Clinical trial report synopsis

Trial registration ID-number NCT01181050	**UTN:** U1111-1114-9194 **EudraCT number:** 2010-019261-28

TITLE OF TRIAL

A randomised, single-dose, double-blind, placebo-controlled, parallel-group trial to assess clinical efficacy of NNC 0142-0000-0002[a] in subjects with active rheumatoid arthritis

[a]*will hereinafter be referred to as NNC0142-0002*

INVESTIGATOR

Signatory/principal investigator: Prof Dr [redacted]

TRIAL SITES

The trial was conducted at 8 trial sites in 3 different countries (Germany, the Russian Federation and Ukraine) as follows: Germany: 1 site, the Russian Federation: 6 sites, and Ukraine: 1 site. All sites enrolled, randomised and dosed at least 1 subject.

PUBLICATIONS

None

TRIAL PERIOD	**DEVELOPMENT PHASE**
Initiation date: 16 August 2010 Completion date: 17 April 2012	Phase 2a

OBJECTIVES

Primary objective

- To evaluate the change in disease activity following a single s.c. dose of NNC0142-0002 compared to placebo in subjects with active RA on background MTX therapy, measured 12 weeks after administration

Secondary objectives

- To assess the following in subjects with active RA on background MTX treatment, up to 24 weeks after administration of a single s.c. dose of NNC0142-0002 compared to placebo:
  - Signs of clinical efficacy, as measured by change in disease activity over time, and clinical responses determined at various time points up to Week 24
  - Signs of effects as assessed by imaging and various PD biomarkers
  - Safety and tolerability, including immunogenicity of NNC0142-0002
  - Quality of life
  - PK of NNC0142-0002 and occupancy of the NKG2D receptor by NNC0142-0002

METHODOLOGY

This was a randomised, single-dose, double-blind, placebo-controlled, parallel-group trial to assess the clinical efficacy of NNC0142-0002 in subjects with active RA concomitantly treated with methotrexate (MTX). The trial included two parallel treatment arms, and subjects were randomised in a 2:1 ratio with 41 subjects allocated to treatment with NNC142-0002 and 22 subjects to placebo treatment. Subjects received a single dose of 4 mg/kg NNC0142-0002 or placebo via subcutaneous injection into the abdominal wall. All subjects were closely monitored for 24 hours after dosing for clinical and laboratory safety assessments, pharmacokinetics and receptor occupancy. Local tolerability was assessed before the subject left the clinic. The dosing visit was followed by regular out-patient visits for 20 weeks (at Weeks 1, 2, 4, 6, 8, 12, 16 and 20), and a final visit at 24 weeks after dosing. An internal safety committee performed ongoing safety surveillance, and all safety laboratory data were reviewed at least every 3 months. The primary endpoint, change in DAS28 (based on C-reactive protein; CRP) from baseline to Week 12, was evaluated in an interim analysis conducted after Week 12-assessments had been performed for the last dosed subject.

NUMBER OF SUBJECTS PLANNED AND ANALYSED

The trial was planned for a total of 160 screened, 63 randomised and exposed, and 51 completing subjects. A total of 86 subjects were screened for the trial and 63 subjects were randomised and exposed (41 subjects to NNC0142-002 and 22 subjects to placebo). All 63 subjects completed the trial and were included in the full analysis set and the safety analysis set.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Men and women (not pregnant and not nursing) between ≥18 and ≤75 years of age, with active RA meeting the ACR1987 diagnosis criteria and characterised by a DAS28-CRP ≥4.5 and at least five tender/swollen joints, including one swollen wrist or at least two swollen ipsilateral metacarpophalangeal joints (second to fifth). Subjects should receive treatment with MTX (7.5–25 mg/week) for at least 12 weeks during the trial, and should have been treated with a stable MTX dose for at least 4 weeks prior to receiving trial product. They should not have failed any biologic therapy for RA and no more than two non-biologic DMARDs (primary or secondary failure to therapy). Further, they should not have any other chronic inflammatory autoimmune disease than RA (except secondary Sjögren's syndrome or stable and appropriately treated hypothyroidism).

Withdrawal criteria

The subject could withdraw at will at any time. The subject could be withdrawn from the trial at the discretion of the investigator or the sponsor due to a safety concern or if judged non-compliant with trial procedures. A subject had to be withdrawn if the following applied: i) Non-compliance with protocol procedures, which in the clinical judgement of the investigator and/or after discussion with the sponsor may invalidate the trial; ii) Sponsor closure of the trial; iii) Withdrawal of informed consent; iv) Pregnancy or intention of becoming pregnant.

