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A Placebo-Controlled, Randomized, Double Blind, Multiple Ascending Dose (MAD), Multiple Cohort Phase 1 Study to Assess Safety, Tolerability & Pharmacokinetics of PNB-001 (Baladol®)

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ABSTRACT:PNB-001, Baladol®, was tested now in 74 healthy subjects in clinical phase 1. Following a SAD study with 42 subjects Baladol® was tested in MAD, (multiple ascending doses) at low, medium and high doses over a period of 14 days in 32 healthy subjects. Overall, Baladol® was tested safe and with an efficient therapeutic window. From 32 subjects 30 completed the MAD trial and only 2 adverse reactions were observed. 1 AE was vomiting in a female subject and at a high dose a >2.5 fold increase of ALT was identified.

KEYWORDS: PNB-001, CCK antagonist, gastrin antagonist, MAD Phase 1, safety/tolerability, pharmacokinetics.

I INTRODUCTION

PNB-001, 3-4-chloro-5-hydroxy-1-phenethyl-5-phenyl-1H-pyrrol-2(5H)-one, is a new chemical entity[1]. Nonclinical data demonstrate PNB-001 is a Cholecystokinin receptor (CCK) antagonist.

Once the in vitro properties were convincingly demonstrated, PNB-001 was extensively tested in preclinical mice and rats efficacy pain models[2]. Various pain models such as simple 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.

Since inflammatory bowel disease (IBD) is one of the indications recommended for CCK antagonists, PNB-001 was tested in indomethacin induced IBD model[3]. 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[4] 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. 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 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 250 µg/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.

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Clinical study

An ascending single dose study (Project No. 315-13) was conducted in healthy adult human volunteers under fasting conditions to assess safety, tolerability,pharmacokineticsandpharmacodynamics of PNB-001.

Doses examined for the SAD study ranged from 25 mg to 1500 mg. The maximum dose tolerated by the subjects was 1500 mg.

Pharmacokinetic profile of PNB-001 was well characterized with reliably estimated pharmacokinetic parameters for all dose levels after single-dose administration. There was no pharmacokinetic comparison carried out in this study. The Pharmacokinetic parameters C_{max} and AUC_{0-t} increased with increasing doses however C_{max} and AUC_{0-t} did not increase in dose proportional manner over the examined dose range of 25-1500 mg.

One of the doses (300 mg) from the selected cohorts was evaluated for food effect on the same subjects of fasting cohort. It was observed that, in presence of high fat high calorie meal, C_{max} and AUC_{0-t} for PNB-001 increased by nearly 5-fold and 4-fold, respectively.

There were no deaths or serious AEs during the conduct of the study. Overall, the active treatment was safe and well tolerated. However, the reported adverse events in single dose study in humans are pyrexia, pain, vomiting, dizziness, sinus tachycardia.

Target indication and pharmacologic activity[5]

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. Though pain is currently treated by opioids, its prolonged use of opioids may lead to resistance and other adverse events, including death. Hence, safer therapeutics to treat pain are required to treat 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.

RATIONALE AND AIMS

The aim of the study[6] was to assess the safety, tolerability and pharmacokinetics of PNB-001 after multiple ascending dose of PNB-001.

It is a descriptive study to evaluate multiple-dose safety and tolerability and to estimate dose-limiting toxicity, if any, of multiple oral doses. Doses selected for the study range from 50 mg to 200 mg BID. Animal models suggest the antiinflammatory and analgesic potential of PNB-001.

TARGET POPULATION AND DURATION OF THE STUDY

As per the protocol, 32 Subjects [(08 subjects x 04 cohorts) - 24 healthy male & 08 healthy surgically sterilized female subjects] non-smoking, normal, healthy, adult, human subjects who complied with all the inclusion and none of the exclusion criteria were the target population in the study. Due to pre-dose discontinuation of one subject in Cohort-II, the study was conducted on 31 subjects instead of 32 subjects.

The duration of the clinical part of the study was about 83 days [11 hours prior to the IMP administration on Day 01 in Cohort-I until the last pharmacokinetic sample collection (24 hours after IMP administration on Day 14) in Cohort-IV].

STUDY OBJECTIVES

Primary objective: To assess the safety and tolerability of study drug.

And

Secondary objective: To assess pharmacokinetics and efficacy of study drug.

II MATERIALS & METHODS

ETHICS INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW**BOARD (IRB)**

Riddhi Medical Nursing Home - Institutional Ethics Committee reviewed the study Protocol[7] (Version 2.0, dated 04 July 2019), Informed Consent Form (ICF-English) (Version 2.0, dated 04 July 2019 – for Cohort I to IV), Amendment Report (Amendment No. 01 - Amendment to the English-ICF, dated 04 July 2019), Investigator's Brochure (Product Name: PNB-001, Edition Number: 1) and gave approval for the study on 03 September 2019.

Informed Consent Form (ICF-Gujarati) (Version 1.0, dated 25 November 2019) (for Cohort 1 to 4), Certificate of Translation Accuracy (dated 25 November 2019) and DCGI Approval Letter (dated 11 November 2019) were submitted separately to the IEC for review, which were approved on 26 November 2019.

Errata No. 01 (Protocol) was notified to IEC on 10 December 2019.

ETHICAL CONDUCT OF THE STUDY

This study was carried out in accordance with the IEC 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

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(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).

SUBJECT INFORMATION AND CONSENT

Subjects willing to participate in the study underwent a screening procedure within 28 days prior to first IMP administration. Written informed consent for the screening procedure was obtained from each subject prior to screening.

Those subjects found eligible for participation in the study underwent a study specific informed consent presentation on the day of check-in for the study. The presentation was carried out in a simple language that was understood by the subjects. All the relevant aspects of the study were clearly explained to the subjects with the help of the ICF.

INVESTIGATIONAL PLAN

OVERALL STUDY DESIGN AND PLAN-DESCRIPTION

This study was a placebo-controlled, randomized, double blind, multiple ascending dose and multicenter study to assess safety, tolerability and pharmacokinetics of PNB-001 in non-smoking, healthy, adult, human subjects under fed conditions.
A sufficient number of volunteers were asked to report on the day of check-in of each cohort in order to ensure that at least 08 subjects were enrolled in the beginning of each cohort (Cohort-I to IV). Due to pre-dose discontinuation of one subject in Cohort-II, the study was conducted on 31 subjects instead of 32 subjects.

Cohort-I

A total of 10 subjects (Subject Nos. 1001-1008, X-1 and X-2) were checked in for the study.

Cohort-II

A total of 08 subjects (Subject Nos. 1009-1016) were checked in for the study.

After being checked in, Subject No. 1016 did not want to continue her further participation in the study due to her personal reason. Hence, she discontinued from the study on her own accord.

