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A Randomized, Comparative Clinical Trial to Evaluate Efficacy and Safety of PNB-001 as Immune Modulator in Moderate COVID-19 Patients

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Abstract

Introduction: Several therapeutic agents are being evaluated for the treatment of coronavirus disease 2019 (COVID-19). PNB-001 is a potent anti-inflammatory agent with immune stimulation properties. It is a first in class CCK A agonists and CCKB antagonist, being responsible for its unique action.

Methods: We conducted a multi-centre, randomized, parallel group, comparative, open label study to assess efficacy and safety of PNB-001 in patients with moderate COVID-19 infection. Patients were randomly assigned to receive PNB-001 100 mg orally with Best Care BC (PNB 001 + BC) or only Best Care (BC). A total of 40 patients (20 in Adjunct and 20 in BC arm) were randomised and received treatment.

Results: The primary endpoint, change in the 8-point WHO Ordinal Scale score for COVID-19 showed significant Clinical Improvement from baseline to day 15 with PNB 001 + BC (P=0.042). Death rate, one patient on PNB 001+BC and two patients in BC arm died (1 Vs 2; HR: 2.0 [95%CI=0.18, 22.05]; P=0.56) by Day 28. Mean Chest X-ray score showed significant improvement (2.05 Vs 1.16; P=0.032) as well as more patients quickly showed complete improvement. Patients needed shorter duration of hospitalization and on day 15, 1 patient was hospitalised on adjunct compared to 5 on BC (P=0.048), thus giving 80% of reduction on the hospitalisation parameter. Mean duration of supplemental oxygen requirement was shorter. 50% of patients were off oxygen on day 6 on adjunct compared to day 8 on BC. Exploratory analysis done for ESR, CRP, IL-6, and N/L ratio and immune parameters showed a statistically significant reduction by Day 15. Lymphocytes were increased into the reference range (P=0.032) and neutrophils were reduced (P=0.013). The role of PNB-001 as immune modulator was clearly established. NLR was reduced significantly for adjunct compared to BC. A total of 24 (11 Vs 13) adverse events were reported in 18 (8 Vs 10) patients and none of the 11, were related to PNB-001. Overall safety profile was found better in test than control arm.

Conclusion: PNB-001 when combined with BC improved the clinical status of patients with moderate COVID-19 infection compared to BC alone. Hospitalisation and dead rate was further reduced by 80% and 50%, respectively. PNB-001 was well tolerated by patients with moderate COVID-19 and acted by stimulation of the immune system.

Key Findings: Anti-inflammatory activity was improved further for test agent PNB-001 even in presence of potent steroids. The immune stimulating properties of PNB-001, analysed in form of NLR, are key to fight COVID-19 infection. NLR was found highly useful as predictive and clinical biomarker and it was significantly reduced by PNB-001.

Keywords: Immune modulator; CCK ligand; Cholecysto-kinin; NLR; Neutrophil; Lymphocyte; Phase 2; endpoints; Clinical; NETosis

Introduction

Coronavirus disease 2019 (COVID-19) has widely spread over the globe since its first case in December 2019 in Wuhan, China. COVID-19 is declared as a pandemic by the WHO. COVID19 is spread via droplets or direct contact and infects the respiratory tract resulting in pneumonia in most of the cases and acute respiratory distress syndrome (ARDS) in about 15% of the cases [1,2]. During COVID-19 infection, finally a large amount of pro-inflammatory cytokines get released resulting in an aggressive inflammatory response in response to fighting the disease.

Severe COVID-19 patients develop acute respiratory distress syndrome that may progress to cytokine storm syndrome, organ dysfunction, and death. Considering, that neutrophil extracellular traps (NETs) have been described as important mediators of tissue damage in inflammatory diseases, it was shown that NETs is highly relevant in COVID-19 pathophysiology [3].

NLR is a predictive biomarker of turning the infection into serious and CCK is required to stimulate the production of lymphocytes (Figure 1). A later stage the overproduction of neutrophils via NETosis leads to the known "cytokine storm", and it shows a direct relation with the increased mortality in COVID-19. Covid-19 is an out of tune of the immune system and key task is to bring neutrophils and lymphocytes levels back into the healthy range [2].

The cholecystokinin via the CCK2 path way is reducing inflammation in addition to the CCK1 cholinergic anti-inflammatory pathway. PNB-001 is a CCK2 antagonist and CCK1 agonist, a first in class. In figure 1, in addition the commonly known role of cholecystokinin in inflammation, the function on the immune system shown.

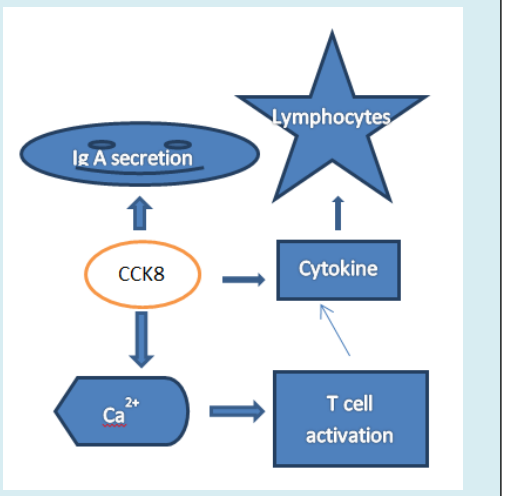

Figure 1: MOA; CCK mediated immunology.

Immuneregulation: High expression of the cholecystokinin receptor (CCKAR and CCKBR) on B lymphocytes in mouse at mRNA and protein levels. In the spleen CCKR is found in high abundance, biologically CCK8-S is supposed a stimulating factor in Ig A and Ig G and IgM to a lesser degree. Key is the increase of the calcium concentration, measured by fura-2 fluorometry, in form of second messengers by the Gq pathway (G protein coupled receptor, GPCR).

Relevant biomarkers for monitoring inflammation at molecular level are C-reactive protein (CRP), for the response of the immune system IL-6, plays a key role. Most relevant neutrophil/lymphocyte (N/L) ratio, platelet/lymphocyte (P/L) ratio, erythrocyte sedimentation rate (ESR) are also associated with more severe disease. Excessive pro-inflammatory cytokines, as part of NETosis aggravates ARDS and cause tissue damage leading to multiple organ failure, and unfavourably affect prognosis of severe COVID-19 [4-11]. PNB-001 as potential neuro-peptidal based immune modulator was used as therapeutic intervention in early hospitalised patients and that therapeutic agent was evaluated in terms of safety and efficacy to improve mortality. hospitalisation and oxygen supplementation [2,11].

