Project protocol

Title: The role of interleukin-1 beta targeted therapy for patients suffering from allergic contact dermatitis: A randomized controlled trial with anakinra vs. placebo.

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Initiation date: August 2022

Duration of study: The study is expected to last no longer than two years from approval.

Eudract-number: 2021-004750-39

NCT Number: NCT05498467

Study drug: Anakinra

Purpose: Assessing the efficacy of anti-IL-1 treatment in patients with known nickel allergy.

1. STUDY BACKGROUND AND RATIONALE

1.1. Background

Allergic contact dermatitis (ACD) is an inflammatory skin disease caused by cutaneous contact with an allergen inducing a cell-mediated type 4 hypersensitivity immune reaction.
ACD is clinically characterized by erythema, pruritus, edema, blistering, thickening, and scaling skin. The estimated prevalence of ACD to at least one contact allergen in North America and North Europe is 21.2%(1), and in recent years the incidence of ACD has been increasing in correlation with an increasing number of environmental detergents(2,3).

ACD can affect the quality of life of patients by causing discomfort, social and occupational limitations(4,5). The current treatment regimen of ACD consists of identification and elimination of the eliciting contact allergen in combination with topical steroids and moisturizing creams. Phototherapy and photochemotherapy can be complementary treatments for ACD patients resistant to topical steroid treatment. In more severe cases of ACD systemic immunosuppressant therapy may be needed such as glucocorticoids, disease-modifying anti-rheumatic drugs, and vitamin A derivatives. Systemic therapy has serious side effects and not all patients with ACD respond well to the current treatments available, therefore there is a need for exploring alternative forms of treatment for ACD(6).

A specific subset of epidermal-resident memory CD8+ T (T_{RM}) cells are generated locally in skin exposed to contact allergens(7). The T_{RM} cells are rapidly activated by re-exposure to the specific allergen. Upon reactivation, T_{RM} cells produce IFN-γ and IL-17A. The presence of T_{RM} cells correlates with an increased epidermal level of IL-1β during the challenge-response to contact allergens, which seems to be mediated by the production of IFN-γ and IL-17A by the T_{RM} cells(7). Within the epidermis, both keratinocytes and Langerhans cells (LC) can produce IL-1β. A central role of IL-1β in the response to contact allergens has been shown in numerous studies, where defects in the IL-1 signaling pathway or blocking of IL-1 resulted in a decreased challenge-response to contact allergens(8–13). IL-1β is involved in many different biological functions central to immune responses to contact allergens. These include induction of the production of various cytokines and chemokines by skin cells, up-regulation of MHC-II on Langerhans cells (LC), and initiation of the migration of LC from the epidermis to the draining lymph nodes(14,15). IL-1β potently increases the capacity of both dermal αβ and γδ T cells to

release IL-17A (16). However, it is not known if IL-1β plays a role in the maintenance and function of epidermal T_{RM} cells.

The recombinant IL-1 receptor antagonist anakinra is a well-explored drug and has been tested in numerous rheumatic diseases such as rheumatoid arthritis, Still's disease, ankylosing spondylitis, psoriatic arthritis, systemic lupus erythematosus, and osteoarthritis(17). The RCT described here will determine the effectiveness of anakinra in the treatment of ACD. A search of the Controlled Trials Register indicates there are no current studies investigating anakinra as a treatment for ACD.

1.2 Details of Investigational Medicinal Product

1.2.1 Description

This study will investigate the effects of anakinra (ATC code L04AC03) on the immune response in humans with ACD. The medication of interest is labeled and used for the treatment of rheumatoid arthritis and Still's disease. Trained personnel at The Department of Dermatology and Allergy, Herlev Gentofte Hospital will inject participants in the treatment group with the medication subcutaneously. The brand of medication used:

- Kineret®, Swedish Orphan Biovitrum, 100 mg/0.67 mL, injectable fluid for s.c. administration.

The pharmaceutical company will be notified about the trial. More details regarding the chemical and pharmacological properties of the study drug can be found in the Summary of Product Characteristics.

The control group will receive placebo in the form of sterile isotonic saline water injections.

- Sodium chloride Fresenuis Kabi 9 mg/ml

1.2.2 Packaging and Labelling

The study drug and placebo will be specially packaged and labeled by Region Hovedstaden's pharmacy and they will be the responsible unit for distribution of the study drug and placebo. The administering personnel are unblinded to treatment but participants and investigators are blinded to the treatment allocation.

