IARC MONOGRAPHS

M

PHARMACEUTICALS

VOLUME 100 A
A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS
ON THE EVALUATION
OF CARCINOGENIC RISKS
TO HUMANS

AZATHIOPRINE

Azathioprine was considered by previous IARC Working Groups in 1980 and 1987 (IARC, 1981, 1987a). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

Chem. Abstr. Serv. Reg. No.: 446-86-6
Chem. Abstr. Name: 9*H*-Purine, 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-
IUPAC Systematic Name: 6-(3-Methyl-5-nitroimidazol-4-yl)sulfanyl-7*H*-purine
Synonyms: 6-(1-Methyl-4-nitroimidazol-5-yl)thiopurine; 6-(1-methyl-4-nitromidazol-5-ylthio)purine; 1*H*-purine, 6-[(1-methyl-4-nitro-1*H*-imidazol-5-yl)thio]-
Description: Pale yellow, odourless powder (Sweetman, 2008)

1.1.1 Structural and molecular formulae, and relative molecular mass
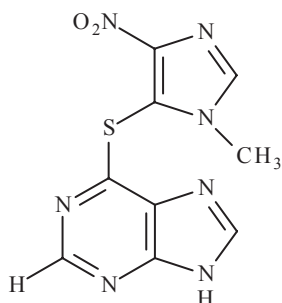
$C_9H_7N_7O_2S$
Relative molecular mass: 277.3

1.2 Use of the agent

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

1.2.1 Indications

Azathioprine is used as an adjunct for prevention of the rejection of kidney allografts. The drug is usually used in conjunction with other immunosuppressive therapy including local radiation therapy, corticosteroids, and other cytotoxic agents. Azathioprine is also used for the management of the signs and symptoms of rheumatoid arthritis in adults. It is considered to be a prodrug which converts into 6-mercaptopurine after absorption. 6-Mercaptopurine is an important component of treatment programmes for acute lymphocytic leukaemia in children and adults.

1.2.2 Dosage

Azathioprine is usually administered orally. Following renal transplantation, azathioprine may initially be given intravenously to patients unable to tolerate oral medication. Oral therapy should replace parenteral therapy as soon as possible.

Azathioprine sodium aqueous solution (10 mg/mL) may be given by direct intravenous injection or further diluted in 0.9% sodium chloride or 5% dextrose injection for intravenous infusion. Intravenous infusions of the drug are usually administered over 30–60 minutes; however, infusions have been given over periods ranging from 5 minutes to 8 hours.

(a) Renal allotransplantation

The usual oral dosage of azathioprine in children and adults undergoing renal transplantation is 3–5 mg/kg daily. Dosage reduction may be necessary due to leukopenia.

(b) Autoimmune disorders

For the treatment of severe active rheumatoid arthritis and autoimmune disorders the usual initial oral adult dosage of azathioprine is 1 mg/kg (approximately 50–100 mg) daily; daily dosage is increased as necessary at 4-week intervals by 0.5 mg/kg up to a maximum of 2.5 mg/kg. Daily maintenance dosage may be reduced to the lowest possible effective level in increments of 0.5 mg/kg (or approximately 25 mg) every 4 weeks, while keeping other therapy constant. The optimum duration of maintenance therapy has not been determined. Azathioprine may be administered in a single dose or twice-daily doses.

Azathioprine is available as 25, 50, 75, and 100 mg tablets for oral administration. Azathioprine sodium is also available as 50 and 100 mg (of azathioprine) powders for reconstitution for injection and intravenous use for parenteral administration.

1.2.3 Trends in use

No information was available to the Working Group.

2. Cancer in Humans

2.1 Transplant recipients

Assessment of the carcinogenicity of single agents used in solid organ transplant recipients can be complex owing to the multiple drugs used in modern transplantation, and to the drugs' respective mechanisms of action.

One large prospective cohort study (Kinlen et al., 1979; also reported in IARC, 1981) on renal transplant recipients that received azathioprine examined the incidence and mortality from different types of cancer compared with expected numbers, based on the incidence and mortality rates for the relevant country (Australia, New Zealand, the United Kingdom). An almost 60-fold increase in risk for non-Hodgkin lymphoma was observed, as well as a 30-fold excess of squamous cell skin cancer in patients from the UK (3 observed, 0.13 expected).

A smaller cohort study in Japan did not find an association between azthioprine use as immunosuppressant in renal transplant patients and the development of all cancers combined (Imao et al., 2007).

Recent reviews on transplant immunosuppression in solid organ recipients reported increased risks for cancers at several sites following azathioprine use (Kauffman et al., 2006; Dantal & Pohanka, 2007).

2.2 Autoimmune disorders

Azathioprine is used in a greater number of autoimmune patients than of transplant recipients. Exposure to immunosuppressive drugs is often sporadical or prolonged, depending on the clinical course (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-15-Table2.1.pdf).

Excesses in non-Hodgkin lymphoma (relative risk (RR), 10.9) and squamous cell skin

cancer (RR, 5.0) were also found in non-transplant patients receiving azathioprine, though to a smaller extent (Kinlen, 1985).

Kandiel et al. (2005) performed a meta-analysis using six published cohort studies on patients with inflammatory bowel disease, and found a meta-relative risk for non-Hodgkin lymphoma associated with the use of azathioprine of 4.18 (2.07–7.51). [The Working Group noted that this study maintained its power when any one of the comprised studies was excluded.]

