2 Summary

Title
Assessment of Neublastin-Induced Skin and Sensory Alterations and Headache in Healthy Subjects (Part A) and Migraine Patients (Part B).

Short Title
Neublastin Challenge in Healthy Subjects and Migraine Patients

Principal investigator & Trial Site
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Background & Rationale
Glial cell-line derived neurotrophic factor receptor alpha-3 (GFRα3) is 1 of 4 members of the glial cell line-derived neurotrophic factor (GDNF) receptor alpha family. These receptors are glycosylphosphatidylinositol (GPI)-linked proteins expressed in the central and peripheral nervous systems and are involved in the sensitization of pain-sensing neurons known as nociceptors (Baloh et al., 1998; Naveilhan et al., 1998; Worby et al., 1998). Artemin, the only known ligand for GFRα3 (Baloh et al., 1998), is a member of the family of GDNF ligands, which also includes GDNF, neurturin, and persephin (Baudet et al., 2000). In adults, GFRα3 receptors are localized in dorsal root, trigeminal and sympathetic ganglia, as well as peripheral nerves and gut (Bespalov & Saarma, 2007). Preclinical findings suggest that modulating the artemin-GFRα3 pathway may provide an analgesic effect in patients with chronic pain conditions.

While the relevance of the GFRα3-artemin pathway outside of pain is poorly understood, the clinical experience from Neublastin (a recombinant human artemin protein of 102/103 amino acid homodimer; BG00010, Biogen, Inc.) provides clues to the supraphysiological effects of this pathway. Clinical studies in which Neublastin was administered intravenously (IV) and/or subcutaneously (SC) in healthy volunteers and patients with sciatica or painful lumbosacral radiculopathy resulted in reports of pruritus, headache, rash, and abnormal sensation of feeling hot in those who received Neublastin compared to placebo. Healthy volunteers who received systemically administered Neublastin also reported significantly higher incidence of headache compared to the placebo group (Okkerse et al., 2016; Rolan et al., 2015). These observations suggest a role for the GFRα3-artemin pathway in patients with intractable pruritus and headache states such as migraine. Thus, there is rationale to consider artemin and a GFRα3 antagonist to treat migraine (Backonja, Williams, Miao, Katz, & Chen, 2017; Okkerse et al., 2016; Rolan et al., 2015).

This study was a Neublastin-challenge in healthy subjects and in patients suffering from episodic migraine to confirm and to quantify phenotypic effects associated with activation of the artemin/GFRα3 pathway with a focus on headache, hypoalgesia, pruritus, and rash. These results may improve the understanding of the mechanism of action and to identify pharmacodynamic biomarkers and potential indications for clinical follow-up studies with a GFRα3 antagonist.

Objective(s)

Primary Objectives

1. To assess pruritus and rash after administration of Neublastin or placebo in healthy subjects and migraine patients (Parts A and B)

2. To assess headache and other migraine-associated symptoms after administration of Neublastin or placebo in migraine patients (Part B)

Secondary Objectives

1. To assess Neublastin-induced mechanical and thermal sensory responses using nociceptive thresholds in healthy subjects and migraine patients (Parts A and B)
2. To assess headache and other migraine-associated symptoms in healthy subjects upon administration of Neublastin or placebo (Part A)
3. To assess changes in temperature perception upon administration of Neublastin or placebo in healthy subjects and migraine patients (Parts A and B)
4. To characterize the pharmacokinetics (PK) profile of a single IV dose of Neublastin in healthy subjects and in migraine patients (Parts A and B)
5. To characterize the safety and tolerability of a single IV dose of Neublastin in healthy subjects and in migraine patients (Parts A and B)
6. To characterize the safety and tolerability of Neublastin administered intradermally (ID) in healthy subjects (Part A)

Design
This was a single-center, randomized, double-blinded, placebo-controlled study of IV and ID administered Neublastin in healthy volunteers (Part A) and episodic migraine patients (Part B).

- **Part A** enrolled 36 subjects in 2 different groups. In Group 1, 24 healthy volunteers were randomized (1:1) to a single IV administration of up to 150 µg/kg Neublastin or placebo. In Group 2, 12 healthy subjects were randomized (1:1) to left/right leg (ID Neublastin or placebo) or left/right leg (ID placebo or Neublastin) over tibialis anterior (single administration of 5 µg, 20 µg or 100 µg). All subjects in Group 2 also received an ID dose of Neublastin in the flank or upper back area.
- **Part B** had a cross-over design. A total of up to 12 migraine patients were randomized to 1 of 2 IV treatment sequences of a single 50 µg/kg IV dose (first 6 patients) or a single IV dose of 150 µg/kg (remaining 6 patients) of Neublastin or placebo. Six patients received Neublastin first and placebo second, and 6 patients received placebo first and Neublastin second.

Investigational drug
Neublastin (also known as BG00010) acts as a selective ligand for the GFRa3 receptor. Neublastin was supplied as a liquid drug product in vials containing 5.0 mL of 1.6 mg/mL Neublastin. The formulation was as follows: 1.6 mg/mL Neublastin in 10 mM Succinate, 75 mM Sodium Chloride, 10 mM L-Arginine HCl, pH 5.5.

Comparative drug
Placebo consisted of a 0.9% sodium chloride solution for IV infusion or ID administration. Placebo was not visually distinguishable from the active investigational drug.

Statistical methodology
The study was exploratory in nature and intended to provide estimates of the primary and secondary endpoints in response to Neublastin challenge. All safety and statistical programming was conducted with SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Study population

Part A – Healthy Subjects
Eighty-nine (89) healthy subjects were screened of which 44 subjects were included into Part A of the study. Six (6) subjects were reserve subjects and never got dosed, 2 subjects were replaced prior to first dosing. In total, 36 subjects completed the treatment and follow-up period per protocol: 24 subjects were randomized to Group 1 in which 12 subjects received a single IV administration of Neublastin and 12 subjects received single IV administration of placebo. The remaining 12 subjects were randomized to Group 2 in which 6 subjects received an ID administration of Neublastin in the left leg and flank or upper back area (1 subject received 5 µg, 1 subject 20 µg and 4 subjects 100 µg) and placebo in the right leg. The other 6 subjects received an ID administration of Neublastin on the right leg and flank or upper back area (2 subjects received 5 µg, 2 subjects 20 µg and 2 subjects 100 µg) and placebo in the left leg. Demographics and baseline characteristics were comparable across both groups.

Part B – Migraine patients
Forty-three (43) subjects with migraine were screened of which 17 subjects were included into Part B of the study. Five (5) subjects were excluded prior to first dosing due to a migraine attack.
In total, 12 subjects completed the treatment and follow-up period per protocol: 6 subjects were randomized to receive a single 50 µg/kg IV dose of Neublastin and the other 6 subjects to receive a single 150 µg/kg IV dose of Neublastin. As per the crossover design, all subjects also received a placebo administration. Demographics and baseline characteristics were comparable across both groups.

Safety results
No deaths or treatment-related SAEs occurred and no subjects withdrew from the study due to AEs, with the exception of 5 excluded migraine patients (Part B) who experienced a migraine attack prior to first dosing. There were no safety concerns from routine chemistry, haematology and glucose parameters, urinalysis or ECG recordings after a single IV dose of Neublastin (50 or 150 µg/kg) or single ID dose of Neublastin (5µg, 20 µg or 100µg). In both study parts, all AEs were mild or moderate of severity and abated either without medication or with symptom-guided treatment, with the exception of 1 non-treatment related SAE reported in Part A.

