The effect of oral folinic acid rescue therapy on pemetrexed induced neutropenia: A randomized open-label trial.

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CLINICAL TRIAL TITLE

FLEX trial. The effect of oral folinic acid rescue therapy on pemetrexed-induced neutropenia: A randomized open-label trial.

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CONFIDENTIALITY STATEMENT

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigative team, regulatory authorities, and members of the Research Ethics Committee.

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ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
ATMP	Advanced Therapy Medicinal Product
AxMP	Auxiliary Medicinal Product
CA	Competent Authority
CCMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee on Research Involving Human Subjects)
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CT	Clinical Trial
CTA	Clinical Trial Authorisation
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
e-CRF	Electronic Case Report Form
EU	European Union
EMA	European Medicines Agency
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
IB	Investigator's Brochure
IC	Informed Consent
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
MS	Member State
PI	Principal Investigator
RSI	Reference Safety Information
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction

1. SYNOPSIS

FLEX trial. The effect of oral folinic acid rescue therapy on pemetrexed-induced neutropenia: a randomized open-label trial.

1.1 Rationale

Pemetrexed is a cytostatic antifolate drug and a cornerstone in the treatment of advanced non-small cell lung cancer (NSCLC), mesothelioma, and thymic epithelial tumors. Pemetrexed affects the folate cycle by inhibiting different enzymes which results in disrupted DNA synthesis and apoptosis of malignant cells.

Although pemetrexed is generally well tolerated, a substantial part of the patient population receiving pemetrexed experiences dose-limiting or even treatment-limiting toxicities. These include mucositis, skin problems, fatigue, renal toxicity, and neutropenia. Reported incidences for grade III/IV neutropenia are up to 26%, making this the main dose-limiting toxicity and the main focus of this trial.

A strategy to prevent toxicity is by treating patients with folinic acid rescue therapy. Folinic acid is readily incorporated in the folate cycle in healthy cells and prevent toxic effects of pemetrexed. The European label of pemetrexed also suggests the use of folinic acid as a treatment option in case of severe haematological toxicity. Since folinic acid can be used to treat toxicity it is also rational that folinic acid can be used to prevent toxicity. Different in-vitro and preclinical data already show the beneficial effects of folinic acid on pemetrexed-induced toxicity.

Pemetrexed is renally excreted and its predominant toxic effect, neutropenia, could be best predicted by the time of pemetrexed plasma concentrations above a certain toxicity threshold. For a typical patient (creatinine clearance (CrCl) 90 ml/min, dosed 500mg/m^2 pemetrexed, according to label) the median time above threshold is 27 hours. This is considered a safe time above threshold, since patients with this threshold are effectively and safely treated with pemetrexed. From the same analysis, for patients with an CrCl of 45ml/min the time above threshold is 46 hours (95%-CI 24-84 hours) after pemetrexed infusion. This longer time above threshold is associated with increased toxicity and the probability to develop of grade III/IV neutropenia is approximately 46% (compared to 19% for patients with an CrCl of 90 mL/min).

The strategy to reduce toxicity with folinic acid is already used for methotrexate. Folinic acid is dosed 30-45mg orally every 6 hours, starting 24-36 hours after the administration of methotrexate until the plasma concentration of methotrexate is <0.1 µM/L. Since methotrexate is a more potent inhibitor of cell proliferation than pemetrexed the same dose can arguably be used to prevent pemetrexed induced toxicity. Based on the knowledge on methotrexate we suggest that rescue therapy for pemetrexed can be safely started after 24 hours (as a proxy for 27 hours as a safe time above threshold) without affecting the efficacy. It seems rational to continue suppletion up to at least 84 hours after the administration, as this was the upper limit of the 95% confidence interval for the time above the threshold concentration in patients with an CrCl of 45 mL/min and pemetrexed is contra-indicated in patients with an CrCl < 45 mL/min.

1.2 Objective & endpoints

The main objective is to evaluate the haematological toxicity in patients who use pemetrexed with and without rescue therapy with folinic acid.

1.2.1 Primary endpoint

Difference between treatment groups in neutrophil count (*10^9/L) at day 8-10 after administration of pemetrexed (nadir).

1.2.2 Secondary endpoints

The grade neutropenia (according to the CTCAE version 5, 2017) at day 8-10, the homocysteine plasma levels at baseline (predictor for developing toxicity), the efficacy of chemotherapy treatment based on response CT after cycle 2 and 4 and the incidence of discontinuation, dose delays and dose reductions of pemetrexed.

1.3 Trial design

The FLEX-trial is a multi-centre, open label, double arm, randomized trial to compare neutropenia in patients with and without folinic acid rescue therapy where subjects are participating for 4 treatment cycles.

1.3.1 Population

In total 50 patients (25 in each arm), >18 years with stage IV non-small cell lung cancer (NSCLC) or mesothelioma treated with pemetrexed (in combination with other chemo- or immunotherapy) are eligible for inclusion.

1.3.2 Interventions

Follow-up will take place during the first 4 cycles of chemotherapy with pemetrexed. Patients in the intervention-arm will receive oral folinic acid orally 4 times 45mg / day for 3 days, starting 24 hours after the administration of pemetrexed.

1.4 Ethical considerations

Folinic acid already is an approved drug for the reduction of haematological toxicity, It generally is well tolerated and has a high and extensively described safety profile. Folinic acid is shown to selectively rescue host cells, with tumor cells being unaffected. This is also supported by the fact that folinic acid is already registered and used after administration of high dose methotrexate, which is a more potent inhibitor of cell proliferation than pemetrexed. Since timely dosing of folinic acid does not affect the efficacy of methotrexate it can be expected that folinic acid will not influence the efficacy of pemetrexed either. Since folinic acid itself already is approved and widely applied drug for prevention of neutropenia with a well-established safely profile, and since the pemetrexed drug label already we consider this a clinical trial with minimal risk.

