Effective long-term solution to therapeutic remission in Inflammatory Bowel Disease: Role of Azathioprine

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Abstract

Azathioprine (AZA) is a well-known immunosuppressant used for many years for its ability to ensure long term disease remission in inflammatory bowel diseases (IBD) at an affordable cost to the public. However, the side effect profile has raised many concerns with numerous investigations into the risk, cause and prevention of these effects. Much of the side effect profile of AZA can be linked to a single nucleotide polymorphism (SNP) in the thiopurine methyltransferase (TPMT) gene which ensures the breakdown and efficacy of AZA. Mutated TPMT alleles result in low or deficient TPMT levels which directly correlate to cytotoxity. This is a review of the role of AZA in the treatment of IBD. Knowing a patient's TPMT status allows the prescribing doctor to make an informed decision about dosage and be more alert to the signs of cytotoxicity. It is essential to include "early warning" SNP testing into common practice to ensure therapeutic efficacy.

Keywords
Azathioprine; Thiopurine methyltransferase; Inflammatory bowel disease

1. Introduction

Azathioprine (AZA) is an immunosuppressant drug that was first produced in 1957 and is included in the World Health Organization's (WHO) List of Essential Medicines. AZA is a purine analogue which interrupts the synthesis of purine ribonucleotides guanine and adenine, causing mis-incorporation of bases and preventing deoxyribonucleic acid (DNA) repair mechanisms [1-3]. It has a most notable effect on fast dividing cells such as T- lymphocytes; at low doses AZA works as an anti-inflammatory while at high doses it has immunosuppressant and cytotoxic characteristics [4, 5]. The drug class of thiopurines is commonly used to treat dermatological conditions, malignancies, rheumatic diseases, prevention of rejection after organ transplant or for the treatment of inflammatory gastrointestinal disorders such as Inflammatory Bowel Disease (IBD). Thiopurine drugs have a very narrow therapeutic index and can cause life threatening toxicity [6].

2. Mechanism of Action

As shown in Figure 1, AZA is a prodrug which once administered, is converted to 6-mercaptopurine (6-MP). 6-MP undergoes methylation via the key enzyme thiopurine methyltransferase (TPMT) to form an inactive methylated metabolite of 6-mercaptopurine (6-Me-MP) [7]. In the absence of methylation by TPMT, 6-MP is converted into 6-thioguanine (6-TG) by xanthine oxidase, where after hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) converts 6-TG into 6-thioguanine nucleotide (6-TGN) metabolites. 6-TGN is the active metabolite that determines cytotoxicity or efficacy. TPMT competes with xanthine oxidase and HGPRT to determine how much of the 6-MP is catalyzed to 6-TGN [8, 9]. TPMT enzyme activity varies greatly in patients due to the presence of polymorphic variation in the TPMT gene [3].

At normal levels of TPMT activity, 6-TGN inhibits intracellular signalling pathways and induces lymphocytic apoptosis. An increase in TPMT enzyme activity above normal results in decreased 6-TGN and hence a decrease in drug efficacy. The decrease or absence of TPMT activity (such as that seen in the TPMT polymorphisms) results in increased levels of 6-TGN which incorporates into the DNA and trigger cytotoxicity [9-11].

3. Pharmacology

Azathioprine can be administered intravenously or orally in both a delayed release oral (DRO) capsule, or tablet form. A study by Van Os *et al* (1996) compared the bioavailability of 50mg AZA when administered in the form of a DRO tablet, oral capsule, rectal hydrophilic foam (HBF), rectal hydrophobic foam (HPF) and intravenously. The oral bioavailability was 41.6%, DRO 9.6%, HBF 5.9% and HPF 1.8%, assuming the intravenous bioavailability was 100% [12]. Oral tablets are considered to be a "local" approach as it delivers a lower bioavailability and thus less risk of toxicity [13].