1.2.3 Storage and administration

The study drug will be stored as per the manufacturer's instructions at The Department of Dermatology and Allergy, Herlev Gentofte Hospital. The clinical trials personnel at the hospital will administer the medication and keep detailed administration records. Double checking of medication will be performed.

1.2.4 Known Side Effects

Known side effects to anakinra are listed below:

>10%: Headache, local reaction at the site of injection

1-10%: Serious infections, neutropenia

0.1-1%: Allergic reactions (including angioedema and anaphylaxis)

2. STUDY PURPOSE AND OBJECTIVES

2.1. Purpose

The purpose of this study is to characterize the clinical effect of inhibiting IL-1β in ACD, and to investigate the role of IL-1β in the maintenance and function of epidermal T_{RM} cells. We expect that this knowledge can lead to the development of more specific treatments and potential cures for patients with ACD.

2.2. Hypothesis

IL-1β is a key cytokine in immune responses to contact allergens. Epidermal-resident memory CD8+ T (T_{RM}) cells lead to an increased production of IL-1β in skin cells upon challenge of the skin with contact allergens. However, the effect of IL-1β on epidermal T_{RM} cells is not known. We hypothesis that a positive feedback loop exists where IL-1β from skin cells boost the epidermal T_{RM} cells to ensure their maintenance and increase their pathological effector function in form of production of IFN-γ and IL-17A production that again stimulate the skin cells to produce IL-1β.

2.3. Primary Objective

- To assess if there is a clinical difference in severity of ACD in participants treated with anakinra compared to placebo measured by different scoring systems for ACD.

2.4. Secondary Objective

- To assess if there is a decrease in T_{RM} function and inflammation after treatment with anakinra compared to the control group, using following methods: flow cytometry, single cell RNA sequencing, immunohistochemistry, ELISA, digital spatial profiling, and qPCR.

3. STUDY DESIGN

3.1. Overview

This study is a prospective. two-arm, double-blinded, parallel group, randomized controlled clinical trial. Participants with known nickel allergy, but with minimal signs of dermatitis, will be recruited to the study until 20 has completed the study. Before enrollment, participants will be screened for eligibility (blood samples (20-25 ml), Quantiferon TB Gold test, and urine test for infection/pregnancy and chest x-ray to ensure that there is no evidence of infections and/or malignancy). Participants will be blinded to treatment and randomly allocated into two equally sized groups. At baseline (day 0) all participants will be exposed to a two-chamber patch test on the lower part of their back, with one chamber containing 5 % NiCl2 in petrolatum and one chamber containing petrolatum. The patch testing will be repeated twice at the exact same skin area, with an interval of 21 days between tests (day 21 and 42). One group (the control group) will be treated with placebo (saline water) and the second group (the treatment group) will be treated with anakinra before and during the second patch test (day 21) for six consecutive days (day 20 to 25). Skin biopsies will be collected with 4 mm punch biopsies right before and 48 hours after the third patch test (day 42 and 44). The wounds will be closed with non-absorbable sutures, and stitches will be removed after eight to ten days.
The study will be comprised of 12 visits in total, including screening.

3.2. Outcome Measures

3.2.1. Primary Outcome Measure

Photographs and clinical evaluation of the skin patch reactions will be performed on-site and blinded, using the Investigators Globals Assessment (IGA) and International Contact Dermatitis Research Group (ICDRG) scoring systems as instruments for classifying the severity of dermatitis. Patient reported outcomes will include Patient's Global Assessment Score (PtGA). Treatment effect will be defined as a difference in score between the two groups on the scoring systems used.

3.2.2. Secondary Outcome Measure

From each group, blood and biopsies from seven individuals will be used for single cell RNA sequencing (sc-RNAseq), and/or digital spatial profiling. Skin biopsies from the remaining three individuals will be used for immunohistochemistry, flowcytometry (staining with anti-CD3, anti-CD8, Ki67, anti-IL-1β) and/or qPCR (IFN-y, IL-17A and IL-1B). Treatment effect will be defined as a reduction in number of T-cells, cytokines and proliferative markers in the treatment group compared to placebo.

3.3. Stopping Rules and Discontinuation

The Department of Dermatology and Allergy, Herlev and Gentofte Hospital can discontinue the trial at any time for failure to meet expected enrolment goals, for safety or any other administrative reasons. Participants and The Regional Committee on Health Research Ethics will be informed in given case. The following may result in the trial being terminated

- Major safety concerns.
- Insurmountable issues with trial conduct (e.g. poor recruitment, loss of resources).

