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A Placebo-Controlled, Randomized, Double Blind, Single Ascending Dose Study to Assess Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of Pnb-001 in Healthy, Adult, Human Male Subjects

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ABSTRACT:Objectives: To assess in a Phase-I (Single Ascending Dose Study) the safety/ tolerability, pharmacokinetics and pharmacody namics of PNB-001 in healthy, adult, human male subjects under fasting conditions and fed conditions.
Methodology: In the study, 19 blood samples for pharmacokinetic assessment and 05 blood samples for pharmaco-dynamic assessment, including one pre-dose blood sample, were collected from each subject to analyze the pharmacokinetic and pharmacodynamic profile of the active treatment.
Safety results:A dose range from 25 mg to 1500 mg was tested in 7 cohorts in 42 subjects and three (03) subjects experienced AEs, which were mild in nature. One (02) AEs was moderate in nature and the subject was withdrawn from the SAD trial.
Pharmacokinetics: Pharmacokinetic profile of PNB-001 was well characterized with reliably estimated pharmacokinetic parameters for all dose levels after single-dose administration.
A food effect was found in presence of high fat high calorie meal, in which C_{max} and AUC_{0-t} for PNB-001 increased approximately 5-fold and 4-fold, respectively. Dose proportionality was also studied in this trial for PNB-001 and concluded that both C_{max} and AUC_{0-t} are not increasing in dose proportional manner over the dose range of 25-1500 mg. No loss of CCKBR selectivity was observed.
Overall, the active treatment was found very safe and very well tolerated in a SAD phase 1 study over a very wide dose range.
KEYWORDS:PNB-001, CCK antagonist, gastrin antagonist, SAD Phase 1, safety/tolerability, pharmacokinetics.

I. INTRODUCTION

Pain and IBD are major problems affecting millions of people. While more than 100 million people in the world suffer from chronic pain, 33% of the world population suffers from IBD. Although pain is currently treated with opioids, prolonged use of opioids will lead to resistance and a variety of adverse events, including death. Hence, safer therapeutics to treat pain will proof highly relevant in these patients. Similarly, currently severe forms of IBD are treated with corticosteroids, such as dexamethasone or prednisolone. Prolonged use of these corticosteroids leads to diabetes, muscle wasting, osteoporosis and others.

PNB-001, 3-4- chloro- 5-hydroxy- 1-phenethyl - 5- phenyl-1H-pyrrol-2(5H)-one, is a new chemical entity. Non-clinical data demonstrate PNB-001 is a Cholecystokinin receptor (CCK) antagonist, which binds to the CCKBR at 20 nM.
PNB-001 was extensively tested in preclinical mice and rats efficacy pain models. Various pain models such as the hot plate paw withdrawal latency, formalin induced inflammation, neuropathic pain, and surgical experiments were performed with PNB-001. Strikingly, PNB-001 was very effective in all these models, indicating its capability to combat pain induced by variety of pathological conditions.

In the first experiment to determine the efficacy in a hot plate paw withdrawal model, 0.5 mg/kg PNB-001 was administered intraperitoneally or 40 mg/kg tramadol. PNB-001 was as effective as tramadol in increasing the latency of paw withdrawal from the hot plate. PNB-001 was also tested in another model of pain, tail flick assay. In this experiment, 0.5 mg/kg PNB-001 or 40 mg/kg tramadol was administered to mice and the latency period in a tail flick assay was measured. The results demonstrate the significant increase in latency of mice treated with PNB-001 and this effect was comparable to that observed with tramadol. In order to confirm that PNB-001 is orally available, mice were administered with 5 mg/kg P.O. of PNB-001

and were compared to 40 mg/kg S.C. dose of tramadol in a rat hot plate pain model. The results clearly demonstrate that PNB-001 at 5 mg/kg P.O. was very effective and superior to tramadol, indicating that it is orally bioavailable and also effective at low dose.

Since PNB-001 was very effective under various conditions in simple hot plate or tail flick pain models, we then evaluated the efficacy of PNB-001 in a more complex model of pain. Rats were grouped and a dose response curve of PNB-001 (P.O. or i.p.) was performed and compared to morphine in a formalin induced neuropathic (phase I) and inflammation pain (phase II) models. PNB-001 was extremely effective in this model of pain. Interestingly, PNB-001 was effective only at 1.5 mg/kg I.P. in phase I of the formalin induced pain condition. However, in the inflammatory pain stage phase II of the model, it is effective at all doses and was superior to morphine.

Since inflammatory bowel disease (IBD) is one of the indications recommended for CCK antagonists, PNB-001 was tested in indomethacin induced IBD model. Interestingly, PNB-001 at 5 mg/kg dose was extremely effective in significantly reducing the IBD caused by indomethacin and this effect was comparable to the positive control of 10 mg/kg prednisolone. This study increases the potential of PNB-001 as IBD is an unmet medical need and a drug that can alleviate IBD is positioned for fast approval.

Toxicology studies with PNB-001 demonstrated very high level of safety. The Maximum Tolerated Dose (MTD) of PNB001 in SD rats for both the sexes, was found to be 2000 mg/kg body weight when administered orally and LD_{50} was found to be >2000 mg/kg/day, under the conditions of this study.

Twenty eight day toxicity studies demonstrated no treatment related clinical signs, body weight gain, feed intake and incidence of mortality were recorded in rats exposed to the PNB001 at and up to a dose of 300 mg/kg body weight during the study period. No treatment related changes were observed in any of the hematology including coagulation, clinical chemistry and urine parameters in rats treated with PNB001 at and up to 300 mg/kg body weight. At termination, there were no treatment related changes in the organ weight, gross and histopathological findings in rats at and up to 300 mg/kg body weight. Based on the findings of this study, the No Observed Adverse Effect Level (NOAEL) of PNB001, following 28 day oral administration in SD rats was found to be 300 mg/kg/day.

In bioanalytical analysis, dose related increase in C_{max} was observed in females, however no dose response was noticed in males treated with 75, 150 and 300 mg/kg respectively. T_{max}, was found to be 0.5h both in males and females at all the doses. The C_{max} ranged between 20-24, 31-58 and 36-39 ng/mL in males and 81-100, 121-188 and 175-266 ng/mL in females at doses at 75, 150 and 300 mg/kg respectively. AUC was found to be 181-189, 229-526 and 368-412 ng.h/mL in males and 463-653, 1105-1107 and 1345-1517 ng.h/mL in females for 75, 150 and 300 mg/kg treated groups respectively.

