NOR-GRASPALL 2016

Clinical Research Protocol

SINGLE-ARM PHARMACOKINETIC/PHARMACODYNAMIC AND SAFETY STUDY OF ERYASPASE (GRASPA®) FOR PATIENTS WITH HYPERSENSITIVITY TO PEG-ASPARAGINASE, DIAGNOSED WITH PH(-) ACUTE LYMPHOBLASTIC LEUKEMIA

Protocol Number:	NOR-GRASPALL 2016
Version Date:	5.0 Aug2019
Investigational Product:	ERYASPASE (GRASPA®)
N° EUDRACT:	2016-004451-70
Development Phase:	Investigator Sponsored Trial
Sponsor:	**NOPHO - Nordic Society of Pediatric Hematology & Oncology** Birgitte Klug Albertsen Children and Adolescent Medicine Aarhus University Hospital Palle Juul-Jensens Boulevard 99 8200 – Aarhus N Denmark
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Approval:

Birgitte Hug Albertsen

Coordinating Investigator Signature

19.09.2019

Date

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing NOPHO with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted ICH GCP principles and to abide by the terms of this protocol.

Protocol Number: NOR-GRASPALL 2016

Protocol Title: Single-Arm Pharmacokinetic/Pharmacodynamic and safety study of ERYASPASE (GRASPA®) for patients with hypersensitivity to PEG-Asparaginase, diagnosed with Ph(-) Acute Lymphoblastic Leukemia

Protocol Date: TBD

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Investigator Signature *Date*

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Print Name and Title

Site # _____

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LIST OF ABBREVIATIONS

AE	adverse event
ALL	Acute Lymphoblastic Leukemia
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	Area under the curve
CL	Clearance
CRF	case report form
CSF	Cerebrospinal Fluid
DI	Dose Intensification
DMC	Data Monitoring Committee
GCP	Good Clinical Practice
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IV	Intravenous
ISF	Investigator site file
LDH	lactate dehydrogenase
mEq	Milliequivalent
MRT	Mean Residence Time
MTX	Methotrexate
NOPHO	Nordic Society of Paediatric Hematology & Oncology
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
RBC	Red blood cells
SAE	serious adverse event
SUSAR	Suspected unexpected serious adverse reaction
Vss	Distribution Volume at steady state

PROTOCOL SYNOPSIS

TITLE	Single-Arm Pharmacokinetic/Pharmacodynamic and Safety Study of eryaspase (GRASPA®) for Patients with Hypersensitivity to PEG-Asparaginase, Diagnosed with PH(-) Acute Lymphoblastic Leukemia
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RATIONALE	As previous trials with eryaspase (GRASPA®) evidenced a good overall safety and activity profiles. The current study is thus set out by NOPHO (the sponsor) to evaluate the PK/PD and safety of eryaspase at the dose of 150 IU/kg in combination with multi-agent chemotherapy, as a promising alternative option to allow ALL patients who have exhibited hypersensitivity reactions with pegylated E.coli, L- asparaginase formulation (clinical allergy or silent inactivation) to maximize the delivery of planned asparaginase therapy. Asparaginase treatment is a very important part of the ALL treatment and it is not possible to continue treatment after a hypersensitivity reaction. Truncated asparaginase therapy is associated with inferior event-free survival outcomes, in particular CNS relapse. Optimal treatment is essential and there is no other optimal substitution for asparaginase in case of hypersensitivity.
STUDY DESIGN	This is a single arm, multicenter, multinational study to assess the biological activity, safety, and immunogenicity of eryaspase in combination with the NOPHO ALL 2008 or ALLTogether pilot protocol administered as second line treatment to children or adult patients from 1 to 45 years old with ALL who experience hypersensitivity reactions to PEG-asparaginase (clinical allergic reaction or silent inactivation).

PRIMARY OBJECTIVE	Main objectives of this study are to evaluate the pharmacological (pharmacokinetic and pharmacodynamic) profile of eryaspase administered to patients who experience a PEG-asparaginase hypersensitivity event during induction, consolidation or subsequent phases of the multi-agent chemotherapy according to NOPHO ALL 2008 or ALLTogether pilot protocol for the treatment of children and adult patients with ALL; The pharmacological profile of eryaspase will be determined by: • The pharmacokinetic (PK) parameters for asparaginase activity will be assessed: Cmax; Tmax; AUC (Area Under the Curve); T1/2 (half-life); Vss (Distribution Volume at steady state), MRT (Mean Residence Time) and CL (Clearance). • The PD parameters for cerebrospinal fluid (CSF) concentrations of asparagine, aspartate, glutamine, and glutamate will be assessed.
SECONDARY OBJECTIVES	• The immunogenicity of eryaspase as determined by assessment of anti-L-asparaginase-antibodies and neutralizing antibodies. • The overall safety and tolerability of eryaspase in combination with multi-agent NOPHO ALL 2008 chemotherapy according to the PdL toxicity consensus definitions for asparaginase related toxicities (hypersensitivity, pancreatitis, hyperlipidemia, SOS, thrombosis, osteonecrosis, and fungal infections)(1) • Evaluate the effect of eryaspase on the concurrent maintenance therapy with 6-mercaptopurine and methotrexate. Levels of cytotoxic methotrexate (MTX polyglutamates) and 6-mercaptopurine metabolites (6-thioguanine nucleotides, 6TGN, and methylated metabolites, MeMP) and of DNA-TGN levels in circulating leukocytes.
EXPLORATORY OBJECTIVE	• The pharmacodynamic (PD) parameters for plasma and whole blood: Concentration of amino acids.
NUMBER OF SUBJECTS	45 children and 5 adults
SUBJECT SELECTION **CRITERIA**	<u>Inclusion Criteria</u>: A patient is eligible for the study if all of the following criteria are met: 1. Male or female aged 1-45 years at diagnosis of ALL 2. First line non-high risk (HR) ALL patients enrolled in NOPHO ALL 2008 or ALLTogether pilot protocol including PEG-asparaginase regimen 3. Documented hypersensitivity reaction to PEG-asparaginase with either:

- Clinical allergy to PEG-Asparaginase of any grade(mild/severe) OR
- Serum asparaginase activity below the lower level of quantification

4. Karnofsky/Lansky score ≥ 50.
5. Ability to understand, and willingness to sign, a written informed consent document and to comply with the scheduled visits, treatment plans, laboratory tests, and other study procedures. For patients under 18 years of age, either both parents or the legally appointed representatives will need to provide consent.

Exclusion Criteria:

1. Philadelphia chromosome positive ALL.
2. Participation in another clinical trial interfering with the study therapy with exception of NOPHO ALL-2008 or ALLTogether pilot protocol. Patients can participate in other clinical trials not interfering with the study drug. In case of doubt this is assessed by the PI.
3. Uncontrolled intercurrent illness including, but not limited to, patients receiving combination antiretroviral therapy or patients with severe or systemic infection, or psychiatric illness/social situations that would limit compliance with study requirements.
4. Other severe acute/chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, and in the judgment of the investigator would make the patient inappropriate for entry into this study.
5. Pregnant or lactating females. (serum human chorionic gonadotropin pregnancy test at screening). Use of a highly effective contraceptive measure in women of child-bearing potential and sexually active girls that are of child-bearing potential is required (contraceptive measures are specified in section 6.0).
6. Inadequate organ functions, which prohibit further asparaginase administration;
    a. History of pancreatitis
    b. History of serious hemorrhage or serious thrombosis with prior asparaginase therapy
    c. Severe hepatic impairment at the time of administration (bilirubin >3 times ULN, transaminases >10 times ULN)
    d. Pre-existing known coagulopathy (e.g. hemophilia)

	7. History of grade 3 or higher transfusion reactions or any contraindication to receive blood transfusion. Presence of specific anti-erythrocytes antibodies (auto-antibodies or anti-public antibodies) preventing from getting a compatible packed Red Blood Cells for the patient. 8. Patient under concomitant treatment likely to cause hemolysis.
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	Eryaspase will be administered intravenously (IV) at a dose of 150 IU/kg every 2 weeks for a maximum of 4 doses and then for a maximum of 3 doses at 6-week intervals for patients treated in the NOPHO 2008 protocol. For patients in the ALLTogether pilot protocol Eryaspase will be administered intravenously (IV) at a dose of 150 IU/kg every 2 weeks for a maximum of 7 doses. Eryaspase will substitute remaining doses of PEG-asparaginase according to the NOPHO ALL 2008 or ALLTogether pilot protocol.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	None.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for approximately 1.5 years including follow-up. **Screening:** up to 8 days **Treatment:** approximately 6 months **Follow-up:** Primary trial assessments for each patient will continue for 1 month following the end of planned eryaspase treatment phases. Each patient will be followed for up to one (1) year maintenance follow-up as defined in the NOPHO ALL 2008 protocol or ALLTogether pilot protocol. Each patient will receive cytostatic treatment in about 22 months after last administration of eryaspase according to the NOPHO ALL 2008 protocol. Follow-up after cessation should be done according to guidelines on each country and site and includes no assessments for this study. Patient enrolment will start in Denmark in January 2017. It is estimated that up to 50 patients could be enrolled by the end of February 2020. The total duration of the study is expected to be 4 years. 3 years for subject recruitment and 1 year for final subject follow-up.

CONCOMITANT MEDICATIONS	Allowed: Standard therapy for ALL is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. Blood transfusions, antibiotic therapy, and other form of supportive care are allowed. Prohibited: • Concomitant vaccination with live vaccines as it may increase the risk of serious infection • Other L-asparaginase marketed products • Highly hemolytic agents that may lead to RBCs hemolysis: Acetanilide, Antipyrine Phenazone, and Chloroquin and derivatives **NOTE**: Benzodiazepine may be given if indicated.
PRIMARY ENDPOINT	The study endpoints are to evaluate: • Pharmacokinetic parameters: ○ Total activity (at time points listed in Section 9.4) will be measured. The main pharmacokinetic parameters will be assessed: Cmax; Tmax; T1/2 (half-life time); Vss (Distribution Volume at steady state), MRT (Mean Residence Time) and clearance. ○ Percentage of patients with continuous asparaginase activity >100 IU/L. ○ Duration of enzyme activity >100 IU/l at 6 w dosing intervals • Pharmacodynamic profile: ○ CSF concentrations of amino acids: (asparagine, aspartate, glutamine, glutamate), at time points listed in the Study Procedures and Guidelines section. • Immunogenicity: titers of anti-asparaginase antibodies and neutralizing antibodies • Safety: Incidence of hypersensitivity (allergic reactions, any grade, and silent inactivation) at the end of asparaginase treatment All parameters defined in the Study Procedures and Guidelines section will be analyzed.
SECONDARY ENDPOINTS	Secondary endpoints are to evaluate: • The safety and tolerability of eryaspase in combination with standard multi-agent chemotherapy in NOPHO ALL 2008 or ALLTogether pilot

	protocol assessed during eryaspase treatment period according to potential toxicities defined in the Adverse Experience section. • Toxicity of the eryaspase treatment compared to the conventional PEG-asparaginase treatment. Toxicities and SAE's will be evaluated according to the PdL consensus definitions. • Levels of maintenance metabolites
EXPLORATORY ENDPOINT	• Plasma and whole blood concentrations of amino acids: asparagine, aspartate, glutamine, glutamate
SAFETY EVALUATIONS	• Only AEs/SAEs that are potentially related to eryaspase will be collected at start of treatment until 30 days after last administration. • SAEs will be registered according to the NOPHO ALL 2008 toxicity registration and classified and described according to the PdL consensus paper on ALL treatment related toxicity(1).
PLANNED INTERIM ANALYSES	An interim analysis will be performed when 5 patients have been included.
STATISTICS **Primary Analysis Plan**	All endpoints will be analyzed with usual descriptive statistics: number of observations, missing data, mean and its 95% CI (Confidence Interval), standard deviation, median, 25% and 75% quartiles, minimum and maximum for continuous variables; number of observations, missing data, and percentage for categorical and ordinal variables. Missing data will not be replaced. Two-sided 95% CI (Wald's method) will also be provided for percentages of interest.
Rationale for Number of Subjects	The sample size of this study is based on the estimated potential number of patients; up to 50 patients (45 children, 5 adults) could be enrolled into the study.

1.0 BACKGROUND

Childhood acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Each year approximately 200 children and adolescents (<18.0 years of age) are diagnosed with ALL in the five Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) (2).

The Nordic Society of Pediatric Hematology/Oncology (NOPHO) was established in 1981, and since then all children with ALL diagnosed within the Nordic region have been registered in the NOPHO Leukemia Registry. Common Nordic ALL protocols have existed since 1992 covering two protocol periods: 1992-2001 and 2002-2008.