3.4. Randomization and Blinding

3.4.1. Randomization

The randomization will be based on a computer-generated code using random permuted blocks of varying size or by sealed envelope. Access to the sequence will be confined to Region Hovedstadens Pharmacy. Participants will be allocated in a 1:1 ratio to the anakinra and placebo treatment arms. The sequence of treatment allocations will be concealed until interventions have all been assigned and recruitment, data collection, and all other trial-related assessments are complete.

3.4.2. Blinding

The investigators and assessors will be blinded to treatment allocation until the results of the outcome measurements are recorded. Only the administering personnel will be unblinded to treatment allocation and know the allocation sequence. Trial participants will also be blinded to treatment allocation until the end of trial.

3.5. Trial Management

The trial and all clinical procedures will be conducted at The Department of Dermatology and Allergy, Herlev and Gentofte Hospital. All laboratory analyses of tissue specimens will be

analyzed at The LEO Foundation Skin Immunology Research Center, University of Copenhagen.

3.6. Duration of Study and Participant Involvement

3.6.1. Duration of Participant Involvement in the Study

All participants will be in the study for two months, and the study will be comprised of 12 visits in total ranging from 30 minutes to an hour per visit.

3.6.2. Duration of the Study

Recruitment will commence on Februar 2022 and is planned to continue until twenty participants are recruited and randomized. The study is expected to last no longer than two years.

3.6.3. End of the Study

The end of study is defined as the last participant's last visit.

3.7. Selection, Recruitment and Withdrawal of Participants

3.7.1. Participants

Participants over the age of 18 and with Fitzpatrick skin type 1 to 4 with known nickel allergy, but with minimal signs of dermatitis, will be enrolled to the study.

3.7.2. Setting

The Department of Dermatology and Allergy, Herlev and Gentofte Hospital will be the primary recruitment site.

3.7.3. Inclusion criteria

- Aged at least eighteen years old.
- Able to provide written informed consent.
- Have at least a ?+ reaction on the ICDRG scoring system when patch tested with nickel on the lower back.
- Fitzpatrick skin type 1-4.
- Able to speak and understand Danish

3.7.4. Exclusion criteria

The following criteria will exclude the participants from the study:

- Received any topical immunomodulating or immunosuppresive treatment on the lower back two weeks prior, or applied crème/lotion on the lower back 24 hours prior to day 0
- Received systemic immunomodulating or immunosuppressive treatment four weeks prior to day 0
- Any skin lesions at the area of interest such as nevi, scar tissue or pigment changes.
- Dermatitis and/or infection on their lower back
- Recent (3 months or less) administration of a live virus vaccine.
- Women of childbearing potential who are not taking adequate contraception or who are pregnant, plan to become pregnant during the study duration or lactating.
- Taking part in any other intervention study.
- Has any other condition which would, in the Investigator's opinion, deem the patient unsuitable for participation in the study (e.g. condition requiring long term or frequent oral steroid use).
- Presence of any condition or use of any medication which precludes the use of the study drug.
- Allergy to any of the ingredients in the drug.

3.7.5. Retention of participants

To help retain participants in the study, in addition to being able to speak to the investigator, participants will be able to telephone the investigator if they wish to discuss any aspect of the study. For medical queries, the participant will be directed to a medical member of staff.

3.7.6. Withdrawal of participants from treatment and the study

Participants may be withdrawn from the trial either at their own request or at the discretion of the Investigator. The participants will be made aware that this will not affect their future care. Participants will be made aware (via the information sheet and consent form) that should they withdraw the data collected to date may still be used in the final analysis. The reason for and date of withdrawal will be recorded on the case report form. All participants who withdraw from study treatment will be followed up for two weeks providing they have not withdrawn consent. Participants may be withdrawn from the study for the following reasons:

- Withdrawal of consent.
- Later found to be ineligible.

3.7.7. Recruitment

The Department of Dermatology and Allergy, Herlev and Gentofte Hospital will recruit 20 participants with known nickel allergy by reviewing patient journal files and/or by advertisements in newspapers, bulletin boards, social media platforms or relevant webpages. Recruitment of participants will continue until 20 participants with a positive nickel allergy test (at least a +2 reaction on the ICDRG scoring system) have been included and completed the study. Prior to enrollment participants will be screened for acute, chronic or recurring infections, malignant diseases, latent or prior history of tuberculosis, granulocytopenia or conditions that increase the risk of infection.