Cohort-III

A total of 10 subjects (Subject Nos. 1017-1024, X-3 and X-4) were checked in for the study.

Cohort-IV

A total of 10 subjects (Subject Nos. 1025-1032, X-5 and X-6) were checked in for the study.
08 (06 active + 02 placebo) subjects were dosed in each cohort. The dose was selected for each cohort as below:
Cohort-I and II: 50 mg, Cohort-III: 100 mg and Cohort-IV: 200 mg

The treatment was followed as given in Table 1.

Active Treatment:	PNB 001 Capsules 50 mg (Cohort I and II)
Table 1	PNB 001 Capsules 100 mg (Cohort III and IV)
	Manufactured by: Syngene International Limited, Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase, Jigani Link Road, Bangalore 560 099, India.
Placebo:	PNB 001 Placebo Capsules 50 mg (Cohort I and II)
	PNB 001 Placebo Capsules 100 mg (Cohort III and IV)
	Manufactured by: Syngene International Limited, Biocon Park, Plot No. 2 & 3, Bommasandra IV Phase, Jigani Link Road, Bangalore 560 099, India.

The screening phase was carried out within 28 days prior to the scheduled dosing day in each cohort. The subjects were administered the study drug during each cohort except for the discontinued/withdrawn subjects (Subject Nos. 1001, 1013, 1026 and 1027). The treatment was determined by the randomization schedule (Appendix No. 16.1.7). The duration of the clinicalpart of the study was about 83 days [11 hours prior to the IMP administration on Day 01 in Cohort-I until the last pharmacokinetic sample collection (24 hours after IMP administration on Day 14) in Cohort-IV].
The schedule of events planned is depicted in the following table[8].

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Table 2. Schedule of Events

Phase	**Screening (Within 28 days prior to dosing)**	**Cohort I to IV Table 2**						
Day		**-1**	**1**	**2**	**3-11**	**12-13**	**14**	**15**
Attendance		X						
Urine Drug Scan		X						
Breath test for alcohol		X						
Informed consent	X (Consent for screening)	X (Study specific consent)						
Serum Pregnancy Test (For female subjects)	X	X						X[#]
Compliance Assessment		X						
Baggage and Body search		X						
Clinical Lab Investigation (Hematology /Immunology/ Biochemistry/ Urine analysis)	X							
Clinical Lab Investigation (Hematology/ Biochemistry)[#]								X
Chest X-ray[$]	X							
ECG	X		X	X	X	X	X	X
Detailed Clinical examination#	X	X						X
Pre-dose vital sign			X	X	X	X	X	
Dosing			X	X	X	X	X	
Blood sampling**			X			X	X	X
Post dose Vitals^			X	X	X	X	X	
Standardized vegetarian breakfast			X	X	X	X	X	

** Blood sampling for pharmacokinetic assessment[9]:

Pre-dose samples On Day 01 (morning), Day 12 (morning and evening), 13 (morning and evening) and 14 (morning) prior to dosing Post dose sample (Day 01 - after morning dose) (Total 22 samples):

The venous blood samples were withdrawn at 0.500, 1.000, 1.500, 2.000, 2.500, 3.000, 3.500, 4.000, 4.333, 4.667, 5.000, 5.333, 5.667, 6.000, 6.333, 6.667, 7.000, 7.500, 8.000, 9.000, 10.000 and 12.000 hours following drug administration.

Post dose sample (Day 14 - morning dose) (Total 25 samples):

The venous blood samples were withdrawn at 0.500, 1.000, 1.500, 2.000, 2.500, 3.000, 3.500, 4.000, 4.333, 4.667, 5.000, 5.333, 5.667, 6.000, 6.333, 6.667, 7.000, 7.500, 8.000, 9.000, 10.000, 12.000, 16.000, 20.000 and 24.000 hours following drug administration.

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^Post-dose vitals: Vitals (sitting blood pressure and radial pulse) was recorded during each clinical examination, on Day 01 to day 13: each pre-dose and at 01, 02, 03, 04, 06 and 10 hours each post-dose in each cohort and on Day 14: at pre-dose and at 01, 02, 03, 04, 06 and 10 hours post-dose in each cohort. Oral body temperature was measured at screening, after check-in and before checkout in each cohort.

12-lead ECG: A standard 12-lead ECG was obtained during screening, on Day 01 to day 13: at each pre-dose and at 01, 02, 03, 04, 06 and 10 hours each post-dose in each cohort and on Day 14: at pre-dose and at 01, 02, 03, 04, 06, 10 and 12 hours post-dose in each cohort & at the end of the study (at the time of check-out).

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

Clinical Laboratory Investigations#

SubjectsTable 3	Cohort	Dose (mg)
08 (06 active + 02 placebo)	I	50
07 (05 active + 02 placebo)	II	50
08 (06 active + 02 placebo)	III	100
08 (06 active + 02 placebo)	IV	200

Hematology, biochemistry, urine analysis and immunology were performed during screening.

The post-study safety assessments included tests for hematology and biochemistry (except random glucose, sodium, potassium and chloride).

For Cohort 3 and 4: Liver function test (SGPT, SGOT and bilirubin) was performed at prior to morning dosing on Day 7.

Serum pregnancy test was performed at the time of screening, prior to check-in and at the end of the study (at the time of check-out). The laboratory reports were reviewed by a physician and were found to be negative for all the subjects. Meals:

Day 1 to Day 14 - Morning dose:

After an overnight fast of at least 08 hours, the subjects were served standardized vegetarian breakfast, which they consumed within 30 minutes.
Day 1 to Day 13 - Evening dose:

After an overnight fast of at least 02 hours, the subjects were served standardized vegetarian breakfast, which they consumed within 30 minutes. Lunch was served after 04 hours post-dose. Further meals after 04 hours post-dose were served at appropriate interval then on until check-out. #At the end of the study (at the time of check-out).
Check-in day (Day -1)
Dosing Day [Day 1 to 13 (Morning & evening) Day 14 (Morning)]
Check out day (24 hours after receiving last dose of the IMP) 4. Post study (at the time of check-out) – Day 15

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.

Inclusion Criteria

The inclusion criteria as per the protocol were as follows:

- Non-smokers, healthy, adult, human volunteers between 18 and 45 years of age (both inclusive).

Having a Body Mass Index (BMI) between 18.5 to 29.9 (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.
- Able to understand and comply with the study procedures, in the opinion of the investigator.
- Able to give voluntary written informed consent for participation in the study.
- In case of female subjects:

Surgically sterilized at least 6 months prior to study participation.
Serum pregnancy test must be negative.

Exclusion Criteria

The exclusion criteria as per the protocol were as follows:

- Known hypersensitivity or idiosyncratic reaction to cholecystokinin receptor antagonist or to any of its excipients or any drug or any substance.
- 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 mmHg and/or more than 140/90 mmHg at the time of screening.

