2 Synopsis

Trial Registration ID-number	**EudraCT number**
NCT00523380	2007-001506-25

Title of Trial

An open–label Phase 2 Trial of Pegylated Liposomal Doxorubicin and rIL-21 in Ovarian Cancer Patients with Persistent or Progressive Disease after, or Relapse within One Year of, Completion of Standard First Line Therapy

This report includes results from the Dose Escalating Part of the Trial

Investigators

There was one principal investigator for each of the trial sites in Germany and France.

Dr [redacted] was appointed as signatory investigator.

Trial Sites

The trial was conducted at 4 sites in Germany and 4 site in France; 8 trial sites in total.

Publications

None

Trial Period	**Development Phase**
04 October 2007 to 07 January 2009	Phase 2

Objectives

Primary Objective:

- To determine a safe dose of human recombinant interleukin (rIL-21) in combination with pegylated liposomal doxorubicin (PLD), and to assess the efficacy of this combination in terms of overall response rate (RR)

Secondary Objectives:

- To evaluate the pharmacokinetics (PKs) of rIL-21 and PLD in subjects treated with a combination of rIL-21 and PLD
- To evaluate the pharmacodynamics and pharmacogenomics in subjects treated with a combination of rIL-21 and PLD
- To assess safety of the treatment in terms of toxicity assessed through clinical adverse events (AEs) and Common Terminology Criteria for Adverse Events (CTCAE) laboratory and non-laboratory toxicities
- To assess progression free survival (PFS)
- To assess health related quality of life (HRQL)
- To assess if antibodies against rIL-21 are induced during treatment

The trial was prematurely terminated due to sponsor's decision to outlicence the further development of rIL-21 and therefore, only the dose escalation and tolerability part of the trial was completed and not the efficacy part. The assessment of the efficacy of the combination of rIL-21 and PLD in terms of overall RR and PFS was not part of the dose escalating part of the trial and therefore not presented in this Clinical Trial Report.

Methodology

This was a 2-stage sequential, open-label, multinational phase 2 trial with an initial dose escalation and tolerability component of rIL-21 combined with PLD followed by an efficacy component. Because the trial was prematurely discontinued after dose escalation up to 30 µg/kg rIL-21 due to the sponsor's decision to out licence the further developments of IL-21, only the dose escalating part of the trial was performed and not the efficacy part. Both rIL-21 and PLD were given intravenously (i.v.) to subjects with ovarian cancer (OC) with persistent or progressive disease (PD) after, or relapse within 1 year of, completion of standard first-line therapy to explore efficacy. Subjects were treated with one PLD dose administered i.v. at Day 1 every 4 weeks, combined with rIL-21 i.v. doses at Days 1-5 and Days 15-19 in every treatment cycle (1 cycle = 4 weeks). Each treatment cycle had 2 treatment periods of 5 days and 2 periods of 9 days off drug (5+9) schedule. Days 1 and 15 in each cycle were always on a Monday. All doses of PLD and rIL-21 were administered at the trial site. At Visit 2 in Cycle 1, the subjects were hospitalised for 24 h after the first dose administration of rIL-21 and PLD for observation of safety, PK and pharmacodynamic. At Days 2-5 and 15-19 (Visits 3-11), the subjects were observed for 1 h after administration of rIL-21. At Visit 12 in Cycle 2 the

subject were observed for 4 h after administration of PLD and rIL-21. At subsequent cycles the subject could leave the hospital after 1 h following completion of treatment.

Subjects were tumour assessed every 2 cycles and continued in the trial, if treatment was safely tolerated, until objective disease progression according to the Response Evaluation Criteria in Solid tumours (RECIST) criteria or the Gynaecologic Cancer Intergroup (GCIG) criteria, or up to a maximum of 6 treatment cycles. Safety and tolerability were monitored throughout the trial, and up until 4 weeks after last dose of rIL-21.

Number of Subjects Planned and Analysed

Planned numbers of subjects to be started on trial products were 95-100. The numbers of subjects included in the trial are shown below.

Subjects		**Full Analysis Set N (%)**
Screened		14
Included	Eligible	9 (90.0)
	Not Eligible	1 (10.0)
Trial	Exposed	10 (100.0)
	Withdrawn	7 (70.0)
	Adverse Event	2 (20.0)
	Non-compliance	0 (0.0)
	Withdrawal Criteria	5 (50.0)
	Other	0 (0.0)
	Completed	3 (30.0)

Diagnosis and Main Criteria for Inclusion

Subjects with advanced epithelial OC (stage IIB-IV), histologically confirmed according to American Joint Committee on Cancer (AJCC); persistent or PD after, or relapse within 1 year of completion of first line platinum-containing chemotherapy; at least 1 measurable lesion ≤ 5 cm in the largest diameter according to RECIST criteria or assessable disease defined as: a one-dimensional measurable lesion, mass with margins not clearly defined, lesions with both diameters 0.5 cm or less on radiographic imaging, palpable lesions under 2 cm or malignant ascites respectively combined with a 2 x Upper normal limit (UNL) (in the absence of cirrhosis) of cancer antigen-125 (CA-125) measured within 2 weeks prior to inclusion in the trial and Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 were included in the trial.