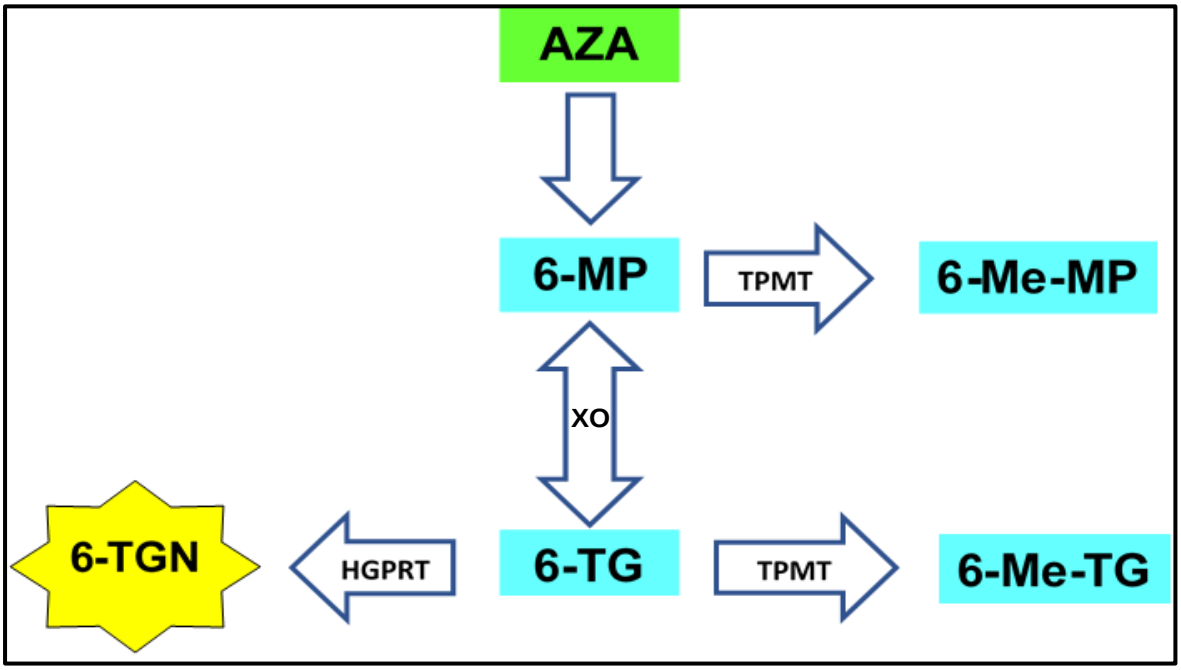

Figure 1: Mechanism of action of thiopurine drugs and potential pathways of metabolism [8]. **AZA** (azathioprine), **6-MP** (6-mercaptopurine), **HGPRT** (hypoxanthine guanine phosphoribosyl transferase), **XO** (xanthine oxidase), **TPM**T (thiopurine S-methyltransferase), **6-Me-MP** (6-methyl-mercaptopurine), **6-TG** (thioguanine), **6-TGN** (6-thioguanine nucleotides).

Direct delivery to the colon is ideal as it surpasses first pass metabolism by the liver. However, direct delivery of rectal foam to the colon produces a lower bioavailability due to reduced absorption by the colon mucosa when compared to that of the gastric mucosa [12]. In a recent animal study by Helmy *et al* (2017), a new therapeutic strategy was developed by loading AZA into colon-targeted chitosan beads and inserting the beads into an acid-resistant capsule [14].

The half-life of AZA is reported to be 26-80 min, or 3-5 hours if metabolites are included [15, 16]. The half-life of TGN in erythrocytes is reported to be 5 days, and months may be needed to reach a steady state [17]. This may explain why a prolonged treatment period is needed before a clinical response occurs.

Therapeutic dosage concentrations range from less than 1 mg/kg bodyweight/day, to 3 mg/kg bodyweight, depending on the severity of the disease and the side effect profile. On average it takes 17 weeks for a therapeutic response to appear in most patients, but it has been suggested that the response time can be sped up by administering a loading dose of 5 mg/kg bodyweight/day to achieve a greater cumulative concentration [3, 12, 18]. Van Os *et al* (1996) observed that the patient response time can also be decreased by administering a loading dose of AZA intravenously, thus providing a portion of the cumulative dose more swiftly [12]. On average 47% of orally administered AZA reaches systemic circulation and just more than 80% of AZA is converted to 6-MP, which has a 16% bioavailability [12, 13].