Bernatsky et al. (2008) examined a nested case-control study of 246 cancer cases and 538 controls selected from an original cohort of approximately 5500 patients with systemic *lupus erythaematosus*. The adjusted hazard ratio for developing all types of cancer after treatment with azathioprine only was 0.75 (0.43–1.31), and for haematological malignancies, 1.19 (0.48–2.92). Taking into account a 5-year lag exposure did not change the results.

Fraser et al. (2002) performed an analysis on 626 patients who received azathioprine, selected from a retrospective cohort of 2204 patients with inflammatory bowel disease. Treatment with azathioprine did not significantly increase the risk for cancer at all sites. For patients with ulcerative colitis, risk for colorectal cancer was not increased.

Matula et al. (2005) examined 96 patients who received 6-mercaptopurine and azathioprine from a larger cohort of 315 patients with inflammatory bowel disease. The hazard ratio for progression to high-grade dysplasia or colorectal cancer was 1.30 (0.45–3.75).

3. Cancer in Experimental Animals

Azathioprine was tested for carcinogenicity by oral administration in one study in mice and one study in rats, by intraperitoneal administration in two studies in mice and one study in rats, and also by subcutaneous or intramuscular injection in five studies in mice (see Table 3.1).

Oral administration of azathioprine caused increased incidences of systemic lymphomas and uterine haemangioendotheliomas in female mice (Ito et al., 1989). Lymphohaematopoietic malignancies were also observed in mice after intraperitoneal, subcutaneous, or intramuscular injections of azathioprine. However, these studies did not report full experimental details, the age-adjusted comparisons, and/or the early death from autoimmune disease of the hybrid mice used, precluding direct comparison with treated mice. Studies in rats were negative though squamous cell ear-duct carcinomas were observed in male and female rats treated orally (IARC, 1981, 1987a).

Several studies were also conducted in which azathioprine was administered in conjunction with other chemical or biological agents. Mice treated with azathioprine in drinking-water, alone or in combination with antigenic stimulation in the form of a single intraperitoneal injection of lactic-dehydrogenase-elevating virus, subcutaneous injections of complete Freund's adjuvant, intramuscular injections of HeLa cells, or vaccination with smallpox vaccine, developed an increase in malignant lymphomas (IARC, 1981, 1987a). Azathioprine also enhanced the photocarcinogenesis of ultraviolet radiation in hairless mice (Kelly et al., 1987). Oral administration of azathioprine did not enhance the hepatocarcinogenesis of *N*-hydroxy-*N*-2-fluorenyl-acetamide- or of 7,12-dimethylbenz[*a*]anthracene-induced tumours when tested in rats (IARC, 1981, 1987a).

Table 3.1 Studies of cancer in experimental animals exposed to azathioprine

| Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F1 (M, F) 94 wk Ito et al. (1989)	Feed 0, 5 or 20 ppm azathioprine 50 sex/treatment group	Lymphomas: F–1/50, 5/49, 6/48 Uterine haemangioendotheliomas: F–0/50, 0/49, 7/48	$P < 0.05$ for high dose $P < 0.01$ for high dose	
Mouse, C57BL (M, F) 65 wk Imamura et al. (1973)	s.c. 100 mg/kg bw azathioprine in basic saline twice/wk for 2 wk and then once/wk for 7 mo. Surviving animals kept for an additional 33 wk 25 M, 21 F	Haematopoietic tumours: 11/38 treated animals (sex NR) – Thymic lymphoma ($n = 8$) Reticulum cell neoplasm ($n = 1$) Non-thymic lymphoma ($n = 2$) No leukaemia in the 16 untreated controls (10 M, 6 F) evaluated	NR	No data on survival provided
Mouse, NZB/NZW (F) 15–17 mo Mitrou et al. (1979a, b)	s.c. 0.2 mg azathioprine in basic saline 5 ×/wk 25 mice (120-d-old), 23 mice (180-d-old) 0.4 mg azathioprine 5 ×/wk 24 mice (120-d-old), 24 mice (180-d-old) 2.0 mg azathioprine once/wk 15 mice (120-d-old), 12 mice (180-d-old). 96 untreated or saline- or solvent-treated mice served as controls. Study terminated at the beginning of the 21st month of age for each group	Incidence of neoplasms: Low dose–10/48 High dose–29/48 2.0 mg/wk dose–4/27 Controls–3/96	$P < 0.05$ for high and low dose compared to controls	Tumours were observed in 43 treated animals in all groups and consisted of 33 lymphomas, 3 undifferentiated sarcomas, 4 squamous cell carcinomas of the anal region and 2 adenocarcinomas of the lung. However, they were not tabulated according to individual groups and there were no age-adjusted comparisons provided. The early deaths of NZB hybrid mice from autoimmune disease precluded direct comparison with treated mice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, NZB/NZW (F) 7–8 mo Mitrou et al. (1979b)	s.c. 0.2 mg azathioprine in basic saline 5 ×/wk Four groups of 9=10 mice. The same control group as that described in a previous study (Mitrou et al., 1979a) was used	Incidence of neoplasms: Treated animals–10/39 Controls–3/96		10 treated animals developed 11 tumours, which were, histologically, 9 lymphomas, 1 mammary adenocarcinoma and 1 adenocarcinoma of the lung. However, they were not tabulated according to individual groups and there were no age-adjusted comparisons provided. The early deaths of NZB hybrid mice from autoimmune disease precluded direct comparison with treated mice
Mouse, NZB (M) 6 mo Casey (1968a, b)	i.m. 0 or 100 mg/kg bw azathioprine in basic saline 3 ×/wk for 4 wk followed by twice/wk for 1 wk and then once/wk, total treatment period 6 mo 8, 8	Thymic lymphoma: Treated–6/8 Controls–0/6	NR	No age-adjusted comparisons were provided and the early deaths of NZB hybrid mice from autoimmune disease precluded direct comparison with treated mice
Mouse, NZB/NZW (F) 6 mo Casey (1968b)	i.m. 0 or 100 mg/kg bw azathioprine in basic saline 3 ×/wk for 3 wk followed by twice/wk for 1 wk and then once/wk, total treatment period 10 mo 12, 12	Thymic lymphoma: Treated–7/12 Controls–0/6	NR	Mean survival times and latent periods were not specified. No age-adjusted comparisons were provided and early death of NZB hybrid mice from autoimmune disease precluded direct comparison with treated mice