3.7.8. Informed consent

All participants will provide written informed consent. Informed consent will be obtained from each participant before they undergo any study procedure. Participants will be given details of the study verbally in an undisturbed environment. Prior to the visit participants will be informed that they have the right to bring an assessor of their choosing to the visit. If the participant is interested in taking part they will be given time to read the full Participant Information Leaflet and the investigator will answer any questions. Participants are allowed to bring an assessor and to have a sufficient period of reflection of at least 24 hours and will be given an opportunity to ask questions before signing the consent declaration. If participants wish, they can be included in the study during the first visit. Those wishing to participate will be required to sign a consent form before any study procedures are carried out. Participants will be randomised to receive one of two treatments. Participants will be reminded that they are free to withdraw their consent at any time. Should there be any subsequent amendment to the final protocol, which might affect a participant's participation in the trial, continuing consent will be obtained using an amended Consent form, which will be signed by the participant.

3.7.9 Informed Consent and Participant Information

The process for obtaining participant informed consent will be in accordance with the REC guidance, and Good Clinical Practice (GCP) and any other regulatory requirements that might be introduced.

The investigator or designee will discuss the study with the participant and also give them a copy of the Participant Information Leaflet which will contain all the details of the study. There

will be opportunity for the participant to ask questions about the study and the investigator or designee will ensure the questions are fully answered.

The investigator or their nominee and the participant shall both sign and date the Informed Consent Form before the person can participate in the study. The original will be kept in the Investigator Site File, one copy given to the participant and a copy will be retained in the participant's hospital records.

The decision regarding participation in the study is entirely voluntary. The investigator or their nominee shall emphasize to them that consent regarding study participation may be withdrawn at any time without penalty or affecting the quality or quantity of their future medical care, or loss of benefits to which the participant is otherwise entitled. No trial specific interventions will be done before informed consent has been obtained.

The investigator will inform the participant of any relevant information that becomes available during the study, and will discuss with them, whether they wish to continue with the study. If applicable they will be asked to sign revised consent forms.

If the Informed Consent Form is amended during the study, the investigator shall follow all applicable regulatory requirements pertaining to approval of the amended Informed Consent Form by the REC and use of the amended form (including for ongoing participants).

Informed consent declaration S3 from The National Committee on Health Research Ethics will be used.

3.8. Study Treatment and Regimen

3.8.1. Study Treatment

At day 20 (week 3) participants will be randomized to receive either:

Anakinra 100 mg/0,67 ml	**OR**	NaCl solution 9 mg/ml

Investigator and participants will be blinded to treatment allocation. The study drug and placebo will be administered by unblinded personnel. The study drug or placebo will be administered subcutaneously for six consecutive days, starting on day 20.
At any point, if the participant experiences a life-threatening adverse reaction, treatment will be terminated.

3.8.2. Rescue Medication

Use of any systemic or topical immunosuppressant/immune-modulating drugs is strongly discouraged to prevent potential systemic effects not related to study intervention. Accidental use of immunosuppressant or immune-modulating drugs during the study period will be classified as a protocol deviation.

3.8.3. Prohibited concomitant medication

The administration of live virus vaccines and any form of immunosuppressive drug is not permitted for all participants. Participants should continue to take medications for other conditions as normal. However, if it is anticipated that the participant will need a live virus vaccine during the intervention phase, they will be ineligible for entry into the study.

3.8.4. Caution with other concomitant medications

Concomitant use of other biological pharmaceuticals is not recommended due to an increased risk of infection.

3.8.5. Adherence

To ensure adherence, designated personnel will administer the study drug. If a participant does not adhere to the administration schedule, it will be classified as a protocol deviation.

3.8.6. Accountability

Personnel at the Department of Dermatology and Allergy, Herlev and Gentofte Hospital will keep detailed records, including participant study number and name, treatment given, batch number, expiry date, dose and date of administration.

3.8.7. Management of blood donation and risk of infection

During the study period, participants cannot donate blood as blood donors. Administration of the study drug will be terminated if a participant gets an infection during the study period.

3.9. Study Visit Schedule and Procedures

3.9.1. Visit Schedule

The visit schedule ensures proper administration of study drug, and that the relevant data and clinical procedures are collected and performed at appropriate time points.