In 28 day toxicity studies in dog, no treatment related mortality, clinical signs, changes in body weight gain, food intake and ophthalmology were noticed in any of the treated dogs at and up to a dose of 200 mg/kg body weight. No gross pathological changes were noticed in any of the treated groups at and up to a dose of 200 mg/kg were noticed.

Genetic toxicity studies and in vitro and in vivo chromosomal aberration studies indicated that PNB-001 did not induce any mutations or chromosomal rearrangements at 2500 µg/plate and 250µg/ml, respectively.

All safety pharmacology studies conducted at 80 mg/kg dose demonstrated high level of safety with no adverse events.

PNB-001 was also tested for its potential to inhibit CYP enzyme and the results indicate that PNB-001 did not inhibit the tested CYP enzymes even at 10 µM concentration.

Overall, the nonclinical studies conducted for PNB-001, a CCK antagonist, show it alleviates pain and IBD. PNB-001 exhibits a favorable safety profile in rats and dogs and represents a viable candidate for continued clinical development as a potential oral CCK antagonist for treatment of pain and IBD.

RATIONALE AND AIMS

The aim of the study was to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of PNB-001 after single ascending dose of PNB-001 in healthy, adult, human male subjects under fasting conditions as well as fed conditions.

This study was the first administration of PNB-001 to man. It is a descriptive study to evaluate single-dose safety and tolerability and to estimate dose-limiting toxicity, if any, of a single oral dose.

Doses selected for the study range from 25 mg to 1500 mg. Safety margins for the 100 mg planned dose in humans were estimated using

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standard surface area ratios. Comparable doses in humans were calculated based on the favourable tolerability and toxicity profile of a 300 mg/kg dose to rats and 200 mg/kg dose to dogs in the 28-day oral gavage toxicity studies. For the 100 mg planned dose in humans, the safety margin based on the 200 mg/kg dose to rats was greater than 100-fold. Based on the 200 mg/kg dose to dogs, the safety margin for the 100 mg planned dose to humans was greater than 200-fold. The planned first dose for the study was further lowered to 25 mg followed by the second cohort of 50 mg dose. At the highest planned dose in the single ascending dose study in humans (1500 mg), the safety margin compared to dogs would approximately 10-fold.

PNB-001 was administered via oral capsule formulation to healthy male volunteers.

II MATERIALS & METHODS

SELECTION OF STUDY POPULATION

All the subjects willing to participate in the study were screened prior to their enrolment, in order to assess their eligibility by satisfying all of the inclusion and exclusion criteria. During screening, the medical history of the subjects was elicited and they underwent a general clinical examination, measurement of blood pressure, radial pulse rate, oral body temperature, respiratory rate, clinical laboratory evaluations, 12-lead ECG, chest X-ray (posterior-anterior view),immunological tests for HIV (Human Immunodeficiency Virus), cardiac monitoring, HBsAg (Hepatitis B Surface Antigen) and HCV (Hepatitis C Virus). This procedure was conducted within 28 days prior to the dose administration of the study in each cohort.

Inclusion Criteria

The inclusion criteria as per the protocol were as follows:

- Non-smoking, healthy, adult, human male volunteers between 18 and 45 years of age (both inclusive) living in and around Ahmedabad city or western part of India.
- Having a Body Mass Index (BMI) between 18.5 to 24.9 (both inclusive), calculated as weight in kg / height in m^2.
- Not having any significant diseases or clinically significant abnormal findings during screening, medical history, clinical examination, laboratory evaluations, 12-lead ECG and chest X-ray (posterior-anterior view) recordings.

Exclusion Criteria

The exclusion criteria as per the protocol were as follows:

- History or presence of any disease or condition which might compromise the haemopoietic, renal, hepatic, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system.
- Sitting blood pressure less than 110/70 mm Hg and/or more than 140/90 mm Hg at the time of screening.
- The presence of abnormal laboratory values which are considered clinically significant. In addition, no subject with liver enzymes (SGOT or SGPT) above 1.25 times the upper limit of normal, total bilirubin above the upper limit of normal by at least 10%, or serum creatinine above the upper limit of normal will be admitted to the study at the discretion of the in investigator.
- Values of hematologic function (hemoglobin, hematocrit, white blood cells or platelets) below the lower limit of normal will be admitted to the study at the discretion of the investigator.
- Positive screen for Hepatitis B consisting of HBsAg (Hepatitis B Surface Antigen), anti-HCV (Hepatitis C Antibody) and HIV.
- Any history or presence of asthma (including aspirin induced asthma) or nasal polyp or NSAIDs induced urticaria.
- The presence of clinically significant abnormal laboratory values during screening.
- Use of any recreational drugs or history of drug addiction or testing positive in pre-study drug scans.
- History or presence of psychiatric disorders.
- Difficulty in swallowing solid dosage forms like tablets or capsules.
- A history of difficulty with donating blood.
- Donation of blood (1 unit or 350 mL) or receipt of an investigational medicinal product or participation in a drug research study within a period of 90 days prior to the first dose of study medication. Elimination half-life of the study drug should be taken into consideration for inclusion of the subject in the study.

Note: In case the blood loss is ≤200 mL; subject may be enrolled 60 days after blood donation or last sample of the previous study.

- An unusual diet, for whatever reason (e.g. low-sodium), for four weeks prior to receiving the study drug. In any such case subject selection will be at the discretion of the Principal Investigator.

Removal of Subject from Therapy or Assessment As per the protocol the investigator

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could withdraw a subject from the study for any of the following reasons:

- The subject suffers from significant inter-current illness or undergoes surgery during the course of the study or the subject has any significant symptoms or signs during the course of the study.
- If it is felt in Principal Investigator's opinion that it is not in the subject's best interest to continue.

Phase	**Screening (Within 28 days prior to dosing)**	**Table 1. Schedule of Events Cohort-I to VII**				
Day		**-1**	**1**	**2**	**3**	**4**
Attendance		X				
Urine Drug Scan		X				
Breath test for alcohol		X				
Informed consent	X (Consent for screening)	X (Study specific consent)				
Compliance Assessment		X				
Baggage and Body search		X				
Clinical Laboratory Investigation (Hematology /Immunology/ Biochemistry/ Urine analysis)	X					
Clinical Lab Investigation (Hematology/ Biochemistry)[#]						X
Chest X-ray[$]	X					
ECG	X		X	X	X	
Detailed Clinical examination#	X	X				X
Pre-dose vital sign			X			
High fat high calorie breakfast[@]			X			
Dosing			X			
Blood sampling**			X	X	X	X
Post dose Vitals^			X	X	X	

- If any subject is found to hide important medical history which in opinion of PI may compromise his safety during participation in this study.
- Any subject experience emesis within 3 hours after dosing of the study drug.

