Azathioprine and chlorambucil: mechanism of action and use in dermatology

Dr. Fiona Bateman BVSc MACVSc

Introduction
Systemic immune moderators have been used in both human and veterinary dermatology for the treatment of a wide variety of immune mediated and allergic conditions. While their use is off label (even within the human field), a large amount of evidence attests to their safety and efficacy. Both azathioprine and chlorambucil have been used as single agent or, more commonly, in combination therapy of a wide range of dermatological conditions.

Azathioprine
历史
Azathioprine was first developed as an anti-rejection drug for renal transplantation and was first used in combination with cortisone for this purpose in 1962. The discovery of azathioprine is one of the first examples of what is now termed ‘rational drug design’. George Hitchings and Gertrude Elion, researchers at Burroughs Wellcome Research Laboratories (now GlaxoSmithKline) were pioneers in drug development and attempted to identify cellular and molecular targets for which they then developed targeted drugs. One such drug was 6-MP (6-mercaptopurine), the precursor of azathioprine.

Hitchings and Elion hypothesized that the development of synthetic purine analogs may halt the growth of rapidly dividing cells. Thus they synthesized a variety of purine analogs, one of which was 6-MP. However, 6-MP proved to be rapidly metabolized in vivo, so in an attempt to increase its efficacy with the addition of an imidazole ring to the sulphur atom at position 6 (see Figure 1). The resulting compound, azathioprine, was more active and had a better safety profile than 6-MP. 1

![Figure 1. Chemical composition of azathioprine, showing addition of imidazole ring to sulphur at position 6 of 6-MP](image_url)

The long collaboration of Hitchings and Elion led to the development of some of the most successful drugs still used today, including allopurinol, pyramethamine (used to treat malaria), trimethoprim, acyclovir, and azidothymidine (AZT). Their discoveries were awarded (along with James Whyte Black) when in 1988, they received the Nobel Prize in Medicine.

Pharmacology
Thiopurines are prodrugs that exert their cytotoxicity after they have been metabolized intracellularly. Despite over 50 years of use in human medicine, their exact mechanism of action remains incompletely characterized. The most biologically active end product of the thiopurines (including azathioprine) are 6-thioguanine nucleotides (6-TGNs). 6-TGNs inhibit de novo synthesis of purines and are incorporated into DNA as a false base, which triggers cell cycle arrest and apoptosis via the DNA mismatch repair mechanism.2

Azathioprine is rapidly absorbed from the gastrointestinal tract following oral administration, with a half life of approximately 3 hours due to the rapid metabolism to 6-mercaptopurine (6-MP). The active
metabolites have a much longer half life, which allows for once daily dosing. Azathioprine is extensively metabolized (Figure 2), with only 2% excreted unchanged in the urine.

Azathioprine is reduced through non-enzymatic degradation to 6-MP \textit{in vivo}. This process occurs through nucleophilic attack by sulphahydryl compounds present in erythrocytes and body tissues. \(^3\) 6-MP is then metabolized by one of four pathways:

1. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT) – conversion to 6-thioinosine-5’-monophosphate (TIMP).
2. Thiopurine methyltransferase (TPMT) – catalyses S-methylation to 6-methyl mercaptopurine (inactive compound)
3. Xanthine oxidase (XO) – catalyses oxidation to 6-thiouric acid (inactive compound)
4. Aldehyde oxidase (AO) – conversion to 6-TGN hydroxylated metabolites (inactive compound)

The enzymatic competition for the 6-MP substrate is vigorous, with the effects of XO and AO activity leaving only 16% of the total dose of 6-MP for systemic distribution. \(^4\) Note this does not include TPMT activity, which will decrease the amount of 6-MP converted to TIMP further. The competing pathways are important in that blocking a metabolic pathway involved in the degradation of 6-MP (for example, through the use of a xanthane oxidase inhibitor such as allopurinol, or through low endogenous TPMT activity) can dramatically increase the available amount of 6-MP to be converted to active 6-TGNs, thereby drastically increasing the risk of severe adverse effects.