INVESTIGATIONAL MEDICINAL PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

All doses of NNC0142-0002 were administered s.c. in the abdominal wall, at 4 mg/kg. NNC0142-0002 was provided as freeze-dried powder in 12 mL vials, which was reconstituted with sterile water to a final concentration of 100 mg/mL per vial. Batches of NNC0142-0002 used in the trial were VLDP031 and YLDP017.

DURATION OF TREATMENT

Subjects received a single dose of trial product.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

All doses of placebo were administered s.c. in the abdominal wall. Placebo was provided as a liquid formulation in 12 mL vials. The batch of placebo used in the trial was VLDP033.

CRITERIA FOR EVALUATION – EFFICACY

Primary efficacy assessments

- tender joint count (28 joints assessed) (TJC28)
- swollen joint count (28 joints assessed) (SJC28)
- C-reactive protein (CRP) level
- subject's global assessment of disease activity (VAS) (PtGA)

Secondary efficacy assessments

- swollen joint count (SJC66)
- tender joint count (TJC68)
- subject's assessment of pain (VAS)
- subject's global assessment of disease activity (VAS) (PtGA)
- physician's global assessment of disease activity (VAS) (PhGA)
- subject's self-assessed disability using a health-assessment questionnaire-disability index (HAQ-DI) questionnaire
- CRP level
- patient-reported outcomes: HAQ-DI, short form 36 (SF-36) health survey, RA quality of life (RAQoL), multidimensional assessment of fatigue (MAF)
- MRI assessment of synovitis, oedema and erosion
- ultrasonography

Secondary pharmacodynamic assessments

- NKG2D receptor occupancy by NNC0142-0002
- CRP level
- Cell markers
- Cytokines, chemokines and other proteins, including anti-cyclic citrullinated peptide (a-CCP), rheumatoid factor (RF), soluble MICA (sMICA) and Granzyme B
- Genomic biomarkers: mRNA expression levels

Secondary pharmacokinetic assessments

- Serum concentrations of NNC0142-0002

CRITERIA FOR EVALUATION – SAFETY

- Adverse events (AEs) including local tolerability
- Physical examination including vital signs
- Clinical laboratory safety (haematology, biochemistry, urinalysis, coagulation, lipids and viral screening)
- Electrocardiogram (ECG)
- Antibodies against NNC0142-0002

STATISTICAL METHODS

The sample size calculation was based on the primary endpoint. Under the assumption of a standard deviation of 1.3 and based on a two-sided t-test, a significance level of 5%, a difference in DAS28 score of 1.2 and a 2:1 randomisation ratio, completion of a total of 51 subjects with a DAS28 ≥4.5 in the trial was expected to ensure 85% power to detect a difference between treatment with NNC0142-0002 and placebo at Week 12. Accounting for drop outs, 63 subjects were to be enrolled in the trial. The following analysis sets were defined:

- The safety analysis set: All randomised patients exposed to at least one dose of the trial product
- The full analysis set (FAS): All randomised subjects exposed to at least one dose of trial product and with at least one post-treatment measurement

All tests were two-sided and a significance level of 5% was used. No adjustment for multiplicity was made, as all secondary endpoints were regarded as supportive. The treatment effect was quantified in terms of the estimated difference (NNC0142-0002 – placebo) or, if the endpoint was log transformed, the ratio (NNC0142-0002 / placebo), together with the 95% confidence interval and p-value.

Primary endpoint – change in DAS28-CRP from baseline to Week 12

The primary model was a mixed-effect model repeated measures (MMRM). The effect at Week 12 (active – placebo) was estimated from this model and presented together with the 95% confidence interval and the p-value for testing no treatment effect.

Supportive efficacy endpoints

- Change in DAS28-CRP from baseline to Weeks 6 and 24
- ACR20 response at Weeks 6, 12 and 24
- ACR50 and ACR70 responses at Week 12
- EULAR responses at Weeks 6, 12 and 24
- Change in patient-reported outcome from baseline to Week 12, using Health Assessment Questionnaire – Disability Index (HAQ-DI), Short Form 36 (SF-36), Rheumatoid Arthritis Quality of Life (RAQoL) questionnaire and Multidimensional assessment of fatigue (MAF) scale

The analyses of change in DAS28 from baseline to Weeks 6 and 24 was based on the MMRM model used for the primary analysis of the primary endpoint, and the ANOVA model using LOCF imputation.

