Effects of allopurinol on 6-mercaptopurine metabolism in unselected patients with pediatric acute lymphoblastic leukemia: a prospective phase II study

Jonatan Källström,[1,2] Riitta Niinimäki ,[3] Johan Fredlund,[4] Hartmut Vogt,[5] Laura Korhonen,[6] Anders Castor,[7] Josefine Palle,[8] Arja Harila,[8] Magnus Borssén,[9] Jonas Abrahamsson[1,2] and Torben Ek[1,2]

[1]Children´s Cancer Center, Sahlgrenska University Hospital, Gothenburg, Sweden; [2]Department of Pediatrics, Institute for Clinical Sciences, University of Gothenburg, Gothenburg, Sweden; [3]Department of Pediatrics and Adolescents, Oulu University Hospital, Oulu, Finland; [4]Department of Pediatrics, Halmstad County Hospital, Halmstad, Sweden; [5]Department of Pediatrics, Linköping University Hospital, Linköping, Sweden; [6]Department of Pediatrics, University of Turku, Turku, Finland; [7]Department of Pediatrics, Skåne University Hospital, Lund, Sweden; [8]Department of Woman's and Children's Health, Uppsala University, Uppsala, Sweden and [9]Department of Pediatrics, Norrlands University Hospital, Umeå, Sweden

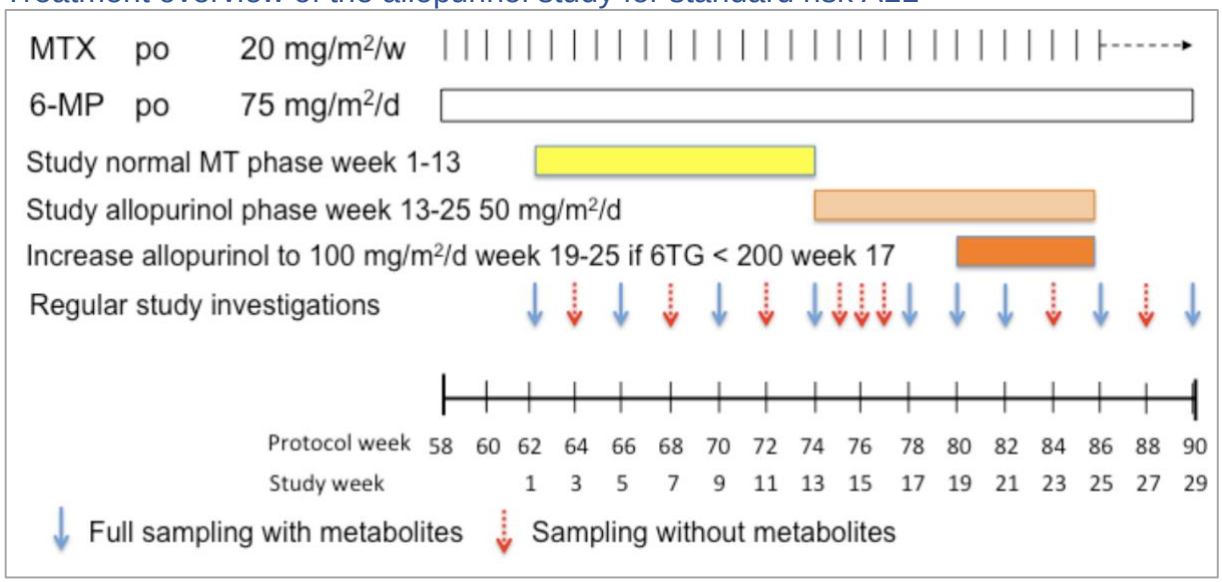
Correspondence: T. Ek
torben.ek@mac.com

Received: September 30, 2023.
Accepted: February 2, 2024.
Early view: February 15, 2024.

https://doi.org/10.3324/haematol.2023.284390

Supplementary files

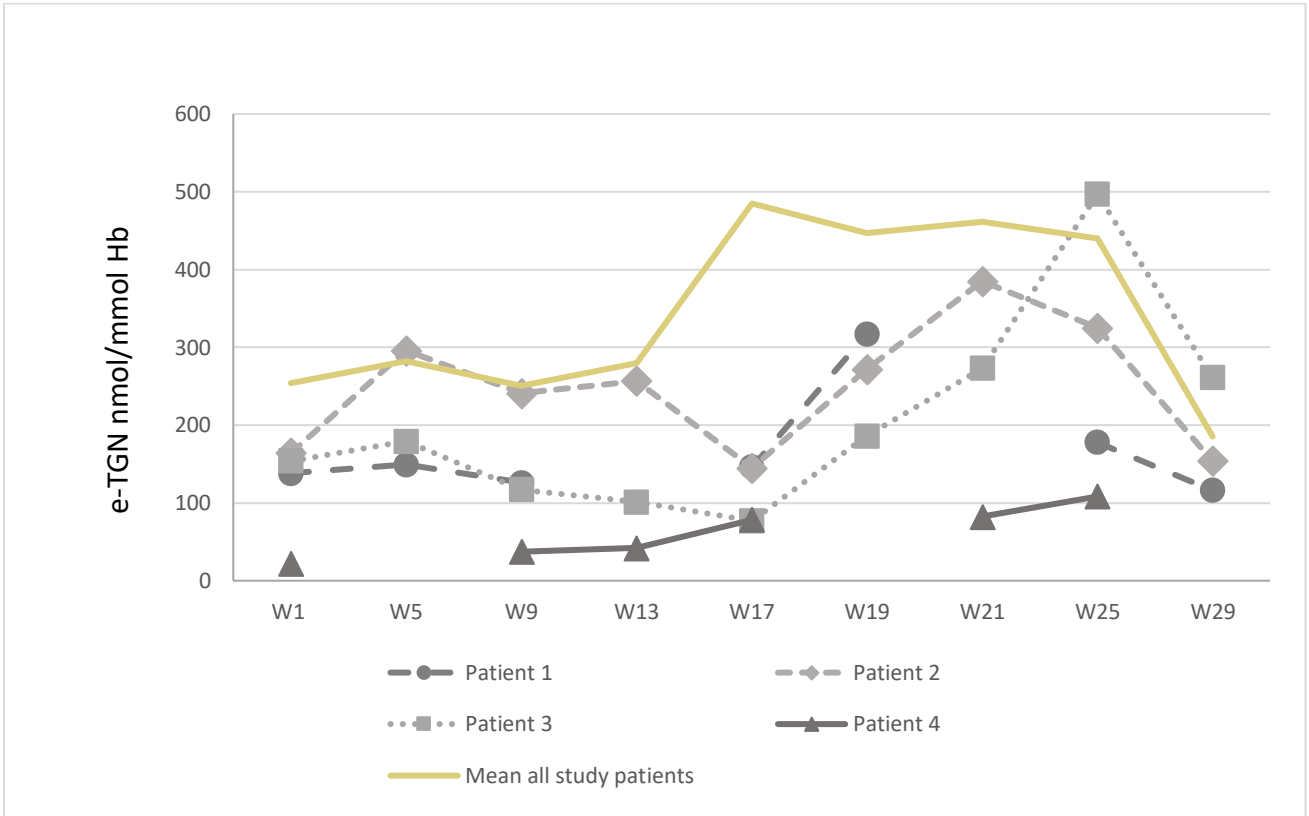
Treatment overview of the allopurinol study for standard risk ALL



MTX = methotrexate, 6-MP = 6-mercaptopurine, MT = Maintenance therapy
6TG = erythrocyte level of thioguanine nucleotides (nmol/mmol Hb)

Patients increasing allopurinol dose

Figure displaying levels of thioguanine nucleotides in erythrocytes (e-TGN) for the four patients who increased allopurinol dose from 50 to 100 mg/m^2 study week 19 due to e-TGN <200 nmol/mmol Hb study week 17 in accordance with study protocol. See results section for details.



Optimizing 6-mercaptopurine therapy in pediatric acute lymphoblastic leukemia by using allopurinol

Clinical study in children 1-19 years on maintenance therapy for acute lymphoblastic leukemia.

Sponsor- Coordinating Investigator	Jonas Abrahamsson Childrens' Cancer Centre Queen Silvias Childrens and Adolescents Hospital 416 85 Gothenburg, Sweden E-mail vobjab@gmail.com (or jonas.abrahamsson@vgregion.se) Phone +46 707 695159 Fax +46 31 215486
Sponsor	Västra Götaland Regionen Regionens Hus 462 80 Vänersborg Sweden
Data Manager	Torben Ek Childrens' Cancer Centre Queen Silvias Childrens and Adolescents Hospital Rondvägen 10 416 85 Gothenburg, Sweden E-mail torben.ek@mac.com Phone +46 706 169284 Fax +46 31 215486

Protocol version: 3.0
Protocol date: 2016-11-28
EUdract number: 2016-003409-33

1 Administration

The study is a cooperative protocol for countries within the Nordic Society for Pediatric Hematology and Oncology (NOPHO)

Sponsor-coordinating investigator Jonas Abrahamsson

National principal investigators

Sweden	Jonas Abrahamsson ☎+46 707695159	Childrens' Cancer Centre, Queen Silvias Childrens and Adolescents Hospital, Rondvägen 10, 416 85 Gothenburg, Sweden Fax +46 31215486 ✉ vobjab@gmail.com
Finland	Riita Niinimäki ☎+358 8 3155832	Dept of Pediatrics and Adolescents, Oulu University Hospital, Box 23, 90029 OYS, Finland Fax +358 8 3155397 ✉ riita.niinimaki@ppshp.fi

