Supplementary material

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1: Participating centres

Clinical site and number of patients	AZA/ALLO number of patients	AZA number of patients
University Hospital Hvidovre	21	21
Herlev University Hospital	15	11
Aalborg university Hospital: 9 patients	5	4
Viborg University Hospital: 5 patients	3	2
Køge University Hospital: 4 patients	3	1
Odense University Hospital: 3 patients	1	2
Esbjerg University Hospital: 1 patient	1	
Vejle University Hospital: 1 patient	1	

2**: Endoscopic Assessment**

Endoscopic remission at week 52 was centrally scored by two blinded investigators (AMN and KT). Disagreements occurred in seven cases (two in the L-AZA/ALLO group and five in the AZA group). These videos were re-evaluated blindly for the investigator and a consensus was obtained before further analyses. In six of the cases the score was reduced by one point and in one case it was reduced by two. For endoscopies with missing videos (n=5), the score from the endoscopist as noted in the CRF was used.

3: Prednisolone tapering table

Week	50 mg	45 mg	40 mg	35 mg	30 mg	25 mg	20 mg	15 mg
1	50	45	40	35	30	25	20	15
2	45	45	40	35	30	25	20	15
3	40	40	35	30	30	25	20	15
4	35	35	35	30	25	25	20	15
5	30	30	30	25	25	20	15	10
6	25	25	25	25	25	20	15	10
7	20	20	20	20	20	20	15	10
8	15	15	15	15	15	15	15	10
9	10	10	10	10	10	10	10	5
10	5	5	5	5	5	5	5	5
11	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
12	0	0	0	0	0	0	0	0

4: Laboratory test

The table shows the median values (25th to 75th percentiles) at baseline (week 0) and end of follow up (week 52) for patients allocated to L-AZA/ALLO versus AZA.

Patients allocated to Low dose AZA/allopurinol								
	Mean Corpuscular volume		C-reactive protein		Haemoglobin		Albumin	
Percentile/week	0	52	0	52	0	52	0	52
50	88	95	3	3	8	8	39	41
25	84	90	0·8	0·9	8	8	33	39
75	90	100	4	4	9	9	41	44
Patients allocated to AZA								
50	89	94	3	3	9	9	39	39
25	86	90	0·6	0·7	8	8	36	37
75	94	96	4	4	9	9	41	41
P-value	0·07	0·55	0·55	0·71	0·790	0·17	0·54	0·11

5: Logistic regression analyses of remission

In a univariable regression, clinical remission at week 52 was not predicted by age (OR 0·99 [0·963 to 1·03]; *p*=0·696), sex (OR 1·007 [0·413 to 2·455]; *p*=0·988), BMI (OR 0·882 [0·767 to 1·013]; *p*=0·077), smoking (OR 1·619 [0·661 to 3·967]; *p*=0·292), disease duration (OR 0·998 [0·926 to 1·075]; *p*=0·956), calprotectin at baseline (OR 0·100 [0·999 to 1·001]; *p*=0·308) or use of IFX before week 26 (OR 1·29 [0·50 to 3·31]; *p*= 0·6021. The only significant predictor was calprotectin at week 26 without adjustment (OR 0·100 [0·994 to 0·998]; *p*=0·040) and after adjusting for age and sex (OR 0·997 [0·994 to 0·999]; *p*=0·028). In total, 23 patients who did not achieve clinical remission by week 52 had a positive calprotectin (higher than 200 mg/kg) at week 26 compared with seven patients who did achieve remission (*p*=0·004). In a logistic regression, a normal calprotectin level at week 26 was a significant predictor of clinical remission at week 52 in unadjusted analyses (OR 0·035 [0·010 to 0·114]; *p*=0·001).

Univariable analysis	Odds Ratio [95% CI]
Treatment group (L-AZA/allo vs AZA)	2·54 [95% CI 1·00 to 6·78]
Age	0·99 [0·96 to 1·03]
Sex	1·01 [0·41 to 2·46]
BMI	0·88 [0·77 to 1·01]
Smoking	1·62 [0·66 to 3·97]
Disease duration	1·00 [0·93 to 1·08]
Baseline calprotectin	1·00 [0·99 to 1·01]
Calprotectin mg/kg > 200 at week 26	0·100 [0·994 to 0·998]; *p*=0·040
Calprotectin mg/kg < 200 at week 26	0·035 [0·010 to 0·114]; *p*=0·001
Infliximab	1·29 [0·50 to 3·31]
Multivariable analysis with all variables entered	

Treatment group AZA/ALLO	1·84 [0·59 to 5·72]
Age	0·99 [0·95 to 1·03]
Sex	0·77 [0·25 to 2·36]
BMI	1·05 [0·89 to 1·23]
Smoking	1·78 [0·92 to 3·47]
Disease duration	1·03 [0·92 to 1·14]
Baseline calprotectin	1·00 [0·99 to 1·01]
Infliximab	0·93 [0.27 to 3·20]

6: Copy of study protocol

Low-dose azathioprine and allopurinol- versus azathioprine monotherapy in patients with ulcerative colitis: An investigator-initiated, open, multicentre, parallel-arm, randomised controlled trial