We consider the extra burden of participating in the described study limited. In the intervention group the additional burden compared to routine care consists of taking 3 days of folinic acid after each cycle of pemetrexed and sampling extra blood twice, 9 days after both the first and second administration of pemetrexed for a neutrophil count. In the control group the additional burden only consists of sampling extra blood twice, 9 days after the first and second administration of pemetrexed. Patients may benefit from participating in this study, as they will be treated with a potentially safe and effective drug that prevents toxic exposure.

2. INTRODUCTION AND RATIONALE

2.1 Pemetrexed and the folate cycle

Pemetrexed is a cytostatic antifolate drug and a cornerstone in the treatment of advanced non-small cell lung cancer (NSCLC), mesothelioma, and thymic epithelial tumors. By interruption of the folate cycle, DNA synthesis is disrupted and apoptosis of malignant cells is induced. Pemetrexed is used in different stages of NSCLC, either alone or in combination with platinum agents and/or immunotherapy, or radiotherapy. In all modalities pemetrexed is dosed based on body surface area (BSA) at 500mg/m^2. According to the label, pemetrexed is contra-indicated in patients with a creatinine clearance < 45 ml/min. [1-4]

Pemetrexed affects the folate cycle by inhibiting different enzymes. The primary inhibited enzyme is thymidylate synthase. Other enzymes that are also inhibited to lesser extend are dihydrofolate reductase, glycinamide ribonucleotide formyltransferase (GARFT), aminoimidazole carboxamide ribonucleotide formyltransferase (AICARFT), and C1 synthase. This is also shown in figure 1 below. [7]

After administration pemetrexed is rapidly and extensively polyglutamated by folylpolyglutamate synthase, which causes drug retention within the cell and ensures a durable effect [25]. Since polyglutamation mainly takes place in malignant cells and in a lesser extent in healthy cells, pemetrexed specifically targets malignant cells. Polyglutamated pemetrexed mainly targets thymidylate synthase which makes this enzyme the primary target. However, pemetrexed monoglutamate also inhibits dihydrofolate reductase which is also present, and can cause damage in healthy cells. [16, 25]

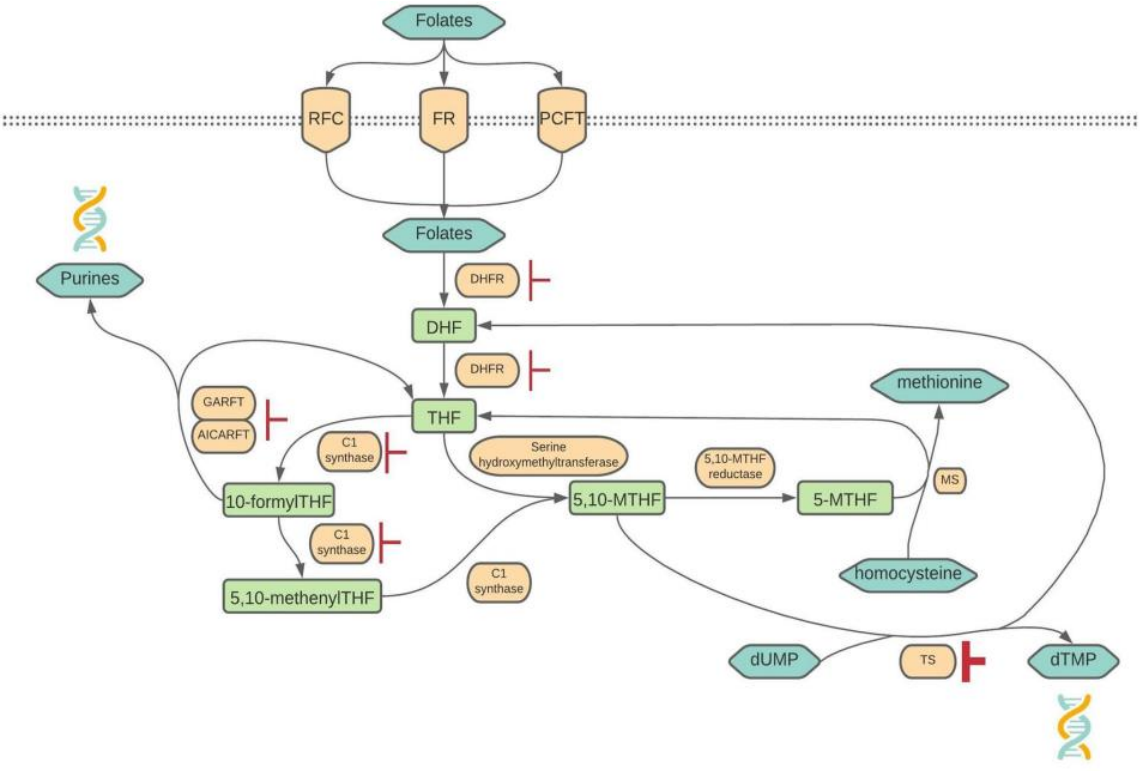

Figure 1 [7]: Folate cycle and target enzymes of pemetrexed. Enzymes inhibited due to pemetrexed are marked with red. The primary site of action of pemetrexed is thymidylate synthase (TS). Secondary targets include dihydrofolate reductase (DHFR), glycinamide ribonucleotide formyltransferase (GARFT), aminoimidazole carboxamide ribonucleotide formyltransferase (AICARFT), and C1 synthase. DHF dihydrofolate, dTMP deoxythymidine monophosphate, dUMP deoxyuridine monophosphate, FR folate receptor, MS methionine synthase, MTHF methylenetetrahydrofolate, PCFT protein coupled folate transporter, RFC reduced folate carrier, THF tetrahydrafolate.

2.2 Pemetrexed-related toxicity

Although pemetrexed is generally well tolerated, a substantial part of the patient population receiving pemetrexed experiences dose-limiting or even treatment-limiting toxicities. These include mucositis, skin problems, fatigue, nephrotoxicity, and neutropenia. Reported incidences for grade III/IV neutropenia are up to 26%, making this the main dose-limiting toxicity. [5] In one study nephrotoxicity led to discontinuation of the treatment in 8.1% of the patients. [6] Pemetrexed can lead to a decline in renal function, which in turn can lead to dose limitations or discontinuation of treatment, since pemetrexed is contraindicated in patients with impaired renal function. Adverse effects caused by pemetrexed (monotherapy or combined with other therapies) can influence the quality of life and, lead to dose-limitation, delay or cessation of treatment, hospitalization and even death. [5-7]

Pemetrexed-induced neutropenia is directly associated with the antifolate effect of pemetrexed. Since pemetrexed disturbs DNA synthesis and apoptosis in all dividing cells (malignant but to some extent also in healthy cells) toxicity is mainly seen in rapidly dividing cells like stem cells and mucosal cells.

