion. The furan monoadduct can absorb a second UVA photon leading to a second four-center photocycloaddition at the pyrone end of the molecule and hence the formation of a diadduct or cross-link. Pyrone monoadducts do not absorb in the UVA range and hence cannot form cross-links with further UVA irradiation. Another important feature of this class of compounds is their ability to generate singlet oxygen, although this process is in direct competition with adduct formation and may be an alternate

pathway for the dissipation of excited state energy. Research on psoralen has historically focused on interactions with DNA and RNA (in particular, ICL formation). Psoralen, however, has also been shown to block signaling of the ErbB2 receptor which is overexpressed in certain aggressive types of breast cancer. A synthetic derivative of 5-MOP, 5-(4-phenoxybutoxy)psoralen, shows promise as an immunosupressent by inhibiting a specific potassium channel. Its structure prevents intercalation into DNA, and it only very weakly produces singlet oxygen, majorly reducing unwanted toxicity and mutagenicity in vivo. This has implications for the treatment of various autoimmune diseases (e.g. multiple sclerosis, type-1 diabetes, and rheumatoid arthritis). While cell-surface modification and ion channel blocking are two newly discovered mechanisms of action, much research remains to be done. Psoralen originates from coumarins in the shikimate pathway; its biosynthesis is shown in the figure below. The aromatic ring in 6 is activated at positions ortho to the hydroxyl group, and is alkylated by 5, an alkylating agent. The dimethylallyl group in 7 then undergoes cyclization with the phenol group to give 8. This transformation is catalysed by a cytochome P-450-dependent monooxygenase17 (psoralen 5-monooxygenase), and cofactors (NADPH) and molecular oxygen. A second P-450-dependent monooxygenase enzyme (psoralen synthase) then cleaves off 10 (in the form of 11) from 8 to give 1. This pathway does not involve any hydroxylated intermediate, and cleavage is postulated to be initiated by a radical reaction.[6]



2-chloro-*N*-(2-chloroethyl)-*N*-ethylethanamine

Figure-10: Alkylating agent HN1

Alkylating agent HN1: Bis(2-chloroethyl)ethylamine

Bis(2-chloroethyl)ethylamine is the organic compound with the formula $C_2H_5N(CH_2CH_2Cl)_2$. Often abbreviated HN1, it is a powerful vesicant and a nitrogen mustard gas used for chemical warfare. HN1 was developed in the 1920s and 1930s to remove warts and later as a military agent. Because of the latter use, it is a Schedule 1 chemical within the Chemical Weapons Convention and therefore use and production is strongly restricted. It has never been used in warfare. It is an oily liquid with a colorless to pale yellow appearance and a faint fishy or musty odor. HN1 is also an alkylating agent. Nitrogen mustards react via an initial cyclization to the corresponding aziridinium salt. The rate of this reaction is pH dependent

because the protonated amine cannot cyclize. The aziridinium ion reacts with water in a slower reaction. At pH 8, the nitrogen mustards are essentially quantitatively converted to the aziridinium ion for subsequent slow reaction with water. In contrast, at pH 4 cyclization and hydrolysis show the classic form of reactions in series. Hydrolysis of HN1 produces toxic intermediates.

HN1 reacts with iron alloys, corroding them at and above 65°C (149°F) and reacts with metals in general, producing hydrogen gas. This can potentially cause explosions. Because HN1 is an alkylating agent, it damages DNA, causes immunosuppression and causes injury to areas that come into contact with it. Exposure to HN1 can be fatal, and its effects on skin and mucous membranes are worsened when they are moist. The alkylation effects cause damage to the spleen, bone marrow, and lymph nodes, which causes anemia, low white cell counts, and internal bleeding. The vesicant effects cause blistering and damage to the skin. The symptoms of exposure depend on the route of exposure.