PROTOCOL NUMBER: VERSION 6

DATE 15.MAJ 2019

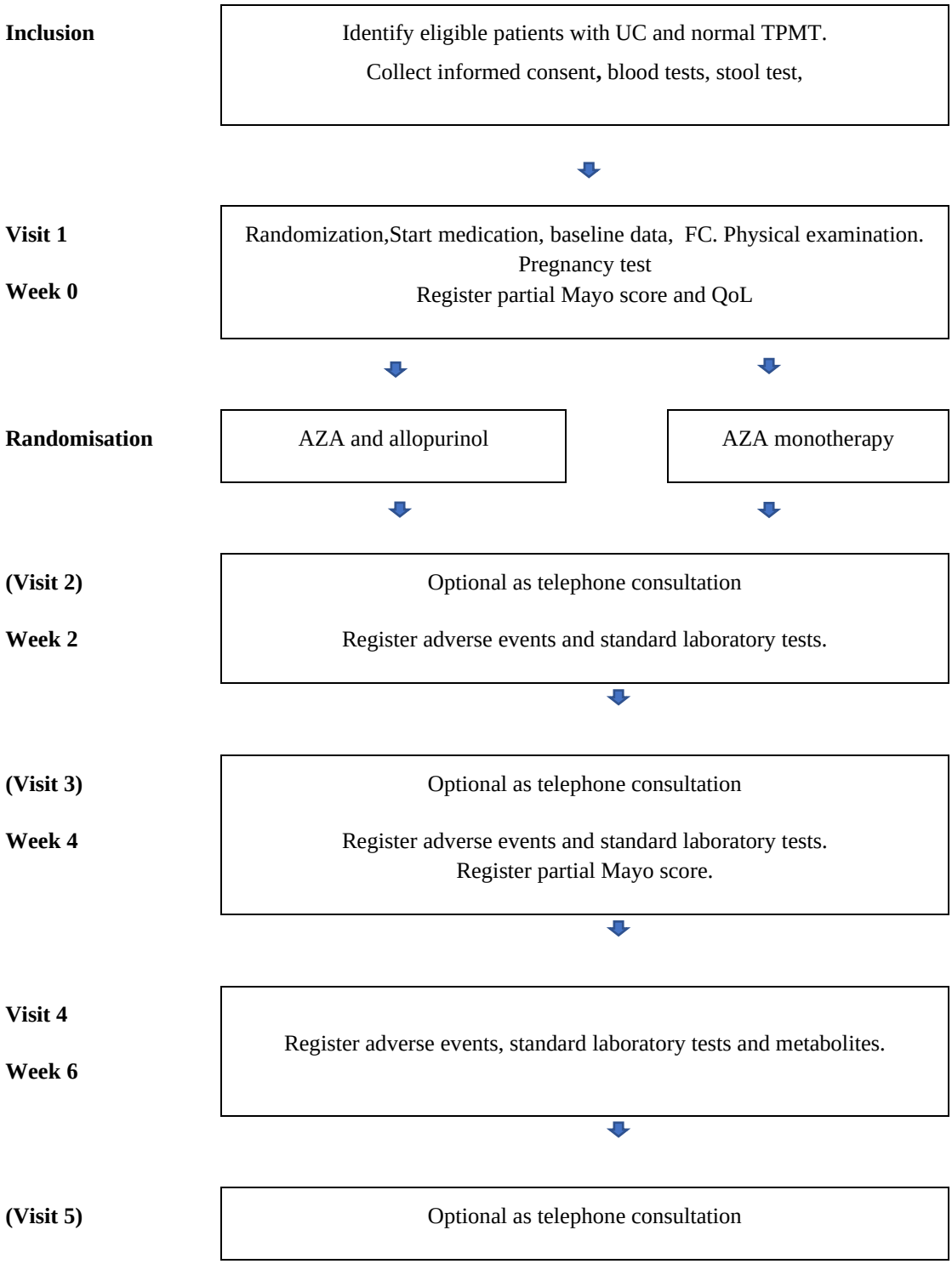
AAUC STUDY GROUP

PROTOCOL SUMMARY

Background	Azathioprine is considered first line immunomodulatory therapy for patients with ulcerative colitis. Up to 50% are treatment failures or experience adverse events leading to treatment withdrawal. Recent evidence suggests that the combination of allopurinol and low dose azathioprine increases the proportion of treatment responders and reduce the risk of adverse events.
Objectives	To evaluate the beneficial and harmful effects of low dose azathioprine and allopurinol versus standard azathioprine monotherapy in patients with ulcerative colitis.
Design	Investigator initiated, multicentre, parallel arm, open, randomised controlled trial with blinded assessment
Experimental Intervention	Azathioprine combined with allopurinol 100 mg once daily orally. The initial dose of azathioprine will be approx. 0.8 mg/kg once daily.
Control Intervention	Azathioprine administered with an initial dose of approx. 2.5 mg/kg once daily.
Population	Adult patients with ulcerative colitis requiring maintenance of remission with thiopurine.
Sites	Hvidovre University Hospital, Herlev University Hospital, Zealand University Hospital in Køge, Odense University Hospital, Århus University Hospital, Aalborg University Hospital, Regionshospital i Viborg, South Jutland Hospital ,Esbjerg, Vejle Sygehus
Inclusion period	01.12.2016 – 01.06.2021.