Test Product, Dose and Mode of Administration, Batch Number

The trial product (rIL-21, 10 mg/mL for injection, 0.8 mL per vial) and sterile saline (sodium chloride 0.9% w/v) for dilution of rIL-21 at the 3 µg/kg dose level were supplied. Recombinant human interleukin-21 (rIL-21, 10 mg/mL) was administered as a single i.v. bolus injection over a few seconds in the morning. At the dose of 30 µg/kg, the trial product was used undiluted. In case of a dose reduction, a lower rIL-21 dose (10 µg/kg) was administered and the trial product was diluted to 1 mg/mL in sodium chloride (0.9% w/v, delivered by the pharmacies at the trial sites) prior to injection. Batch no.: PD06050.

Duration of Treatment

Subjects were treated with one PLD dose administered i.v. at Day 1 every 4 weeks, combined with rIL-21 i.v. doses at Days 1-5 and Days 15-19 in every treatment cycle (1 cycle = 4 weeks). Each treatment cycle had 2 treatment periods of 5 days and 2 periods of 9 days off drug (5+9) schedule. Days 1 and 15 in each cycle were always on Monday. All doses of PLD and rIL-21 were administered at the trial site. At Visit 2 in Cycle 1, the subjects were hospitalised for 24 h after the first dose administration of rIL-21 and PLD for observation of safety, PK and pharmacodynamic. At Days 2-5 and 15-19 (Visits 3-11) the subjects were observed for 1 h after administration of rIL-21. At Visit 12 in Cycle 2 the subject were observed for 4 h after administration of PLD and rIL-21. At subsequent cycles the subject could leave the hospital after 1 h following completion of treatment.

Reference Therapy, Dose and Mode of Administration, Batch Number

Pegylated liposomal doxorubicin was supplied as 2 mg/mL doxorubicin hydrohloride in a pegylated liposomal

formulation in vials containing a withdrawable volume of 10 mL. The batch number of PLD was 62517117.

Criteria for Evaluation – Efficacy

- PK of rIL-21 and PLD
- Biomarkers - soluble IL-21 receptor (CD25), cell markers on T cells, regulatory T cells, natural killer (NK) cells, NK cell cytotoxicity and markers and genotyping of the rIL-21 receptor
- Tumour response
- Anti-rIL-21 antibodies
- Health-related quality of life

Criteria for Evaluation – Safety

- Adverse events
- Physical examination
- Vital signs
- Electrocardiogram (ECG) monitoring and Multiple Gated Acquisition Scan (MUGA)
- Clinical laboratory tests (haematology, biochemistry and urine analysis)

Statistical Methods

Primary endpoint:

The primary endpoint was AEs/toxicity to the combination of rIL-21 and PLD, assessed using the CTCAE version 3.0.

Secondary endpoints:

- Safety: effect of rIL-21 on vital signs (BP and heart rate), ECG, MUGA scan, body temperature, haematology, biochemistry and urine analysis, body weight and physical examination
- PK profiles of rIL-21 and PLD: AUC, AUC_{0-24}, AUC_{0-24} (only for PLD), C_{5min} CL, MRT, $t_{½}$, V_z, V_{ss}
- Biomarkers: effect of rIL-21on sCD25 and genotyping of the IL-21 receptor
- Efficacy: effect of rIL-21 on tumour size, determined as Complete Response (CR), Partial Response (PR), Stable Disease (SD) or PD according to the RECIST criteria
- Changes in anti-rIL-21 antibody serum concentration
- Health-related quality of life

Statistical analyses of the primary endpoint:

No formal statistical analysis was performed. The primary endpoint is presented by summary tables and listings.

Statistical analyses of the secondary endpoint:

All PK parameters were derived using non-compartmental methods. The hypothesis of dose proportionality for AUC, AUC_{0-24h} and C_{5min} of rIL-21 at treatment Cycles 1 and 2 were tested using a linear normal model for the logarithm to the endpoint including logarithm to dose as a covariate, cycle and the interaction cycle*logarithm to dose as fixed effects and subject as a random effect. The PK endpoints for rIL-21 and PLD at treatment Cycles 1 and 2 and the ratios of AUC obtained in treatment Cycle 2 versus 1 for rIL-21 and PLD were summarised and listed by dose level. The individual and mean serum/plasma concentration-time profiles for rIL-21 and PLD were presented graphically.