The NOPHO ALL 2008 protocol was developed by a working group that represented all five Nordic countries. In addition the countries of Lithuania and Estonia, as well as adult hematologists have joined this collaboration since July 2008. Approximately 20 Baltic children and 25 Nordic/Baltic adults are diagnosed with ALL annually.

The ALLTogether consortium is a collaboration between several international study groups and consist of NOPHO, UKALL (Great Britain), DCOG (The Netherlands), COALL (Northern Germany), BSPHO (Belgium), SHOP (Portugal), PHOAI (Ireland) and SFCE (France). The ALLTogether-1 is the first common treatment protocol and is for children and young adults (1-45 years of age) with newly diagnosed acute Lymphoblastic leukemia (ALL). The background for this collaboration is to make easier and faster to perform clinical trials for the benefit of the patients, especially for small subgroups of patients with a poorer prognosis.

In contemporary protocols, overall survival of childhood ALL has reached 90%, because of the increased survival, the focus has expanded to include that all patients will receive the optimal treatment in order to maintain the good prognosis. Furthermore focusing on exploration of the toxic treatment burden, which constitute a significant cause of leukaemia-associated mortality is important (3).

Asparaginase has a central part of the treatment of childhood ALL. Asparaginase is an enzyme that hydrolyses asparagine to aspartic acid and ammonia and, to some extent, glutamine to glutamic acid and ammonia (4). In the extra cellular space this results in asparagine depletion and a reduction of glutamine. The lymphoblasts, which cannot effectively synthesize *de novo* asparagine, have their protein biosynthesis inhibited, and they undergo apoptosis (5). Cerebrospinal fluid (CSF) asparagine depletion is considered as a marker of asparaginase effect in the central nervous system (CNS) and asparaginase play an important role in CNS-directed anti-leukemia therapy(6).

In the current NOPHO ALL 2008 protocol, PEG-asparaginase (pegylated E.coli asparaginase) is for non-high risk (non-HR) patients given from treatment week 5 to 35, being given at a dose of 1000 IU/m2 i.m. every two weeks. Children and adults (aged 1-45 years) in five Nordic and two Baltic countries (Lithuania and Estonia) are treated according to ALL2008. HR patients receive PEG-asparaginase once at the end of induction, once in each HR treatment block (7-9 blocks) and twice during DI. From January 2009 until March 2016 at treatment day 92 (after 5 doses of PEG-asparaginase at 2 weeks intervals) non-HR Nordic children (85% of all Nordic children with ALL) were offered a randomization

between continuous and intermittent PEG-asparaginase treatment (given every 2nd or every 6th weeks, respectively).

The randomization was closed March 1st 2016 and non-HR patients are now treated according to the previous experimental arm (i.e. PEG-asparaginase every 6w from day 92). Manuscript of the results of the randomization is in preparation.

In ALLTogether pilot protocol PEG-asparaginase (1500 I.E./m^2 i.v.<16 years, and 1000 I.E./m^2 i.v. ≥16 years) is given from treatment day 4 and repeated at 2-week intervals. The non-high risk group consists of three treatment groups after stratification day 29, i.e. very-low risk, intermediate low risk and intermediate high risk and receiving 4, 5 and 8 doses, respectively. Therapeutic drug monitoring are done routinely to identify enzyme inactivation (silent or in combination with hypersensitivity reactions). Optimization of asparaginase treatment is considered one of the major focus areas in the ALLTogether protocol.

The use of PEG-asparaginase are compromised by frequent and significant toxicities, such as clinical allergic reactions, pancreatitis, and thrombosis. Clinical allergy is the most frequent serious adverse event observed. In total, 13% of the patients (~30/year) treated on the NOPHO ALL 2008 protocol develop allergy to PEG-asparaginase (7). The majority (85%) of the Nordic ALL2008 childhood ALL patients who developed allergy, did so after the 2nd or 3rd dose and are thus potentially undertreated because further doses are impossible.

Truncated asparaginase therapy is associated with inferior event-free survival outcomes, in particular CNS relapse. It is well known that insufficient CSF asparagine depletion is a concern and is correlated with ineffective killing of leukemic cells in the CNS. CNS may serve as a sanctuary for leukemic cells, and consequently this may increase the risk of CNS relapse despite other CNS-directed therapy. Truncated L-asparaginase treatment has been associated with increased risk of CNS relapse (8-10).

The impact of asparaginase truncation due to clinical allergy may depend on the timing of the allergic event (10). Data from NOPHO ALL 2008 protocol (manuscript in preparation) indicates that there retrospectively is no asparaginase-activity from start of treatment in patients who later develop clinical allergy. This means that patients with hypersensitivity reactions have no effect of the treatment with asparaginase at all.

In addition, loss of enzymatic efficacy - "silent inactivation" are found in 5-6% of the patients who receive conventional PEG-asparaginase treatment. It is observed as a consequence of the production of symptom-free serum antibodies directed to the asparaginase and blocking its pharmacological activity. These patients have no effect of the asparaginase treatment as well. (Manuscript in preparation).

After an allergic reaction, non-HR patients are treated with Erwinase (20.000 IU/m^2 3 times a week for two weeks) during Delayed Intensification, and HR patients during the subsequent blocks and DI. Erwinase has a short half-life (7-16 hrs), thus frequent administration is necessary. There is a need for an effective alternative preparation with a longer half-life for PEG-asparaginase allergic patients. In the current NOPHO ALL 2008, protocol PEG-asparaginase treatment is only replaced for 2 weeks and further scheduled doses are cancelled. The importance of the treatment with asparaginase is so

essential that all asparaginase-doses must be substituted with Erwinase in the future NOPHO-protocols in case of allergy or "silent inactivation" unless a better alternative is found. This induce a huge number of hospitals visit for the patients.

According to the NOPHO ALL 2008 protocol, maintenance treatment phases include thiopurines e.g. 6-mercatopurine (6-MP) and methotrexate (MTX). 6-MP is a prodrug and its cytotoxicity depends on conversion to 6-thioguanine nucleotides (TGN). TGN are incorporated into DNA (DNA-TGN) and are occasionally mismatched to thymidine, causing cell death by post-replicative mismatch repair (11).

It has been suggested that asparaginase may reduce the metabolism and thus increase the toxicity of other drugs. Furthermore, asparaginase-associated hypoproteinemia, which leads to higher free drug levels of other chemotherapeutics, has also been proposed as a contributing factor of in this context (12).

Analysis of data (publication under preparation) from the NOPHO-protocol indicates increments of 6MP and MTX metabolites levels during the treatment of PEG-asparaginase. This emphasize that asparaginase can have a major impact on 6MP and MTX metabolism and thus their toxic effect. Accordingly, PEG- asparaginase has been previously associated with increased myelotoxicity and higher frequency of 6MP/MTX dose reductions during maintenance therapy for childhood ALL (13). Furthermore, it is shown that the higher levels of 6MP metabolite levels were associated with an increased risk of hepatic sinusoidal obstruction syndrome (SOS). Data from NOPHO ALL 2008 indicates that the hepatotoxicity is increased when 6MP is given in combination with continuous PEG-asparaginase (14). Further studies are planned and in progress, but it is important to investigate in studies with alternative formulations of asparaginase.

1.1 Investigational Product

1.1.1 ERYASPASE (GRASPA®), new formulation of L-asparaginase

Eryaspase is a dispersion of homologous erythrocytes encapsulating recombinant L-asparaginase for infusion.

Additional descriptive information can be found in the Investigator's Brochure (IB).

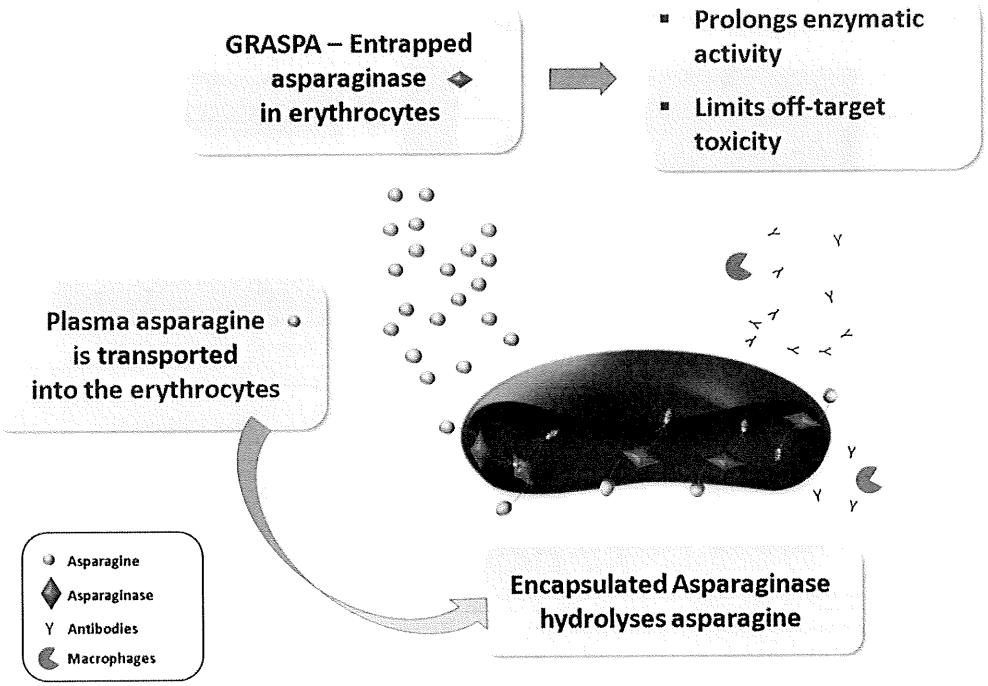
1.1.2 Mechanism of Action

As demonstrated by several teams using different bioactive entrapment technologies, L-asparaginase remains catalytically active while entrapped inside erythrocytes (15-18).

Eryaspase acts as a "cellular circulating bioreactors" leading to asparagine depletion in blood. As depicted in Figure 1, plasma asparagine is actively pumped through sodium-coupled neutral amino acid transporters of the erythrocyte membrane into the intracellular compartment where it is cleaved by entrapped L-asparaginase. The erythrocyte membrane protects L-asparaginase against fast degradation and elimination processes, hence allowing for a long circulating activity and a longer half-life than with free L-asparaginase. Thus, eryaspase combines the capacity of erythrocytes to actively 'pump' asparagine from blood plasma and the enzymatic activity of entrapped L-asparaginase to cleave asparagine into aspartic acid and ammonia, leading to plasma asparagine depletion. This encapsulation concept is not considered as a 'slow release' of the L-asparaginase, since the activity of

asparagine degradation takes place within the erythrocyte, until the latter is removed in the same manner as normally transfused erythrocytes, by macrophages or dendritic cells in the liver or spleen(18-20). The encapsulation of L-asparaginase eliminates the direct somatic contact with the L-asparaginase, and it is hypothesized that this provides the potential to i) prolong the activity of the enzyme and ii) reduce toxicities associated with the parent L-asparaginase notably by preventing the recognition of its active site by the immune surveillance of the host.

FIGURE 1. MECHANISM OF ACTION



1.2 Overview of Clinical Studies

Since 2006, an extensive clinical program has been conducted to investigate the efficacy and safety profile of eryaspase in combination with multi-agent chemotherapy for the treatment of ALL as the primary indication and for other cancers including acute myeloid leukemia and pancreatic adenocarcinoma (21).

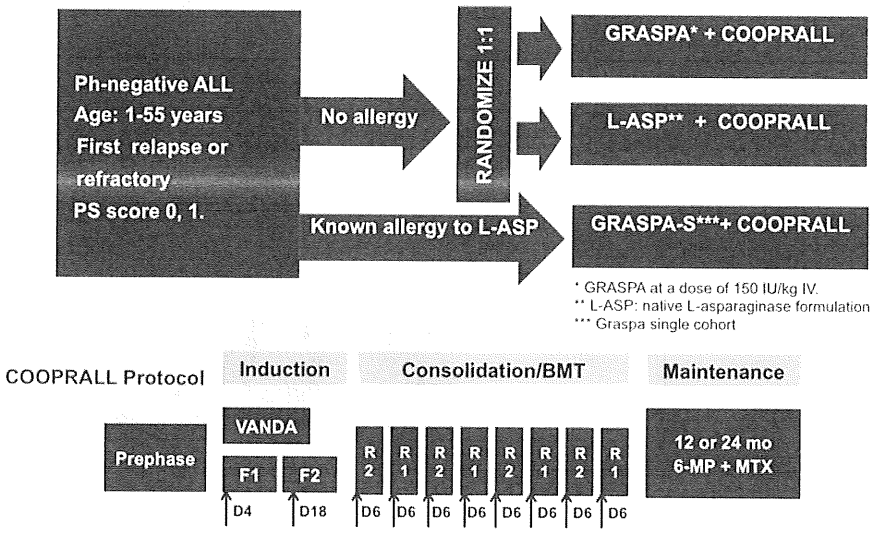
Five clinical trials with eryaspase have been performed or are still ongoing in Europe in pediatric and adult (1st relapse) or elderly (1st line) patients with ALL. One clinical trial with eryaspase is currently ongoing in the US in 1st line adult ALL patients.