ACR20/50/70 responders at Week 12 were compared between the two treatment groups using the Fisher's exact test. The odds ratios for achieving ACR20/50/70 for active versus placebo (active/placebo) were estimated by fitting a logistic regression model. The same analysis was also implemented to ACR20 at Week 6 and Week 24, respectively.

The EULAR response at each timepoint (Weeks 6, 12 and 24) was compared between the two groups by fitting a proportional odds model. A supplementary analysis not specified in the trial protocol was implemented for comparing the two treatment arms using a Cochran-Mantel-Haenszel test. The change from baseline to Week 12 in patient-reported outcome using HAQ-DI and SF-36 questionnaires was each compared by fitting an ANOVA. The same analysis was conducted for the RAQoL questionnaire and MAF scale (not specified in the protocol).

Supportive efficacy endpoints not specified in the protocol

- Remission scores according to various criteria: DAS28-CRP ≤2.6 and ≤2.0, SDAI ≤3.3, CDAI ≤2.8 and $DA_{All\ comp \leq 1}$ (the latter being a Boolean-based definition stating that at any time point, a subject had to satisfy all of the following criteria to be in remission: TJC28 ≤1, SJC28 ≤1, CRP ≤1 [mg/dL] and PtGA ≤1[cm])

The number and percentage of subjects reaching remission scores were summarised by treatment groups.

Supportive efficacy endpoints associated with imaging

- Change in the synovitis RAMRIS from baseline to Week 12
- Change in the oedema RAMRIS from baseline to Week 12 (endpoint not specified in the trial protocol)

- Change in the erosion RAMRIS from baseline to Week 12 (endpoint not specified in the trial protocol)
- Change in ultrasound assessment of joint inflammation from baseline to Week 12

Change in the RAMRIS score from baseline to Week 12 was analysed using an ANOVA model. No statistical analysis was performed for the ultrasound assessment, as this was done only for subjects at the trial site in Germany.

PD endpoints

- Level of NNC0142-0002 occupancy of NKG2D receptor on circulating leukocyte subsets, up to Week 24
- Biomarkers: CRP; ESR; cell markers; cytokines and chemokines; genomic biomarkers (polymorphic DNA sequences [optional] and gene expression); and levels of anti-CCP, RF, sMICA and MMP-3.

PD effect was evaluated by fitting an ANOVA to the maximum concentration (E_{max}), and the minimum concentration (E_{min}) of the respective parameter.

Changes in NKG2D receptor occupancy, CRP, ESR, cell markers, cytokines, anti-CCP, RF, sMICA and MMP-3 after 12 weeks were each analysed using an ANOVA, where serum concentrations of CRP, anti-CCP, RF, sMICA and MMP-3 were logarithmic-transformed before analysis (analysis not specified in the trial protocol).

Safety endpoints

- AEs including local tolerability
- Physical examination, incl. vital signs; ECG
- Clinical laboratory safety (haematology, biochemistry, urinalysis, lipids and viral screening)
- Antibodies against NNC0142-0002

All safety endpoints were summarised descriptively based on all collected data. The PD effect based on the safety parameters was investigated using the same statistical methods as described for the PD effect based on the efficacy parameters (analysis not specified in the trial protocol).

PK endpoints

- AUC, $C_{max,}$ t_{max} and $t_{½}$
- $AUC/AUC_{(0–W12)}$ (endpoint not specified in the trial protocol)

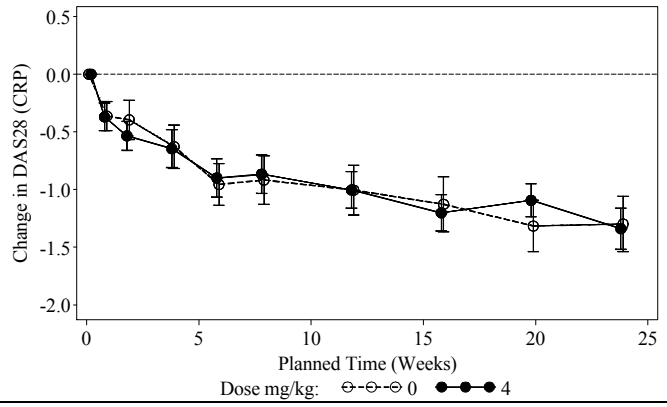
All PK endpoints were summarised descriptively.