- Ingestion of a medicine [prescribed & over the counter (OTC) medication including herbal remedies] at any time within 2 weeks before first dosing (4 weeks for any drug which may affect hepatic drug metabolism). In any such case subject selection will be at the discretion of the Principal Investigator.
- Any history or presence of asthma (including aspirin induced asthma) or nasal polyp or NSAIDs induced urticaria.
- Consumption of Grapefruits or its products within a period of 72 hours prior to first IMP administration.
- A recent history of harmful use of alcohol (less than 2 years), i.e. alcohol consumption of more than 14 standard drinks per week for men and more than 7 standard drinks per week for women (A standard drink is defined as 360 mL of beer or 150 mL of wine or 45 mL of 40% distilled spirits, such as rum, whisky, brandy etc.) or Consumption of alcohol or alcoholic products within 48 hours prior to first IMP administration.
- Smokers or who have smoked within last 06 months prior to start of the study.
- Difficulty in swallowing solids dosage forms like capsule or tablets.
- 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.
- A history of difficulty with donating blood.
- Donation of blood (1 unit or 350 mL) within a period of 90 days prior to the first dose of study medication.
- 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**.

** If investigational medicinal product is received within 90 days where there is no blood loss except safety lab testing, subject can be included considering 10 half-lives duration of investigational medicinal product received.

- A positive hepatitis screen including hepatitis B surface antigen and/or HCV antibodies.
- A positive test result for HIV antibody (I &/or II).
- An unusual diet, for whatever reason (e.g. low-sodium), for four weeks prior to first IMP administration. In any such case subject selection will be at the discretion of the Principal Investigator.
- Nursing mothers (females).
- Females of child bearing potential.

All the checked in subjects satisfied all the above inclusion and exclusion criteria.

Removal of Subject from Therapy or Assessment

As per the protocol the investigator 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.
- Any subject found to have entered the study in violation of the protocol. This would include pre-study directions regarding alcohol and drug use, fasting/fed or if the subject is non-compliant during the study or uncooperative with study procedures.
- Any subject who requires the use of an unacceptable concomitant medication (prescribed & over the counter (OTC) medication including herbal remedies).
- If it is felt in Principal Investigator's opinion that it is not in the subject's best interest to continue.
- If any subject cross-participates in other drug trial or trial screening.
- 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 who wishes to withdraw his/her consent.
- Any other justifiable reason.
- Any subject experience emesis within 12 hour after dosing of the study drug (not applicable for cohort 5 and 6).
- Found positive in serum pregnancy test (for females).

TREATMENTS

Treatments Administered

Day 1 to Day 14 - Morning dose:

After an overnight fast of at least 08 hours, the subjects were served standardized vegetarian breakfast, which they consumed within 30 minutes.

Day 1 to Day 13 - Evening dose:

After an overnight fast of at least 02 hours, the subjects were served standardized vegetarian breakfast, which they consumed within 30 minutes.

The investigational medicinal products (either active treatment or placebo treatment) were administered

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at 30 minutes after serving of the standardized vegetarian breakfast to the subjects in sitting posture with 240 ± 02 mL of drinking water. The IMP administration was as per the randomization schedule and under double blinded conditions.
The capsule was swallowed whole without chewing or crushing.
Note: Due to pre-dose discontinuation of one subject in Cohort-II, the study was conducted on 31 subjects instead of 32 subjects.

The IMP (either active treatment or placebo treatment) was administered twice daily from Day 01 to Day 13 (at 12 hours apart) and once on Day 14 (morning) to each subject. The time of dose administration on Day 01 was the reference time for all subsequent dosing. All the subsequent doses were administered at an interval of 12 hours with reference to the previous dose. Based on the safety assessment, the next cohort was dosed.

Subjects were not enrolled for next dose panel until safety data for all subjects from the previous dose panel was reviewed by Data Safety Monitoring Board (DSMB). If the studied dose cohort was safe and tolerated, then the succeeding dose panel received the next higher dose.

This procedure for dose escalation was applied for all dose cohorts until the maximum tolerated dose was achieved, or up to a maximum dose level of 200 mg BID.

The time of administration of the investigational medicinal product was the time at which the subject completed drinking 240 ± 02 mL of water and that was captured in the respective source data forms.

This activity was followed by a mouth check to assess the compliance to dosing. The subjects remained in sitting posture or ambulatory position up to 02 hours post-dose unless medically necessary due to adverse event or procedurally required or natural exigency, in such cases it was not considered as protocol deviation. Thereafter, the subjects were allowed to engage only in normal activities while avoiding any strenuous physical activity.

They refrained from drinking water from 01 hour prior to dosing till 01 hour after dosing during the study, except for the water given at the time of drug administration. Prior to and thereafter, water was allowed at all times.
Selection and Timing of Dose for Each Subject

Day 1 to Day 14 - Morning dose:

Dosing was carried out in the morning at 30 minutes after serving of the standardized vegetarian breakfast after an overnight fast of at least 08 hours.

Day 1 to Day 13 - Evening dose:

Dosing was carried out in the morning at 30 minutes after serving of the standardized vegetarian breakfast after an overnight fast of at least 02 hours.
Blinding

The study design incorporated randomization and used double blind design. Subjects, Investigator and analysts were blinded to the identity of the treatment in each cohort.

Pharmacy custodian at the clinical unit who prepared and verified the individual unit doses and QA personnel who witnessed dispensing activity were the only staff members at the clinical unit who were not blinded.

Two sets (one for Investigator and one for DSMB) of sealed, opaque individual codebreak envelopes were provided to the Investigator/Designee for emergency unblinding. The randomization envelopes were maintained in a secure location with access limited to authorized personnel. The subjects and the medical staff were not aware of which treatment was being administered to each subject enrolled in the study. The sponsor remained blind during the study.

PHARMACOKINETIC AND SAFETY VARIABLES

Pharmacokinetics: The following pharmacokinetic parameters[10] were computed for PNB-001 using non-compartmental model of Phoenix®WinNonlin® Version 8.1
(Certara L.P.) for cohort 1 to cohort 4. Day 01:

Actual time-points of the sample collection were used for the calculation of pharmacokinetic parameters.

All concentration values below the lower limit of quantification are set to zero for the pharmacokinetic and statistical calculations.

Safety: The following measures were taken to monitor and assess the safety of the subjects during the study:

- Subjects were monitored throughout the study period for adverse events. They were instructed to bring to the notice of the nurse, clinical custodian or the doctor, any adverse event that occurred during their stay at the clinical facility.
- A physician was available within the clinical facility whenever the subjects were housed (from check-in to check-out). A consultant physician was always available on call during the study period.
- Clinical examination of the subjects including recording of vital signs (blood pressure and radial pulse) and oral body temperature was

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done at screening, after check-in and before check-out in each cohort. The clinical examination before check-out started within 120 minutes prior to the scheduled time of check-out of each subject.