Once 6-MP is converted to TIMP, TIMP is then converted to 6-thioguanosine-5’-monophosphosphate (TGMP) in a 2 step process. TGMP is further metabolized through a series of reductases and kinases to form the 6-TGN metabolite, deoxy-6-thioguanosine-5’-triphosphate (dGS). dGS is then incorporated into DNA as a false base and triggers cell cycle arrest and apoptosis.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Figure2.png}
\caption{Metabolism of azathioprine to active 6-TGN metabolites.}
\end{figure}

Key: 6-thioguanine nucleotides (6-TGN), aldehyde oxidase (AO), xanthine oxidase (XO), azathioprine (AZA), 6-methyl mercaptopurine (6-MMP), 6-mercaptopurine (6-MP), hypoxanthine-guanine phosphoribosyl transferase (HGPRT), 6-thioinosine-5’-monophosphate (TIMP), methyl-6-thioinosine monophosphate (MeTIMP), Thiopurine methyltransferase (TPMT), to 6-thioguanosine-5’monophosphate (TGMP)

\section*{Mechanism of action}

The 6-TGN active metabolites disrupt the function of endogenous purines. While all cells are theoretically affected by this unstable base incorporated into DNA, RNA and proteins, lymphocytes are preferentially targeted by thiopurines. Lymphocytes rely on \textit{de novo} synthesis of purines and lack a purine salvage pathway. Thus they are most affected by the action of azathioprine on purine synthesis and metabolism. \(^5\) Azathioprine has a wide range of short and long term effects on the immune system, including:

- reversible reduction of monocyte numbers in circulation and tissues \(^6\)
- impaired synthesis of gamma globulin (IgM, IgG) in patients with rheumatoid disorders \(^7\)
- long term immunosuppression decreases the number of cutaneous Langerhans cells \(^8\)
- impaired responses of helper T cell dependent B cells \(^9\)
- impaired function of T suppressor cells \(^9\)
- impaired T cell lymphocyte function and IL-2 production \(^10\)
- interaction with Rac1, a triphosphate binding protein on T lymphocytes that mediates a costimulatory signal for T cell activation. Azathioprine inhibits Rac1, blocking the costimulatory signal and inducing Fas-associated apoptosis.\(^{11,12}\)

In addition to the effects of 6-TGN metabolites, purine *de novo* synthesis is also inhibited by methyl-6-thioinosine monophosphate (Me-TIMP). Inhibition of *de novo* purine synthesis contributes to immunosuppression and blocks proliferation of various lymphocyte lines, thereby contributing to the cytotoxic action of azathioprine.\(^{13}\)

**Azathioprine and TMPT activity**

TMPT is the predominant inactivation pathway of thiopurines in haemopoetic cells.\(^{13}\) The end product of AZA degradation through the TMPT pathway is 6-methyl mercaptopurine (6-MMP), an inactive and non-toxic molecule. Erythrocyte levels of TPMT have been found to correlate well with levels in lymphocytes, platelets, kidney and liver cells in humans.\(^1\) TMPT activity is genetically controlled, with several polymorphisms identified in humans. In a recent study of over 3000 patients, approximately 80% had normal TPMT activity 9% had above normal enzymatic activity and 10% had low TPMT activity. Additionally, 0.45% of patients had no detectable TPMT activity.\(^{14}\) Low activity is associated with an increased risk of leukopenia, intermediate levels are associated with the development of late onset leukopenia and high TPMT activity results is less immunosuppression by azathioprine. Determination of pre-treatment TPMT level has been advocated in human medicine to detect patients at risk for early onset neutropenia (i.e. those with no or low TPMT activity). However the significance of TPMT activity in dogs and cats remains poorly characterized.

Studies of TPMT activity in the dog indicate that average levels of erythrocyte TPMT activity are similar to that in humans, but that marked variation exists in the distribution of TPMT activity when compared to the human studies.\(^{15,17}\) Interestingly, no dog was found with deficient TPMT activity (comparable to the human ‘low activity’ group) and that the 6 of 299 dogs in one study that experienced marked leukopenia associated with azathioprine use, all of these dogs had intermediate to high TPMT activity.\(^{16}\) This suggests that there exists a different mechanism by which azathioprine-induced myelotoxicity is induced in the canine population when compared with humans.

