CLINICAL STUDY PROTOCOL

A Phase 1, Partially-Blinded, Placebo-Controlled, Randomized Single Ascending Dose (SAD) with a Food Effect Cohort Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of TBI-223 in Healthy Adult Participants

PROTOCOL NUMBER

TBI-223-CL-001

Version: 3.0

21 Jan 2020

CONFIDENTIALITY STATEMENT
The confidential information in this document is provided to you as an Investigator or consultant for review by you, your staff, and the applicable Institutional Review Board/Independent Ethics Committee. Your acceptance of this document constitutes agreement that you will not disclose the information herein to others without written authorization from Worldwide Clinical Trials Early Phase Services, LLC, except to the extent necessary to obtain informed consent from persons who participate as subjects in this study.

PROTOCOL APPROVAL PAGE

A Phase 1, Partially-Blinded, Placebo-Controlled, Randomized, Single Ascending Dose (SAD)with a Food Effect Cohort Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of TBI-223 in Healthy Adult Participants

Protocol Number:	TBI-223-CL-001
Sponsor:	Global Alliance for TB Drug Development
Sponsor Contact and Medical Monitor:	Paul Bruinenberg MD, MBA Global Alliance for TB Drug Development 40 Wall Street, 24th Floor New York, NY 10005, United States of America Telephone: +1 646 616 8629 Mobile: +1-201-312-0988 Facsimile: +1 212 227 7541 Email: Paul.Bruinenberg@tballiance.org

DocuSigned by:
Paul Bruinenberg
Signer Name: Paul Bruinenberg
Signing Reason: I approve this document
Signing Time: 21-Jan-2020 | 10:51:10 EST
3B496C3B3A5A4B0AAE32731255B7116B

21-Jan-2020 | 10:51:14 EST

(Signature) (Date)

Principal Investigator: Cynthia A. Zamora, MD
Worldwide Clinical Trials Early Phase Services, LLC
2455 N.E. Loop 410, Suite 150
San Antonio, Texas 78217
Telephone: 210.635.1500
Fax: 210.635.1646

DocuSigned by:
Cynthia Zamora
Signer Name: Cynthia Zamora
Signing Reason: I approve this document
Signing Time: 21-Jan-2020 | 16:11:56 GMT
FA8D8484FD8647B3A4A33071357CA7F7

21-Jan-2020 | 16:12:01 GMT

(Signature) (Date)

SUMMARY OF CHANGES

The primary reasons for this amendment are to incorporate the changes from the protocol clarification letter dated 18 Oct 2019 regarding subject replacement of subjects who experience emesis, how replacement subjects will be numbered, and that replacement subjects will receive the same dose allocation of study drug.

This amendment also includes clarifying Part 1 and Part 2 and updates whereby Part 1 captures adding a capsule cohort (3b, TBI-223, 300 mg), updating the dose of cohort 7 (TBI-223, 2600 mg) and whereby Part 2 consists of 2 additional cohorts (Cohort 8 and 9) that will be added to the study in relation to evaluating tablet formulations of TBI-223.

Part 1: (TBI-223 oral suspension)

Cohort 3b: Is a cohort that was added to evaluate a capsule formulation of TBI-223 150 mg in oral enteric capsules, dosed at 300 mg

Cohort 7: Is the final TBI-223 oral suspension cohort at a dose of 2600 mg

Part 2: (TBI-223 tablet formulation)

Cohort 8: Is a single dose cohort that investigates 3 different TBI-223 Sustained Release (SR) tablet formulations (Prototypes 1, 2, and 3) and one TBI-223 Immediate Release (IR) tablet formulation. The cohort includes 4 arms with 6 subjects per arm. The 3 SR tablet formulations (Prototypes 1, 2, and 3) will be evaluated under fed conditions (Arms 1-3) and the IR tablet formulation will be evaluated under fasting conditions (Arm 4).

Cohort 9: 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation will be brought back following a 7-day washout and receive the IR tablet formulation under fed conditions. If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by healthy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).

Additional minor changes to format, grammar, and spelling have been applied.

The sections of protocol version 2.0 dated 12 Jul 2019 affected by these changes are indicated below.

Section Number (s)	Section Title(s)	Description of Change (s)
Synopsis	Study period (maximum duration, from screening to study exit):	*From:* Single ascending dose (SAD) cohort (fasting): 40 days Food-effect cohort: 50 days The maximum duration of the study will depend upon the final number and timing of each cohort.