Part A – *Healthy Subjects*
- The most evident AEs reported are headache and skin reactions (erythema and pruritus), more prominently in the patients receiving IV medication compared to those receiving ID medication.
    - Pruritus was reported by 9/12 subjects (75%) receiving Neublastin IV compared to none in the placebo group. Erythema was reported by 5/12 subjects (41.7%) receiving Neublastin IV, compared to 3/12 subjects (25%) receiving placebo IV.
    - Headache was reported by 4/12 subjects (33.3%) receiving Neublastin IV, compared to 3/12 (25%) of subjects receiving placebo IV.

Part B – *Migraine patients*
- The most prevalent AEs reported were headache, migraine and skin reactions (erythema and pruritus).
    - Erythema was reported by 5/6 subjects (83.3%) after 50µg/kg Neublastin compared to 4/6 subjects (66.6%) after 150µg/kg Neublastin and none after Placebo.
    - Pruritus was reported by 3/6 subjects (50%) after 50µg/kg Neublastin compared to 5/6 (83.3%) after 150µg/kg Neublastin and none after Placebo.
    - Headache was reported by 3/6 subjects (50%) after 50µg/kg Neublastin compared to 4/6b subjects (66.7%) after 150µg/kg Neublastin and 9/12 subjects (75%) after

Placebo.

- Migraine was reported by 4/6 subjects (66.7%) after 50µg/kg Neublastin compared to 3/6 subjects (50%) after 150µg/kg Neublastin and 9/12 subjects (75%) after Placebo.

Pharmacodynamic results

Overall no significant differences were observed in the pharmacodynamic measurements after Neublastin administration in both study parts, except for the (self-reported) itch, rash and headache questionnaire data (Part A and B) and for a minor change in Pressure PTT and TSA Heat Pain Threshold (Part A, IV group). No clear effect on the other questionnaire data (temperature sensations) for either study part was observed. Furthermore, no sensitization of the capsaicin response was observed after Neublastin administration.

Part A - Healthy Subjects

A higher incidence of itch was observed for the Neublastin IV group where 11/12 patients (91.7%) responded yes during the study with 124/335 (37%) days of itch reported, compared to 2/12 patients (16.7%) with 7/329 (2%) days of itch in the placebo group. In addition, slightly more patients were observed experiencing headache with Neublastin IV (8/12 (66.7%) patients with 30/323 (9%) headache days reported with Neublastin IV versus placebo 6/12 (50%) patients with 11/316 (3%) headache days).

Part B – Migraine patients

A higher incidence of itch was observed for the Neublastin 150 µg/kg group where 5/6 patients (83.3%) responded yes during the study with 69/169 (40.8%) days of itch reported, compared to 1/12 patients (8.3%) with 4/326 (1.2%) days of itch in the placebo group. In addition, slightly more rash was observed compared to placebo (5/6 (83.3%) patients with 36/169 (21.3%) rash days reported with Neublastin 150 µg/kg versus placebo 1/12 (8.3%) patients with 6/326 (1.8%) rash days. Furthermore, the incidence of headache observed in the two Neublastin dosing groups were comparable (6/6 (100%) patients with 34/117 (50 µg/kg) and 33/163 (150 µg/kg) (29.1% and 20.2%) headache days reported) and slightly higher than placebo (11/12 patients with 43/316 (13.6%) headache days).

Overall conclusion:

A single IV or ID dose of Neublastin was generally well tolerated with no findings of clinical concern. We concluded that the most reported AEs were headache, migraine and skin reactions after Neublastin administration. Data from this study showed moderate increase in pruritus and rash after Neublastin administration with no alteration of nociceptive thresholds and headache/migraine incidence.

11 Pharmacodynamics Evaluation

11.1 Data Sets Analysed

The analysis population was defined as all subjects who were validated (randomized), received at least one dose of study treatment, and have at least one post-baseline assessment of the parameter being analyzed.

11.2 Demographics and other Baseline Characteristics

Individual demographics and baseline information including medical history information are provided in Appendix 15.1 (Part A) and 15.2 (Part B). No important differences were noted between treatment groups.

11.3 Measurements of Treatment Compliance

Treatments were administered to the subjects under supervision at the clinical research unit, therefore there was full treatment compliance.

11.4 Analysis of Pharmacodynamics

The pharmacodynamic analyses were conducted after completion of the study. The analysis was performed according to the SAP (see Appendix 15.10) and the changes in the planned analysis are described in section 9.7.2. A full overview of the pharmacodynamic results together with a more comprehensive list of summary descriptive statistics can be found in Appendix 5.8 (Part A) and Appendix 15.9 (Part B). The missing questionnaire data that occurred due to questionnaire non-compliance are reported with an “M”. Self-reported questionnaire data on skin and headache complaints were considered PD data.

11.4.1 Pharmacodynamic results

All pharmacodynamics results are provided in the Statistics Reports (15.8 (Part A), 15.9 (Part B)). No overall significant differences were observed in the pharmacodynamic measurements after Neublastin administration for both study parts, except for the questionnaire data (Part A and B) and for a minor change in Pressure PTT and TSA Heat Pain threshold (Part A, IV group). Furthermore, no sensitization of the capsaicin response was observed after Neublastin administration.
Relevant results include:

Part A – Healthy volunteers
A higher incidence of itch was observed for the Neublastin IV group where 11/12 patients (91.7%) responded yes during the study with 124/335 (37%) days of itch reported, compared to 2/12 patients (16.7%) with 7/329 (2%) days of itch in the placebo group, as presented in Table 6. In addition, slightly more patients were observed experiencing headache with Neublastin IV (8/12 (66.7%) patients with 30/323 (9%) headache days reported with Neublastin IV versus placebo 6/12 (50%) patients with 11/316 (3%) headache days).
The levels of itch and headache rated by the subjects during the study period, rated each 24 hours presented in Figures 1 and 2 respectively. The highest level of itch was observed around Day 3 after dosing and the highest level of headache at its worst was observed around Day 16 after dosing. Figure 3 shows a bar-graph of the incidence and intensity of itch in healthy volunteers (IV group). The height of each bar represents the number of subjects that reported itch while each bar is colored according to the intensity. The highest incidence of itch was observed around Day 7-9 after dosing for the Neublastin 150 µg/kg group with the highest level observed at Day 7. In addition, a high level was also observed around Day 19 and 21 after dosing. No clear effect on the other questionnaire (rash and temperature sensations) data for both IV and ID groups were observed. Most of the questions were answered with a “No”

Table 6. Frequency of itch and headache in the IV group during the previous 24 hours.

Treatment	No	Yes	M

Did you itch during the last 24 hours?			
Placebo	322 (98%) (n=12)	7 (2%) (n=2)	19
Neublastin 150 µg IV	211 (63%) (n=12	124 (37%) (n=11)	13
Did you experience a headache during the last 24 hours?			
Placebo	305 (97%) (n=12)	11 (3%) (n=6)	20
Neublastin 150 µg IV	293 (91%) (n=12)	30 (9%) (n=8)	13

N (Placebo)=12, N (Neublastin 150 µg)=12
M=Missing, n= number of subjects experiencing event

Figure 1. Levels of itch in healthy volunteers during the last 24 hours (N (placebo) = 12, N (Neublastin 150µg) =12).