In cats, average levels of TPMT activity were significantly lower than both humans and dogs, and cats also displayed large individual variations in the level of TPMT activity.\(^{18}\) This is consistent with the high level of myelosuppression and leukopenia seen when azathioprine is administered to cats, and further supports the recommendation that azathioprine not be used in this species.

Studies of azathioprine use in the horse are limited, but TPMT activity is reported to be lower than both dogs and cats.\(^{19}\) Interestingly, marked myelosuppression (which would be expected to be marked given the low TPMT activity) is infrequently seen in the horse.\(^{20}\) This may indicate that erythrocyte TPMT activity may not correlate to TPMT activity in other tissues (particularly the liver) or that other degradation pathways, such as XO or AO may be more important for the metabolism of 6-Mp to inactive compounds in this species.

**Indications in dermatology**

Dermatologic use of azathioprine remains off label in both human and veterinary literature. Azathioprine has been used in dermatological conditions for over 50 years, and its use is supported by numerous studies, case reports and expert opinion. However, by the strictest evidence-based medicine standards, the support for its use is not as strong as for a variety of newer medications, such and cyclosporine.\(^1\)
**Human**

### Table 1. Selected dermatologic diseases where azathioprine has shown to be of benefit

<table>
<thead>
<tr>
<th>Immunobullous disease</th>
<th>Photodermatitis</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bullous pemphigoid</td>
<td>Eczema</td>
<td>Contact dermatitis</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cicatricial pemphigoid</td>
<td>Actinic reticuloid</td>
<td>Chronic actinic dermatitis</td>
</tr>
<tr>
<td>Juvenile pemphigus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemphigus foliaceous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraneoplastic pemphigus</td>
<td></td>
<td>Erythema multiforme</td>
</tr>
<tr>
<td>Pemphigus erythematosus</td>
<td></td>
<td>Cutaneous lupos erythematousus</td>
</tr>
</tbody>
</table>

**Eczematous diseases**
- Psoriasis
- Atopic dermatitis

*Adapted from Patel, et. al.*

### Table 2. Selected dermatologic diseases where azathioprine may be of benefit

<table>
<thead>
<tr>
<th>Pemphigus foliaceous</th>
<th>Lupoid onychitis ** 23</th>
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<tr>
<td>Pemphigus vulgaris</td>
<td>Uveodermatologic syndrome</td>
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<tr>
<td>Superficial pemphigus complex</td>
<td>Epidermolysis bullosa acquisita 24</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Perianal fistula **</td>
</tr>
<tr>
<td>Vesicular cutaneous lupus erythematosus</td>
<td>Erythema multiforme 26</td>
</tr>
<tr>
<td>Cutaneous reactive histiocytosis ** 27</td>
<td>? Atopic dermatitis** 28</td>
</tr>
<tr>
<td>Idiopathic sterile granuloma and pyogranuloma 22</td>
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**Veterinary**

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**Adverse effects**

**Haematologic**

Cytopenias and severe bone marrow suppression have been reported in both the human and veterinary literature, and may occur weeks to months after initiating treatment. Humans may suffer from bone marrow suppression years after initiating therapy, as steady state levels of azathioprine in the blood may take months to years to achieve. While neutropenia is the most common cytopenia noted, anaemia and thrombocytopenia may also occur. TPMT genotype testing is advocated in human medicine to identify those individuals at risk for developing acute or delayed onset neutropenia. While TPMT activity has been measured in dogs, cats and horses, its relevance to the development of adverse effects is unknown. While pretreatment TPMT activity is a useful indicator of susceptible individuals in humans, continuous hematological monitoring is still mandatory in humans and animals. Leukopenia induced by azathioprine, while potentially life threatening, is usually reversible with discontinuation of treatment or a dose reduction.