Procedures, data collection, and drug administration	**Day 0 (screening)[2]**	**Day 2**	**Day 3**	**Day 20**	**Day 21**	**Day 22**	**Day 23**	**Day 24**	**Day 25**	**Day 42**	**Day 44**	**Day 45**
Clinical diagnosis of nickel allergy	X											
Obtain informed consent	X											
Ascertain the participant is eligible for the study	X											
Collect medical history and demographic data	X											
Samples taken for routine blood and urine tests[1]		X										
Routine chest X-ray		X										
Record adverse reactions					X	X	X	X	X			
Patch testing	X				X					X		
Removal of patch test		X					X				X	
Clinical evaluation of patch test reaction		X	X	X	X	X	X	X	X	X	X	X
Clinical photography	X	X	X		X		X	X		X	X	X
Collect skin biopsies										X	X	
Collect blood samples										X	X	
Give study diary to participant	X											
Dispense either anakinra or placebo according to randomization schedule				X	X	X	X	X	X			
Start study medication: anakinra or placebo				X								
Stop study medication: anakinra or placebo									X			

[1]Tests will include (but will not be restricted to) full red and white blood cell count, liver function tests, creatinine, HIV, hepatitis B and C serology, urea and urine screen.

[2]And baseline if the patient wish to participate immediately

3.9.2. Day 0: Screening

The initial visit will cover screening for eligibility and baseline assessments. For clarity, the screening visit will be described here as one clinic visit. The following procedures will be carried out at the screening visit:

- Give the participant information leaflet. Discuss the study and answer any initial questions the participant may have. Allow patient sufficient time to consider their participation in the study. Discuss the study with the participant further and answer any questions about the study.
- Obtain consent from the patient before proceeding with the study.
- Physical examination and confirmation of clinical diagnosis of nickel allergy.
- Check patient meets all eligibility criteria.
- Record relevant medical history and demographic data.
- Give study diary to participant to record self-reported outcome measures, any medical problems, health service usage and adherence with study protocol.
- Apply first set of patch tests containing nickel.

3.9.3. Day 2 and 3 visit

At the day 2 visit patch tests will be removed and clinical evaluation of patch test reactions including photography will be performed. Following procedures will be performed during day 2 and 3.

- Removal of patch tests.
- Clinical evaluation of patch test reactions.
- Clinical photography of skin sites.

If positive patch test:

- Take samples for full blood cell count, kidney and liver function tests, plus any others that are clinically indicated. Collect sample for urine screen (sediment, protein, hCG and glucose). The results of these tests will not be collected or analysed but they should be checked by the clinician as per routine practice and any clinically significant findings treated accordingly and recorded on the medical history if appropriate.
- Perform routine chest X-ray to exclude any latent infectious disease.

3.9.4. Day 20 visit

The purpose of the day 20 visit is to initiate the study medication treatment and randomize participants into the anakinra or placebo group.

The following procedures will be carried out at the day 20 visit:

- Physicial examination.
- Randomise participant. The participant will be randomised to receive either anakinra or placebo.
- Initiate treatment 24 hours prior to the second set of patch tests with nickel chloride.

3.9.5. Day 21 visit

At this visit, participants will receive their second dose of study medication and a second set of patch tests containing nickel chloride will be applied.

The following procedures will be carried out at the day 21 visit:

- Clinical evaluation of patch test reactions.
- Administration of the second dose of study medication right before patch testing.
- Application of second set of patch tests containing nickel chloride.
- Record adverse events.

3.9.6. Day 22

At this visit, participants will receive their daily dose of study medication.

The following procedures will be carried out at this visit:

- Administration of study medication.
- Record adverse reactions.

3.9.7. Day 23

This visit will comprise of patch test removal, clinical evaluation of patch test reactions and administration of the daily dose of study medication.

The following procedures will be carried out at this visit:

- Administration of study medication.
- Record adverse reactions.
- Patch test removal.
- Clinical evaluation of patch test reactions.
- Clinical photography.

3.9.8. Day 24

At this visit participants will receive their daily dose of study medication and clinical evaluation of patch test reactions and clinical photography of skin sites will also be performed.

The following procedures will be carried out at these visits:

- Administration of study medication.
- Record adverse events.
- Clinical evaluation of patch test reactions.
- Clinical photography.

3.9.9. Day 25

This visit will be the last visit where participants receive study medication.

The following procedures will be carried out at these visits:

- Administration of study medication.
- Record adverse events.
- Stop study medication after administration.

3.9.6. Day 42, 44 and 45 visits

The remaining visits are at day 42, 44, 45.