PNB-001 is a dual action cholecystokinin (CCK) receptor ligand acting on the CCK-B / gastrin as antagonist and CCKAR agonist. It has shown analgesic, antipyretic and anti-inflammatory properties in animal models. PNB-001 was approved for IBD in India and was redirected into COVID19 once the outbreak became significant [12,13]. The inflammatory immune response of PNB-001 was studied in Dengue infected mice and a reduced death rate. In single ascending dose study, PNB-001 was administered at a range of 25 to 1500 mg under fasting condition [14]. The PK (C_{max} and AUC_{0-t}) of PNB-001 increase well in dose proportional manner and the target therapeutic concentration range was reached [15].

In multiple ascending dose study (50, 100 and 200 mg for 14 days), mean half-lives of PNB-001 ranged from 2.4 to 6.8 hours on Day 1 and from 5.3 to 7.9 hours on Day 14. PNB001 was well tolerated in both the studies without any incidence of death or serious adverse events and 100 mg was found to be the suitable choice [16]. The objectives of this clinical trial is to assess efficacy and safety of PNB-001 plus standard of care (SOC) in comparison to BC alone in patients with moderate COVID-19 Infection [15,16].

Methods

The study was conducted in compliance to International Conference on Harmonization (ICH) guidance for Good Clinical Practice (GCP) and CDSCO (Schedule-Y) guidance of New Drugs and Clinical Trials Rules, 2019. The study

protocol was approved by independent ethics committee. All the patients provided written informed consent prior to study enrolment. The study was conducted at two sites in India (Study Registration No. CTRI/2020/10/028423) and the 8 score ordinal scale was applied to evaluate efficacy (Table 1).

Patient State	Descriptor	Score
Uninfected	No clinical or virological evidence of infection	0
Ambulatory	No limitation of activities	1
	Limitation of activities	2
Hospitalized Mild disease	No oxygen therapy	3
	Oxygen therapy by mask or nasal prongs	4
Hospitalized Severe disease	Non-invasive ventilation or high-flow oxygen	5
	Intubation and medical ventilation	6
	Ventilation + additional organ support – pressors, RRT, ECMO	7
Dead	Death	8
ECMO=extracorporeal membrane oxygenation; RRT=renal replacement therapy		

Table 1: Ordinal Scale for Clinical Improvement Eligibility.

The inclusion criteria of the study were laboratory-confirmed SARS-CoV-2 infection as determined by PCR within 2 days of randomization; patients having pneumonia with no signs of severe disease with SpO_2 ≤94% (range 90-94%) on room air; patients with any two of the following signs or symptoms suggestive of COVID-19: fever, cough, dyspnoea, or hypoxia; respiratory rate more or equal to 24 per minute; radiographic infiltrates as confirmed by imaging (chest x-ray). Patients were excluded if they required invasive mechanical ventilation; had the following clinically significant laboratory abnormalities: SGOT, SGPT, serum bilirubin > 2.5 times the Upper Limit Normal (ULN); had abnormal serum creatinine value of ≥ 2 mg/dl; had Type 1 diabetes mellitus or uncontrolled Type 2 diabetes mellitus with random sugar ≥ 200 mg/dL; or had uncontrolled hypertension (systolic blood pressure > 160mmHg, or diastolic blood pressure >100mmHg) or previous history of hypertension crisis or hypertensive encephalopathy.

Characteristics	Statistics	PNB-001 (N=20)	Standard of Care (N=20)	All (N=40)	p-value
Age	n	20	20	40	0.8426
	Mean (SD)	52.10 (12.83)	52.80 (8.98)	52.45 (10.94)	
	Median (Min – Max)	54.0 (30.0, 73.0)	56.50 (34.0, 64.0)	55.0 (30.0, 73.0)	
Height	n	20	20	40	0.4762
	Mean (SD)	158.30 (6.97)	156.0 (7.94)	157.45 (7.42)	
	Median (Min – Max)	156.0 (150.0, 176.0)	156.50 (146.0, 179.0)	156.50 (146.0, 179.0)	
Weight	n	20	20	40	0.1592
	Mean (SD)	61.90 (13.94)	56.75 (7.94)	59.33 (11.49)	
	Median (Min – Max)	57.0 (45.0, 95.0)	58.50 (45.0, 70.0)	58.0 (45.0, 95.0)	
Sex	Male	14 (70.00%)	12 (60.00%)	26 (65.00%)	0.5073
	Female	06 (30.00%)	08 (40.00%)	14 (35.00%)	
Race	Indian	20 (100%)	20 (100%)	40 (100%)	-
Medical History					

Characteristics	Statistics	PNB-001 (N=20)	Standard of Care (N=20)	All (N=40)	p-value
Diabetes Mellitus		04(20.00%)	03 (15.00%)	07 (17.50%)	-
Asthma		00 (00.00%)	01 (05.00%)	01 (02.50%)	-
Hypertension		02 (10.00%)	04 (20.00%)	06 (15.00%)	-
Hypothyroidism		01 (05.00%)	00 (00.00%)	01 (02.50%)	-
DVT		02 (10.00%)	00 (00.00%)	02 (05.00%)	-
Pulmonary embolism		01 (05.00%)	00 (00.00%)	01 (02.50%)	-
SOC = Standard of Care					

Table 2: Summary of Demographics and Baseline Characteristics.

Study Design and Treatment

This was a multi-centre, randomized, parallel group, comparative, open label, clinical study to evaluate efficacy and safety of PNB-001 in patients with moderate COVID-19 infection. Patients were randomized in 1:1 ratio to receive either PNB-001 along with Standard of Care (PNB001 + BC) or only best clinical Care (BC). Patients, who were randomized to PNB001 + BC arm, received PNB-001 100 mg capsule orally thrice daily for 14 days with Standard of care. Patients randomized to other arm received only BC. Standard of Care BC was considered as per Clinical Management Protocol: COVID-19 Ministry of Health and Family Welfare Directorate General of Health Services and PI discretion.