Section Number (s)	Section Title(s)	Description of Change (s)
		To: **Part 1:** Single ascending dose (SAD) cohort (fasting): 40 days Food-effect cohort: 50 days **Part 2:** **Cohort 8: Single dose cohort of the 4 TBI-223 tablet formulations (Prototypes 1, 2, and 3 SR tablet formulations under fed conditions and one IR tablet formulation under fasting conditions): Duration 40 days.** **Cohort 9: The IR tablet formulation will be evaluated under fed conditions** **Single dose: 40 days** The maximum duration of the study will depend upon the final number and timing of each cohort.
Synopsis	Duration of treatment	*From:* Fasting cohorts: One single dose of TBI-223 oral suspension or placebo for TBI-223 oral suspension Food-effect cohort: Two single doses of TBI-223 oral suspension or placebo for TBI-223 oral suspension separated by a washout period of at least 7 days *To:* **Part 1:** Fasting cohorts: One single dose of TBI-223 oral suspension or placebo for TBI-223 oral suspension Food-effect cohort: Two single doses of TBI-223 oral suspension or placebo for TBI-223 oral suspension separated by a washout period of at least 7 days **Part 2:** **Cohort 8: Single dose cohort of 4 TBI-223 tablet formulations (three SR tablet formulations as prototypes 1, 2, and3 and one IR tablet formulation). The SR tablet formulations will be evaluated under fed conditions and the IR tablet formulation will be evaluated under fasting conditions** **Cohort 9: The IR tablet formulation will be evaluated under fed conditions**
Synopsis	Number of subjects (planned)	*From:* Fasting Cohorts: Planned to enroll up to 48 subjects in a total of 6 single ascending dose (SAD) cohorts of 8 subjects each (6 to receive active drug

Section Number (s)	Section Title(s)	Description of Change (s)
		and 2 to receive placebo). Food-effect Cohort: 1 cohort with 10 subjects (8 active and 2 placebo) who will receive TBI-223 or placebo under both fasting and fed conditions. Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. These decisions on additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach. *To:* **Part 1:** Fasting Cohorts: Planned to enroll up to 48 subjects in a total of 6 single ascending dose (SAD) cohorts of 8 subjects each (6 to receive active drug and 2 to receive placebo). Food-effect Cohort: 1 cohort with 10 subjects (8 active and 2 placebo) who will receive TBI-223 or placebo under both fasting and fed conditions. **Part 2:** **Cohort 8: Planned to enroll 24 subjects in a total of 4 arms with 6 subjects in each. Subjects will receive a single dose of 1 of the 3 prototypes of TBI-223 SR tablets under fed conditions (Arms 1-3) or a single dose of TBI-223 IR tablets under fasted conditions (Arm 4).** **Cohort 9: Plan to bring back the 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation, following a 7-day washout to receive the IR tablet formulation under fed conditions.** **If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).** Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. These decisions on additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach.
Synopsis and 2	Objective	*From:* The primary objective of the study is:

Section Number (s)	Section Title(s)	Description of Change (s)
		• To evaluate the safety and tolerability of single ascending doses of TBI-223 oral suspension in healthy adult subjects. The secondary objectives of the study are: • To determine the pharmacokinetics of TBI-223 and its metabolite M2 after single doses of TBI 223 oral suspension in healthy adult subjects; and • To compare the rate and extent of absorption of a single dose of TBI-223 oral suspension when administered in healthy adult subjects either after a high-calorie, high-fat meal or in the fasting state. *To:* The primary objective of the study is: • To evaluate the safety and tolerability of single ascending doses of TBI-223 oral suspension**, TBI-223 oral enteric capsules, and TBI-223 tablet formulations** in healthy adult subjects. The secondary objectives of the study are: • To determine the pharmacokinetics of TBI-223 and its metabolite M2 after single doses of TBI-223 oral suspension**, TBI-223 oral enteric capsules, and TBI-223 tablet formulations** in healthy adult subjects; and • To compare the rate and extent of absorption of a single dose of TBI-223 oral suspension**, TBI-223 oral enteric capsules, and TBI-223 tablet formulations** when administered in healthy adult subjects either after a high-calorie, high-fat meal or in the fasting state.
Synopsis	Section before Test product, dosage and mode of administration	*From:* This is a partially-blinded, placebo-controlled, randomized SAD study with a food effect cohort. The study will be conducted at one study center in the United States. This study will have up to 6 planned dose levels. Based on interim pharmacokinetic data obtained during the dose escalation, a dose cohort will be selected to return for additional dosing after a high-calorie, high-fat meal (food-effect cohort). Sentinel Cohort: The first cohort will be separated into 2 groups. A sentinel group of 3 subjects (2 active and 1 placebo) will be dosed at least 24 hours before the remaining 5 subjects (4 active and 1 placebo). The remaining cohorts will be dosed in 2 groups of 4 subjects each (3 active and 1 placebo), at least 24 hours apart.