Statistical analyses of biomarkers were performed using a linear normal model including time, dose level and the interaction between time and dose level as fixed effects and subject as a random effect. In addition to these general statistical analyses contrasts (comparisons) describing specific changes over time, like variation between rIL-21 dosing periods and between time points within dosing periods were also explored.

The sum of longest diameter (SLD) of target lesions and the percentage change in SLD of target lesions after start of treatment were summarised by dose level and visit. The overall tumour response was also summarised by dose level and visit. In addition, the percent change in SLD of target lesions from baseline to Week 8 and the best overall percent change from baseline in SLD of target lesions during the entire trial were presented graphically by subject with information on the dose level and the overall tumour response. The FACT-O and FACT-BRM assessments were listed by dose level and subject.

No formal statistical analyses were performed for the secondary safety endpoints but they were analysed using

descriptive statistics and presented graphically.

Demography of Trial Population

All 10 exposed subjects were White (2 from Germany and 8 from France). Subjects were between 42 and 74 years of age (median was 64 years) and body weight for the subjects ranged from 46 to 106 kg (mean was 67.6 kg).

Efficacy Results

Pharmacokinetics

- A linear increase in AUC was observed with escalating doses of rIL-21 following single and multiple dosing with 3 to 30 µg/kg rIL-21 in combination with PLD but dose proportionality was only demonstrated for multiple dosing with rIL-21 with wide CIs
- No apparent interaction between the PK profile of rIL-21 and PLD following multiple rIL-21 dosing was observed
- There was no unexpected accumulation of rIL-21 after multiple dosing compared to single dosing when rIL-21 was administered in combination with PLD
- Slightly lower mean $t_{½}$ was observed after single dosing with 3 to 30 µg/kg rIL-21 (ranging from 1.3 to 2.3 h) than after multiple dosing with rIL-21 (ranging from 2.6 to 4.5 h)
- The AUC and C_{max} of PLD remained unchanged following single and multiple dosing with rIL-21 at doses up from 3 to 30 µg/kg

Tumour Response

- Of the 9 subjects evaluable for tumour response, the best overall tumour response was PR in 1 subject, SD in 4 subjects and PD in 4 subjects

Biomarkers

- Serum levels of sCD25 increased significantly upon rIL-21 treatment. In addition, baseline levels of sCD25 steadily and significantly increased between dosing periods following the '5+9' dose regimen
- Statistically significant redistributions of NK-cells, cytotoxic T-cells, B-cells, and monocytes as assessed by:
  - Statistically significant drops in absolute numbers of NK-cells ($CD45^+/CD3^-/CD56^+$), cytotoxic T-cells ($CD45^+/CD3^+/CD4^-/CD8^+$), and T-helper cells ($CD45^+/CD3^+/CD4^+/CD8^-$)
  - A minor but statistically significant increase in the fraction of regulatory T cells (%$CD25^+/FoxP3^+$ of $CD4^+$)
  - A minor but statistically significant acute increase in the absolute numbers of B-cells ($CD45^+/CD19^+$) on Day 5 of dosing
  - A statistically significant increase in the absolute number of monocytes ($CD45^+/CD14^+$)
- Statistically significant induction of apoptosis in NK-cells and cytotoxic T-cells but not in T-helper cells as assessed by:
  - Statistically significant increase in fraction of apoptotic NK-cells (%$Caspase3^+$ of $CD3^-/CD16^+/CD56^+$) and cytotoxic T-cells (%$Caspase3^+$ of $CD3^+/CD8^+$)
  - No statistically significant effects of rIL-21 dosing on apoptosis of T-helper cells (%$Caspase3^+$ of $CD3^+/CD8^-$)
- Statistically significant increase in NK-cell mediated kill of tumour cells
- No conclusions on pharmacogenomic data were drawn due to the low number of subjects included in the trial

Anti-rIL-21 Antibodies

- None of the subjects developed anti-rIL-antibodies

Health-related Quality of Life

- No conclusions on HRQL were drawn due to the low number of subjects included in the trial

Safety Results

- In the current trial, 10 µg/kg rIL-21 administered i.v. in combination with PLD was the highest tolerated dose tested
- Two subjects exposed to 30 µg/kg rIL-21 experienced DLTs. One subject reported increased GGT as a DLT; and another subject reported pruritus, [redacted] as DLTs
- During the entire trial, 224 AEs were reported in 10 subjects. The majority of the AEs were of severity grade 1 (131 in total) followed by grade 2 (75 in total). Seven (7) subjects reported 17 AEs of grade 3 severity and 1 subject experienced an AE of grade 4 severity