Interestingly, an Extended Access Program is ongoing in France providing eryaspase in combination with multi-agent chemotherapy in patients under 55 years old with ALL unable to receive other

formulations of asparaginase. Fifteen patients have been included in this program since March 30th, 2016, evaluating safety of eryaspase.

The main efficacy and safety data on eryaspase arise from the pivotal phase III trial (GRASPALL 2009-06). This trial was conducted to confirm the benefit/risk profile of eryaspase at 150 IU/kg in combination with the COOPRALL regimen, which consisted of successive blocks of chemotherapy of 14 to 21 days each, in pediatric and adults patients with relapsed or refractory ALL with or without known prior history of hypersensitivity reactions to native or pegylated E.Coli-asparaginase. The study design of this trial is depicted in Figure 2.

FIGURE 2. **GRASPALL2009-06** STUDY DIAGRAM



Two co-primary endpoints were evaluated in this trial:

- the duration (in days) of whole blood L-asparaginase activity >100IU/L and
- the occurrence of any grade study drug-related allergic reactions during the induction phase.

The mean duration of asparaginase activity > 100 IU/L measured in whole blood was significantly higher in the eryaspase arm compared to control arm, with a mean (± SD) of 20.5 (5.2) days and 9.4 (7.4) days, respectively (p = 0.001).

As a secondary endpoint, patients in the eryaspase arm achieved a higher CR (65.4%, 95% CI: [51.6:89.8]) as compared to the control arm (39.3%, 95% CI: [23.3:63.1]), p=0.026.

Safety analysis of the overall study period revealed a significantly lower overall incidence of drug-related AEs and SAEs in the eryaspase arm (73.1%, and 15.4%, respectively) compared to the control arm (100% and 35.7%, respectively). Similarly, the incidence of drug-related Grade 3 or 4 AEs was lower with eryaspase (57.7%) than native E.Coli asparaginase (89.3%).

The key adverse events of interest are generally considered to be class-effects relevant to L-asparaginase, including hypersensitivity reactions, coagulopathic events, pancreatic events and hepatic events. The majority of these events occurred at a lower frequency with eryaspase compared to native *E.Coli* asparaginase during overall study period (Table 1).

TABLE 1: SUMMARY OF KEY ADVERSE EVENTS OF INTEREST THAT ARE DRUG-RELATED AEs IN ≥ 10% OF PATIENTS ARRANGED BY MEDDRA PREFERRED TERM

Preferred Term	ERYASPASE N=26 (%)	Control native asparaginase N= 28 (%)
At least one AE	**19 (73.1)**	**28 (100)**
Hypofibrinogenemia	7 (26.9)	18 (64.3)
Elevated amylase and/or lipase enzyme elevation	7 (26.9)	15 (53.6)
Transaminase increased	5 (19.2)	6 (21.4)
Hypoalbuminemia	4 (15.4)	9 (32.1)
Anti-thrombin III decreased	3 (11.5)	20 (71.4)
Hyperbilirubinemia	2 (7.7)	5 (17.9)
Drug hypersensitivity reactions	2 (7.7)	16 (57.1)
Hepatotoxicity	1 (3.8)	5 (17.9)
GGT increased	1 (3.8)	4 (14.3)
Activated partial thromboplastin time prolonged	1 (3.8)	3 (10.7)

Extras of Table 14.3.1.10.1 from clinical study report of the GRASPALL 2009-06 trial

Altogether, eryaspase was shown to demonstrate improved clinical efficacy and a better overall safety profile compared to native *E.Coli*-asparaginase in patients with relapsed or refractory ALL (22). Noteworthy, history of hypersensitivity reactions due to prior exposure to native or pegylated *E.Coli* asparaginase and anti-L-asparaginase antibodies status does not adversely impact the pharmacological activity and the safety profile of eryaspase in this patient population. Focusing on the key adverse events of interest, it has to be noted that only (3) 12% of patients who had previous allergic reactions to asparaginase experienced drug-related hypersensitivity reactions. The encouraging results with eryaspase in this pivotal trial provides a potential alternative treatment for these patients who might be at risk of hypersensitivity reactions and/or other asparaginase-related toxicities.

Further studies indicate a good safety profile of GRASPA. A multicentre randomized controlled trial, investigated three doses of GRASPA for the duration of asparagine depletion in a phase I/II study in adults and children with acute lymphoblastic leukemia (ALL) in first relapse showed a reduction in the number and severity of allergic reactions and a trend towards less coagulation disorders. Other expected adverse events were comparable to those observed with E. coli asparaginase (17).

Furthermore a Phase II trial evaluated the safety and efficacy GRASPA in patients >55 years with Philadelphia chromosome-negative acute lymphoblastic leukemia without increased toxicity and associated with durable asparagine depletion (19, 23).
Eryaspase has been approved by the national authorities in Norway and Finland and Ethics Committee to be studied in a multinational, randomized, controlled Phase 2b trial evaluating its efficacy and tolerability in the treatment of newly diagnosed AML patients over 65 years of age and unfit for intensive chemotherapy. The study completed enrolment, and patients are currently followed up for 1 year. Two safety assessments had been performed by an external Data and Safety Monitoring Board when 30, 60 and 105 patients were treated in the study with no safety concerns identified. The inclusion of 123 patients is fulfilled.

2.0 STUDY RATIONALE

As previous trials with eryaspase evidenced promising overall safety and activity profiles, the current study is thus set out by NOPHO (the sponsor) to evaluate the PK/PD and safety of eryaspase at the dose of 150 IU/kg in combination with multi-agent chemotherapy, as a promising alternative option to allow ALL patients who have exhibited hypersensitivity reactions with pegylated E.coli L-asparaginase formulation (clinical allergy or silent inactivation) to maximize the delivery of planned asparaginase therapy.

2.1 Risk / Benefit Assessment

2.1.1 Potential Benefit for the Patients

PEG-asparaginase in combination with polychemotherapy is used in consolidation, delayed intensification, and maintenance phases of ALL treatment, mainly in children and young adults. However, its use has been hampered by frequent and/or significant toxicities, of which immunologic reactions take a prominent place. In addition, loss of enzymatic efficacy, the so called "silent inactivation" may be observed as a consequence of the production of symptom-free serum antibodies directed to the asparaginase moiety and blocking its pharmacological activity.

Eryaspase has been proposed as a new approach to maintain an activity of L-asparaginase while reducing its antibody mediated toxicity and improve its safety profile. Eryaspase at a dose of 150 IU/kg of L-asparaginase, according to previous study designs in combination with standard chemotherapy, was shown to be effective and well tolerated in children and adults with relapsed ALL (17) or in elderly (23). Previous study showed that eryaspase efficacy/safety profile of both doses 100 and 150 IU/Kg achieved sustained asparaginase activity with no excessive limiting toxicities.

This trial will explore the tolerability and the biological activity of eryaspase in patients with previous hypersensitivity to PEG-asparaginase formulation. The expected benefit for those patients is to provide them an additional alternative therapy with eryaspase.

This Nordic Society of Pediatric Haematology and Oncology (NOPHO) protocol NOR-GRASPALL 2016 is a Multi-agents protocol with eryaspase expected to be as effective as NOPHO ALL 2008 and ALLTogether pilot protocol Multi-agents protocol with pegylated E.coli L- asparaginase and better tolerated.

2.1.2 Potential Risks for the Patients

Adverse effects resulting from the administration of asparaginase or erythrocytes are well known. Toxicity data come from clinical studies carried on since 2005 with eryaspase as add-on therapy with protocols combining several chemotherapies that have their own toxicity profile.

In study GRASPALL 2009-06, the incidence of allergic reactions was in both eryaspase arms in the non-allergic (7.7%) and allergic patients (11.5%). There were no withdrawals due to hypersensitivity reactions, either in the allergic or non-allergic patient subsets. The most common drug-related events with eryaspase were hypofibrinogenemia (26.9%), asymptomatic pancreatitis (26.9%), elevated transaminases (19.2%), hypoalbuminemia (15.4%), and decreased anti-thrombin III (11.5%).

The risk of asparaginase related toxicities is not increased compared to the treatment with other formulations of asparaginase e.g. PEG-asparaginase.

Detailed safety information is summarized in the Investigator Brochure.

The risk of transfusion complications are very low when national guidelines for blood transfusions are followed, the risk rate is 3.7/100000 transfusions of red blood cells which include transfusion with incorrect blood components.(24)

The patients will not have any inconvenience and risk because of extra hospital visits, blood tests, lumbar punctures for CSF colleting and clinical assessments because it all will be done according to the conventional investigations in the NOPHO ALL 2008 or ALLTogether pilot protocol.

Previous studies have shown a good safety profile and the risk of toxicity is not increased compared to the conventional treatment with PEG-asparaginase. According to that it is assessed that getting eryaspase and completing the asparaginase treatment are beneficial compared to the risk. The risk is considered reasonable in proportion to the severity of the diagnosis and the conventional treatment according to the NOPHO ALL 2008 or ALLTogether pilot protocol.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

Main objectives of this study are to evaluate the pharmacological (pharmacokinetic and pharmacodynamic) profile of eryaspase administered to patients who experience PEG-asparaginase hypersensitivity event during induction, consolidation or subsequent phases of the multi-agent chemotherapy in NOPHO ALL 2008 or ALLTogether pilot protocol for the treatment of children and adult patients with ALL;

The pharmacological profile of eryaspase, determined by:

- The pharmacokinetic (PK) parameters for asparaginase activity will be assessed: C_{max}; T_{max}; AUC (Area Under the Curve); $T_{1/2}$ (half-life); V_{ss} (Distribution Volume at steady state), MRT (Mean Residence Time) and CL (Clearance).

- The pharmacodynamic (PD) parameters for cerebrospinal fluid (CSF) concentrations of asparagine, aspartate, glutamine, and glutamate will be assessed.

3.2 Secondary Objectives

- To assess the immunogenicity of eryaspase as determined by assessment of anti-L-asparaginase antibodies and neutralizing antibodies.

- To assess the overall safety and tolerability of eryaspase in combination with multi-agent NOPHO ALL 2008 chemotherapy according to the PdL toxicity consensus definitions for asparaginase related toxicities (hypersensitivity, pancreatitis, hyperlipidemia, SOS, thrombosis, osteonecrosis, and fungal infections)(25).

- Evaluate the effect of Eryaspase on the concurrent maintenance therapy with 6-mercaptopurine and methotrexate. Levels of cytotoxic methotrexate (MTX polyglutamates) and 6-mercaptopurine metabolites (6-thioguanine nucleotides, 6TGN, and methylated metabolites, MeMP) and of DNA-TGN levels in circulating leukocytes.

3.3 Exploratory Objective

- The pharmacodynamic (PD) parameters for plasma and whole blood: Concentration of asparagine, aspartate, glutamine, and glutamate will be assessed.

4.0 CRITERIA FOR EVALUATION

4.1 Primary Endpoints

The study endpoints are to evaluate:

Pharmacokinetic parameters:

- Total-activity (at time points listed in Section 8.4.1) will be measured. The main pharmacokinetic parameters will be assessed: Cmax; Tmax; T1/2 (half-life time); Vss (Distribution Volume at steady state), MRT (Mean Residence Time) and clearance.
- Percentage of patients with continuous asparaginase activity >100 IU/L.
- Duration of enzyme activity >100 IU/l at 6 w dosing intervals

Pharmacodynamic profile:

- CSF concentrations of amino-acids: (asparagine, aspartate, glutamine, glutamate), at time points listed in the Study Procedures and Guidelines section.

Immunogenicity: titers of anti-asparaginase antibodies and neutralizing antibodies.

Safety: Incidence of hypersensitivity (allergic reactions, any grade, and silent inactivation) at the end of asparaginase treatment

All parameters that will be taken account are those defined in the Study Procedures and Guidelines section.

4.2 Secondary Endpoints

The secondary endpoints are to evaluate:

- The safety and tolerability of eryaspase in combination with standard multi-agent NOPHO ALL 2008 or ALLTogether pilot protocol chemotherapy assessed during eryaspase treatment period according to potential toxicities defined in the Adverse Experience section.
- Toxicity of the eryaspase treatment compared to the conventional PEG-asparaginase treatment. Toxicity and SAE's will be evaluated according to the PdL toxicity consensus definitions.
- Levels of maintenance metabolites

4.3 Exploratory Endpoint

o Plasma and whole blood concentrations of amino acids: asparagine, aspartate, glutamine, glutamate. Time points listed in the study Procedures and Guidelines section.