The site team or the patient recorded the details of the study medication administration in the drug dosing card including date, time, dose, frequency, and missed dose information. During the treatment period of 14 days, the subjects were assessed daily for the improvement in clinical signs and symptoms, concomitant medications, and adverse events. If there were no improvement in clinical signs and symptoms of the subject, the patients were discontinued from the study as treatment failure and were provided with other effective therapy, if available.

Endpoints

Primary endpoints of the study were change in the 8-point WHO Ordinal Scale score for COVID-19 from baseline to day 15 and mortality rate by Day 28. Secondary endpoints were (i) percentage of patients showing change in clinical status using the ordinal scale from baseline; (ii) percent of patients showing improvement in inflammatory segments in X-ray chest from baseline; (iii) reduction of days of hospitalization; (iv) duration of supplemental oxygen (if applicable) (v) improvement in oxygen saturation from baseline; and (vi) days to negative PCR for COVID-19. The endpoint improvement in oxygen saturation from baseline was removed since it was identified as not a useful parameter. Exploratory analysis was done for IL-6, CRP, N/L ratio, P/L ratio, and ESR to assess mean change in these markers from baseline to Day 15.

Safety Assessments

The assessment of safety parameters was based on the frequency of AEs, changes in laboratory values and abnormal vital signs. Safety assessment were evaluated by assessing laboratory parameters from baseline till day 15 which included complete blood counts, erythrocytes sedimentation rate, liver function tests, total bilirubin, SGOT, SGPT, serum creatinine, CRP, and IL6 with X-ray Chest and ECG. Vital signs were measured at all visits.

Statistical Analysis

Continuous efficacy and safety variables were presented using descriptive statistics which included number, mean, standard deviation, median, and range. Categorical data were presented using counts and percentages. Mean change in the ordinal scale from baseline to end of study was analysed using Wilcoxon rank sum test. Mortality through Day 28 were analysed as a time to event endpoint. Differences in time-to-event endpoints by treatment were summarized with Kaplan-Meier curves. Secondary efficacy parameters were analysed using descriptive statistics and appropriate statistical test. Changes in (IL-6, CRP) inflammatory markers from baseline to end of study were analysed using two sample t-test using descriptive statistics. Exploratory analysis for IL-6, CRP, N/L ratio, P/L ratio, and ESR were done using two sample t-test and descriptive statistics. All the statistical analyses were performed using SAS 9.2 or higher version software.

Results

Patient Disposition

A total of 42 patients were screened of which one failed during screen and one withdrew consent before randomization. Hence, 40 patients were randomized into

two arms, i.e. 20 patients/treatment arm. In PNB-001 plus standard of care (BC) arm, 18/20 patients completed the study; one patient withdrew consent and one patient was discontinued from the study at the discretion of investigator. In BC arm, 17/20 patients completed the study; one patient withdrew consent and two patients were discontinued from the study at the discretion of investigator. Thus, total 35 patients completed the study. Patients, who discontinued the study at the discretion of investigator, had worsening severity of the disease, and these patients succumbed to the disease. Figure 2 depicts the patient disposition flow.

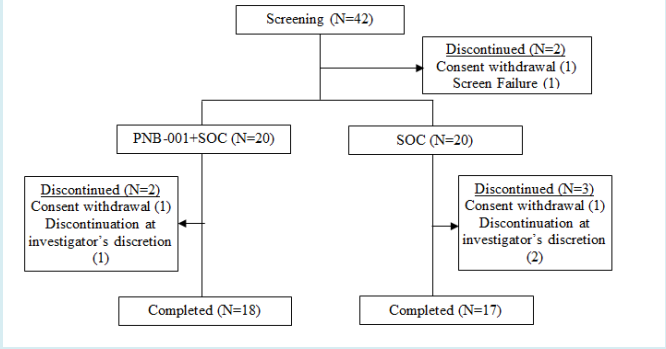

Figure 2: Patient Disposition by Treatment Arms.

Patients Demography and Baseline Characteristics

There were 14 males in the PNB-001 arm and 12 males in the Standard of Care arm. The mean patient age (52.10 years) in PNB-001+BC arm was comparable with (52.8 years) BC arm. The mean body height (158.30 cm) in PNB-001+BC arm was comparable with (156.0 cm) SOC arm. The average body weight (61.90 kg) in PNB-001+BC arm was comparable with (56.75 kg) BC arm. All patients were Indian. The demographics of patients were balanced between two treatment arms (Extended Data Table 1).

Two patients in PNB-001+BC arm had history of tobacco use; one was an occasional user and other consumed 10-15 packs of tobacco every week. The concomitant medication usage was comparable between the treatment arms. Commonly used concomitant medication were remdesivir, steroids, heparins, aspirin, different antibiotics, ivermectin, zinc, vitamin C, drugs for acidity, antiemetics, etc. A few patients had comorbid disease such as diabetes mellitus, asthma, hypertension, hypo-thyroidism, deep vein thrombosis, pulmonary embolism, and obesity. The baseline characteristics of patients were comparable between the treatment arms (Extended Data Table 1).

Efficacy primary efficacy endpoints

Ordinal scale: Patients in PNB-001+SOC (test) arm showed significant improvement in WHO Ordinal Scale for COVID-19 Clinical Improvement compared to patients in BC (control) arm at the end of the treatment (Figure 3).

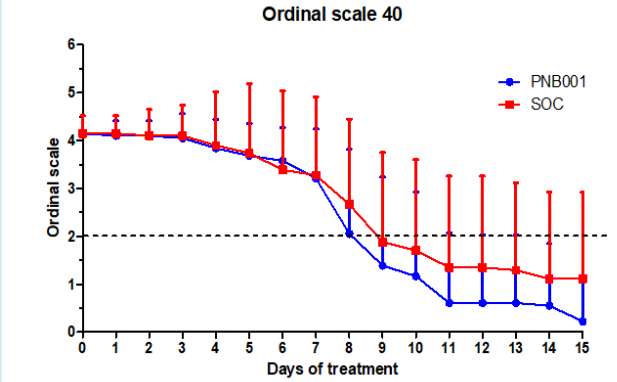

Figure 3: Ordinal Scale kinetics from day 0 to day 15, SOC=Standard of Care.