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, Swiss-Webster (M, F) 15 mo Weisburger (1977)	i.p. 7.5 (M or F), 15 (M) or 30 (F) mg/kg bw azathioprine in basic saline 3 ×/wk for 6 mo Two groups of 25 males and females 254 untreated mice as controls. Animals that survived over 100 d observed for up to 12 mo	Incidence of neoplasms: M–9/21 (43%) F–25/40 (63%) [reported as 1.5–2 times higher than that in controls (26%)] Histology: M– Lymphosarcomas (n = 4) Lung tumours (n = 5) F– Lymphosarcomas (n = 11) Lung tumours (n = 6) Uterine tumours (n = 2)	NR	Inadequate reporting of survival times, the amalgamation of various experimental groups and tumour types, as well as the lack of age-adjustment in the analyses precluded a complete evaluation of this study
Rat, Sprague-Dawley CD (M, F) 15 mo Weisburger (1977)	i.p. 18 or 37 mg/kg bw azathioprine in basic saline 3 ×/wk for 6 mo Two groups of 25 males and females Approximately 180 untreated rats of each sex served as controls. Animals that survived over 100 d observed for up to 12 mo	Incidence of neoplasms: M–15/34 F–22/44 (reported as similar to that seen in controls)	NR	Inadequate reporting of survival times, the amalgamation of various experimental groups and tumour types, as well as the lack of age-adjustment in the analyses precluded a complete evaluation of this study
Rat, F344 (M, F) 52 wk Frankel et al. (1970)	Feed 100 mg/kg feed azathioprine/d for 52 wk 50 sex/group 10 males and females (6–8-wk-old) as controls	Squamous cell carcinomas of the ear-duct: Controls–0/20 M–3/17 F–3/25	[NS]	Toxicity observed in females, 40% died within first 12 wk of study. All surviving animals killed after 52 wk. No difference in incidence of other tumour types

bw, body weight; d, day or days; F, female; i.m., intramuscular; i.p., intraperitoneal; M, male; mo, months; NR, not reported; NS, not significant; s.c., subcutaneous; wk, week or weeks; yr, year or years

Fig. 4.1 Conversion of azathioprine to 6-mercaptopurine

Azathioprine   Glutathione-*S*-transferase(s)   6-Mercaptopurine
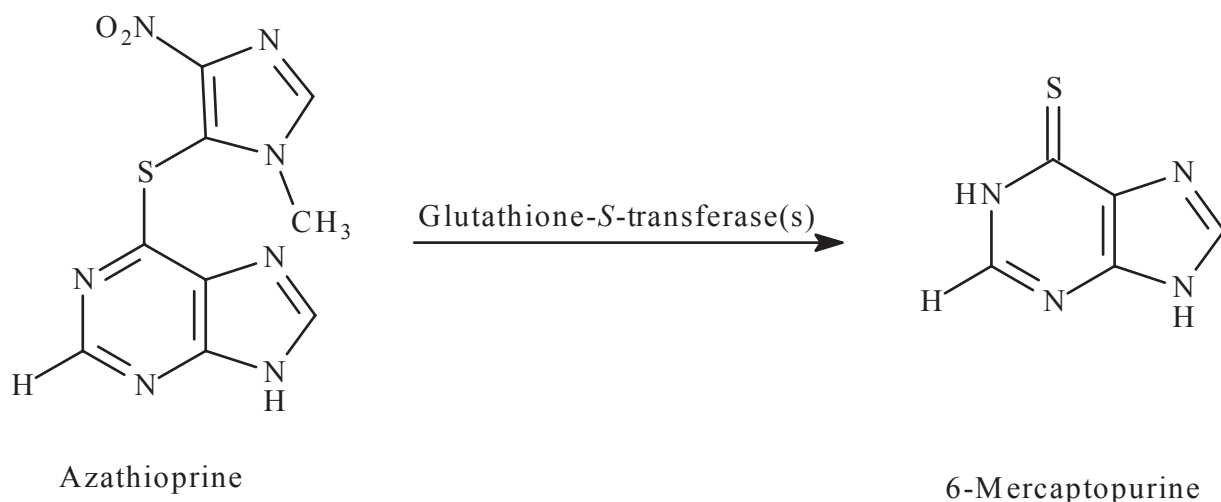
4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Azothioprine is essentially a prodrug for 6-mercaptopurine (see Fig. 4.1; for review, see Aarbakke *et al.*, 1997).