4.4 Safety Evaluations

All AEs/SAEs will be collected at start of treatment until 30 days after last administration.

SAE's will be registered according to the NOPHO ALL 2008 or ALLTogether pilot protocol toxicity registration and classified and described according to the PdL consensus paper on ALL treatment related toxicity(1).

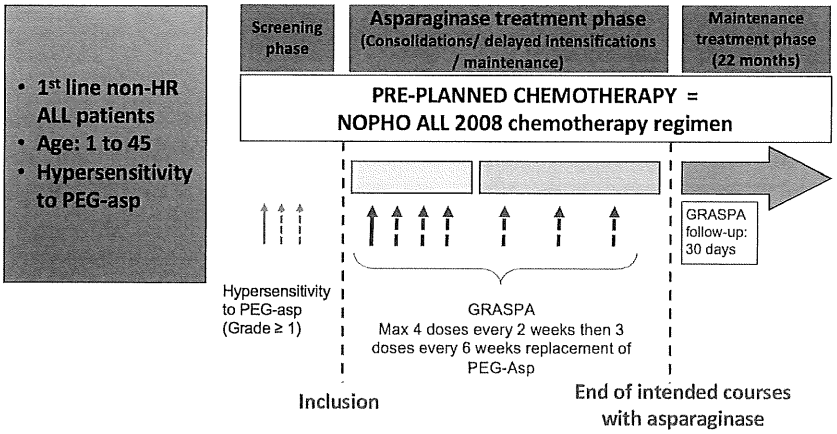
5.0 STUDY DESIGN

5.1 Study Overview

This is a single arm, multicenter, multinational study to assess the pharmacological activity, safety and immunogenicity of eryaspase in combination with the NOPHO ALL 2008 or ALLTogether pilot protocol chemotherapy protocol administered as second line treatment to children or adult patients from 1 to 45 years old with ALL who experience hypersensitivity reactions (clinical allergic reaction or silent inactivation) to PEG-asparaginase.

All patients will receive the NOPHO ALL 2008 or ALLTogether pilot protocol chemotherapy regimen, which consists of an induction treatment, consolidation, delayed intensification phase and maintenance phases, a CNS prophylaxis and interim maintenance for a total of 30 months' treatment duration. Once a patient presents with hypersensitivity to PEG-asparaginase, eryaspase will be given as a replacement therapy for remaining scheduled PEG-asparaginase administrations (Figure 3).

FIGURE 3A. STUDY DIAGRAM – PATIENTS TREATED ACCORDING TO NOPHO ALL 2008:



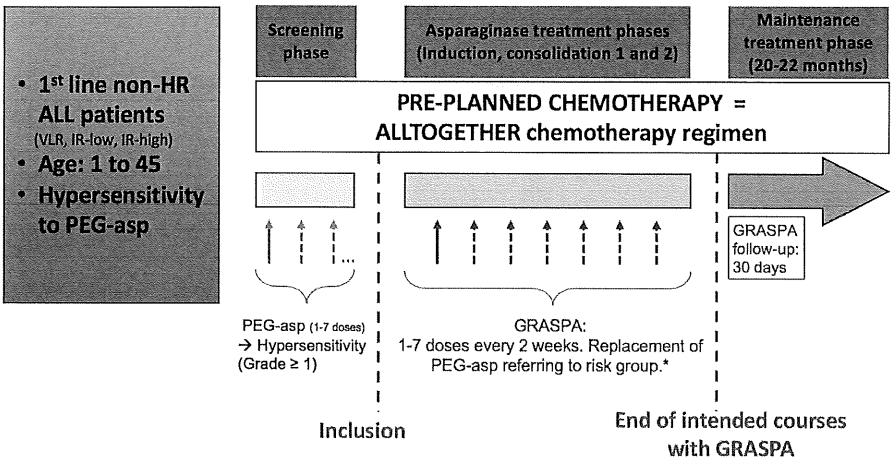
Once GRASPA is initiated, PEG-Asparaginase regimen should be replaced by scheduled GRASPA during all remaining chemotherapy course

Peg-Asparaginase

GRASPA dose: 150 IU/kg IV infusions will be administered every 2 weeks for maximum 4 doses then every 6 weeks for 3 doses up to the end of planned chemotherapy protocol

Erytech internal code GRASPALL 2016-12

FIGURE 4B. STUDY DIAGRAM – PATIENTS TREATED ACCORDING TO ALLTOGETHER PILOT PROTOCOL:



Once GRASPA is initiated, PEG-Asparaginase regimen should be replaced by scheduled GRASPA during all remaining chemotherapy course

Peg-Asparaginase

GRASPA dose: 150 IU/kg IV infusions will be administered every 2 weeks for maximum 3-7 doses up to the end of planned chemotherapy according to protocol and risk group.

*According to ALLTOGETHER protocol: VLR: 4 doses IR low: 5 doses IR high: 8 doses

Erytech internal code GRASPALL 2016-12

Eryaspase will be administered every 2 weeks for a maximum of 4 doses. Then administered every 6 weeks for 3 doses to the end of planned treatment according to the NOPHO ALL 2008 or ALLTogether pilot protocol.

Assessments include overall safety profile of eryaspase, PK/PD parameters of eryaspase, immunogenicity of eryaspase, during successive courses of treatment with eryaspase.

Primary trial assessments for each patient will continue for 1 month following the end of the planned eryaspase treatment phases (approximately 6 months in case of 7 doses). Each patient will receive cytostatic treatment in about 22 months after last administration of eryaspase according to the NOPHO ALL 2008 or ALLTogether pilot protocol. After cessation of therapy patients will be followed for recurrence of disease or the development of late effects. The follow-up after cessation of therapy should be done according to guidelines on each country and site and includes no assessments for this study. Further details regarding the study assessments during the study are provided in section 9.0.

The duration of treatment with eryaspase will be dictated by the remaining number of intended courses following the occurrence of hypersensitivity to PEG-asparaginase, and therefore ranges between 1-7 doses of eryaspase.

An interim analysis will be performed when 5 patients have been included.

It is estimated to include 45 children and 5 adults in this study. Patient enrolment will start in Denmark in January 2017. It is estimated that 50 patients will be enrolled by end of February 2020.

5.2 Definition of End of Trial

The trial can be prematurely stopped (definitely or temporarily) when:

- The treatment is considered as too noxious to continue with further clinical investigations
- Occurrence of new fact that can modify NOPHO Competent Authority approval on the trial, or for any unethical reason

In case of any reason motivating such withdrawal, the Investigator should promptly inform the patients, ensure appropriate therapy and follow-up, and complete the CRF with all available data at the time of trial arrest.

Trial withdrawal with the reason will be declared to Competent Authority (and EC if applicable) in accordance with local requirements.

NOPHO or Erytech Pharma may decide to stop the study at any time.

5.3 Inclusion Criteria

A patient is eligible for the study if all of the following criteria are met:

1. Male or female aged 1-45 years at diagnosis of ALL
2. First line non-high risk (HR) ALL patients enrolled in NOPHO ALL 2008 or ALLTogether pilot protocol including PEG-asparaginase regimen

3. Documented hypersensitivity reaction to PEG-asparaginase with either:
    - Clinical allergy to PEG-Asparaginase (mild/severe) OR
    - Serum asparaginase activity below the lower level of quantification
4. Karnofsky/Lansky score ≥ 50.
5. Ability to understand, and willingness to sign, a written informed consent document and to comply with the scheduled visits, treatment plans, laboratory tests, and other study procedures. For patients under 18 years of age, either both parents or the legally appointed representatives will need to provide consent.

5.4 Exclusion Criteria

1. Philadelphia chromosome positive ALL.
2. Participation in another clinical trial interfering with the study therapy with exception of NOPHO ALL-2008 or ALLTogether pilot protocol. Patients can participate in other clinical trials not interfering with the study drug. In case of doubt this is assessed by the PI.
3. Uncontrolled intercurrent illness including, but not limited to, patients receiving combination antiretroviral therapy or patients with severe or systemic infection, or psychiatric illness/social situations that would limit compliance with study requirements.
4. Other severe acute/chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, and in the judgment of the investigator would make the patient inappropriate for entry into this study.
5. Pregnant or lactating females. (serum human chorionic gonadotropin pregnancy test at screening). Use of a highly effective contraceptive measure in women of child-bearing potential and sexually active girls that are of child-bearing potential is required (contraceptive measures are specified in section 6.0).
6. Inadequate organ functions, which prohibit further asparaginase administration;
    a) History of pancreatitis
    b) History of serious hemorrhage or serious thrombosis with prior asparaginase therapy
    c) Severe hepatic impairment at the time of administration (bilirubin >3 times ULN, transaminases >10 times ULN)
    d) Pre-existing known coagulopathy (e.g. haemophilia)
6. History of grade 3 or higher transfusion reactions or any contraindication to receive blood transfusion. Presence of specific anti-erythrocytes antibodies (auto-antibodies or anti-public antibodies) preventing from getting a compatible packed Red Blood Cells for the patient.

7. Patient under concomitant treatment likely to cause hemolysis.

6.0 Concurrent Medications

All patients should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

6.1 Contraception

All chemotherapeutic agents can be teratogenic and may be excreted in breast milk. Inclusion of women of child-bearing potential and sexually active girls that are of child-bearing potential will have a pregnancy test before entering the study. Additional pregnancy testing should be performed in case of delayed menstrual period and is recommended to be performed monthly in case of sexual activity. Furthermore, a pregnancy test should be performed at the end treatment period.

Inclusion of women of child-bearing potential and sexually active girls that are of child-bearing potential requires use of a highly effective contraceptive measure in the study period and should be continued for at least 3 months. Highly effective contraceptive methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (intravaginal or transdermal)
- progestogen-only hormonal contraception associated with inhibition of ovulation (injectable or implantable)
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- sexual abstinence

Be aware that since an indirect interaction between components of the oral contraceptives and asparaginase cannot be ruled out, oral contraceptives are not considered acceptable as contraceptive measures in the current clinical trial.

6.2 Infection prophylaxis

According to the NOPHO ALL-2008 and ALLTogether pilot protocol it is recommended that the patients receive prophylaxis against Pneumocystis carinii Pneumonia (trimethoprim/sulphamethoxazole at a dose of 5 mg/kg of trimethoprim on 2 consecutive days per week). Antifungal prophylaxis and further antibacterial prophylaxis is not recommended for patient of SR and IR in the NOPHO ALL-2008 and ALLTogether pilot protocol or in this study.

6.3 Allowed Medications and Treatments

Standard therapy according to NOPHO ALL 2008 is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. Blood

transfusions, antibiotic therapy, and other supportive care are allowed and will in general be given according to the guidelines of the treating institution.

6.4 Prohibited Medications and Treatments

The following medications are prohibited during the study period (from inclusion until 30 days after last administration), and administration will be considered a protocol violation:

- Concomitant vaccination with live vaccines as it may increase the risk of serious infection
- Other L-asparaginase marketed products
- Highly hemolytic agents that may lead to RBCs hemolysis: Acetanilide, Antipyrine Phenazone, and Chloroquin and derivatives

NOTE: Benzodiazepine may be given if indicated.

7.0 STUDY TREATMENTS

7.1 Formulation of Test and Control Products

7.1.1 Formulation of Test Product

Eryaspase is dispersion for Infusion of L-asparaginase encapsulated in erythrocytes, packed in transparent PVC bag designed for blood product, containing a single dose for administration.

Each bag of eryaspase contains:

- Active substance: recombinant L-asparaginase encapsulated in erythrocytes (or red blood cells), equivalent dose of L-asparaginase according to medical prescription and patient weight, 150 IU/kg
- Preservative solution: AS3

The Red Blood Cell (RBC) source material is from leuko-reduced packed red blood cells, prepared and qualified by a blood bank. RBC source material is selected based on compatibility with the patient (phenotype and results of irregular antibodies screening test).

The encapsulation technique (i.e. reversible hypotonic swelling and resealing of the RBCs), is a controlled technique enabling asparaginase to be encapsulated in a safe, reproducible, controlled automated manner. The encapsulation process is GMP compliant.

The minimal volume of a bag of eryaspase is 50 mL. The final volume of the eryaspase bag(s) depends on patient weight. Depending on patient's weight, additional eryaspase bags may be administered to comply with the prescribed total dose.

7.1.2 Packaging and Labelling

Eryaspase is packaged in PVC bags designed for blood product. Three removable segment-tubes are attached for cross-matching before administration.

