CLINICAL STUDY PROTOCOL

1 TITLE PAGE

Study Title:	Multicenter Open-Label Randomized Active-Comparator Study to Evaluate Early Bactericidal Activity, Safety, and Pharmacokinetics of PBTZ169 in Patients with Newly Diagnosed Microbiologically Proven Respiratory Tuberculosis with Preserved Susceptibility to Isoniazid and Rifampicin
Studied indication:	Newly Diagnosed Respiratory Tuberculosis
Study design:	Open-label randomized parallel-group active-comparator study to evaluate efficacy (based on the early bactericidal activity), safety, and pharmacokinetics of the study medication.
Study drug:	PBTZ169 80 mg oral capsules
Comparator:	Isoniazid 300 mg tablets
Sponsor Name:	NEARMEDIC PLUS LTD.
Sponsor Address:	12 Aviakonstruktora Mikoyana St., Moscow 125252, Russia Tel.: +7(495)741-4989; Fax: +7(499)193-4350
Sponsor Name:	NEARMEDIC PLUS LTD.
Protocol Number/Code:	PBTZ169-A15-C2A-1
Study Phase:	IIa
Medical Expert appointed by the Sponsor:	Nikolay V. Yemelyanov, Head of the Medical Studies and Information Section of the Project Development Department at NEARMEDIC PLUS LTD.
POA to sign for the Sponsor:	Roman N. Bolgarin, Director of Development of NEARMEDIC PLUS LTD
Statement on Conducting the Clinical Study in Accordance with the Good Clinical Practice:	The given study is carried out in accordance with the Good Clinical Practice (GCP) provided by the International Harmonization Council (ICH), including retention of the essential documents
Report Version and Date:	5.0 dated 20.07.2017

SIGNATURE PAGES

Sponsor's Signature Page

Reviewed and approved by:

Head of Project Development Department

Roman N. Bolgarin, Director of Development of NEARMEDIC PLUS LTD	Date

Head of the Medical Studies and Information Section

Nikolay V. Yemelyanov, Head of the Medical Studies and Information Section of the Project Development Department NEARMEDIC PLUS LTD.	Date

Head of the Medicinal Products Sector

Nataliya A. Shevkun, Head of the Medicinal Product Sector of the Project Development Department NEARMEDIC PLUS LTD.	Date

Head of Pharmacovigilance

Yelena I. Zotova, Head of Pharmacovigilance of the Project Development Department NEARMEDIC PLUS LTD.	Date

Signature Page of the Contract Research Organization (CRO)

Reviewed and approved by:

Project Manager (CSI)

Irina V. Ivushkina, Project Manager, OCT Rus LLC 48 Myasnitskaya St., Moscow Tel.: +7(495)660-6550, ext. 4017	Date

Biostatistician

Irina B. Bondareva, lead biostatistician, Data MATRIX LLC 5 Kovenskiy Pereulok, Saint Petersburg	Date

Medical writer

Boris D. Yeliseyev, medical writer, OCT Rus LLC, 48 Myasnitskaya St., Moscow Tel.: +7(495)660-6550, ext. 4032	Date

2 SUMMARY (SYNOPSIS)

Name of active substance:
PBTZ169 Hydrochloride

Study Title: Multicenter Open-Label Randomized Active-Comparator Study to Evaluate Early Bactericidal Activity, Safety, and Pharmacokinetics of PBTZ169 in Patients with Newly Diagnosed Microbiologically Proven Respiratory Tuberculosis with Preserved Susceptibility to Isoniazid and Rifampicin

Protocol Number/Code: PBTZ169-A15-C2A-1

Primary investigators and study centers:

Study Center 1:

Federal State Budgetary Institution Central Tuberculosis Research Institute of the Russian Academy of Medical Sciences Address: 2 Yauzskaya Alleya, Moscow 107564, Russia Principal investigator: Tatevik R. Bagdasaryan.