Pemetrexed is almost completely excreted as unchanged drug in urine. [7] Although the exact mechanism of pemetrexed induced renal toxicity is not fully understood, different folate carriers and receptors are able to transport pemetrexed into kidney cells which in turn can cause kidney injury. [8]

2.3 Strategies to prevent toxicity

In the systematic review of de Rouw et al. [7] different strategies are described to prevent pemetrexed-induced toxicity, of which some are already part of standard care. Since a poor folate status and vitamin B12 (cobalamin) deficiency are associated with increased toxicity, patients receive a low dose of folic acid (350 to 1000 µg/day) and vitamin B12 as standard of care. Moreover, in preclinical studies, it was suggested that optimization of folate could possibly augment therapeutic activity of folate antagonists. [7, 9-10]

Sufficient folate is necessary to continue the folate cycle in healthy cells after the administration of pemetrexed. Folic acid is a synthetic form of folate and has the same function as folate. Since folic acid can only enter the folate cycle after reduction by dihydrofolate reductase the inhibiting effect of pemetrexed on this enzyme has to be worn out. [7]

Vitamin B12 is supplemented since this is an import co-factor in the folate pathway. To estimate the folate and vitamin B12 status, the total plasma homocysteine concentrations can be measured. A high plasma concentration of homocysteine is an indication for potential folate of vitamin B12 deficiencies and a higher risk of pemetrexed-induced toxicity. [7,11]

2.4 Folinic acid to reduce toxicity

A strategy to prevent toxicity is by treating patients with folinic acid rescue therapy. Folinic acid is the reduced form of folic acid. Since folinic acid is further downstream in the folate cycle, other than folic acid, folinic acid does not depend on dihydrofolate reductase and can directly be used in the folate cycle in healthy cells. Administration of folinic acid after pemetrexed can be an effective strategy to prevent toxicity. [7] The European label of pemetrexed also suggests the use of folinic acid as a treatment option in case of severe toxicity. [7,22-24] This proves the concept of administering folinic acid to reduce pemetrexed induced toxicity. High doses can be used to treat toxicity whereas a lower dose of folinic acid might be used to prevent toxicity.

The effect of folinic acid on the folate cycle after the use of pemetrexed was extensively studied in vitro. In one cell line (leukaemia CCRF-CEM), it was shown that the addition of folinic acid completely reversed pemetrexed-induced growth inhibition [7,18,19]. A preclinical study on the effect of folinic acid on raltitrexed-induced toxicity also showed a beneficial effect of folinic acid. Raltitrexed is, like pemetrexed also a thymidylate synthase inhibitor. [7,20] In another preclinical study of pemetrexed toxicity in dogs, the beneficial effects of folinic acid were confirmed. Administration of folinic acid between days 4 and 10 after pemetrexed administration reversed clinical signs of toxicity as well as myelosuppression [7,21]. In phase I study protocols of pemetrexed intravenous administration of folinic acid was also included as a possibility to treat grade IV haematological toxicity (loading dose 100 mg/m2 followed by 50 mg/m2 every 6 hours for 8 days).

Pemetrexed and specifically the pemetrexed monoglutamate can inhibit the folate cycle in healthy cells through dihydrofolate reductase. Since folinic acid has already been approved as rescue therapy after methotrexate is seems rational to also examine the prophylactic effect of folinic acid rescue after the use of pemetrexed in humans. Different in-vitro and preclinical data already show the beneficial effects of folinic acid on pemetrexed-induced toxicity.

2.5 The pharmacological rationale for folinic acid dosing

Based on a recent thorough analysis of the relationship between pemetrexed pharmacokinetics and myelotoxicity, it was shown that development of neutropenia could be best predicted by the time of pemetrexed plasma concentrations above a certain toxicity threshold. This threshold was shown to be 0.110 mg/ml. For a typical patient (CrCl 90 ml/min, dosed 500mg/m^2 pemetrexed, according to label) the median time above threshold is 27 hours. [26] This is considered a safe time above the threshold, since patients with this threshold are effectively and safely treated with pemetrexed. From the same analysis, for patients with an CrCl of 45ml/min the time above threshold is 46 hours (95%-CI 24-84 hours) after pemetrexed infusion. This longer time above threshold is associated with increased toxicity and the probability to develop of grade III/IV neutropenia is approximately 46% (compared to 19% for patients with an CrCl of 90 mL/min). [26]

It was shown that with decreasing renal function, the risk for grade III or higher haematological toxicity dramatically increased to 93% in patients with an CrCl of 5 ml/min. Since pemetrexed currently is contra-indicated for patients with an CrCl of 45ml/min or lower these patients are not included in this trial.

In case of methotrexate, folinic acid is dosed 30-45mg orally every 6 hours starting 24-36 hours after the administration of methotrexate until the plasma concentration of methotrexate is <0.1 µM/L. Since methotrexate is a more potent inhibitor of cell proliferation than pemetrexed, and folinic acid is already relatively overdosed in this situation as there is competitive inhibition, the same dose can arguably be used to prevent pemetrexed induced toxicity. [7] Based on the knowledge on methotrexate we suggest that rescue therapy for pemetrexed can be safely started after 24 hours (as a proxy for 27 hours as a safe time above threshold) without affecting the efficacy. It seems rational to continue suppletion up to at least 84 hours after the administration, as this was the upper limit of the 95% confidence interval for the time above the threshold concentration in patients with a CrCl of 45 mL/min.