Protocol group: centre representatives and site principal investigators

Gothenburg	Lene Karlsson ☎+46 313435610	Childrens' Cancer Centre, Queen Silvias Childrens and Adolescents Hospital, Rondvägen 10, 416 85 Gothenburg, Sweden Fax +46 31215486 ✉ lene.karlsson@vgregion.se
Linköping	Hartmut Vogt ☎+46 101031343	Linköping University Hospital, Dept of Pediatrics, 58185 Linköping, Sweden. Fax +46 101034747 ✉ hartmut.vogt@regionostergotland.se
Lund	Anders Castor ☎+46 46178064	Skåne University Hospital, Lund, Dept of Pediatrics, Getingevägen 4 22241, Lund, Sweden. Fax +46 46130573 ✉ anders.castor@skane.se
Stockholm	Arja Harila-Saari ☎+46 851770000	Astrid Lindgrens Barnsjukhus, Karolinska Universitetssjukhuset, 17176 Stockholm, Sweden. Fax ✉ arja.harila-saari@karolinska.se
Umeå	Ulrika Norén-Nyström ☎+46 907850250	Norrlands Universitetssjukhus, Barnkliniken NUS, 90185 Umeå, Sweden. Fax +46 907851634 ✉ ulrika.norennystrom@pediatri.umu.se
Uppsala	Josefine Palle ☎+46 18-6110000	Dept of Woman´s and Children´s Health, Uppsala University, Uppsala, Sweden. Fax +46 18508680 ✉ josefine.palle@akademiska.se

Data Manager Torben Ek

2 Abbreviations and Acronyms

6MMP	6-methylmercaptopurine
6MP	6-mercaptopurine
6TG	6-thioguanine
ALL	Acute lymphoblastic leukemia
BSA	Body surface area
CRF	Case Report Form
DNA-6TG	DNA incorporated 6-thioguanine
DMC	Data monitoring committee
GCP	Good clinical practice
Hb	Hemoglobin level
HR	High risk
IBD	Inflammatory bowel disease
IR	Intermediate risk
MCV	Mean corpuscular volume
MT	Maintenance therapy
Mtx	Methotrexate
S-GPT	Serum glutamic pyruvic transaminase (ALT - alanine aminotransferase)
S-GT	Serum gamma-glutamyl transpeptidase
SAE	Serious adverse event
SJS	Steven Johnson syndrome
SR	Standard risk
SUSAR	Suspected unexpected serious adverse reaction
TEN	Toxic epidermal necrolysis
UNL	Upper normal limit

3 Summary of protocol

Background: Oral maintenance therapy (MT) with 6-mercaptopurine (6MP) and methotrexate is an essential part of therapy for acute lymphoblastic leukemia. It is well-known that the anti-leukemic effect of MT correlates with levels of neutrophils and in NOPHO guiding of MT aims at keeping WBC between 1.5-3.0 x 10^9/L. The metabolism of 6MP is complicated and there is a large inter-individual variation in the generation of different metabolites. It is believed that the anti-leukemic effect is mainly mediated by the metabolite 6-thioguanine (6TG) whereas high 6-methylmercaptopurine (6MMP) levels are associated with hepatotoxicity and myelosuppression. Patients, heterozygous for thiopurine methyltransferase have higher levels of 6TG and lower 6MMP and have a lower relapse risk but a higher incidence of secondary malignancy.

Steering of MT can be challenging in some patients. One subset of patients has difficulties in achieving adequate 6TG levels despite high dosing of 6MP and others experience severe side effects, such as hepatotoxicity and hypoglycemia, that require dose reduction despite having suboptimal levels of 6TG or WBC . In many of these patients, analysis of 6MP metabolites have shown that they have low 6TG and high 6MMP levels.
Several studies of 6MP in inflammatory bowel disease (IBD) have confirmed that there is a subset of patients that are preferential "6MMP metabolizers". Since the therapeutic effect of 6MP in IBD also depends on 6TG levels, several methods of skewing 6MP metabolism towards 6TG generation have been attempted. These studies have shown that addition of allopurinol in the majority of patients leads to higher 6TG, lower 6MMP and lower hepatotoxicity.

Aim: The aim of this study is to investigate if addition of allopurinol during MT for ALL gives the same effects as in IBD, i.e. higher 6TG, lower 6MMP and reduced hepatotoxicity. More specifically we will investigate if allopurinol increases the proportion of patients with 6TG levels above 200 nmol/mmol Hb and reduces the proportion with 6MMP levels in excess of 15000 nmol/mmol Hb. Secondary aims are to document how allopurinol affects laboratory parameters for hepatotoxicity and metabolic function after fasting.

Design: All children with standard or intermediate risk acute lymphoblastic leukemia, aged 0-18 years at time of diagnosis of leukemia and treated according to ALL2008 protocols and with TPMT wild type who start the maintenance 2 phase are eligible.
The patients are excluded if any of the following criteria exist.

- Mature B cell lymphoblastic leukemia; t(9;22) positive acute lymphoblastic leukemia
- Unknown TPMT status or presence of TPMT mutation (both heterozygous and homozygous)
- Known intolerance to any of the chemotherapeutic drugs in the protocol
- Major organ failure precluding administration of planned chemotherapy
- Severe liver toxicity defined as persistent (≥ two weeks) elevation of either S-bilirubin > 50 µmol/l or S-GPT > 20 x UNL (upper normal limit) or P-Protrombin complex > 1.5
- Reduced kidney function defined as S-creatinine ≥ 2 x UNL
- Lactating female or female of childbearing potential not using adequate contraception.

The primary endpoint is to compare 6TG levels with or without allopurinol therapy. Secondary aims are 6MMP levels, laboratory measures of hepatotoxicity and metabolic status, 6MP doses, myelosuppression and incidence of serious adverse reactions.

The study is planned to recruit 60 patients beginning four (-12) weeks after the start of maintenance two in ALL2008. The study is planned to commence in January 2017 and end in December 2018.

1. Patients will for 12 weeks be on normal MT and at one to four week intervals a sample for analysis of 6MP metabolites (6TG, 6MMP, DNA-6TG), Hb, WBC, platelet count, differential count, S-bilirubin, S-GPT, S-GT, S-albumin, S-creatinine, S-PK will be obtained. In patients who receive intrathecal therapy (IR and HR patients) sampling for metabolic studies will be obtained after fasting for general anesthesia. These will include P-glucose, P-cortisol, P-3-hydroxybutyrate, P-acetoacetate, P-free fatty acids and P-insulin.
2. After 12 weeks, providing MT is not put on hold for excess side effects, allopurinol at a dose of 50 mg/m^2 once daily will be initiated and 6MP dose will be reduced by 50%. Samples will be obtained as described above, including sampling after fasting in association with intrathecal therapy.
3. If, after 6 weeks of allopurinol therapy, 6TG levels are below 200 nmol/mmol Hb, the dose of allopurinol will be increased to 100 mg/m^2.

During both treatment periods patients will have a diary in which prescribed and ingested doses of 6-mercaptopurine, methotrexate and allopurinol will be recorded. Patients/guardians will also record if nausea or symptoms related to hypoglycemia occur. Serious adverse reactions (SAEs) are normally rare during this treatment period and all SAEs will be registered per GCP guidelines within 24 hours except for febrile episodes which will be registered after each 12 week period. After the end of the study period (12+12 weeks), patients will go back to normal MT without allopurinol.

4 Objective of study

The primary objective of the study is to investigate if addition of allopurinol in patients with TPMT wild type skews the distribution of metabolites towards an increase in 6TG and reduction in 6MMP levels without increasing myelosuppression or other untoward side effects. Secondary objectives are to investigate how blood levels of DNA-TGN and Mtx are affected by allopurinol treatment and if allopurinol reduces laboratory signs of hepatotoxicity as well as metabolic disturbance after fasting.

4.1 Primary endpoint

The primary endpoint is the fraction of patients with 6TG levels above 200 nmol/mmol Hb after 12 weeks of allopurinol treatment (week 25) vs. after 12 weeks of standard maintenance therapy (week 13).

4.2 Secondary endpoints

Secondary endpoints are to compare the following parameters with or without allopurinol treatment:

- The mean level of 6TG and DNA-TGN at week 13 and 25
- The mean level of 6MMP at week 13 and 25
- The weighted mean level of Hb, WBC, platelets and ANC during the respective treatment phases
- Laboratory measures of hepatotoxicity (weighted mean of S-bilirubin and S-GPT) during treatment phases
- Laboratory measures of hypoglycemia and metabolic dysfunction (cumulative incidence of hypoglycemia during the treatment phases)
- Serious adverse events (cumulative incidence during the treatment phases)
- The mean cumulative dose of 6MP and Mtx and days with treatment interruption during the two treatment phases

5 Background

Maintenance therapy with 6-mercaptopurine (6MP) and methotrexate is a cornerstone of current therapy for acute lymphoblastic leukemia. Both the anti-leukemic effects and side effects are dose dependent and most treatment protocols adjust doses individually to achieve a target white blood cell level of 1.5-3.0 (-3.5) x 10^9/L. The metabolism of 6MP is complicated including alternative pathways and metabolites with variable anti-leukemic as well as hepatotoxic and myelosuppressive effects. It has been shown that the relapse rate is correlated to erythrocyte levels of 6MP metabolites(1) Since there is a large inter-individual difference in the metabolism of 6MP, patients exhibit large differences in metabolite levels but also in how well the white blood cell target range is achieved and the extent of side effects, particularly regarding hepatotoxicity and hypoglycemia.

5.1 6-mercaptopurine metabolism

Figure 1 shows a simplified scheme on the metabolization of 6MP.