Study Center 2:

Federal State Budget Educational Institution of Higher Professional Education Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation Address: 8/2 Trubetskaya St., Moscow 119991, Russia Principal investigator: Pavel V. Senchikhin.

Study Center 3:

Moscow State Budgetary Health-Care Institution Moscow Municipal Research and Practical Center for Tuberculosis Control of the Moscow City Health-Care Department Address: 10 Stromynka St., Moscow 107014, Russia Principal investigator: Taisiya N. Ivanushkina.

Study Center 4:

State Budgetary Health-Care Institution of the Orel Region Orel Tuberculosis Dispensary. Address: 15 Tsvetayeva St., Oryol 302027, Russia Principal investigator: Boris Ya. Kazyonnyy

Study Center 5:

State Health-Care Institution of the Voronezh Region Pokhvisneva Voronezh Regional Clinical Tuberculosis Dispensary Address: 1 Teplichnaya St., Voronezh 394070, Russia. Detached building A and A1, entrance A and A1. Principal investigator: Galina A. Batishcheva

Study Center 6:

State Budgetary Health-Care Institution of the Vladimir Region Center for Phthisiatric and Pulmonologic Specialty Care Address: 63 Sudogodskoye Shosse, Vladimir 600023, Russia Principal investigator: Grigoriy V. Volchenkov

Study Center 7:

Name of active substance:
PBTZ169 Hydrochloride

State Budgetary Health-Care Institution of the Arkhangelsk Region Arkhangelsk Clinical Tuberculous Dispensary Address: 28 Novgorodskiy Prospekt, Arkhangelsk 163002, Russia Principal investigator: Andrey O. Maryandyshev

Study Center 9:

Regional State Budgetary Health-Care Institution Tomsk Phthisiatric and Pulmonologic Medical Center Address: 17 Rozy Luksemburg St., Tomsk 634009, Russia Principal investigator: Yelena V. Vorobyova

Study Center 10:

State Health-Care Institution of the Samara Region Tolyatti Municipal Tuberculous Dispensary Address: 34 Telegrafnaya St., Tolyatti, Samara Region 445013, Russia Principal investigator: Yelena A. Borodulina

Study Center 11:

Federal State Budgetary Institution Novosibirsk Tuberculosis Research Institute of the Ministry of Health Care of the Russian Federation Address: 81A Okhotskaya St., Novosibirsk 630040, Russia Principal investigator: Yekaterina V. Kulchavenya

Study Center 12:

State Autonomous Health-Care Institution Republican Clinical Tuberculosis Dispensary Address: 27A Sibirskiy Trakt St., Kazan 420029, Russia Principal investigator: Marat F. Yaushev

Study Center 13:

Federal State Budgetary Institution Saint Petersburg Research Institute of Phthisiopulmonology of the Ministry of Health Care of the Russian Federation Address: 2/4 Ligovskiy Prospekt, Saint Petersburg 197758, Russia Principal investigator: Pyotr K. Yablonskiy

Publications (references): none

Phase of drug development: IIa

Goals: to study the efficacy of PBTZ169 in the daily doses of 160, 320, and 640 mg based on its early bactericidal activity, safety, and pharmacokinetics of PBTZ169 p.o. as monotherapy for 14 days in patients with newly diagnosed microbiologically proven respiratory tuberculosis with preserved susceptibility to Isoniazid and Rifampicin.

Design: this study is a randomized open-label study of efficacy (in terms of early bactericidal activity), safety, and pharmacokinetics of PBTZ169 in parallel groups with active control in patients with newly diagnosed respiratory tuberculosis. The study comprises the following stages:

Name of active substance:
PBTZ169 Hydrochloride

- Screening, up to 10 days
- Monotherapy, 14 days
- Follow-up, 7 days

Screening procedures are carried out within 10 days before randomization. Screening procedures can be carried out on an outpatient or inpatient basis. If the screening procedures are performed outpatiently, subjects are admitted to hospital on the day before the first dose of the study drugs. If the screening procedures are performed inpatiently, subjects stay hospitalized for the purpose of participation in the clinical study.