In humans, azathioprine is readily absorbed from the gut. Following oral administration of ^{35}S-azathioprine, 12% of the radioactivity is found in faeces as unabsorbed material, and 50% in the urine over 24 hours. After oral administration of ^{14}C-azathioprine, about 30% is bound to serum proteins in the blood, but it appears to be dialysable (Elion, 1972).

In vivo, azathioprine is split primarily by chemical conversion (Elion & Hitchings, 1975) but possibly also by enzymatic conversion (Watanabe *et al.*, 1978), with the release of 6-mercaptopurine. In addition, splitting occurs such that the sulfur is part of the methylnitroimidazole ring.

In humans, azathioprine is catabolized to a variety of oxidized and methylated derivatives, which are excreted by the kidneys; very little azathioprine or 6-mercaptopurine are excreted intact. At least 11 different metabolites have been identified, with the major one, 6-thiouric acid, found in urine. Other metabolites resulting from the biotransformation of the methylnitroimidazole moiety include 5-mercapto-1-methyl-4-nitroimidazole, 1-methyl-4-nitro-5-thioimidazole, 8-hydroxyazathioprine, and inorganic sulfate (Chalmers *et al.*, 1967; Elion, 1972; Elion & Hitchings, 1975). Some of these metabolites are also found in the urine of rodents and dogs. The profile of the methylnitroimidazole urinary metabolites in the dog is similar to that in humans but different from that in the rat (de Miranda *et al.*, 1973, 1975; IARC, 1981).

Most of the biological and biochemical effects of azathioprine depend on its in-vivo conversion to 6-mercaptopurine. Following removal of the protective methylnitroimidazole group in reactions involving glutathione (mediated most likely by glutathione-*S*-transferase) (Watanabe *et al.*, 1978), 6-mercaptopurine enters the purine salvage pathway. It is converted to thioinosine monophosphate (TIMP) by hypoxanthine(guanine)phosphoribosyl transferase (HPRT), and then by a series of enzymatic

**Fig. 4.2 Salvage pathway of 6-mercaptopurine, metabolite of azathioprine**

| 6-MP | TIMP | dthioGTP (TGN) |
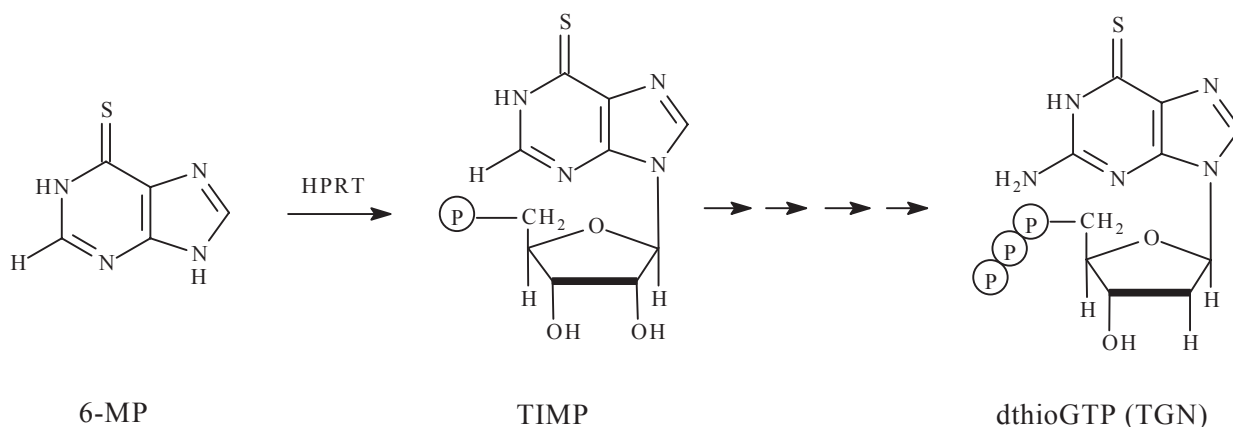
6-MP, 6-mercaptopurine; dthioGTP, 6-thioguanine deoxynucleoside triphosphate; HPRT, hypoxanthine(guanine)phosphoribosyl transferase; TGN, thioguanine nucleotide; TIMP, thioinosine monophosphate

steps to the thioguanine nucleotide (TGN), 6-thioguanine deoxynucleoside triphosphate (dthioGTP) (see Fig. 4.2). Thioguanine nucleotide is a precursor of DNA synthesis, and a good substrate for replicative DNA polymerases, which also copy template 6-thioguanine with reasonable efficiency and accuracy (Spratt & Levy, 1997). In an important catabolic step (see Fig. 4.3), thiopurine methyltransferase (TPMT) methylates thioinosine monophosphate to methylthioinosine monophosphate (meTIMP), a potent inhibitor of purine biosynthesis (Stet et al., 1993; Dervieux et al., 2002). The balance between thioguanine nucleotide and methylthioinosine monophosphate formation is the key to the pharmacological action of the thiopurine drugs. There are several polymorphic variants of thiopurine methyltransferase (Weinshilboum & Wang, 2006). These vary in their efficiency and when patients who are homozygous (or compound heterozygous) for alleles encoding inefficient thiopurine methyltransferase are treated with azathioprine, they experience severe and often life-threatening myelotoxicity – essentially an azathioprine overdose. The past 20 years or so has seen a significant body of research into the occurrence and effects of polymorphisms