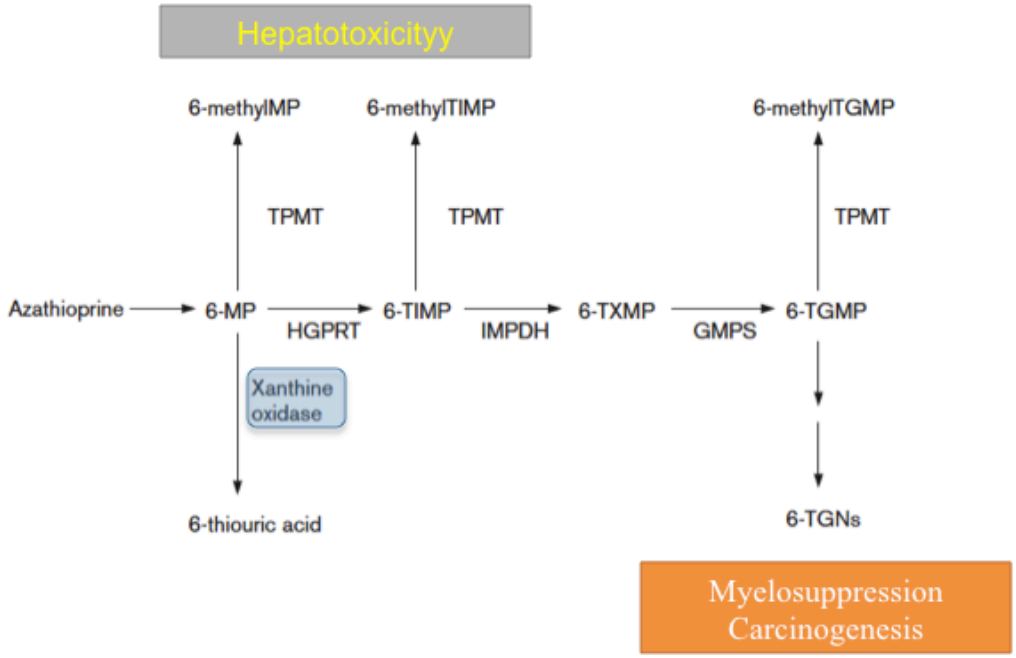


Figure 1. Thiopurine metabolism. 6-MP 6-mercaptopurine, 6-methylMP 6 methylmercaptopurine, 6-TIMP 6 methyl-thioinosine monophosphate, 6-methylTIMP methyl-thioinosine monophosphate, 6-TXMP thioxanthosine monophosphate, 6-TGMP thioguanosine monophosphate, 6-methylTGMP 6-methyl-thioguanosine monophosphate, 6-TGN 6-Thioguanine, TPMT thiopurine methyltransferase, HGPRT hypoxanthine-guanine phosphoribosyl transferase, IMPDH inosine monophosphate dehydrogenase, GMPS guanosine monophosphate synthethase

Through first-pass metabolism a large proportion of 6MP is converted to inactive 6-thiouric acid. The remaining 6MP is either methylated by TPMT to methylated thiopurine metabolites or converted into thioguanine nucleotides. The anti-leukemic effect is primarily attributed to incorporation of the thioguanin metabolites into DNA but also to inhibition of purine biosynthesis by methylated thiopurine metabolites. Hepatotoxicity is believed to be caused mainly by the methylated thiopurines. Around 10% of patients are heterozygous for one non-functional TPMT allele and they have reduced TPMT activity

with a 15-20 fold increased ratio of erythrocyte 6TG/erythrocyte 6MMP compared to patients with wild type TPMT(2). These patients with heterozygous TPMT mutations show higher 6TG levels, have more profound myelosuppression and a lower relapse risk whereas patients with high TPMT activity experience more hepatotoxicity.
NOPHO has since 1992 performed three large clinical treatment studies in children with ALL (NOPHO92, NOPHO2000 and NOPHO2008). Several studies of maintenance therapy including measurement of 6MP and Mtx metabolite levels have been included in these studies. Therefore, we know that both 6TG and 6MMP metabolite levels are stable over time during the maintenance phase and that the median level of 6TG is around 200 nmol/mmol hemoglobin(3).

5.2 Rationale for manipulation of 6MP metabolism

There are several major problems that may be influenced by an unfavorably low ratio between 6TG and 6MMP levels attained during maintenance therapy.
Firstly, low levels of 6TG may lead to an increased risk of relapse. Apart from findings in childhood ALL, studies in patients with inflammatory bowel disease (IBD) treated with azathioprine or 6MP have shown that there is a subset of patients that seem to be preferential 6MMP metabolizers having low 6TG and high 6MMP levels. IBD patients with low 6TG have much less effect of 6MP medication on disease activity. In accordance with some patients with high TPMT activity in ALL, increasing the 6MP dose in IBD patients in order to elevate 6TG often only results in a further increase in 6MMP with more hepatotoxicity(4). In IBD, several strategies have been tried to manipulate the ratio between 6TG and 6MMP. One obvious method has been to administer 6-thioguanine instead of 6MP. However, due to fear of side effects, particularly nodular regenerative hyperplasia, the use of 6TG in these patients has been limited(5). A meta-analysis of three large randomized studies in pediatric ALL, demonstrated that administration of 6TG had an anti-leukemic effect equal to or better than 6-mercaptopurine but was more toxic including an up to 20% risk of veno-occlusive disease of the liver during prolonged therapy(6).
A more promising strategy has been to administer allopurinol in conjunction with 6MP. Allopurinol inhibits xanthine oxidase and administration of allopurinol reduces the degradation of 6MP to the inactive metabolite thiouric acid(7). Theoretically, this would also cause increased 6MMP levels but the main effect of allopurinol co-administration seems to be increased 6TG and reduced hepatotoxicity(7).
Secondly, a substantial proportion of children experience episodes of hypoglycemia during maintenance therapy, particularly during fasting. The incidence is not well defined but one study with continuous glucose monitoring found that 8/18 patients had glucose levels below 3.2 mmol/L(8). This study found an association between hypoglycemia with low body mass index and elevated liver enzymes. In a small series of four patients with hypoglycemia during MT for ALL, very high levels of 6MMP was observed and we have own data showing the same findings in five patients(9).
Thus, administration of allopurinol together with 6MP may augment efficacy of maintenance therapy, particularly in patients with high TPMT activity, and reduce hepatotoxicity and incidence of hypoglycemia. A study in IBD in adults showed that a dose of 50 mg was safe and effective in increasing 6TG levels(10). A study in children with IBD showed that azathioprine in combination with 50 mg allopurinol to patients below 30 kg

and 100 mg to those ≥ 30 kg was safe and led to both increased 6TG levels and reduced 6MMP(11). Two small studies including five children with ALL has also demonstrated safety in adding allopurinol to 6MP therapy. These studies used a dose of 50 mg/m^2 and 4 mg/kg (corresponds to approximately 120 mg/m^2), respectively(12, 13).

6 Study population

All children with TPMT wild type treated on the ALL2008 or on the Swedish ALL2008 Infant protocol in the treatment phase maintenance 2 are eligible for inclusion. All Swedish centers and all Finnish centers except Helsinki (see below) will recruit patients to the study. Patients from other Nordic countries can also be included but there will be a complementary clinical study investigating the effect of addition of 6TG to MT performed in larger centers in these countries. Therefore we expect only few patients to be included from other Nordic countries.

6.1 Inclusion criteria

Patients are eligible for the study if they fulfil all five criteria below

- Confirmed diagnosis of acute lymphoblastic leukemia
- Treatment according to NOPHO ALL2008 based protocols
- Age 0-18y at time of initial diagnosis
- TPMT wild type
- Written informed consent

6.2 Exclusion criteria

Patients are excluded if any of the criteria below are present

- Mature B cell lymphoblastic leukemia
- t(9;22) positive acute lymphoblastic leukemia
- Unknown TPMT status or presence of TPMT mutation (both heterozygous and homozygous)
- Known intolerance to any of the chemotherapeutic drugs in the protocol
- Major organ failure precluding administration of planned chemotherapy
- Severe liver toxicity defined as persistent (≥ two weeks) elevation of either S-bilirubin > 50 µmol/l or S-GPT > 20 x UNL (upper normal limit) or P-Protrombin complex > 1.5.
- Reduced kidney function defined as S-creatinine ≥ 1.5 x UNL.
- Lactating female or female of childbearing potential not using adequate contraception[·]. Recommended methods of contraception are condoms with contraceptive foam, oral, implantable or injectable contraceptives, contraceptive patch, intrauterine device or diaphragm with spermicidal gel.

6.3 Withdrawal of consent

Patients participating in the study can at any time choose to leave the study without being required to specify a reason. These patients are recommended to continue treatment according to their original treatment plan i.e. standard maintenance therapy with 6-mercaptopurin and methotrexate.

Table 1. Overview of study procedures.

Flow sheet of allopurinol study		**Study Treatment period**																												
		Normal MT phase												**Allopurinol phase** Allopurinol dose⇧?**												**Follow up**				
Protocol week																														
Study week	**Prestudy period**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19****	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**
Informed consent	X																													
Inclusion/Exclusion evaluation	X																													
Psysical examination		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X				X
TPMT activity	X																													
Medical history with special reference to side effects to maintenance therapy		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X				X
Height and weight		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X				X
Ultrasonography liver- spleen see appendix 1		X												X																X
Hemoglobin, platelet count, white blood cell count, differential count including absolute neutrophil count		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X		X		X
S-creatinine		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X				X
S-bilirubin, SGPT (ALAT), S-GT, P-Protrombin complex (INR), S-albumin		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X				X
Standard mercaptopurin metabolites (6TG, 6MMP), See appendix 2 for sampling instructions		X				X				X				X				X*		X		X				X				X
Additional MT metabolites (DNA-TGN, Ery-Mtx), see appendix 2 for sampling instructions		X				X				X				X				X		X		X				X				X
Pregnancy test (U-HCG) for females of childbearing potential		X																												
Check patient diary and fill in prescribed doses for next study period		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X		X		
Fill in the CRF		X		X		X		X		X		X		X	X	X	X	X		X		X		X		X		X		X
Examinations before intrathecal therapy																														
Documentation of length of fasting for patient receiving intrathecal injections***																														
P-glucose, S-cortisol, P-3-hydroxybutyrate, P-acetoacetate, P-free fatty acids, P-insuline***																														

* Check that the result of the 6TG analysis is available at week 19 ** If 6TG week 17<200 nmol/mmol Hb the allopurinol dose should be increased from week 19

*** These additional examinations should be performed on visits where patients are fasting for intrathecal therapy

7 Study plan

After checking that inclusion criteria are fulfilled and no exclusion criterion is present and obtaining written informed consent the study begins four weeks after start of maintenance 2 in NOPHO ALL2008 or ALL2008 Infant. Patients are however allowed to enter the study at a later time point, but this should not exceed three months from the start of MT2. MT2 starts after approximately 58 weeks of ALL therapy for patients with SR and 66 weeks of therapy for IR. Table 1 shows a comprehensive overview of study procedures.
An overview for study patients with SR therapy is shown in figure 2, and for patients with IR in figure 3.