Plasma separation for pharmacokinetic assessment

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The blood samples were centrifuged at 3000 ± 100 rcf for 5 minutes below 10°C to separate plasma. The blood samples were kept in ice cold water bath before centrifugation and during separation. The separated plasma was transferred to pre-labelled polypropylene tube.

The samples were stored upright in a freezer at a temperature -65 ± 10°C for interim storage till transfer of the same to the bio-analytical department. During transfer, the samples were kept in a box containing adequate amount of dry iceand stored in a bioanalytical freezer at -65 ± 10° C until completion of analysis.

Serum separation for pharmacodynamic markers

The blood collected in serum separator tubes was allowed to clot for 30 minutes before centrifugation at 3000 ± 100 rcf for 10 minutes.The separated serum was transferred to pre-labelled polypropylene tubes into two aliquots [around 0.4 mL into the first aliquot for lipase and amylase estimation and stored at 2-8°C until completion of analysisand rest of the volume transferred in to second aliquot as back-up sample. Back-up aliquot was stored at 2-8°C.

All the received samples were transferred to the freezer maintained at -65±10°C at the bioanalytical facility. Before analysis, all the samples were verified.

ETHICAL CONDUCT OF THE STUDY

This study was carried out in accordance with the EC approved protocol, all relevant SOPs and was compliant with all the requirements regarding the obligations of investigators and all other pertinent requirements of the Schedule Y (subsequent amendments) of CDSCO (Central Drugs Standard Control Organization), Ministry of health and family welfare, Government of India, 'National Ethical Guidelines for Biomedical and Health Research Involving Human Participants', ICMR [Indian Council of Medical Research (2017)], ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) 'Guideline for Good Clinical Practice' 2016 and Declaration of Helsinki (Brazil, October 2013).

** Blood sampling for pharmacokinetic assessment: The venous blood samples were withdrawn pre-dose (0.000) and at 0.333, 0.667, 1.000, 1.333, 1.667, 2.000, 2. 333, 2.667, 3.000, 4.000, 6.000, 8.000, 10.000, 12.000, 24.000, 36.000, 48.000 and 72.000 hours post-dose.

Blood sampling for pharmacodynamic assessment: The venous blood samples were withdrawn pre-dose (0.000) and at 6.000, 12.000, 24.000 and 72.000 hours post-dose.

^Post-dose vitals: Vitals (blood pressure and radial pulse in supine position after resting for 5 minutes) were recorded prior to administration of dose and at approximately at 01, 02, 03, 04, 06, 12, 24, 36 and 48 hours post-dose in each cohort.

[@]In case of food effect study only: Cohort-VII: Dosing was carried out in the morning at 30 minutes after serving of the high fat high calorie vegetarian breakfast after an overnight fast of at least 10 hours and for 05 hours post-dose.

Laboratory assessment (hematology, biochemistry and urine analysis only) was performed within three working days prior to dosing for Cohort-VII only.

[$]Chest X-ray (during the last 6 months) (posterior-anterior view) was performed during screening.

[#]At the end of the study (at the time of check-out).

1. Check-in day (Day -1)
2. Dosing Day (Day 1)
3. Check-out day (72 hours after receiving of the investigational medicinal product)
4. Post-study (at the time of check-out)
5. Enrolment for next dose panel was after safety data review for all subjects from the previous dose panel by Data Safety Monitoring Board (DSMB) was completed.
6. IMP administration into each cohort was separated by at least 10 days between any two consecutive cohorts (at least 14 days between fasting and fed cohort).

Table 2. Number of subjects (planned and analysed).

Planned for inclusion		07 Cohorts (07 subjects/each cohort)
Pre-dose discontinued/withdrawn		06
Dosed	Cohort-I	07 (05 active + 02 placebo)
	Cohort-II	07 (05 active + 02 placebo)
	Cohort-III	07 (05 active + 02 placebo)
	Cohort-IV	07 (05 active + 02 placebo)
	Cohort-V	07 (05 active + 02 placebo)
	Cohort-VI	07 (05 active + 02 placebo)
	Cohort-VII	06 (04 active + 02 placebo)
Post-dose withdrawn		01

Analyzed	42 (In which, withdrawn Subject Nos. 1027 and 1034 were also analysed as per protocol requirements)
Considered for pharmacokinetic, pharmacodynamic and dose proportionality analysis	5 (Cohort-1, Cohort-2, Cohort-3, Cohort-4 and Cohort-6) 4(Cohort-5 and Cohort-7)

Diagnosis and main criteria of inclusion:

Non-smoking, normal, healthy, adult, human male volunteers between 18 to 45 years of age (both inclusive), having a Body Mass Index (BMI) between 18.5 and 24.9 kg / m^2 (both inclusive), were able to understand and comply with the study procedures and having given their written informed consent were checked in for the study.

They did not have any significant diseases or clinically significant abnormal findings during screening, medical history, clinical examination, laboratory evaluations, 12-lead ECG and chest X-ray (posterior-anterior view) recordings.

Volunteers who complied with all the inclusion criteria were checked in for the study.

Table 3. Active Treatment and Placebo

.**Active Treatment-A:**	PNB 001 Capsules 25 mg
	PNB 001 Capsules 50 mg
Treatment B = Placebo	PNB 001 Capsules 100 mg
	PNB 001 Capsules 300 mg
Placebo capsules contained no API and only excipients, excipients are not disclosed	Manufactured by: Syngene International Limited, Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase, Jigani Link Road, Bangalore 560 099, India.

Note: The IMP was available in 25 mg, 50 mg, 100 mg and 300 mg Capsules form. For 1000 mg dose, 3 capsules of 300 mg and 1 capsule of 100 mg was administered. For 1500 mg dose, 5 capsules of 300 mg were administered.

Table 4.Subjects with allocated cohort and dosing requirements.

Dose and mode of administration:

For Cohort-I to Cohort-VI (Fasting Condition):

After an overnight fast of at least 10 hours, the investigational medicinal products (either active treatment or placebo treatment) were administered with 240 ± 02 mL of drinking water at ambient temperature to the subjects in sitting posture. The IMP administration was as per the randomization schedule and under double-blinded conditions.