Label statements are specific to the clinical trial, complying with legal requirements for medicinal product. In addition, the label displays specific items necessary for traceability of source cell material

and medicinal product (phenotype, patient's identification number, etc.) to allow for verification of the patient identity and blood group before administration.

Additional descriptive information for eryaspase can be found in the Investigator's brochure (IB).

7.2 Supply of Study Drug at the Site

ERYTECH will ship eryaspase directly to the clinical site. Study drug can be shipped after site activation (i.e., all required regulatory documentation has been received by the Sponsor and a contract has been executed). Study drug shipments are made after site request for resupply according to Section "Dispensing/Allocation of ERYASPASE" below.

In order to ship the study drug to the clinical site, the following information is required:

- Name (s) of recipient
- Location: pharmacy or blood bank,
- Address and contact details

Eryaspase will be shipped to the investigator in a qualified container by a specialized carrier, who will ensure that the cold chain is maintained between +2-8°C (35-46°F).

The time of manufacturing will be noted on the product label. Please refer to the IMP Manual for stability of the IMP. If a temperature excursion outside of +2°C to +8°C temperature range occurs, the product should be quarantined and ERYTECH Pharma should be contacted to determine usability of the product. The investigational drug will be delivered directly to the investigator's institutional pharmacy or blood bank per the provided shipping details.

Instructions for the receipt and administration of eryaspase are detailed in IMP Manual and provided with the shipment.

7.3 Storage Conditions

Eryaspase may be stored at room temperature up to 6 hours prior to administration, including infusion time. If not used immediately after receipt, it is **mandatory** to store eryaspase at temperatures between +2 and +8°C. Eryaspase must not be stored at room temperature for more than 6 hours.

7.4 Treatments Administered

7.4.1 Dosage/Dosage Regimen

The dose of eryaspase prescribed in this study protocol is equivalent to 150 IU/kg of recombinant L-asparaginase activity.

7.4.2 Treatment Duration

NOPHO ALL 2008:

Eryaspase will be administered at a dose of 150 IU/kg every 2 weeks for a maximum of 4 doses and then for a maximum of 3 doses at 6-week intervals. Assuming that first asparaginase treatment at day

29 from the NOPHO ALL 2008 protocol regimen corresponds to PEG-asparaginase, patients will receive a maximum of 7 doses.

Depending on the timing of development of hypersensitivity reactions with prior PEG-asparaginase, treatment with eryaspase may start during consolidation, delayed intensification or maintenance phase of NOPHO ALL 2008 treatment.

The total duration of treatment with eryaspase will therefore vary among patients according to the time of switch to eryaspase during their respective chemotherapy protocol and end of eryaspase treatment.

ALLTogether pilot protocol:

Eryaspase will be administered at a dose of 150 IU/kg every 2 weeks. Patients will receive maximum 3 to7 doses according to risk group defined in the ALLTogether pilot protocol.

- Very low risk: maximum of 4 doses
- Intermediate risk – low: maximum of 5 doses
- Intermediate risk – high: maximum of 8 doses

First dose of PEG-asparaginase is at day 4 in induction phase for all risk groups.

Depending on the timing of developing hypersensitivity reaction to PEG-asparaginase, treatment with eryaspase may start during induction or in consolidation 1 or 2 described in the ALLTogether pilot protocol.

The total duration of treatment with eryaspase will therefore vary among patients according to the time of switch to eryaspase during their respective chemotherapy protocol and end of eryaspase treatment.

7.4.3 Dispensing/Allocation of ERYASPASE

Once a patient has consented and is determined to be eligible for this study, ERYTECH Pharma must be notified and the process below followed:

To initiate eryaspase manufacturing and allocation of eryaspase, NOPHO or designee must be provided with the documents listed below:

- **At Screening:** a validated phenotype and ABO status (double determination is mandatory), as well as IAST results are required. The blood group form should be completed, and the lab results should be attached to this form. A template of the blood group form along with instructions is available in IMP Manual.
- **5 working days prior to each eryaspase injection:** Prescription Sheet indicating patient identifiers, weight, and estimated time of delivery. A template of the prescription sheet is available in IMP Manual.

- **Within 72 hours of each eryaspase injection:** Documentation of the IAST (Irregular Antibody Screening Test) performed at the local immuno-hematology laboratory.

- **In case of positive IAST** or previous history of positivity, a compatibility test is mandatory in order to appropriately select the packed Red Blood Cells. In cases of a positive IAST where compatibility testing is required, the administration of eryaspase may be delayed depending on the timeline for the compatibility results.

Instructions for blood sampling management sent to French blood bank (EFS) for selecting compatible packed Red Blood Cells are detailed in IMP Manual.

7.4.4 Administration Instructions

Instruction for the receipt and administration of eryaspase are detailed in the IMP manual. Should any issues occur, please contact Erytech Pharma for immediate appropriate action.

Prior to Administration

Prior to each infusion, the following must be performed and recorded on the "ERYASPASE Shipment and Administration Form" (see IMP manual):

- Consistency with prescription
- Check patient identification compared to information stated on the product label
- Carry out a final blood test to check compatibility between the patient's blood and eryaspase (removable segment tubes provided)

ERYASPASE MUST NOT BE ADMINISTERED IN EVENT THERE IS ANY DISCREPANCY IN INFORMATION

Administration

Eryaspase is administered by intravenous route using the administration line provided or equivalent under medical responsibility. Eryaspase is a ready to use dispersion designed to be fully infused.

Eryaspase must be administered only by trained staff. He/she must record the date and duration (start and stop time) of the IV infusion on the eryaspase "Shipment and Administration Form" (cf. IMP Manual).

The administration must take place within the expiry time stated on the label of eryaspase.

CAUTION: Although no incompatibility has been demonstrated, eryaspase should not be transferred to another container before injection. Since no data regarding incompatibility are available, eryaspase should not be mixed or administered simultaneously with any other product, solution or medicinal product during the infusion.

The mean duration for administration is approximately 45-60 minutes. If a patient has impairments, such as cardiac impairment, the infusion timeline may be lengthened and the flow rate of the bag adjusted to meet the patient's clinical conditions.

- The entire content of the bag(s) must be administered

- The patient must remain awake and should be constantly monitored for occurrence of any adverse reactions during the infusion and for a minimum 1 hour post completion of infusion
- Should a major medical event occur (e.g. malaise), eryaspase injection should be stopped immediately and the principal investigator informed
- At the end of the infusion, the lines should be rinsed with 20-40 ml of physiological saline solution (0.9% NaCl).
- **CAUTION: If a bag of eryaspase is partially transfused or not transfused to the patient, this must be documented accordingly with full explanation of why transfusion was incomplete or not done. This information is documented on the Shipment and Administration Form and within the patient's chart.**

Post Administration

The responsible person of administration should record on "Shipment and Administration Form" the below information after completion of administration:

- Date and duration of administration (start and stop time)
- Name and position, signature
- Whether the entire content was administered and the lines accurately rinsed, with full explanation if it was not the case.

The completed document should be sent immediately following infusion to Erytech to confirm eryaspase administration. Please refer to the IMP Manual for instructions.

7.4.5 The monitoring during and immediately after the infusion of IMP.

The patient must remain awake and should be constantly under medical supervision **during the administration and for at least one hour after the end of administration** for occurrence of any adverse reactions. The infusion must be stopped **immediately in case of occurrence of any adverse reactions and the PI (or designee) must be informed.**

Eryaspase administration should be performed **under medical responsibility** and only by trained staff. The staff must be trained in handling blood products and in diagnosing and treatment of acute allergic reactions. Medication and equipment to treat acute allergic reactions in relation to infusion must be placed in the room of administration and the personnel must have immediate access.

The management of adverse reactions should be based on clinical presentation and is at investigator's discretion. Should the patient exhibit symptoms suggestive of hypersensitivity reactions to eryaspase, the general treatment of such reactions will follow the guidelines of the department.

7.5 Study Drug Accountability

An accurate and current accounting of the dispensing and return of study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The study monitor will verify these documents throughout the course of the study.

A common monitoring plan created by the sponsor and the GCP authorities in Aarhus will be distributed to the responsible monitor authorities in the Nordic and Baltic countries for them to apply to during monitoring.

7.6 Dose interruption and modification

Temporarily dose interruption in case of following:

- Severe hepatic impairment (transaminases >10 times ULN).
- Bilirubin > ULN and other clinical signs of SOD (according to the PdL consensus definitions)
- Serious infectious disease

Treatment can be resumed when parameters are normalized and the clinical condition allows further treatment. Impact of hematological parameters will not induce dose interruption.

Eryaspase treatment is discontinued in case of:

- Clinical allergy of any grade
- Pancreatitis
- Serious hemorrhage or serious thrombosis
- No asparaginase activity

There will be no dose-modification due to haematological or non-hematological toxicities only temporarily dose interruption or discontinuation of the eryaspase treatment.

7.7 Treatment after end of trial

Simultaneously with this study the patient receives multi-agent chemotherapy according to the NOPHO ALL-2008 or ALLTogether pilot protocol. After the end of this trial the patient will continue the remaining part of the maintenance therapy for patients with SR or IR ALL according to the NOPHO ALL-2008 or ALLTogether pilot protocol.

8.0 STUDY PROCEDURES AND GUIDELINES

8.1 Schedule of Events

The following schedules in Table 2 below illustrate a 2-week course (NOPHO ALL 2008 - induction and intensification phase and ALLTogether pilot protocol) and 6-week course (NOPHO ALL 2008 protocol - delayed intensification and consolidation phase) of eryaspase and the events in the period. The schedules must be repeated for every dose so that "schedule of events, 2-week course" must be repeated 1-4 times and the "schedule of events, 6-week course" must be repeated 1-3 times.

The schedule is a worksheet and presents an overview of the events. All data, events and exact time points have to be registered in the CRF.

Trial assessments will continue for one (1) month following the end of planned eryaspase courses for each patient. Then patients will be followed for up to one (1) year maintenance follow-up according to the NOPHO ALL 2008 or ALLTogether pilot protocol.

In case of eryaspase treatment termination, patients will be followed at least one month after the last administration of eryaspase for safety evaluation.

TABLE 2: SCHEDULE OF EVENTS

Study calendar, 2 weeks courses

			Week 1								Week 2						
Time/day	Screening	≥ 5 days (COURSE 1)	Baseline [i] (DAY 1)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Assessment																	
Eligibility check	X [b]																
Signed written informed Consent	X [b]																
Baseline data (Demographics, medical history, ALL disease history)	X [b]																
Physical examination and vital signs incl. Karnofsky/Lansky Score	X		X														
ALL clinical assessment	X																
Pregnancy test[o]	X																
Weight		X											X				
Ordering/Quality check																	
Blood phenotyping		X [c,d]															
Prescription/ordering		X [e]											X [e]				
Irregular Antibody Screening Test		X [f] (< 72 hours)													X [f]		
Serologic crossmatch [g]			X														
Eryaspase administration				X													
Sampling																	
Amino acids sample [h,i]			X	X	X		X			X				X			
Asparaginase activity [h,j]			X[1]	X[2]	X[3]		X[4]			X[5]				X[6]			X [s]
Antibodies Titres [h,j]			X	X	X		X			X				X			X [s]
CSF amino acids [l]				(X) [k]													
Maintenance metabolites				(X) [k]													
Hematological and liver parameters [n]				(X) [m]													
Biological safety parameters [p]			X [t]														X[t,s]
Registration																	
Adverse event assessment and registration			X [q]	Ongoing registration													X [q]
Concominant treatment			X [r]	Ongoing registration													X [r]