**Gastrointestinal**

Nausea and vomiting, though widely reported in the human literature, are uncommonly encountered in veterinary medicine. Symptoms usually occur in the first few weeks of therapy and may self resolve. Administration of azathioprine with food or in a divided dose may help to reduce the incidence of gastrointestinal upset.

Hepatotoxicity is the second most common gastrointestinal side effect and is independent of TPMT activity. In most cases, hepatotoxicity is an unpredictable side effect and the mechanism of hepatocyte injury is poorly characterized. In dogs, elevation of alkaline phosphatase, alanine aminotransferase and bilirubin may be transient, in other cases they may be persistent and progressive leading to hepatic failure and death. In a small pilot study using azathioprine for the treatment of canine atopic dermatitis, serum levels of alanine aminotransferase and alkaline phosphatase rose in 83% of dogs by the second week of daily treatment, and clinical signs of hepatitis were reported in 3 of 12 dogs (25%) necessitating the need for removal from the study.
Pancreatitis has been associated with azathioprine use in both the human and veterinary literature. In humans, pancreatitis mostly occurs in patients with concurrent gastrointestinal disorders. In dogs, azathioprine is commonly used in combination therapy (usually with corticosteroids) so a direct causal relationship may be difficult to determine.

**Opportunistic infections**

Long term immunosuppression has been associated with an increased risk of the development of opportunistic infections. This may occur even in the absence of leukopenia and herpes simplex, herpes zoster and verrucae have been reported at a higher incidence in humans receiving combination azathioprine and cortisone therapy.

Opportunistic infections have been reported in dogs, but the overall prevalence of opportunistic infections is animals on immunosuppressive doses is low.

**Carcinogenesis**

Controversy exists as to the potential link between long term immunosuppressive therapy with azathioprine and increased risk of malignancy. While some authors suggest that there is currently no evidence that thiopurine therapy is associated with an increased risk of malignancy, other studies indicate that dermatology patients on long-term azathioprine therapy may be at risk of developing aggressive squamous cell carcinoma, particular where the patient has had excessive exposure to UV light. Finally, yet further studies have indicated that while an increase risk of squamous cell carcinoma is present in renal transplant patients, it appears to be independent of the drug used. In this study, either long term cyclosporine and azathioprine with or without corticosteroids showed no difference in cancer risk between the groups.

The method by which carcinogenesis is proposed to occur (if at all) is due to the incorporation of 6-TGN metabolites into DNA. Once this process occurs, the DNA becomes prone to oxidation due to the high reactivity of the thiobase. Exposure to UVA light destabilises the double helix and sensitises the cell to the mutagenic effect of UV light, which is believed to be one of the causes of azathioprine-related malignancies, in particular squamous cell carcinoma. Additionally, azathioprine causes inactivation of the mismatch repair system in myeloid precursor cells which can lead to development of drug-resistant cells.

**Hypersensitivity reactions**

Rare reports exist of azathioprine hypersensitivity syndrome in humans. Clinical signs may include hypotension, shock, urticarial or vasculitic eruption, fever and rhabdomyolysis. To the authors knowledge, similar reactions have not been reported in the veterinary literature.

**Miscellaneous**

When administered with isotretinoin, azathioprine has been reported to induce curling of the hair in humans.

**Contraindications and drug interactions**

Azathioprine should not be used in patients with a known hypersensitivity to the drug. In addition dosage adjustments may need to be made in cases of renal or hepatic insufficiency. As the safety margin for use in cats is extremely low, it is not recommended for use in this species. Use in pregnant animals should be with care as azathioprine is both mutagenic and teratogenic in lab animals, though no clear-cut relationship between the drug and sporadic reports of human congenital anomalies has been accepted. Use should only be considered where the benefits clearly outweigh the risk and clients should be adequately counselled. There is no evidence that azathioprine produces gonadotoxicity or infertility in humans.