The subjects were served lunch after 04 hours post-dose. Further meals after 04 hours post-dose were served at appropriate interval then on until check-out.

For Cohort-VII (Fed condition of Cohort-IV):

After an overnight fast of at least 10 hours, the subjects were served high fat high calorie vegetarian breakfast, which they consumed within 30 minutes.

The investigational medicinal products (either active treatment or placebo treatment) were administered at 30 minutes after serving of the high fat high calorie vegetarian breakfast to the subjects in sitting posture with 240 ± 02 mL of drinking water at ambient temperature. The IMP administration wasas per the randomization schedule and under double-blinded conditions.The subjects were served lunch after 05 hours post-dose. Further meals after 05 hours post-dose were served at appropriate interval then on until check-out.

The capsule was swallowed whole without chewing or crushing.

DISPOSITION OF SUBJECTS

As per the protocol, the study was to be conducted in 42 subjects (07 subject x 06 cohorts).

07 (05 active + 02 placebo) subjects were dosed in each cohort. The dose was selected for each cohort as below:

Cohort-I: 25 mg, Cohort-II: 50 mg, Cohort-III: 100 mg, Cohort-IV: 300 mg, Cohort-V: 1000 mg, Cohort-VI: 1500 mg and Cohort-VII 300 mg.

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Subjects	Cohort	Dose (mg)
07 (05 active + 02 placebo)	I	25
07 (05 active + 02 placebo)	II	50
07 (05 active + 02 placebo)	III	100
07 (05 active + 02 placebo)	IV	300
07 (05 active + 02 placebo)	V	1000
07 (05 active + 02 placebo)	VI	1500
07 (05 active + 02 placebo)	VII	300

Cohort-I

A total of 09 subjects (Subject Nos. 1001-1007, X-1 and X-2) were checked in for the study. Subject Nos. X-1 and X-2 were checked in for the study, in order to compensate for any dropouts prior to dosing.

Both the extra subjects were checked out of the facility as none of the subjects discontinued / were withdrawn from the study prior to dosing.

Hence, 07 subjects (Subject Nos. 1001-1007) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-II

A total of 08 subjects (Subject Nos. 1008-1014 and X-3) were checked in for the study. Subject No. X-3 was checked in for the study, in order to compensate for any dropout prior to dosing.

Subject No. 1012 had clinically significant pre-dose ECG pattern at 08:55 hours on 31 October 2018, which was suggestive of arrhythmia. Hence, he was withdrawn from the study on medical grounds. He was replaced with Subject No. X-3, who was later, allotted Subject No. 2012.

Hence, 07 subjects (Subject Nos. 1008-1011, 2012, 1013 and 1014) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-III

A total of 09 subjects (Subject Nos. 1015-1021, X-4 and X-5) were checked in for the study. Subject Nos. X-4 and X-5 were checked in for the study, in order to compensate for any dropouts prior to dosing.

Subject No. 1021 had clinically significant pre-dose ECG pattern at 08:59 hours on 15 November 2018, which was suggestive of arrhythmia. Hence, he was withdrawn from the study on medical grounds. He was replaced with Subject No. X-4, who was later, allottedSubject No. 2021.

Subject No. 2021 had clinically significant pre-dose ECG pattern at 09:12 hours on 15 November 2018, which was suggestive of bradycardia. Hence, he was withdrawn from the study on medical grounds. He was replaced with Subject No. X-5, who was later, allotted Subject No. 3021.

Hence, 07 subjects (Subject Nos. 1015-1020 and 3021) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-IV

A total of 09 subjects (Subject Nos. 1022-1028, X-6 and X-7) were checked in for the study. Subject Nos. X-6 and X-7 were checked in for the study, in order to compensate for any dropouts prior to dosing.

After being checked in, Subject No. 1022 did not want to continue his further participation in the study. Hence, he discontinued from the study on his own accord. He was replaced with Subject No. X-6, who was later, allotted Subject No. 2022.

Subject No. X-7 was checked out of the facility as no more subject discontinued / was withdrawn from the study prior to dosing.

Hence, 07 subjects (Subject Nos. 2022 and 1023-1028) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-V

A total of 09 subjects (Subject Nos. 1029-1035, X-8 and X-9) were checked in for the study. Subject Nos. X-8 and X-9 were checked in for the study, in order to compensate for any dropouts prior to dosing.

After being checked in, subjectNo. 1035 did not want to continue his further participation in the study. Hence, he discontinued from the study on his own accord. He was replaced with Subject No. X-8, who was later, allotted Subject No. 2035.

After being checked in, Subject No. 2035 did not want to continue his further participation in the study. Hence, he discontinued from the study on his own accord. He was replaced with Subject No. X-9, who was later, allotted Subject No. 3035.

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Subject No. 1034 was withdrawn from the study on emesis grounds.
Hence, 07 subjects (Subject Nos. 1029-1034 and 3035) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-VI

A total of 11 subjects (Subject Nos. 1036-1042,X-10 to X-13) were checked in for the study. Subject Nos. X-10to X-13 were checked in for the study, in order to compensate for any dropouts prior to dosing.
The extra subjects X-10 to X-13 were checked out of the facility as none of the subjects discontinued / were withdrawn from the study prior to dosing.

Hence, 07 subjects (Subject Nos. 1036-1042) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

Cohort-VII

Food effect on PNB-001 pharmacokinetics and pharmacodynamics was evaluated in this cohort based on concentration profile of subjects of Cohort-IV.

A total of 06 subjects (Subject Nos. 2022, 1023-1026 and 1028) were checked in for the study. SubjectNo. 1027 did not report for fed condition cohort.

Hence, 06 subjects (Subject Nos. 2022, 1023-1026 and 1028) were dosed in the study and all the dosed subjects completed the clinical phase of the study successfully.