Although AZA has been proven to increase miscarriages by 20 fold in mice, conflicting studies and reports have been published with regards to the teratogenic nature of the drug in humans, with some studies [4, 19] finding that the drug only causes low birth weight and premature birth while other studies [20, 21] claim the drug is definitely teratogenic. It is difficult to relate AZA directly to foetal abnormalities as majority of the females taking the medication are on combination therapy and are advised to stay on treatment during the pregnancy to prevent disease relapse, many studies therefore conclude that any other birth abnormalities are due to underlying disease rather than the medication itself [13, 19]. Yet, AZA has been listed as a class D teratogen according to the Food and Drug Administration (FDA), and is not recommended while breastfeeding [12].

AZA has been associated with a variety of side effects ranging from general nausea to myelosuppression, in very rare cases red-cell aplasia or death (in cases of TMPT polymorphism) [22, 23]. Most side effects can be separated into two reaction groups, namely dose-independent (DI) and dose-dependent (DD) reactions. Drug Induced reactions tend to be hypersensitive, allergy-like reactions which tend to occur within the first few weeks after initial dosing. Symptoms such as pancreatitis, fever, joint pain, gastrointestinal disturbances and rash are common in DI reactions [17, 24, 25].

DD reactions tend to appear at later stages of therapy due to metabolite build up and often present as leukopenia, cholestatic jaundice, uncommon bacterial infections, hepatitis, nausea and myelosuppression. DD related side effects will generally disappear once the dosage is decreased, while DI reactions will continue until therapy is discontinued [12, 13, 17]. Many studies have reported patients developing toxicity long after therapy was initiated, most notably two studies totalling 1135 patients observed the onset of toxicity ranging from immediately after first dose to patients developing toxicity after 11 years of therapy [26-28]. In a more recent study by Björnsson *et al* (2017), it was noted that nearly three-quarters of patients who had developed thiopurine-induced hepatotoxicity, also developed cholestatic hepatitis within 3 months of starting the dose or increasing the dosage [29].

There are known associations between thiopurine treatment for rheumatoid arthritis or renal transplant and the increased risk of developing a malignancy, however there are differing opinions regarding the relationship when it comes to IBD patients [30, 31]. Some studies claim there is no significant association between thiopurine therapy for IBD patients and the risk of malignancies [30], while other studies claim that there is a significant association with an increased risk of malignancies [32, 33]. These malignancies include urinary tract cancer in older men, non-melanoma skin cancer (NMSC) in younger patients and lymphoma in the general population [34, 35]. However, both study groups agree that there is limited data to establish causality [2].

Kandiel *et al* (2005) argued that a 4-fold increase in lymphoma is observed in IBD cases, but again whether this is due to the underlying disease, as a result of thiopurines or a combination of the two factors, remains unclear [2, 36]. A study by Lewis *et al* (2000) pointed out that despite the 4-fold increase in risk of lymphoma, AZA is still a vital component of immunomodulatory therapy in IBD management and that the increased risk would have to be greater than 9.8-fold for the benefit of alternative therapies to outweigh the benefit of AZA therapy [17, 37]. This statement has been reiterated recently in a study by Clowry *et al* (2017) on the relationship between thiopurine therapy and the risk of NMSC where, as previously concluded, due to the rising prevalence of IBD in young patients with little other alternative to immunosuppressive therapy, the benefit of this treatment outweighs the risk. Furthermore, it was noted that combination therapy for long periods of time also increased the risk factors [35].

Individuals using AZA are commonly on a combination therapy with a corticosteroid; where the steroid will slowly be weaned off once disease remission status is achieved. A study by de Jong *et al* (2014) has found that patients who are on a combination therapy with a higher dosage of steroid when starting the AZA tend to have fewer and less severe adverse events [38]. It is estimated that 20-30% of all patients taking AZA monotherapy will discontinue use due to an adverse drug reaction (ADR) of some kind [9, 17].