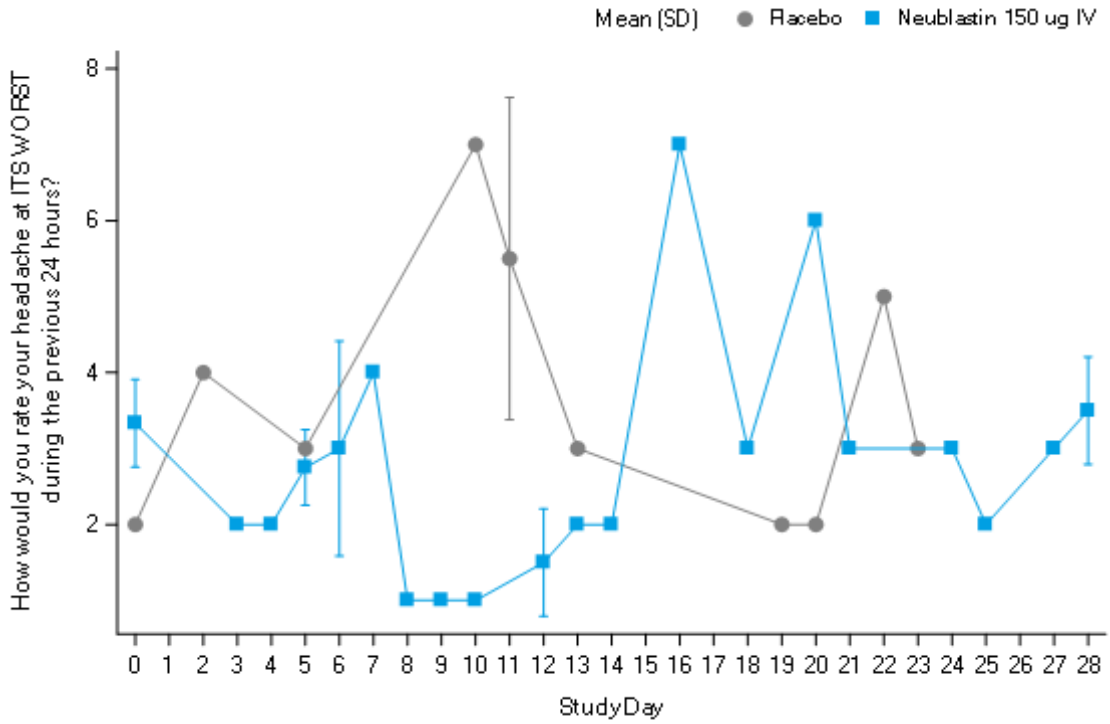

Figure 2. Levels of headache at ITS WORST in healthy volunteers during the last 24 hours (N (placebo) = 12, N (Neublastin 150µg) =12).

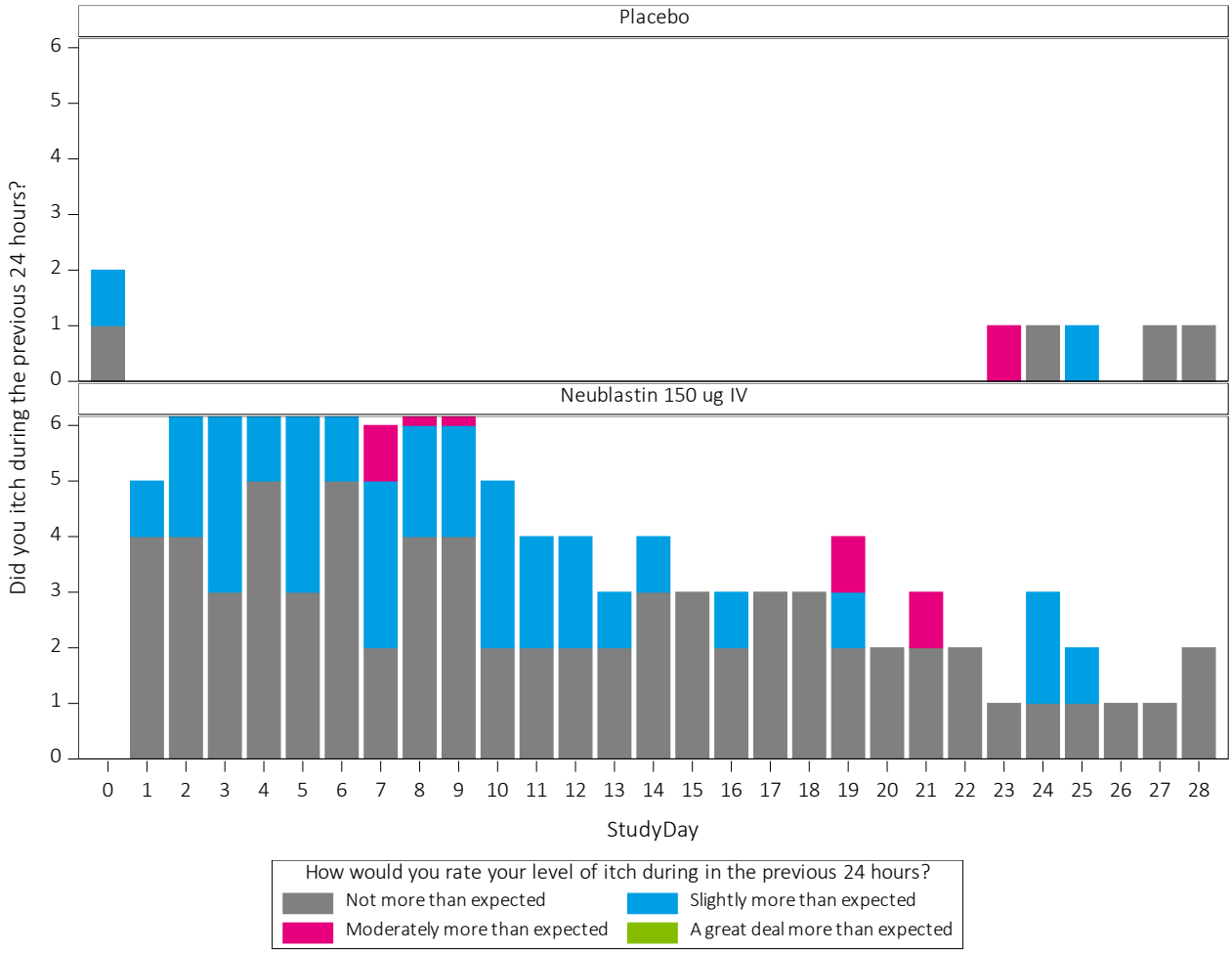

Figure 3. Bargraph incidence and intensity itch in healthy volunteers (IV group)

Part B – Migraine patients

A higher incidence of itch was observed for the Neublastin 150 µg/kg group where 5/6 patients (83.3%) responded yes during the study with 69/169 (40.8%) days of itch reported, compared to 1/12 patients (8.3%) with 4/326 (1.2%) days of itch in the placebo group., as presented in Table 7. The highest level of itch was observed around Day 3 after dosing (150 µg/kg) as presented in Figure 4. In addition, slightly more rash was observed compared to placebo (5/6 (83.3%) patients with 36/169 (21.3%) rash days reported with Neublastin 150 µg/kg versus placebo 1/12 (8.3%) patients with 6/326 (1.8%) rash days. The incidence of headache observed in the two Neublastin dosing groups were comparable (6/6 (100%) patients with 34/117 (50 µg/kg) and 33/163 (150 µg/kg) (29.1% and 20.2%) headache days reported) and slightly higher than placebo (11/12 patients with 43/316 (13.6%) headache days), see Figure 5.

Figure 6 shows a bar-graph of the incidence and intensity of itch in migraine patients. The height of each bar represents the number of subjects that reported itch while each bar is colored according to the intensity. The highest incidence of itch was observed around Day 2-4 after dosing for the Neublastin 150 µg/kg group with the highest level observed at Day 3. Moreover, from the stacked bar graphs, a shift in intensity can also be observed as indicated by an increase of "moderately more than expected" and "a great deal more than expected" answers for Neublastin 50µg/kg and 150µg/kg compared to placebo. This effect was most evident around Day 2-8 after dosing.

No clear effect on the other questionnaire data (temperature sensations) for both IV groups were observed. Most of the questions were answered with a "No".

Table 7. Frequency of itch, rash and headache in the IV group during the previous 24 hours.