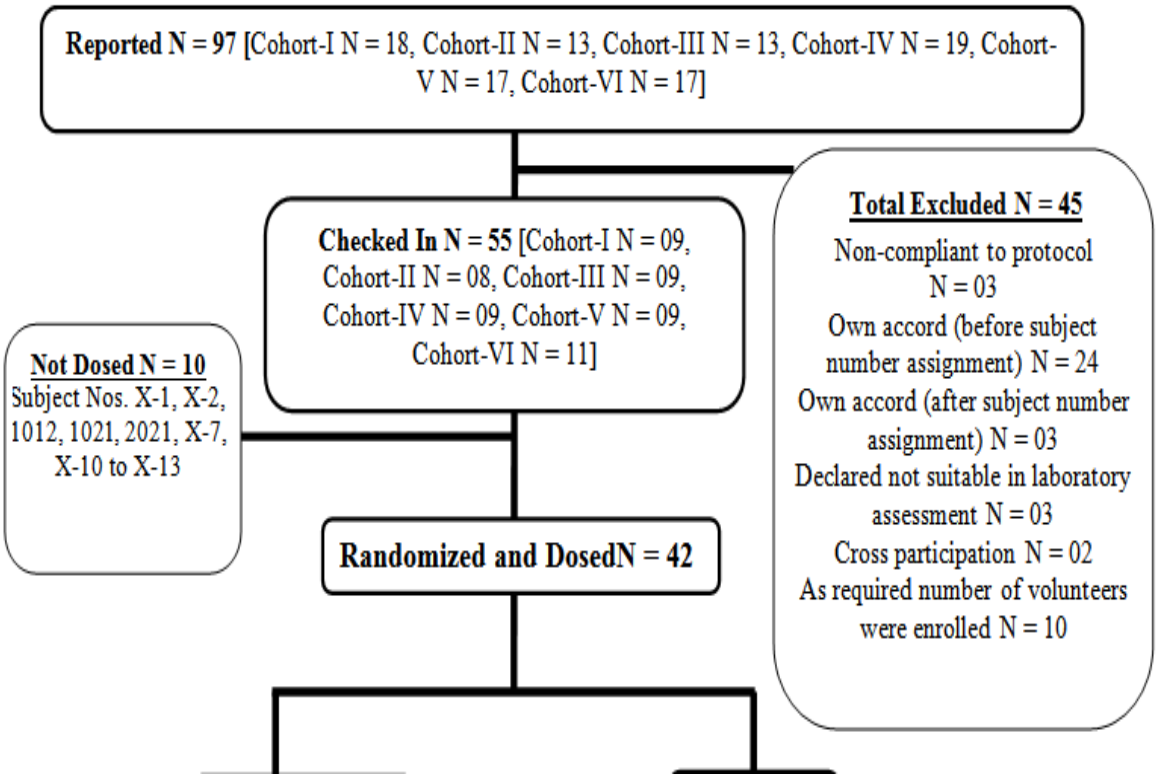
In all the seven cohorts, 41 subjects (Subject Nos. 1001-1011, 2012, 1013-1020, 3021, 2022, 1023-1033, 3035 and 1036-1042) completed the clinical phase of the study successfully.

Statistical Analysis

To evaluate food effect for PNB-001 in Cohort-7 against Cohort-4, the ratio of geometric least squares means is calculated using SAS® Version 9.4 (SAS Institute Inc., USA) and is summarized in the table 8.

Pharmacokinetic analysis

The pharmacokinetic parameters are derived individually for each analyzed subject from the plasma concentration vs. time profiles of PNB-001. Dataset for the calculation of pharmacokinetic parameters has been prepared using Phoenix® Win Nonlin® Version 6.4 (Certara L.P.).



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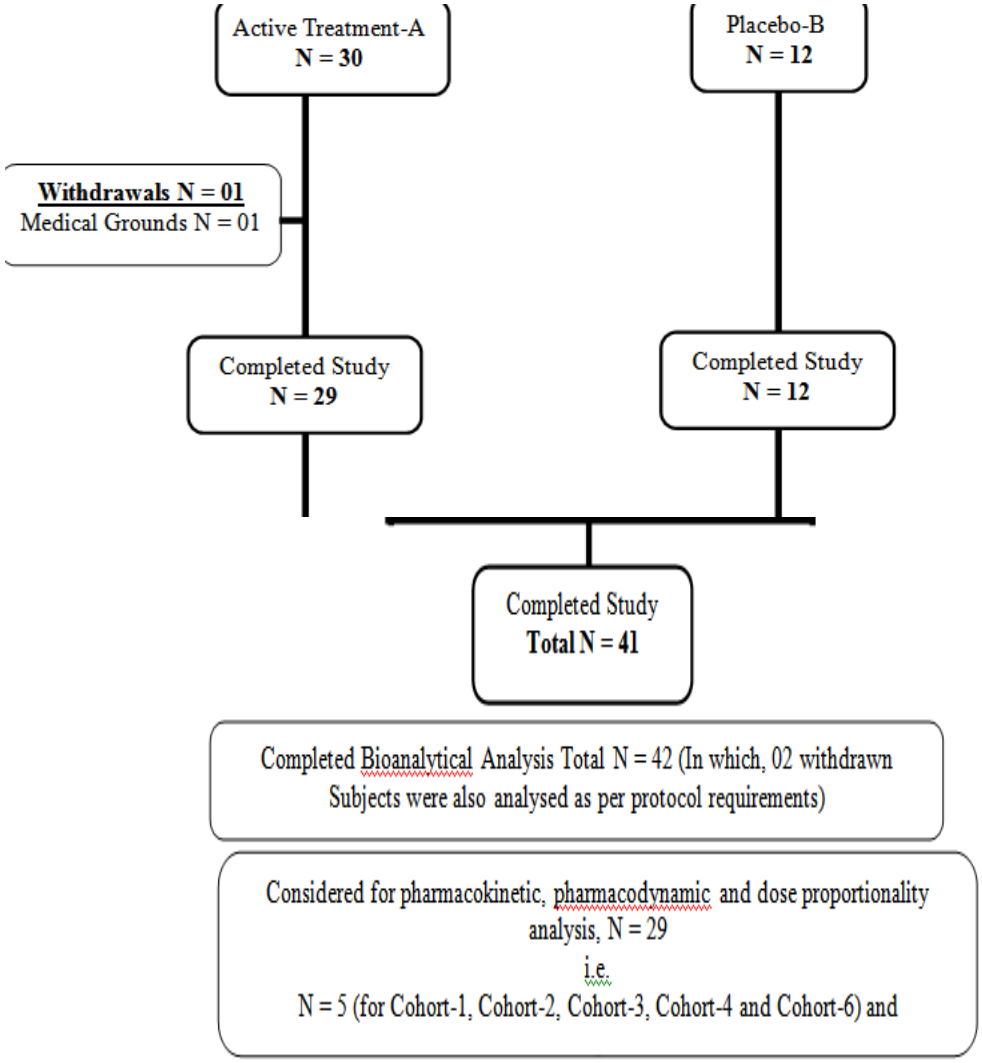