3. STRUCTURED RISK ANALYSIS

3.1 Folinic acid as the investigational medicinal product

Folinic acid (Rescuvolin®, Leucovorin®) is an approved drug for the reduction of haematological toxicity. Folinic acid is a reduced form of folic acid, also known as vitamin B11. Folinic acid has been a registered since the early 1980's. Based on studies and experience folinic acid has proven to be a safe drug to use. The mechanism of action is already elucidated and the adverse effect profile of folinic acid is well characterized. [30]

Folinic acid can be used to reduce pemetrexed induced toxicity by nullifying the effect of pemetrexed through regeneration of the folate cycle. [30] Folinic acid is shown to selectively rescue host cells after methotrexate treatment, while the anti-tumour effect remains. [12, 13] As described earlier, pemetrexed toxicity in healthy cells is mainly caused by monoglutamated pemetrexed which inhibits dihydrofolate reductase. The main mechanism of pemetrexed in malignant cells is caused by polyglutamated pemetrexed which inhibits the further downstream enzyme thymidylate synthase. [7,16] The effect of high dose folic acid, the precursor of folinic acid, has been studied in vitro. It was shown that folic acid does not affect the efficacy of pemetrexed. [31]

This is also supported by the fact that folinic acid is already licensed for use after administration of high dose methotrexate, which is a more potent inhibitor of cell proliferation than pemetrexed and gets its effectivity by inhibiting dihydrofolate reductase. [7, 14] Since timely dosing of folinic acid does not affect the efficacy of methotrexate, it can be expected that folinic acid does not influence the efficacy of pemetrexed either. [32] The first dose of folinic acid is administered 24 hours after pemetrexed. This is the same interval used after high dose methotrexate. Since pemetrexed is faster and more extensively polyglutamated then methotrexate is it even less likely to influence the efficacy. [33]

As stated before, based on a pharmacokinetic model pemetrexed induced neutropenia is mainly caused by plasma levels being above a certain threshold for a period of time. For efficacy, it is postulated that AUC or total exposure is the main driver. [7] Therefore, the exposure in the first 24 hours assures efficacy, while the time above the threshold after this period of time is the driver for neutropenia. No study directly examined the effect of folinic acid on thymidylate synthase.

All patients who are eligible for this trial are diagnosed with stage IV lung cancer and will be treated with pemetrexed whether they participate in this study or not. The addition of folinic acid to the treatment of these patients is expected to have a positive effect on pemetrexed-induced toxicity.

Based on the information above we assume that rescue therapy with folinic acid can be safely started after 24 hours without affecting the efficacy of pemetrexed. Folinic acid itself already is approved and widely applied drug for prevention of neutropenia with a well-established safely profile. However, since no study did directly assess the effect of folinic acid on the efficacy of pemetrexed we decided to classify the study as a clinical trial.

4. OBJECTIVES AND ENDPOINTS

Objective(s)	**Endpoint(s)**
Primary objective	**Endpoint for the primary objective**
To evaluate the haematological toxicity (continuous measure) in patients who use pemetrexed with and without rescue therapy with folinic acid.	Difference in neutrophil count (*10^9/L) at day 8-10.
Secondary objectives • To evaluate the difference in haematological toxicity based on the CTCAE criteria for neutrophil count • To evaluate the influence of baseline homocysteine plasma levels on occurrence of haematological toxicity. • To evaluate the efficacy of the treatment with pemetrexed. • To evaluate the incidence of treatment delay or dose reduction of pemetrexed.	**Endpoints for secondary objectives** • Grade neutropenia (according to the CTCAE version 5, 2017) at day 8-10 • Homocysteine plasma levels at baseline (µmol/L) • Efficacy based on response CT after cycle 2 and 4 (categorical: response, partial response, progression) • Incidence of discontinuation, dose delays and dose reductions.

Table 1: Summary table with primary and secondary study objectives and endpoints.

5. STUDY PLAN AND DESIGN

5.1 Trial Design

The FLEX-trial is a multi-centre, open label, double arm, randomized trial to compare toxicity in patients with and without folinic acid rescue therapy where subjects are participating for 4 treatment cycles. (see figure 1 for an overview)

5.2 Number of Patients

In total 50 patients (25 in each arm) with stage IV non-small cell lung cancer (NSCLC) or mesothelioma treated with pemetrexed (in combination with other chemo- or immunotherapy) will be included. Only patients who start with their first chemotherapy treatment are eligible for inclusion.

The sample size calculation is explained in the statistical chapter.

5.3 Randomization & blinding

All patients who start treatment that contains pemetrexed are eligible for this study. Since patients can receive different concomitant chemotherapy or immunotherapy, patients will be divided in strata based on chemotherapy protocol. Stratification will also be performed for the use of proton pump inhibitors since different studies show a relation between the use of proton pump inhibitors and haematological toxicity induced by pemetrexed. [27, 28] Within these strata patients will be randomly assigned to the treatment or non-treatment group with folinic acid in a 1:1 ratio.

5.4 Overall study duration and follow-up

All included patients will follow a study period of 4 treatment cycles.

The expected inclusion period will be one year (or shorter if 50 patients have been included). Approximately 100 – 120 patients in the Amphia hospital and 60 patients in the Albert Schweitzer Hospital start with pemetrexed every year. With 160-180 eligible patients in a year we expect this to be a realistic time to include 50 patients.

5.5 Patient participation

Patients are screened and informed by the physician. Subsequently, an informed consent procedure will start. Patients will receive understandable written and oral patient information in Dutch. Patients can only be included when they fully understand the impact and extent of this trial. Follow-up and the registration of information can only start after a signed informed consent. A trial nurse will perform the informed consent procedure. See chapter 13 Ethical Considerations for more details.

6. STUDY PARAMETERS AND PROCEDURES

All patients are will be treated with 4 cycles of pemetrexed (repeating every 3 weeks) according to the standard of care. Follow-up will take place during the first 4 cycles of chemotherapy with pemetrexed. Patients in the intervention-arm will receive oral folinic acid orally 4 times 45mg / day for 3 days, starting 24 hours after the administration of pemetrexed. Patients in the control group will not receive folinic acid. For the schematic procedure, see figure 2.