Follow up	52 weeks.
Primary outcomes	Steroid- and biologic treatment free remission at 52 weeks defined as total Mayo score ≤1 without rectal bleeding.
Secondary outcomes	To compare • Time to remission (defined as described above) • Clinical response after 52 weeks (defined as a Mayo score between ≤1 to < 3) • Endoscopic remission after 52 weeks (defined as a Mayo subscore of 0) • Fecal calprotectin levels after 52 weeks. • Quality of life after 26 and 52 weeks using the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) and short health scale (SHS). • Correlation between E-6TGN and clinical, laboratory, endoscopic and histological indices. • Histological mucosal healing after 52 weeks. • Adverse events
Analyses	Patients will be compared using t-tests with inclusion of all patients randomised (intention-to-treat). Binary outcomes will be analysed using multivariable and univariable logistic regression analysis. Time to event data will be analysed in Kaplan Meier Plots with log-rank tests to compare groups.
Sample size	The required sample size is 84 evaluable patients (42 patients in each arm).

"Timeline of events"



Week 8	Register adverse events and standard laboratory tests. Register partial Mayo score.
(Visit 6) **Week 12**	Optional as telephone consultation Register adverse events, standard laboratory tests, FC and metabolites. Control prednisone tapering. Partial mayo score
(Visit 7) **Week 16**	Optional as telephone consultation Register adverse events, standard laboratory tests. Register partial Mayo score
Visit 8 **Week 26**	Register adverse events, standard laboratory tests and metabolites. Control anti-TNFα therapy has ended. FC. Register partial Mayo score and QoL.
(Visit 9) **Week 38**	Optional as telephone consultation Register adverse events and standard laboratory tests. Register partial Mayo score.
Visit 10 **Week 52**	Final visit. Register adverse events, standard laboratory tests FC and metabolites. Register total Mayo score and QoL. Sigmoidoscopy

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Background

The thiopurines azathioprine and mercaptopurine have been established as first line immunomodulatory therapy for Crohn's disease and ulcerative colitis. Up to 60% of patients with inflammatory bowel disease (IBD) are treated with thiopurines during the course of their disease.[1] Thiopurines also play an increasing important role as concomitant immunomodulation alongside biologic treatment to avoid immunogenicity.[2] The yearly costs of azathioprine amounts to less than 200 € whereas biologic treatments are associated with costs exceeding 13.500 €/year. Unfortunately, about 50% of patients are classified as thiopurine treatment failures due to an inadequate response or adverse events leading to

withdrawal of therapy.[3] Potential alternatives to treatment failure include less-established immunosuppressive drugs such as methotrexate (in Crohn's disease) or biologics, or surgery.

Allopurinol was originally developed to optimize azathioprine metabolite profiles and improve treatment response.[4] The combination of azathioprine and allopurinol was abandoned after studies in leukemia failed to demonstrate additional benefit from the co-intervention. The treatment was re-introduced after subsequent studies found improved azathioprine metabolite profiles and graft survival in patients undergoing renal transplantation.[5] Recently, the combination of low-dose azathioprine and allopurinol was assessed in IBD patients with an inadequate response or adverse events during treatment with azathioprine monotherapy. Most patients failing azathioprine monotherapy have a "predominant methylator" phenotype (thiopurine shunt) associated with normal thiopurine methyltransferase (TPMT) activity, a key enzyme in the thiopurine metabolic pathway.[6] The co-intervention, reverses the unfavorable metabolite profile leading to higher erythrocyte 6-Thioguanin-Nucleotide (6-TGN) levels and lower methylated metabolites, which reduces the risk of hepatotoxicity and other adverse events.[6-10] In non-responders to azathioprine monotherapy, the co-intervention appears to increase clinical remission rates and provide safe long-term immunosuppression.[7-12] In a recent randomized pilot study including 46 IBD patients, we found that azathioprine/allopurinol therapy increased remission rates from 35% to 70% compared to azathioprine monotherapy.[13] The results suggest that for every three patients treated with azathioprine/allopurinol, one additional patient will achieve remission and that the annual costs associated with alternative biologic treatment would be reduced with about 472.000€ for every 100 patients. These estimates are based on a small number of patients and the included patients were relatively heterogeneous as the trial included both patients with ulcerative colitis and Crohn's disease. Crohn's disease is a more heterogeneous disease as it can affect the entire digestive tract, so it is more easy to perform studies in ulcerative colitis. We therefore plan to conduct a large, randomized, open, controlled trial evaluating the benefits and harms of low dose azathioprine/allopurinol versus standard azathioprine monotherapy in patients with ulcerative colitis.

Hypothesis

In patients with ulcerative colitis and normal TPMT, the combination of azathioprine and allopurinol increases the proportion of patients achieving remission and reduces the risk of adverse events

Study design

Randomised, investigator-initiated, parallel-arm, open trial in patients with ulcerative colitis. Participants will be allocated to low dose azathioprine/allopurinol - versus standard azathioprine monotherapy (Timeline). The primary and secondary endpoints will be evaluated by a blinded assessor.

Experimental intervention: Low azathioprine administered once daily with an initial dose of approx. 0.8 mg/kg combined with allopurinol once daily (appendix 1).

Control intervention: Standard azathioprine administered once daily with an initial dose of approx. 2.5 mg/kg once daily (appendix 1).

Inclusion criteria

In order to be eligible to participate in this trial, all of the following criteria must be met:

- Age 18 - 80 years at the time of inclusion;
- Willingness to comply with all trial procedures and being available for the duration of the trial.
- Clinically and histologically verified ulcerative colitis.
- Eligible for treatment with thiopurines are patients with severe Ulcerative Colitis with the need for therapy with prednisolone or/and anti-TNFα.
- A sigmoidoscopy or colonoscopy showing active inflammation during the present disease flare
- Negative stool test for pathogen bacteria incl. C. difficile
- Informed consent.
- Normal TPMT TPMT can be measured as genotype or phenotype. A normal TPMT genotype is ‘wild type’. A normal TPMT phenotype is > 14 U/ml ery.Oral 5-ASA dose stable for 2 weeks

Exclusion criteria

Patients are excluded according to the following criteria:

- Kidney disease with a GRF < 50 ml/min.