Section Number (s)	Section Title(s)	Description of Change (s)
		Food-effect Cohort: Based on exposure levels, subjects in one of the dose levels will return after a 7-day minimum washout or 5 half-lives of the drug (whichever is longer) to receive the same dose under fed conditions. Safety will be assessed throughout the study for all subjects. Blood will be collected for pharmacokinetic analysis. Plasma samples will be analyzed for TBI-223 and TBI-223 metabolite (M2). Dose escalation to the next cohort (i.e., dose level) will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort has been demonstrated to permit proceeding to the next cohort. Additional cohorts may be enrolled if deemed appropriate (e.g. if bioavailability is lower than expected) by the Sponsor to repeat a dose level, to study other dose levels, change proposed cohorts, or to study a different dosage formulation. These decisions regarding changed or additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator and dose escalating committee, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach for review and approval. *To:* This is a partially-blinded, placebo-controlled, randomized SAD study with a food effect cohort. The study will be conducted at one study center in the United States. Safety will be assessed throughout the study for all subjects. Blood will be collected for pharmacokinetic analysis. Plasma samples will be analyzed for TBI-223 and TBI-223 metabolite (M2). **Part 1:** There will be up to 6 planned dose levels. Based on interim pharmacokinetic data obtained during the dose escalation, a dose cohort will be selected to return for additional dosing after a high-calorie, high-fat meal (food-effect cohort). Sentinel Cohort: The first cohort will be separated into 2 groups. A sentinel group of 3 subjects (2 active and 1 placebo) will be dosed at least 24 hours before the remaining 5 subjects (4 active and 1 placebo). The remaining cohorts will be dosed in 2 groups of 4 subjects each (3 active and 1 placebo), at least 24 hours apart. Food-effect Cohort: Based on exposure levels, subjects in one of the dose

Section Number (s)	Section Title(s)	Description of Change (s)
		levels will return after a 7-day minimum washout or 5 half-lives of the drug (whichever is longer) to receive the same dose under fed conditions. Dose escalation to the next cohort (i.e., dose level) will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort has been demonstrated to permit proceeding to the next cohort. **Cohort 8:** **There will be 4 arms each consisting of 6 subjects. Subjects will be assigned to one of the following arms and dose:** • **Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 2 – TBI-223 SR Tablets (Prototype 2) - 1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed conditions** • **Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions** **Cohort 9:** **The 6 subjects from Cohort 8, Arm 4, who were received IR tablet formulation will be brought back following a 7-day washout – TBI-223 IR Tablets – 2000 mg (2 x 1000 mg tablets), under fed conditions.** **If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).** Additional cohorts may be enrolled if deemed appropriate (e.g. if bioavailability is lower than expected) by the Sponsor to repeat a dose level, to study other dose levels, change proposed cohorts, or to study a different dosage formulation. These decisions regarding changed or additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator and dose escalating committee, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach for review and approval.
Synopsis	Test product, dosage and mode of	*From:* TBI-223 oral suspension, orally administered Dose level 1 = single dose of 50 mg TBI-223

Section Number (s)	Section Title(s)	Description of Change (s)
	administration	Dose level 2 = single dose of 100 mg TBI-223 Dose level 3 = single dose of 300 mg TBI-223 Dose level 4 = single dose of 600 mg TBI-223 Dose level 5 = single dose of 1200 mg TBI-223 Dose level 6 = single dose of 2000 mg TBI-223 Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or a different dosage formulation. *To:* **Part 1:** TBI-223 oral suspension, orally administered Dose level 1 = single dose of 50 mg TBI-223 Dose level 2 = single dose of 100 mg TBI-223 Dose level 3 = single dose of 300 mg TBI-223 ***Dose level 3 includes a Cohort 3a with TBI-223 (oral suspension) and the same subjects participated in Cohort 3b with TBI-223 within oral enteric capsules*** Dose level 4 = single dose of 600 mg TBI-223 Dose level 5 = single dose of 1200 mg TBI-223 Dose level 6 = single dose of 2000 mg TBI-223 **Dose level 7= Single dose of 2600 mg TBI-223** Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or a different dosage formulation. **Part 2:** **Cohort 8:** **Arm 1 - TBI-223 SR Tablets 600 mg (Prototype 1). Dose of 1800 mg (3 tablets of 600 mg), under fed conditions** **Arm 2 - TBI-223 SR Tablets 600 mg (Prototype 2). Dose of 1800 mg (3 tablets of 600 mg), under fed conditions** **Arm 3 - TBI-223 SR Tablets 900 mg (Prototype 3). Dose of 1800 mg (2 tablets of 900 mg), under fed conditions** **Arm 4 - TBI-223 IR Tablets 1000 mg. Dose of 2000 mg (2 tablets of**