Further, patients showed significant improvement from baseline from Day 5 onwards till end of the study in the adjunct arm. Patients who received only BC, showed significant improvement from baseline from Day 8 onwards. At the end of the treatment (Day 15), mean ordinal scale was 0.22 in PNB-001+BC arm and 1.12 in BC arm, and the mean change in ordinal scale from baseline to Day 15 was statistically significant (P=0.042). For selected data see table 3.

Mortality: During the study, one patient in PNB-001+SOC arm died on Day 4. Total two patients died in BC arm; one patient on Day 6, the other patient on Day 10. These patients were discontinued from the study by the investigator as per the protocol due to worsening of clinical condition prior to the event. Mortality rate were reduced by 50% between the test (1) and control arms (2), being not significant due to too low patient numbers. For a statistically significant reduction of death rate, not N=40, but N=400 would be required. With a 50% reduction and 10/190 versus 20/180, a P value of P=0.06 would be calculated. The patient who died following randomization to PNB-001+BC is unlikely to have derived benefit from test treatment as onset of action is found after 1 week.

Visit	Scale	PNB-001 (N=20) n (%)	Standard of Care (N=20) n (%)	Total (N=40) n (%)
Baseline	4-Oxygen by mask or nasal prongs	17 (85.00%)	17 (85.00%)	34 (85.00%)
	5-Non-invasive ventilation or high flow oxygen	03 (15.00%)	03 (15.00%)	06 (15.00%)
Day 1	4-Oxygen by mask or nasal prongs	18 (90.00%)	17 (85.00%)	35 (87.50%)
	5-Non-invasive ventilation or high flow oxygen	02 (10.00%)	03 (15.00%)	05 (12.50%)
Day 2	3-Hospitalized, no oxygen therapy	00 (00.00%)	02 (10.00%)	02 (05.00%)
	4-Oxygen by mask or nasal prongs	18 (90.00%)	14 (70.00%)	32 (80.00%)
	5-Non-invasive ventilation or high flow oxygen	02 (10.00%)	04 (20.00%)	06 (15.00%)
Day 12	0-No clinical or virological evidence of infection	15 (83.33%)	11 (64.71%)	26 (74.29%)
	3-Hospitalized, no oxygen therapy	01 (05.56%)	01 (05.88%)	02 (05.71%)
	4-Oxygen by mask or nasal prongs	02 (11.11%)	05 (29.41%)	07 (20.00%)
Day 13	0-No clinical or virological evidence of infection	15 (83.33%)	11 (64.71%)	26 (74.29%)
	3-Hospitalized, no oxygen therapy	01 (05.56%)	02 (11.76%)	03 (08.57%)
	4-Oxygen by mask or nasal prongs	02 (11.11%)	04 (23.53%)	06 (17.14%)
Day 14	0-No clinical or virological evidence of infection	15 (83.33%)	12 (70.59%)	27 (77.14%)
	3-Hospitalized, no oxygen therapy	02 (11.11%)	01 (05.88%)	03 (08.57%)
	4-Oxygen by mask or nasal prongs	01 (05.56%)	04 (23.53%)	05 (14.29%)
Day 15	0-No clinical or virological evidence of infection	17 (94.44%)	12 (70.59%)	29 (82.86%)
	3-Hospitalized, no oxygen therapy	00 (00.00%)	01 (05.88%)	01 (02.86%)
	4-Oxygen by mask or nasal prongs	01 (05.56%)	04 (23.53%)	05 (14.29%)

Table 3: Summary of Ordinal Scale from Baseline to Day 15.

Secondary Efficacy Endpoints

A summary of change in ordinal scale from baseline to all the visits is presented in Table 3. Higher number of patients achieved ordinal scale of zero in adjunct arm compared to BC arm from Day 8 onwards. At the end of the treatment, 17 out of 18 patients have achieved zero ordinal scale in adjunct arm, whereas, 12 out of 17 patients have achieved zero ordinal scale in BC arm (P=0.07). On day 1 the mean OS was 4.2 in A / BC, on day 15 it was reduced to 1.1 for BC and 0.25 for adjunct treatment (Figure 3). In terms of ordinal scale kinetics the OS 4, requiring oxygen was reduced to OS=1, having no limitations of activities. This was reached on day 10 for adjunct treatment with PNB-001 and day 15 for BC (Figure 3). Other secondary efficacy results are presented in Table 4.

Parameters	Statistics	PNB-001+SOC (N=20)	SOC (N=20)	P-value between groups
Improvement in inflammatory segments in X-ray chest from baseline to day 15	Screening			
	0 – (0%)	00 (00.00%)	00 (00.00%)	-
	1 – (1-25%)	02 (10.00%)	04 (20.00%)	-
	2 – (Up to 50%)	06 (30.00%)	05 (25.00%)	-
	3 – (Up to 75%)	04 (20.00%)	05 (25.00%)	-
	4 – (>75%)	08 (40.00%)	06 (30.00%)	-
	Day 15*			
	0 – (0%)	10 (50.00%)	07 (36.84%)	
	1 – (1-25%)	06 (30.00%)	05 (26.32%)	-
	2 – (Up to 50%)	02 (10.00%)	02 (10.53%)	-
	3 – (Up to 75%)	01 (05.00%)	02 (10.53%)	-
	4 – (>75%)	01 (05.00%)	03 (15.79%)	-

Parameters	Statistics	PNB-001+SOC (N=20)	SOC (N=20)	P-value between groups
Time to complete improvement in inflammatory segments in X-ray chest by Day 15	HR: 1.48	95% CI (0.64,3.44)		0.4309
Chest X-ray score improvement from baseline to Day 15	Mean (SD)	2.05 (1.19)	1.16 (1.21)	0.0321
Time to discharge after hospitalization by Day 15	N (%)	19 (95%)	15 (75%)	0.0486
Duration of supplemental oxygen, Days	Mean (SD)	5.45 (2.96)	7.10 (4.38)	0.1708
No. of patients not needing external oxygen support on day 15	N (%)	17 (42.50%)	13 (32.50%)	0.1441
Days to Negative PCR	Median	7.6 (1.90)	7.0 (2.03)	0.6265
* N=19 for SOC arm.				

Table 4: Secondary Efficacy Assessments.

X-Ray: X-ray assessment of inflammatory segments of lungs showed improvement in higher number of patients in adjunct arm compared BC arm (50.00% vs. 36.84%, HR: 1.48 [95% CI: 0.64, 3.44]; P=0.43). Mean change in the chest X-ray score was 2.05 (SD=1.19) in PNB-001+BC arm and 1.16 (1.21) in BC arm (P=0.032).