- Persistent alanine aminotransferase U/L (ALT) twice above upper limit of the normal range.
- Participation in other interventional clinical trials.
- Pregnancy or breastfeeding.
- Previous thiopurine treatment.
- Previous or current treatment with other biologics than anti-TNFα
- Not being able to comply with the study, assessed by investigator

Withdrawal criteria

- Partial Mayo score ≥ 3 at week 16 and during the remaining study
- Subjects with intolerable side effects to medication.
- Subjects with laboratory test showing persistent liver affection (ALT > 100 U/L), increasing s-amylase (twice of normal values) or leukopenia (total leukocyte count < 3.0), irrespective of patient's condition.
- Change in oral 5-ASA dose during study
- Use of topical steroids during study
- Abdominal surgery.
- Severe infection.

Concomitant medication

Per oral steroid has to be administered following the table below or using budesonide mutimatrix 9 mg/day until week 8. Per oral steriods has to be stopped at week 12 regardless of the starting dose following the table below.

Uge	50 mg	45 mg	40 mg	35 mg	30 mg	25 mg	20 mg	15 mg
1	50	45	40	35	30	25	20	15
2	45	45	40	35	30	25	20	15
3	40	40	35	30	30	25	20	15
4	35	35	35	30	25	25	20	15
5	30	30	30	25	25	20	15	10
6	25	25	25	25	25	20	15	10
7	20	20	20	20	20	20	15	10
8	15	15	15	15	15	15	15	10
9	10	10	10	10	10	10	10	5
10	5	5	5	5	5	5	5	5
11	2,5	2,5	2,5	2,5	2,5	2,5	2,5	2,5
12	0	0	0	0	0	0	0	0

In the case of a relapse, the per oral steroid can be increased/restarted with 20 mg and decreased with 5 mg/week. Likewise, budesonide multimatrix can be restarted at 9 mg until week 16.

Not being able to stop steroids at week 16 is regarded a failure to study medication.

Infliximab can be used as a rescue therapy in week 0, 2, 6 and every 8 week until week 26 where it has to be stopped if the patients have clinical response.

The dose of per oral 5-ASA must be stable in 2 weeks before inclusion and during the entire study.

It is allowed to use topical 5-ASA during the study.

Topical steroids are not allowed during the study

It is allowed to continue concomitant medication for other diseases, including inhalation steroids, throughout the study.

Sigmoidoscopy

Sigmoidoscopy (or colonoscopy) at week 52 is performed, as in clinical practice, to evaluate treatment efficacy. The endoscopic degree of inflammation is evaluated using the Mayo Endoscopic Subscore, and the disease extent is evaluated according to the Montréal criteria and divided into proctitis, left-sided or extensive colitis. The endoscopy will be recorded and/or photographed

The recording will start from the left flexure and continuously until the anus and have a duration of at least 3 minutes. If video endoscopy is not available at the participating center, 4 photos from each segment will be taken; 4 from the left colon/sigmoid and 4 from the rectum.

The videos/photos will be assessed blinded by 2 specialized endoscopists at Hvidovre University Hospital. The patient information will be pseudo anonymized stored on an encrypted USB device and kept in a locked locker in a locked room. 4 biopsies shall be taken from the rectum and in case of inflammation from the most inflamed area, according to standard department procedures. The biopsies will be stored at the local pathology storage facility (patobank) and will be collected at the end of the study for evaluation of histological mucosal healing by the pathologist at Hvidovre Hospital as a second opinion. After examination the biopsies will be returned, within 7 days, to the local pathology storage facility (patobank). The samples will be reviewed by 2 experienced gastrointestinal pathologists unaware of any clinical information. The pathologists will grade histological inflammatory activity into 4 groups: (0) No neutrophil inflammation, (1) cryptitis or crypt abscesses in ,10% of the illustrated crypts, (2) cryptitis or crypt abscesses in 10%–50% of the illustrated crypts, and (3) cryptitis or crypt abscesses in .50% of the illustrated crypts or presence of erosions or ulcers. The tissue samples showing the highest grade of inflammation determines the final score. Interpretative doubts will be solved by consensus

Study duration and study visits

The duration of the inclusion period will be one year. Participants will be followed for 52 weeks (see timeline below). Visits will be planned at baseline (time 0) and after (2), (4), 6, (8), 12, (16), 26, 38, and 52 weeks. Visits with ( ) are blood test, and a visit for registration of adverse events. The visit is optional as telephone consultation.

It is allowed to reschedule the visits ± 5 days.

Primary outcome

Steroid and biologic treatment free remission at 52 weeks defined as total Mayo score ≤1 without rectal bleeding.