Eligible subjects are randomized. During randomization, each subject is assigned a randomization number generated by the IWRS. Each patient is assigned to one of the following four groups based on the randomization number:

- Group 1: subjects receive PBTZ169 capsules at a daily dose of 160 mg for 14 days as a monotherapy, after which the drug is discontinued, and standard antituberculosis therapy is prescribed (hereinafter, standard antituberculosis therapy is defined as the tuberculosis therapy provided for under Order of the Ministry of Health of the Russian Federation No. 951 dated December 29, 2014). Up to 15 subjects are planned to be randomized to this group.
- Group 2: subjects receive PBTZ169 capsules at a daily dose of 320 mg for 14 days as a monotherapy, after which the drug is discontinued, and standard antituberculosis therapy is prescribed. Up to 15 subjects are planned to be randomized to this group.
- Group 3: subjects receive PBTZ169 capsules at a daily dose of 640 mg for 14 days as a monotherapy, after which the drug is discontinued, and standard antituberculosis therapy is prescribed. Up to 15 subjects are planned to be randomized to this group.
- Group 4: subjects receive isoniazid tablets at a standard daily dose of 600 mg for 4 days as a monotherapy, after which standard antituberculosis therapy follows. Up to 7 subjects are planned to be randomized to this group.

All study procedures are conducted in the subjects and documented under the randomization number.

Name of active substance:
PBTZ169 Hydrochloride

Nocturnal sputum is collected from all subjects in the morning at V1. After that, every patient received the study drug (PBTZ169 or isoniazid) as a monotherapy for 14 consecutive days. After 14 days of monotherapy (Day 15 of the study, V8), PBTZ169 is discontinued in Groups 1 to 3 (Day 14 being the day of the last PBTZ169 dose). Standard therapy is initiated 3 days after the monotherapy end date (Day 18 of the study, V10). Standard therapy drugs are resumed in Group 4 (standard dose of isoniazid) without a break—that is, the day following the monotherapy end date (Day 15 of the study). Patient monitoring during clinical study is continued on a hospital basis and ended 7 days following the monotherapy end date.

Venous blood samples are taken from all subjects in Groups 1, 2, and 3 (PBTZ169 monotherapy) to measure PBTZ169 levels. Blood samples are drawn before the first doze of PBTZ169 (0 min time point) and later at the following time points after the first dose: in 0:20, 0:40, 1:00, 1:30, 2:00, 2:30, 3:00, 4:00, 6:00, 8:00, 10:00, 12:00, and 24:00 (hr:min). Blood samples are also taken before the last doze of PBTZ169 (0 min time point) and later at the following time points after the last dose: after 0:20, 0:40, 1:00, 1:30, 2:00, 2:30, 3:00, 4:00, 6:00, 8:00, 10:00, 12:00, 24:00, 48:00, and 72:00 (hr:min).

All venous blood samples are kept in case any additional parameters have to be measured.

Subjects are hospitalized throughout the monotherapy and follow-up periods.

Number of subjects: It's planned to randomize 52 eligible subjects for the study, namely 15 subjects in each PBTZ169 group and 7 subjects in the isoniazid group.

The data of subjects who will receive at least one dose of the study drug and whose study drug concentrations are known will be processed in the final statistical analysis of efficacy and pharmacokinetics.

The data of subjects of ITT population will be processed in the final statistical analysis of safety.

Diagnosis and key inclusion criteria:

Subjects complying with all of the following criteria will be included into the clinical study:

1. Written informed consent received from the subject.
2. Men and women aged 18 to 45 years old, inclusive.
3. Newly diagnosed active pulmonary tuberculosis confirmed by characteristic radiological findings (infiltration, dissemination, and destruction) in chest X-ray or computed tomography not involving other

organs or involving one or more of the following: the larynx, the trachea, the bronchi, and lymph nodes.