Section Number (s)	Section Title(s)	Description of Change (s)
		1000 mg), under fasting conditions **Cohort 9:** **TBI-223 IR Tablets 1000 mg. Dose of 2000 mg (2 tablets of 1000 mg), under fed conditions.** **Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or a different dosage formulation.**
Synopsis	Control product, dosage and mode of administration:	*Add the following to the beginning of the section:* **Part 1 only:**
Synopsis	Criteria of evaluation	*Second paragraph. Delete the following pharmacokinetic parameters and definitions:* • AUC_{tau} – area under the concentration time curve during the dosing interval • C_{avg} – average stead state concentration • CLss/F - apparent total clearance at steady state • C_{min} minimum concentration • C_{trough}- trough plasma concentration • R_{AUC} accumulation ration for AUC • R_{Cmax}- accumulation ratio for C_{max} *Add the following to the definitions of CL/F and Vz/F* **(TBI-223 only)** *Delete the third paragraph:* Noncompartmental pharmacokinetic parameters of AUC_{last}, AUC_{inf}, C_{max}, T_{max}, CL/F, Vz/F, λ_z, and $T_{1/2}$ will be calculated from plasma concentrations of TBI-223 and M2. Additional pharmacokinetic parameters may be calculated if deemed appropriate. *Add the following to the fourth paragraph from:* Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters under fed versus fasting conditions using an analysis of variance (ANOVA) approach.

Section Number (s)	Section Title(s)	Description of Change (s)
		To: Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters **(C_{max}, AUCs)** under fed versus fasting conditions using an analysis of variance (ANOVA) approach.
3	Study Design Summary	*Add the following before the first paragraph of the section* **Part 1:** *Add the following to the end of the section, after Table 2* **Part 2:** **Cohort 8: A minimum of 24 subjects in 4 dosing arms are planned to be enrolled; within each arm, 6 subjects will be assigned to receive a single dose treatment. Subjects will be assigned to one of the following arms and dose:** • **Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 2 – TBI-223 SR Tablets (Prototype 2) -1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed conditions** • **Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions** **Procedures and analyses will be conducted as outlined in Appendix 6.** **Cohort 9:** **The 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation fasted will return after a 7-day washout and receive a single dose of TBI-223 IR Tablets, 2000 mg dose (2 x 1000 mg tablets), under fed conditions.** **If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).** **Procedures and analyses will be conducted as outlined in Appendix 6.**
3.1	Dose Escalation	*Change the title of the section from:*

<table>
<tr><th>Section Number (s)</th><th>Section Title(s)</th><th>Description of Change (s)</th></tr>
<tr><td></td><td></td><td>Dose Escalation
To:
Part 1 Dose Escalation</td></tr>
<tr><td>3 (pg 12</td><td>Table 2</td><td>Update Table 2
From:
Table 2 presents the planned dose cohorts for the study.
Table 2 Planned Dose Cohorts
<table>
<tr><th>Cohort[a] (Group[b])</th><th colspan="2">Dose[c]</th></tr>
<tr><td>1 (sentinel group)</td><td>Single dose of 50 mg TBI-223 (n=2)</td><td>Placebo (n=1)</td></tr>
<tr><td>1 (remainder of cohort)</td><td>Single dose of 50 mg TBI-223 (n=4)</td><td>Placebo (n=1)</td></tr>
<tr><td>2 (Group 1)</td><td>Single dose of 100 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>2 (Group 2)</td><td>Single dose of 100 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>3 (Group 1)</td><td>Single dose of 300 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>3 (Group 2)</td><td>Single dose of 300 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>4 (Group 1)</td><td>Single dose of 600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>4 (Group 2)</td><td>Single dose of 600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>5 (Group 1)</td><td>Single dose of 1200 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>5 (Group 2)</td><td>Single dose of 1200 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>6 (Group 1)</td><td>Single dose of 2000 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>6 (Group 2)</td><td>Single dose of 2000 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
</table>
To:</td></tr>
</table>

<table>
<tr><th>Section Number (s)</th><th>Section Title(s)</th><th>Description of Change (s)</th></tr>
<tr><td></td><td></td><td>Table 2 presents the actual dose cohorts for Part 1 of the study.
Table 2 Actual Dose Cohorts for Part 1
<table>
<tr><th>Cohort[a] (Group[b])</th><th colspan="2">Dose[c]</th></tr>
<tr><td>1 (sentinel group)</td><td>Single dose of 50 mg TBI-223 (n=2)</td><td>Placebo (n=1)</td></tr>
<tr><td>1 (remainder of cohort)</td><td>Single dose of 50 mg TBI-223 (n=4)</td><td>Placebo (n=1)</td></tr>
<tr><td>2 (Group 1)</td><td>Single dose of 100 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>2 (Group 2)</td><td>Single dose of 100 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>3a (Group 1)</td><td>Single dose of 300 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>3a (Group 2)</td><td>Single dose of 300 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>3b (Capsule cohort)</td><td>Single dose of 300 mg (TBI oral in oral enteric) (n=4)</td><td>N/A</td></tr>
<tr><td>4 (Group 1)</td><td>Single dose of 600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>4 (Group 2)</td><td>Single dose of 600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>5 (Group 1)</td><td>Single dose of 1200 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>5 (Group 2)</td><td>Single dose of 1200 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>6 (Group 1)</td><td>Single dose of 2000 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>6 (Group 2)</td><td>Single dose of 2000 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>7 (Group 1)</td><td>Single dose of 2600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
<tr><td>7 (Group 2)</td><td>Single dose of 2600 mg TBI-223 (n=3)</td><td>Placebo (n=1)</td></tr>
</table></td></tr>
<tr><td>4</td><td>Identity of Investigational</td><td>Add the following to Table 3, Identify of Investigational Product</td></tr>
</table>