Secondary outcomes

To compare

- Time to remission (defined as described above)
- Clinical response after 52 weeks (defined as a Mayo score between ≤1 to < 3)
- Endoscopic remission after 52 weeks (defined as a Mayo subscore of 0)
- Fecal calprotectin levels after 52 weeks.
- Quality of life after 26 and 52 weeks. We will assess the quality of life using the Short Inflammatory Bowel Disease Questionnaire (SIBDQ) and short health scale (SHS).[1]
- Histological mucosal healing after 52 weeks.
- Correlation between E-6TGN and clinical, laboratory, endoscopic and histological indices after 52 weeks
- Adverse events defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. The subjects who develop an adverse event will be followed until complete resolution. A serious adverse event is one that meets one or more of the following criteria:

    i. Results in death

    ii. Is life-threatening (places the subject at immediate risk of death from the event as it occurred)

    iii. Results in inpatient hospitalization or prolongation of existing hospitalization

    iv. Results in a persistent or significant disability or incapacity

    v. Results in a congenital anomaly or birth defect

    vi. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Randomization

The patients will be randomised 1:1 based on computer generated random numbers. The allocation sequence will be kept by a research nurse who is not involved in the trial. The trial is open.

Trial medication

Trial medication will be shelf medicine.

Every patient will have an individual medicine registration form according to GCP guidelines. Data includes: patient ID, name-, dose and amount of medicine, batch-number, amount of medicine extradited, expiration date, initials and date of extraditing personnel, amount of medicine returned from patient, date and initials for return of medicine.

Labels are applied to trial medicine at each site. Investigators at each site will teach the local nurses and doctors involved in the project, to apply labels according to procedures that fulfill the GCP guidelines.

Pregnancy

Women of childbearing age must have a negative pregnancy test prior to inclusion. Effective contraception must be used during the study. The Danish Medicines Agency considers the following contraceptives as effective contraceptive methods in drug trials: Contraceptive coil, birth control pills, hormonal implants, transdermal patches, vaginal ring or transdermal injection.

Potential Risks and Adverse effects

The risk of side effects from treatment with combination therapy with ALLO and low-dose AZA is not expected to be larger than AZA therapy alone.

Adverse events azathioprine

There is no currently accepted clinical documentation for azathioprine that can be used to determine the frequency of undesirable effects. Undesirable effects may vary in their incidence and severity depending on the indication; occasionally these adverse effects may have a fatal outcome, particularly in patients receiving several immunosuppressive drugs. The following convention has been utilised for the classification of frequency: Very common, ≥ 1/10; common, ≥1/100 and < 1/10; uncommon, ≥1/1000 and < 1/100; rare, ≥ 1/10000 and < 1/1000; very rare, < 1/10000.

Infection and infestations

Transplant patients receiving azathioprine in combination with other immunosuppressants.

Very common: Viral, fungal and bacterial infections

Other indications.

Uncommon: Viral, fungal and bacterial infections

Frequency unknown: Meningitis

Patients receiving Azathioprine alone, or in combination with other immunosupressants, particularly corticosteroids, have shown increased susceptibility to viral, fungal and bacterial infections, including severe or atypical infection with varicella, herpes zoster and other infectious agents (see also section 4.4 Special Warnings and Precautions for Use).

Neoplasms benign and malignant (including cysts and polyps)

Rare: Neoplasms including non-Hodgkin's lymphomas, skin cancers (melanoma and non-melanoma), sarcomas (Kaposi's and non-Kaposi's) and uterine cervical cancer in situ, acute myeloid leukemia and myelodysplasia (see also section 4.4).

The risk of developing non-Hodgkin's lymphomas and other malignancies is increased in patients who receive immunosuppressive drugs, particularly in transplant recipients receiving aggressive treatment and such therapy should be maintained at the lowest effective levels. The increased risk of developing non-Hodgkin's lymphomas in immunosuppressed rheumatoid arthritis patients compared with the general population appears to be related at least in part to the disease itself.

There have been rare reports of acute myeloid leukemia and myelodysplasia (some in association with chromosomal abnormalities).

Blood and lymphatic system disorders

Very common: Bone marrow depression; leucopenia.
Common: Thrombocytopenia.
Uncommon: Anemia.
Rare: Agranulocytosis, pancytopenia, aplastic anemia, megaloblastic anemia, erythroid hypoplasia.
Hematological changes are dose-related and generally reversible. Bone marrow depression occurs particularly in patients predisposed to myelotoxicity, such as those with TPMT deficiency and renal or hepatic insufficiency and in patients failing to reduce the dose of azathioprine when receiving concurrent allopurinol therapy. Reversible dose-related increases in mean corpuscular volume and red cell content have occurred in association with azathioprine therapy.
Immune system disorders
Uncommon: Hypersensitivity reactions
Several different clinical syndromes, which appear to be idiosyncratic manifestations of hypersensitivity, have been described occasionally following administration of azathioprine. Clinical features include general malaise, dizziness, nausea, vomiting, diarrhoea, fever, rigors, exanthema, rash, vasculitis, myalgia, arthralgia, hypotension, renal dysfunction including interstitial nephritis, hepatic dysfunction and cholestasis.
In many cases, re-challenge has confirmed an association with azathioprine. Immediate withdrawal of azathioprine and institution of circulatory support where appropriate have led to recovery in the majority of cases.
Other marked underlying pathology has contributed to the very rare deaths reported. Following a hypersensitivity reaction to azathioprine, the necessity for continued administration of the drug should be carefully considered on an individual basis.
Respiratory, thoracic and mediastinal disorders
Very rare: Reversible pneumonitis.
Gastrointestinal disorders
Uncommon: Pancreatitis.
Pancreatitis has been reported in a small percentage of patients receiving azathioprine, particularly in renal transplant patients and those diagnosed as having inflammatory bowel disease. There are difficulties in relating the pancreatitis to the administration of one particular drug, although re-challenge has confirmed an association with azathioprine on occasions.
Rare: Colitis, diverticulitis, gastrointestinal ulceration, gastrointestinal hemorrhage, intestinal necrosis and bowel perforation, severe diarrhea.
Serious gastrointestinal complications have been described in transplant recipients receiving immunosuppressive therapy. However, the aetiology is not clearly established and high-dose corticosteroids may be implicated. Severe diarrhea, recurring on re- challenge, has been reported in patients treated with azathioprine for inflammatory bowel disease. The possibility that exacerbation of symptoms might be drug-related should be borne in mind when treating such patients.
Frequency unknown: Nausea, anorexia, vomiting
The reported incidence of gastrointestinal intolerance to oral administration of azathioprine is variable. In some instances, it seems to be a dose-related phenomenon and after a brief interruption administration may often be successfully reinstituted, at a lower dose. Doses should, where possible, be taken with food.