Patients normally continuously use folic acid (5mg/day) during all cycles of chemotherapy with pemetrexed. Since folinic acid is the reduced form of folic acid it is not necessary to take both these drugs at the same time. During the 3 days of folinic acid, patients temporary stop the use of folic acid. Patients in the control group continue folic acid according to the regular chemotherapy protocol.

Neutrophil count

In both the intervention and control group, routine white blood cell count (WBC) including differentiation will be assessed pre-treatment cycle in line with the drug label. During cycle 1 and 2, on day 8-10 an WBC is performed to assess the effect on nadir.

Homocysteine

At baseline before start of the first treatment cycle, a baseline homocysteine will be assessed in all included patients from a blood sample that is taken as a part of routine care.

Dose delays or reduction

Dose delays or dose reductions will be checked and registered during the study period.

Efficacy assessment

In both the intervention and control group, standard care response CTs after 2 and 4 cycles of chemotherapy will be assessed. Based on the conclusion of the radiologist (independent from the trial) response will be categorized in tumor progression, no progression but also no reduction or tumor reduction.

Safety assessment folinic acid

The most common adverse effects (>1:100 according to the SmPC) of folinic acid will be assessed.

Other parameters to be collected are:

- Age
- Weight
- Length
- Sex
- Use of concomitant proton pump inhibitors and NSAIDs
- Concomitant chemotherapy or immunotherapy
- Renal function

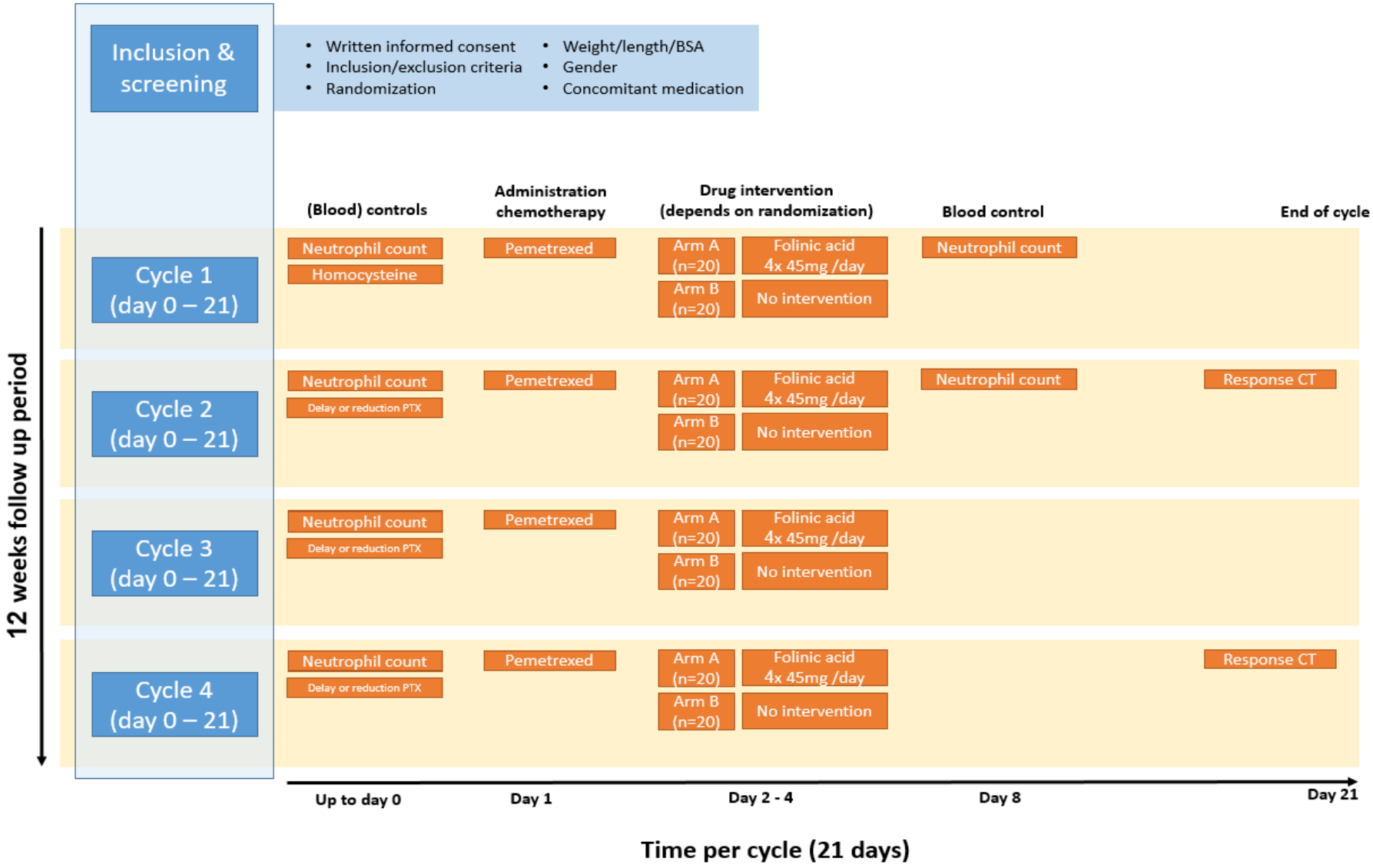

Figure 2: Schematic study procedure.

7. STUDY POPULATION

7.1 Population

All patients with stage IV non-small cell lung cancer (NSCLC) or mesothelioma who start their treatment with pemetrexed-based induction therapy are eligible for participation in the trial. In general practice, new patients will start pemetrexed combined with carboplatin or cisplatin (chemotherapy) or with pembrolizumab (immunotherapy).

The physician or physician assistant determines if a patient may be eligible to participate in this trial using the in- and exclusion criteria.

7.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. ≥18 years old
2. Eligible for treatment with pemetrexed-based chemotherapy based on indication.
3. ECOG performance score of 0-2.
4. Subject is able and willing to sign the Informed Consent Form

7.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Contraindications for treatment with folinic acid in line with the SmPC.
    a. Hypersensitivity to the active substance or to any of the excipients.
    b. Anaemia caused by vitamin B12 deficiency.
2. The presence of clinically relevant drug-drug interactions, according to the current SmPC of folinic acid.