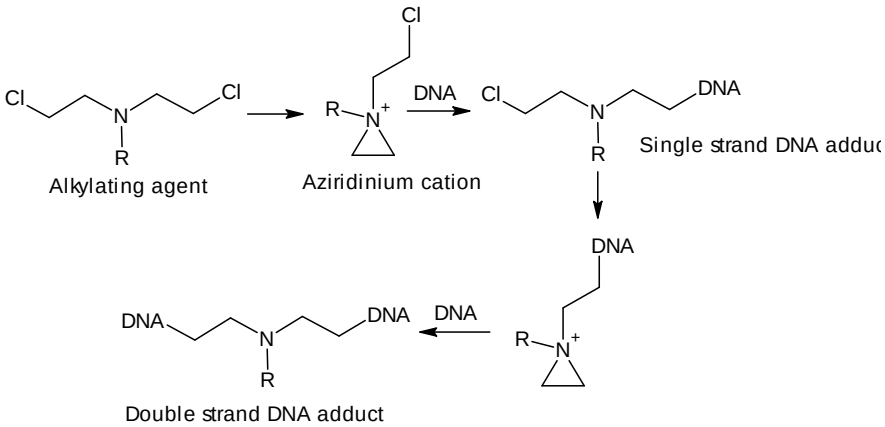


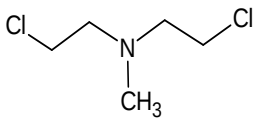
Figure-11: HN1/HN2/HN3 converting into aziridinium cation

Eye exposure to vapor can cause lacrimation (tears), blepharospasm (eyelid twitching), irritation, itching, burning pain, dry feeling, and sometimes miosis (pinpoint pupils). More severe vapor exposure can also cause swelling and fluid buildup (edema) in the eyelids, increased pain and redness. Very severe exposure to vapor or exposure to liquid can cause photophobia (aversion to light), corneal ulceration, and blindness. Inhaling the vapor causes symptoms that begin in the upper airway and expand to the lower airway. Increased concentrations cause worse symptoms. Mild inhalation exposure causes rhinorrhea (runny nose), sneezing, barking cough, epistaxis (nosebleed), dyspnea (shortness of breath) that affects smokers and asthmatics, hoarseness that turns into toneless voice, ageusia (loss of taste) and anosmia (loss of smell); later on, sinus and nose pain develops. With more severe

inhalation exposure, the airway becomes inflamed, pneumonia develops, and the respiratory epithelium can begin to have necrosis and slough off, forming a pseudomembrane that can occlude the airway. This occlusion can be fatal, as can the pneumonia. Skin contact with nitrogen mustard in low concentrations causes symptoms beginning with redness, then moving to blistering, itching and burning pain. More severe exposure can cause necrosis (cell death) in the blisters, and systemic toxicity, which causes malaise, vomiting, exhaustion, and fever. Skin exposure that causes symptoms over more than 25% of the body area is often fatal. Though ingestion is uncommon, nitrogen mustard can burn the GI tract and cause nausea, vomiting, hemorrhagic diarrhea and abdominal pain. Nitrogen mustard exposure does not cause symptoms until several hours to several days afterwards, but more severe exposure causes symptoms sooner. With severe exposure, eye injury can manifest within 1-2 hours, airway damage within 2-6 hours, and skin damage within 6-12 hours (sooner in hot or humid weather). Mild exposure takes longer to manifest symptoms: eye injury within 3-12 hours, airway damage within 12-24 hours, and skin damage up to 48 hours post-exposure. Long-term sequelae. Effects of nitrogen mustard exposure can be long-term or permanent; it is also a known carcinogen, reprotoxin, and developmental toxin after chronic and acute exposure, causing skin cancer and airway cancers in particular. Blindness from an acute exposure is usually temporary, resolving in days to months depending on severity. Chronic respiratory and eye infections are also common after acute nitrogen mustard exposure. Other consequences of acute exposure include ageusia, anosmia, pulmonary fibrosis, scarring, bronchitis, chronic respiratory disease, mental illness and central nervous system damage. Consequences of chronic exposure beyond cancer include permanent kidney damage and immunosuppression. Treatment for HN1 exposure is primarily supportive, since there is no antidote. First aid involves decontamination, irrigation, removing the affected person from the source of exposure, immediate medical attention, airway management (in cases of inhalation exposure) and medical monitoring of respiratory and cardiac function. If the affected person has trouble breathing (dyspnea) or stops breathing (apnea), ventilatory support and oxygen therapy can be helpful. If HN1 has been ingested, emetics (agents that induce vomiting) and gastric lavage are contraindicated, and nothing should be consumed by mouth because they could damage the gastrointestinal system.[7]

Alkylating agent HN2: Bis(2-chloroethyl)methylamine: Chlormethine (INN, BAN), mechlorethamine (widely used in the US, not the USAN, however) also known as mustine and HN2 and in former USSR known as Embichin is a nitrogen mustard sold under the brand

name Mustargen. It is the prototype of alkylating agents, a group of anticancer chemotherapeutic drugs. It works by binding to DNA, crosslinking two strands and preventing cell duplication. It binds to the N7 nitrogen on the DNA base guanine. As the chemical is a blister agent, its use is strongly restricted within the Chemical Weapons Convention where it is classified as a Schedule 1 substance.