- Vitals (sitting blood pressure and radial pulse) was recorded during each clinical examination, on Day 01 to day 13: each pre-dose and at 01, 02, 03, 04, 06 and 10 hours each post-dose in each cohort and on Day 14: at pre-dose and at 01, 02, 03, 04, 06 and 10 hours post-dose in each cohort. Oral body temperature was measured at screening, after check-in and before checkout in each cohort.
- Chest X-ray (during the last 6 months) (postero-anterior view) was performed during screening.
- A standard 12-lead ECG was obtained during screening, on Day 01 to day 13: at each pre-dose and at 01, 02, 03, 04, 06 and 10 hours each post-dose in each cohort and on Day 14: at pre-dose and at 01, 02, 03, 04, 06, 10 and 12 hours post-dose in each cohort & at the end of the study (at the time of check-out).

Note: All the ECG during housing could start within ± 20 minutes of the scheduled time; ECG before check-out could started within 120 minutes of the scheduled time.

- Sitting blood pressure was at least 110/70 mm Hg and less than or equal to 140/90 mm Hg at the time of screening.
- Day 01 to day 14: Continuous cardiac monitoring was done from each pre-dose until 6 hours each post-dose in each cohort.
- Day 01 to day 14: Cardiologist/MD Physician remained available inside the facility from first dose administration on day 1 till end of the study (at the time of check-out) in each cohort.
- Subjects were questioned for well-being at the time of clinical examination and during recording of vital signs in each cohort.
- Serum pregnancy test for female subjects were carried out at the time of screening, prior to check-in and at the end of the study (at the time of check-out).
- Subjects were instructed not to participate in other clinical trial or donate blood anywhere else during the study.
- For Cohort 3 and 4: Liver function test (SGPT, SGOT and bilirubin) was performed prior to morning dosing on Day 7
- Laboratory assessment was done at the time of screening. Urine scan for drug of abuse and breath test for alcohol consumption were carried out prior to check-in in each cohort.
- Hematology and biochemistry tests (except random glucose, sodium, potassium and chloride) were done at the end of the study (at the time of check-out) to assess the post-study safety.

Note: After completion of each cohort, there was a DSMB (Data Safety Monitoring Board) meeting for discussion regarding safety of the investigational medical product. Recruitment for the next cohort was performed only after product was found safe by majority of DSMB members.

Drug Safety Monitoring Board (DSMB)

An Independent Data Safety Monitoring Board[11] (DSMB) evaluated safety and tolerability data obtained in the study. The safety and tolerability data (adverse event, electrocardiogram, vital signs and clinical laboratory test results) obtained for each cohort were reviewed by an independent DSMB to allow escalation to the next dose level. Data review and decision for dose escalation by DSMB was based on blinded data. The chairperson of the DSMB was provided with the randomization list, in order to have access to individual treatment assignment. All DSMB meetings and decisions were documented. The roles and responsibilities of DSMB were guided by DSMB Charter which was mutually agreed by Sponsor, CRO and DSMB Members.

Bioanalytics

Plasma concentration measurements are often used for the calculation of pharmacokinetic parameters. The sampling time-points were planned based on the reported pharmacokinetics of the drug. These time-points were chosen to assess C_{max}, Tmax, AUC0-t, AUC0-$_{\infty}$, λz, AUC_% Extrap_obs, Cτ,ss, Cmax,ss, Tmax,ss, AUC0-$_{\tau,ss}$, Cav,ss and %Fluctuation and $t_{1/2}$ appropriately.

Blood samples were collected through an indwelling intravenous cannula (Venflon) placed in a forearm vein of the subjects. Immediately after collection of blood, the collection tube (vacutainer) was inverted gently several times to ensure the mixing of tube contents (i.e. anticoagulant). For Cohort I to IV, pre-dose blood samples on Day 12 (morning and evening) and Day 13 (morning and evening) were collected via fresh vein puncture. A blood sample for liver function test (SGPT, SGOT and bilirubin) prior to morning dosing on Day 7 was collected through a fresh vein puncture (for Cohort 3 and 4). On Day 01 and 14, cannula was removed after 12.000 hours post-dose blood sample collection. Blood samples after 12.000 hours post-dose were collected through a fresh vein puncture. For precaution purpose, vacutainers were placed

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upright in a rack kept in ice cold water bath until centrifugation.

As per the protocol, the pre-dose blood samples were collected within 05 minutes (except pre-dose sample on day 01 within 60 minutes) before the dosing and the postdose in-house blood samples were to be collected within ± 02 minutes from the scheduled time. Post-dose samples not collected within this time frame from the scheduled time were documented as sampling deviations. The actual time of collection of each blood sample was recorded immediately after blood collection. For precaution purpose, the blood samples were kept in ice cold water bath during collection.

Deviations in this regards are appended in Appendix No. 16.3.2 (Other CRFs submitted).

On Day 01 & 14: Intravenous indwelling cannula was kept in situ as long as possible by injecting 0.5 mL of normal saline solution to maintain the cannula patent for collection of all blood samples up to 12.000 hours post-dose. In such cases blood samples were collected after discarding the first 0.5 mL of normal saline containing blood from the tubing. The blood samples were collected using syringe and transferred into pre-labelled vacutainers containing K_2EDTA as the anticoagulant.

Sample Processing The blood samples were centrifuged at 3000 ± 100 rcf for 5 minutes below 10°C to separate plasma. For precaution purpose, the blood samples were kept in ice cold water bath before centrifugation and during separation. The separated plasma was transferred to pre-labeled polypropylene tube in two aliquots (around 0.5 mL in first aliquot and remaining volume in second aliquot). 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 ice and stored in a bioanalytical freezer at -65 ± 10° C until completion of analysis.

Initially first aliquot samples were transferred to bio-analytical site and once the samples were received at bio-analytical site, the second aliquot was transferred from clinical site separately.

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.

III RESULTS and DISCUSSION

DISPOSITION OF SUBJECTS

As per the protocol, the study was to be conducted in 32 subjects (08 subjects x 06 cohorts). Due to pre-dose discontinuation of one subject in Cohort-II, the study was conducted on 31 subjects instead of 32 subjects.

08 (06 active + 02 placebo) subjects were dosed in each cohort. The dose was selected for each cohort as below:

Cohort-I and II: 50 mg, Cohort-III: 100 mg and Cohort-IV: 200 mg

Cohort-I

A total of 10 subjects (Subject Nos. 1001-1008, 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.