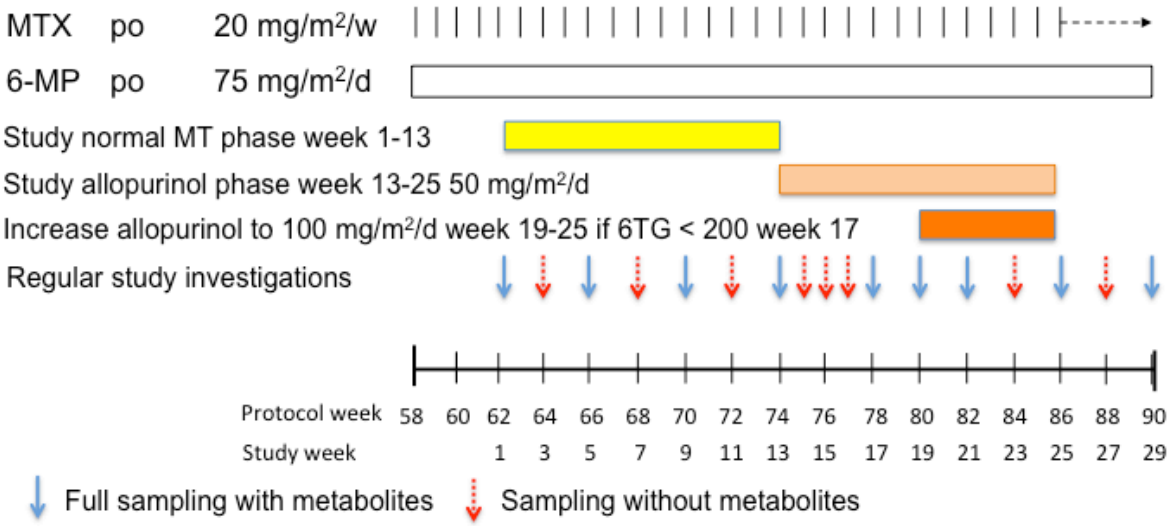


Figure 2. Overview of therapy and study milestones for patients with standard risk ALL.

7.1 Procedures on day one

On day one of the study, baseline investigations should be performed. These include

- Taking up patient history with special reference to side effects to maintenance therapy
- Physical examination
- Height and weight
- Ultrasonography of the abdomen with documentation of liver size, texture and blood flow and presence of ascites (may be performed up to one week earlier). See appendix 1 for guidelines on ultrasonography.
- Hematological values (Hemoglobin, platelet count, white blood cell count (WBC) Differential count including absolute neutrophil count (ANC))
- S-creatinine
- S-bilirubin, S-GPT (ALAT), S-GT, P-Protrombin complex (INR), S-albumin

- Standard mercaptopurin metabolites (6TG, 6MMP). See appendix 2 for sampling instructions
- Additional MT metabolites (DNA-TGN, Erythrocyte-Methotrexate concentration) See appendix 2 for sampling instructions.
- TPMT activity
- Pregnancy test (U-human chorionic gonadotropin) for females of childbearing potential

The patients and guardians should obtain the diary (appendix 3). Thereafter, at each visit, the local investigator should prescribe the dose of 6MP and Mtx until the next scheduled visit. The family should be reminded to fill into the diary the dose that was given, time-point of administration and also to record any specific side effects. It is recommended that the weekly methotrexate dose is scheduled so that it not is given within 48 hours prior to laboratory sampling. After each scheduled visit, when laboratory results have arrived, the investigator must fill in the case report form corresponding to the study time point (CRFw1, CRFw3.....CRFw29).

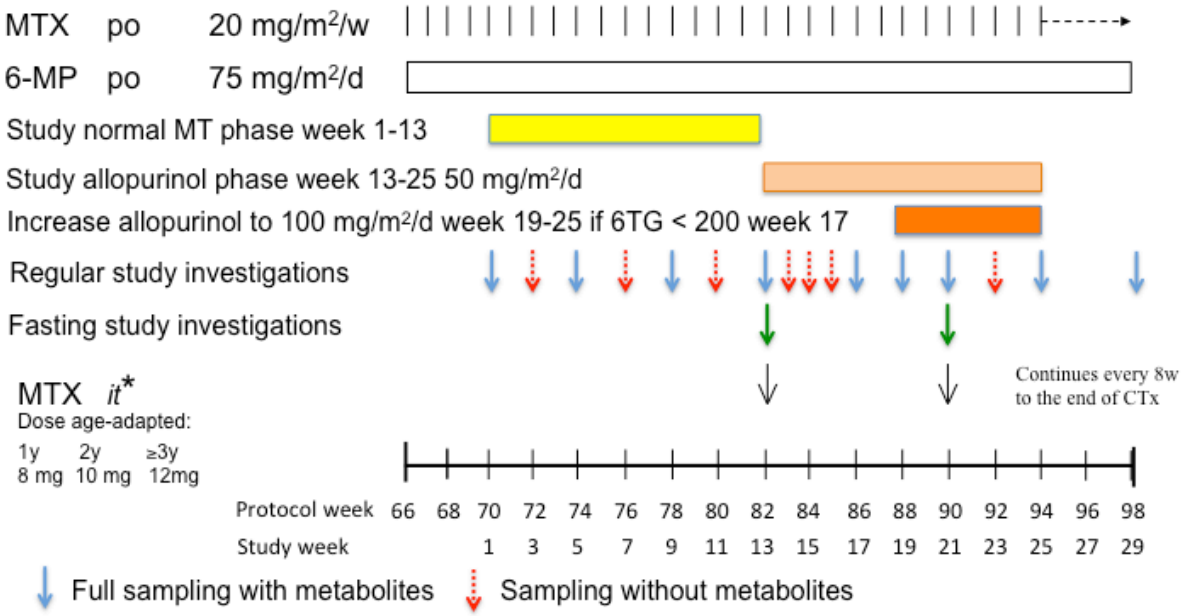


Figure 3. Overview of therapy and study milestones for patients with intermediate risk ALL.

7.2 Steering of maintenance therapy during the first 12 weeks of the study

During this period dose adjustment of 6MP and Mtx should be done according to the NOPHO ALL-2008 protocol.
The target level of WBC counts is 1.5-3.0 x10^9/L.

7.2.1 Dose reduction

- **WBC**: If the WBC is 1.0 – 1.5 x10^9/L the dose of 6MP and MTX should both be reduced by 50%.
  If the WBC is <1.0 x10^9/L oral 6MP and MTX should be withheld and restarted with 75% of the dose when the WBC is ≥1,5 x 10^9/L.

- **ANC:** If ANC drops and stays < $0.5x10^9$/L on at least two consecutive weeks consider modifying/withholding 6MP and MTX dosage (preferably reduce both, instead of omitting one of these totally). Note that unnecessary withholding MTX/6MP may increase the risk of relapse, which is a greater threat to the patient than the risk of toxic death. In cases of persistent ANC <$0.5x10^9$/L a leukemic relapse should be considered.
- **Platelet counts:** The 6MP/MTX therapy may be continued at stable platelet counts as long as they are ≥ 50 $x10^9$/L. If the platelet count is <50 $x10^9$/L oral 6MP/MTX should be withheld (also consider leukemic relapse).
- **Aminotransferases:** A rise in aminotransferase levels does not in itself warrant dose adjustment.
- **Coagulation factors**: Specific coagulation factors do not in general need monitoring unless S-bilirubin and/or S-prothrombin complex are elevated. If coagulation factors II-VII-X fall below <0.5 of normal lower limit oral 6MP/MTX should be reduced or withheld.
- **Bilirubin:** If bilirubin level rises to >50 µmol/l, oral 6MP/MTX should be reduced or withheld. Once bilirubin levels fall below 50 µmol/l the study drugs can be re-instituted. The dose of 6-MP should initially be reduced by 50% but can be increased if subsequent bilirubin levels are satisfactory.
- **Other organ toxicities:** The substitution of oral 6MP/Mtx maintenance therapy may be recommended in rare cases of other severe organ toxicities. Such changes in the therapy should be discussed with the principal investigator. Note that no alternative maintenance therapy regimens (except substitution of 6MP with 6TG) have been shown to have equal effect as MTX/6MP.

7.2.2 Dose increments:

- If WBC count remains ≥3.0 $x10^9$/L after ≥2 weeks of an appropriate dosage, increase the doses of MTX and/or 6MP by approximately 20%. Lack of titration to the target WBC may increase the risk of relapse. High aminotransferase levels (for TPMT wild type patients) and/or high MCV indicate that the high WBC is not due to poor treatment compliance. In a small subset of patients it is not possible to obtain a stable WBC within the target range without inducing severe hepatotoxicity or intermittent bone-marrow aplasia. Discuss such patients with the principal investigator.

7.3 Biweekly examinations during the first 12 week study period

Study patients are scheduled once every other week for study procedures. At each visit the following procedures should be performed.