Hospitalisation: Patients receiving PNB-001 + BC were hospitalized for lesser duration compared to BC (Mean 9.45 vs. 9.80 days). Significantly higher number of patients were discharged from the hospital by Day 15 in adjunct arm compared to BC arm (19 vs. 15, P=0.048).

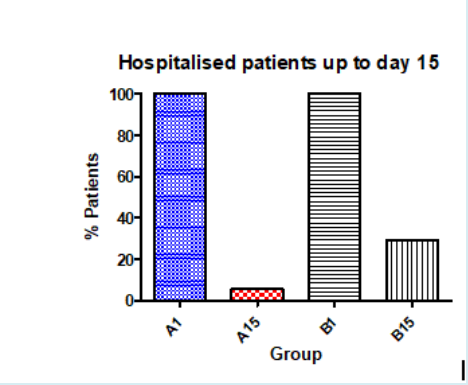

Figure 4: Hospitalised patients on day 1 and day 15 in percentage.

On day 1 in both arms 100% are hospitalised, on day 15 on the best care arm 5 of 17 patients are hospitalised (29%) compared with 1 of 18 (5.5%) on the adjunct treatment with 100 mg PNB-001. This is a reduction of hospitalisation on day 15 of 81%.

Oxygen Support: Mean duration of requirement of supplemental oxygen was shorter for patients in the adjunct test arm (5.45 vs. 7.10 days, P=0.17). In PNB-001+BC arm, 17 (94.44%) patients were off Oxygen support on Day 14 whereas only 13 (76.47%) patients were off Oxygen support with BC (P=0.14).

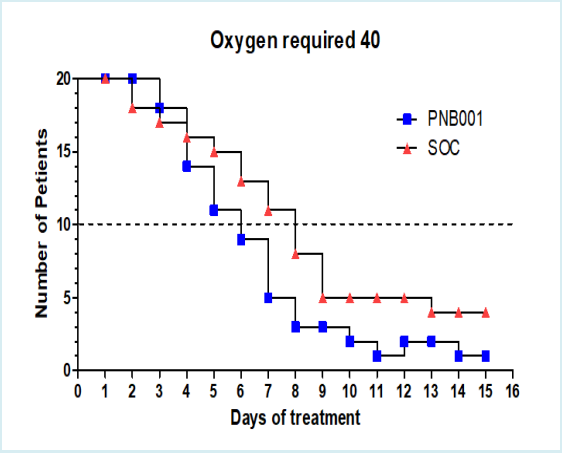

Figure 5: Oxygen supplementation.

On adjunct treatment 50% of patients were off oxygen supplementation on day 6 compared to day 8 for best care. By day 15 1 patient was on oxygen support in the adjunct arm, compared to 5 patients in best care. This is a reduction of 80% for the PNB-001 treatment. These days are clinically significant to prevent further damage of the lung tissue. A summary of different methods of oxygen administered during the study is provided in Extended Data Table 3.

Exploratory Efficacy Endpoints

Exploratory analysis was performed for ESR as inflammation biomarker at the cellular level and CRP at the molecular level. IL-6, as immune marker, was analysed and effects of cytokines on neutrophils and lymphocytes as N/L

ratio were evaluated and data are shown in Extended Data Table 4. Treatment of cancer and viral infection require a full functioning immune system [17]. Covid-19 hypothesis had been postulated with respect to the involvement of the immune system. The reference value for CRP is within the range of only 1-5 mg/L for healthy patients. Therefore, at molecular level, inflammation at 20 mg/ml is classified as mild and moderate about 60 mg/ml.

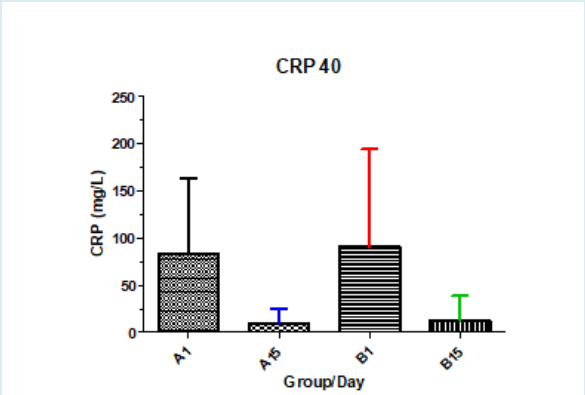

Figure 6: Reduction of molecular inflammation marker CRP.

Overall, CRP was reduced in adjunct group by >90% and less than 80% in the best care group. This finding is larger than any known anti-inflammatory agent. IL-6 is a key inflammation- /immune bio-marker and immune response will require the presence of cytokines. Excess cytokines lead to sepsis in the last stage and organ damage within the entire body. Organ damage in the lung, kidney, liver, arteries and the brain is present in Covid-19 infections.

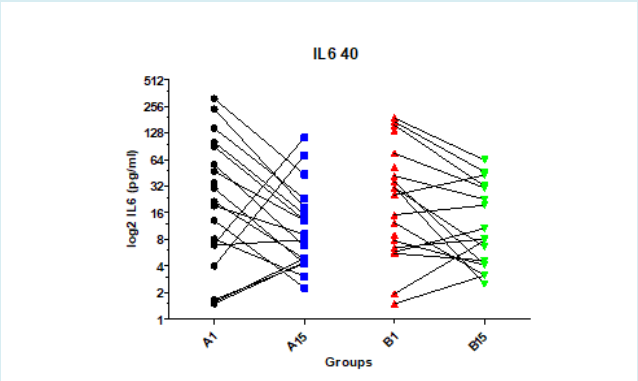

Figure 7: Change of cytokine IL-6; A =adjunct, B=BC on day 1 and day 15.

High IL-6 levels in the hundred pg/ml range are reduced. This finding for cytokine IL-6 is in line with a reduction of inflammation marker IL-6 in Dengue fever, rat paw test and Baker's yeast induced fever. For high inflammation a reduction of cytokines is observed. For low IL-6 levels in the single digits the increase is observed, thus leading to the stimulation of the immune system via CCK8, by starting the proliferation of lymphocytes. Covid-19 infection and the probability of death are directly linked with the absolute lymphocyte count.

