4 Synopsis

Trial Registration ID-number	**EudraCT number**
NCT00601861	2006-005350-79
Title of Trial A single arm, single-centre, open-label, exploratory trial of recombinant interleukin-21 administered subcutaneously for four weeks as neo-adjuvant treatment prior to sentinel lymph node/complete lymph node dissection followed by eight weeks of adjuvant treatment in subjects with stage III malignant melanoma	
Investigator PD Dr [redacted]	
Trial Site [redacted] Germany	
Publications None	
Trial Period 4th of March 2008 to 25th of June 2008	**Development Phase** Phase 2

Objectives

Clear signs of rIL-21 anti-tumour activity have been recorded in phase I trials for a few subjects with stage IV malignant melanoma and stage IV renal cell carcinoma. Experiences with lymphatic mapping suggest that local injections in the area of the primary tumour consistently target the draining lymph nodes. It is hypothesised that local s.c. administration of rIL-21 in the area of the primary tumour will result in relatively high concentration of rIL-21 within the tumour draining lymph nodes, and that this local high concentration of rIL-21 will have immuno-modulatory effects and potentially anti-tumour effects (for more details, please refer to Appendix 16.1.1).

Primary Objective:

- To estimate the complete response rate of the sentinel lymph node(s) as assessed by histopathology in subjects with stage III malignant melanoma measured after four weeks of local neo-adjuvant treatment with recombinant human interleukin-21 (rIL-21)

Secondary Objectives:

Efficacy

- To describe the frequency of cells staining for markers of T cells, B cells and dendritic cells in the affected lymph nodes after rIL-21 treatment as measured by immunohistochemistry staining
- To describe cytotoxicity of natural killer (NK) cells as well as frequency and absolute numbers of blood cells staining for cell markers related to the pharmacodynamic responses to rIL-21 in peripheral blood before, during and after treatment with rIL-21, as measured by flow cytometry of peripheral blood mononuclear cells
- To describe levels of soluble CD25 (sCD25) and other serum markers
- To compare fine needle aspirates in the affected lymph nodes before and after rIL-21 treatment by quantitative reverse transcriptase polymerase chain reaction of a selected panel of genes specific for T cells and B cells, as well as various cytokines, effectors molecules, chemokines and receptors
- To investigate the importance of variation in genes that transmit/transduce signals of rIL-21 on the effects of rIL-21 treatment
- To describe morphological and functional changes in the affected lymph nodes during treatment as assessed by ultrasonography

Relapse-free survival

- To evaluate relapse-free survival

Safety

- To describe the safety of neo-adjuvant and adjuvant subcutaneous administration of rIL-21 in subjects with stage III malignant melanoma
- To assess if antibodies against rIL-21 are induced during therapy

Methodology

The trial was designed as a single-centre, open-label, single-arm exploratory trial of human rIL-21 administered subcutaneously as local neo-adjuvant treatment for four weeks prior to sentinel lymph node biopsy/complete lymph node dissection, followed by eight weeks of systemic adjuvant treatment.

Administration of rIL-21 was planned to take place three times weekly during 4 weeks, followed by 2 weeks of recovery and a repeated exposure period of 8 weeks, arriving at a total treatment period of 14 weeks.

A total of 6 mg rIL-21 (0.6 mg/mL) was delivered at each injection event, corresponding to 80 µg/kg body weight (Appendix 16.2.8). During the first treatment period, injections were given in the area where the primary tumour had been located before surgery. During the second treatment period, injections were given in the abdominal wall with the exception of one occasion when the injection was given in the [redacted] (Appendix 16.2.8).

During the trial blood samples were drawn for the assessment of safety parameters, effect of rIL-21 on various biomarkers, and anti-rIL-21 antibodies (Appendix 16.2.8).

Number of Subjects Planned and Analysed

The decision to cancel the trial was made by Novo Nordisk as part of a strategic decision for the company to exit oncology as a therapeutic area. This communication was made shortly after one subject had been enrolled. Hence the trial included only one subject, who was withdrawn before completion due to a serious adverse event (hepatitis toxic) (please refer to the Safety Results section).