2-chloro-*N*-(2-chloroethyl)-*N*-methylethanamine

Figure-12: Alkylating agent HN2

Mechlorethamine belongs to the group of nitrogen mustard alkylating agents. It has been derivatized into the estrogen analogue estramustine, used to treat prostate cancer. It can also be used in chemical warfare where it has the code-name HN2. This chemical is a form of nitrogen mustard gas and a powerful vesicant. Historically, some uses of mechlorethamine have included lymphoid malignancies such as Hodgkin's disease, lymphosarcoma, chronic myelocytic leukemia, polycythemia vera and bronchogenic carcinoma. Mechlorethamine is often administered intravenously, but when compounded into a topical formulation it can also be used to treat skin diseases. There have been studies demonstrating that topical administration of mechlorethamine has efficacy in mycosis fungoides-type cutaneous T cell lymphoma. Another important use of chlormethine is in the synthesis of meperidine. Mechlorethamine is a highly toxic medication, especially for women who are pregnant, breastfeeding, or of childbearing age. At high enough levels, exposure can be fatal.[8]

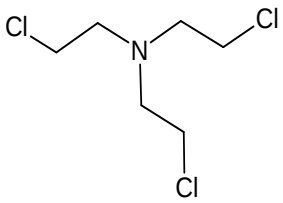
The adverse effects of mechlorethamine depend on the formulation. When used in chemical warfare, it can cause immunosuppression and damage to mucous membranes of the eyes, skin, and respiratory tract. Mucous membranes and damp or damaged skin are more affected by exposure to HN2. Though symptoms of exposure are generally delayed, the DNA damage it causes occurs very quickly. More serious exposures cause symptoms to develop sooner. Eye symptoms develop first, in the first 1–2 hours (severe exposure) or 3–12 hours (mild to moderate exposure) followed by airway (2-6/12–24 hours) and skin symptoms (6–48 hours). Hot, humid weather shortens the latent (symptom-free) period. Symptoms of toxic exposure to HN2 vary based on the route of exposure. Eye exposure causes lacrimation (tear production), burning, irritation, itching, a feeling of grittiness or dryness, blepharospasm

(spasms of the eyelid), and miosis (pinpoint pupils). More severe cases cause edema (swelling from fluid accumulation) in the eyelids, photophobia (extreme sensitivity to light), severe pain, corneal ulceration, and blindness. Inhalation of chlormethine damages the upper and lower airways sequentially, with more severe exposures causing faster damage that afflicts lower parts of the respiratory tract. Early symptoms include rhinorrhea (runny nose), epistaxis (nosebleed), toneless voice, sneezing, barking cough, and dyspnea (in smokers and asthmatics). Later symptoms include pain in the nose/sinuses and inflammation of the airway. In severe cases, there may be epithelial necrosis throughout the respiratory tract, causing pseudomembrane formation, which can obstruct the airway. Pneumonia may develop and prove fatal.[9]

Skin exposure mainly causes erythema (redness) and vesication (blistering) at first, but absorption through the skin causes systemic toxicity. In cases where more than 25% of the skin is affected, fatal exposure is likely to have occurred. Though ingestion is uncommon, if mechlorethamine is swallowed it causes severe chemical burns to the gastrointestinal tract and concomitant nausea, vomiting, diarrhea, abdominal pain, and hemorrhage. Long-term effects of acute or chronic chlormethine exposure are caused by damage to the immune system. White blood cell counts drop, increasing the risk of infection, and red blood cell and platelet counts may also drop due to bone marrow damage. Chronic eye infections may result from exposure, but blindness is temporary. Long-term effects on the respiratory system include anosmia (inability to smell), ageusia (inability to taste), inflammation, chronic infections, fibrosis, and cancer. Skin that has been damaged by HN2 can change pigmentation or become scarred, and may eventually develop cancer. Successful clinical use of chlormethine (mechlorethamine) resulted in development of the field of anticancer chemotherapy, led by Cornelius P. Rhoads at Memorial Sloan-Kettering. The drug is a nitrogen-based analogue of mustard gas (which is sulfur-based) and was derived from chemical warfare research. Secret clinical trials of the agent for Hodgkin's disease and several other lymphomas and leukemias in humans began in December 1942. Because of wartime secrecy restrictions, it was not until 1946 that the results of these trials were published openly. Chlormethine is combustible and under extreme conditions becomes explosive. It can react with metals to form gaseous hydrogen.