Treatment	No	Yes	M
Did you itch during the last 24 hours?			
Placebo	322 (93%) (n=12)	4 (1%) (n=1)	22 (6%)
Neublastin 50 µg IV	140 (80%) (n=6)	15 (9%) (n=5)	19 (11%)
Neublastin 150 µg IV	100 (57%) (n=6)	69 (40%) (n=5)	5 (3%)
Did you experience rash during the last 24 hours?			
Placebo	320 (92%) (n=12)	6 (2%) (n=1)	22 (6%)
Neublastin 50 µg IV	147 (84%) (n=6)	8 (5%) (n=4)	19 (11%)
Neublastin 150 µg IV	133 (76%) (n=6)	36 (21%) (n=5)	5 (3%)
Did you experience headache during the last 24 hours?			
Placebo	273 (81%) (n=12)	43 (13%) (n=11)	20 (6%)
Neublastin 50 µg IV	117 (70%) (n=6)	34 (20%) (n=6)	17 (10%)
Neublastin 150 µg IV	130 (77%) (n=6)	33 (20%) (n=6)	5 (3%)

N (Placebo)=12, N (Neublastin IV 50 µg)=6, N (Neublastin IV 150 µg)=6,
M=Missing, n=number of subjects experiencing event

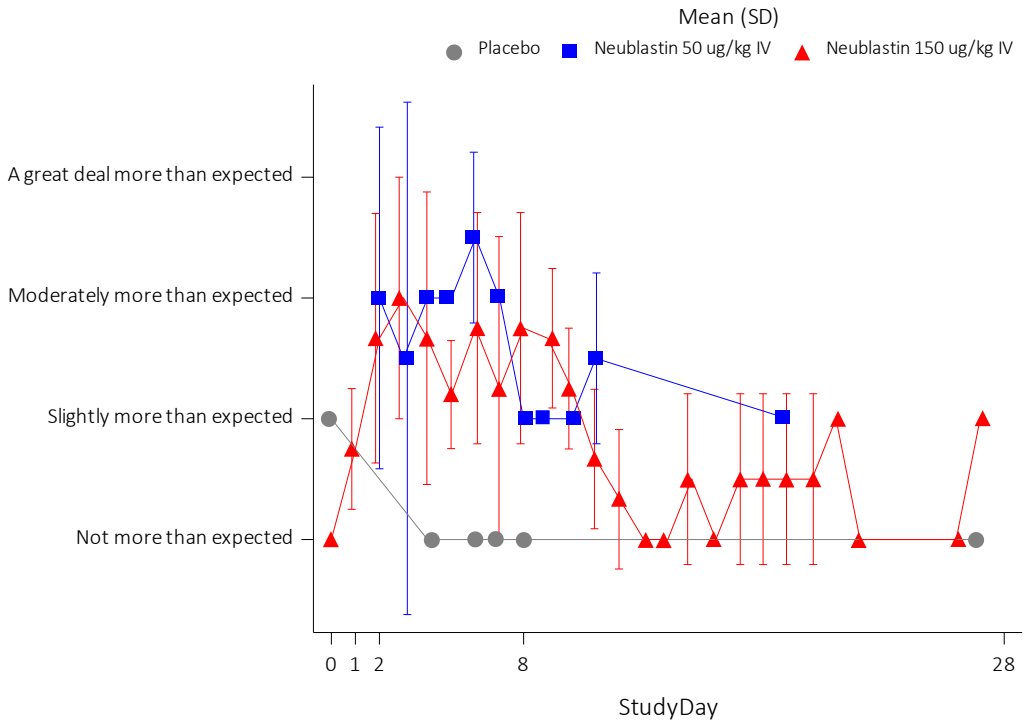

Figure 4. Levels of itch in migraine patients during the last 24 hours: combined graphs 50µg/kg IV Neublastin, 150 µg/kg IV Neublastin and placebo.

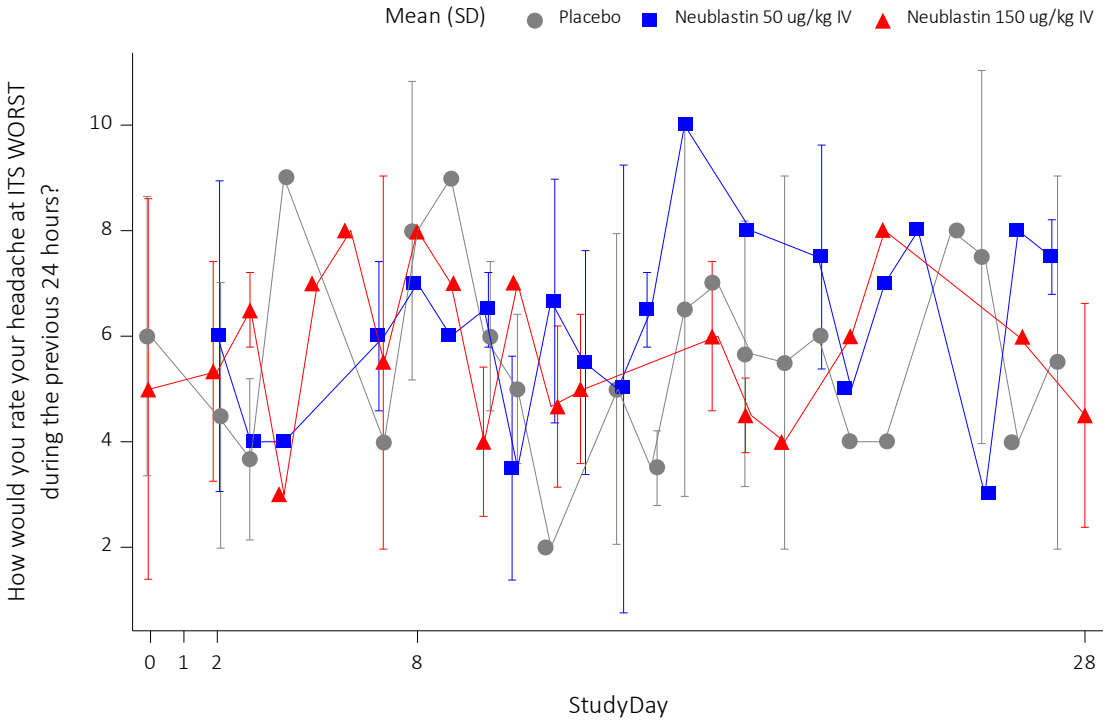

Figure 5. Levels of headache at ITS WORST in migraine patients during the last 24 hours.