- Patient history with special reference to side effects to maintenance therapy
- Physical investigation
- Height and weight
- Hematological tests (Hemoglobin, platelet count, white blood cell count, differential count including absolute neutrophil count)
- S-creatinine

- S-bilirubin, S-GPT (ALAT), S-albumin (every second week)
  S-GT, P-Protrombin complex (INR) (only week 1, 5, 9, 13)
- Check patient diary and fill in prescribed doses for the next week.

Some children will not have a central line at this time point. Capillary samples are usually sufficient. If problems in obtaining adequate sample volumes occur P-hematological testing, bilirubin, S-GPT and metabolite analysis should be prioritized.

7.4 Analysis of metabolites during the study

Analysis of metabolites should be performed at start of study followed by analysis at week 5, 9, 13, 17, 19, 21, 25 and finally at week 29. We recommend that oral methotrexate is not given within 48 hours of sampling preferably scheduling the drug on either the day of sampling or the following two days.

- Standard mercaptopurin metabolites (6TG, 6MMP). These will be continuously analyzed at Bonkolab, Copenhagen free of charge. Sampling, transport instructions and referral forms are found in appendix 2.

- Additional metabolites (DNA-TGN, Erythrocyte-Methotrexate concentration). These tests can be performed on the same sample with the same referral form as the standard metabolites. Sample and form will be sent to Bonkolab, Copenhagen where they will be frozen for subsequent batch analysis. Sampling, transport instructions and referral forms are found in appendix 2.

7.5 Procedures at start of 12 week allopurinol treatment

After 12 weeks on study with normal protocol maintenance therapy, treatment with allopurinol should be instituted at a dose of approximately 50 mg/m^2 once daily. See section 11.3.1 for guidelines on individual dosing to children with different body surface area (BSA). Since allopurinol is likely to increase myelosuppression it is **important to reduce the 6MP dose by 50% at start of allopurinol therapy.** The dose of allopurinol will be the same during the first six weeks. At this time, patients with low 6TG levels (<200 nmol/mmol Hb), as determined by the sample obtained after four weeks of allopurinol therapy (week 17), will increase the allopurinol dose to 100 mg/m^2 once daily.

If 6MP and Mtx are on hold at the scheduled time for beginning allopurinol then the start of the allopurinol phase should also be withheld until 6MP and Mtx can be reinstituted. If 6MP and Mtx subsequently are withheld for some period due to myelosuppression allopurinol should not be given during the same period.

On the day of starting allopurinol the following procedures are required:

- Patient history with special reference to side effects to maintenance therapy.
- Physical investigation.
- Height and weight.

- Ultrasonography of the abdomen with documentation of liver size, texture and blood flow and presence of ascites (may be performed up to one week earlier). See appendix 1 for guidelines on ultrasonography.
- Hematological values (Hemoglobin, platelet count, white blood cell count, differential count including absolute neutrophil count).
- S-creatinine.
- S-bilirubin, S-GPT (ALAT), S-GammaGT, P-Protrombin complex (INR), S-albumin.
- Standard mercaptopurin metabolites (6TG, 6MMP).
- Additional metabolites (DNA-TG, Erythrocyte-Methotrexate concentration)
- Check patient diary and fill in prescribed doses until the next visit.

For safety reasons hematological, creatinine and liver function tests will be measured weekly during the first four weeks of allopurinol treatment. At each visit the investigator should prescribe the dose of 6MP and Mtx until the next visit. The family should be reminded to fill into the diary the dose that was given, time-point of administration and also to record any specific side effects.
Some children will not have a central line at this time point. Capillary samples are usually sufficient. If problems in obtaining adequate sample volumes occur P-hematological testing, bilirubin, S-GPT and metabolite analysis should be prioritized.

7.6 Steering of maintenance therapy during the 12 weeks with allopurinol

In principle, during the 12 week allopurinol treatment phase the 6MP and Mtx doses are adjusted according to the same guidelines according to the NOPHO ALL-2008 protocol. However when introducing allopurinol (dose 50 mg/m^2) it is **essential to reduce 6MP dose by 50% in order to avoid excessive myelosuppression**. Thereafter dose adjustments should be made as given below.
The target level of WBC counts is 1.5-3.0 x10^9/L.

7.6.1 Dose reduction

- **WBC**: If the WBC is 1.0 – 1.5 x10^9/L the dose of 6-MP and MTX should both be reduced by 50%.
  If the WBC is <1.0 x10^9/L oral 6-MP and MTX should be withheld and restarted with 75% of the dose when the WBC is ≥1,5 x 10^9/L. The dose of allopurinol should not be modified depending on WBC but if 6MP and MTX are on hold allopurinol should also be paused.
- **ANC:** If ANC drops and stays < 0.5x10^9/L on at least two consecutive weeks consider modifying/withholding 6-MP and MTX dosage (preferably reduce both, instead of omitting one of these totally). Note that unnecessary withholding MTX/6MP may increase the risk of relapse, which is a greater threat to the patient than the risk of toxic death. In cases of persistent ANC <0.5x10^9/L a leukemic relapse should be considered. The dose of allopurinol should not be modified depending on ANC levels but if 6MP and MTX are on hold allopurinol should also be paused.
- **Platelet counts:** The 6-MP/MTX therapy may be continued at stable platelet counts as long as they are ≥ 50 x10^9/L. If the platelet count is <50 x10^9/L oral 6-

MP/MTX should be withheld (also consider leukemic relapse). The dose of allopurinol should not be modified depending on platelet levels but if 6MP and MTX are on hold allopurinol should also be paused.

- **Aminotransferases:** A rise in aminotransferase levels does not in itself warrant dose adjustment.
- **Coagulation factors**: Specific coagulation factors do not in general need monitoring unless S-bilirubin and/or S-prothrombin complex are elevated. If coagulation factors II-VII-X fall below <0.5 of normal lower limit oral 6MP/MTX should be reduced or withheld. If 6MP and Mtx are withheld, allopurinol should also be paused. Once coagulation factors exceed half of the lower limit all study drugs can be re-instituted.
- **Bilirubin:** If bilirubin level rises to >50 µmol/l, oral 6-MP/MTX should be reduced or withheld. If 6MP and Mtx are withheld, allopurinol should also be paused. Once bilirubin levels fall below 50 µmol/l the study drugs can be re-instituted. The dose of 6-MP should initially be reduced by 50% but can be increased if subsequent bilirubin levels are satisfactory.
- **Creatinine:** If the S-creatinine level rises to ≥ 1.5 x UNL, allopurinol should be temporary discontinued. Until study week 25, allopurinol can be re-started when creatinine is below the UNL. If, upon reinstitution of allopurinol, the S-creatinine again increases to ≥ 1.5 x UNL the drug will be permanently discontinued.
- **Other organ toxicities:** The substitution of oral 6MP/Mtx maintenance therapy may be recommended in rare cases of other severe organ toxicities. Such changes in the therapy should be discussed with the principal investigator and in general such patients should go off study. Note that no alternative maintenance therapy regimens have been shown to be superior to MTX/6MP.

7.6.2 Dose increments:

- If WBC count remains ≥3.0 x10^9/L after ≥2 weeks of an appropriate dosage, increase the doses of MTX and/or 6MP by approximately 20%. Since 6MP is reduced when allopurinol is introduced start by increasing 6MP alone. The dose of allopurinol should not be modified.
  Lack of titration to the target WBC may increase the risk of relapse. High aminotransferase levels (for TPMT wild type patients) and/or high MCV indicate that the high WBC is not due to poor treatment compliance. In a small subset of patients it is not possible to obtain a stable WBC within the target range without inducing severe hepatotoxicity or intermittent bone-marrow aplasia. Discuss such patients with the principal investigator.

7.7 Examinations during the 12 week allopurinol study period

Study patients are scheduled once weekly for the first four weeks followed by every second week for study procedures. At each visit the following procedures should be performed:

- Patient history with special reference to side effects to maintenance therapy
- Physical investigation
- Height and weight

- Hematological tests (Hemoglobin, platelet count, white blood cell count, differential count including absolute neutrophil count)
- S-creatinine
- S-bilirubin, S-GPT (ALAT), S-albumin on each time point
  S-GT, P-Protrombin complex (INR) only on weeks 13, 17, 19, 21, 25 and 29
- Check patient diary and fill in prescribed doses until the next visit.
- Note that after six weeks of allopurinol therapy (i.e. on the visit at week 19) the investigator should check the 6TG level measured at week 17. If 6TG is below 200 nmol/mmol Hb the allopurinol dose should be increased to 100 mg/m^2 which is given during the final six weeks of therapy. Note that this dose adjustment should only be performed at the visit after six weeks of allopurinol therapy. See section 11.3.1 for guidelines on individual dosing to children with different body surface area (BSA)
- Note that mercaptopurin metabolites (6TG, 6MMP, DNA-TG) and erythrocyte methotrexate are measured at study week 13, 17, 19, 21 and 25 during the allopurinol treatment phase (see figure 3) and finally at week 29.

Some children will not have a central line at this time point. Capillary samples are usually sufficient. If problems in obtaining adequate sample volumes occur P-hematological testing, bilirubin, S-GPT and metabolite analysis should be prioritized.

Remember to discontinue allopurinol at the visit on week 25. Note that the standard maintenance drugs should be prescribed in the patient diary and recorded in case report forms also for week 25-26. This is important in order to see the effect on hematological parameters after cessation of allopurinol.

7.8 Examinations on week 27

Patients should be scheduled for a visit on week 27. At this time point only standard hematological parameters need to be assessed (Hemoglobin, WBC, platelet count and ANC) in order to allow adjustment of maintenance therapy as appropriate. 6MP and Mtx doses should be prescribed in the patient diary also for week 27-28 and recorded in the case report forms.