Diagnosis and Main Criteria for Inclusion

Key inclusion criteria:

1. Confirmed histologically stage III malignant melanoma according to the American Joint Committee on Cancer (AJCC) 2006, within the following classification: T1-4b
2. 18 years of age or above, an Eastern Cooperative Oncology Group performance status of 0 or 1
3. At least one lymph node with only partial malignant involvement as assessed by ultrasonography and verified by fine needle aspiration cytology
4. Kidney, liver and haematology status: S-creatinine ≤ 1.8 mg/dL. Alanine transaminase (ALT) and alkaline phosphatase (ALP) ≤ 2.5 times the upper limit of normal (ULN); lactate dehydrogenase (LDH) ≤ 2 times the ULN. White blood cell count ≥ $2.5x10^9$/L; absolute neutrophil count ≥ $1.5x10^9$/L; platelet count ≥ $100x10^9$/L; haemoglobin ≥ 100 g/L

Key exclusion criteria:

1. Any signs of stage IV disease according to AJCC 2006 as assessed by routine radiographic staging
2. Bulky lymph nodes metastases larger than 2 cm in longest diameter as assessed by ultrasonography
3. Clinically significant infection in the area of the primary tumour
4. Prior wide excision of the primary lesion
5. Documented positive serologic testing for hepatitis B or C
6. History of or active presence of auto-immune diseases (except vitiligo and treated pernicious anaemia)
7. History of any other active malignancy incl. ocular malignant melanoma (except basal cell carcinoma of the skin and *in situ* cervical cancer) within five years of enrolment
5. Cardiac disease within the last 12 months defined as: decompensated heart failure (New York Heart Association class III or IV), unstable angina pectoris, serious arrhythmias or myocardial infarction

Test Product, Dose and Mode of Administration, Batch Number

Recombinant human IL-21 (0.6 mg/mL) was administered by subcutaneous injections (Appendix 16.2.8). A total of 6 mg rIL-21 was delivered at each injection event, corresponding to 80 µg/kg body weight. Batch number: PD06050.

Duration of Treatment

Recombinant IL-21 was administered three times weekly during 4 weeks, followed by 2 weeks of recovery and a

repeated exposure period of 4 weeks. Thus, the total treatment duration was 10 weeks (Appendix 16.2.8).

Reference Therapy, Dose and Mode of Administration, Batch Number

No reference therapy was applied.

Criteria for Evaluation – Efficacy

- Size of malignant involvement in the sentinel lymph nodes (SLN)
- Relapse-free survival
- Leukocyte count; distribution, differentiation and activation of lymphocyte subpopulations
- NK cell cytotoxicity
- T cell cytokine expression of interferon-gamma (IFN-γ), interleukin (IL)-13 and IL-17
- Serum concentration of antibodies against rIL-21

Criteria for Evaluation – Safety

- Adverse events
- Physical examination
- Vital signs
- Electrocardiogram (ECG) monitoring
- Clinical laboratory tests (haematology, biochemistry and urinalysis)

Statistical Methods

Primary efficacy endpoint:

- Effect of rIL-21 on tumour size within the SLN

Secondary efficacy endpoint:

- Effect of rIL-21 on the following biomarkers: leukocyte count; distribution, differentiation and activation of leukocyte subpopulations; T cell cytokine expression; NK cell toxicity

Safety endpoints:

- Adverse events, vital signs (blood pressure and heart rate), ECG, clinical laboratory variables (haematology, biochemistry and urine analysis), body temperature, body weight and physical examination

Statistical analyses:

Since only one subject was involved in the trial, no formal statistical analyses were applied.

Demography of Trial Population

The subject was a [redacted] at the age of [redacted], weighing [redacted] kg (Appendix 16.2.4), diagnosed with a stage IIIA [redacted] melanoma (Appendix 16.2.8).

Deviation from the exclusion criteria:

In accordance with standard procedures at the trial site, a wide excision of the primary tumour was performed on the enrolled subject. This took place before treatment with trial product was initiated, thereby violating exclusion criterion #4 listed above (Appendix 16.2.8). However, in the present case, it was not anticipated that the excision should [redacted]. Furthermore, the wide excision would not compromise the safety of the subject and it was therefore decided that the subject could continue participating in the trial (Appendix 16.2.2).