4. The patient's sputum volume must be enough for any tests provided for under the Protocol but not less than 4 to 5 mL at screening.
5. Acid-fast mycobacteria found in sputum smear microscopy (1+ and more using the luminescent dye microscopy according to Order of the Russian Ministry of Health No. 109 dated March 21, 2003, latest revision) and tuberculosis mycobacterial DNA found in molecular genetic tests.
6. A body weight of at least 51 kg.
7. Body mass index of 18.5–25 kg/m^2.
8. The ability (according to the investigator's opinion) to comply with all requirements of the Protocol.
9. Agreement to use reliable contraception methods during the study and 3 months after the last dose of the drug, including temporary abstinence from sex or double contraception combining male condoms with at least one of the following:
   - Using hormonal contraception
   - Using aerosols, creams, suppositories, and other agents containing spermicides
   - Using an intrauterine device

Subjects who meet any of the following criteria will not be included in the clinical study:

1. Extrapulmonary localization of tuberculosis.
2. Resistance to rifampicin and/or isoniazid found in molecular -genetic tests on sputum samples.
3. Administration of any antituberculosis drugs from the moment of tuberculosis diagnosis to enrolment in the study.
4. Absolute indications for surgical treatment of tuberculosis at screening.
5. Testing positive for syphilis or HIV infection serological markers at screening, active hepatitis, or decompensated liver cirrhosis.
6. History of allergic reactions.
7. Laboratory renal and/or hepatic parameters measured as follows (with due regard to the laboratory's reference range):
   - AST > 2.0 x upper limit of normal values (ULN)

Name of active substance:
PBTZ169 Hydrochloride

- ALT > 2.0 x ULN
- Total bilirubin > 1.5 x ULN
- Creatinine > 1.5 x ULN

8. Individual intolerance to any ingredients of the study drug.
9. History of malignant tumors, except for basal cell carcinoma.
10. Decompensated severe chronic somatic diseases, including cardiovascular, bronchopulmonary, neuroendocrine, ENT, -gastrointestinal, hepatic, renal, blood, skin diseases, and other somatic or mental diseases that the investigator believes to prevent the subject's enrolment.
11. Gastrointestinal- surgery (except for appendectomy performed more than 1 year before the screening).
12. Any mental disorders that can affect the patient's compliance with the Protocol.
13. Diabetes mellitus.
14. Acute viral and bacterial infections at enrolment or within 2 weeks prior to enrolment.
15. Alcoholism (unless the investigator believes the subject to be able to abstain from alcohol consumption during the study) or substance abuse.
16. A positive test for narcotic and psychotropic products.
17. Regular administration (including topical) of any hormonal drugs for over 1 week within 30 days before the screening (except for oral hormonal contraceptives and hormonal intrauterine devices).
18. Use of cytotoxic drugs within 30 days before the screening.
19. Repeated administration of any drugs where the instruction for medical use mentions nervous system, hemodynamics, and liver function adverse events as "very frequent" (≥10%) or "frequent" (≥1% and <10%) within 21 days before the screening.
20. Pregnancy or lactation.
21. Planned conception or sperm donation during the study after the study drug administration or within 3 months after the date of the last drug administration.
22. Participation in other clinical trials of drugs within less than 3 months before the screening.

Name of active substance:
PBTZ169 Hydrochloride

Study drug, dose, administration, and batch number:
Study drug code: PBTZ169
Brand name: none
International nonproprietary name: none
Dosage form: 80 mg capsules.
Pharmacotherapeutic group: antituberculosis drug
ATC code: J04A
Dose and administration route: patients receive PBTZ169 orally once a day in the morning in the fasting state in the following doses:

- Group 1: 160 mg daily
- Group 2: 320 mg daily
- Group 3: 640 mg daily

Name of active substance:

PBTZ169 Hydrochloride

Duration of treatment with the study drug:

Monotherapy for 14 consecutive days.