At day 42, the following procedures will be carried out:

- Clinical evaluation of patch test reactions.
- Application of third set of patch tests containing nickel chloride.
- Collection of blood samples and skin biopsies for analysis.

- Clinical photography.

At day 44, the following procedures will be carried out:

- Removal of patch tests 48 hours after application.
- Clinical evaluation of patch test reactions.
- Clinical photography.
- Collection of blood samples and skin biopsies for analysis and closure of wounds.
- Collect the completed study diary and record self-reported outcome measures, medical problems, health service usage and adherence with study protocol.

At day 45, the following procedures will be carried out:

- Clinical evaluation of patch test reactions.
- Clinical photography.

4. STATISTICS

4.1. Methods

The analysis plan and statistical analysis will be carried out by the trial's investigators. Any deviations from the original plan will be documented and justified in the final report.
The results will be analyzed based on treatment code using a statistical analysis plan finalised prior to revealing the coded allocation sequence. Only after the analysis is complete will the actual treatment arms corresponding to the treatment codes be revealed. The analysis will be conducted by per protocol.

4.2. Sample size

A total of 20 participants will be recruited to the study (10 per arm). This should be sufficient to detect a reduction in clinical score of at least one scoring point at 80% power and 95% significance level.

5. ADVERSE EVENTS

5.1. Definitions

An **adverse event (AE)** is any unfavourable and unintended sign, symptom, syndrome or illness that develops or worsens during the period of observation in the study.

An AE does include a / an:

1. Exacerbation of a pre-existing illness.
2. Increase in frequency or intensity of a pre-existing episodic event or condition.
3. Condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.
4. Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.

An AE does not include a / an:

1. medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion); but the condition that lead to the procedure is an AE.
2. pre-existing disease or conditions present or detected at the start of the study that did not worsen.
3. situations where an untoward medical occurrence has not occurred (e.g., hospitalisations for cosmetic elective surgery, social and / or convenience admissions).
4. disease or disorder being studied or sign or symptom associated with the disease or disorder unless more severe than expected for the participant's condition.
5. overdose of concurrent medication without any signs or symptoms.

A **Serious Adverse Event (SAE)** is any adverse event occurring that results in any of the following outcomes:

- Death
- life-threatening
- Inpatient hospitalisation or prolongation of existing hospitalisation
- Persistent or significant disability / incapacity
- A congenital anomaly or birth defect in the offspring of a participant
- Other medical events may be considered to be a SAE if they require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All adverse events will be assessed for seriousness, expectedness and causality (not related, possibly related, probably related or definitely related). An adverse event whose causal relationship to the study drug is assessed by the Chief Investigator as "possible", "probable", or

"definite" is an Adverse Reaction (AR). If the AR is classed as serious then it is a Serious Adverse Reaction (SAR). A distinction is drawn between serious and severe AEs. Severity is a measure of intensity whereas seriousness is defined using the criteria above. Hence, a severe AE need not necessarily be serious. All adverse events will be recorded and closely monitored until resolution, stabilisation, or until it has been shown that the study medication or treatment is not the cause. The summary product characteristics for Kineret® will be used as a reference document to assess if an AE or SAE is expected or unexpected.

5.2. Causality

Not related or unlikely: a clinical event including laboratory test abnormality with temporal relationship to trial treatment administration which makes a causal relationship incompatible or for which other drugs, chemicals or disease provide a plausible explanation**.** This will be counted as "unrelated" for notification purposes.

Possible: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, but which could also be explained by other drugs, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Probable: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and is unlikely to be due to other drugs, chemicals or concurrent disease. This will be counted as "related" for notification purposes.

Definite: a clinical event, including laboratory test abnormality, with temporal relationship to trial treatment administration which makes a causal relationship a reasonable possibility, and which can definitely not be attributed to other causes. This will be counted as "related" for notification purposes.

An AE whose causal relationship to the study investigational medical product (IMP) is assessed by the Chief Investigator as "possible", "probable", or "definite" is an Adverse Drug Reaction.

With regard to the criteria above, medical and scientific judgment shall be used in deciding whether prompt reporting is appropriate in that situation.

5.3. Reporting of Adverse Events

Participants will be asked to contact the study site immediately in the event of any significant adverse event. The Chief Investigator shall be informed immediately of any reportable serious adverse reactions and shall determine seriousness and causality in conjunction with any treating medical practitioners.