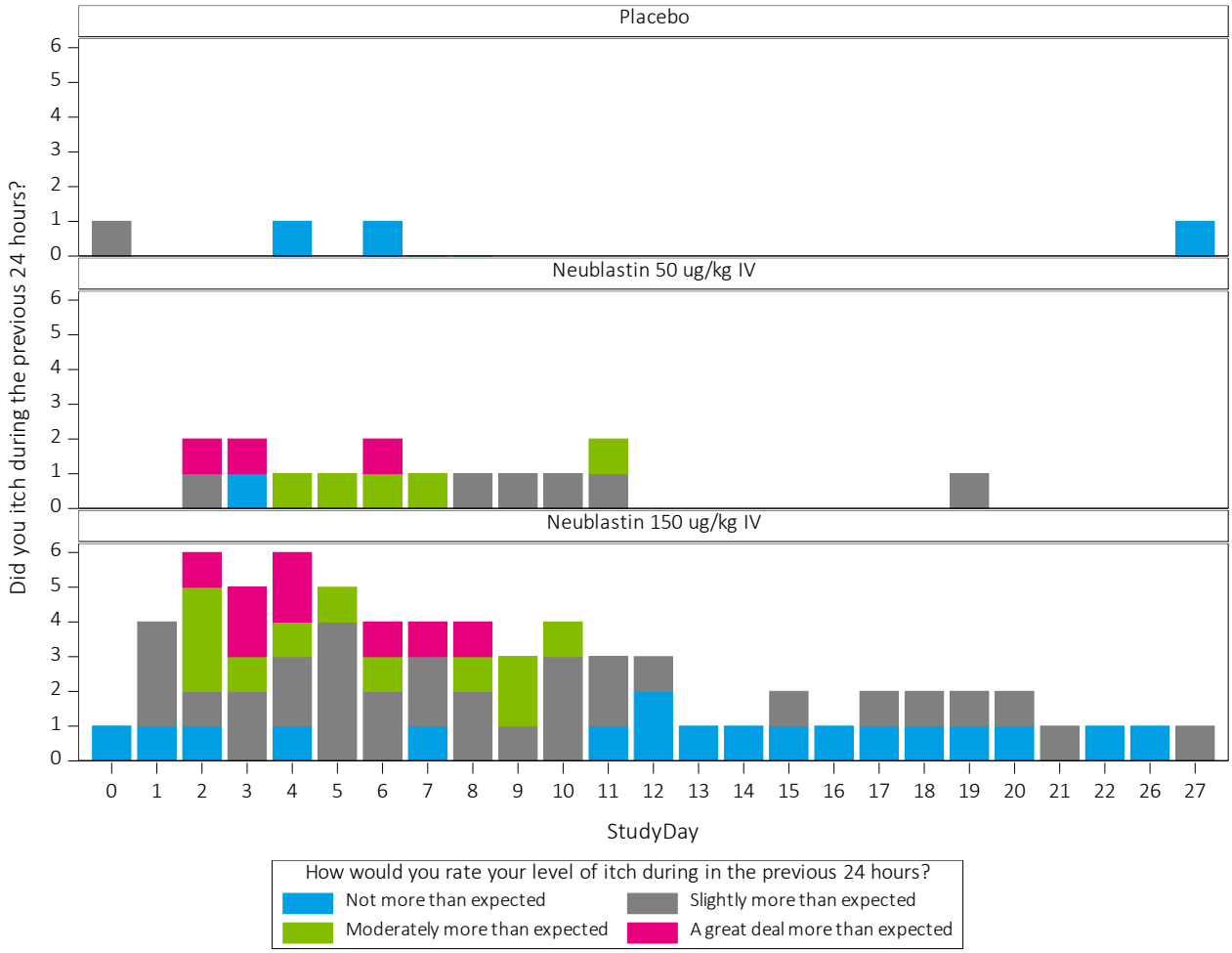

Figure 6. Bargraph incidence and intensity itch in migraine patients.

11.4.2 Statistical/Analytical issues

There were changes in the planned analysis. See section 9.7.2.

11.4.3 Adjustments For Covariates

For all PD analyses, the average of the pre-value has been used as covariate.

11.4.4 Handling Of Dropouts Or Missing Data

Missing data were not imputed before summary or analysis. Data below the limit of quantification (LOQ) was set to 50% of the LOQ for summary purposes only, and was entered as missing in the analysis.

11.4.5 Interim Analyses And Data Monitoring

No safety interim analysis were performed during the conduct of the study. Blinded pharmacodynamic data were reviewed by the study team comprised of members of CHDR and Regeneron during the study. Monitoring visits and contacts occurred at regular intervals. A close-out visit was performed after study closure.

11.4.6 Multicentre Studies

Not applicable, this was a single centre study.

11.4.7 Multiple Comparison/Multiplicity

There was no adjustment for multiplicity due to the exploratory nature of the study.

11.4.8 Use Of An "Efficacy Subset" Of Patients

Not applicable.

11.4.9 Active-Control Studies Intended To Show Equivalence

Not applicable, this study investigated the effects of Neublastin compared to a placebo. No active control was used.

11.4.10 Examination Of Subgroups

Not applicable.

11.4.11 Tabulation Of Individual Response Data Active-Control Studies Intended To Show Equivalence

Listings of individual response data are provided in the Statistics Report.

11.4.12 Drug dose, Drug concentration, and relationships to response

Not applicable.

11.4.13 Drug-drug and drug-disease interactions

Not applicable.

11.4.14 By-patient display

Not applicable.

12 Safety Evaluation

12.1 Extent of Exposure

Part A
Two types of dosing were applied during this study:

- IV (intravenous) group (duration 12 minutes (500 mL/h))
  - Neublastin 150 µg/kg (N=12)
  - Placebo (N=12)
- ID (intradermal) group
  - 5µg to left/right leg (Neublastin/placebo) or left/right leg (placebo/Neublastin) and Neublastin 5µg in flank or upper back area (N=3)
  - 20µg to left/right leg (Neublastin/placebo) or left/right leg (placebo/Neublastin) Neublastin and Neublastin 20µg in flank or upper back area (N=3)
  - 100µg to left/right leg (Neublastin/placebo) or left/right leg (placebo/Neublastin) and Neublastin 100µg in flank or upper back area (N=6)

Additionally, capsaicin (1% cream) was applied to a 3x3cm2 area on the forearm of healthy subjects in Part A (IV administration only).

Part B
In total, 12 otherwise healthy subjects diagnosed with migraine were enrolled and randomized for study participation. All subjects received either 50 or 150 µg/kg IV Neublastin (duration 12 minutes (500 mL/h)) and placebo according to the randomization schedule.

12.2 Adverse Events (AEs)

12.2.1 Brief Summary of Adverse Events

In Part A, a total of 42 AEs were reported by 12 subjects (100%) after administration of Neublastin IV (N=12), compared to 21 AEs reported by 9 subjects (75%) receiving placebo IV (N=12), see Table 8. In the ID dosing groups, a total of 6 AEs were reported in 3 subjects (100%) receiving 5 µg Neublastin (N=3), see Table 9. 4 AEs were reported by 3 subjects (100%) receiving 20 µg Neublastin (N=3). In the 100 µg Neublastin group (N=6), 11 AEs were reported by 4/2 subjects (66.7%).

In Part B, A total of 41 AEs were reported by 6 subjects (100%) after administration of 50 µg/kg Neublastin IV (N=6), compared to 43 AEs reported by 6 subjects (100%) receiving 150 µg/kg Neublastin IV (N=6), see Table 10. All the subjects (N=12, 100%) reported a total of 43 AEs during the placebo IV administration period. All AEs in this study were of mild or moderate severity.