Comparator, dose and administration, batch number:

Brand name: Isoniazid

International nonproprietary name: isoniazid

Dosage form: 300 mg tablets.

Pharmacotherapeutic group: antituberculosis drug

ATC code: J04AC01

Dose and administration route: Isoniazid is prescribed to subjects randomized into Group 4 in the daily dose of 600 mg of orally in the morning after meals.

Comparator treatment duration:

Monotherapy for 14 consecutive days. Isoniazid is then continued as part of the combined antituberculosis therapy.

Assessment parameters:

Efficacy:

Primary efficacy endpoint:

- Early bactericidal activity 14 days from the monotherapy start date (EBA_{0-14}).

Variables for the secondary efficacy endpoint:

- Early bactericidal activity 2 days from the monotherapy start date (EBA_{0-2}).
- Early bactericidal activity 7 days from the monotherapy start date (EBA_{0-7}).

Safety:

Variables for the safety endpoint

- Adverse events
- Changes in vital signs
- Results of laboratory tests (complete blood count, biochemical blood analysis, urinalysis)
- ECG findings
- Physical examination results

Name of active substance:
PBTZ169 Hydrochloride

Pharmacokinetics:

Variables for pharmacokinetics evaluation:

- Maximum concentration (C_{max}) within the dosing interval of 24 hours after single and multiple dosing
- Minimum concentration (C_{min}) within the dosing interval after single and multiple dosing
- Trough concentration (C_{trough}) (measured 24 hours after the first dose administration, prior to the last dose, and 24 hours after the last dose)
- Time to maximum concentration (T_{max}) within the dosing interval after single and multiple dosing
- Area under the concentration–time curve within the dosing interval (AUC_{0-24}) after single and multiple dosing
- Area under the concentration–time curve within the interval from 0 to the last measurement value above LLoQ (AUC_{0-t}) after single and multiple dosing
- Area under the concentration–time curve within the interval from 0 to infinity ($AUC_{0-\infty}$) after single and multiple dosing
- Mean concentration ($C_{ss,av}$) within the dosing interval after multiple dosing
- Fluctuations (%) within the dosing interval after multiple dosing ($[C_{max}-C_{min}]\times 100\%/C_{ss,av}$)
- Apparent total clearance following oral administration (Cl_t/F) as measured after single and multiple dosing
- Distribution volume (V_d) measured after single and multiple dosing
- Half-life ($T_{1/2}$) measured after single and multiple dosing
- Elimination constant k_{el} measured after single and multiple dosing.

Pharmacodynamics:

- $C_{max,ss}$ and $AUC_{0-24,ss}$ measured after multiple dosing after 14 days of therapy are used for early bacterial activity EBA_{0-14} and EBA_{2-14} as parameters for PK comparison.

  C_{max}, C_{min}, and AUC_{0-24} measured after the first dose administration are used for early bacterial activity EBA_{0-2} as parameters for PK comparison.

Name of active substance:
PBTZ169 Hydrochloride

Statistical methods:

Descriptive statistics will be provided for all demographics and other baseline characteristics, efficacy and safety parameters, and changes in them (if applicable) in the course of the study by means of assessment time points and treatment groups. Descriptive statistics for quantitative variables include mean value, standard deviation (SD), minimum and maximum values, and the number of valid observations. Qualitative data is presented as rates and percentages. 95% confidence intervals around point estimations are provided as well (where applicable).

Efficacy analysis

According to the EMA Guidance, early bactericidal activity (EBA) assessment should be based on determining the count of live mycobacteria in the patient's sputum during monotherapy with the drug. The primary method of determining mycobacterial count used is inoculating agar with sputum to count CFU/mL. The method is chosen as it is used as the primary method in nearly all studies assessing early bactericidal activity, which means it can be considered conventional [16-–19].

The method of quantitative PCR for counting mycobacterial cells in sputum is used as a secondary method for secondary analysis [23].

All assessments of $\log_{10}$ CFU/mL sputum, mycobacterial cell count estimates obtained with PCR, and their changes from baseline are tabulated by measurement points and treatment groups.