Figure 1. Disposition of subjects

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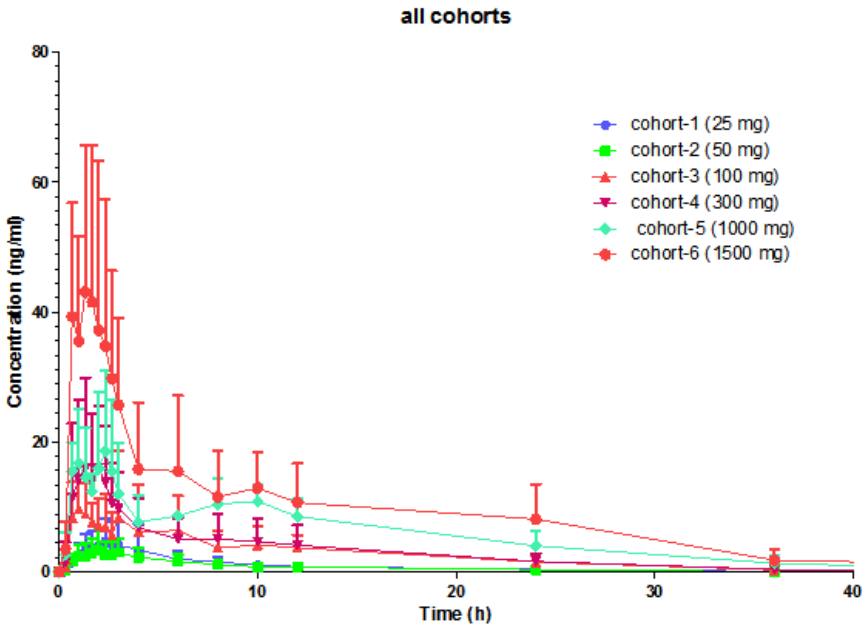

Figure 2.Plasma concentrations in ng/ml for cohort 1-6 under fasting conditions.

Table 6: Pharmacokinetic analysis under fasting conditions

Parameters (Units)	Mean ± SD (untransformed data) Table 6					
	Cohort-1 (N=5)	**Cohort-2 (N=5)**	**Cohort-3 (N=5)**	**Cohort-4 (N=5)**	**Cohort-5 (N=4)**	**Cohort-6 (N=5)**
T_{max} (h)[#]	2.333 (2.000 - 8.000)	1.667 (1.000 - 4.000)	1.667 (1.333 - 3.000)	1.333 (1.000 - 2.333)	1.509 (0.667 - 2.333)	1.333 (0.667 - 2.000)
C_{max} (ng/mL)	5.182 ± 3.4434	4.062 ± 3.4194	11.984 ± 8.7758	20.543 ± 13.0280	26.524 ± 6.6554	50.054 ± 19.7238
AUC_{0-t} (ng.h/mL)	33.785 ± 29.9942	27.166 ± 22.4194	107.038 ± 70.0868	129.113 ± 67.6316	240.477 ± 64.2657	420.062 ± 189.1681
$AUC_{0-\infty}$ (ng.h/mL)	43.514 ± 31.5237^	39.359 ± 18.9754	112.607 ± 67.6243	134.334 ± 66.9795	248.922 ± 64.5890	427.926 ± 191.3543
λ_z (1/h)	0.108 ± 0.0798^	0.090 ± 0.0789	0.098 ± 0.0281	0.106 ± 0.0297	0.106 ± 0.0392	0.079 ± 0.0232
$t_{½}$ (h)	8.436 ± 3.7286^	18.497 ± 20.9841	7.826 ± 3.2609	6.958 ± 1.9545	7.720 ± 4.3895	9.314 ± 2.4694
AUC_% Extrap_obs (%)	12.200 ± 8.2674^	29.668 ± 36.2972	8.527 ± 11.6331	4.669 ± 5.0562	3.364 ± 4.1305	1.936 ± 1.7086

III RESULTS AND DISCUSSION

In Table 6, descriptive statistics of formulation means for PNB-001 under fasting condition are outlined. The pharmacokinetic parameters of PNB-001 for Cohort-1, Cohort-2, Cohort-3, Cohort-4, Cohort-5and Cohort-6 under fasting condition (Figure 2) and Cohort-7 under fed condition are summarized in the following table 6 and table 7.

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Parameters (Units)	Cohort-7 (N=4)
T_{max} (h)#	6.000 (4.000 - 6.000)
C_{max} (ng/mL)	90.289 ± 50.0347
AUC_{0-t} (ng.h/mL)	495.027 ± 284.7537
$AUC_{0-\infty}$ (ng.h/mL)	497.956 ± 285.2635
λ_z (1/h)	0.137 ± 0.0497
$t_{½}$ (h)	5.551 ± 1.8369
AUC%Extrap_abs (%)	0.720 ± 0.4313

Table 7. Descriptive statistics of (right column) formulation means for PNB-001under fed condition

Fasting conditions, cohort 1-6

T_{max} values obtained for all dose levels are almost comparable under fasting condition with median T_{max} ranging from 1.333 – 2.333 hours post-dose. Half-life of all dose levels were also comparable for Further dose proportionality for C_{max} and AUC_{0-t} has been evaluated by statistical analysis.

The half-life was analysed in the range of 8 h and this resulted in a MAD design of 2-3 daily oral doses. Remarkable is the accurate prediction of this longer half-life in man compared to rats using in vitro liver microsome preparations.PNB-001 is a class 2 drug using the biophysical classification system. As predicted for a molecule with a high membrane penetration and a low water solubility, the absorption is poor in absence of food / lipids.
PNB-001 is active in the nanomolar range and here, it was confirmed, that protein binding, which is high with >90%, is no issue in a clinical setting.

In conclusion using fatty food, the efficient plasma concentration is achieved with food about a quarter of the dose required under fasting conditions.

Thus, a dose reduction was applied in MAD design, starting under fed conditions with 50 mg capsule in male healthy subjects. This would be equivalent to a 200 mg dose range, which was tolerated very well in SAD, followed by ascending doses by factor 3 and factor 2.Potentially the presence of proteins in food could reduce plasma concentrations by protein binding, but the opposite was observed here, as lipids (a lipid matrix) are enabling the molecule to be absorbed in the GI tract.In the SAD study 1 group was added to evaluate the food effect and the dose, which was selected was 300 mg. In order to reduce variance the same group of subjects, who previously completed the study under fasting conditions, was re-enrolled for the fed conditions.