DEMOGRAPHY OF TRIAL POPULATION

All subjects were White with a mean age of 52 years and a mean BMI of 27.5 kg/m^2. The majority of subjects (63.5%) were enrolled from the Russian Federation, followed by Ukraine (19.0%) and Germany (17.5%). More females than males (90% versus 10%) were included. Subjects had been diagnosed with RA for a mean of 7.1 years, had a mean DAS28 of 5.4, and approximately 80% of the subjects were seropositive (positive for RF or antibodies against CCP). Subjects had been treated with stable doses of MTX for a mean of 3.3 years (range: 0.3 to 13.4 years), and the time from RA diagnosis to initiation of MTX therapy was 3.8 years (range: 0 to 24 years). The two dose groups were comparable with respect to demography, disease profile and MTX treatment.

EFFICACY RESULTS

- A single subcutaneous administration of 4 mg/kg NNC0142-0002 did not result in a statistically significant reduction in disease activity in terms of DAS28-CRP at 12 weeks after treatment, when compared to placebo. Neither was any reduction observed at 6 or 24 weeks after treatment.



- No statistically significant difference in ACR20/50/70 or EULAR responses was observed during the trial, when compared to placebo.
- No statistically significant difference in change in patient-reported outcomes (HAQ-DI, SF-36 and MAF) was observed during the trial when compared to placebo, with the exception of significant improvements in the RA quality of life (RAQoL) for the placebo group at 12 and 20 weeks after treatment.
- Numbers of subjects achieving remission according to the different remission criteria were comparable between the two treatment groups. 3 (7%) actively treated subjects and 1 (5%) placebo-treated subject achieved remission according to the DAS28 ≤ 2.6 remission criterion. Single or no subjects achieved remission according to the other remission criteria applied (DAS28 ≤2.0, SDAI ≤3.3, CDAI ≤2.8 and $DA_{[All\ comp \leq 1]}$). The single remissions occurred in the group with actively treated subjects.
- No statistically significant difference in change in MRI (RAMRIS) scores on synovitis, oedema or erosion were observed during the trial, when compared to placebo.
- Full (i.e., above 95%) mean NKG2D receptor occupancy by NNC0142-0002 was observed at Week 1 and maintained throughout the 12 weeks after dosing. A mean NKG2D receptor occupancy of above 90% was maintained in blood for 13.2 weeks (SD: 3.3 weeks). For 85% of the subjects, the occupancy had declined below 20% by Week 24. For 15% of the subjects, occupancies were ≥20% at Week 24.
- No statistically significant difference in changes in biomarker parameters (anti-CCP, sMICA, MMP-3, CRP or ESR) was observed at Week 12 or in the follow-up period when compared to placebo, with the exception of a reduction in RF at Week 12 (ratio: 1.2; 95% CI: 1.02, 1.42; p=0.032) in placebo-treated subjects.
- Statistically significant reductions in the mean E_{min} of NKG2D receptor-expressing fraction (%) of $CD8^+$ T cells and NK cells, respectively, were observed when compared to placebo (overall cell numbers remained within normal range). In addition, a statistically significant reduction in the E_{min} of the absolute (MEF) mean NKG2D receptor expression was evident for the pooled $CD8^+$ T cell and NK cell fraction when compared to placebo. At Week 24, the proportion of NKG2D receptor-expressing NK cells approached normalisation.
- No statistically significant reduction of the mean fraction (%) $NKG2D^+CD28^-$ T cells of $CD4^+$ T cells was observed when compared to placebo. However, $NKG2D^+CD28^-CD4^+$ T cells levels were relatively low throughout the trial.
- No statistically significant differences in surface expression of CD69, CCR3/CCR5, CD45RA/CCR7 or VLA-4 at E_{min} or E_{max} were observed when compared to placebo. However, most cell markers were measured only in subjects enrolled at the German trial site because of lacking cell-marker stability during shipment.
- No effect of treatment with NNC0142-0002 on peripheral blood gene-expression profiles was observed. No genotyping was performed.
- The mean observed maximum serum concentration (C_{max}) was 27.8 µg/mL (95% CI: 25.0, 30.9), and the median time to maximum observed serum concentration (t_{max}) was approximately 7 days (95% CI: 6.9, 7.0). An average of 94% of NNC0142-0002 was eliminated at 12 weeks after treatment.