In the primary analysis of treatment efficacy, changes from baseline in $\log_{10}$ CFU/mL sputum (for agar inoculation) 14 days after the therapy start date are estimated. Changes in $\log_{10}$ CFU/mL sputum 2 and 7 days after the therapy start date are also estimated in the secondary analysis of treatment efficacy. Early bactericidal activity (EBA) is assessed as a change in $\log_{10}$ of CFU/mL of sputum specified for the treatment day [24] with a two-sided 95% confidence interval.

Though sample size is estimated empirically disregarding formal comparisons between the two dose levels, the Kruskal–-Wallis test is used to compare the values for different dose levels of the study drug at a two-sided 5% significance level.

The history of mean changes in $\log_{10}$ CFU/mL from baseline by assessment points is presented graphically by treatment groups.

The Jonckheere- –-Terpstra test is used to identify the trend for the primary efficacy endpoint depending on the increase in dose level (increasing $EBA_{0–14}$ for increasing doses).

A similar efficacy analysis (based on changes in $\log_{10}$ cells/mL) is carried out by determining mycobacterial cell count with quantitative PCR.

Graphical methods and calculation of correlation coefficients between individual measurements obtained using the two quantification methods are used to analyze the

consistency of the results between the two methods of determining mycobacterial count.

Only available measurements are used in efficacy analysis. No missing values are restored. The analysis is based on average values for each measurement point. An additional analysis at the maximum of two measurements is carried out (with the highest number of CFU or MBT DNA copies using agar inoculation and quantitative PCR, respectively).

Safety analysis

The MedDRA dictionary is used to code adverse events. The number (percentage) of patients with AEs and the number of AEs are tabulated by system organ class and preferred term as well as by study treatment, severity, and treatment group. Each patient is included within each system organ class and preferred term only once regarding the study treatment and maximum severity.

Laboratory test values and their changes from baseline (at screening) following the administration of the study drug and "shift tables" based on reference values are tabulated by treatment groups and measurement points.

The findings of ECG, vital sign assessment, and body temperature measurements are tabulated by study group for each measurement point, including changes from the most recent measurement made prior to dosing.

Physical examination findings are presented descriptively by treatment group and measurement point.

Pharmacokinetic analysis

All concentration measurements after single and multiple dosing are tabulated by time point and treatment group and listed. Reasons are explained for any missing measurements and measurements not included in the pharmacokinetic analysis.

Descriptive statistics for concentration measurements and PK parameters include the mean, standard deviation, the median, the minimum, the maximum, coefficient of variation (%), and the number of valid cases (N). Geometric means and geometric coefficient of variation are also presented for concentrations and PK parameters such as C and AUC.

The actual values of sampling time are used to calculate individual values of PK parameters using the noncompartmental approach. The dependence of drug concentration on time is presented graphically for each individual patient based on actual sampling time. The descriptive statistics and average PK curves are presented based on the estimated sampling time points. Average and individual PK curves are presented on the arithmetic and logarithmic scale.

Name of active substance:
PBTZ169 Hydrochloride

Pharmacokinetic and pharmacodynamic analysis

An additional analysis is carried out to compare pharmacokinetic and pharmacodynamic parameters. $C_{max,ss}$ and $AUC_{0–24,ss}$ measured after multiple dosing after 14 days of therapy are used for early bacterial activity $EBA_{0–14}$ and $EBA_{2–14}$ as parameters for PK comparison. C_{max}, $C_{min,}$ and $AUC_{0–24,}$ measured after the first dose administration are used for early bacterial activity $EBA_{0–2}$.

PK/PD indicators are compared using graphical representation (plotting pairs of individual values against on X and Y for each comparison of parameters X and Y) and by calculating coefficients of correlation within treatment group as well as in general.

An interim analysis is to be carried out when at least 15 patients across all groups have completed the study. The interim analysis is not carried out since the study is terminated early.