4. PHARMACOGENETICS

The varying degrees of therapeutic response and potential toxicity can be attributed to genetic variations of genes which encode key enzymes for the metabolism of thiopurines. These allelic variants are known as single nucleotide polymorphisms (SNP's), which are single base-pair change at a specific site, found in the human deoxyribonucleic acid (DNA) sequence. SNP's differ from normal genetic mutations in that they are only defined as a SNP when the specific allele switch is observed in more than 1% of the population[39]. The key enzymes involved in the metabolism of AZA include TPMT, HGPRT, Inosine Triphosphatase (ITPase) and Xanthine Oxidase. The polymorphisms result in the formation of variable active metabolites, of which ITPase and TPMT metabolites are the most studied [40].

4.1 Inosine Triphosphate and Azathioprine

Inosine triphosphate (ITP), a median in the purine metabolic pathway, is catalysed by ITPase to form inosine monophosphate (IMP). Deficiency of ITPase is due to a SNP on the ITP encoding gene on chromosome 20 and is characterised by irregular accumulation of ITP in erythrocytes, but it is a benign condition and under normal circumstances thought to be of no detriment to patient health [41]. However, in the presence of AZA or 6-MP, ITPase deficiency results in the accumulation of 6-thio-inosine triphosphate (6-tITP). 6-tITP is described as a "rogue nucleotide" with the potential to cause cell toxicity [42]. Currently there are five known ITPase SNP's of which three are silent and two have been linked to a decrease in ITPase activity when present in a homozygous state. It has been estimated that 6% of the population is heterozygous for a decreased activity SNP, and even less so for a homozygous SNP [17]. Despite three studies [41-43] having reported an association between ITPase deficiency and thiopurine toxicity, no consistent toxicity pattern has

been reported; thus a conclusion was made by Lennard (2002) that ITP genotype testing or observation does not provide a clue as to whether a patient is at risk for cell toxicity [44, 45]. This conclusion was supported by a more recent study by Citterio-Quentin *et al* (2017) in which it was shown that ITPase activity is rarely influenced by factors other than genetic parameters and has no influence on the concentration of AZA metabolites (6-TGN and 6-me-MP) [46].

4.2 Thiopurine methyltransferase and AZA

Thiopurine methyltransferase is encoded by the TPMT gene which is located on chromosome 6 [1]. This gene exhibits an autosomal codominant genetic polymorphism which can lead to an absent or low level of TPMT activity in individuals who are heterozygous or homozygous for this genetic polymorphism [8]. Currently there are 41 allele variants of the TPMT gene, with studies observing that allele genotype may be specific to race or ethnicity [47, 48].

The most common functional allele is TPMT*1, while TPMT*2, *3A, *3B, *3C and *4 are well documented polymorphism alleles associated with heterozygous or homozygous variant genotypes as listed in **Table 1** and **Table 2**. TPMT*2, *3A and *3C account for over 90% of the inactive alleles [49].

Individuals can be homozygous normal, meaning they inherited both wild-type normal metabolizing alleles, or they can be homozygous variant meaning both inherited alleles contained SNP's and there is little to no TPMT activity. Lastly an individual can be a combination, or heterozygous, implying that they inherited both a functional and a nonfunction allele resulting in a moderate enzyme activity level.

Table 1: Nomenclature of common TPMT allele mutations, issued by the TPMT Nomenclature Committee [50].

Common Allele Name	Coding DNA	Protein
TPMT*2	238G>C	Ala80Pro
TPMT*3A	460G>A / 719A>G	Ala154Thr / Tyr240Cys
TPMT*3B	460G>A	Ala154Thr
TPMT*3C	719A>G	Tyr240Cys

There have been numerous studies on the TPMT activity in Caucasian and Asian individuals but less so in the African population. A study by Fong *et al* (2017) found the *3A allele to be most common in the Caucasian and Indian population and the

*3C allele to be most common in the Asian population [1]. Studies in Nigeria and Libya have shown majority of the African population to have the *3C allele, a finding supported in a study on TPMT activity in African-Americans by Hon *et al* (1999) [8, 51, 52].

Updated recommendation for AZA dosing based on TPMT genotyping are frequently published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), an association created to meet the need for specific guidance of pharmacogenetic testing for both laboratories and clinicians. This dosing is based on the phenotype and genotype of the individual [3].