Study calendar, 6 weeks courses

			Week 1								Week 2							3	4	5	6
Time/day	Screening	≥ 5 days (COURSE 1)	Baseline [a] (DAY 1)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	22	29	36
Assessment																					
Eligibility check	X [b]																				
Signed written informed Consent	X [b]																				
Baseline data (Demographics, medical history, ALL disease history)	X [b]																				
Physical examination and vital signs incl. Karnofsky/Lansky Score	X		X																		
ALL clinical assessment	X																				X
Pregnancy test[o]	X																				
Weight		X											X								
Ordering/Quality check																					
Blood phenotyping		X [c,d]																			
Prescription/ordering [e]		X [e]																			X [e]
Irregular Antibody Screening Test		X [f] (<72 hours)																			X [f]
Serologic crossmatch [g]			X																		
Eryaspase administration				X																	
Sampling																					
Amino acids sample [h,i]			X	X	X		X			X				X				X	X		
Asparaginase activity [h,j]			X[1]	X[2]	X[3]		X[4]			X[5]				X[6]				X[7]	X[8]		
Antibodies Titres [h,j]			X	X	X		X			X				X				X	X		
CSF amino acids [l]				(X) [k]																	
Maintenance metabolites				(X) [k]																	
Hematological and liver parameters [n]				(X) [m]																	
Biological safety parameters [p]			X[t]														X[t]		X[t]		
Registration																					
Event assessment and registration			X [q]	Ongoing registration																	X [q]
Concomitant treatment			X [r]	Ongoing registration																	X [r]

a. At the day of administration, before administration (Day 1)

b. Complete section 3 and 4 in the CRF and please send it to: Line.stensig@rm.dk
c. Complete "Blood group form" (appendix 3 in the IMP manual) and attach lab results → send to Erytech pharma: shipment@erytech.com (or *Fax*: +33 478 789 305)
d. Only before the first administration
e. Complete the "prescription form" (appendix 4 in the IMP manual) → Send the documents to Erytech Pharma **at the latest 5 working days prior to each eryaspase administration:** shipment@erytech.com (or *Fax*: +33 478 789 305)
f. 72-48 hours before administration → send lab results for Erytech Pharma. In case of positive results, contact Erytech pharma immediately → blood sample must be shipped to Erytech pharma/French blood bank for serologic cross match → follow guidelines in IMP manual.
g. A serologic crossmatch test between the patient's blood and the IMP is required to ensure IMP administration under safe conditions. This test can be done at the local lab or blood bank using one of the removable segment-tubes attached to the IMP bag. Complete Safety and administration form (appendix 5 in IMP manual). Erytech will now be testing blood in case of incompatibility with IMP, regardless of IAST positivity.
h. Blood sampling will be modified by local conditions and activities planned in the NOPHO ALL 2008 protocol. Aimed time slot is marked in this study calendar (1-7) and is described in the research protocol in section 8.4 and below here (#1-7) Date and time of blood sampling must be registered in the CRF.
i. Every effort will be made to collect these samples. Guidelines to the measurements of whole blood and plasma amino acids is found in the Trial master file.
j. Samples must be send to: *Pediatric research Laboratory, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99,8200 Aarhus N, Denmark. Att. Jane Knudsen. Please notify by email before the shipment:* janeknen@rm.dk
k. When planned in NOPHO ALL 2008 protocol.
l. Must be send on dry ice to: *Pediatric research Laboratory, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99,8200 Aarhus N, Denmark. Att. Jane Knudsen. Please notify by email before the shipment:* janeknen@rm.dk. Samples can be send at the end of study period-
m. In connection with measurements of maintenance metabolites. Lab results must be attached the CRF. Send the samples to Bonkolab, Copenhagen according to the NOPHO protocol.
n. Blood samples includes: Hematological parameters (Hemoglobin, thrombocytes, Differentiated White Blood Cell Count) and liver parameters (bilirubin, alanine aminotransferase (ALAT), Lactate dehydrogenase (LDH).
o. Serum human chorionic gonadotropin pregnancy test at screening in women of child-bearing potential and sexually active girls that are of child-bearing potential is required.
p. Biological safety parameters are recommended and include: Hematological parameters (Hemoglobin, thrombocytes, Differentiated White Blood Cell Count) and liver parameters (bilirubin, alanine aminotransferase (ALAT), Lactate dehydrogenase (LDH), amylase, lipase, triglycerides and cholesterol, urinary glucose and coagulation parameters (partial thromboplastin time (PTT), prothrombin time (PT), antithrombin III and fibrinogen)
q. Be attentive to events from last course at baseline of next course. Please complete the CRF and send it after each course (Line.stensig@rm.dk). Guidelines to registration is found in the CRF.
r. Registration of other cytostatic treatment including maintenance therapy (according to the NOPHO ALL 2008 protocol) in the CRF.
s. Blood samples at day 14 coincide with baseline of next course. If applicable examinations not need to be repeated at both time points.
t. Additional blood counts for biological safety can be performed due to clinical conditions and as standard of care.

1. 15 min (± 5 min) before eryaspase administration
2. End of infusion (0-10 min)
3. 24 hours post-infusion (± 2 hrs)
4. 3 days (± 1 day) post-infusion
5. 6 days (± 1 day) post-infusion
6. 10 days (± 2 days) post-infusion
7. 14 days (± 2 days) post-infusion
8. 21 days (± 2 days) post-infusion

8.2 Clinical Assessments

8.2.1 Demographics

Demographic information (date of birth, gender, race) will be recorded at Screening.

8.2.2 Medical History and ALL Disease History

Relevant medical history, including history of current disease and information regarding underlying diseases, will be recorded at Screening and from the NOPHO database.

8.2.3 Physical Examination and Vital Signs

A complete physical examination will be performed by either the investigator or a sub-investigator who is a physician at Screening, during Course Evaluation and at the End of Study Visit. Qualified staff may complete the abbreviated physical exam at all other visits. New abnormal physical exam findings must be documented and will be followed by a physician or other qualified staff at the next scheduled visit.

- Full physical examination including height (at baseline only), weight and vital signs (body temperature, blood pressure and pulse rate)
- Karnofsky/Lansky score performance status.

8.2.4 ALL Clinical Assessment

ALL clinical assessment will be performed at Screening, and at the end of the study.

8.2.5 Adverse Events/Serious Adverse Events Assessment

See Section 10.0 "ADVERSE EVENT REPORTING AND DOCUMENTATION."

8.3 Clinical Laboratory Measurements

Performed as standard of care.

8.3.1 Biological safety parameters

Before initiating therapy, bilirubin, hepatic transaminases, and coagulation parameters (e.g., partial thromboplastin time (PTT), prothrombin time (PT), antithrombin III and fibrinogen) should be determined.

After administration of eryaspase, monitoring of bilirubin, hepatic transaminases, urinary glucose, coagulation parameters (e.g., PTT, PT, anti-thrombin III, fibrinogen, and D-dimer), amylase, lipase, triglycerides, and cholesterol is recommended. Plasma glucose must be measured regularly if the patient has concomitant treatment corticosteroids and if the urine shows sign of glucose and managed as clinically indicated.

In 2 weeks courses, baseline sampling (before administration) is used as a control after the preceding administration. In 6 weeks courses, parameters will be determined at least once in the treatment period.

Additional blood counts can be performed due to clinical conditions and as standard of care.

8.3.2 Maintenance Metabolites

Maintenance metabolites will be measured once a month during first year of maintenance. Blood samples will be collected at local institutions and sent to: The Laboratory for Pediatric Oncology, Bonkolab 5704, The National University Hospital, Rigshospitalet, Copenhagen, Denmark. Here it will be stored and analysed.

Hematological parametres (Hemoglobin, thrombocytes, White Blood Cell Count) and liver parameters (bilirubin, alanine aminotransferase (ALAT), Lactate dehydrogenase (LDH)) have to be measured at the same time points. Furthermore, details about maintenance therapy have to be filled in the CRF.

8.4 Pharmacokinetic/Pharmacodynamic and Immunogenicity Assessments

A laboratory manual describing sample collection, processing and shipment will be provided to investigational center. Blood samples will be collected at local institutions and sent to pediatric research laboratory, Aarhus University Hospital (AUH), Denmark, where it will be stored and analyzed.

Detailed procedures and logistic for the management of samples are detailed in the lab manual provided to the sites.

8.4.1 Total Asparaginase Activity

Blood samples will be drawn to determine asparaginase activity as follows:

Courses with dose every 2 weeks:

- 15 min (± 5 min) before eryaspase administration
- End of infusion (0-10 min)
- 24 hours post-infusion (± 2 hrs)
- 3 days (± 1 day) post-infusion
- 6 days (± 1 day) post-infusion
- 10 days (± 2 days) post-infusion
- 14 days (± 2 days) post-infusion. The 14-day post-infusion sampling corresponds with the pre-eryaspase for next dose (i.e., the Day 15 sample for dose 1 = the pre-dose sample for dose 2, etc.)

Courses with dose every 6 weeks:

- 15 min (± 5 min) before eryaspase administration
- End of infusion (0-10 min)
- 24 hours post-infusion (± 2 hrs)
- 3 days (± 1 day) post-infusion
- 6 days (± 1 day post-infusion
- 10 days (± 2 days) post-infusion

- 14 days (± 2 days) post-infusion
- 21 days (± 2 days) post-infusion

8.4.2 Amino acids: asparagine, aspartate, glutamine, and glutamate

8.4.2.1 Amino-acids in CSF

Samples for asparagine, aspartate, glutamine, and glutamate levels in CSF will be drawn when intrathecal therapy occurs according to the time points in the NOPHO ALL 2008 or ALLTogether pilot protocol schedule.

8.4.2.2 Amino-acids in whole blood and plasma

Samples for asparagine, aspartate, glutamine, and glutamate levels in whole blood and plasma will be drawn at the time points for "Total asparaginase activity" according to section 8.4.1. Every effort will be made to collect these samples.

8.4.3 Anti-Asparaginase Antibodies

Anti-asparaginase antibodies will be measured **within 3 days before each eryaspase administration** and at the time points for blood samples for asparaginase activity at the end of the course.

8.5 Blood volumes

The blood volume for each blood count:

- Enzyme activity and asparaginase antibodies: 4mL
- Whole blood and plasma amino acids: 1,2 mL
- Maintenance metabolites: 2 mL
- coagulation parameters: 2 mL
- Hematological parameters: 2 mL
- Liver enzymes, billirubin, amylase, lipase: 2 mL
- Irregular antibody screening test: 4 mL

Blood volume drawn at individual visit:

- Baseline visit: 11,2 mL
- Visit including measurement of enzyme activity, antibodies, whole blood and plasma amino acids: 5,2 mL
- Visit including biological safety parameters: 6 mL
- Visit including maintenance metabolites: 6 mL

Some of the blood samples will be drawn at the same visit, the maximum blood volume for an individual visit is: 11,2 mL.

Blood volume drawn at each course:

- The maximum total extra blood volume for a 2 weeks course is: 53,2 mL
- The maximum total extra blood volume of a 6 weeks course is: 69,6 mL

The total blood volume for the total duration of the trial would be individual according to the number of doses and courses.
The blood sampling for maintenance metabolites would be measured even if the patient was not included in this study according to the NOPHO protocol. Furthermore, the hematological parameters and Liver enzymes would be performed as standard of care and will not be extra blood counts as well. Both these blood counts are included in the volume above.

The Investigator will contact the Medical Monitor regarding any pediatric patients who require adaptation of the blood sample schedule due to the patient's body weight in consideration of ICH Topic E11 Clinical Investigations of Medicinal Products in the Pediatric Population(26).

8.6 Setting up a Research Biobank

A research biobank will be set up for storing of the samples collected from each study participant for the analysis mentioned in the section above. Peripheral blood for analysis of asparaginase activity and antibody measurements (4 mL per sampling) and CSF (2 mL from each sampling) for analysis of amino acids will be stored at "Pediatric research laboratory", Aarhus University Hospital. Peripheral blood for measurements of maintenance metabolites (2 mL per sampling) will be stored at Bonkolab, Rigshospitalet. The biobank will terminate at 01. January 2021 and excess biological material will be stored for future studies in a biobank established for the purpose at two departments mentioned above. Separate approval from the Committee on Health Research Ethics will be procured, if the material is to be used. Samples will be destroyed in year 2036 at the latest.

9.0 EVALUATIONS BY VISIT

9.1 Screening

Informed consent form will be obtained from the patient prior to any study specific procedures. The screening visit must be performed at the latest 8 days before start of eryaspase treatment. The following assessments and procedures will be performed and documented:

1. Informed consent form signature
2. Check of inclusion and exclusion criteria (to be repeated in case of occurrence of an adverse event)
3. Demographic data (patient initials, date of birth, gender)
4. Risk group according to treatment protocol (NOPHO ALL 2008 or ALLtogether pilot protocol).
5. Physical examination including height, weight and vital signs according to the normal procedure at the centers.
6. Karnofsky/Lansky score
7. ALL clinical assessment according to the normal procedure at the centers.
8. Record all Concomitant Medications

9. Blood phenotype and ABO status
10. IAST Results

The above data will be collected on the relevant page of the CRF.

9.2 Day 1 of each eryaspase administration (±2 days)

1. Physical examination if clinician finds it necessary.
2. Perform and record vital signs (temperature, blood pressure, pulse rate) and weight.
3. Evaluate Karnofsky/Lansky score
4. Evaluate ALL Clinical Assessment according to the normal procedure at the centers.
5. Record any AEs/SAEs
6. Record any newly added concomitant medications
7. Collect blood for PK/PD testing
8. Collect blood for Antibodies Titers
9. IAST Results within 72 hours prior to each administration
10. Administer eryaspase via IV 150 IU/kg

9.3 Study Termination Visit

30 days after the last administration the patients will enter planned course of treatment and the follow-up program described in the NOPHO ALL 2008 protocol. A separate study termination visit is not required for the patients enrolled in the study.