Xanthine oxidase inhibitors such as allopurinol, should not be used in combination with azathioprine where possible. Allopurinol inhibits the metabolism of azathioprine to inactive metabolites, thereby increase the amount of azathioprine available for metabolism to 6-TGNs. If allopurinol must be used, the dose of azathioprine should be reduced by at least 2/3rds, however the risk of myelotoxicity remains.

Angiotensin-converting enzyme inhibitors (ACEIs) have been shown to potentiate the effects of azathioprine in humans. Additionally, trimethoprim-sulfamethoxazole is an antimetabolite that has a synergistic effect in inhibiting bone marrow proliferation. However, the clinical significance of the interaction of these drugs with azathioprine has not been seen in the non-renal transplant setting. Sulfasalazine is an inhibitor of TPMT activity and may potentiate azathioprine toxicity. Current therapeutic guidelines advise against concurrent use of these medications with azathioprine where possible.

Warfarin resistance has been reported in the humans, however azathioprine toxicity is not enhanced by warfarin, rather the effects of warfarin are reduced. While this is a notable drug interaction in the human
literature, warfarin is rarely used therapeutically in animals and may be less of a concern in veterinary medicine.

**Dosage and monitoring**

Azathioprine (Imuran®, GlaxoSmithKline) is available in 25mg and 50mg tablets and as a sodium salt for injection (50mg vial). The recommended dose for azathioprine in dermatology in humans and dogs is reported to be 1-2.2 mg/kg orally daily, reducing to every other day administration after 1-2 weeks of daily therapy. However, many clinicians advocate the use of a body surface area derived dose rate (50mg/m²) in animals over 20kg.

In humans, a new dosing system (Table 3) has been proposed based on pretreatment TPMT activity. While TPMT is a useful indicator of potential risk groups for adverse haematologic effects, the role of TPMT activity in dogs, cats, and horses remains unclear so dosing based on TPMT activity in these species is not recommended.

### Table 3. Proposed new dosing schedule (human) – adapted from Patel, et al.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>TPMT activity (U/ml rbcs)</th>
<th>Suggested max. dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No activity (homozygous mutation)</td>
<td>&lt; 5</td>
<td>Recommend not using</td>
</tr>
<tr>
<td>Low activity (heterozygous)</td>
<td>5-13.7</td>
<td>1</td>
</tr>
<tr>
<td>Normal activity (homozygous wild type)</td>
<td>13.8-19.5</td>
<td>2.5</td>
</tr>
<tr>
<td>High activity (high homozygous)</td>
<td>&gt; 19.5</td>
<td>3</td>
</tr>
</tbody>
</table>

Therapeutic response to azathioprine occurs in 6-8 weeks. If a response is not seen, then the azathioprine dose may be increased by 0.5 mg/kg at 4 week intervals with reference to white cell counts and clinical response, but a total dose of 3mg/kg should not be exceeded. 3 If there is no response to treatment after 12-16 weeks, azathioprine should be discontinued.

No formal guidelines exist for hematological or biochemical monitoring of azathioprine in dermatology patients in the human or veterinary fields. Baseline complete blood count (CBC) and serum biochemistry panels should be run prior to the initiation of therapy. The author then repeats the CBC at weekly intervals for the first 4 weeks of therapy, then fortnightly to 8 weeks of therapy, monthly to 16 weeks then every 3 months thereafter. Biochemical analysis (with particular reference to liver function testing) is repeated 2 weeks after initiation of therapy, and then if any evidence of gastrointestinal upset, inappetence, fever, malaise or icterus is present. Ideally, urine culture and sensitivity should be performed prior to the initiation of therapy and then every 3 months, due to long term immunosuppression and the risk of occult urinary tract infections (note that this risk is not specific to azathioprine).