Subject No. 1005 did not want to continue his further participation in the study due to his personal reason. Hence, he discontinued from the study on his own accord. He was replaced with extra available Subject No. X-1, who was later, allotted Subject No. 2005.

Subject No. 1007 did not want to continue his further participation in the study due to his personal reason. Hence, he discontinued from the study on his own accord. He was replaced with extra available Subject No. X-2, who was later, allotted Subject No. 2007.

Hence, 08 subjects (Subject Nos. 1001-1004, 2005, 1006 and 2007) were dosed in the study. In all, 07 subjects (Subject Nos. 1002-1004, 2005, 1006 and 2007) completed clinical phase of the study successfully.

Cohort-II

G2, 50 mg,female subjects: A total of 08 subjects (Subject Nos. 1009-1016) were checked in for the study.

On Day 1 after the morning dose subject No. 1016 discontinued on her own accord without medical reason.

On day 2 in the evening, subject No. 1013 discontinued based on a mild emesis ground.

In all, 06 subjects (Subject Nos. 1009-1012, 1014 and 1015) completed clinical phase of the study successfully.

Cohort-III

G3 PNB-001 CAPSULE 100 MG, medium dose: 8 subjects were enrolled into the study G3 from 17-Jan-2019 to 01-Feb-2020 and all 8 subjects completed without any adverse events.

Cohort-IV

A total of 10 subjects (Subject Nos. 1025-1032, X-5 and X-6) were checked in for the study. Subject Nos. X-5 and X-6 were checked in for the study, in order to compensate for any dropouts prior to dosing.

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Parameters (Units)	Mean ± SD (untransformed data) Table 4			
	Cohort-1 (N=6)	**Cohort-2 (N=4)**	**Cohort-3 (N=6)**	**Cohort-4 (N=6)**
Tmax (h)#	3.917 (2.000 - 4.667)	4.500 (3.000 - 4.667)	4.333 (3.500 - 4.333)	4.333 (3.000 - 4.667)
C_{max} (ng/mL)	17.310 ± 12.2410	24.584 ± 13.2606	55.277 ± 31.5769	57.939 ± 23.2387
AUC_{0-t} (ng.h/mL)	45.386 ± 32.9743	64.012 ± 33.6046	134.112 ± 58.2673	156.973 ± 82.3717
$AUC_{0-\infty}$ (ng.h/mL)	51.789 ± 34.2404	77.215 ± 41.6995	152.473 ± 56.5509	170.404 ± 84.7862
λ_z (1/h)	0.281 ± 0.1551	0.228 ± 0.0884	0.249 ± 0.1455	0.318 ± 0.0938
$t_{½}$ (h)	6.840 ± 11.2662	3.686 ± 2.2238	4.945 ± 4.5391	2.399 ± 0.9049
AUC_%Extrap_obs (%)	12.759 ± 15.9511	16.011 ± 6.0290	12.807 ± 9.6600	8.527 ± 6.9238

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, 08 subjects (Subject Nos. 1025-1032) were dosed in the study. In all, 06 subjects (Subject Nos. 1025 and 1028-1032) completed clinical phase of the study successfully.

In all the four cohorts, 27 subjects (Subject Nos. 1002-1004, 2005, 1006, 2007, 10081012, 1014, 1015, 1017-1025 and 1028-1032) completed the clinical phase of the study successfully. G4 PNB-001 CAPSULE 200 mg, high dose:The trial ware performed from 14-Feb-2020 to 29-Feb-2020 and 8 healthy subjects were enrolled and 6 completed the trial, having 2 adverse event recorded:

Subject No. 1026 (Discontinued ground: Medical Ground).

Standardized vegetarian breakfast deviation (Subject No. 1004) - The requirement of standardized vegetarian breakfast[12] calories shall be between 400-600 kcal, and on particular day for Subject No. 1004 calorie consumption are above 400 kcal in Cohort-I. Hence, considering this, it would unlikely to have any significant impact on study outcome in general, but here due to the large food effect, attention to detail was essential. The average C_{max} was 17ng/ml and the deviation due to food reduction was found to be within the SD, so not significant.

Standardized vegetarian breakfast deviation (Subject Nos. 1010-1012) - The requirement of standardized vegetarian breakfast calories shall be between 400-600 kcal, and on particular day for Subject Nos. 1010-1012 calorie consumption are above 400 kcal in Cohort-II. Hence, considering this, no increase or decrease of plasma level was found. In conclusion variation of food consumption is unlikely to have any significant impact on study outcome and bioavailability (absorption).

Pharmacokinetic Analysis

The pharmacokinetic parameters[13] are derived individually for each analyzed subject from the plasma concentration vs. time profiles of PNB-001. Dataset for the calculation of pharmacokinetic

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parameters has been prepared using Phoenix®WinNonlin® Version 8.1 (Certara L.P.). C_{pd}on day 12 (morning and evening), day 13 (morning and evening) and day 14 (morning) are reported for PNB-001.

Actual time points of the sample collection are used for the calculation of pharmacokinetic parameters. Sampling time points deviations used in the pharmacokinetic evaluation are tabulated in Table No. 14.2.2 (Sampling time point deviations used for pharmacokinetic evaluation). The pharmacokinetic parameters of PNB-001 for Cohort-1 to Cohort-4 are summarized in the following table 4 and table 5.

Descriptive Statistics of Formulation Means for PNB-001 (Day 01)

$^{\#}T_{max}$ is represented as median (min-max) value.