Alkylating agent HN3: Tris(2-chloroethyl)amine: Tris(2-chloroethyl)amine is the organic compound with the formula $N(CH_2CH_2Cl)_3$. Often abbreviated HN3, it is a powerful blister

agent and a nitrogen mustard gas (although it is not a gas) used for chemical warfare. HN3 was the last of the nitrogen mustard agents developed. It was designed as a military agent and is the only one of the nitrogen mustards that is still used for military purposes. It is the principal representative of the nitrogen mustards because its vesicant properties are almost equal to those of HD and thus the analogy between the two types of mustard is the strongest. As a vesicant the use and production is strongly restricted within the Chemical Weapons Convention where it is classified as a Schedule 1 substance.



2-chloro-*N*,*N*-bis(2-chloroethyl)ethanamine

Figure-13: Alkylating agent HN3

Nitrogen mustards react via an initial cyclization to the corresponding quaternary aziridine salt. The rate of this reaction is pH dependent because the protonated amine cannot cyclize. HN3 has found some applications in chemotherapy, e.g., for Hodgkin's disease, and in some compound Semiconductor research but it is mainly of interest for its military uses and is the only one of these agents that remains anywhere as a military agent. These agents are more immediately toxic than the sulfur mustards. HN3 can be absorbed into the body by inhalation, ingestion, eye contact, and skin contact (though inhalation is the most common). The chemical is extremely toxic and may damage the eyes, skin, and respiratory tract and suppress the immune system. HN3 penetrates and binds quickly to cells of the body, but its health effects develop slowly. The full extent of cellular injury may not be known for days. Nor-mustard can be used in the synthesis of piperazine drugs. For example, mazapertine, aripiprazole & fluanisone. Canfosfamide was also made from normustard.

Nitrogen mustards (NMs) form cyclic aminium ions (aziridinium rings) by intramolecular displacement of the chloride by the amine nitrogen. This aziridinium group then alkylates DNA once it is attacked by the N-7 nucleophilic center on the guanine base. A second attack after the displacement of the second chlorine forms the second alkylation step that results in the formation of interstrand cross-links (ICLs) as it was shown in the early 1960s. At that time it was proposed that the ICLs were formed between N-7 atom of guanine residue in a 5'-d(GC) sequence. These kinds of lesions are effective at forcing the cell to undergo apoptosis

via p53, a protein which scans the genome for defects. Note that the alkylating damage itself is not cytotoxic and does not directly cause cell death. Later it was clearly demonstrated that NMs form a 1,3 ICL in the 5'-d(GNC) sequence.[10]

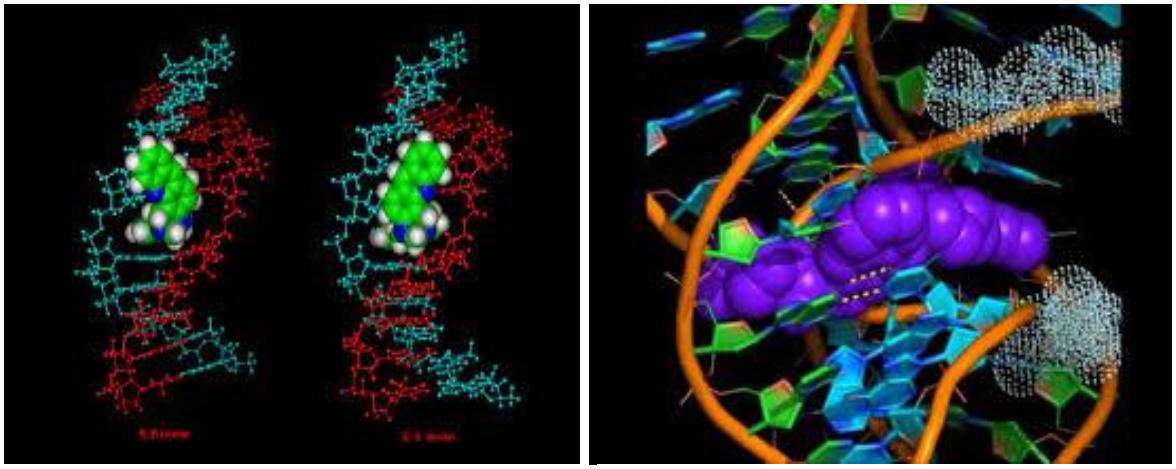


Figure-14: DNA intercalation

The strong cytotoxic effect caused by the formation of ICLs is what makes NMs an effective chemotherapeutic agent. Other compounds used in cancer chemotherapy that have the ability to form ICLs are cisplatin, mitomycin C, carmustine, and psoralen. Nitrogen Mustard, Mustine and Mechlorethamine Hydrochloride are other names for Mechlorethamine. In some cases, health care professionals may use the trade name Mustargen or other names Nitrogen Mustard, Mustine, and Mechlorethamine Hydrochloride when referring to the generic drug name Mechlorethamine.