12.2.2 Display of Treatment Emergent Adverse Events (TEAE's)

Part A - IV

System Organ Class/ Preferred Term	Neublastin 150ug/kg (N=12) Events N	Neublastin 150ug/kg (N=12) Subjects N (%)	Placebo (N=12) Events N	Placebo (N=12) Subjects N (%)
ANY EVENTS	42	12 (100.0)	21	9 (75.0)
GASTROINTESTINAL DISORDERS	3	2 (16.7)	1	1 (8.3)
Abdominal pain	2	2 (16.7)	-	-
Nausea	-	-	1	1 (8.3)
Toothache	1	1 (8.3)	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	5	4 (33.3)	3	2 (16.7)
Burning sensation	1	1 (8.3)	-	-
Fatigue	1	1 (8.3)	3	2 (16.7)
Feeling hot	2	2 (16.7)	-	-
Injection site pain	1	1 (8.3)	-	-
IMMUNE SYSTEM DISORDERS	-	-	1	1 (8.3)
Dermatitis contact	-	-	1	1 (8.3)
INFECTIONS AND INFESTATIONS	1	1 (8.3)	1	1 (8.3)
Nasopharyngitis	1	1 (8.3)	1	1 (8.3)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	-	-	1	1 (8.3)
Incision site pruritus	-	-	1	1 (8.3)
INVESTIGATIONS	-	-	1	1 (8.3)
Body temperature increased	-	-	-	-
White blood cells urine positive	-	-	1	1 (8.3)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	2	2 (16.7)	1	1 (8.3)
Myalgia	2	2 (16.7)	1	1 (8.3)
NERVOUS SYSTEM DISORDERS	7	5 (41.7)	4	3 (25.0)
Dizziness	-	-	-	-
Headache	6	4 (33.3)	4	3 (25.0)
Presyncope	1	1 (8.3)	-	-
PSYCHIATRIC DISORDERS	1	1 (8.3)	-	-
Nightmare	1	1 (8.3)	-	-
RENAL AND URINARY DISORDERS	-	-	1	1 (8.3)
Polyuria	-	-	1	1 (8.3)
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	1	1 (8.3)	1	1 (8.3)
Menstrual discomfort	1	1 (8.3)	1	1 (8.3)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1	1 (8.3)	-	-
Oropharyngeal pain	-	-	-	-
Pneumothorax spontaneous	1	1 (8.3)	-	-
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	21	10 (83.3)	6	3 (25.0)
Dermatitis contact	1	1 (8.3)	-	-
Eczema	-	-	1	1 (8.3)
Erythema	7	5 (41.7)	5	3 (25.0)
Pruritus	12	9 (75.0)	-	-
Rash	1	1 (8.3)	-	-

Table 8. Summary of number of subjects with TEAEs by treatment SOC and PT, IV group Part A.

Part A – ID

System Organ Class/ Preferred Term	Neublastin 5ug (N=3) Events N	Neublastin 5ug (N=3) Subjects N (%)	Neublastin 20ug (N=3) Events N	Neublastin 20ug (N=3) Subjects N (%)	Neublastin 100ug (N=6) Events N	Neublastin 100ug (N=6) Subjects N (%)
ANY EVENTS	6	3 (100.0)	4	3 (100.0)	11	4 (66.7)
GASTROINTESTINAL DISORDERS	-	-	-	-	-	-
Abdominal pain	-	-	-	-	-	-
Nausea	-	-	-	-	-	-
Toothache	-	-	-	-	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	-	-	1	1 (33.3)	-	-
Burning sensation	-	-	-	-	-	-
Fatigue	-	-	1	1 (33.3)	-	-
Feeling hot	-	-	-	-	-	-
Injection site pain	-	-	-	-	-	-
IMMUNE SYSTEM DISORDERS	-	-	-	-	-	-
Dermatitis contact	-	-	-	-	-	-
INFECTIONS AND INFESTATIONS	-	-	-	-	1	1 (16.7)
Nasopharyngitis	-	-	-	-	1	1 (16.7)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	-	-	-	-	-	-
Incision site pruritus	-	-	-	-	-	-
INVESTIGATIONS	-	-	-	-	1	1 (16.7)
Body temperature increased	-	-	-	-	1	1 (16.7)
White blood cells urine positive	-	-	-	-	-	-
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	-	-	-	-	-	-
Myalgia	-	-	-	-	-	-
NERVOUS SYSTEM DISORDERS	1	1 (33.3)	1	1 (33.3)	3	2 (33.3)
Dizziness	-	-	1	1 (33.3)	-	-
Headache	1	1 (33.3)	-	-	2	2 (33.3)
Presyncope	-	-	-	-	1	1 (16.7)
PSYCHIATRIC DISORDERS	-	-	-	-	-	-
Nightmare	-	-	-	-	-	-
RENAL AND URINARY DISORDERS	-	-	-	-	-	-
Polyuria	-	-	-	-	-	-
REPRODUCTIVE SYSTEM AND BREAST DISORDERS	-	-	-	-	-	-
Menstrual discomfort	-	-	-	-	-	-
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	2	2 (66.7)	-	-	-	-
Oropharyngeal pain	2	2 (66.7)	-	-	-	-
Pneumothorax spontaneous	-	-	-	-	-	-
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	3	3 (100.0)	2	2 (66.7)	6	2 (33.3)
Dermatitis contact	-	-	-	-	-	-
Eczema	-	-	-	-	-	-
Erythema	3	3 (100.0)	2	2 (66.7)	2	1 (16.7)
Pruritus	-	-	-	-	3	2 (33.3)
Rash	-	-	-	-	1	1 (16.7)

Table 9. Summary of number of subjects with TEAEs by treatment SOC and PT, ID group Part A

Part B

System Organ Class/ Preferred Term	Neublastin 50 ug/kg IV (N=6) Events N	Neublastin 50 ug/kg IV (N=6) Subjects N (%)	Neublastin 150 ug/kg IV (N=6) Events N	Neublastin 150 ug/kg IV (N=6) Subjects N (%)	Placebo (N=12) Events N	Placebo (N=12) Subjects N (%)
ANY EVENTS	41	6 (100.0)	43	6 (100.0)	43	12 (100.0)
EAR AND LABYRINTH DISORDERS	1	1 (16.7)	-	-	-	-
Tinnitus	1	1 (16.7)	-	-	-	-
GASTROINTESTINAL DISORDERS	2	2 (33.3)	-	-	2	2 (16.7)
Gastroenteritis	-	-	-	-	1	1 (8.3)
Nausea	-	-	-	-	1	1 (8.3)
Toothache	1	1 (16.7)	-	-	-	-
Vomiting	1	1 (16.7)	-	-	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	1	1 (16.7)	4	3 (50.0)	7	4 (33.3)
Fatigue	-	-	-	-	4	3 (25.0)
Feeling abnormal	-	-	1	1 (16.7)	-	-
Feeling hot	1	1 (16.7)	3	3 (50.0)	1	1 (8.3)
Influenza like illness	-	-	-	-	1	1 (8.3)
Injection site pain	-	-	-	-	1	1 (8.3)
INFECTIONS AND INFESTATIONS	3	2 (33.3)	-	-	3	2 (16.7)
Nasopharyngitis	-	-	-	-	1	1 (8.3)
Pharyngitis	1	1 (16.7)	-	-	-	-
Upper respiratory tract infection	1	1 (16.7)	-	-	-	-
Urinary tract infection	-	-	-	-	1	1 (8.3)
Vaginal infection	1	1 (16.7)	-	-	1	1 (8.3)
INVESTIGATIONS	2	2 (33.3)	-	-	-	-
Body temperature increased	1	1 (16.7)	-	-	-	-
Menstruation normal	1	1 (16.7)	-	-	-	-
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	-	-	-	-	2	2 (16.7)
Arthritis	-	-	-	-	1	1 (8.3)
Back pain	-	-	-	-	1	1 (8.3)
NERVOUS SYSTEM DISORDERS	13	5 (83.3)	19	5 (83.3)	24	12 (100.0)
Bradyphrenia	-	-	1	1 (16.7)	-	-
Dizziness	1	1 (16.7)	-	-	-	-
Headache	4	3 (50.0)	7	4 (66.7)	11	9 (75.0)
Migraine	8	4 (66.7)	10	3 (50.0)	13	9 (75.0)
Paraesthesia	-	-	1	1 (16.7)	-	-
PSYCHIATRIC DISORDERS	1	1 (16.7)	1	1 (16.7)	-	-
Nervousness	-	-	1	1 (16.7)	-	-
Nightmare	1	1 (16.7)	-	-	-	-
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	1	1 (16.7)	-	-	1	1 (8.3)
Oropharyngeal pain	1	1 (16.7)	-	-	-	-
Sneezing	-	-	-	-	1	1 (8.3)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	16	6 (100.0)	19	5 (83.3)	4	3 (25.0)
Dermatitis	-	-	2	2 (33.3)	2	2 (16.7)
Dermatitis contact	1	1 (16.7)	-	-	1	1 (8.3)
Dry skin	2	1 (16.7)	-	-	-	-
Ecchymosis	-	-	-	-	1	1 (8.3)
Erythema	5	5 (83.3)	6	4 (66.7)	-	-
Photosensitivity reaction	-	-	1	1 (16.7)	-	-
Pruritus	6	3 (50.0)	9	5 (83.3)	-	-
Rash	2	2 (33.3)	-	-	-	-
Rash pruritic	-	-	1	1 (16.7)	-	-
VASCULAR DISORDERS	1	1 (16.7)	-	-	-	-
Syncope	1	1 (16.7)	-	-	-	-

Table 10. Summary of number of subjects with TEAEs by treatment SOC and PT, IV group Part A.