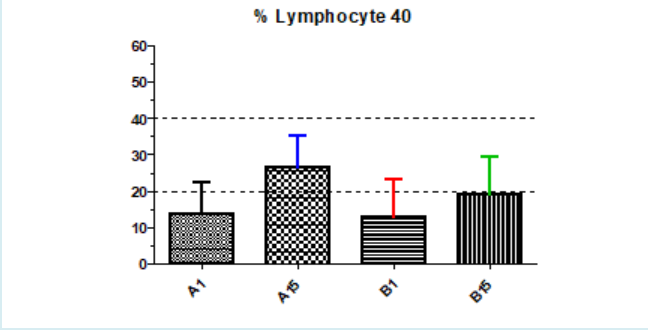

Figure 8: Lymphocytes of both arms in beginning and end of treatment.

An increase of lymphocytes from 14% to 27% into the middle of the reference range is found for the adjunct treatment after 14 days. For best care the increase is only from 14% to 19%. The change between the arms on day 15 for Adjunct with PNB-001 versus BC is significant with a P=0.032. Reduction of neutrophils is linked with reduction of NEtosis and subsequently prevention of cytokine storm. Here, the number of neutrophils is reduced by the cholinergic inflammatory pathway and the infiltration of neutrophils is reduced by the inhibition of the CCK-B gastrin pathway with involvement of histamine.

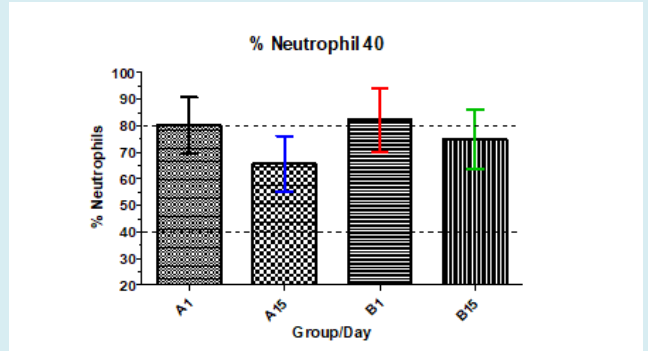

Figure 9: Neutrophil count in group A & B for day 1 and day 15.

The percentage of neutrophils was reduced from 81% to 65% on adjunct treatment and from 72% to 76% on best care, giving a significant reduction of neutrophils between

the arms on day 15 with P=0.013%.

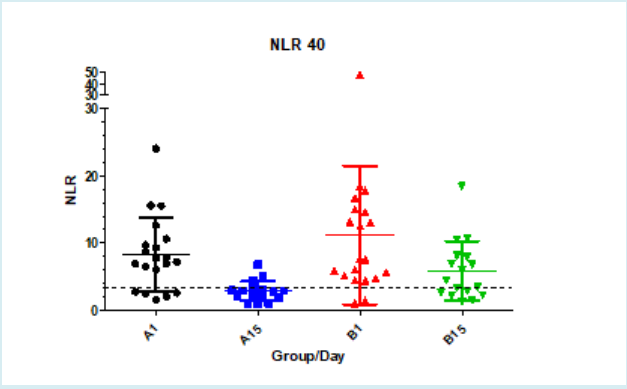

Figure 10: NLR Bar chart converted into scattering plot for N=40 NLR ratio for A and BC on day 1 and day 15.

For a deeper analysis, in addition to ALC the NLR, the Ratio of Neutrophils over Lymphocytes is calculated [18]. The enrolled patients had an elevated NLR ratio as a result of Covid-19 infection.

The Neutrophil-to- Lymphocyte ratio (NLR) was reduced from 8.5 to 2.8 for the adjunct treatment and from 11.1 to 5.9 for best care. This is a significant reduction of this most relevant Covid-19 biomarker. The difference between the arms was significant with a P=0.010. A lower than 3.3 ratio was clearly linked with a better, less severe outcome in patients. Here, a reduction of death rate, as well as all other parameters was found. The neutrophil-lymphocyte ratio (NLR) has been shown to serve as the most reliable indicator of progression to severe COVID-19, as well as a clinical biomarker to monitor treatment. In conclusion, NLR was useful as predictive and as clinical biomarker to assess treatment response.

Category	PNB-001 (N=20)	Standard of Care (N=20)	All (N=40)
	N_1 (%) n	N_1 (%) n	N_1 (%) n
OVERALL	11 (55.00%) 11	13 (65.00%) 13	24 (60.00%)24
Cardiac Disorders			
Tachycardia	05 (25.00%) 5	08 (40.00%) 8	13 (32.50%) 13
Bradycardia	00 (00.00%) 0	01 (05.00%) 1	01 (02.50%) 1
Ear and Labyrinth Disorders			
Ear pain	01 (05.00%) 1	00 (00.00%) 0	01 (02.50%) 1
Investigations			
Hepatic enzyme increased	01 (05.00%) 1	00 (00.00%) 0	01 (02.50%) 1
Metabolism and Nutrition Disorders			
Hyperglycaemia	01 (05.00%) 1	00 (00.00%) 0	01 (02.50%) 1
Respiratory Thoracic and Mediastinal Disorders			
Acute respiratory distress syndrome	01 (05.00%) 1	03 (15.00%) 3	04 (10.00%) 4
Vascular Disorders			
Rectal haemorrhage	01 (05.00%) 1	00 (00.00%) 0	01 (02.50%) 1
Hypotension	01 (05.00%) 1	01 (05.00%) 1	02 (05.00%) 2

N: Total sample size, N1: Subject count, n: Event count, All AEs are represented as: Subject count (Percentage of subjects) Event count.

Table 5: Summary of AEs by BC and PT– Safety Population.

Safety and Tolerability

PNB-001 (100 mg) by oral administration was well tolerated in patients with moderate COVID-19 infection. Most AEs were mild to moderate and considered not related to the study treatment. There were no abnormal laboratory findings observed during the study and less instance of hyperglycaemia documented as adverse event. Abnormal vitals included tachycardia and increased respiratory rate which were considered due to disease physiology. No clinically meaningful differences were observed in the safety profiles between PNB001+SOC and SOC treatment arms.