In the event of a pregnancy occurring in a trial participant or the partner of a trial participant monitoring shall occur during the pregnancy and after delivery to ascertain any trial related adverse events in the mother or the offspring. Where it is the partner of a trial participant consent will be obtained for this observation from both the partner and her medical practitioner.

All serious adverse reactions will be recorded and reported to the Danish Medicines Agency (DMA) and regional ethics committees (REC) as part of the regular mandatory reports. SUSARs will be reported by e-form within the statutory timeframes to the DMA and REC as stated below. The Chief Investigator shall be responsible for all adverse event reporting.

5.4. Suspected Unexpected Serious Adverse Reaction (SUSARs)

A serious adverse reaction (SAR) that is unexpected (i.e. not a known side effect of the study drug) is classed as Suspected Unexpected Serious Adverse Reaction. All SUSARs will be reported immediately to the Chief Investigator and will require expedited reporting to the authorities as described below.

The Chief Investigator will:

- Assess the event for seriousness, expectedness and relatedness to the study IMP.
- Take appropriate medical action, which may include halting the trial.
- If the event is deemed a SUSAR, shall, within seven days, complete the CIOMS form and send to the DMA.
- Shall inform the REC using the relevant reporting form found on the REC's web page within seven days of knowledge of the event.
- Shall, within a further eight days send any follow-up information and reports to the DMA and REC.
- Make any amendments as required to the study protocol and inform the ethics and regulatory authorities as required.

5.5. Collection of Adverse Event Data

This study investigates a widely used drug, anakinra, for which the side-effect profile is well established. For this reason, only adverse events that are known side-effects will be collected (detailed in section 4.2.4, Known side effects) i.e. only adverse reactions will be collected.

At each mandatory study visit, the investigator will record whether the participant had experienced any of the reportable adverse events. The investigator can also record any other significant adverse events that are not listed if deemed clinically relevant.

All available sources of information will be used to collect information on adverse events including hospital notes, the participant diary and discussion with the participant. Any adverse events reported will be graded according to a modified version of the Common Terminology Criteria for Adverse Events v3.0.

6. Ethics Committee and Regulatory Aspects

6.1. Ethical and Regulatory Approval

The trial will not be initiated before the protocol, informed consent forms and participant and information sheets have received approval / favourable opinion from the DMA and REC.

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, 1996; the principles of Good Clinical Practice, in accordance with the Medicines for Human Use Regulations, Statutory Instrument 2004, 1031 and its subsequent amendments.

6.3. Case Report Forms

Each participant will be assigned a trial identity code number, allocated at randomisation, for use on the case report forms (CRFs), other trial documents and the study database. The documents and database will also use their initials (of first and last names separated by a hyphen or a middle name initial when available) and date of birth (dd/mm/yy).

CRFs will be treated as confidential documents and held securely in accordance with regulations. The investigator will make a separate confidential record of the participant's name,

date of birth, CPR-number, and Participant Trial Number, to permit identification of all participants enrolled in the trial, in case additional follow-up is required.

All paper forms shall be filled in using black/blue ballpoint pen. Errors shall be lined out but not obliterated by using correction fluid and the correction inserted, initialled and dated.

6.4. Direct access to source data / documents

The CRF and all source documents, including progress notes and copies of laboratory and medical test results shall be made available at all times for review by the Chief Investigator and inspection by relevant regulatory authorities (e.g. DMA).

6.5. Data Protection

Due respect for data protection and confidentiality will be maintained.

All trial staff and investigators will endeavour to protect the rights of the trial's participants to privacy and informed consent, and will adhere to the Data Protection Regulation and the Data Protection Act. The CRF will only collect the minimum required information for the purposes of the trial. CRFs will be held securely, in a locked room, or locked cupboard or cabinet. Access to the information will be limited to the trial staff and investigators and relevant regulatory authorities. Computer held data including the trial database will be held securely and password protected. All data will be stored on a secure dedicated web server. Access will be restricted by user identifiers and passwords (encrypted using a one way encryption method).

Information about the trial in the participant's medical records / hospital notes will be treated confidentially in the same way as all other confidential medical information. Following information will be extracted from participants' medical records: blood sample results, x-ray description, urine sample result, age, gender, skin type, current and prior medical diseases, medical disease predispositions and medical list.

Electronic data will be backed up every 24 hours to both local and remote media in encrypted format.