8. STUDY TREATMENTS

8.1 Investigational Medicinal Product(s) (IMP(s))

8.1.1 Name and description of the IMP

In this study folinic acid (Rescuvolin) is prescribed to reduce pemetrexed induced toxicity. The IMP, Folinic acid, already has an EU marketing authorisation to reduce methotrexate induced toxicity and to increase the efficacy of 5-fluorouracile (5-FU). An oral formulation (tablets of 15mg) is used. More detailed information can be found in the SmPC.

8.1.2 Status of development of the IMP

See chapter 5.1 of the Summary of Product Characteristics (SmPC) for detailed information on findings from clinical and in vitro studies and chapter 5.3 for preclinical safety data.

8.1.3 Description and justification of dosage and route of administration

In this trial folinic acid will be orally administered for 3 days, starting 24 hours after the administration of pemetrexed. This will be repeated for 4 cycles (also see figure 2). All patients will receive a dose of 45mg, 4 times a day. Oral administration is preferable as it is low invasive and better for patient convenience. See rationale for a detailed description of the justification of the dosage.

8.2 Preparation and labelling of the study treatment(s)

8.2.1 Preparations

Registered folinic acid 15mg tablets will be used.

8.2.2 Labelling

In compliance with the Clinical Trial Regulation (EU) 536/2014 bullet 57, as a general rule no additional labelling is required for investigational medicinal products that have already been placed on the market as an authorized medicinal product. Folinic acid is an authorized medicinal product and will be labelled in line with the national guidance “KNMP richtlijn ter hand stellen”.

9. TRACEABLILITY, STORAGE, ACCOUNTABLILITY AND COMPLIANCE

9.1 Traceability and storage of the study treatment(s)

Folinic acid will be supplied from site stock, where it is stored according to GDP practices, as is best practice in Dutch hospital pharmacies. Accurate records of all drugs dispensed will be maintained by the pharmacist. The traceability of all dispersed folinic acid will be assured.

10. STUDY DISCONTINUATION AND COMPLETION

10.1 Definition End of Trial

The inclusion will start on the 16st of January 2022 until the 31th of December 2022. The end of the trial is reached at the end of the inclusion period or sooner, when 50 patients (25 in each arm) are included for follow-up. Each patient will be followed-up for 4 cycles. The end of the trial is defined as the last visit of the last subject.

10.2 Criteria for temporary halt and early termination of the clinical trial

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

10.3 Discontinuation/withdrawal of individual subjects

Patients are allowed to withdrawal for the study at any given time.

10.4 Arrangements for subjects after their participation in the clinical trial ended

No arrangements are made after ending of the clinical trial.

11. SAFETY REPORTING

This study is classified as a Clinical trial since the investigational product already has market authorisation. The use of the investigational product for this indication is not yet directly examined, however, the use can be supported by published scientific evidence and the additional diagnostic procedures do not pose more than minimal additional risk and burden. This is also addressed in the structured risk analysis. Safety reporting will be performed in accordance with article Chapter VII and Annex III of the EU trial regulation 16th of April 2014.

11.1 Definitions

11.1.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to folinic acid. All adverse events that occur after signing informed consent reported spontaneously by the subject or observed by the investigator or his staff will be recorded. An elective hospital admission will not be considered as a serious adverse event.

11.1.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- Is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due
- to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible to pursue and obtain adequate information both to determine the outcome and to assess whether the event meets the criteria for classification as an SAE or caused the participant to discontinue the study intervention.

The investigator will report the SAEs through the web portal of CTIS within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the investigator has first knowledge of the serious adverse events.

11.1.3 Suspected unexpected serious adverse reactions (SUSARs)

The investigator will report all SUSARs with exception of SUSARs which are directly caused by the concomitant chemotherapy. The investigator will report the SUSARs through the web portal

of CTIS within 7 days of first knowledge for SUSARs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the investigator has first knowledge of the serious adverse events.

11.2 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until end of study within the Netherlands, as defined in the protocol.

11.3 Annual safety report

In addition to the expedited reporting of SUSARs, the investigators will submit, once a year throughout the clinical trial, a safety report to the accredited METC and competent authority.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

11.4 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

11.5 Urgent safety measures and other relevant safety reporting

Where an unexpected event is likely to seriously affect the benefit-risk balance, the sponsor and the investigator will take appropriate urgent safety measures to protect the subjects. In addition the sponsor will notify the Member States concerned, through CTIS, of the event and the measures taken. That notification will be made without undue delay but no later than **7 days** from the date the measures have been taken (CTR: Article 54).

11.6 Data Safety Monitoring Board (DSMB)/Data Monitoring Committee (DMC)

For this study, no DSMB will be appointed. Using the NFU guideline on quality assurance on research involving human subjects we classified the risk of this study intermediate. [29] This is based on a small change and slight severity of damage. Therefore a Data Safety Monitoring Board is not required. In the past years extensive experience has been gained with the use of folinic acid. The adverse effect profile of folinic acid is mild, well characterized, therefore adequate safety monitoring is already a part of routine clinical care. See the structured risk analysis for a detailed explanation. We estimate the chance for occurrence of unknown effects to be negligible. The study will be monitored by a qualified study monitor, see chapter '11.2 Monitoring and quality assurance'.

12. STATISTICAL ANALYSIS

12.1 Description of statistical methods

All data will be analysed using the software program IBM SPSS Statistics 25.

12.2 Participant demographics and other baseline characteristics

Relevant baseline characteristics are age, weight, length and sex will be recorded. Also renal function, stage NSCLC will be recorded.

12.3 Randomisation

All patients who start treatment with pemetrexed are eligible for this study. Since they can use different concomitant chemotherapy of immunotherapy it is important that patients receive these concomitant treatments are equal in both groups. For this reason patients will be divided in strata based on chemo therapy protocol. Within these strata patients will be randomly assigned to the treatment or non-treatment group with folinic acid in a 1:1 ratio. The same applies to the use of proton pump inhibitors.