7.9 Investigations in patients receiving intrathecal injections

Patients with IR ALL receive intrathecal injections at two month intervals during maintenance 2. In most patients this will coincide with week 13 and 21 in this study. However, it may occur that the time for intrathecal injections are scheduled at other time points and it is allowed that fasting samples are obtained at other time points. The time-point (week in study) should be documented on the case report forms.
In order to investigate if allopurinol reduces hypoglycemia and metabolic acidosis after fasting an extended sampling will be performed in these patients before they are anaesthetized. Apart from the procedures given by the actual study week at the time of intrathecal therapy the following additional examinations should be performed

- Documentation of length of fasting

- P-glucose
- S-cortisol
- P-3-hydroxybutyrate
- P-acetoacetate
- P-free fatty acids
- P-insulin

7.10 Investigations at the end of study on week 29

After the end of allopurinol treatment on week 25 the following examinations should be performed at week 29

- Taking up patient history with special reference to side effects to maintenance therapy
- Physical examination.
- Height and weight.
- Ultrasonography of the abdomen with documentation of liver size, texture and blood flow and presence of ascites (may be performed up to one week earlier). See appendix 1 for guidelines on ultrasonography.
- Hematological values (Hemoglobin, platelet count, white blood cell count, Differential count including absolute neutrophil count).
- S-creatinine.
- S-bilirubin, S-GPT (ALAT), S-GT, P-Protrombin complex (INR), S-albumin.
- Standard mercaptopurin metabolites (6TG, 6MMP).
- Additional metabolites (DNA-TG, Erythrocyte-Methotrexate concentration).

Some children will not have a central line at this time point. Capillary samples are usually sufficient. If problems in obtaining adequate sample volumes occur P-hematological testing, bilirubin, S-GPT and metabolite analysis should be prioritized.

8 Quality control and toxicity registration

The study will be conducted according to the declaration of Helsinki and ICH Guidelines for Good Clinical Practice (GCP). GCP is a scientific and ethical standard for the design, conduction and reporting of clinical trials involving human subjects. Compliance with these standards will assure that the rights, safety and well-being of the subjects are protected and that the trial data are credible.

The trial will be conducted in accordance with the protocol, legal requirements, and GCP quality assurance and control procedures. To obtain the necessary documentation and monitoring, each National Principal Investigator will establish an agreement with national independent GCP units to perform GCP monitoring.
The national coordinators are responsible for that each participating trial centre has the facilities to conduct the study according to protocol guidelines and meet any emergency that may arise during the study. Data retention including clinical trial records and other essential documents will follow national laws.

8.1 Monitoring of the study

The overall monitoring of the trial will be performed at four levels

- GCP monitoring
- Supervision by the protocol committee
- Supervision by a data monitoring committee (DMC)
- Monitoring of essential laboratory investigations

To facilitate monitoring and to comply with EU-Directive 2001/20/EC and ICH Guidelines for Good Clinical Practice a standardised toxicity registration will be established including reporting of severe adverse events (SAEs) and suspected unexpected severe adverse reactions (SUSARs).

8.2 GCP monitoring

The GCP monitoring provides a systematic and independent examination of trial-related activities and documents to determine, whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. To achieve these goals the monitoring should evaluate essential protocol issues. Therefore the following will be monitored in all patients:

1. Documentation of informed consent for the allopurinol maintenance study. These will be filed by the investigator at each centre and/or in the individual patients charts.
2. Documentation and validity of the inclusion and exclusion criteria. This information will be held in the study files at each centre.
3. Verification that all variables on the case report forms are registered with documentation of variables with missing data. This will ensure that study procedures are performed and that adverse events are reported.
4. Documentation and reporting of all SAEs as defined in the protocol.

5. Verify all source data for all variables on the case report forms on week 1,13,17 and 25. This includes documentation in medical files of side effects and drug prescription as well as results of laboratory and radiological analyses. The analyses at these time points contain the full source data for the primary endpoint and several secondary endpoints. It also allows for control of appropriate dose adjustment of allopurinol.
6. Verify that all patient diaries are completely filled in and that the information is correctly transferred to the case report forms. This will verify that patients have received correctly prescribed doses of all study drugs and that AEs are registered.
7. Verify that all variables on the case report forms are filled in at visits when patients have extended laboratory sampling in connection with fasting and intrathecal therapy. Verify these data against source data.

Most of the source data in the study (patient reports including information on inclusion/exclusion criteria, documentation of side effects, drug prescriptions, results of laboratory and radiological tests) are included in the medical files of the patients. At some centres consent forms are scanned into the medical file system and there considered to be original documents whereas at other centres these are kept in paper files. The monitor will have access to all source data and this fact is included in the consent forms.

8.3 Supervision by protocol committee

The protocol committee will convene every three months and discuss progress of the trial including accrual, events and toxicity. An interim analysis investigating outcome of primary endpoint and toxicity during allopurinol treatment will be performed after 25 patients have been treated. The sponsor-coordinating investigator is responsible to initiate additional meetings in case of unexpected problems with the protocol. Principal and sub-investigators are encouraged to contact the sponsor-coordinating investigator and/or national coordinators for guidance.

8.4 Data monitoring committee (DMC)

The DMC will annually receive a full report of the outcome data of the treatment arms and a toxicity report. The DMC will together with the sponsor-coordinating investigator assess the overall toxicity and outcome measures of the trial. While the study is running, only the data manager, the sponsor-coordinating investigator of the protocol and the members of the DMC will have access to compiled outcome data. None of the DMC members are involved in the treatment of study patients. When obtaining written informed consent for the study, an authorisation from the trial subject will be acquired to allow third party (monitor, auditor or inspector from the authorities) to access information on the trial person's health data.

8.5 Toxicity

8.5.1 Adverse events (AE)

An adverse event is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal (investigational) or medical product, whether or not it is considered to be related to the medicinal (investigational) product. Adverse events encompass illness, signs of illness (including pathological laboratory findings) and symptoms that initiate during the trial or previous conditions that become worse. AEs could be diseases, signs or symptoms which occur or worsen after enrolment of the patient in the clinical trial.
The intensity of AEs are graded according to Common Terminology Criteria for Adverse Events v4.0 (https://ctep.cancer.gov). They are further divided according to seriousness (serious or non-serious, see section 8.7)

In this study, AEs deemed relevant will be documented in the case report forms. These include laboratory measures of hematological, liver and kidney toxicity and anamnestic or clinical signs of skin toxicity, infection, liver toxicity, nausea and vomiting. Other AEs can be reported at the discretion of the investigators but this is not required.

8.5.2 Toxicity registration

The main purpose of the registration is to early detect unacceptably high and/or unexpected toxicity from the therapy in order to ensure the safety of the trial subjects. In compliance with EU-Directive 2001/20/EC and ICH Guidelines for Good Clinical Practice (GCP), deaths and SUSARs must be documented and reported to the sponsor coordinating investigator immediately (within 24 hours). The SUSAR report form is in appendix 4. This study is performed during a treatment phase in which deaths are not expected to occur. Should any toxic death occur the protocol study group will have an immediate meeting to investigate if allopurinol treatment may have contributed.
The toxicity reporting will be divided into three main categories. Reporting of

- SUSARs and deaths
- SAE
- AE according to section 8.5.1 on case report forms

8.6 Suspected unexpected severe adverse reactions (SUSARs) and deaths

A SUSAR is defined as a serious adverse reaction which is not consistent with the product information and either

- Results in death.
- Is life threatening or requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Causes congenital malformation.

Thus, severe SAE that are well-known side effects of the anti-leukemic therapy are not to

be registered as SUSARs.

All SUSARs or deaths, unrelated to death from progressive leukaemia, must be reported on the SUSAR/death form and sent by fax to the sponsor-coordinating investigator and national principal investigators. In case of SUSAR, the sponsor-coordinating investigator will inform the national medicine agency in Sweden and the ethics committee in Gothenburg, Sweden, and the national investigators will contact their national regulatory agencies if required by national laws. The sponsor-coordinating investigator will also notify all principal investigators at the study sites of the SUSAR.

8.7 Registration of SAE

The criteria used for defining adverse events will be those defined by the US National Cancer Institute Terminology Criteria for Adverse Events Common Toxicity Criteria (CTC) http://ctep.cancer.gov/reporting/ctc.html.

A serious adverse event (SAE) is defined as any untoward medical occurrence (adverse event) that at any dose either

- Results in death.
- Is life threatening or requires inpatient hospitalisation or prolongation of existing hospitalisation.
- Results in persistent or significant disability/incapacity.
- Causes congenital malformation

During this treatment phase, the incidence of SAE is normally very low. The only SAE expected to occur with some frequency is neutropenic or non-neutropenic fever. All SAEs should be reported on the SAE report form (appendix 5) and sent to the sponsor-coordinating investigator by fax or email within 24 hours from the time the investigator was aware of the SAE. On this form the investigator must document if the SAE was judged related to the investigational drug (not related, possibly related, likely related).

8.8 Stopping rules

No toxic deaths are expected during this treatment phase. Therefore, any death is considered a potential cause to stop the study. Should a death occur this will immediately and carefully be evaluated by the study group to determine if allopurinol treatment may have contributed to death.

8.9 Withdrawal from study

Individual patients are excluded from the study in case of any of the following situations:
- Withdrawal of consent
- Pregnancy
- Significant noncompliance
- New medical conditions not allowing for continuation of protocol treatment.

A patient can at any time, without specifying the reason, choose to withdraw from the

study. In this case no further study related procedures will be performed and no further data registered. These patients will continue their MT according to the treatment guidelines in their original treatment protocol which for the majority of patients will be NOPHO ALL2008.