of the thiopurine methyltransferase gene. A screening test has been introduced (reviewed in Evans & Relling, 1999; Relling & Dervieux, 2001; Evans, 2004). In general, patients with high thiopurine methyltransferase levels have clinically poor responses to azathioprine therapy. Despite this, the formation of methylthioinosine monophosphate and potential inhibition of de novo purine biosynthesis are considered by some to be the basis of the pharmacological effects of azathioprine. This paradoxical position may have arisen from the choice of mismatch repair (MMR)-deficient cell lines in which the effects of azathioprine were examined in vitro; in the absence of MMR, purine depletion may be a significant factor in toxicity (Karran, 2006). Nevertheless, since many bona fide inhibitors of de novo purine biosynthesis have similar effects on purine biosynthesis (Shipkova et al., 2005), a contribution of deoxynucleotide pool imbalance to the immunosuppressive and anti-inflammatory activities of azathioprine has not been formally excluded.

Thioguanine nucleotide levels are an alternative determinant of the effects of azathioprine (Lennard et al., 1987). There is a correlation between thioguanine nucleotide levels and

Fig. 4.3 Catabolism of 6-mercaptopurine
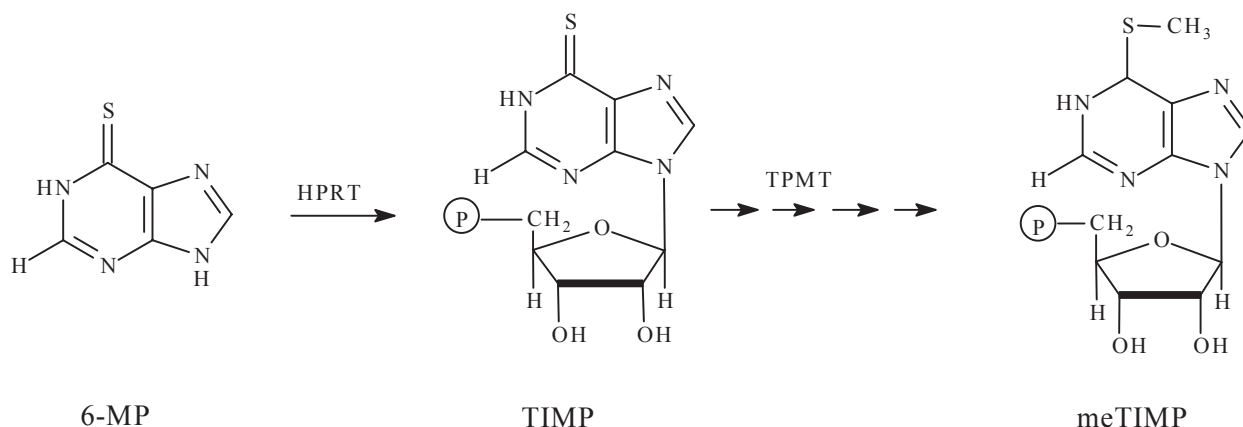
6-MP, 6-mercaptopurine; meTIMP, methyl-TIMP; TIMP, thioinosine monophosphate; TPMT, thiopurine methyltransferase; HPRT, hypoxanthine (guanine) phosphoribosyl transferase

clinical effectiveness in inflammatory bowel disease (Osterman *et al.*, 2006). Thioguanine nucleotides enter the deoxynucleotide pool and are used as substrates for replicative DNA polymerases. This results in the incorporation of 6-thioguanine in place of DNA guanine, and levels of around 0.5% (around 10^5 to 10^6 per cell) replacement have been reported (Warren *et al.*, 1995; Cuffari *et al.*, 2004). DNA-6-thioguanine forms reasonable base pairs (Bohon & de los Santos, 2003) and is not, in itself, cytotoxic. The biological effects of this substitution require an active DNA MMR system and depend on the particular chemical reactivity of DNA thioguanine. The thiol group of DNA-6-thioguanine is more reactive than the corresponding oxygen of guanine. DNA-6-thioguanine is susceptible to chemical methylation – most likely by *S*-adenosylmethionine. This reaction converts a small fraction of DNA-6-thioguanine, approximately one in 10^4 or 10^5 incorporated 6-thioguanine bases, into *S*-methylthioguanine (me6-TG) which, unlike unmodified DNA-6-thioguanine, is highly miscoding during replication; DNA-6-methylthioguanine preferentially base pairs with thymine (T) during replication (Swann *et al.*, 1996). The formation of me6-TG:T base

pairs triggers recognition by MMR which leads to cell death. Therefore, cells in which MMR is inactive are highly resistant to 6-mercaptopurine and 6-thioguanine. Importantly, loss of MMR confers a significant selective advantage; and in the laboratory, thiopurine treatment of human cells *in vitro* can be used to isolate rare variants with defective MMR from populations of cells with proficient MMR (Offman *et al.*, 2004).

4.2 Genotoxic effects

There are conflicting reports of effects on the incidence of chromosomal aberrations in lymphocytes and bone-marrow cells of patients treated with azathioprine. In one study, the incidence of sister chromatid exchange in lymphocytes of treated patients was not increased. In animals treated *in vivo*, azathioprine induced dominant lethal mutations in mice, chromosomal aberrations in rabbit lymphocytes and Chinese hamster bone-marrow cells, and micronuclei in mice, rats and hamsters; it did not induce sister chromatid exchange in Chinese hamster bone-marrow cells.