- Most commonly reported AEs related to rIL-21 were pyrexia, asthenia and headache, which were all observed in more than 50% (5 or more of the 10 subjects) exposed. Nausea was observed in 40% of subjects. Most commonly reported AEs related to PLD were asthenia, nausea and headache which were all observed in more than 50% of the subjects exposed
- A total of 2 SAEs (reported as pyrexia and pleural effusion) were observed in 1 subject dosed with 3 µg/kg rIL-21. Both SAEs were of grade 3 severity. Pyrexia was evaluated as possibly related to both rIL-21 and PLD and pleural effusion was evaluated as unrelated to both rIL-21 and PLD. [redacted]
- No deaths were reported in the trial
- Decreases in haemoglobin, platelets, lymphocytes and neutrophils and sporadic increases in ALT and AST were observed. Changes in laboratory values appeared to be related to rIL-21 administration as values returned to or approached baseline levels during the 9-days rest and tended not to sustain after the treatment. The CA-125 levels remained unchanged and above normal range for most subjects throughout the entire trial
- No clinically relevant changes in vital signs and ECG were observed during the trial
- Abnormal, clinically significant physical examination findings were observed in 9 subjects throughout the trial but all were reported as AEs unless the findings were evaluated as a progression of disease

Conclusions

- The MTD was not determined according to the protocol, as it was decided not to include more subjects at the 10 µg/kg dose level. The 10 µg/kg rIL-21 in combination with PLD was, however, tolerated in 3 subjects. The efficacy of rIL-21 in combination with PLD was not determined in terms of overall RR due to premature termination of the trial but was, however, determined in terms of best overall tumour response. Of the 9 subjects evaluable for tumour response, the best overall tumour response was PR in 1 subject, SD in 4 subjects and PD in 4 subjects
- A linear increase in AUC was observed with escalating doses of rIL-21 following single and multiple dosing with 3 to 30 µg/kg rIL-21 in combination with PLD but dose proportionality was only demonstrated for multiple dosing with rIL-21 with wide CIs. No apparent interaction between the PK profile of rIL-21 and PLD following multiple rIL-21 dosing was observed. There was no unexpected accumulation of rIL-21 after multiple dosing compared to single dosing when rIL-21 was administered in combination with PLD. Slightly lower mean $t_{½}$ was observed after single dosing with 3 to 30 µg/kg rIL-21 (ranging from 1.3 to 2.3 h) than after multiple dosing with rIL-21 (ranging from 2.6 to 4.5 h). The AUC and C_{max} of PLD remained unchanged following single and multiple dosing with rIL-21 at doses from 3 to 30 µg/kg
- Serum levels of sCD25 increased significantly upon rIL-21 treatment. In addition, baseline levels of sCD25 steadily and significantly increased between dosing periods following the '5+9' dose regimen. Key findings of biomarker data were:
  - Statistically significant redistributions of NK-cells, cytotoxic T-cells, B-cells, and monocytes as assessed by:
    - Statistically significant drops in absolute numbers of NK-cells ($CD45^+/CD3^-/CD56^+$), cytotoxic T-cells ($CD45^+/CD3^+/CD4^-/CD8^+$), and T-helper cells ($CD45^+/CD3^+/CD4^+/CD8^-$)
    - A minor but statistically significant increase in the fraction of regulatory T cells (%$CD25^+/FoxP3^+$ of $CD4^+$)
    - A minor but statistically significant acute increase in the absolute numbers of B-cells ($CD45^+/CD19^+$) on Day 5 of dosing
    - A statistically significant increase in the absolute number of monocytes ($CD45^+/CD14^+$)
  - Statistically significant induction of apoptosis in NK-cells and cytotoxic T-cells but not in T-helper cells as assessed by:
    - Statistically significant increase in fraction of apoptotic NK-cells (%$Caspase3^+$ of $CD3^-/CD16^+/CD56^+$) and cytotoxic T-cells (%$Caspase3^+$ of $CD3^+/CD8^+$)
    - No statistically significant effects of rIL-21 dosing on apoptosis of T-helper cells (%$Caspase3^+$ of $CD3^+/CD8^-$)
  - Statistically significant increase in NK-cell mediated kill of tumour cells

- No conclusions on pharmacogenomic data were drawn due to the low number of subjects included in the trial
- A total of 2 subjects exposed to 30 µg/kg rIL-21 experienced DLTs. One (1) subject reported increased GGT as a DLT; and another subject reported pruritus, [redacted] as DLTs. No deaths were reported in the trial. No clinically relevant changes in vital signs and ECG were observed during the trial
- The PFS was not determined according to the protocol due to premature termination of the trial
- No conclusions on HRQL were drawn due to the low number of subjects included in the trial
- None of the subjects developed anti-rIL-21-antibodies

The trial was conducted in accordance with the Declaration of Helsinki (amended 2004) and ICH Good Clinical Practice (May 1996).