12.2.3 Analysis of Adverse Events

In Part A, the most prominent AEs were headache and skin reactions, which are listed below. Other AEs were evenly distributed across treatment groups and are therefore are not discussed here.

- Headache:
  - IV: In the 150µg/kg Neublastin group (N=12), headache was reported 6 times by 4 subjects (33.3%) during the study, compared to 4 times by 3 subjects (25%) in the Placebo IV group (N=12). These AEs were mild or moderate of severity.
  - ID: Headache was reported by 2 subjects (33.3%) in the 100 µg Neublastin group (N=6), compared to none (0%) in the 20 µg group (N=3)and once by 1 subject (33.3%) in the 5 µg Neublastin group (N=3).These AEs were of mild severity.
- Skin reactions:
  - IV: In the 150µg/kg Neublastin group (N=12), skin reactions were reported 21 times by 10 subjects (83.3%) during the study, compared to 6 times by 3 subjects (25%) in the Placebo IV group (N=12). These AEs were of mild severity.
    - Pruritus was reported 12 times reported by 9 subjects (75%) in the Neublastin group, compared to no reporting of pruritus in the placebo IV group
    - Erythema: in the Neublastin group erythema was reported 7 times by 5 subjects (41.7%), compared to 5 times by 3 subjects (25%) in the placebo group.
    - Rash was reported once by one subject (8.3%) in the Neublastin group, compared to none in the placebo group.
  - ID: Skin reactions were reported 6 times by 2 subjects (33.3%) in the 100 µg Neublastin group (N=6). In the 20 µg Neublastin group (N=3) skin reactions were reported by 2 subjects (66.7%). In the 5 µg group (N=3), skin reactions were reported 3 times by 3 subjects (100%). These AEs were of mild severity.
    - Pruritus: in the Neublastin 100 µg group, pruritus was reported 3 times by 2 subjects (33.3%%) compared to none in the 5 µg and 20 µg group.
    - Erythema: In the 100 µg Neublastin group, erythema was reported twice by 1 subject (16.7%) in the Neublastin group. Erythema was reported twice by 2 subjects (66.7%) in the 20 µg Neublastin group. Erythema was reported 3 times by 3 subjects (100%) in the 5 µg Neublastin group.
    - Rash was reported once by one subject (16.7%) in the Neublastin 100 µg group, compared to none in the 5 µg and 20 µg group.
- Additional AEs:
  - Additional AEs that stand out in the safety report are discussed below. There were AEs which are evenly distributed across treatment groups that are not discussed here. Those that were considered unrelated to IMP administration in a blinded fashion are also not mentioned. All AEs in this study were of mild or moderate severity, with the exception of the aforementioned SAE, spontaneous pneumothorax, classified as severe.
    - A generalised hot feeling was reported by 2 subjects (16.7%) in the Neublastin IV group (N=12), compared to none in the placebo IV group. In one AE, this hot feeling was considered possibly related to IMP administration, while in the other it was considered unrelated. This AE was not reported by the ID therapy groups.
    - Mild abdominal pain was reported by 2 subjects (16.7%) in the Neublastin IV group (N=12), compared to none in the placebo IV group. This AE was not reported by the ID therapy groups.

In Part B,

- Headache and migraine:
  - After receiving 50µg/kg Neublastin (N=6), headache symptoms were reported a total of 4 times by 3 subjects (50%). Migraine was reported a total of 8 times by 4 subjects (66.7%).

- After receiving 150µg/kg Neublastin (N=6), headache symptoms were reported a total of 7 times by 4 subjects (66.7%). Migraine was reported a total of 10 times by 3 subjects (50%).
- After receiving Placebo (N=12) headache symptoms were reported a total of 11 times by 9 subjects (75%). Migraine was reported a total of 13 times by 9 subjects (75%).

- Skin reactions:
  - After receiving 50µg/kg Neublastin (N=6), erythema was reported a total of 5 times by 5 subjects (83.3%). Pruritus was reported a total of 6 times by 3 subjects (50%). Rash was reported a total of 2 times by 2 subjects (33.3%).
  - After receiving 150µg/kg Neublastin (N=6), erythema was reported a total of 6 times by 4 subjects (66.7%). Pruritus was reported a total of 9 times by 5 subjects (83.3%). Pruritic rash was reported a total of once by 1 subject (16.7%).
  - After receiving Placebo (N=12), erythema and pruritus were not reported by any subjects. Other skin reactions were not considered related to study drug administration, with the exception of 1 report of ecchymosis by 1 subject (8.3%) .

- Other AEs of note:
  - A generalized hot feeling following IV administration was reported by 1 subject (16.7%) in the Neublastin IV 50 µg/kg group (N=6), by 3 subjects (50%) receiving Neublastin 150 µg/kg (N=6) and by 1 subject (8.3%) after Placebo IV (N=12).

This numerical description of the AEs spans the whole 28 day period following IV administration of either Neublastin or Placebo. The graphical description of the duration, intensity and moment of onset of the headache and skin reaction symptoms are shown in Appendix 15.8 (Part A) and 15.9 (Part B).

12.2.4 Listing of Adverse Events by Subject

Listings of adverse events by subject are provided in the Safety Report (Appendix 15.1 (Part A_ and 15.2 (Part B)).

12.3 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

12.3.1 Listing of Deaths, other Serious Adverse Events and Other Significant Adverse Events

There were no deaths or other significant adverse events in this study.

- In Subject 103 in Part A, who received 150µg/kg IV Neublastin, one SAE occurred (spontaneous pneumothorax), which was considered unrelated to IMP administration.

12.3.2 Deaths

There were no deaths in this study.

12.3.3 Other Serious Adverse Events

One serious adverse event, spontaneous pneumothorax, was reported by Subject 103 of Part A (see section 12.3.5). There were no other serious adverse events during Part A or Part B of the study.

12.3.4 Other Significant Adverse Events

There were no other significant adverse events in this study.

12.3.5 Narratives of Deaths, Other Serious Adverse Events and Certain Other Significant Adverse

Events

Subject 103 of Part A was a healthy, tall and slender 20 year old male subject who reported a spontaneous pneumothorax on the left, starting at 10:30 AM on 3 January 2020 (he had been dosed on 17 Dec 2019 with 150µg/kg IV of Neublastin). The subject did not have a history of pneumothorax. Subject was hospitalized and received a thoracic drain, as well as analgesia with paracetamol and morphine. The symptoms were resolving by the time subject contacted the investigator around 19:00 PM on 3 Jan 2020. Other AE's previously reported by subject during the study were mild headache and mild pruritus and rash on hands. Specifically, no haematomas were reported or observed. The subject was discharged from the hospital on the 5th of January without complications and after a complete recovery. Subject has been scheduled for a pleural abrasion operation as a preventive measure for future pneumothorax. The blinded analysis of the causal relationship of this SAE was done in consultation with the PI and it was concluded that this spontaneous pneumothorax was considered unrelated to Neublastin or placebo administration. After unblinding, it was revealed that the subject had received 150µg/kg of Neublastin.