Fed conditions, cohort 7

Median T_{max} under fed condition was 6.000 hours post-dose. Hence, in presence of high-fast high-calorie breakfast T_{max} is delayed by ~ 4 hours as compared to fasting condition. Further food effect for C_{max} and AUC_{0-t} has been evaluated by statistical analysis.

Under fed conditions for the 300 mg dose, administered orally as a capsule, a Cmax of 90ng/ml was observed. Based on preclinical data a 20 ng/ml concentration is required for efficacy, and based on these data 75mg 3 times a day will be tested for pain in a MAD design, considering additionally the shorter half-life about 6h under these conditions.
Additionally, dose proportionality was also evaluated in this trial for the dose range of 25-1500 mg after single-dose administration.

Parameters	Geometric Least Squares Means		
	Cohort-7 (Fed Condition) (N=4)	Cohort-4 (Fasting Condition) (N=5)	Ratio(Fed/Fast)%
lnC_{max}	81.148	16.080	504.7
$lnAUC_{0-t}$	429.181	113.503	378.1

Table 8. Food effect summary for PNB-001 (fedcondition vs. fasting condition)

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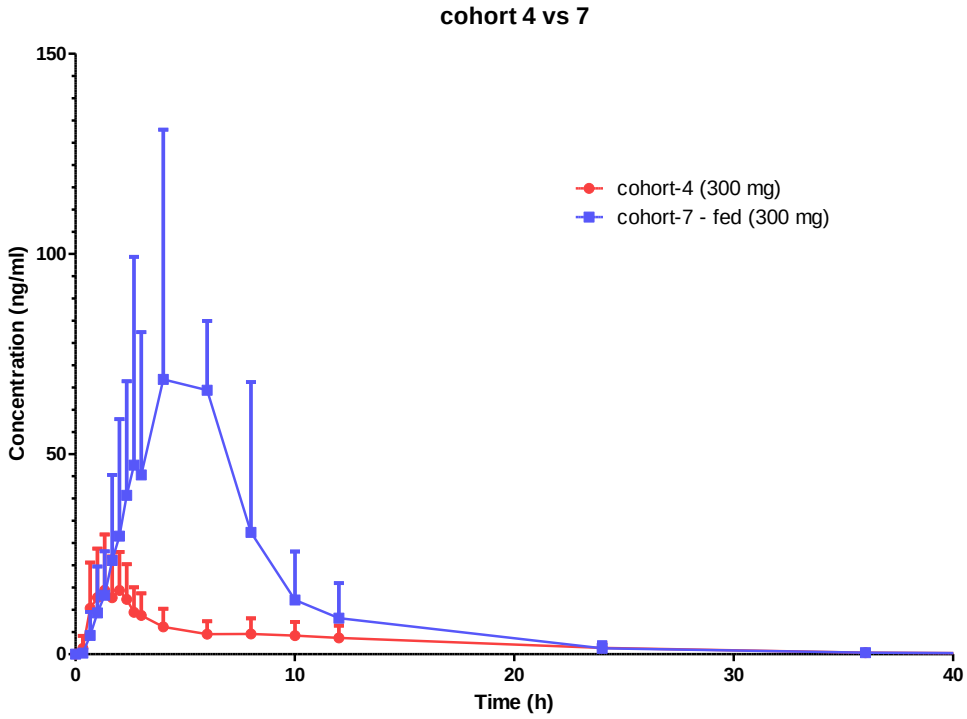
The 90% CI obtained for C_{max} and AUC_{0-t} for PNB-001 does not contain unity. Therefore, it can be concluded that both C_{max} and AUC_{0-t} for PNB-001 are not increasing in dose proportional manner over the dose range of 25-1500 mg. Point estimate were ~0.64 and ~0.67 for C_{max} and AUC_{0-t} for PNB-001, respectively, which represent that both C_{max} and AUC_{0-t} were increased in less than dose proportional manner over the dose range of 25-1500 mg.

Figure 2: Food effect for PNB-001



In presence of high fat high calorie meal, C_{max} and AUC_{0-t} for PNB-001 was increased by approximately 5 fold and 4 fold, respectively.

In summary, dose proportionality was studied in this trial for PNB-001 and concluded that both C_{max} and AUC_{0-t} are not increasing in dose proportional manner over the dose range of 25-1500 mg under fasting conditions.

In rats without food restriction a linear kinetics was observed. The slowest rate determining step is the absorption in the GI tract, which obviously required lipid food in order to facilitate the absorption of the lipophilic drug molecule.

SAFETY EVALUATION

ADVERSE EVENTS (AEs) in summary five (05) significant adverse events (AEs) were reported by three (03) subjects during the conduct of the study. Two (02) AEs were reported in Cohort-III, 100mg. AEs were reported in Cohort-V and one (01) AE was reported in Cohort-VII of the study and apparently all the AEs were reported in subjects after administration of Active Treatment-A.

Three (03) AEs were mild in nature and two (02) AEs were moderate in nature. All the subjects were treated appropriately and were followed up until resolution of their AEs. There were no deaths or serious AEs during the conduct of the study.

Five (05) significant adverse events (AEs) were reported by three (03) subjects during the conduct of the study. There were no deaths or serious AEs during the conduct of the study.

There were no clinically significant findings in the vital signs assessment, 12-lead ECG recording or the laboratory tests in any of the subjects in the study.

<u>Subject No. 1017 (Pyrexia and Pain, Cohort-III, 100 mg)</u>

The subject had complaint of fever and bodyache since approximately 14:25 hours on 17 November 2018. He had no other complaints. The adverse events were gradual at onset, continuous in occurrence and mild in severity.

Plasma concentration levels of PNB-001 for Subject No. 1017 were minimal within the

cohort. The plasma concentration within this group was in the range from 10-20 ng/mg, and with a $C_{max}$of only 3 ng/ml the observed AEs might not be related to PNB-001.

However, he was treated appropriately and was followed up till resolution of his AE. His AE of bodyache was resolved since 21:40 hours on 17 November 2018 and AE of pain was resolved since 07:20 hours on 18 November 2018.

The adverse events were mild in nature and the relationship of the adverse events to the study drug was considered but unlikely, as there was nearly no investigational drug present; and the incidence of colds during this time was manifold higher in the general population.