**Chlorambucil**

**History**

Chlorambucil is a potent alkylating agent used in a range of neoplastic and non-neoplastic dermatological conditions. The development of the alkylating agents, including chlorambucil, have revolutionised cancer chemotherapy. Alkylating agents are derivatives of mustard gas (nitrogen mustards), which were extensively used as chemical warfare agents in both World War I and II. Top secret studies carried out in the early to mid 1940s revealed that when these agents were administered systemically they were highly cytotoxic, with the degree of cytotoxicity positively correlated with the proliferative capacity of the cells – thus nitrogen mustards and their derivatives preferentially killed highly proliferative organs such as the gastrointestinal tract, bone marrow and lymphoid tissues. 37

The first clinical report of nitrogen mustards using in cancer chemotherapy was published by Goodman et al in 1946, 38 when 67 patients with Hodgkin’s lymphoma, leukaemia and lymphosarcoma were treated with a nitrogen mustard derivative. Significant improvement was seen in a number of patients, but the margin of safety of the drug in these individuals was narrow.

Chlorambucil was developed in 1953 by Everett et al, 39 with the addition of an aryl group to the nitrogen mustard molecule known as bis-(2-chloroethyl)amine, Addition of other active moieties onto this base molecule led to the development of a number of more targeted drugs such as melphalan and cyclophosphamide. 40

**Pharmacology and mechanism of action**

Alkylating agents exert their effect directly on DNA, RNA and proteins, usually by non specific means. The chlorine groups on the nitrogen mustard facilitate nucleophilic attack of nitrogen to form an immininium ion (R₂N). This highly reactive ion undergoes alkylation at N7 of guanine to form a
monomonoalkylated product on the DNA strand. Repetition of this cycle causes cross-linking of DNA. In the case of chlorambucil, two complementary strands of DNA are cross-linked. Cross-linking of DNA prevents separation of DNA strands for transcription and subsequent failure of transcription leads to apoptosis. Chlorambucil can also covalently bond to RNA and proteins through a similar mechanism.

**Figure 4. Chemical composition of chlorambucil**

Chlorambucil is considered cell cycle non-specific. Following oral administration, chlorambucil is rapidly and nearly completely absorbed from the gastrointestinal tract. It is highly protein bound in plasma. The major route of metabolism is spontaneous hydrolysis, though chlorambucil is also metabolized in the liver to form phenylacetic acid mustard (active compound). Phenylacetic acid mustard is further metabolized to inactive products which are excreted in the urine and faeces.

### Indications in Dermatology

#### Human

<table>
<thead>
<tr>
<th>Neoplastic disease</th>
<th>Non-neoplastic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous T cell lymphoma[^45]</td>
<td>Pemphigus vulgaris[^53]</td>
</tr>
<tr>
<td>Sézary syndrome[^46]</td>
<td>Pemphigus foliaceus[^53]</td>
</tr>
<tr>
<td>Cutaneous B cell lymphoma[^50]</td>
<td></td>
</tr>
</tbody>
</table>

[^44]: Pyoderma gangrenosum[^44]
[^45]: Behçet’s disease[^47],[^48]
[^46]: Necrobiosis xanthogranuloma[^49]
[^50]: Cutaneous sarcoidosis[^51]
[^53]: Sweet’s syndrome[^72]
[^54]: Bullous pemphigoid[^54]
[^55]: Dermatomyositis[^51]

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<td></td>
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</tbody>
</table>

[^45]: Sezary syndrome[^46]
[^49]: Cutaneous B cell lymphoma[^50]
[^53]: Pemphigus vulgaris[^53]
[^55]: Cutaneous T cell lymphoma[^59]

### Veterinary

<table>
<thead>
<tr>
<th>Neoplastic disease</th>
<th>Non-neoplastic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mast cell tumour[^56],[^57]</td>
<td>Discoid lupus erythematosus[^22]</td>
</tr>
<tr>
<td>Feline eosinophilic granuloma complex[^58]</td>
<td>Systemic lupus erythematosus[^22]</td>
</tr>
<tr>
<td>Pemphigus foliaceus[^21],[^22]</td>
<td>Immune-mediated vasculitis[^22]</td>
</tr>
<tr>
<td>Pemphigus vulgaris[^21],[^22]</td>
<td>( ) Cold agglutinin disease[^22]</td>
</tr>
<tr>
<td>Superficial pemphigus complex[^21],[^22]</td>
<td>Urticaria pigmentosa[^56]</td>
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### Adverse effects

#### Haematologic

Bone marrow toxicity is the most common side effect of chlorambucil therapy. It may be mild and transient, or severe and progressive. Myelosuppression is manifested by anaemia, leukopenia and thrombocytopenia. Leukocyte nadir may take 7-14 days, with recovery from 7-28 days. Severe pancytopenia may take months to years to achieve full recovery.