12.3.6 Analysis and Discussion of Deaths, Other Serious Adverse Events and Other Significant

See section 12.3.5. for the analysis of the serious adverse event in Part A. No deaths, other serious adverse events or other significant adverse effects occurred during the study.

12.4 Clinical Laboratory Evaluation

12.4.1 Listing of Individual Laboratory Measurements by Subject And Each Abnormal Laboratory Value

Listings of laboratory measurements by subject are provided in the Safety Report.

12.4.2 Evaluation of Each Laboratory Parameter

A complete listing of individual laboratory measurements and a summary of each parameter is provided in the Safety Report. Review of the summary statistics revealed no clinically significant changes from pre-dose values in any of the clinical chemistry or hematology safety tests following administration of IMP compared to placebo. Some subjects showed one or more laboratory parameters outside the normal range. These observations were incidental, not treatment-related, and judged to be of no clinical relevance by the investigator.

Notable observations in Part A, not considered clinically significant and/or related to IMP Administration. No such observations were found in Part B.

- In Part A, Subject 126 (ID 5 µg Neublastin) had a transient decrease in platelet count (predose value 270, decreased to 170) on Day 8 after dosing, which had recovered on Day 12 after dosing. The subject experienced no symptoms or haematomas. This laboratory finding was not considered as clinically significant.
- Subject 131 (ID 100 µg Neublastin) was a 56 year old male whose urinalysis repeatedly showed traces of blood throughout the study. As it was present at screening, it is not considered related to study drug administration. However, considering his age and the continuous presence of blood in his urine, the subject and his general practitioner was duly informed of this observation for follow up.

12.5 Vital Signs and Physical Findings and Other Observations Related to Safety

Subject 203 had an increase in body temperature to 38.6 degrees Celcius 3 h and 57 min following IV dosing of Neublastin 50 µg/kg. This had normalised after 6 hours. It is possible that this was a result of IV dosing of Neublastin.

There were no other clinically relevant changes or unexpected treatment-related trends observed in vital signs, supine systolic and diastolic blood pressure, heart rate, and temperature following administration of IMP.

Data and change from baseline values of vital signs are listed and summarized in the Safety Report.

ECG recordings

In the current study, there were no clinically significant changes observed in the 12-lead ECG derived parameters heart rate, PR interval, QRS interval, QT interval, and QTc interval following administration of IMP

12-lead ECG data including individual listings and summaries are presented in the Safety Report (Appendix 15.1 (Part A) and 15.2 (Part B)).

13 Discussion And Overall Conclusions

The main objective of this study was the assessment of Neublastin-induced skin and sensory alterations and headache in healthy subjects and migraine patients.

During the study, all subjects had to fill in questionnaires on pruritus, rash, headache/migraine and temperature sensations throughout the entire study period. From the (self-reported) questionnaire data, we concluded that 150 µg/kg Neublastin led to an increase in frequency and severity of itch in both healthy volunteers and migraine patients. The severity was considerably higher in the migraine group. These results are consistent with results from previous Neublastin studies (BG00010, Biogen, Inc.), in which patients treated with Neublastin experienced pruritus (78%) In addition, an increase in rash was observed in migraine patients who received 150 µg/kg Neublastin.

Headache was also reported in both healthy volunteers and migraine patients. However, no significant increase in the incidence of migraine headaches was observed after administration of Neublastin in migraine patients compared to the placebo group, where a similar incidence of headache was observed. For migraine patients, the patients' known history of migraine may well explain the relatively high incidence of reported headache episodes in the placebo group, however this may also have been triggered by the stress induced by study participation and change of daily routine during the stay in the clinical unit.. Aside from itch, rash and headache, no clear effects on the other questionnaire data (temperature sensations) could be observed in both study parts (overall "no" answers).

Furthermore, no clear effect of Neublastin could be observed on the nociceptive thresholds (pressure pain, local pressure pain, thermal pain) in both study parts. In Part A, a small decrease in pressure PTT and TSA Heat Pain was observed in the IV group, however this was not significant. No sensitization of the capsaicin response was observed after Neublastin administration. After ID application of Neublastin 5 µg (ID group, Part A), an increase was observed in the total and secondary area of mechanical allodynia as assessed using Von Frey filaments. However, this effect was not observed for the higher doses (20 and 100µg) and no dose dependent trends were observed.. We did not further elaborate on these PD results in the results section of this clinical study report, since no clear overall effect on nociceptive thresholds could be observed.

Presented safety data indicated that a single IV or ID dose of Neublastin was generally well tolerated with no findings of clinical concern.

In Part A, Neublastin IV (150 µg/kg) or ID (5, 20 or 100 µg) did not result in clinically relevant changes in vital signs, ECG, urinalysis or blood chemistry, haematology or glucose parameters. One SAE occurred in this study, consisting of a spontaneous pneumothorax, which was considered unrelated to IMP administration. All other AEs were mild or moderate of severity and abated either without medication or with symptom-guided treatment. The most evident AEs reported were headache and skin reactions, more prominently in the patients receiving IV medication compared to those receiving ID medication. Pruritus was reported by 75% of subjects receiving Neublastin IV compared to none in the placebo group. Erythema was reported by 41.7% of subjects receiving Neublastin IV, compared to 25% receiving placebo IV. Headache was reported by 33.3% of subjects receiving Neublastin IV, compared to 25% of subjects receiving placebo IV.

In Part B, Neublastin IV (50 or 150 µg/kg) did not result in clinically relevant changes in vital signs, ECG, urinalysis or blood chemistry, haematology or glucose parameters. No SAEs were observed in this Part B study. All AEs were mild or moderate of severity and abated either without medication or with symptom-guided treatment. The most prevalent AEs reported were headache, migraine and skin reactions (erythema and pruritus). Erythema was reported by 83.3% of subjects after 50µg/kg Neublastin compared to 66.6% after 150µg/kg Neublastin and none after Placebo. Pruritus was reported by 50% of subjects after 50µg/kg Neublastin compared to 66.6% after 150µg/kg Neublastin

and none after Placebo. Headache was reported by 50% of subjects after 50µg/kg Neublastin compared to 66.7% after 150µg/kg Neublastin and 75% after Placebo. Migraine was reported by 50% of subjects after 50µg/kg Neublastin compared to 50% after 150µg/kg Neublastin and 75% after Placebo. The safety description of the AEs spans the 28 day period following IV administration of either Neublastin or Placebo. We overall concluded that the most reported AEs in this study were headache, migraine and skin reactions after Neublastin administration.

In this study there was an inconsistency between the AEs reported when subjects visited the clinical research unit and the symptoms reported by the subjects using the questionnaire app at home, in particular the data on skin and headache complaints. Subjects reported more events in the diary. This inconsistency may be explained by the fact that the symptoms self-reported by the subjects on daily basis were also reported when subjects visited the clinical research unit, however this was done by recall.
Contrary to previous studies, it was possible for study participants in this study to distinguish between “normal” headache and migraine-like headache. However, the most characteristic symptoms associated with migraine, such as photophobia, phonophobia, nausea and vomiting were not assessed. Adding information on these typical migraine symptoms, in addition to patients’ baseline symptoms and migraine presentation may have helped in further distinguishing headache from migraine. Another limitation of this study was the limited sample size, particularly in the ID group and migraine group. However, the sample size was small as the study was exploratory in nature and was intended to provide estimates of the primary and secondary endpoints.

The current study was intended to validate a Neublastin challenge model to investigate the role of the GFRalpha 3 pathway in pain, pruritus and migraine and support an ongoing clinical development program with anti-GFRalpa 3 antibodies. Data from this study showed only moderate increase in pruritus and rash after Neublastin administration in addition to no reliable alteration of nociceptive thresholds and headache/migraine incidence. These results supported the sponsor decision to pause further evaluations in this program.