10.0 ADVERSE EVENT REPORTING AND DOCUMENTATION

Adverse event (AE) are defined as any untoward medical occurrence in the patient after infusion of eryaspase, which does not necessarily have a causal relationship with this treatment. Adverse reactions (AR) are defined as any untoward and unintended response to the investigational medicinal product related to any dose administered.

All AEs must be registered until 30 days after last administration. The Investigator should ensure that adequate medical care is provided to the subject for any adverse event.

If there are several events and different symptoms combined to a main AE, then the only main AE (diagnostic preferable if available) should be recorded in the CRF. Where there is no link between different clinical symptoms occurring at the same time, each sign should be recorded as a separate AE

The following should not be recorded as AEs:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject has signed the informed consent.
- Pre-existing conditions found as a result of screening procedures.
- Other events, any grade, not related to eryaspase, will not be recorded in the CRF.

If seriousness criterion is met, a SAE should be declared to NOPHO drug safety, completing SAE report form.

10.1 Serious Adverse Events (SAE)

An adverse event is defined as "serious" when at least one the following criterion is met:

- Results in death: be aware that a death cannot be an event but always the outcome of an event. Exception is when death occurs suddenly, without the exact cause is stated. In this case only, the event should be recorded as "sudden death" with the seriousness criterion "fatal" indicated.
- Is life-threatening: life-threatening in the definition of a serious adverse event refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital anomaly or birth defect.
- Is considered as serious according investigator judgment in other situation. Important adverse events/ reactions that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above, should also be considered serious.

10.2 AEs/SAEs not to be reported

When treating children with cancer, the strategy is generally to treat to the limit of toxicity. Thus, all patients will be at a significant risk of experiencing AE's and SAEs, and all patients and families are informed hereof, when they enter therapy. Most patients will experience febrile neutropenia with or without confirmed bacteraemia, and these are potentially life threatening or may require inpatient hospitalization or prolongation of existing hospitalization. Thus, these AE's and several other SAEs reflect the overall treatment intensity of the current protocols for childhood ALL and not only the

treatment of asparaginase. In addition, treating to neutropenia is in principle the goal of several of the treatment phases and will frequently lead to infections (febrile neutropenia and other infections).

A number of toxicities are so well-known and frequent during therapy that they will not be reported. These includes:

- Since leukopenia is the target toxicity (monitoring parameter), this side-effect will not be regarded as a SAE. This also includes febrile neutropenia leading to hospitalization or prolongation of ongoing hospitalization if the patient's condition otherwise is good with no signs of septic shock.
- Since thrombocytopenia is the target toxicity (monitoring parameter) this side-effect will not be regarded as a SAE.
- A rise in aminotransferases with normal liver function tests (i.e. bilirubin and INR (or coagulation factor 2-7-10) is a well-known side effect of HD-MTX, 6MP and asparaginase and will not be regarded as a SAE.
- A rise in bilirubin to less than 5x UNL.
- Hyperglycemia will not be reported.
- Infection/fever leading to hospitalisation or prolongation of existing hospitalisation.

The overall safety and tolerability of eryaspase in combination with multi-agent NOPHO ALL 2008 or ALLTogether pilot protocol chemotherapy will be evaluated according to the PdL toxicity consensus definitions for asparaginase related toxicities (hypersensitivity, pancreatitis, hyperlipidemia, SOS, thrombosis, osteonecrosis, and fungal infections)(1). Table 3 contains the toxicities potentially related to treatment with asparaginase. All of these toxicities must be reported to PI's and on the CRF.

TABLE 3. POTENTIAL RELATED TOXICITY TO ASPARAGINASE (1)

Toxicity	Consensus definition
Hypersensitivity to asparaginase	An adverse local or general response from exposure to Asparaginase characterised by flushing, rash, urticaria, drug fever, dyspnoea, symptomatic bronchospasm, oedema/angiooedema, hypotension and/or anaphylaxis. Grading: 1.Mild: transient flushing or rash, drug fever <38° C, 2. Severe: drug fever >38° C; allergy-related edema/angiooedema; dyspnoea and/or symptomatic bronchospasm with or without urticarial; and/or hypotension and anaphylaxis with indication for Asp infusion interruption and parenteral medication (e.g. antihistamines, glucocorticosteroids).
Silent inactivation of asparaginase	No clinical allergy, but trough Asparaginase activity levels below lower level of quantification (preferably measured in two successive independent samples). In case of biweekly PEG-Asparaginase, a day 7 Asparaginase activity level <100 IU/L and/or a day 14 level <LLQ.
Hyperlipidemia	Triglycerides/cholesterol above ULN. Grading: 1.Mild: triglycerides/cholesterol <10 times ULN, 2.Moderate: triglycerides/cholesterol 10-20 times ULN, 3. Severe: triglycerides/cholesterol >20 times ULN. Note: Measurements only as part of research protocols. Dose modification based only on laboratory findings is not recommended.
Osteonecrosis	Osteonecrosis results from the temporary or permanent loss of the blood supply to the bones, which can cause pain, limitation in activity of daily living, and may result in collapse of an articulating surface with enhanced pain and development of arthritis. It should be confirmed by magnetic resonance imaging. Grading: 1. Asymptomatic with findings only by MRI, 2. Symptomatic, not limiting or only slightly limiting self-care ADL. Lesions only outside joint lines in non-weight-bearing bones, 3. Symptomatic, not limiting or only slightly limiting self-care ADL. Lesions in weight-bearing bones or affecting joint lines in non-weight-bearing bones, 4. Symptomatic with deformation by imaging of one or more joints and/or significantly limiting self-care ADL.
Asparaginase-associated pancreatitis	At least two of three features must be fulfilled: i) abdominal pain strongly suggestive of pancreatitis, ii) serum lipase or amylase ≥3 times UNL, iii) characteristic imaging findings of pancreatitis (US/CT/MRI). Re-exposure should only be considered in mild cases. Grading: 1.mild: symptoms and enzyme elevations >3 times ULN that last <72 hours, 2.severe: symptoms and enzyme elevations above >3 times ULN that last ≥72 hours or haemorrhagic pancreatitis or pancreatic abscess or cyst, 3.death from pancreatitis.
Sinusoidal obstruction syndrome	Fulfilment of at least three out of five criteria: i) hepatomegaly, ii) hyperbilirubinaemia >ULN), iii) ascites, iv) weight gain of at least 5%, and v) thrombocytopenia (transfusion-resistant and/or otherwise unexplained by treatment, e.g. myelosuppression). Doppler ultrasound may document changes in hepatic portal

	venous flow and rule out alternative causes, but normal findings do not exclude sinusoidal obstruction syndrome. Grading: 1.mild: bilirubin <103 µM and weight gain <5%; 2.moderate: bilirubin 103-342 µM and/or weight gain ≥5% or ascites; 3.severe: bilirubin ≥342 µM and/or respiratory or renal failure or hepatic encephalopathy; 4. death due to SOS.
Thrombo-embolism	Venous and/or arterial TE. Confirmation by imaging or by autopsy is required for grade 2 and higher. Grading: 1. superficial thrombophlebitis or central venous line VL-associated deep venous thrombosis with neither symptom (e.g. pain, shortness of breath) nor objective signs (e.g. swelling, discoloration, collaterals); or causing only CVL dysfunction. Systemic anticoagulation not given, 2A. asymptomatic TE (including asymptomatic cerebral thrombosis). Systemic anticoagulation is usually given (not evidence-based), 2B. symptomatic DVT, systemic anticoagulation indicated, 3. symptomatic pulmonary embolism or cardiac mural thrombus without cardiovascular compromise or symptomatic cerebral sinovenous thrombosis or arterial ischaemic stroke; all grade 3 require systemic anticoagulation/antiaggregation. 4. life-threatening TE, including arterial insufficiency, haemodynamic or neurologic instability. Urgent intervention needed, 5. death due to TE.

10.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

This means a serious adverse reaction, the nature of which is not consistent with the product information.

- 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 and asparaginase are not to be registered as SUSARs. SUSARs will be reported within 48 hours to the principal investigator and sponsor.

10.4 Serious Adverse Event Reporting

In addition to be recorded in patient's CRF, Serious Adverse Events and SUSARs will be recorded in a separate SAE form, and declared to the sponsor, whether attributed to the protocol or not. The

investigator is responsible for reporting the SAE's in accordance with the European Directive 2001/20/EC.

Therefore, a SAE report must be completed and faxed without delay and in any case within **24-hours of obtaining knowledge about the event to**:

Birgitte Klug Albertsen, MD
Children and Adolescent medicine
Aarhus University Hospital
Palle Juul-Jensens Boulevard 99
8200 Aarhus N, Denmark
Email: biralber@rm.dk (email is preferable)
Fax: +45 7845 1710

If necessary, Investigator will be asked for additional information. SAE Follow-up report will be then completed and faxed to the sponsor in the same way as the initial report.

Any documentation regarding SAE, initial and follow-up report, should be archived together with patient's source documents.

The sponsor is responsible for immediately reporting all Unexpected Serious Adverse Reactions (treatment related events) to Competent Authorities and Erytech Pharma (and Ethical Committee(s) as needed), in accordance with the European Directive 2001/20/EC and mutual agreement between Erytech Pharma and the sponsor and the local requirements. Investigators will be informed as well.

The sponsor must ensure that all relevant information about suspected unexpected serious adverse reactions, which are fatal or life-threatening, is recorded and reported to the national competent authorities as soon as possible and no later than 7 days after the sponsor is informed of such a suspected adverse reaction. No later than 8 days after the reporting, the sponsor must inform the national competent authorities of relevant follow-up information on the sponsor's and the investigator's follow-up action to the reporting.

Any other suspected unexpected serious adverse reactions must be reported to the authorities no later than 15 days from the time when the sponsor is informed about them.

Furthermore, it is the sponsor's responsibility to prepare an annual safety reporting to national competent authorities and Ethics Committee.

Sponsor will keep detailed records of all adverse events which are reported to her by the investigator or investigators. These records shall be submitted to the Member States in whose territory the clinical trial is being conducted, if they request according to local regulations.

10.4.1 SAE follow-up

The Investigator should ensure that adequate medical care is provided to the subject for any adverse event.

All SAEs must be followed until the subject has recovered, stabilized, recovered with sequelae or died. The Investigator must forward follow-up information on the SAE to the sponsor without delay and in any case within 48 hours of obtaining the new information. In accordance with the standard operating procedures and policies of the local Independent Ethics Committee (IEC), the site investigator will report SAEs to the IEC.

Reporting of Adverse Events to the IEC

Any observed or volunteered adverse event that is determined to be 1) associated with the investigational drug or study treatment(s); 2) serious; and 3) unexpected will be reported to all local IEC involved in this clinical study if applicable according the local law.

The AE and Follow-up will be reported in accordance with the standard operating procedures and policies of the site investigators' local IEC.

Investigators

Details of all SUSARs and any other safety issue which arise during the course of the trial will be reported to the Principal Investigator and Sponsor. A copy of any such correspondence should be filed in the ISF.

10.5 Medical Monitoring

Dr. Birgitte Klug Albertsen should be contacted directly at this number to report medical concerns or questions regarding safety.

Phone: +45 20224643

10.6 Data Safety Monitoring

Results of the study will be discussed continuously at the NOPHO meetings and at a minimum twice per year. An interim analysis will be planned on safety and pharmacological assessment after 5 patients have been enrolled.

11.0 DISCONTINUATION

11.1 Early Discontinuation of Study Drug

A patient may be discontinued from study treatment at any time if the patient, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for study treatment discontinuation:

Lack of asparaginase activity (in scheduled blood samples after a dose of eryaspase)

Disease progression

Subject withdrawal of consent (or assent)

Subject is not compliant with study procedures as judged by the investigator and/or sponsor

Adverse event that in the opinion of the investigator would be in the best interest of the subject to discontinue study treatment

Lost to follow-up

Sponsor and/or Erytech request for early termination of study

Patients who stop treatments from the study for toxicity or lack of activity will continue to be monitored for 1 month after last administration of eryaspase for safety follow up.

In addition, the patient will be requested to continue assessments until 1-year maintenance follow up.

In case of early withdrawal, the reason and the date of withdrawal must be documented in the patient's medical chart as well as in the study CRF.

11.2 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents. Patients who discontinue study treatment early should have an early discontinuation visit. Refer to Section 9.3 for early termination procedures.

11.3 Replacement of Subjects

Subjects who withdraw from the study treatment will not be replaced.

12.0 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

12.1 Data Sets Analysed

12.1.1 Safety Population

The safety population will consist of all patients enrolled in the study (including patients who do not meet the required inclusion/exclusion criteria or patients who deviate from the protocol procedures) who receive at least one dose of frontline eryaspase in combination with standard multi-agent NOPHO chemotherapy. Safety data will be analyzed using actual dose initially received.