Efficacy Results

Tumour response

A high-resolution ultrasonography (US) and fine needle aspiration cytology (FNAC) was performed at Visit 1 and identified the presence of malignant cells in the sentinel lymph nodes (SLN). The presence of malignant cells in the fine needle aspirate was confirmed by a trained histopathologist prior to enrolment. Experience with high-resolution US and FNAC from the clinical site has shown that the specificity of a positive result is very high[1] (99%; 317/321 patients), meaning that malignant cells identified by high-resolution US and FNAC will predict presence of malignancy within the SLN biopsy performed subsequently. Complete lymph node dissection was performed on the subject after 4 weeks of neoadjuvant treatment and [redacted] lymph nodes were examined by immunohistochemistry. No malignant cells could be identified, and pathology assessments thereby classified the patient as a complete responder (Appendix 16.2.8).

Biomarkers

Flow cytometry analyses were performed on blood samples obtained at three occasions: prior to rIL-21 exposure (base level sampling), on the day of the third administration or rIL-21 (first sampling), and five days after completion of the first four-week treatment period (second sampling). For further details, please refer to Appendix 16.2.8.

- In line with previous results obtained in clinical trials with rIL-21, absolute numbers of leukocytes and lymphocytes were initially decreased (first sampling), but returned to levels above baseline (second sampling).
- The absolute numbers of $CD4^+$ T-cells and $CD8^+$ T-cells decreased (first sampling), but returned to levels above baseline (second sampling).
- The absolute numbers of NK-cells decreased (first and second sampling).
- The absolute numbers of B-cells increased slightly (first and second sampling)

Anti-rIL-21 antibodies

- No antibodies against rIL-21 were detected (Appendix 16.2.8)

Relapse-free survival

- The subject presented no signs of tumour relapse between surgery and the final visit (Appendix 16.2.8)

Safety Results

- A total number of 14 adverse events, of which 1 was defined as serious (hepatitis toxic), were reported in the 1 subject enrolled. A total of 8 adverse events were stated as probably, possibly or definitely related to the administration of rIL-21. Of these, only the single serious adverse event was graded as ≥3 according to the Common Terminology Criteria for Adverse Events scale (Appendix 16.2.7).
- The vital signs remained virtually unchanged with only minor fluctuations. Temporary reductions of creatinine, bicarbonate and LDH were recorded but not classified as adverse events (Appendix 16.2.8). Non-serious adverse events reported as possibly, probably or definitely related to the administration of rIL-21 were: injection site reaction and erythema, platelet and lymphocyte count decrease, lymphopenia, blood calcium decrease and nasopharyngitis (Appendix 16.2.7).
- The onset of the serious adverse event (SAE) hepatitis toxic was observed after [redacted] days of treatment ([redacted]), during which a total of [redacted] doses of rIL-21 was administered. No administration of trial product took place hereafter. The SAE was evaluated as possibly related to the trial product (Appendix 16.2.7). [redacted] (Appendix 16.2.8). [redacted] (Appendix 16.2.8).
- [redacted]

Conclusions

- The trial was terminated early and only one subject was included in the trial.
- Complete lymph node dissection was performed after 4 weeks of neoadjuvant treatment and [redacted] lymph nodes were examined by immunohistochemistry. No malignant cells could be identified, and pathology assessments classified the subject as complete responder.
- Observed immunomodulatory effects of rIL-21 comprised variations in the absolute numbers, distribution and activation of lymphocyte subpopulations, NK cell toxicity, HLA-DR surface expression on monocytes and dendritic cells, and T-cell production of IL-13 and IFN-γ
- Measurements of sCD25 were not performed due to the low number of trial participants
- Genetic analyses (genotyping and gene expression profiling) were not performed [redacted]
- Ultrasonography assessment of morphological changes of lymphnodes could not identify any malignant lymph nodes after 4 weeks of neoadjuvant treatment.
- The subject presented no signs of tumour relapse between surgery and the final visit
- The subject was withdrawn from the trial due to a serious adverse event reported as hepatitis toxic. Non-serious adverse events reported as possibly or definitely related to the administration of rIL-21 were: injection site reaction and erythema, platelet and lymphocyte count decrease, lymphopenia, blood calcium decrease and nasopharyngitis. [redacted]

• No antibodies against rIL-21 were detected
References 1 Schafer-Hesterberg G, Schoengen A, Sterry W, Voit C. Use of ultrasound to early identify, diagnose and localize metastases in melanoma patients. Expert Rev Anticancer Ther 2007; 7(12):1707-1716.
The trial was conducted in accordance with the Declaration of Helsinki (amended 2002) and ICH Good Clinical Practice (May 1996).