12.4 Sample size, trial power and level of significance used

The sample size is calculated using a two-sample t-test, based on the primary endpoint, the difference in neutrophil count between both groups at day 9 (continuous data).

The pharmacokinetic/pharmacodynamic model for pemetrexed used by Boosman et al. [26] was used to estimate the effect of folinic acid on the neutrophils. Using 500 simulated patients, the mean neutrophil count on day 9 (log-transformed) was assessed without folinic acid supplementation (full model) and with supplementation (cutting of the 'drug effect' on neutrophil count 24 hours after pemetrexed administration).
The observed difference was used as the effect size, resulting in a Cohens D of 1.2 D (mean difference divided by the standard deviation). With an alpha defined as 0.05 and the 1-beta (power) as 0.8; this yields a required sample size of 20 patients (10 in both groups).
This estimation is based on optimal conditions and equal response in all patients, while other factors such as concomitant other chemotherapy are also present. Also, we need to account for drop-out and loss to follow-up. Thus, although it is physiologically assumable that the effect will occur in all patients, if we assume that the effect will take place in 2/3 of the patients (Cohens D = 0.8), this yields a sample size of 42 patients (21 in both groups). To account for drop-out or loss to follow-up 25 patients in each group will be included (total n=50).

12.5 Planned analysis

To investigate if there is a significant difference ($p<0.05$) different tests are used for all endpoints according to table 2. The secondary endpoints will be presented as explorative results.

For the continuous primary endpoint, difference in neutrophil count, and secondary continuous endpoint homocysteine, an unpaired t-test or Mann Whitney U test will be performed depending on the distribution of the data. For categorical data like grade toxicity, effectivity, dose reduction, delay and discontinuation a chi-squared test or Fishers' exact will be performed.

Patients who drop out of the study because of toxicity will not be excluded and taken into account in the analysis and results. For these patients the last observed outcome value is included in the analysis (last observation carried forward principle). Moreover, a sensitive analysis is performed to study the robustness of the conclusion.

However, in case of missing data for the primary endpoint due to other reasons, patients will be excluded. Since this study is powered based on 25 patients in each group these excluded patients will be replaced in the study. In case of missing data for the secondary endpoints the patients will not be excluded, however will not be taken into account for the specific missing endpoint.

Primary endpoints	**Type of data**	**Statistic test**
• Neutrophil count (*10^9/L)	Continuous	Unpaired t-test or Mann Whitney U test.
Secondary endpoints	**Type of data**	**Statistic test**
• Grade neutropenia (according to the CTCAE version 5, 2017)	Categorical	Chi-squared test or Fishers' exact test
• Homocysteine plasma levels at baseline (µmol/L)	Continuous	Unpaired t-test or Mann Whitney U test.
• Efficacy based on response CT	Categorical	Chi-squared test or Fishers' exact test
• Incidence of discontinuation, dose delays and dose reductions.	Binary	Chi-squared test or Fishers' exact test

Table 2: Overview of the primary and secondary endpoints and corresponding statistical test.

13. ETHICAL CONSIDERATIONS

13.1 Declaration of Helsinki

This study will be conducted according to the principles of the Declaration of Helsinki (19-10-2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

13.2 Recruitment and informed consent procedures

Eligible patients will be identified at the multi-disciplinary thoracic oncology meeting in each participating hospital. The supervising physician will inform the patient about the study. Inclusion will be performed by the research nurse, nurse practitioner or physician and ask for the patients' consent. After receiving information, potential participants have time to decide to participate in the trial until the start of treatment with pemetrexed. Patients will be informed as soon as possible after diagnosis and treatment strategy. In general the time between diagnosis and start of treatment is approximately 1 week. Consent will be signed by the patient and the physician.

Informed consent will be obtained prior to any study related procedures will be undertaken. Informed consent will be written, dated and signed by the subject and the person performing the interview. The investigator or his/her representative will explain the nature of the study to the subject, and answer all questions regarding this study. In the interview it will be verified that the subject has understood the information. The subject will be provided with a copy of the document (or the record) by which informed consent has been given. The informed consent will be documented and filed in the site master file. (CTR: Article 29).

13.3 Benefits and risks assessment, group relatedness

We consider the extra burden of participating in the planned study, limited. In the intervention group the additional burden compared to routine care consists of taking 3 days of folinic acid after each cycle of pemetrexed and sampling extra blood twice, 9 days after the first and second administration of pemetrexed for a neutrophil count. In the control group the additional burden only consists of sampling extra blood twice, 9 days after the first and second administration of pemetrexed. Patients may benefit from participating in this study, as they will be treated with a potentially safe and effective drug that prevents toxic exposure.

13.4 Compensation for injury

The sponsor has an insurance that is in accordance with the legal requirements in the Netherlands (Article 7 WMO, under 1). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

The sponsor/investigator has a liability insurance that is in accordance with article 7, under 9, of the WMO.

14. ADMINISTRATIVE ASPECTS, MONITORING AND CONFIDENTIALITY

This trial will be conducted in compliance with the protocol, with Clinical Trials Regulation N536/2014 and with the principles of good clinical practice.

14.1 Approval initial application and substantial modifications

The trial protocol, informed consent form, subject information leaflet, investigational medicinal product dossier, investigators brochure and any other documents required by the Regulation will be submitted for the regulatory approval before the clinical trial is started via CTIS.

The sponsor will also submit and obtain approval for substantial modifications to the original approved documents via CTIS.

A 'substantial modification' is defined in the CTR as any change to any aspect of the clinical trial which is made after notification of a decision referred to in Articles 8, 14, 19, 20 or 23 and which is likely to have a substantial impact on the safety or rights of the subjects or on the reliability and robustness of the data generated in the clinical trial.

14.2 Monitoring

Monitoring will be performed in line with NFU guideline on quality assurance on research involving human subjects(64). The monitor will work independently and has no involvement in the set-up of the study, the conduct of the study and interpretation of the results. In accordance with International Conference on Harmonisation Good Clinical Practice (ICHGCP) guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs at regular intervals throughout the study, to verify adherence to the protocol, and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to any subject records needed to verify the entries on the CRFs. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

Prior to first screening an initiation meeting will be performed and after the trial a close out meeting will be performed. The monitor will work according to Monitoring Guidelines and Checklists that will be written especially for this study.