In more detail, a total of 24 AEs were reported by 18 patients during the study: 11 AEs in 8 patients in PNB001+SOC arm and 13 AEs in 10 patients in SOC arm. The adverse

events reported were tachycardia, bradycardia, hypotension, pain in ear, hyperglycaemia, liver enzymes increased, acute respiratory distress syndrome (ARDS) and bleeding per rectum. The most common AEs were tachycardia and ARDS. Three AEs of ARDS (1 in PBN-001+SOC arm and 2 in SOC arm) were severe while all other AEs were mild to moderate in severity; none of the AEs were considered related to the study treatments. One SAE of ARDS was reported in PNB-001+SOC arm. The SAE was severe, not related to the study treatment, and resulted to the death of the patient. The death in PNB-001 plus standard of care arm was not considered as related to the study treatment.

Overall, there was no clinically meaningful difference in the safety profile between adjunct and BC arms. In MAD for the highest dose (200 mg) liver toxicity was observed in one patient (1 in 42). Here, no change of ALT and AST was observed in conjunction with gluco-steroids. For glucose the protocol had a cut-off value of 200 mg/dl. From preclinical experiments, efficacy is higher at higher glucose concentrations and therefore, violation of the protocol was justified with clear clinical benefits to this subgroup.

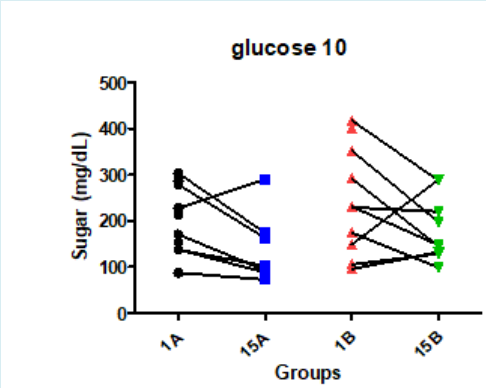

Figure 11: Glucose. N=20; Tuning of glucocorticoid profile by 100 mg of PNB-001 via PO administration.

Deviations from the protocol were filed and clearly a reduction of glucose blood concentration was found on adjunct treatment. This is of particular importance as pre-diabetic and diabetic patients have a much worse Covid-19 prognosis and are in much higher treatment need.

Discussion

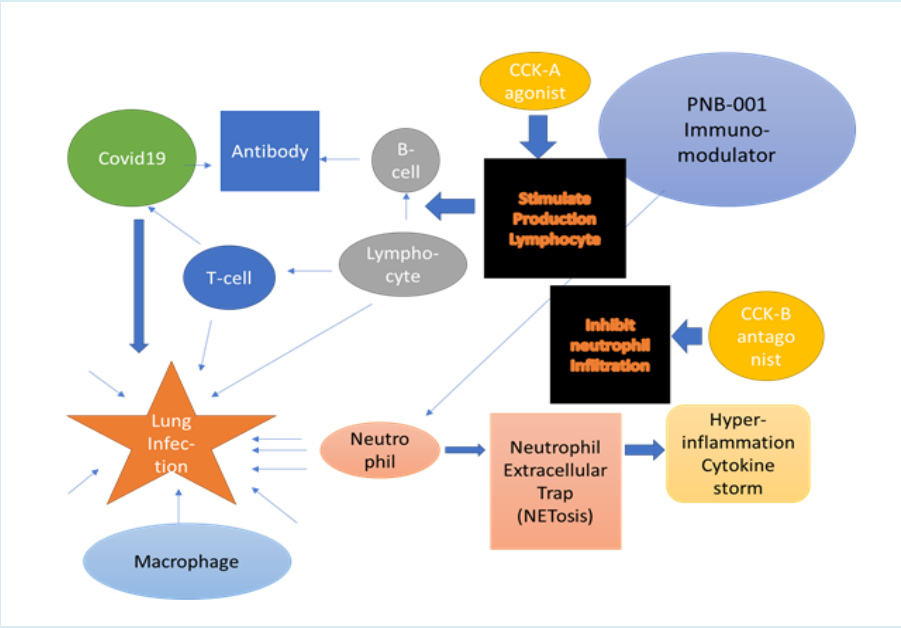

Figure 12: Working hypothesis of COVID-19 invention and therapeutic interventions.

CCK1R, the CCK A receptor, is directly and indirectly via acetylcholine Ach involved in the stimulation the number of lymphocytes. The T lymphocytes, T-cells, act in form of killer lymphocytes in fighting the virus.

B-lymphocytes, B-cells, form specific antibodies to fight the viral infection. CCK 2 is modulating the formation and the infiltration of neutrophils. This pathway is blocked by PNB-001 the gastrin antagonist, resulting in less neutrophils and

less infiltration of neutrophils for example in the lung. The NETosis = neutrophil extracellular trap is causing hyper-inflammation. This damage is directly via the CCK pathway or indirectly via histamin, similar to the release of gastric acid in the stomach. Overall the role of GI neuromodulators was discovered in the GI tract, then the CNS and more recently the role of CCK in the immune system was understood.

The current COVID-19 pandemic compelled the healthcare industry and other stake-holders to congregate sources to evaluate novel or existing therapeutic intervention for the treatment of COVID-19. The efforts of pharmaceutical companies' coalition are swiftly and decisively progressing to provide promising therapeutics agents as an effective treatment option for prevention and treatment of COVID-19. WHO recommends that a randomised controlled trials in combination with supportive care is the suitable way to evaluate the efficacy and safety of treatments for COVID-19. In terms of prediction, recent studies on COVID-19, have focused on Neutrophil-to-lymphocyte ratio (NLR) as an independent risk factor of the in-hospital death and a significant prognostic biomarker of outcomes in critically ill patients [19,20].

In recent years, the bidirectional relationship between the nervous and immune system has become increasingly clear, and its role in both homeostasis and inflammation has been well documented over the years [21]. Since the introduction of the cholinergic anti-inflammatory pathway, there has been an increased interest in parasympathetic regulation of both innate and adaptive immune responses, including T helper 2 responses.

A remarkable link of the anti-panic study with aging of the CCK receptor and the analysis in lymphocytes concluded, that CCK receptor aging is aging in the CNS, brain and now aging of the immune system [22]. Baladol was developed for IBD and the link IBD and immune system is featured [23]. In vitro, we have shown the release of Ach and Ach regulates immunity [24]. The subtypes of CCKR were discussed and in particular the role of CCKR1, CCK and ACH gives an insight. Cholecystokinin 1-receptor (CCK1-R) activation stimulates vago-vagal reflex pathways in the brain stem [25]. The cholinergic anti-inflammatory pathway is a pathway known to modulate cytokine release. F. Tian, et al. studied the effects of cholecystokinin pathways on acute lung injury in rats and proposed in addition to mediator NO, hydrogen sulfide involvement [26].