6.6 Biobank

Handling of biological samples and personal information will be kept in accordance to the GCP standard and the Data Protection Regulation and the Data Protection act. Surplus material from the biopsies will be kept in a research biobank for up to five years after study termination in case it will be necessary to do any further analyses. After this period the samples will be destroyed. All extra analyses will only be performed after approval from The National Committee on Health Research Ethics. Biological material will not be sent to any other countries.

6.7 Procedures for breaking randomisation code

Sponsor will receive a set of coded envelopes with all randomization numbers and another set of coded envelopes will be delivered seperatly together with the study drug/placebo. In case that the treatment allocation needs to be revealed, the envelopes can be opened. The envelopes are stored and available at the department 24/7. The investigator can be reached by telephone 24/7.

7. Quality Assurance and Audit

7.1. Insurance and Indemnity

Insurance and indemnity for trial participants and trial staff is covered within the hospital's liability insurance

7.2. Trial Conduct and Monitoring

This trial will be conducted in adherence with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (ICH-GCP) and the applicable regulatory requirements. All study documents and standard operating procedures will be prepared to ensure compliance with GCP.

The investigators will ensure that all study procedures are followed and any deviations documented and investigated.

The study will be performed in accordance with GCP. The GCP-unit, Copenhagen (Frederiksberg hospital, Nordre Fasanvej 57, Skadestuevej 1, parterre, 2000 Frederiksberg) will monitor the study. The Danish Data Protection Agency will be notified about this study.

7.3. Record Retention and Archiving

In compliance with the ICH-GCP guidelines, the Principal Investigator will maintain all records and documents regarding the conduct of the study. These will be retained for at least 7 years or for longer if required. If the responsible investigator is no longer able to maintain the study records, a second person will be nominated to take over this responsibility.

7.4. Risk Assessment

There is little additional risk or benefit to the individual participant by entering this study. The study drug is a standard treatment option for certain rheumatoid diseases and the risks associated with the treatment are well documented with the most common being infection. During the study we will be giving the study drug at the daily recommended dose. Risks related to the clinical procedures in the study can be minor pain, bruise and infection when taking blood samples. Minor pain and bruising during blood pressure measuring. Redness and irritation of skin related to patch testing. Redness, irritation, minor risk of bleeding, infection and minor scarring related to the biopsies. Minor radiation risk exposes the participant to about 0.1 mSv (corresponding to 1-2 weeks of background radiation during chest x-ray. Background radiation is 3 mSv/year). Participants in the study will receive either an active drug or placebo. The results of this study will help inform clinical treatment decisions that will benefit society.

7.5. Confidentiality

Individual participant medical information obtained as a result of this study is considered confidential. Participant confidentiality will be further ensured by utilising identification code numbers to correspond to treatment data in the computer files.

Data generated as a result of this trial will be available for inspection on request by the participating physicians, the ethics committees, host institutions and the regulatory authorities.

7.6. Ethical Considerations

Participants will be provided with written and oral information on this study and will only be enrolled when given their written informed consent. The study will be designed in accordance to the Helsinki-declaration. Herlev and Gentofte Hospital will ensure that collection and processing of personal data comply with national legislation on data protection and privacy. Participants will

not directly benefit from the study. All procedures (biopsy, blood tests and allergies) are performed routinely in the department, anakinra is already used for individual patients with autoinflammatory diseases. However, it will extend our knowledge about mechanisms involved in ACD, which may lead to new treatments of the disease. Severe ACD is associated with known reduced life quality, disruption of sleeping and affected work life. Thus, studies investigating ACD are needed.

8. PUBLICATION AND DISSEMINATION POLICY

The results of the study will be submitted for publication in a peer review journal as soon as possible after analysis. All investigators will be named in the acknowledgments, detailing their role in the study. Participants will not be identified in any publications.

9. STUDY FINANCES

9.1. Funding source

This study is fully funded by the LEO Foundation and Aage Bang Foundation (DKK 1.070.000). The University of Copenhagen administrates the economy. None of the investigators have any commercial interests in the study. The LEO Foundation has no influence on this study. The research conducted is fully independent of the LEO Foundation.

9.2. Participant stipends and payments

Participants will be economically compensated to participate in the trial since they will not directly benefit from the study. Travel expenses will be covered for any costs incurred for hospital visits. Participants will be compensated with 10.000 DKK for disadvantage, medical treatment, visits, and skin biopsies upon completion of the study. The economical compensation is taxable income. Participants will receive 500 DKK as compensation for their time if they’re found non-eligible after the screening visit and participants who drop out prior to completion will be economically compensated according to the number of visits and biopsies they have participated in.

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