12.2 Demographic and Baseline Characteristics

Demography and baseline data will be summarized using descriptive statistics.

12.3 Analysis of Study Endpoints

All endpoints will be analyzed with usual descriptive statistics: number of observations, missing data, mean and its 95% CI (Confidence Interval), standard deviation, median, 25% and 75% quartiles, minimum and maximum for continuous variables; number of observations, and percentage for categorical and ordinal variables. Missing data will not be replaced. Two-sided 95% CI (Wald's method) will also be provided for percentages of interest.

Adverse event rates will be coded by body system and MedDra classification term. Adverse events will include the number of patients for whom the event occurred, the rate of occurrence, and the severity and relationship to study drug.

12.4 Analysis of Pharmacokinetics Endpoints

Pharmacokinetics profile of eryaspase will be analyzed for each patient, and for all patients.

The scheme administration being repeated administration, analysis of PK will depend on the concentration of the product at the time of the second and third injection. The dose and frequency of administration will determine the steady state concentration and oscillations between Cmin and Cmax.

Non-compartmental methods will be used to determine pharmacokinetic parameters without deciding on a particular compartmental model. T. The first moment is calculated as concentration times x time (Cp • t). The AUMC is the area under the concentration times time versus time curve.

Both the AUC and AUMC will be calculated using the trapezoidal rule.

The following parameters will be calculated:

- The Area Under the Curve (AUC) represents the amount of study treatment available over time. AUC will be approximated by a sum of series of trapezoids at each time point (trapezoidal method).
- The Area Under the first moment Curve (AUMC) represents the amount of study treatment available over time. AUC will be approximated by a sum of series of trapezoids at each time point (trapezoidal method).
- The constant C0, being the concentration at time t = 0, considered in the further analyses as the time of the injection.
- MRT (Mean Residence Time) will be defined as AUMC (infty)/AUC(infty). This corresponds to the average time that the drug stays in the plasma.
- The apparent Elimination Rate will be defined as 1/MRT
- The total clearance will be defined as CL=Dose/AUC
- The Volume of Distribution at Steady State (Vss) will be defined as CLxMRT

- The half-life of elimination, defined as the time interval necessary (T1/2) to move from a concentration Cmax at a concentration Cmax / 2 during the elimination phase. This time T1/2 can be read directly from the plasma concentration curve.
- The concentration at steady state Css: it has been proved that Css is reached after 5 half-lives.

Evaluation of steady-state

Individual concentrations measured at each time point will be presented graphically by patient and dose level. Corresponding mean values will be also graphically displayed.

With regard to the limited number of subjects, steady-state may be evaluated by visual inspection of the different figures.

12.5 Interim Analysis

An informal interim analysis will be performed following the enrolment of the first 5 patients in the study. The purpose of this interim look is review the safety and tolerability of GRASPA in combination with chemotherapy and assess the preliminary results for PK and PD. Data from interim analysis may be used confidentially by Erytech Pharma to support their ongoing effort for Marketing Authorization Application of GRASPA in relapsed and refractory ALL setting. There will be no planned sample size re-estimation nor study discontinuation.

12.6 Sample Size and Randomization

The sample size of this study is based on the estimated potential number of patients needed to establish the feasibility; 50 patients (45 children, 5 adults) could be enrolled into the study.

12.7 Protocol Violations

A protocol violation occurs when the subject, investigator, or Sponsor fails to adhere to the protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication

Failure to comply with Good Clinical Practice (ICH GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation will result in withdrawal of a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files.

13.0 DATA COLLECTION, RETENTION AND MONITORING

A full list of all included trial subjects will be kept at the NOPHO Leukemia Registry, Stockholm including full name and personal identification number (CPR-number). All information is confidential. Case Report Forms (CRFs) will not be available for third parties, except monitor, auditor, or inspectors from authorities. Case Report Forms will be maintained for every trial subject. Any data to be recorded directly on the CRFs (i.e. no prior written or electronic record of data), are considered to be source data and will be pre-defined. Corrections in CRFs can be made only by investigators, co-investigators, doctors and specialized nurses who have the adequate training and only by drawing lines through erroneous data (to maintain readability) and hereafter noting the correct data with date and initials. A list of initials for sponsor, chair of the trial, and the investigators will be kept. Unless otherwise approved, blood and CSF samples will be destroyed when the last patient that entered the study has been followed 20 years from the time of diagnosis of leukemia. Information on other medication can be obtained from an Electronic Patient Module, if available. Data linked to the blood samples taken will by uninformative patient numbers that are linked to the NOPHO leukemia Registry in Stockholm.

Before initiation of the trial, permission to keep the database will be obtained from the National Data Protection Agencies.

13.1 Data Collection

The CRFs must be completed within reasonable period of time. After monitoring is complete and all queries are resolved the Principal investigator, or designee, must sign each CRF book.

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Templates of trial forms may be amended by the National co-ordinating centre, NOPHO, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

13.2 Data Management Procedures

The data will be entered into a validated database. The Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting ICH GCP guidelines for the handling and analysis of data for clinical trials.

13.3 Data Quality Control and Reporting

After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Query reports (Data

Clarification Requests) pertaining to data omissions and discrepancies will be forwarded to the Investigators and study monitors for resolution. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

13.4 Archival of Data

The database is safeguarded against unauthorized access by established security procedures; appropriate backup copies of the database and related software files will be maintained. Databases are backed up by the database administrator in conjunction with any updates or changes to the database.

At critical junctures of the protocol (e.g., production of interim reports and final reports), data for analysis is locked and cleaned per established procedures.

It is the responsibility of the Principal Investigator at each site to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 20 years after the end of the trial.

Study documents should not be destroyed without prior approval from Birgitte Klug Albertsen or NOPHO.

13.5 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IEC, and Regulatory Agency, inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (patient files, signed informed consent forms, copies of CRFs, Study File Notebook, etc.) must be kept secured for a period of 20 years following marketing of the investigational product. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

13.6 Monitoring

Monitoring visits will be conducted by representatives of the Sponsor according to the ICH Guidelines for GCP (E6). By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

13.6.1 Site Set-up and Initiation

All sites will be required to sign a *"Clinical Study Site Agreement"* prior to participation. In addition, all participating Investigators will be asked to sign the necessary agreements and supply a current CV to the Sponsor.

All members of the site research team will also be required to sign a site signature and delegation log which should be returned to the Sponsor

Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and reporting of data and record keeping.

Sites will be provided with an Investigator Site File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The Sponsor must be informed immediately of any change in the site research team.

13.6.2 On-site Monitoring

When a monitoring visit is required the Sponsor or its designee will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the trial staff access to source documents as requested.

During the course of the trial, a dedicated CRA will visit investigational sites to monitor study conduct as it progresses. The purpose of these visits is to ensure:

- Patients enrolled were duly informed and gave their consent,
- Adherence to protocol and ICH GCP guidelines are followed,
- CRF completion is accurate (consistency of data recorded versus source document),
- AE/SAE are notified accordingly if any,
- Drug accountability is well documented, and documentation regarding investigational product is present and complete,
- Any problems detected in the course of the monitoring visits are resolved.

Therefore, the Monitor must be given direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyze, verify and reproduce any record(s) and report(s) that are important for clinical trial evaluation.

Investigator must be available for discussions and clarifications as well.

The following data can be recorded directly on the CRF and will be considered as source data: Vital signs.

Computerized data controls will be performed as well and may raise queries where there are inconsistencies. Investigator will be then requested to answer queries in due time.

13.6.3 Central Monitoring

Trial staff will be in regular contact with the site research team to check on progress and address any queries that they may have. Trial staff will check data received for compliance with the protocol, data consistency, missing data and timing. Sites will be sent requests for missing data or clarification of inconsistencies or discrepancies.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or ICH GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to the Sponsor and the relevant regulatory bodies. This includes reporting serious breaches of ICH GCP and/or the trial protocol to the IEC(s) and competent authorities.

13.7 Subject Confidentiality and Data Protection

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the Competent Authorities. The Investigator must also comply with all applicable privacy regulations (e.g., EU Data Protection Directive 95/46/EC). Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the National Data Protection Laws.

Patients will be identified using only their unique *<study ID number>*, on the Case Report Form and correspondence between the Trials Office and the participating site.

The Investigator must maintain documents not for submission to the Sponsor (e.g. Patient Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that patient confidentiality is protected.

The Sponsor will maintain the confidentiality of all patient's data and will not disclose information by which patients may be identified to any third party Representatives of the NOR GRASPALL 2016 trial team may be required to have access to patient's notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

13.8 Audit and Inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents.

Sites are also requested to notify the Sponsor of any CA inspections.

13.9 Notification of Serious Breaches

Serious breaches will prompt lead to action of the sponsor to secure the compliance. The sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of:

- The conditions and principles of ICH GCP in connection with that trial or;
- The protocol relating to that trial.
- A "serious breach" is a breach, which is likely to effect to a significant degree:

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

Sites are therefore requested to notify the National co-ordinating centre, Sponsor of a suspected trial-related serious breach of ICH GCP and/or the trial protocol. Where the National co-ordinating centre, Sponsor is investigating whether or not a serious breach has occurred sites are also requested to cooperate with the National co-ordinating centre, Sponsor in providing sufficient information to report the breach to the CA where required and in undertaking any corrective and/or preventive action.

13.10 Finance

This is a clinician-initiated and clinician-led trial funded by Erytech Pharma and Orphan Europe. Pr. Birgitte Klug Albertsen, MD is the sponsor on behalf of NOPHO. Erytech Pharma and Orphan Europe will fund the trial with 1.840.000 Euro.

No individual per patient payment will be made to healthcare providers, Investigators or patients.

14.0 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996.

The trial will be conducted in accordance with the relevant legislation in the nation state and the International Conference on Harmonisation Guidelines for Good Clinical Practice (ICH GCP).

Before any patients are enrolled into the trial, the Principal Investigator at each site is required to obtain all applicable regulatory approval(s). Sites will not be permitted to enroll patients until written confirmation of such approval(s) have been received by the National Co-ordinating Centre/Sponsor.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

14.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IEC approval and Competent Authority approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IECs are notified within five working days.

14.2 Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IEC of each participating center prior to study initiation. Serious adverse events regardless of causality will be reported to the IEC in

accordance with the standard operating procedures and policies of the IEC, and the Investigator will keep the IEC informed as to the progress of the study. The Investigator will obtain assurance of IEC compliance with regulations.

Any documents that the IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IEC. The IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IECs unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

The IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse events occurring during the study in accordance with the standard operating procedures and policies of the IEC; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

14.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, EU Data Protection Directive 95/46/EC and local regulations.

The Investigator will send an IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

It is the responsibility of the Investigator or individuals delegated this responsibility on the delegation of authority log by the Investigator, to obtain written informed consent from the patient or an approved guardian prior to performing any trial related procedure.

Parent/guardian and age-specific Patient Information Sheets are provided to facilitate this process, these documents are prepared in accordance with the national guidelines. Investigators must ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator will also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time. The parent/approved guardian and /or patient should be given ample time (e.g. 24 hours) to read the Information Sheet and to discuss their participation with others outside of the site research team. They must be given an opportunity to ask

questions which should be answered to their satisfaction. The right of the parent/approved guardian and/or patient to refuse to participate in the trial without giving a reason must be respected.

If the parent/approved guardian and/or patient expresses an interest in the patient participating in the trial they will be asked to sign and date the latest version of the Informed Consent Form. The Investigator or designate must then sign and date the form. A copy of the Informed Consent Form should be given to the parent/approved guardian and/or patient, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into the trial the patient's trial number should be entered on the Informed Consent Form maintained in the ISF.

It should be recorded in the patient's medicinal records that the patient and/or the parents are informed about the study and have signed the ICF.

Throughout the trial the parent/approved guardian and/or patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion, it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log. The investigator should obtain the consent of the patient and notify/inform their General/Medical Practitioner that they are taking part in the trial. A Letter to their General/Medical Practitioner should be provided for this purpose.

14.4 Insurance and Indemnity

Patients will be covered by the hospital's insurance in accordance with requirements of the law in the different countries.

14.5 Publication Policy

Results of this trial will be submitted for publication in a peer reviewed journal. The results will be published regardless of being positive, negative or inconclusive. The manuscript will be prepared by the Trial Management Group (PhD student Line Stensig Lynggaard and Sponsor/PI Birgitte Klug Albertsen on behalf of NOPHO), Orphan Europe and Erytech Pharma. Authorship will be determined by mutual agreement.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of Erytech Pharma and Orphan Europe Intellectual property rights will be addressed in the "Clinical Study Site Agreement" between Sponsor and site.

There will not be published information that can identify the individual patients.

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