<table>
<tr><th>Section Number (s)</th><th>Section Title(s)</th><th>Description of Change (s)</th></tr>
<tr><td></td><td>Product</td><td>
From:
<table>
<tr><td>Test Product:</td><td>TBI-223 oral suspension 25 mg/mL</td></tr>
<tr><td></td><td>Manufactured for Global Alliance for TB Drug Development</td></tr>
<tr><td>Control Product:</td><td>Placebo for TBI-223 oral suspension</td></tr>
<tr><td></td><td>Manufactured for Global Alliance for TB Drug Development</td></tr>
</table>
To:
<table>
<tr><td>Part 1</td><td></td></tr>
<tr><td>Test Product:</td><td>TBI-223 oral suspension 25 mg/mL</td></tr>
<tr><td></td><td>Manufactured for Global Alliance for TB Drug Development</td></tr>
<tr><td>Control Product:</td><td>Placebo for TBI-223 oral suspension</td></tr>
<tr><td></td><td>Manufactured for Global Alliance for TB Drug Development</td></tr>
<tr><td>Test Product: Cohort 3b</td><td>TBI-223 Oral Enteric Capsules, 150 mg</td></tr>
<tr><td></td><td>Manufactured for Global Alliance for TB Drug Development</td></tr>
<tr><td>Part 2</td><td></td></tr>
<tr><td rowspan="8">Test Products: Cohort 8 (Arms 1-4)</td><td>TBI-223 SR Tablets 600 mg (Prototype 1)</td></tr>
<tr><td>Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited</td></tr>
<tr><td>TBI-223 SR Tablets 600 mg (Prototype 2)</td></tr>
<tr><td>Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited</td></tr>
<tr><td>TBI-223 SR Tablets 900 mg (Prototype 3)</td></tr>
<tr><td>Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited</td></tr>
<tr><td>TBI-223 IR Tablets 1000 mg</td></tr>
<tr><td>Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited</td></tr>
<tr><td rowspan="2">Test Products: Cohort 9</td><td>TBI-223 IR Tablets 1000 mg</td></tr>
<tr><td>Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited</td></tr>
</table>
</td></tr>
</table>

Section Number (s)	Section Title(s)	Description of Change (s)
6.1	Subjects Assignment	*From:* Up to 50 subjects are planned to be enrolled in the study. Additional subjects may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. The number of subjects selected for the study was based on the adequate number considered to provide sufficient safety data. This study has not been formally powered. Each subject will receive an assigned treatment (active or placebo) based on the randomization schedule prepared by the unblinded statistician. The unblinded pharmacy staff will ensure compliance with the randomization schedule. Note: the repeat cohort using a different dosage formulation will be non-randomized and unblinded; see Appendix 5. *To:* **For Part 1,** up to 50 subjects are planned to be enrolled in the study. Additional subjects may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. **For Part 2, Cohort 8, 24 subjects will be enrolled. For Part 2, Cohort 9, the 6 subjects from Cohort 8 who received the IR tablet formulation fasted will return after a 7-day washout and receive the IR tablet formulation under fed conditions. If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).** The number of subjects selected for the study was based on the adequate number considered to provide sufficient safety data. This study has not been formally powered. **For Part 1,** each subject will receive an assigned treatment (active or placebo) based on the randomization schedule prepared by the unblinded statistician. The unblinded pharmacy staff will ensure compliance with the randomization schedule. Note: the repeat cohort using a different dosage formulation **in Part 1 and subjects in Part 2** will be non-randomized and unblinded; see Appendix 5 **and Appendix 6**.
6.3	Check-in Procedures	*Update the first sentence from:* Subjects will check into the clinic on Day -2. *To:* Subjects will check into the clinic on Day -2 **for Part 1 and Day -1 for Part 2.**