Azathioprine induced chromosomal aberrations but not sister chromatid exchange in human

lymphocytes *in vitro*. It induced chromosomal aberrations in *Drosophila*, was weakly mutagenic to fungi, and was mutagenic to bacteria (IARC, 1987b).

4.3 Mechanisms of carcinogenesis in organ-transplant patients

Until recently, azathioprine was the most commonly prescribed immunosuppressant in solid organ transplant patients. It was usually given combined with steroids and/or ciclosporin. Organ transplant patients get two types of cancer: post-transplant lymphoproliferative disorders and skin cancer, predominantly squamous cell carcinoma.

4.3.1 Post-transplant lymphoproliferative disorders

After skin cancer, these are the most common malignancies associated with transplantation. They tend to affect around 5% of transplant recipients (LaCasce, 2006). Most are associated with the Epstein-Barr virus (EBV). Most arise in the first year after transplant during which immunosuppression is intense, and are of recipient origin.

The development of post-transplant lymphoproliferative disorders almost certainly reflects immunosuppression. EBV is incorporated into B-lymphocytes during primary infection, and immunocompetent hosts mount both antibody and cellular immune responses. Post-transplant lymphoproliferative disorders develops as a consequence of compromised immunosurveillance. EBV-naïve transplant recipients typically acquire the infection from donor B cells, and post-transplant lymphoproliferative disorders is more than 20 times more common in this group (LaCasce, 2006). Because azathioprine is an immunosuppressant, it may contribute to the development of post-transplant lymphoproliferative disorders.

4.3.2 Squamous cell skin carcinoma

The frequency of squamous cell skin carcinoma in transplant patients is up to 250-fold higher that in the normal population (Euvrard *et al.*, 2003). There is no association with defective MMR in transplant-related squamous cell skin carcinoma. Although immunosuppression *per se* undoubtedly contributes to this increased incidence, the identification of sunlight as a cofactor (Bavinck *et al.*, 1993) suggests a possible alternative contributory mechanism.

The skin of patients taking azathioprine contains DNA-6-thioguanine (O'Donovan *et al.*, 2005). Unlike the canonical DNA bases, 6-thioguanine acts as an endogenous ultraviolet A (UVA) chromophore. UVA comprises more than 95% of the UV radiation that reaches the Earth's surface. 6-Thioguanine has been shown to be a UVA photosensitizer, and to generate reactive oxygen species, including singlet oxygen $^1O_2$, when exposed to UVA. $^1O_2$ is highly damaging for DNA and oxidizes DNA guanine to the promutagenic 8-oxoguanosine, which has been implicated in the development of cancer (Cadet *et al.*, 2003, 2006). DNA-6-thioguanine itself is also susceptible to oxidation leading to DNA lesions that block replication and transcription, and are likely to be promutagenic (O'Donovan *et al.*, 2005). Patients taking azathioprine are selectively sensitive to erythema induction by UVA (Perrett *et al.*, 2008). This suggests that these potentially hazardous photochemical reactions of DNA-6-thioguanine, which may generate promutagenic DNA changes, occur in the skin of patients.

4.4 Synthesis

Azathioprine is carcinogenic via two mechanisms:

- as an immunosuppressant, it is associated with post-transplant lymphoproliferative disorders that generally have a viral etiology;

- because it causes 6-thioguanine to accumulate in patients' DNA, it also contributes to cancer development by DNA damage.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of azathioprine. Azathioprine causes cancer of the skin (squamous cell carcinoma), and non-Hodgkin lymphoma.

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

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

References

Aarbakke J, Janka-Schaub G, Elion GB (1997). Thiopurine biology and pharmacology. *Trends Pharmacol Sci*, 18: 3–7. doi:10.1016/S0165-6147(96)01007-3 PMID:9114722

Bavinck JN, De Boer A, Vermeer BJ *et al.* (1993). Sunlight, keratotic skin lesions and skin cancer in renal transplant recipients. *Br J Dermatol*, 129: 242–249. doi:10.1111/j.1365-2133.1993.tb11841.x PMID:8286220

Bernatsky S, Joseph L, Boivin JF *et al.* (2008). The relationship between cancer and medication exposures in systemic lupus erythaematosus: a case-cohort study. *Ann Rheum Dis*, 67: 74–79. doi:10.1136/ard.2006.069039 PMID:17545189

Bohon J & de los Santos CR (2003). Structural effect of the anticancer agent 6-thioguanine on duplex DNA. *Nucleic Acids Res*, 31: 1331–1338. doi:10.1093/nar/gkg203 PMID:12582253

Cadet J, Douki T, Gasparutto D, Ravanat JL (2003). Oxidative damage to DNA: formation, measurement and biochemical features. *Mutat Res*, 531: 5–23. PMID:14637244

Cadet J, Ravanat JL, Martinez GR *et al.* (2006). Singlet oxygen oxidation of isolated and cellular DNA: product formation and mechanistic insights. *Photochem Photobiol*, 82: 1219–1225. doi:10.1562/2006-06-09-IR-914 PMID:16808595

Casey TP (1968a). The development of lymphomas in mice with autoimmune disorders treated with azathioprine. *Blood*, 31: 396–399. PMID:5640634

Casey TP (1968b). Azathioprine (Imuran) administration and the development of malignant lymphomas in NZB mice. *Clin Exp Immunol*, 3: 305–312. PMID:4297669