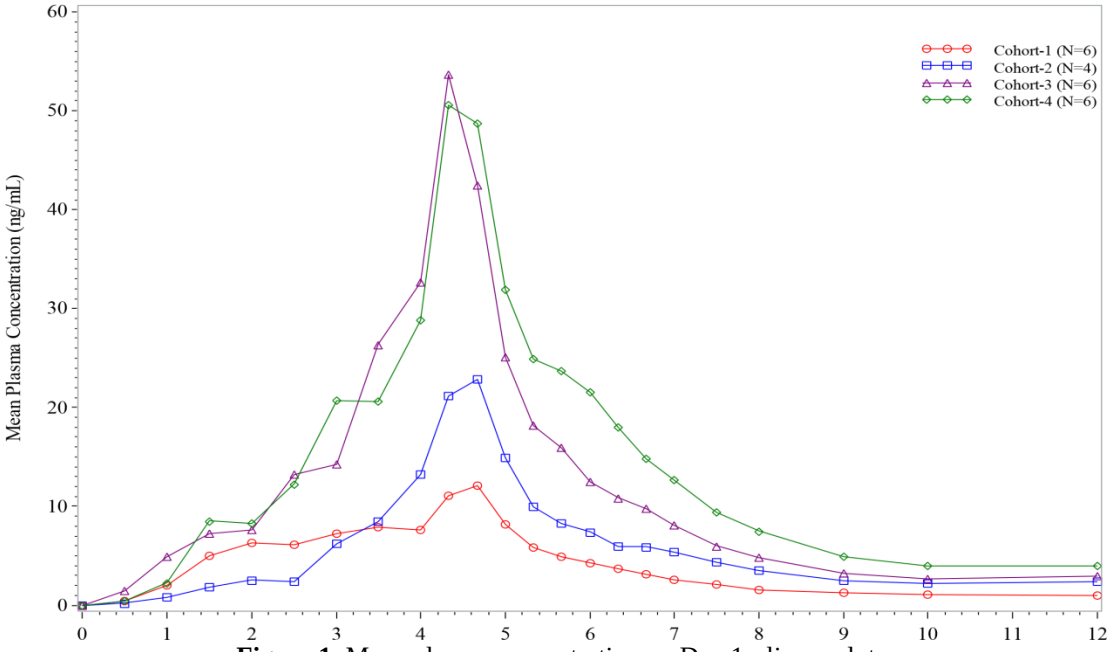

Figure 1. Mean plasma concentration on Day 1 - linear plot

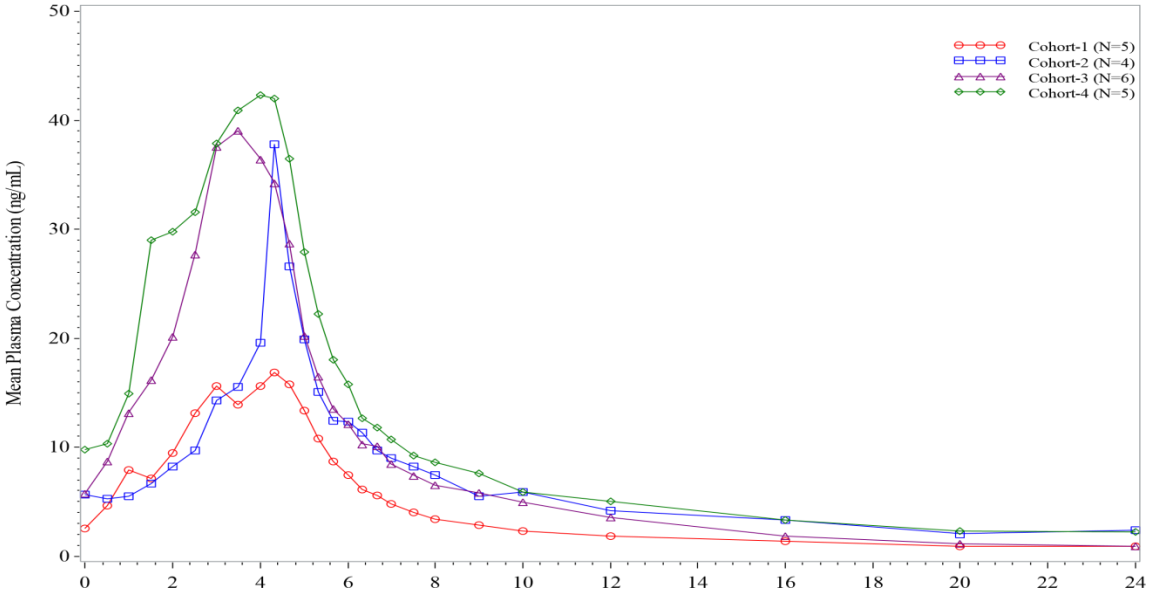

Figure 2. Mean plasma concentration on Day 14 - linear plot

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Descriptive Statistics of Formulation Means for PNB-001 (Day 14)

Parameters (Units)	Mean ± SD (untransformed data) Table 5			
	Cohort-1 (N=5)	**Cohort-2 (N=4)**	**Cohort-3 (N=6)**	**Cohort-4 (N=5)**
Tmax,ss (h)[#]	3.000 (2.500 - 4.700)	4.342 (4.333 - 4.667)	3.250 (3.000 - 4.000)	3.500 (3.000 - 4.333)
Cmax,ss (ng/mL)	24.724 ± 13.7479	38.696 ± 21.1751	47.169 ± 18.3947	49.267 ± 16.1197
$AUC_{0-\tau,ss}$ (ng.h/mL)	85.973 ± 61.1394	122.607 ± 56.0912	175.253 ± 67.1760	216.544 ± 101.9205
Cτ,ss (ng/mL)	1.844 ± 1.6908	4.196 ± 1.9110	3.533 ± 0.9169	5.026 ± 3.1759
Cav,ss (ng/mL)	7.164 ± 5.0949	10.217 ± 4.6743	14.604 ± 5.5980	18.045 ± 8.4934
%Fluctuation (%)	349.567 ± 78.0850	320.267 ± 86.6760	303.925 ± 52.8341	274.623 ± 99.7303
AUC_{0-t} (ng.h/mL)	100.525 ± 77.5354	157.092 ± 74.5950	196.208 ± 73.8779	253.524 ± 124.9794
$AUC_{0-\infty}$ (ng.h/mL)	112.448 ± 98.1653	183.783 ± 95.5100	205.653 ± 78.2705	281.365 ± 149.6530
λ_z (1/h)	0.151 ± 0.0566	0.100 ± 0.0245	0.125 ± 0.0523	0.090 ± 0.0157
$t_{½}$ (h)	5.319 ± 2.6649	7.228 ± 1.7753	6.598 ± 3.1765	7.929 ± 1.6621
AUC_%Extrap_obs (%)	6.434 ± 6.9977	13.216 ± 6.3698	4.675 ± 3.6265	8.353 ± 4.5239

[#]T_{max} is represented as median (min-max) value.

Based on above pharmacokinetic data presented for Day 1 under fed condition, median peak concentration of PNB-001 was achieved from the range of 3.9 to 4.5 hours post-dose irrespective of dose level and gender. Approximately 41-49% higher mean Cmax or AUCs were observed in female subjects (Cohort-2) as compared to male subjects (Cohort-1).

Increase in Cmax, AUC0-t and AUC0-∞ for Cohort-3 (100 mg) in comparison of Cohort-1 (50 mg) were higher than the proportional values while for Cohort-4 (200 mg) same was lower than the proportional values. Some variation is also observed in mean half-lives which were ranging from 2.4 to 6.8 hours. Based on above pharmacokinetic data presented for Day 14 under fed condition, median peak concentration of PNB-001 was achieved from the range of 3.0 to 4.3 hours post-dose irrespective of dose level and gender. Approximately, 43-63% higher mean $C_{max,ss}$, $C_{av,ss}$ or AUC_s were observed in female subjects (Cohort-2) as compared to male subjects (Cohort-1). Mean Cτ,ss was increase more than 2 fold in female subjects (Cohort-2) as compared to male subjects (Cohort-1).