Section Number (s)	Section Title(s)	Description of Change (s)
		Add the following to the last bullet point: **(Part 1 only)**
6.4	Confinement	*First paragraph, change from:* Fasting cohorts: Subjects will be admitted to the research center on Day -2 and remain confined until after completion of the 48-hour procedures on Day 3. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) and receive a follow up phone call on Day 11 (+1 day). *To:* **Part 1** fasting cohorts **and Part 2, Cohort 8, Arm 4 only**: Subjects will be admitted to the research center on Day -2 **(Part 1) or Day -1 (Part 2)** and remain confined until after completion of the 48-hour procedures on Day 3. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) and receive a follow up phone call on Day 11 (+1 day). *Second paragraph, change from:* Food-effect cohort: Subjects will be admitted to the research center on Day -2 of Period 1 and Period 2 and remain confined until after completion of the 48-hour procedures on Day 3 of each study period. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) in each study period. Subjects will receive a follow up phone call on Day 11 (+1 day) of Period 2. *To:* **Part 1** food-effect cohort, **Part 2, Cohort 8, Arms 1-3 and Cohort 9**: Subjects will be admitted to the research center on Day -2 of Period 1 and Period 2 **(Part 1) or Day -1 (Part 2)** and remain confined until after completion of the 48-hour procedures on Day 3 of each study period. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) in each study period. Subjects will receive a follow up phone call on Day 11 (+1 day) of Period 2. **Please note that Cohort 9 is the same 6 subjects that were in Cohort 8, Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8, Arm 4, subjects fasted.**
6.5.1	Fasting/Meals	*First paragraph, change from:* Fasted cohorts: Optional meals (lunch, snack, and dinner) may be served the day of check-in. All subjects will then be required to fast for at least 10 hours before dosing. The subjects will fast for 4 hours after dosing. Standard meals will be provided at appropriate times after dosing. *To:*

Section Number (s)	Section Title(s)	Description of Change (s)
		Part 1 fasted cohorts **and Part 2, Cohort 8, Arm 4 only**: Optional meals (lunch, snack, and dinner) may be served the day of check-in. All subjects will then be required to fast for at least 10 hours before dosing. The subjects will fast for 4 hours after dosing. Standard meals will be provided at appropriate times after dosing. *Second paragraph, change from:* Food-effect cohort: Optional meals (lunch, snack, and dinner) may be served the day of check-in. *To:* **Part 1** food-effect cohort, **Part 2, Cohort 8, Arms 1-3 and Cohort 9**: Optional meals (lunch, snack, and dinner) may be served the day of check-in. *Fourth paragraph, first sentence, change from:* In the fed study period. Subjects will be required to fast for at least 10 hours overnight before consuming a required FDA standard high-fat, high-calorie breakfast. *To:* In the fed study period **of Part 1 and Part 2 (Cohort 8, Arms 1-3 and Cohort 9), subjects** will be required to fast for at least 10 hours overnight before consuming a required FDA standard high-fat, high-calorie breakfast. *Add the following to the end of the section:* **Please note that Part 2, Cohort 9 is the same 6 subjects that were in Cohort 8 Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8 Arm 4, subjects fasted.**
6.6	Drug administration	*Add the following subsection:* *6.6.3 Administration of TBI-223 SR and IR Tablets* *Add the following to the new section:* **For Part 2, Cohort 8 and Cohort 9, each subject will receive the oral dose of the assigned study treatment (TBI-223 SR tablet formulations [Prototypes 1, 2, or 3] or TBI-223 IR tablet formulation) with approximately 240 mL (8 fl. oz.) of room temperature water. Subjects must swallow the study medication intact. The medication should NOT be crushed or chewed. A hand and mouth check will be performed immediately after dose to ensure that the medication has been appropriately swallowed.** **The subjects will remain seated, except as otherwise required for study procedures or personal needs, for the first 4 hours after dosing. Should the need to move about occur during the first 4 hours after each dose,**

Section Number (s)	Section Title(s)	Description of Change (s)
		subjects may be escorted to such procedures or activities by research personnel as deemed medically necessary. Subjects will not be allowed to lie down, except as directed by clinical staff secondary to AEs, for the first 4 hours after dosing.
6.7.1	Fasting cohort	*Change title of section from:* Fasting cohort *To:* **Part 1** fasting cohorts**, Part 2, Cohort 8, Arm 4 only**
6.7.2	Food-effect cohort	*Change title of section from:* Food-effect cohort *To:* **Part 1** food-effect cohort**, Part 2, Cohort 8, Arms 1-3 only, and Cohort 9** *Add the following after the first paragraph:* **Please note that Part 2, Cohort 9 is the same 6 subjects that were in Cohort 8 Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8 Arm 4, subjects fasted.**
6.9.7	Cardiac Holter Monitoring, and Cardiodynamic Assessment	*Add the following sentence to the end of the section:* **Part 2 of the study will not have cardiac Holter monitoring.**
8.4	Indications for Subject Withdrawal	*Add the following paragraphs to the end of the section:* **Subjects who experience emesis may be replaced if the emesis could potentially impact drug absorption and therefore the pharmacokinetic data. Cases of emesis will be evaluated by the Sponsor and Principal Investigator to determine if subject replacement is needed.** **For Part 1, replacement subjects will be numbered 17XX where XX is 10 plus the original subject's last 2 assigned numbers (e.g. if subject 1702 needs to be replaced due to emesis, the replacement subject number would be 1712). Replacement subjects will receive the same dose allocation of study drug.**
9.2	Pharmacokinetic Analysis	*Add the following to the definitions of CL/F and Vz/F:* **(TBI-223 only)** *Deleted the first sentence from third paragraph, from:* Noncompartmental pharmacokinetic parameters of AUC_{last}, AUC_{inf}, C_{max}, T_{max}, CL/F, Vz/F, λ_z, and $T_{1/2}$ will be calculated from plasma concentrations of TBI-223 and M2. Additional pharmacokinetic parameters may be