Chalmers AH, Knight PR, Atkinson MR (1967). Conversion of azathioprine into mercaptopurine and mercaptoimidazole derivatives in vitro and during immunosuppressive therapy. *Aust J Exp Biol Med Sci*, 45: 681–691. doi:10.1038/icb.1967.68 PMID:4867621

Cuffari C, Li DY, Mahoney J *et al.* (2004). Peripheral blood mononuclear cell DNA 6-thioguanine metabolite levels correlate with decreased interferon-gamma production in patients with Crohn's disease on AZA therapy. *Dig Dis Sci*, 49: 133–137. doi:10.1023/B:DDAS.0000011614.88494.ee PMID:14992447

Dantal J & Pohanka E (2007). Malignancies in renal transplantation: an unmet medical need. *Nephrol Dial Transplant*, 22: Suppl 1i4–i10. doi:10.1093/ndt/gfm085 PMID:17456618

de Miranda P, Beacham LM 3rd, Creagh TH, Elion GB (1973). The metabolic fate of the methylnitroimidazole moiety of azathioprine in the rat. *J Pharmacol Exp Ther*, 187: 588–601. PMID:4770400

de Miranda P, Beacham LM 3rd, Creagh TH, Elion GB (1975). The metabolic disposition of 14C-azathioprine in the dog. *J Pharmacol Exp Ther*, 195: 50–57. PMID:1181404

Dervieux T, Brenner TL, Hon YY *et al.* (2002). De novo purine synthesis inhibition and antileukemic effects of mercaptopurine alone or in combination with methotrexate in vivo. *Blood*, 100: 1240–1247. doi:10.1182/blood-2002-02-0495 PMID:12149204

Elion GB (1972). Significance of azathioprine metabolites. *Proc R Soc Med*, 65: 257–260. PMID:5083313

Elion GB, Hitchings GH (1975). *Azathioprine.* In: *Handbook of experimental pharmacology,* 38. Berlin: Springer-Verlag, pp. 404–425.

Euvrard S, Kanitakis J, Claudy A (2003). Skin cancers after organ transplantation. *N Engl J Med*, 348: 1681–1691. doi:10.1056/NEJMra022137 PMID:12711744

Evans WE (2004). Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. *Ther Drug Monit*, 26: 186–191. doi:10.1097/00007691-200404000-00018 PMID:15228163

Evans WE & Relling MV (1999). Pharmacogenomics: translating functional genomics into rational therapeutics. *Science*, 286: 487–491. doi:10.1126/science.286.5439.487 PMID:10521338

Frankel HH, Yamamoto RS, Weisburger EK, Weisburger JH (1970). Chronic toxicity of azathioprine and the effect of this immunosuppressant on liver tumor induction by the carcinogen N-hydroxy-N-2-fluorenylacetamide. *Toxicol Appl Pharmacol*, 17: 462–480. doi:10.1016/0041-008X(70)90203-6 PMID:5471562

Fraser AG, Orchard TR, Robinson EM, Jewell DP (2002). Long-term risk of malignancy after treatment of inflammatory bowel disease with azathioprine. *Aliment Pharmacol Ther*, 16: 1225–1232. doi:10.1046/j.1365-2036.2002.01297.x PMID:12144571

IARC (1981). Some antineoplastic and immunosuppressive agents. *IARC Monogr Eval Carcinog Risk Chem Hum*, 26: 1–411. PMID:6944253

IARC (1987a). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

IARC (1987b). Genetic and related effects: An updating of selected IARC monographs from Volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 6: 1–729. PMID:3504843

Imamura N, Nakano M, Kawase A *et al.* (1973). Synergistic action of N-nitrosobutylurea and azathioprine in induction of leukemia in C57BL mice. *Gann*, 64: 493–498. PMID:4588868

Imao T, Ichimaru N, Takahara S *et al.* (2007). Risk factors for malignancy in Japanese renal transplant recipients. *Cancer*, 109: 2109–2115. doi:10.1002/cncr.22636 PMID:17407138

Ito A, Mori M, Naito M (1989). Induction of uterine hemangioendothelioma and lymphoma in (C57BL/6N x C3H/2N)F1 mice by oral administration of azathioprine. *Jpn J Cancer Res*, 80: 419–423. PMID:2502517

Kandiel A, Fraser AG, Korelitz BI *et al.* (2005). Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. *Gut*, 54: 1121–1125. doi:10.1136/gut.2004.049460 PMID:16009685

Karran P (2006). Thiopurines, DNA damage, DNA repair and therapy-related cancer. *Br Med Bull*, 79-80: 153–170. doi:10.1093/bmb/ldl020 PMID:17277075

Kauffman HM, Cherikh WS, McBride MA *et al.* (2006). Post-transplant de novo malignancies in renal transplant recipients: the past and present. *Transpl Int*, 19: 607–620. doi:10.1111/j.1432-2277.2006.00330.x PMID:16827677

Kelly GE, Meikle W, Sheil AG (1987). Effects of immunosuppressive therapy on the induction of skin tumors by ultraviolet irradiation in hairless mice. *Transplantation*, 44: 429–434. doi:10.1097/00007890-198709000-00021 PMID:3629691

Kinlen LJ (1985). Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. *Am J Med*, 78: 1A44–49. doi:10.1016/0002-9343(85)90245-1 PMID:3970040

Kinlen LJ, Sheil AG, Peto J, Doll R (1979). Collaborative United Kingdom-Australasian study of cancer in patients treated with immunosuppressive drugs. *BMJ*, 2: 1461–1466. doi:10.1136/bmj.2.6203.1461 PMID:393355

LaCasce AS (2006). Post-transplant lymphoproliferative disorders. *Oncologist*, 11: 674–680. doi:10.1634/theoncologist.11-6-674 PMID:16794246

Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM (1987). Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations.