Table 2: CPIC recommended azathioprine therapy, according to TPMT phenotype [53, 54]

Phenotype	**TPMT Genotype**	**Example of Diplotypes**	**Recommendations: Therapeutic**	**Recommendations: Steady State**
Homozygous wild-type -High enzyme activity	Two functioning alleles	*1/*1	2-3 mg/kg/day	Allow 2 weeks to achieve steady state between each dose adjustment
Heterozygous -intermediate enzyme activity	One functional allele + One non-functional allele	*1/*2 *1/3A *1/3B *1/3C *1/4	Start at 30-70% of target dose, increase based on tolerance	Allow 2-4 weeks to achieve steady state between each dose adjustment
Homozygous variant -low/deficient enzyme activity	Two non-functioning alleles	*2/3A *3A/3A *3A/4 *3C/2 *3C/3A	Dose 3 x weekly or reduce daily dose by 10-fold. - Consider alternative treatment	Allow 4-6 weeks to achieve steady state between each dose adjustment

World-wide 11% of all Caucasian individuals will have the polymorphism, of these 1/300 individuals will be homozygous for the variant allele and thus completely TPMT deficient [6]. Measuring the TPMT activity level is an effective method of determining an individual's risk of toxicity and allows for dose adjustment or drug avoidance, to prevent the risk of the individual developing bone marrow suppression, leukopenia, thrombocytopenia or neutropenia [28, 55-60]. TPMT polymorphisms have only been associated with overall thiopurine-induced adverse drug reactions and not with the less severe side effects such as gastrointestinal upsets, skin

reactions and pancreatitis; which are instead attributed to the underlying disease [61]. None the less, in 2003, the FDA made it compulsory to add that the status of TPMT be determined prior to dosing, to the AZA package insert [1].

TMPT status can be determined via phenotypic enzyme-level testing using red blood cells, or by creating a white blood cell genetic profile [9, 55]. Determining the TPMT genotype of the individual will enable the clinician to better understand and predict variation in ADR's [62]. Abnormalities in other AZA metabolizing enzymes are extremely rare [58].

5. GASTROINTESTINAL THERAPY

5.1 Inflammatory Bowel Disease

One and a half million Americans suffer from IBD- an umbrella term used to represent a group of intestinal disorders affecting all or part of the digestive tract - which are comprised of 2 major diseases: ulcerative colitis (UC) and Crohn's disease (CD). IBD is estimated to affect 0.3% of the westernised world and has a rising prevalence in newly industrialised countries such as Africa, South America and the Middle East[63, 64]. Of these IBD patients at least 20% will suffer from leukopenia and eventually myelosuppression [9, 41, 61].

While UC and CD are very similar is symptoms, they differ vastly in location and treatment. UC only affects the inner lining of the colon whereas CD can affect any component of the gastrointestinal tract from the oesophagus to the rectum, and may also affect the skin, eyes or liver. Individuals suffering from IBD have a chronically inflamed gastrointestinal tract as the body perceives food or bacteria as a foreign substance and will respond by activating T-lymphocytes, triggering inflammation. Leukopenia is a common haematological sign of AZA toxicity, and occurs in up to 20% of IBD patients due to the TPMT polymorphism [57, 65]. In 2012, 100,000 people were admitted to hospital in the United States for various complications arising from IBD resulting in an annual hospitalization cost of over $3 billion [66].

A meta-analysis was conducted by Gisbert *et al* (2009), to review the efficacy of AZA and mercaptopurine in the induction and maintenance of disease remission in UC. This included a selection of randomised clinical trials (RCT) comparing AZA to placebo or aminosalicylates. A total of 1065 patients were included from 15 studies, taken from the year 2000 and onward[67]. The detail of the latter studies are

highlighted in Table 3. Of the 1065 patients, efficacy was observed in 697 patients (65.45%), i.e. they were found to be in disease remission at the end of the respective study period. The majority of these studies AZA was indicated for induction and maintenance purposes.