SAFETY RESULTS

- A single subcutaneous administration of NNC0142-0002 at 4 mg/kg was well tolerated in subjects with active RA concomitantly treated with MTX.
- A total of 53 AEs were reported for 33 (52%) subjects during the trial. Comparable proportions of subjects with AEs were observed for the two treatment groups, with 31 AEs reported for 22 (54%) actively treated subjects, and 22 AEs reported for 11 (50%) placebo-treated subjects. The mean time of trial participation was comparable between actively treated subjects (24.1 weeks; range: 23.1 to 25.3 weeks) and placebo-treated subjects (24.1 weeks; range: 23.3 to 24.3 weeks).
- Comparable proportions of subjects experiencing possibly/probably related AEs were observed for the two treatment groups, with 3 AEs reported for 3 (7%) actively treated subjects (single events of moderate rheumatoid arthritis, moderate stomatitis and moderate headache), and 2 AEs reported for 2 (9%) placebo-treated subjects (single events of moderate herpes zoster and mild viral respiratory tract infection). None of these possibly/probably AEs were evaluated as severe; 1 (rheumatoid arthritis) was classified as serious.
- AEs were mainly mild (49% of events) or moderate (42% of events) in severity. A total of 5 (9% of events) events were evaluated as severe: single events of musculoskeletal [shoulder] pain and [aggravated] RA in 2 (5%) actively treated subjects, and single events of [aggravated] rheumatoid arthritis, portal hypertension and hepatic fibrosis in 2 (9%) placebo-treated subjects. All severe AEs were resolved at end of trial, with the exception of the portal hypertension and hepatic fibrosis ([redacted]). None of the severe events were

evaluated as possibly or probably related to trial product; 2 severe events (portal hypertension and musculoskeletal pain) were classified as SAEs.

- A total of 5 SAEs were reported for 4 (6%) subjects: single events of moderate erosive gastritis, moderate chronic pancreatitis, severe musculoskeletal pain and moderate rheumatoid arthritis, in a total of 3 (7%) actively treated subjects; and severe portal hypertension in 1 (5%) placebo-treated subject. All SAEs were evaluated as unlikely related to the trial product, with the exception of the rheumatoid arthritis event, which was evaluated possibly related to the trial product.
- There were no deaths, AEs leading to withdrawal of a subject or medical events of special interest (such as medication errors or suspected transmission of an infectious agent via a trial product) reported during the trial.
- A total of 2 injection-site reactions were reported for 2 (3%) subjects, both administered placebo: 1 event of redness and 1 event of haematoma.
- Statistically significant decreases in mean E_{min} of B cells and sodium were observed in actively treated subjects when compared to placebo, but were not considered clinically relevant. Infrequent and transient levels outside the normal range were observed for various cell subtypes, but there were no consistent trends indicative of relationship to treatment with NNC0142-0002.
- Urinalysis parameters, viral screen, physical exam and ECG results were without remarks throughout the trial. No statistically significant difference in coagulation, lipid or cytokines, body weight, body temperature, pulse, diastolic or systolic blood pressure was observed for the actively treated group when compared to placebo.
- Treatment-induced anti-drug antibodies of low titre (titre: 1) were observed in samples collected from 3 (7%) of the subjects: at Week 12 for 1 subject, Week 16 for 1 subject and Weeks 20 and 24 for 1 subject. The anti-drug antibodies were not neutralising; i.e. did not interfere with NNC0142-0002 binding to the NKG2D receptor *in vitro*.

CONCLUSIONS

- A single subcutaneous administration of 4 mg/kg NNC0142-0002 did not result in a statistically significant reduction in disease activity in terms of DAS28-CRP at 12 weeks after treatment, when compared to placebo. Neither was any reduction observed at 6 or 24 weeks after treatment. No statistically significant difference in ACR20/50/70 or EULAR responses was observed for subjects treated with NNC0142-0002 when compared to placebo.
- Elimination of NNC0142-0002 in serum was almost complete after 12 weeks. Full (i.e., above 95%) mean NKG2D receptor occupancy by NNC0142-0002 was maintained throughout the 12 weeks after dosing.
- Treatment with NNC0142-0002 reduced the NKG2D receptor-expressing fraction of $CD8^+$ T cells and NK cells, respectively. There were no significant differences in changes of the biomarkers investigated, and no effect of treatment with NNC0142-0002 on peripheral blood gene-expression profiles or MRI scores on synovitis, oedema or erosion.
- No statistically significant improvement in patient-reported outcomes for actively treated subjects was observed for subjects treated with NNC0142-0002 when compared to placebo.
- No safety concerns were raised during the trial, and NNC0142-0002 was well tolerated within the context of the trial. Treatment-induced, non-neutralising anti-drug antibodies of low titres were observed in 3 (7%) subjects.

The trial was conducted in accordance with the Declaration of Helsinki (2008) and ICH Good Clinical Practice (1996). The results presented reflect the data available in the clinical database as of 12-June-2012.