Clin Pharmacol Ther, 41: 18–25. doi:10.1038/clpt.1987.4 PMID:3467886

Matula S, Croog V, Itzkowitz S *et al.* (2005). Chemoprevention of colorectal neoplasia in ulcerative colitis: the effect of 6-mercaptopurine. *Clin Gastroenterol Hepatol*, 3: 1015–1021. doi:10.1016/S1542-3565(05)00738-X PMID:16234048

McEvoy GK, editor (2007). *American Hospital Formulary Service Drug Information*. Bethesda, MD: American Society of Health-System Pharmacists.

Mitrou PS, Fischer M, Mitrou G *et al.* (1979a). The oncogenic effect of immunosuppressive (cytotoxic) agents in (NZB X NZW) mice. I. Long-term treatment with azathioprine and ifosfamide. *Arzneimittelforschung*, 29: 483–488. PMID:314806

Mitrou PS, Fischer M, Mitrou G, Röttger P (1979b). The oncogenic effect of immunosuppressive (cytotoxic) agents in (NZB x NZW) mice. II. Emergence of tumors in young animals treated with azathioprine and ifosfamide, including a histologic assessment of the neoplasms. *Arzneimittelforschung*, 29: 662–667. PMID:582763

O'Donovan P, Perrett CM, Zhang X *et al.* (2005). Azathioprine and UVA light generate mutagenic oxidative DNA damage. *Science*, 309: 1871–1874. doi:10.1126/science.1114233 PMID:16166520

Offman J, Opelz G, Doehler B *et al.* (2004). Defective DNA mismatch repair in acute myeloid leukemia/myelodysplastic syndrome after organ transplantation. *Blood*, 104: 822–828. doi:10.1182/blood-2003-11-3938 PMID:15090454

Osterman MT, Kundu R, Lichtenstein GR, Lewis JD (2006). Association of 6-thioguanine nucleotide levels and inflammatory bowel disease activity: a meta-analysis. *Gastroenterology*, 130: 1047–1053. doi:10.1053/j.gastro.2006.01.046 PMID:16618398

Perrett CM, Walker SL, O'Donovan P *et al.* (2008). Azathioprine treatment photosensitizes human skin to ultraviolet A radiation. *Br J Dermatol*, 159: 198–204. doi:10.1111/j.1365-2133.2008.08610.x PMID:18489587

Relling MV & Dervieux T (2001). Pharmacogenetics and cancer therapy. *Nat Rev Cancer*, 1: 99–108. doi:10.1038/35101056 PMID:11905809

Royal Pharmaceutical Society of Great Britain (2007). *British National Formulary*, No. 54. London: BMJ Publishing Group Ltd./RPS Publishing.

Shipkova M, Armstrong VW, Oellerich M, Wieland E (2005). Mycophenolate mofetil in organ transplantation: focus on metabolism, safety and tolerability. *Expert Opin Drug Metab Toxicol*, 1: 505–526. doi:10.1517/17425255.1.3.505 PMID:16863458

Spratt TE & Levy DE (1997). Structure of the hydrogen bonding complex of O6-methylguanine with cytosine and thymine during DNA replication. *Nucleic Acids Res*, 25: 3354–3361. doi:10.1093/nar/25.16.3354 PMID:9241252

Stet EH, De Abreu RA, Bökkerink JP *et al.* (1993). Reversal of 6-mercaptopurine and 6-methylmercaptopurine ribonucleoside cytotoxicity by amidoimidazole carboxamide ribonucleoside in Molt F4 human malignant T-lymphoblasts. *Biochem Pharmacol*, 46: 547–550. doi:10.1016/0006-2952(93)90534-4 PMID:8347177

Swann PF, Waters TR, Moulton DC *et al.* (1996). Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine. *Science*, 273: 1109–1111. doi:10.1126/science.273.5278.1109 PMID:8688098

Sweetman SC, editor (2008). *Martindale: The Complete Drug Reference*. London: Pharmaceutical Press. Available at: http://www.medicinescomplete.com/mc/

Warren DJ, Andersen A, Slørdal L (1995). Quantitation of 6-thioguanine residues in peripheral blood leukocyte DNA obtained from patients receiving 6-mercaptopurine-based maintenance therapy. *Cancer Res*, 55: 1670–1674. PMID:7712473

Watanabe A, Hobara N, Nagashima H (1978). Demonstration of enzymatic activity converting azathioprine to 6-mercaptopurine. *Acta Med Okayama*, 32: 173–179. PMID:29442

Weinshilboum RM & Wang L (2006). Pharmacogenetics and pharmacogenomics: development, science, and translation. *Annu Rev Genomics Hum Genet*, 7: 223–245. doi:10.1146/annurev.genom.6.080604.162315 PMID:16948615

Weisburger EK (1977). Bioassay program for carcinogenic hazards of cancer chemotherapeutic agents. *Cancer*, 40: Suppl1935–1949. doi:10.1002/1097-0142(197710)40:4+<1935::AID-CNCR2820400827>3.0.CO;2-R PMID:907995