Section Number (s)	Section Title(s)	Description of Change (s)
		calculated if deemed appropriate. *To:* Additional pharmacokinetic parameters may be calculated if deemed appropriate. *Add the following to last paragraph, from:* Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters under fed versus fasting conditions using an analysis of variance (ANOVA) approach. *To:* Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters **(C_{max}, AUCs)** under fed versus fasting conditions using an analysis of variance (ANOVA) approach.
9.3	Statistical Analysis	*Second and third paragraph, change from:* Pharmacokinetic parameters will be summarized by cohort using descriptive statistics. Summary statistics will also be presented by gender within each cohort. Dose proportionality will be assessed using a power model approach. Food-effect cohort: The effect of food will be assessed comparing pharmacokinetic parameters under fed versus fasting conditions using an analysis of variance (ANOVA) approach. *To:* Pharmacokinetic parameters will be summarized by cohort using descriptive statistics. Summary statistics will also be presented by gender within each cohort. Dose proportionality **for exposure parameters (C_{max}, AUCs)** will be assessed using a power model approach. Food-effect cohort: The effect of food will be assessed comparing pharmacokinetic parameters **(C_{max}, AUCs)** under fed versus fasting conditions using an analysis of variance (ANOVA) approach.
11	Drug Supplies	*Add the following to the end of the section:* **Global Alliance for TB Drug Development will supply sufficient quantity of the study drug, TBI 223 SR tablet formulations (Prototypes 1, 2, and 3) and TBI-223 IR tablet formulation for Part 2 of the study.** **Study drug formulations will be shipped to Worldwide Clinical Trials Early Phase Services, LLC pursuant to site standard operating procedures. Upon receipt of the study drug products, the supplies will be inventoried and stored in an environmentally controlled and secure, limited access area. The lot numbers of the drugs along with the**

<table>
<tr><th>Section Number (s)</th><th>Section Title(s)</th><th>Description of Change (s)</th></tr>
<tr><td></td><td></td><td>expiration dates (where available) will be recorded and copies of the Certificate of Analysis (where available) will be maintained on file. Records will be maintained of the receipt and dispensation of the drugs supplied. At the conclusion of the study, any unused study drug will be returned to the sponsor or destroyed by the site pursuant to written authorization by the sponsor and applicable federal and state regulations.</td></tr>
<tr><td>Table 9</td><td>Schedule of Assessments</td><td>Change title of table from:
Schedule of Assessments

To:
Schedule of Assessments, Part 1</td></tr>
<tr><td>Appendix 2</td><td>Description and Composition of Test Product</td><td>Add the following before the first paragraph:
Oral Suspension
Add the following to the end of the section after the fifth paragraph:
Enteric Capsules

Description of the Dosage Form

TBI-223 Oral Enteric Capsules contain 150 mg TBI-223 in Vcaps® Enteric capsules. The capsules are filled at the clinical study site by manually filling TBI-223 into Vcaps® Enteric capsules which are commercially available empty capsule shells.

Composition of the Drug Product

The quantitative composition, function and quality of each ingredient in the drug product (TBI-223 Oral Enteric Capsules, 150 mg) is provided in Table 10.

Table 10 Qualitative and Quantitative Composition of the TBI-223 Oral Enteric Capsules, 150 mg
<table>
<tr><th>Ingredient</th><th>Function</th><th>Quality Standard</th><th>Quantity per 300 mg Dose</th></tr>
<tr><td>TBI-223</td><td>Drug Substance</td><td>In-house</td><td>300 mg</td></tr>
</table>
</td></tr>
</table>

Section Number (s)	Section Title(s)	Description of Change (s)

Vcaps® Enteric Capsules[1], Off-white, Opaque, Size 00	**Capsule shell**	**Commercially available**	**2 capsules**
Total Quantity			**300 mg in 2 capsules**

[1]Capsule shell composition: titanium dioxide 2% in Hypromellose-Hypromellose AS.

Container and Closure System

TBI-223 Oral Enteric Capsules are stored in white HDPE bottles with PP CRC with a liner. These bottles are used for storage prior to administration.

Tablets

Description of the Dosage Form

TBI-223 tablets are supplied as immediate release (IR) or sustained release (SR). The description of the tablets and strengths are presented in Table 11.