Active Treatment:	PNB 001 Capsules 50 mg (Cohort I and II)
Table 6	PNB 001 Capsules 100 mg (Cohort III and IV)
Placebo:	PNB 001 Placebo Capsules 50 mg (Cohort I and II)
	PNB 001 Placebo Capsules 100 mg (Cohort III and IV)

Increase in $C_{max,ss}$, Cτ,ss, $C_{av,ss}$ or AUC_s for Cohort-3 (100 mg) in comparison of Cohort-1 (50 mg) were increased in dose proportional manner while for Cohort-4 (200 mg) same was lower than the proportional values. Mean half-lives at steady state were comparable over the dose range and gender, which were ranging from 5.3 to 7.9 hours. Overall, the experimental drug PNB001 seems to have a saturation pharmacokinetic at the higher doses.

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While comparing the Day 1 pharmacokinetic profile to Day 14, Accumulation Index [assessed as mean $AUC_{0-\tau,ss(Day\ 14)}$/ mean $AUC_{0-t(Day1)}$] were ~1.9 for Cohort-1 and Cohort-2 while same were 1.3 and 1.4 for Cohort-3 and Cohort-4, respectively.

These findings again may support a saturation pharmacokinetic at higher dose levels. Even for

Active Treatment (N=23)Displays of Adverse Events Table 7

Adverse event (Preferred Term)	**Mild**		**Moderate**		**Severe**		**Total**		**Total**
	R	**NR**	**R**	**NR**	**R**	**NR**	**R**	**NR**	**R+NR**
Musculoskeletal and connective tissue disorders									
Pain in extremity	0	1 (4.35%)	0	0	0	0	0	1 (4.35%)	1
Subject No.		1001							
Investigations									
Alanine aminotransferase increased	2 (8.70%)	0	0	0	0	0	2 (8.70%)	0	2
Subject Nos.	1003 and 1026								
Hepatic enzyme increased	1 (4.35%)	0	0	0	0	0	1 (4.35%)	0	1
Subject No.	1026								
Gastrointestinal disorders									
Vomiting	1 (4.35%)	0	0	0	0	0	1 (4.35%)	0	1
Subject No.	1013								
Dyspepsia	1 (4.35%)	0	0	0	0	0	1 (4.35%)	0	1
Subject No.	1028								
General disorders and administration site conditions									
Vessel puncture site pain	0	1 (4.35%)	0	0	0	0	0	1 (4.35%)	1
Subject No.		1028							
Placebo Treatment (N=8)									
Adverse event (Preferred Term)	**Mild**		**Moderate**		**Severe**		**Total**		**Total**
	R	**NR**	**R**	**NR**	**R**	**NR**	**R**	**NR**	**R+NR**
Gastrointestinal disorders									
Abdominal pain	1 (12.5%)	0	0	0	0	0	1 (12.5%)	0	1
Subject No.	1027								

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Cohort-3 and Cohort-4, mean $C_{max,ss}$ at Day 14 were slightly lower than C_{max} at Day 1.

SAFETY EVALUATION

EXTENT OF EXPOSURE

Dosage: Dose variation was allowed in this study. The study comprised of four cohorts. The subjects were administered the active or placebo treatment with multiple ascending dose levels (50, 100 and 200 mg). Treatment regimen consisted one of the following formulations.

Subject Exposure: A total of 38 subjects were checked in for the study. Out of these 38 subjects, 31 subjects were dosed in Cohort-I to IV. The safety assessment includes information for all 31 subjects who were dosed at least one during this study.

ADVERSE EVENTS (AEs): Eight (08) adverse events (AEs) were reported by six (06) subjects during the conduct of the study. Two (02) AEs were reported in Cohort-I, one (01) AE was reported in Cohort-II and five (05) AEs were reported in Cohort-IV of the study.

Seven (07) AEs were reported in the subjects after administration of Active Treatment and one (01) AE was reported in the subject after administration of Placebo Treatment.

All the AEs were mild in nature in nature. All the subjects were followed up until resolution of their AEs.

The causality assessment was judged as possible for six (06) AEs, as unlikely for one (01) AE and as unrelated for one (01) AE.

There were no deaths or serious AEs during the conduct of the study.

Out of the total reported eight (08) AEs, one (01) AE was significant. The subject was withdrawn from the study on medical grounds. He was treated appropriately and followed up until resolution of hisAE. The causality assessment was judged as possible for the significant AE.

Analysis of Adverse Events The analysis of adverse events was carried out treatment-wise. Six (06) subjects reported a total of eight (08) AEs during the conduct of the study.

Two (02) AEs were reported in Cohort-I, one (01) AE was reported in Cohort-II and five (05) AEs were reported in Cohort-IV of the study.

Five (05) subjects in the Active treatment group reported a total of seven (07) AEs and one (01) subject in the Placebo treatment group reported one (01) AE.

The causality assessment was judged as possible for six (06) AEs, as unlikely for one (01) AE and as unrelated for one (01) AE. The causality assessment was judged as possible for all the AEs. All the AEs were mild in nature. Out of eight (08) AEs, three (03) AEs resolved with requiring treatment with medication and three (03) AEs resolved without requiring treatment with medication.

Cohort-I

On day 3, Subject No. 1001 was discontinued on his own accord without medical reason. No AE was reported and all remaining subjects completed the study period.

In all, 07 subjects (Subject Nos. 1002-1004, 2005, 1006 and 2007) completed clinical phase of the study successfully.

R=Related; NR=Not Related

Calculation of % of AEs = Total number of AEs*100 / Total number of subjects who have consumed atleast a single dose of the particular study drug (Active Treatment or Placebo Treatment) during the

Cohort-II

G2, 50 mg,female subjects: A total of 08 subjects (Subject Nos. 1009-1016) were checked in for the study.

On Day 1 after the morning dose subject No. 1016 discontinued on her own accord without medical reason.

On day 2 in the evening, subject No. 1013 discontinued based on a mild emesis ground): Subject has complaint of single episode of vomiting at 13:26 hrs on 20-Dec-2019. Vomiting lasted for about 02 minutes. Vomitus contained fluid mixed with food particles of approx. 150-200 ml. Subject has no associated complaint of headache, abdominal pain, diarrhoea, fever, body ache. Subject has no other complaint at present. As per PI's advice, subject was discontinued from study on emesis grounds as outlined in protocol.

In all, 06 subjects (Subject Nos. 1009-1012, 1014 and 1015) completed clinical phase of the study successfully.

In SAD at 1000 mg in 1 subject vomiting was observed and none vomited in the 1500 mg cohort. The adverse effect may be related to food intake at lunch time. No other AE was reported and all remaining subjects completed the second week of the trial period.