#### Gastrointestinal

Nausea and vomiting are frequent side effects of alkylating agent administration, including chlorambucil. Nausea has been reported within minutes of administration of the drug in humans, but may take hours or days to become clinically apparent. In general, traditional antiemetics are poorly effective in controlling vomiting with these agents. Intractable nausea and/or vomiting may require hospitalization and symptomatic therapy. A dose reduction should be considered in severe cases.

Hepatotoxicity has been documented in humans and animals with alkylating agents, however chlorambucil has a higher safety margin than other alkylating agents (e.g. lomustine) thus fatal hepatoxocity with this drug is rarely reported in the human literature and, to the authors’ knowledge, has not been reported in the veterinary literature.
Carcinogenesis
Due to their mutagenic properties, patients receiving alkylation therapy have been shown to have an increased risk in developing a second malignancy. Acute leukaemia is most frequently described as a second malignancy in humans, and usually develops within 1-4 years after drug exposure. The phenomenon has not yet been documented in veterinary literature, possible due to the short treatment lengths in these species.

Hypersensitivity reactions
Anaphylaxis, urticaria and drug eruptions have been reported with chlorambucil use in humans. Reactions to topically applies alkylating agents may also sensitise to systemically administered compounds.

Miscellaneous
Interstitial pneumonitis and pulmonary fibrosis have been reported in the human literature but not identified in veterinary medicine. Interestingly, a cumulative effect seems to be required as pulmonary fibrosis secondary to chlorambucil therapy has been noted after the discontinuation of therapy.

Alkylating agents have a significant toxic effect on reproductive tissue leading to ovarian atrophy and aspermia. As chlorambucil damages DNA at a fundamental level, it is also considered teratogenic.

Alopecia a delayed regrowth of the hair coat has been reported in dogs, with Poodles and Kerry Blue Terrier more likely to be affected than other breeds. As with azathioprine, the theoretical risk of opportunistic infections may be increased with long term immunosuppression, but little data exists in the human or veterinary literature on the prevalence of opportunistic infections associated with chlorambucil use.

Contraindications and drug interactions
Chlorambucil should not be used in patients with a known hypersensitivity to the drug. In addition dosage adjustments may need to be made in cases of renal or hepatic insufficiency. Use in pregnancy should be avoided unless the benefits clearly outweigh the risk.

The principle concern for development of myelosuppression is concurrent use of antineoplasics, immunosuppressants (e.g. azathioprine, corticosteroids, cyclophosphamide) and other bone marrow suppressive agents (e.g. chloramphenicol, flucytosine, amphotericin B, griseofulvin, colchicine).

Dosage and monitoring
Chlorambucil (Leukeran®, GlaxoSmithKline) is available in a 2mg tablet. Doses range from 0.1-0.2 mg/kg (dog and cat) administered orally every 24-48 hours. Concurrent use of corticosteroids may be required in the induction phase, once clinical response is seen then maintenance on every other day dosing of chlorambucil has been reported.

As with azathioprine, no formal guidelines exist for hematological or biochemical monitoring of chlorambucil in dermatology patients in exist. Baseline complete blood count (CBC) and serum biochemistry panels should be run prior to the initiation of therapy. The author then repeats the CBC at weekly intervals for the first 4 weeks of therapy, then fortnightly to 8 weeks of therapy, monthly to 16 weeks then every 3 months thereafter. biochemical analysis is then only repeated if there is any evidence of gastrointestinal upset, inappetence, fever, malaise or icterus present. Ideally, urine culture and sensitivity should be performed prior to the initiation of therapy and then every 3 months.
References