The study protocol offers clear guidelines for management of severe toxicities including when to stop study drug administration so most patients experiencing such toxicities can safely continue to be included in the study. Allopurinol should be permanently discontinued if signs of Steven-Johnson syndrome or toxic epidermal necrolysis (TEN) occur (e.g. progressive rash often with blisters and skin damage). Other hypersensitivity syndromes (fever, rash, lymphadenopathy, arthralgias, internal organ involvement) has been described in rare cases, and should lead to permanent drug discontinuation. If, renal toxicity occurs such that S-creatinine repeatedly or persistently exceeds 1.5 x UNL, allopurinol should be permanently discontinued.
Patients excluded from the study for reasons of toxicity are further observed and considered for appropriate analyses unless they withdraw their consent for registration within the study. They should continue to be managed according to their original treatment protocol.
It can not be excluded that some patients for logistic or other reasons be withdrawn from the study. If this occurs the patient should be managed according to the treatment guidelines in their original treatment protocol which for the majority of patients will be NOPHO ALL2008.

8.10 Serious breach of protocol

A serious breach is a breach that is likely to effect to a significant degree

- the safety or physical or mental integrity of the subjects in the trial or
- the scientific value of the trial

The principal investigator at the trial sites should report breaches of protocol to the sponsor. It is the responsibility of the sponsor coordinating investigator to judge whether a breach is serious or not. If judged serious, the sponsor is required to notify the competent medical authority within seven days.

8.11 Premature termination of the study

The study is expected to run between January 2017 to December 2018. If the target number of patients (N=60) is reached prior to this time the study will be closed. The study can also be terminated at individual sites if the sponsor judges that the site fails to comply with protocol requirements or with legal or regulatory requirements.

8.12 Amendments

If, for any reason, changes of the study are needed, it is the responsibility of the sponsor to judge if the change in question is significant and if so notify and apply for approval from the competent authority and ethical committee as appropriate.

9 Data management

All patients in this study have through their guardians, and when appropriate for age also themselves, apart from giving written consent for this study, also given written informed consent to data registration for research purposes in the Nordic Society for Pediatric Hematology and Oncology leukemia registry which is located at Karolinska Institute Stockholm. This means that all relevant disease specific and demographic data including outcome data are updated in this leukemia registry and available to the present study. Additional data generated in this study will be registered on case report forms on paper.. These will be forwarded to the study centre in Gothenburg at six week intervals. A copy will be kept at the trial site. The case report forms consist of an inclusion form and separate forms for reporting at each visit. In Gothenburg these papers will be archived and entered into a secure research database. The key code for patient identification will only be available at the individual trial sites. The data generated in the study and the resulting database will be archived, according to specific procedures for archiving of research material at Sahlgrenska University Hospital, for fifteen years after completion of the study.

10 Drug description

10.1 6-mercaptopurine

Formulation 50 mg tablets or oral suspension 20 mg/ml.
Dose Starting dose 75mg/m^2 (but should be adjusted according to protocol guidelines to the WBC target of 1.5-3.0 x10^9/L). Due to interindividual variations in pharmacokinetics and drug tolerance the dosage needed during maintenance to obtain the target myelosuppression may vary significantly among patients. Note that the dose in each individual should be reduced by 50% when commencing allopurinol treatment.
Administration Orally. Doses are to be taken once a day at a regular schedule with respect to food and circadian rhythm.
Storage At room temperature.
Toxicity The main side effect of treatment with 6-mercaptopurine is bone-marrow suppression leading to leucopenia, thrombocytopenia, and anemia. 6-mercaptopurine is also hepatotoxic. The incidence of hepatotoxicity varies considerably and can occur with any dose, but more frequently when a dose of 75 mg/m^2 body surface area per day is exceeded. In general, the incidence and severity of side effects are considered to be dose-related.
Interactions
Methotrexate. Since 6-mercaptopurine and methotrexate work synergistically, it is preferable in case of unacceptable bone-marrow toxicity during maintenance therapy to give both drugs at reduced dosage rather that discontinue therapy with one of the drugs.

10.2 Methotrexate

Methotrexate (MTX) is a folate analogue that inhibits enzyme dihydrofolate reductase, which is important in conversion of folic acid to tetrahydrofolic acid, and several enzymes involved in purine *de novo* synthesis. Tetrahydrofolic acid is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the formation of

tetrahydrofolic acid MTX interferes with DNA, RNA, and protein synthesis. Similar to the natural folates, MTX is polyglutamated by the enzymes folylpolyglutamyl synthetase, and the polyglutamated metabolites increases the affinity for those enzymes that are inhibited by MTX. The propensity for polyglutamation significantly influences cancer cells sensitivity for MTX. MTX cytotoxicity is highly dependent on the absolute drug concentration and the time of exposure. Initial (α) i.v. half-life is 1-2 hours, with a second phase (β) of 6-11 hours. About 50% of MTX is bound to protein. MTX is poorly and variably absorbed orally, not least at high doses. MTX distributes widely to body tissues and fluids with sustained concentrations in the liver and kidneys. MTX may accumulate in the third space fluid collections (e.g. ascites or pleural fluid), which may significantly alter MTX pharmacokinetics. Slow release of MTX from these third spaces may prolong the half-life, and lead to increased toxicity. Methotrexate is excreted primarily by the kidney via glomerular filtration and active secretion into the proximal tubulus.
Formulation Tablets of 2.5 and 10 mg.
Dose Oral administration with starting dose of 20 mg/m^2/week and thereafter adjusted according to blood values.
Storage At room temperature.
Administration. Tablets orally once a week given in the evening.
Toxicity
Common: Transaminase elevations.
Occasional: Vomiting, nausea, anorexia, diarrhea, myelosuppression, photosensitivity.
Rare: Dizziness, malaise, blurred vision, allergic reactions. alopecia, folliculitis, renal toxicity.
Interactions. Methotrexate is partially bound to serum albumin, and toxicity may be increased because of displacement by certain drugs, such as **salicylates, phenylbutazone, phenytoin, and sulfonamides.** Renal tubular transport is also diminished by **probenecid**. **Penicillins** may reduce the renal clearance of methotrexate and lead to increased serum concentrations. **Trimethoprim/sulfamethoxazole** has been reported to increase bone-marrow suppression in patients receiving methotrexate, probably by an additive antifolate effect. Furthermore, it may delay excretion. Methotrexate may decrease the clearance of **theophylline**. Vitamin preparations containing folic acid or its derivatives may decrease responses to systemically administered methotrexate. Liver enzyme inducing antiepileptics (e.g. **phenytoin, phenobarbital, carbamazepin**) may decrease MTX concentrations and impair therapeutic efficacy.

10.3 Allopurinol

Allopurinol exerts it's action by inhibiting xanthin oxidase.
Formulation: Tablets of 100 mg. They may be dissolved in water if the patient is unable to swallow tablets.
Dose: 50 mg/m^2 given orally once daily. If suboptimal response on 6TG levels are observed after 6 weeks (6TG < 200 nmol/mmol Hb) the dose is increased to 100 mg/m^2.
Administration: Orally once daily.
Storage: Room temperature
Toxicity: The risk of side effects is increased in patients with impaired renal function. In case of a newly emerging skin rash, except for facial exanthema often associated with 6-mercaptopurine, discontinuation of allopurinol may be considered.

Both Steven Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) have been described and if signs of SJS or TEN occur (e.g. progressive rash often with blisters and skin damage) the drug should be discontinued. Other hypersensitivity syndromes (fever, rash, lymphadenopathy, arthralgias, internal organ involvement) has been described in rare cases, and should lead to drug discontinuation.
Interactions: **Ampicillin** should not be used together with allopurinol because of increased risk of SJS. **Amoxicillin** may theoretically be excluded for the same reason.

10.3.1 Allopurinol dosing guideline

Allopurinol is marketed by several manufacturers as 100 mg tablets. Most manufacturers have tablets with a central division line so they easily can be divided in two parts. Given the low dose in this study it will be necessary to divide into quarters. Slight variations in daily dosing is acceptable and as long as all quarters are used the total dose over e.g. a week will be correct. The tablets can be dissolved.

Suggested dosing in children of different body surface area at the allopurinol dose level 50 mg/m².

BSA*	Exact daily dose mg	Exact weekly dose mg	1/4 tablets each week**	Practical weekly dosing T. allopurinol 100 mg
0,4	20	140	5,6	1/4 daily
0,5	25	175	7	1/4 daily
0,6	30	210	8,4	1/4 5 days, 1/2 2 days
0,7	35	245	9,8	1/4 4 days, 1/2 3 days
0,8	40	280	11,2	1/4 3 days, 1/2 4 days
0,9	45	315	12,6	1/2 daily
1	50	350	14	1/2 daily
1,1	55	385	15,4	1/2 daily + 1/4 one day
1,2	60	420	16,8	1/2 daily + 1/4 3 days
1,3	65	455	18,2	1/2 daily + 1/4 4 days
1,4	70	490	19,6	1/2 daily + 1/4 6 days
1,5	75	525	21	1/2 daily + 1/4 7 days
1,6	80	560	22,4	1 tablett one day, 1/2 6 days
1,7	85	595	23,8	1 tablett 2 days, 1/2 5 days
1,8	90	630	25,2	1 tablett 4 days, 1/2 3 days
1,9	95	665	26,6	1 tablett daily
2	100	700	28	1 tablett daily

* Body surface area calculated according to the root square of weight (kg) x length (cm) / 3600.
** This column gives the exakt calculated number of quarter tablets. The column to the right shows the recommended practical daily schedule to use.

Suggested dosing in children of different body surface area at the allopurinol dose level 100 mg/m^2.