CONCLUSION

An alkylating antineoplastic agent is an alkylating agent used in cancer treatment that attaches an alkyl group (C_nH_{2n+1}) to DNA. The alkyl group is attached to the guanine base of DNA, at the number 7 nitrogen atom of the purine ring. Since cancer cells, in general, proliferate faster and with less error-correcting than healthy cells, cancer cells are more sensitive to DNA damage—such as being alkylated. Alkylating agents are used to treat several cancers. However, they are also toxic to normal cells (cytotoxic), particularly cells that divide frequently, such as those in the gastrointestinal tract, bone marrow, testicles and ovaries, which can cause loss of fertility. Most of the alkylating agents are also carcinogenic. Hyperthermia is especially effective at enhancing the effects of alkylating agents. When nitrogen mustard is administered systemically, it acts as an alkylating agent with an anti-mitotic effect. The NM used in medicine include mechlorethamine, cyclophosphamide,

ifosfamide, melphalan and chlorambucil. Mechlorethamine is the only NM used as a topical agent in the management of cutaneous lymphomas. The alkylating agents have a common property of becoming strong electrophiles which then result in formation of covalent linkages by alkylation of various nucleophilic moieties. The cytotoxic effects are directly related to the alkylation of DNA. Mechlorethamine is a bifunctional alkylating agent with two 2-chlorethyl side chains. The 7 nitrogen atom of guanine residues in DNA is highly susceptible to the formation of a covalent bond with bifunctional alkylating agents such as NM. The modified guanine leads to DNA damage and ultimately causes cell death. The mechanism of action of topical NM is still uncertain. One theory suggests that NM has a unique effect on the immune mechanisms (e.g. immunostimulation) against MF. The toxic effects of mustard agent depend on its ability to covalently bind to other substances. The chlorine atom is spiked off the ethyl group and the mustard agent is transferred to a reactive sulphonium ion. This ion can bind to a large number of different biological molecules. Most of all it binds to nucleophiles such as nitrogen in the base components of nucleic acids and sulphur in SH-groups in proteins and peptides. Since mustard agent contains two "reactive groups", it can also form a bridge between or within molecules. Mustard agent can destroy a large number of different substances in the cell by means of alkylation and thereby influence numerous processes in living tissue. Dialkylating agents can react with two different 7-N-guanine residues, and, if these are in different strands of DNA, the result is cross-linkage of the DNA strands, which prevents uncoiling of the DNA double helix. If the two guanine residues are in the same strand, the result is called limpet attachment of the drug molecule to the DNA. Busulfan is an example of a dialkylating agent: it is the methanesulfonate diester of 1,4-butanediol. Methanesulfonate can be eliminated as a leaving group. Both ends of the molecule can be attacked by DNA bases, producing a butylene crosslink between two different bases. Monoalkylating agents can react only with one 7-N of guanine. Limpet attachment and monoalkylation do not prevent the separation of the two DNA strands of the double helix but do prevent vital DNA-processing enzymes from accessing the DNA. The final result is inhibition of cell growth or stimulation of apoptosis, cell suicide.

Nitrogen Mustard is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. Nitrogen Mustard is classified as an "alkylating agent." What Nitrogen Mustard is used for: As part of combination regimens in treatment of Hodgkin's disease, non-Hodgkin's lymphoma, As palliative chemotherapy in lung and breast cancers, As a lotion to skin lesions of mycosis fungoides (cutaneous T-cell lymphoma).

How Nitrogen Mustard is given: As an injection into the vein (intravenous, IV). Mechlorethamine is a vesicant. A vesicant is a chemical that causes extensive tissue damage and blistering if it escapes from the vein. The nurse or doctor who gives this drug must be carefully trained. If you notice redness or swelling at the IV site while you are receiving mechlorethamine, alert your health care professional immediately. Diluted solution applied to mycosis fungoides skin lesions. There is no pill form of mechlorethamine. The amount of mechlorethamine that you will receive and route depends on many factors, including your height and weight, your general health or other health problems, and the type of cancer or condition being treated. Your doctor will determine your dose and schedule. Important things to remember about the side effects of Nitrogen Mustard: Most people do not experience all of the side effects listed. Side effects are often predictable in terms of their onset and duration. Side effects are almost always reversible and will go away after treatment is complete. There are many options to help minimize or prevent side effects. There is no relationship between the presence or severity of side effects and the effectiveness of the medication.

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