For the female cohort G2 a higher plasma concentration was analysed for the 50 mg with a C_{max} about 25ng/ml compared to cohort G1 in male subjects, who showed a C_{max} about only 12ng/ml. This finding is in line with preclinical studies in dogs.

Cohort-III

G3 PNB-001 CAPSULE 100 MG, medium dose: 8 subjects were enrolled into the study and all 8 subjects completed without any adverse events.

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Project No. 0218-19 Cohort -IV		Table 8. Elevated liver enzyme ALT and remission			
		SGPT (12.0 - 82.0 U/L)			
ID	Subject No.	Screening	Day 07 (21-Feb-2020)	Day 08 (22-Feb-2020)	Day 11 (25-Feb-2020)
AH14-00899	1026	37	113	115	93

For the 100 mg PO dose a $C_{max}$of 55 ng/ml was observed after 4h and the plasma concentration above 10 ng/ml lasted until 7-8h. For a maximum efficacy effect in rats a C_{max} range from 20-40ng/ml was giving best results in a variety of assays. This

ideal dose had no dropouts and unfortunately a questionnaire could not be completed to evaluate the enhanced well-being of the subjects.

Cohort-IV

08 subjects (Subject Nos. 1025-1032) were dosed in the study. In all, 06 subjects (Subject Nos. 1025 and 1028-1032) completed clinical phase of the study successfully.

Subject No. 1027 (Discontinued ground: Medical Ground): Subject has complain of abdominal pain over left flank region since 15:36 hrs on 17-Feb-2020, which is not associated with vomiting, diarrhea, fever, hiccups, constipation at present. Subject has no any other complain at present. As per PI's advice, subject is discontinued from the study.

CCK antagonists, such as devazepide have shown abdominal cramping as side effect.

However, this is remarkable as Baladol is gastrin selective and the CCK physiology should not be affected at this dose level.

On deeper analysis after un-blinding, is was found, that the subject received placebo.

So, in conclusion no loss of gastrin selectivity was found, PNB001 is highly gastrin selective and the AD was due to a nocebo effect, associated with the informed concent document.

Subject No. 1026 (Discontinued ground: Medical Ground): Subject's day 07 safety laboratory assessment done at 07:10 hrs on 21-Feb-2020 revealed SGPT level 113 U/L which is high and abnormal. Other lab parameters are clinically acceptable. Hence, repeat SGPT was advised. Repeat investigation done at 11:03 on 22-Feb-2020 revealed SGPT level 115 U/L which is high and clinically significant. However, subject is asymptomatic at present. Hence, this AE is documented for raised SGPT levels. As per PI's advice, subject is discontinued from the study on medical ground. At baseline the ALT was about 40 U/L and here, on day 7 a 3x times increase for the liver panel enzyme ALT was observed. For statins, which are considered very safe and, which are taken for life, at a 2.5 fold increased ALT level, it is advised to review risk and benefits.

On day 11 after a break of only 3 days the ALT was reduced to 80% and after 1 week the biomarker was back to baseline.

Baladol, as gastrin antagonist, may have a possible potential for liver injury as seen in recent CGRP receptor antagonists, which is licenced for migraine treatment.

In SAD a sinus tachycardia was observed and this resulted for the MAD trial in extensive ECG monitoring.

Here in this MAD study no cardiotoxicity could be detected at all. So, this is ruled out, but the asymptomatic ALT increase may be noted as possible adverse effect linked to the medication.

IV CONCLUSIONS

Pharmacokinetics

Under fed condition, median T_{max} values of PNB-001 were ranging from 3.0-4.5 hours at Day 1 and Day 14 for all dose levels and irrespective of gender.

Overall a higher rate and extent of exposure of PNB-001 was observed on female subjects as compared to male subjects.

At higher dose levels, PNB-001 seems to follow saturation pharmacokinetics, which leads to an increase in C_{max} and AUCs in less than dose proportional manner. Accumulation Index was determined in Cohort-1 and Cohort-2 and was found about ~1.9.

AI was calculated as 1.3 and 1.4 for Cohort-3 and Cohort-4, respectively.

Mean half-lives were ranging from 2.4 to 6.8 hours on Day 1; while mean half-lives were ranging from 5.3 to 7.9 hours on Day 14.

Safety

The investigational products were safe and well tolerated by healthy subjects, as a multiple dose administration.

In general, the clinical portion of the study was completed with eight (08) AEs, out of which one (01) AE was significant. In SAD a sinus tachycardia was observed and this resulted for the MAD trial in extensive ECG monitoring.

Here in this MAD study no cardiotoxicity could be detected at all. So, this is ruled out, but the unsymptomaticALT increase may be noted as possible adverse effect linked to the medication.

The food effect in SAD was re-confirmed in all 3 doses in multiple ascending doses from 50 via 100 to 200 mg. The current formulation resulted in excellent plasma concentrations in male and even higher bioavailability in female healthy subjects.

- Based on high safety in addition to acute pain chronic pain indications may also be considered.
- Period pain will be extended in further trials towards pain in migraine, knee pain after surgery and dental pain.
- New formulations, such as Baladol rapid® are under development to achieve analgesia faster.
- Chronic pain must be balanced with possible liver injury due to loss of CCK-B gastrin receptor selectivity.

OVERALL CONCLUSIONS

Baladol® (PNB-001) was found extremely safe in a PHASE 1 clinical trials with expected pharmacokinetics, leading to subsequent phase 2 trials in IBD and dysmenorrhea. PNB-001, will be marketed and promoted under Baladol®, which was tested now in 74 healthy subjects in clinical phase 1. Baladol is designed as anti-inflammatory analgesic and subsequent phase 2a trials in dysmenorrhea and inflammatory bowel disease are ongoing. In further trials a pain cohort with 6 female patients on active treatment and 6 on placebo is approved and scheduled. For IBD 6 male patients with active treatment and 2 placebo is approved and scheduled. These trials were stopped due to the current COVID19 pandemic.

In SAD a dose range from 25 mg to 1500 mg was tested and found safe. A large food effect was found resulting in a MAD trial under fed conditions.

The food effect in SAD was re-confirmed in all 3 doses in multiple ascending doses from 50 via 100 to 200mg. The current formulation resulted in excellent plasma concentrations in male and even higher bioavailability in female healthy subjects.

Planned clinical trials to gain market authorisation in India & NDA application:

G5 for IBD and G6 (50 mg) in pain are designed as phase 1b to gain safety data in patients and in addition to evaluate efficacy at the same time, thus equivalent to a phase 2a clinical trial. These trials are granted by the DCGI & scheduled. A further 2a trial with 100 mg will follow with 12+4 patients. Subsequently, a phase 2b trial with one selected dose is planned in patients to gain accelerated market authorisation due to a classified unmet medical need.

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