Monitoring consists of:

- Check essential documents at the site.
- Check eligibility of patients.
- Monitoring for completeness and correctness of the source documents.
- Monitoring of the data in the workbook, and transfer of data from source documents.
- Compliance to the CRF.
- Write monitoring reports.

The investigator should understand that source documents for this study should be made available to regulatory authority or health authority inspectors.

14.3 Recording, handling and storage of information

14.3.1 Handling of data and data protection

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 separate categories: (1) investigator's study file, and (2) patient clinical source documents.

The investigator's study file will contain the protocol/amendments, CRF and query forms, IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

Subject clinical source documents (usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs) would for example include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, electrocardiogram (ECG), electroencephalogram (EEG), X-ray, pathology and special assessment reports, consultant letters, screening and enrolment log, etc.

Sample handling will be done through a validated routine process. Since all white blood cell counts will be performed within routine care the blood samples will be labelled and stored according to the general procedures of the laboratory of the clinical chemistry department of Result lab which stores all monsters for 3 days. The principle investigator is responsible for the storage. After 3 days all material will be destroyed.

For each patient enrolled, a CRF must be completed and approved by the principal investigator or co-/sub-investigator within a reasonable time period after data collection. This also applies to records for those patients who fail to complete the study (even during a screening period if a CRF was initiated). If a patient withdraws from the study, the reason must be noted on the CRF. If a patient is withdrawn from the study because of a treatment limiting adverse event, thorough efforts should be made to clearly document the outcome.

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only subject identification code (i.e., not names) should be recorded on any form submitted to the sponsor and IEC. The investigator must keep a screening log showing codes, for all subjects screened and for all subjects enrolled in the study. The medical record and other source documents are only accessible by the medical staff of the clinical research centre, the investigator and monitor as well as for audits by METC members or authorized government personnel. CRFs do not contain identifiable information and will be coded with subject ID numbers only. Information and study files that are necessary for the evaluation of the research are stored coded and the identification key will not be accessible by unauthorized parties.

All data will be collected through validated software (Castor ODC). The management of all data in Castor will be done according to GCP standards. Castor is equipped with an audit trail to avoid alternation and loss of data and personal information. All employees involved in the study will be trained.

14.3.2 Clinical trial master file and data archiving

The sponsor and the investigator shall keep a clinical trial master file. The clinical trial master file shall at all times contain the essential documents relating to the clinical trial which allow verification of the conduct of a clinical trial and the quality of the data generated (CTR: Article 57).

The sponsor/investigator shall archive the content of the clinical trial master file for 25 years after the end of the clinical trial, unless other EU law requires archiving for a longer period. The medical files of subjects shall be archived in accordance with national law (CTR: Article 58). All data will be stored in the software program Castor.

The content of the clinical trial master file shall be archived in a way that ensures that it is readily available and accessible, upon request (CTR: Article 57).

14.4 Reporting of serious breaches

The sponsor will notify the Member States concerned about a serious breach of the Regulation or of the version of the protocol applicable at the time of the breach through CTIS without undue delay but not later than **seven days** of becoming aware of that breach (CTR: Article 52).

In case of a security breach this will be reported to the authorities within 7 days according to the CTR. Also the general protocol of the Amphia hospital for a security breach of information will be followed. The breach will be reported to the authorities in the Netherlands within 72 hours according to the Algemene Verordering Persoonsgegevens (AVG). To avoid further breaches or new breaches in the future a root-cause analysis and, if necessary, correcting and preventive actions will be carried out.

14.5 Notification of the start and the end of the recruitment

The sponsor will notify within 15 days each Member State concerned of the start of a clinical trial in relation to that Member State through CTIS (CTR: Article 36(1)).

The sponsor will notify within 15 days each Member State concerned of the first visit of the first subject in relation to that Member State through CTIS (CTR: Article 36(2)).

14.6 Temporary halt/(early) termination

The sponsor will notify within 15 days each Member State concerned of the end of a clinical trial in relation to that Member State through CTIS (CTR: Article 37(1)).

14.6.1 Temporary halt/early termination for reasons not affecting the benefit-risk balance

The sponsor will notify with 15 days each Member State concerned of a temporary halt of a clinical trial in all Member States concerned for reasons not affecting the benefit-risk balance through CTIS (CTR: Article 37(5)).

When a temporarily halted clinical trial for reasons not affecting the benefit-risk balance is resumed the sponsor will notify each Member State concerned through CTIS (CTR: Article 37(6)).

Early termination will take place in accordance with the CCMO statement regarding early termination of the trial. (CCMO guideline – assessment research contracts and the CCMO template research contract)

The sponsor will notify to the EU portal CTIS of early termination of the clinical trial for reasons not affecting the benefit-risk balance through CTIS. The reasons for such action and, when appropriate, follow-up measures for the subjects will be provided as well (CTR: Article 37(7))

14.6.2 Temporary halt/early termination for reasons of subject safety

In accordance to article 38 of the CTR, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The temporary halt or early termination of a clinical trial for reasons of a change of the benefit-risk balance will be notified to the Member States concerned through the EU portal CTIS without undue delay but not later than in 15 days of the date of the temporary halt or early termination. It shall include the reasons for such action and specify follow-up measures. The restart of the clinical trial following a temporary halt as referred to in paragraph 1 shall be deemed to be a substantial modification subject to the authorisation procedure laid down in Chapter III of the CTR (CTR: Article 38).

14.7 Summary of the results

Within one year from the end of a clinical trial in all Member States concerned, the sponsor will submit to the EU database CTIS a summary of the results of the clinical trial. The content of the summary of the results is set out in CTR Annex IV. It shall be accompanied by a summary written in a manner that is understandable to laypersons. The content of the summary is set out in CTR Annex V (CTR: Article 37(4)).

14.8 Public disclosure and publication policy

The study will be registered to a publicly accessible registry and results database (ClinicalTrials.gov). Co-investigators will be involved in the publication process.

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