Hepato-biliary disorders
Uncommon: Cholestasis and abnormal liver function tests.
Rare: Life-threatening hepatic damage. Cholestasis and deterioration of liver function have occasionally been reported in association with azathioprine therapy and are usually reversible on withdrawal of therapy. This may be associated with symptoms of a hypersensitivity reaction (see Immune system disorders).
Rare, but life-threatening hepatic damage associated with chronic administration of azathioprine has been described primarily in transplant patients. Histological findings include sinusoidal dilatation, peliosis hepatis, veno-occlusive disease and nodular regenerative hyperplasia. In some cases, withdrawal of azathioprine has resulted in either a temporary or permanent improvement in liver histology and symptoms.
Skin and subcutaneous tissue disorders
Rare: Rashes which may be erythematous, pruritic or pustular and may occur as part of a hypersensitivity reaction (see Immune system disorders), alopecia, photosensitivity.
Hair loss has been described on a number of occasions in patients receiving azathioprine and other immunosuppressive agents. In many instances the condition resolved spontaneously despite continuing therapy. The relationship between alopecia and azathioprine treatment is uncertain.
Very rare: Stevens-Johnson syndrome and toxic epidermal necrolysis
Frequency unknown: Acute febrile neutrophilic dermatosis.

Adverse events allopurinol

The most frequent adverse reaction to allopurinol is skin rash. Skin reactions can be severe and sometimes fatal. Therefore, treatment with allopurinol should be discontinued immediately if a rash develops). Some patients with the most severe reaction also had fever, chills, arthralgias, cholestatic jaundice, eosinophilia and mild leukocytosis or leukopenia. Among 55 patients with gout treated with Allopurinol for 3 to 34 months (average greater than 1 year) and followed prospectively, Rundles observed that 3% of patients developed a type of drug reaction which was predominantly a pruritic maculopapular skin eruption, sometimes scaly or exfoliative. However, with current usage, skin reactions have been observed less frequently than 1%. The explanation for this decrease is not obvious. The incidence of skin rash may be increased in the presence of renal insufficiency. The frequency of skin rash among patients receiving ampicillin or amoxicillin concurrently with allopurinol has been reported to be increased.

Most Common Reactions (more than 1 %):

Gastrointestinal: Diarrhea, nausea, alkaline phosphatase increase, SGOT/SGPT increase.

Metabolic and Nutritional: Acute attacks of gout.

Skin and Appendages: Rash, maculopapular rash.

Incidence Less Than 1%**:**

Body as a Whole: Ecchymosis, fever, headache.

Cardiovascular: Necrotizing angiitis, vasculitis.

Gastrointestinal: Hepatic necrosis, granulomatous hepatitis, hepatomegaly, hyperbilirubinemia, cholestatic jaundice, vomiting, intermittent abdominal pain, gastritis, dyspepsia.

Hemic and Lymphatic: Thrombocytopenia, eosinophilia, leukocytosis, leukopenia.

Musculoskeletal: Myopathy, arthralgias.

Nervous: Peripheral neuropathy, neuritis, paresthesia, somnolence.

Respiratory: Epistaxis.

Skin and Appendages: Erythema multiforme exudativum (Stevens-Johnson syndrome), toxic epidermal necrolysis (Lyell's syndrome), hypersensitivity vasculitis, purpura, vesicular bullous dermatitis, exfoliative dermatitis, eczematoid dermatitis, pruritus, urticaria, alopecia, onycholysis, lichen planus.

Special Senses: Taste loss/perversion. *Urogenital:* Renal failure, uremia

Registration and reporting of adverse events and reactions

All adverse events (AE), adverse drug reactions (AR), serious adverse events (SEA), serious adverse drug reactions (SAR) and unexpected serious adverse reactions (SUSARS) will be registered at every patient visit or in between visits if patients are admitted to the hospital or contact the outpatient clinic with information of an event/reaction.

The reference documents for ARs, SARs and SUSARS is the investigators brochure

All ARs and AEs (including SAEs) will be collected from investigators and submitted to EudraCT at the end of the study.

Investigators will report SAEs, SARs and SUSARs to Sponsor immediately (within 24 hours) and sponsor will determine whether a SAE is drug related. Investigators will report the event using a predefined report form according to the GCP guidelines.

Once a year throughout the trial, the investigator will prepare a list of all **SAR's** that have occurred during the trial, and the list will be submitted to Sundhedsstyrrelsen.