Subject No. 1034 (Vomiting and Dizziness, Cohort-V, 1000 mg)

The subject had single episode of vomiting at approximately 10:12 hours on 12 December 2018 associated with dizziness. The adverse event of vomiting was sudden at onset, intermittent in occurrence and moderate in severity. The subject had a low glucose plasma concentration and glucose was given, which stopped dizziness. His AE of vomiting was resolved since 11:12 hours on 12 December 2018 and AE of dizziness was resolved since 12:30 hours on 12 December 2018. The subject was withdrawn from the study on emesis grounds.

Plasma samples were collected only up to 1.000 hour post-dose due to discontinuation. Plasma concentrations up to 1.000 hour post-dose were in line with concentration data of same cohort.

The observed AEs might be related to PNB-001 and additionally linked with the fastening administration of the test drug. The dizziness seemed a result of the vomiting induced by a greater sensitivity subject 1034 to a high dose. The AE was considered a general irritation caused by a chemical in contact with the GI system.

This non-specific and non-selective general toxicity of the drug substance was discussed and it was considered to accept 1000 mg dose as the maximum tolerated dose. However, as the adverse events were moderate in nature and it was felt, that this is not representing the real toxicity of the test drug, the SAD study was continued with the last 1500 mg dose.

The trial was continued and even in the cohort 7 (1500 mg) no adverse events were recorded, confirming the rationale behind this case.

Subject No. 1024 (Sinus tachycardia, Cohort-VII, 300 mg)

The subject's 02 hours post-dose ECG recorded at 11:03 hours on 04 January 2019, showed sinus tachycardia with an increased heart rate, which was clinically significant.The adverse event was unknown at onset, continuous in occurrence and very mild in severity.

Plasma concentrations levels of PNB-001 were comparatively high for subject No. 1024 about 160 ng/ml (Cohort-VII), compared to other subjects of the same cohort. However, they were within the same plasma range, which was observed for cohort 6 at the 1500 mg dose level. Compared to cohort 7 in cohort 6 no AE were reported at all for the same range of plasma concentrations.

Subject 1024 was treated appropriately and he was followed up till resolution of his AE. His AE was resolved since 11:48 hours on 04 January 2019. The adverse event was mild in nature and the relationship of the adverse event to the study drug was considered possible, but unlikely as he had a pulse of 88 prior to treatment and the pulse rate at maximum only increased to 105 beats per minute. The adverse event may be a response to the hospital setting and a nice example of the important placebo and/or white coat effect.

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Active Treatment-A (N=30) TABLE 9									
Adverse event (Preferred Term)	Mild		Moderate		Severe		Total		Total
	R	NR	R	NR	R	NR	R	NR	R+NR
General disorders and administration site conditions									
Pyrexia	1 (3.33%)	0	0	0	0	0	1 (3.33%)	0	1
Subject No.	1017								
Pain	1 (3.33%)	0	0	0	0	0	1 (3.33%)	0	1
Subject No.	1017								
Cardiac disorders									
Sinus tachycardia	1 (3.33%)	0	0	0	0	0	1 (3.33%)	0	1
Subject No.	1024								
Dizziness	0	0	1 (3.33%)	0	0	0	1 (3.33%)	0	1
Subject No.			1034						
Gastrointestinal disorders									
Vomiting	0	0	1 (3.33%)	0	0	0	1 (3.33%)	0	1
Subject No.			1034						

Table 9: Overview of adverse events, data summary. Calculation of % of AEs = Total number of AEs*100 / Total number of subjects who have consumed Active Treatment-A during the conduct of the study.The five (05) significant AEs were reported during the conduct of the study and the safety summary is outlined in Table 9.
R=Related; NR=Not Related

Overall there were no deaths or serious AEs during Based on this finding the MAD dose range was reduced for fed conditions to 50 mg and the safety evaluation in MAD is continued with 50, 150 and 300 mg of PNB-001 without restriction of food intake, normal under fed conditions.

The investigational products were safe and well tolerated by healthy subjects, as a single dose administration. Subjects were questioned for well-being at the time of clinical examination and during recording of vital signs during the study. None of the subjects had any clinically significant abnormalities.

Pharmacodynamics

Overall, it is supposed and confirmed now experimentally, that the pancreas contains a few CCK-B receptors in human pancreatic tissues and a large majority of CCK-A receptors. In line with the effect of PNB-001 on the release of amylase, is the CCKA and CCKB receptor expression. Only for the highest 1500 mg dose of PNB-001, a reduction of lipase was recorded, which was not statistically significant.

Thus, the biomarkers amylase and lipase were applied as selectivity markers and confirmed no loss of gastrin receptor selectivity towards the cholecystokinin receptor subtype, even at very high concentrations.

IV CONCLUSIONS

Pharmacokineticanalysis: Approximately a 5 fold and 4 fold increase was observed for C_{max} and AUC_{0-t} for PNB-001, respectively, under fed condition as compared to fasting condition after single-dose administration. These findings resulted in a dose reduction in MAD und the investigational drug product containing PNB-001 will be administered under normal fed conditions.

Safety: Five (05) significant mild adverse events were reported by three (03) of the 42 subjects, and the correlation or non-correlation with PK data was analysed and discussed.

PNB-001 was found very safe and only 1 out of 42 subjects had a mild adverse effect, which could not be drug related.

PNB-001 is an inflammatory analgesic and in addition to the serious condition IBD, period pain (dismenorrhoea) is included in the next phase of the clinical trial.

In addition to 3 safety cohorts, cohort 4 will be added to access safety and PK in woman.

In cohort 5 IBD will be tested, an inflammatory disease. In cohort 6 pain will be evaluated in form of period pain under the consideration of plasma concentration, which was found higher in female dogs; and CCK-oxytocin-

interactions in female patients, which are of additional benefit.

The biomarkers for selectivity were analysed in SAD and PNB-001 was found selective at the highest dose of 1500 mg and therefore, for MAD selectivity biomarkers amylase/lipase will be omitted.

Inflammation biomarkers, such as ESR and CRP, which are established pathology standards are included and FC, which is an IBD specific biomarker, is included additionally.

Last not least, the large dose range for the gastrin antagonist PNB-001, was selected at the high end (1500 mg dose) to cover an extra safety margin and potentially include the therapeutic applications of gastrin related cancers.

Initial evaluation of efficacy will generate a meaningful clinical trial, balancing the risks and the benefits of PNB-001.

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