Table 3: Azathioprine monotherapy randomised clinical trials [67]

Author	Year of Publication	Use of Azathioprine	Efficacy = n/N (%)
Bastida *et al*[68]	2007	Induction / Maintenance	21/25 (80%)
Campbell & Ghosh[69]	2001	Maintenance	82/94 (87%)
Christodoulou *et al* [70]	2003	Induction / Maintenance	15/15 (94%)
Cuffari *et al* [71]	2001	Maintenance	14/19 (74%)
Falasco *et al* [72]	2002	Induction / Maintenance	25/58 (43%)
Fraser *et al* [73]	2002	Induction / Maintenance	201/346 (58%)
Gisbert *et al* [74]	2008	Induction / Maintenance	65/156 (42%)
Hibi *et al* [75]	2003	Maintenance	15/17 (88%)
Khan *et al* [76]	2000	Induction / Maintenance	38/53 (72%)
Kull & Beau [77]	2002	Induction / Maintenance	23/30 (77%)
Lopez-Sanroman *et al*[78]	2004	Maintenance	24/34 &71%)
Mantzaris *et al* [79]	2001	Induction	24/40 (60%)
Mantzaris *et al*[80]	2004	Maintenance	28/34 (82%)
Paoluzi *et al*[81]	2002	Induction / Maintenance	22/32 (69%)
Sood *et al*[82]	2006	Induction / Maintenance	101/111 (91%)

In 2017, a 5-year review was published by Cassieri *et al* (2017), documenting the number of IBD patients utilising AZA treatment at the IBD Outpatient Clinic at Cristo Fe Hospital in Italy. There were 260 IBD patients receiving AZA treatment over the 5-year observation period, 145 for CD and 115 for UC. Upon completion of the 5 yeartreatment period, 86 CD patients (59.3%) and 49 UC patients (42.6%) were still in remission[83].

Azathioprine is used in conjunction with steroids, methotrexate or biological agents such as infliximab to induce disease-remission and maintain remission, by reducing the white cell count and neutrophil count [84].

In 2017, Cholapranee *et al* (2017) released a systematic review of RCT's that was conducted to compare the efficacy of AZA monotherapy, Infliximab monotherapy and a combination of AZA and Infliximab in the treatment of CD. This review mentioned 12 RCT's which made use of monotherapy or combination therapy to attain disease remission induction and maintenance as well as mucosal healing [85]. Two of these studies that revealed significant results, were conducted by Lemann *et al* [86] (2006) and Colombel *et al* [87] (2010). Both studies were double-blind and made use of a placebo to maintain the blind.

The study by Lemann *et al* (2006) took into consideration that results may be altered in patients having previously used AZA or being treated with AZA at inclusion or commencement of the study, hence the study made use of two groups within the dosing arms; a failure stratum (previously exposed to AZA) and a naïve stratum [85]. 55 patients were part of the failure stratum and 58 of the naïve stratum. Patients were then treated with AZA and a placebo or AZA and Infliximab. The Crohn's Disease Activity Index (CDAI) was used to evaluate patients at 12 and 24 weeks respectively. A score of CDAI <150 was used as the marker for disease remission. Of the 113 patients partaking in the study, 57% of the combination group had disease remission at 24 weeks, in comparison to the 29% experienced by the monotherapy group. These findings are depicted in Figure 2. No significant effect was seen when comparing the stratum to the treatment group [86].