PNB-001, is a novel first in class small molecule with CCKA agonising and CCKB antagonising properties making it a first anti-inflammatory agent analgesic with immune modulating properties. Significantly high blood levels of cytokines and chemokines were noted in patients with COVID-19 infection and these pro-inflammatory cytokines included IL1-β, IL1RA, IL-7, IL-8, IL-9, IL-10, basic FGF2, G-CSF, GM-CSF, IFN-γ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNF-α, and VEGFA and we consider these cytokines a result of neutrophil overpopulation with NETosis. PNB-001 has a potential to reduce the pro-inflammatory cytokines if values are high and to increase cytokines when low, measured here in form of IL-6. Currently the in-depth analysis of lymphocytes is performed to understand, if T-cells or B-cells are formed and which T-cells are formed in particular to help fighting the infection. PNB-001 is directly acting by stimulation of the immune response indirectly thus preventing the cytokine storm, which is the main cause of fatalities in COVID-19 patients.

This randomised study was conducted to assess and compare efficacy and safety of PNB001 adjunct treatment [27,28]. Patients received 100 mg PNB-001 three times a day along with best standard of care or only best standard of care for 14 days. The best care contained antiviral remdesivir and dexamethasone for anti-inflammatory treatment (+other disease specific treatments). The study efficacy was assessed based on the primary endpoint of WHO Ordinal Scale for COVID-19 Clinical Improvement besides mortality rate. The secondary endpoints were the standard endpoints as recommended by WHO and by Natanegara et al. [29,30].

The patient's demographics, baseline characteristics, and disease severity of patients were comparable between both arms. One exception was a morbidly obese patient in the adjunct treatment arm with a glucose blood concentration of 420 mg/dl, who responded in forming lymphocytes, but more slowly and was the only patients left in hospital on day 15 in the PNB-001 treatment group. The primary efficacy endpoint, mean change in ordinal scale, showed significant clinical improvement in PNB-001+BC arm compared to BC arm on Day 15. Higher number of patients achieved ordinal score zero, i.e. full recovery, in the test arm from Day 8 to End of the Treatment compared to control arm. At the end of the study, all tested patients in either treatment arms were (94.44% Vs 70.59%) free from clinical or virological evidence of infection. Multiple clinical studies are conducted to assess efficacy of a range of therapeutic agents targeting different mechanism of action for moderate to severe COVID-19 using different ordinal scale methods. Ruiz et al. reported clinical improvement using the 6-point ordinal scale in 44.3% (39/88) and 73.9% (65/88) by days 7 and 14, respectively in severe COVID-19 patients. Following remdesivir treatment, an ordinal score of 1 was reported in 50.7% (38/75) patients who had baseline ordinal score of 4 [31,32].

In total 1 patient in the test and 2 in control arm died during the study; giving a reduction of death rate by 50% (2 to 1), which is NS due to low numbers. In the UK the death

rate is about 10 % in hospitalised patients and for N=20 2 are exactly within that range. A reduction of death rate from 10% to 5% would be considered clinical significant, however in terms of statistical significance more numbers are required. For a statistically significant reduction of death rate, not N=40, but N=400 would be required. With a 50% reduction and 10/190 versus 20/180, a P value of P=0.06 would be calculated. The patient who died following randomization to PNB-001+BC is unlikely to have derived benefit from test treatment as onset of action is found after 1 week. Moreover, one patient withdraw from the study to leave for a private hospital. He responded well to treatment, as seen in x-ray of the lung and without immune support in form of PNB-001, he deteriated and died within 28 day period.

The secondary efficacy endpoints, mean Chest X-ray score and speed of discharge from the hospital showed statistically significant difference favouring PNB-001+ BC arm. Duration of hospitalization on day 15 was reduced from 5 to 1 patient, so a reduction of 80%. In terms of number of patients to treat to benefit, 4/20 benefited from adjunctive treatment, giving a 1 in 5 number to treat to benefit. (The patient in hospital should not be enrolled with a glucose of 420 mg/dl and without this deviation from the protocol, every single patient would have left the hospital by day 15).

Duration of supplemental oxygen requirement was significantly reduced (A versus BC P=0.05). It is clinically relevant, that 50% of patients on adjunct were off oxygen from day 6 onwards, compared to day 8 for best care.

Due to the attack on the immune system N/L ratio and P/L ratio are elevated in COVID-19 patients. We have established now to use NLR, as predictive biomarker, to assess who will benefit from treatment and to monitor the regression of the Covid-19 infection, so a clinical biomarker [33]. Persistently elevated level of IL-6 and CRP are also reported in severe cases of corona-infected patients. PNB-001 further reduced inflammation, monitored by CRP, below the level of anti-inflammatory steroid BC group, making PNB-001 a super anti-inflammatory agent [34].

In the current study immune inflammatory marker IL-6 was modulated, reduced when too high and increased when low to stimulate to fight of the immune system. The number of lymphocytes is increased and the number of T-cells, monitored in form of CD4/CD8 ratio and these results will be reported in due course.

P/L ratio, ESR, killer T-cells, helper T-cells, immunoglobulin IGM and IGG are all involved in an orchestrated response to the Covid-19 infection and are part of PART 2 of this series of publications. Due to no observed toxicity, which was even less in the adjunct group than the BC, no value for the number to treat to harm could be calculated, but overall the clear benefits over harm in line with the unique agonist antagonist action, justify urgent approval. Approval for phase 3 is seeked, via the emergency route in an exponentially developing curve of Covid-19 cases in India.

Conclusion

In conclusion, PNB-001 a first in class CCKA agonist and CCKB antagonist with immune stimulation anti-inflammatory properties showed significant clinical improvement in moderate COVID-19 patients, when compared to standard of care, in only 40 patients. PNB-001 was very well tolerated by moderate COVID-19 patients as adjunct treatment and the unique mode of action is already fully established in this phase 2 trial.

Role of the Funding Source

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Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, [EL].

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