Sponsor will ensure that all information about SUSARs that are fatal or life-threatening is recorded and reported to Sundhedsstyrelsen as soon as possible and no later than 7 days after the investigator became aware of such possible side effects. All SUSARS that are not fatal or life-threatening will be reported to Sundhedsstyrelsen as soon as possible and no later than 15 days after the investigator is aware of the possible side effect

All patients will be followed up for one year for late adverse events, whether continuing trial medication or not. After that they will be followed up according to standard department guidelines.

Risks and disadvantages

There will be disadvantages associated with collecting stool samples and blood sampling. The number of visits and blood and fecal tests is usual practice when starting AZA treatment.

The risks associated with sigmoidoscopy include bleeding after biopsies and perforation of the colon (<1:1000).

The risks associated with the additional blood tests include pain and formation of a hematoma after the blood test is drawn. The amount of blood drawn is approx. 100 ml over one year.

Potential benefits

Included patients may receive the combination of low dose azathioprine and allopurinol, which may potentially be associated with a better chance of achieving remission and a lower risk of adverse events.

Premature Termination or Suspension of Study

This trial may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. Circumstances that may warrant termination include, but are not limited to: Determination of unexpected, significant, or unacceptable risk to subjects.

Unscheduled/Intermediate Visits

If patients experience adverse reactions the investigator will decide if an unscheduled visit is needed. At an unscheduled visit or if participants are admitted between scheduled visits, the following data will be collected:

- Information about adverse events;
- Blood tests including hemoglobin, white blood cell count, platelet count, liver blood tests, serum amylase, c-reactive protein, sodium, potassium, and serum creatinine.
- Partial Mayo Score

Prior to enrolment

We will screen potentially eligible patients in the outpatient clinics and departments of included sites. Patients will be screened for inclusion and exclusion criteria. Patients will be contacted if potentially eligible.

Table 1. Study schedule

	Inclusion	Week 0	(Week 2) *	(Week 4) *	Week 6	(Week 8) *	(Week 12) *	(Week 16) *	Week 26	(Week 38) *	Week 52	Unscheduled	Withdrawal
Standard blood tests**	1	1	1	1	1	1	1	1	1	1	1	1	1
Collection of baseline demographic data		1											
Physical examination		1											
Sigmoidoscopy											1		
TPMT genotype	1												
Pregnancy test***		1											
Faeces calprotectin		1					1		1		1		1
Quality of life questionnaires		1							1		1		1
Partial Mayo score		1		1		1	1	1	1	1	1	1	1
Total Mayo score											1		
6-TGN and 6-MeMP					1						1		1
Registration of adverse events			1	1	1	1	1	1	1	1	1	1	1
Faeces pathogens and clostridium difficile	1												1

*Optional as a telephone consultation**Standard blood tests include hemoglobin, MCV, white blood cell count, platelet count, liver blood tests, serum amylase, c-reactive protein, sodium, potassium, and serum creatinine. ***Women of childbearing age.

Inclusion

Collection of informed concent and blood tests including TPMT genotype, standard blood tests (hemoglobin, white blood cell count, platelet count, MCV, liver blood tests, serum amylase, c-reactive protein, sodium, potassium creatinine), pregnancy test, feces for pathogen bacteria and C.difficile.

Week 0

Inclusion criteria will be systematically reviewed. If all criteria are as the protocol subscribes, there will be collected demographic data (sex, height, weight, smoking, medical history, concomitant medication) fecal calprotectin, Mayo score, quality of life questionnaires and performed physical examination. Randomization will be carried out and patients will start study medication.

Inclusion and week 0 visit can be merged as one visit if all information for completing in- and exclusion criteria are present at inclusion.

Etc for remaining visits

Standard blood tests will be analysed at the local hospital laboratory at each site, as in the usual clinincal practice. Metabolites (6-TGN and MeMP) and fecal calprotectin will be analysed at 'Klinisk biokemisk afdeling' at Hvidovre hospital. The results will not be available to sponsor or the investigators until the end of the trial.

The blood tests for the metabolites will be kept in a research biobank at 'Klinisk biokemisk afdeling'. The blood tests will be destroyed according to the guideline at 'Klinisk biokemisk afdeling' immediately after analysation and at the latest 01.06.21, when the study ends.

All other biological material will be analyzed as soon as possible and afterwards destroyed.

Sample size considerations

We conducted the sample size calculation with alpha 5% and power 80%. Based on previous evidence, we set the response rate to 60% in the intervention group (azathioprine/allopurinol) and 33% in the control group (standard azathioprine monotherapy). The required sample size is 84 evaluable patients (42 in each treatment group). Patients who are randomized and fail to be meet inclusion criteria will be replaced. Patients who have severe lack of effect of treatment that results stop of study medication or colectomy within 8 weeks from study start will be replaced. Patient withdrawn due to other conditions than withdrawal criteria will also be replaced.

Statistical considerations

Data will be entered into paper Case Record Forms (CRFs), which will be considered our source data. We plan to conduct our analyses based on the intention to treat principle including all patients randomized irrespective of compliance or follow up. For patients with missing outcome data, we will use carry forward of the last observed response. We will summarize quantitative data using means with standard deviations and binary data using proportions. The intervention and control group will be compared using t-tests or chi-square tests as appropriate. In addition, we will conduct multivariable and univariable logistic regression analyses to identify predictors of primary and secondary binary outcomes at week 52 (as specified under primary and secondary outcomes). We will analyze time to event data using Kaplan Meier plots with log-rank tests comparing groups. We will use two-sided p-values and set the level of significance to 5%. No interim analyses will be performed. The trial will be terminated at the end of the planned follow up.