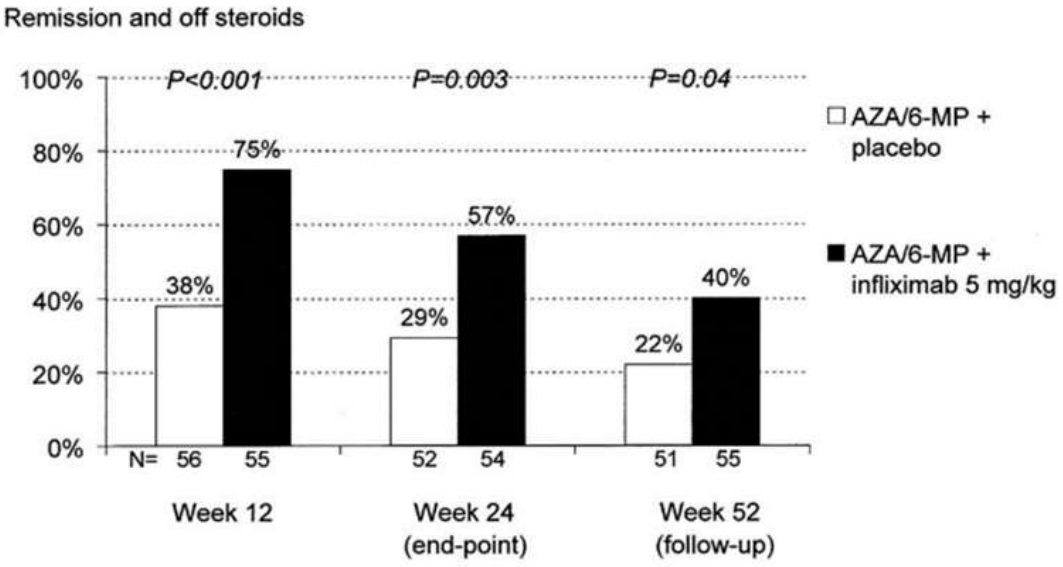

Figure 2: Disease remission in Azathioprine and placebo vs Azathioprine and Infliximab arms from the study by Lemann *et al* [86]

Colombel *et al* (2010) made use of three separate dosing arms in the double-blind study, comparing Infliximab and placebo versus AZA and placebo versus Infliximab and AZA. 508 patients were included in a 26 week study, which as seen in Table 4, showed that the combination therapy had more efficacy in reaching disease remission, than when compared to the other treatment arms (56.8% vs 44.4% vs 30.0%). This pattern was also seen in the mucosal healing within the arms[87]. This study also made use of the CDAI scoring.

Table 4: Details of the double-blind randomised clinical trials by Lemann *et al* [86] and Colombel *et al* [87]

Author	Year of Publication	Drug	Comparison	n=arm	Efficacy = n/N (%)
Lemann *et al*[86]	2006	Azathioprine	Infliximab	57	32/57 (57%)
		Azathioprine	Placebo	56	16/56 (29%)
Colombel *et al*[87]	2010	Azathioprine	Placebo	170	51/170 (30.0%)
		Infliximab	Placebo	169	75/169 (44.4%)
		Infliximab	Azathioprine	169	96/169 (56.8%)

There are a variety of ways to determine therapeutic efficacy, depending on the health care system and patients' standard of living.The most common mean is by biochemical evaluation where C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are assessed. CRP and ESR are both non-specific markers of inflammation that are used in combination to monitor the disease status of IBD patients. Frequent measuring of ESR and CRP counts as well as full blood counts (FBC) should be mandatory in individuals on chronic AZA treatment, as suggested by the FDA and recommended by other studies [3, 18, 61]. Studies suggest that patients should be monitored weekly for the first 8 weeks of therapy, thereafter monthly for the next 6 months and then bi-annually once the patient is on long-term therapy [17]. An accurate ESR count can be affected by numerous factors such as anaemia, infection or a sudden decrease in red blood cells, while CRP fluctuates rapidly and is affected by obesity or pregnancy. CRP values correlate very well to endoscopic findings in CD, but less so in UC [88-90].

Additional therapeutic markers include faecal calprotectin or faecal lactoferrin, which are superior to CRP in detecting active versus inactive IBD, but not superior to endoscopic findings. Using biochemical tests to determine inflammation should be an additive tool used in combination with clinical and endoscopic findings [91].

There is a great deal of debate as to what the exact therapeutic endpoint for disease remission should be, as many of the clinical efficacy markers are symptom based and reflect poorly on endoscopic findings of inflammation. It has recently been suggested that mucosal healing should be considered the therapeutic endpoint for disease remission, as opposed to simply achieving normal ranges of ESR and CRP counts, as emerging data is suggesting that individuals who have achieved mucosal healing have a better long-term prognosis [92, 93]. These CD and UC patients will require less hospitalizations and lower the need for systemic steroids [85].

6. Conclusion

AZA therapy is an affordable, effective solution to maintain disease remission in IBD patients and while genetic testing is expensive, it still outweighs the rate of hospitalisation, and the cost of medication and treatment caused by AZA cytotoxicity. When comparing AZA to the more recent IBD therapies that are available, AZA is a

viable option for the state sector but it is essential to make "early warning" SNP testing, common practice to ensure therapeutic efficacy.

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