BSA*	Exact daily dose mg	Exact weekly dose mg	1/4 tablets each week**	Practical weekly dosing T. allopurinol 100 mg
0,4	40	280	11,2	1/4 2 days, 1/2 5 days
0,5	50	350	14	1/2 daily
0,6	60	420	16,8	1/2 daily + 1/4 3 days
0,7	70	490	19,6	1/2 daily + 1/4 6 days
0,8	80	560	22,4	1 five days, 1/2 2 days
0,9	90	630	25,2	1 six days, 1/2 one day
1	100	700	28	1 daily
1,1	110	770	30,8	1 daily + 1/4 2 days
1,2	120	840	33,6	1 daily + 1/4 5 days
1,3	130	910	36,4	1 daily + 1/2 4 days
1,4	140	980	39,2	1 daily + 1/2 6 days
1,5	150	1050	42	1 daily + 1/2 7 days
1,6	160	1120	44,8	2 four days, one 3 days
1,7	170	1190	47,6	2 five days, one 2 days
1,8	180	1260	50,4	2 six days, 1 one day
1,9	190	1330	53,2	2 six days, 1 one day
2	200	1400	56	2 daily

* Body surface area calculated according to the root square of weight (kg) x length (cm) / 3600.

** This column gives the exakt calculated number of quarter tablets. The column to the right shows the recommended practical daily schedule to use.

11 Statistical considerations

Primary endpoint: This is a phase 2 study of allopurinol employing a cross-over design with repeated measures. The rationale behind using repeated measures and not two independent cohorts with randomization is that the inter-individual variability in 6MP metabolism is very large. Thus, a randomized study would require a larger total number of patients (at least 120). Furthermore, since we know that metabolite levels are stable during standard maintenance therapy over time whereas we do not know if allopurinol treatment would cause a carry-over effect we have chosen to first investigate a 12 week standard therapy followed by the allopurinol treatment phase.

The primary objective is to investigate if addition of allopurinol increases 6TG levels during maintenance therapy of ALL. The primary endpoint is to investigate if the fraction of patients with 6TG levels above 200 nmol/mmol Hb is higher at week 25 (after 12 weeks allopurinol treatment) than at week 13 (after 12 weeks of standard maintenance therapy). 200 nmol/mmol Hb represents the median level of 6TG observed in previous Nordic studies using the same strategy for maintenance therapy (3). Accordingly, we expect 50% of patients to have levels of 6TG below 200 during the first

study phase with only maintenance therapy. Previous studies in IBD patients show that a majority of patients increase 6TG levels with addition of allopurinol. We therefore assume that at least 70% of patients will increase their 6TG levels above 200. With 54 patients a statistical power of 80% to detect this difference will be achieved. To minimize the effect of inter-center variability in steering of maintenance therapy paired analyses will also be performed.

Secondary endpoints: The mean levels of 6TG, 6MMP and DNA-TGN at week 13 and 25 in the respective treatment groups will be compared using repeated measures ANOVA. During each treatment phase a weighted (according to time between sampling points) mean level of WBC, ANC, platelets, Hb, S-GPT and S-bilirubin will be calculated and compared between treatment groups.
The incidence of SAE is expected to be too low for statistical analysis. However, the overall incidence will be compared between treatment groups and also characterized and examined at a qualitative level.
For each individual, the cumulative body-adjusted dose of 6MP, Mtx and number of days with treatment interruption will be calculated during each treatment phase and the respective mean levels compared with repeated measures ANOVA.

12 Ethical considerations

The study will be submitted for approval to the National and/or local Scientific Ethical Committees and the National Medicine Agencies in all participating countries. The patients will be recruited by their attending physician who will always be the principal investigator or a sub-investigator at a trial site. Patients will participate only after receiving proper oral and written information and after their parents/guardians and (when appropriate) also themselves have given oral and written consent.
All sampling related to the study will be obtained in relation to routine sampling as part of their treatment. Trial subjects and families will be able to contact both the study and national coordinators if they require additional information.

12.1 Possible benefits and risks for patients

It is well documented that a subset of patients do not achieve adequate target levels during MT with an increased risk of relapse. This is mainly manifested by failure to increase 6MP dosage to obtain adequate WBC levels due to high hepatotoxicity and/or hypoglycemia. Many of these patients exhibit low 6TG and high 6MMP levels. Previous studies indicate that these difficulties may be overcome by adding allopurinol to MT. Thus there is a clear potential for this study to both increase efficacy of MT, with lower relapse risk and to reduce the toxicity which particularly for hypoglycemia severely impairs the quality of life for the patients.
Experiences in IBD show that addition of allopurinol has been associated with little if any side effects but the studies include only few patients so this has to be confirmed in a larger cohort. Although we do not expect severe toxicity from the study, the patients will be closely monitored by pediatric oncologists with a large experience both in treating ALL and in clinical studies thereby minimizing risks for the patients.

12.2 Informed consent

The parents/guardians/legal representatives of the child and the child will receive both written and oral information of the trial including study objectives, study procedure and possible risks. Only principal or sub-investigators with experience in communicating with children and adolescents and with an in depth knowledge of the study will give information. All information will be given in compliance with guidelines from the national Scientific Ethical Committees. Both oral and written information is adjusted for age and written in an easily understandable language. Information will be given at a dedicated meeting and they will be given ample time to make their decision. This will most often require that the family after the first information are given time to read the written information and think about the study and that the final consent is obtained at a second meeting. Consent will be obtained from the parents and when appropriate from the child. If a child is capable to understand the study and rejects participation while the parents give consent, the child will not be included in the study. Families should be assured that they at any time can choose to withdraw the consent. Information will also include that those that decide not to participate will be treated according to their original standard protocol. The guardians and when appropriate the child will receive a copy of the signed consent form. If new or important information relevant to the study is revealed during the study this will in general require an amendment to the protocol with

revision of the patient information sheets. If this happens the guardians and patient should be informed and given the opportunity to reconsider continued participation and sign a revised consent form.

13 Financial issues

All expenses for submission for approval of the trial to the relevant authorities of a country is the responsibility of the Principal Investigator of the country in question. The National Principal Investigators, the research laboratories, the administrative groups can apply for external funding from research foundations and similar non-profit organization to cover expenses linked to the implementation of the protocol, running of the infrastructure (including registries), and for the research activities.

14 Publication guidelines

The results of the study will be reported to the EudraCT database, in accordance with the European Commission Guideline 2012/C302/03 within 12 months after completion of the study. The overall results of the study will be published in international peer-reviewed journals. The responsibility for writing these publications rests on the sponsor-coordinating investigator and all members of the protocol group will be included as co-authors. Additional co-authors may be included if they have made significant scientific contributions. All authorships should be approved by the protocol study group.

Data from the study can and will be used in other publications, abstracts and presentations after approval of the protocol study group.

15 List of Appendices

1 *Guidelines for ultrasonography of the abdomen*
2 *Instruction and referral form for sampling of metabolites*
3 *Patient diary*. The patient will receive a diary in which, at each visit, the physician fills in planned doses of the study drugs for the upcoming time period The family/patient brings it home and documents intake of study drugs and some specified side effects. At each visit the investigator should discuss the recordings in the diary with the family and make a compilation into the case report forms. When a patient has completed the study the diary should be sent to the sponsor-coordinating investigator in Gothenburg. Before sending it, all patient identifiers except the NOPHO number should be made illegible e.g. by striking over with a non-transparent marker pen.
4 *SUSAR report form*. Should be sent by fax (+46 31 215486) or email (vobjab@gmail.com) to the sponsor-coordinating investigator within 24 hours.

5 *SAE report form.* Should be sent by fax (+46 31 215486) or email (vobjab@gmail.com) to the sponsor-coordinating investigator within 24 hours.

16 References

1. Schmiegelow K., Schroder H., Gustafsson G., Kristinsson J., Glomstein A., Salmi T. and Wranne L. (1995) *J Clin Oncol* **13**(2),345-351
2. Zwaan C.M., Kolb E.A., Reinhardt D., Abrahamsson J., Adachi S., Aplenc R., De Bont E.S., De Moerloose B., Dworzak M., Gibson B.E., Hasle H., Leverger G., Locatelli F., Ragu C., Ribeiro R.C., Rizzari C., Rubnitz J.E., Smith O.P., Sung L., Tomizawa D., van den Heuvel-Eibrink M.M., Creutzig U. and Kaspers G.J. (2015) *J Clin Oncol* **33**(27),2949-2962
3. Nielsen S.N., Grell K., Nersting J., Frandsen T.L., Hjalgrim L.L. and Schmiegelow K. (2016) *Cancer Chemother Pharmacol* **78**(5),983-994
4. Bradford K. and Shih D.Q. (2011) *World J Gastroenterol* **17**(37),4166-4173
5. Dubinsky M.C., Hassard P.V., Seidman E.G., Kam L.Y., Abreu M.T., Targan S.R. and Vasiliauskas E.A. (2001) *Inflamm Bowel Dis* **7**(3),181-189
6. Escherich G., Richards S., Stork L.C., Vora A.J. and Childhood Acute Lymphoblastic Leukaemia Collaborative G. (2011) *Leukemia* **25**(6),953-959
7. Witte T.N. and Ginsberg A.L. (2008) *Can J Gastroenterol* **22**(2),181-185
8. Trelinska J., Fendler W., Szadkowska A., Czerwoniuk D., Mianowska B., Wegner O. and Mlynarski W. (2011) *Leuk Lymphoma* **52**(9),1704-1710
9. Melachuri S., Gandrud L. and Bostrom B. (2014) *Pediatr Blood Cancer* **61**(6),1003-1006
10. Curkovic I., Rentsch K.M., Frei P., Fried M., Rogler G., Kullak-Ublick G.A. and Jetter A. (2013) *Eur J Clin Pharmacol* **69**(8),1521-1531
11. Rahhal R.M. and Bishop W.P. (2008) *Inflamm Bowel Dis* **14**(12),1678-1682
12. Brackett J., Schafer E.S., Leung D.H. and Bernhardt M.B. (2014) *Pediatr Blood Cancer* **61**(6),1114-1117
13. Giamanco N.M., Cunningham B.S., Klein L.S., Parekh D.S., Warwick A.B. and Lieuw K. (2016) *J Pediatr Hematol Oncol* **38**(2),147-151