Economics

This study is investigator initiated. The results are of exclusive scientific interest and do not involve economic and commercial interest. Economic support to Clinical monitoring and steering the study in addition Good Clinical Practice (GCP) monitoring and measurements of metabolites, calprotectin and histologic investigation of the biopsies has been given from Regionernes Medicinpulje (2.3 mill dk see budget). The investigators have no conflicts of interest in the pharmaceutical companies which manufactures the medicine used in this study. There is no fee for trial participants.

Ethics

Patients are recruited from the Department's Out-Patient Clinic as well as from the respective hospital wards.

Patients with UC, who in accordance with the department's general guidelines, is recommended to start AZA as part of the treatment for their chronic bowel disease, will be asked to participate in the project.

Initial patient contact is in association with a visit in the out-patient clinic or hospital submission. Therefore, it cannot always be ensured that the project participant has an accomplice present during the initial consultation. All project participants will be informed - in oral and written form - of the possibility to have an accomplice present. If desired, then only written information will be provided. An appointment will be agreed upon for a discussion with oral information. As a starting point, the time for considering project participation will be 24 hours but can be less as one often must begin treatment as soon as indications have been made. The participant will be accordingly informed. A written letter of consent must be signed for participation in the project. The participant will be informed that they can, whenever desired, withdraw their consent and this without any consequences for further treatment.

One of the doctors responsible for the investigation will perform the information discussion. In order to avoid disturbance, this will occur under the same conditions as the patient experiences when being generally informed and examined in the department or its out-patient clinic.

Oral and written consent is gathered in compliance with the current rules regarding health science research projects. The project will seek approval from the local ethics committees in agreement to the Declaration of Helsinki. If the patient should desire it, they can also receive the written information - "The rights of a trial subject in a health sciences research project".

The project is registered with Eudract number 2016-002433-30, the Data Protection Agency and the Danish Medicines Agency (Sundhedsstyrelsen) and will be monitored by the regional Good Clinical Practice (GCP) unit.

The law regarding the handling of personal information will be complied with in a manner that respects the participant's physical and mental integrity as well as their privacy.

Clinically-relevant data regarding the participant's medical history and previous treatment will only be available to the medical doctor treating the patient and the monitoring staff from the GCP unit. Clinically-relevant data as medical condition and investigations will be used to characterize project participants and may be handed over to the principal investigator. This data will be anonymous and stored electronically on a computer, this including specific removable media and will only be available to the project's medical doctors, GCP monitor and possible control by the health authorities. Patient information will not be sent from patient journals to other parties.

Project participation will not as such incur treatment benefits or disadvantages for the participant in comparison with a standard treatment, with the exception that medication will be delivered without costs.

A positive consequence of the project can imply that the future treatment of patients with ulcerative disease can be modified. Treatment can be given with less risk for side-effects, improved and quicker effect as well as with substantial reduction of economic expenses.

We therefore believe that the disadvantages of project participation, this including possible unforeseen risks, must be weighed against the possible benefits in form of a longer-term improved treatment of inflammatory bowel disease.

Publication

The results (positive, negative or inconclusive) from this study will be submitted to an International peer reviewed journal.

Sponsor and the project coordinating investigator have the exclusive rights to publish the results.

Authorship of publications will follow the Vancouver protocol

1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data;

(2) drafting the article or revising it critically for important intellectual content; and

(3) final approval of the version to be published.

Conditions (1), (2), and (3) must all be met. Acquisition of funding, the collection of data, or general supervision of the research group, by themselves, do not justify authorship. Substantial acquisition of data is defined as inclusion of at least 5 patients.

Participant Confidentiality

The participants will sign a consent form that allows investigators access to hospital records. Participant confidentiality is extended to cover any study information relating to participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party.

Study Records Retention

Study records will be maintained for at least five years from the date that the study is completed.

Respect for the patients physical and mental integrity and privacy

This study will be conducted according to the Danish national legislation on health. The study will be reported to the Regional Science Ethics Committee of the capital region of Denmark. The permission from The Regional Data Protection Agency will be sought and is awaited before the initiation of the study. Authorities will have full access to data, documents and registration procedures during monitoring, audits and inspections. Both oral and written informed consent will be obtained before entering the study according to the Declaration of Helsinki V and the Regional Science Ethics Committee of the capital region of Denmark. The study will comply with the law on personal data.

Appendix 1

Study drug administration

The study drugs will be administered as showed in the tablets in an open design.

In the control group, the first drug of choice is AZA 2.5 mg/kg.

In the treatment group, AZA is given as 0.5-0.8 mg/kg together with ALLO 100 mg.

Study drug will be delivered from the department, labeled as a study drug.

Study drugs can be paused for up to 7 days in case of a fever or a temporary non-severe infection.

Vægt (kg)	**AZA (2,5 mg/kg)**	**AZA/ALLO (0,5-0,75mg/kg)**
≤50,4	100 mg	50 mg
50,5-55,4	125 mg	
55,5-60,4	150 mg	
60,5-65,4	150 mg	
65,5-70,4	175 mg	
70,5-75,4	175 mg	
75,5-80,4	200 mg	
80,5-85,4	200 mg	
≥85,5	200 mg	75 mg

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