Table 11 Strength, Tablet Weight and Appearance of TBI-223 IR and SR Tablets

Tablet Strength	Tablet Weight	Tablet Appearance
TBI-223 Immediate Release (IR) Tablets		
600 mg	**750 mg**	**White to off-white, capsule shaped tablets, debossed with "TBA" on one side and "22" on the other**
1000 mg	**1250 mg**	**White to off-white, oval shaped tablets, debossed with "TBA" on one side and "23" on the other**
TBI-223 Sustained Release (SR) Tablets		
600 mg (Prototype 1)	**1000 mg**	**White to off-white, capsule shaped tablets, debossed with "TBA" on one side and "11" on the other**
600 mg (Prototype 2)	**1000 mg**	**White to off-white,**

Section Number (s)	Section Title(s)	Description of Change (s)

		capsule shaped tablets, debossed with "TBA" on one side and "SR" on the other
900 mg (Prototype 3)	**1200 mg**	**White to off-white, oval shaped tablets, debossed with "TBA" on one side and "900" on the other**

Composition of the Drug Product

The composition of TBI-223 IR tablets, 600 mg and 1000 mg is presented in Table 12. The composition of TBI-223 SR Tablets, 600 mg (Prototype 1), 600 mg (Prototype 2) and 900 mg (prototype 3) is presented in Table 13, Table 14 and Table 15, respectively.

Table 12 Composition of TBI-223 IR Tablets, 600 mg and 1000 mg

Component	Function	Percentage (% w/w)	600 mg/tablet	1000 mg/tablet	Reference to Standard
		Intragranular			
TBI-223 [a]	**Active**	**80.00**	**600.00**	**1000.00**	**In-House**
Microcrystalline cellulose (Avicel PH 302) [b]	**Diluent**	**10.00**	**75.00**	**125.00**	**USP-NF, Ph.Eur.**
Hypromellose (Methocel E5 Premium LV)	**Binder**	**3.00**	**22.50**	**37.50**	**USP-NF, Ph.Eur.**
Crospovidone (Kollidon CL)	**Disintegrant**	**3.00**	**22.50**	**37.50**	**USP-NF, Ph.Eur.**
Purified water [c]	**Granulating solvent**	**q.s**	**q.s**	**q.s**	**USP, Ph.Eur.**
		Extragranular			
Crospovidone (Kollidon CL)	**Disintegrant**	**2.00**	**15.00**	**25.00**	**USP-NF, Ph.Eur.**
Colloidal silicon dioxide (Aerosil 200 Pharma)	**Glidant**	**1.00**	**7.50**	**12.50**	**USP-NF, Ph.Eur.**
Magnesium stearate (Ligamed MF-2-V)	**Lubricant**	**1.00**	**7.50**	**12.50**	**USP-NF, Ph.Eur.**

Section Number (s)	Section Title(s)	Description of Change (s)

Total unit dose	**100.00**	**750.0** **1250.0**

[a] **The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.**

[b] **The quantity of microcrystalline cellulose may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.**

[c] **Removed during processing.**

Table 13 Composition of TBI-223 SR Tablets, 600 mg (Prototype 1)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	**Active**	**60.00**	**600.00**	**In-House**
Lactose monohydrate [b] (Pharmatose 200M)	**Diluent**	**9.90**	**99.00**	**USP-NF, Ph.Eur.**
Microcrystalline cellulose (Avicel PH 200)	**Diluent**	**6.60**	**66.00**	**USP-NF, Ph.Eur.**
Hypromellose (Methocel E5 Premium LV)	**Binder**	**5.00**	**50.00**	**USP-NF, Ph.Eur.**
Purified water [c]	**Granulating solvent**	**q.s**	**q.s**	**USP, Ph.Eur.**
		Extragranular		
Hypromellose (Methocel K100M Premium DC2)	**Control Release Polymer**	**16.00**	**160.00**	**USP-NF, Ph.Eur.**
Colloidal silicon dioxide (Aerosil 200 Pharma)	**Glidant**	**1.50**	**15.00**	**USP-NF, Ph.Eur.**
Magnesium stearate (Ligamed MF-2-V)	**Lubricant**	**1.00**	**10.00**	**USP-NF, Ph.Eur.**
Total unit dose		**100.00**	**1000.0**	

Section Number (s)	Section Title(s)	Description of Change (s)

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.
[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.
[c] Removed during processing.

Table 14 Composition of TBI-223 SR Tablets, 600 mg (Prototype 2)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	Active	60.00	600.00	In-House
Lactose monohydrate [b] (Pharmatose 200M)	Diluent	12.90	129.00	USP-NF, Ph.Eur.
Microcrystalline cellulose (Avicel PH 200)	Diluent	8.60	86.00	USP-NF, Ph.Eur.
Hypromellose (Methocel K4M Premium CR)	Control Release Polymer	5.00	50.00	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	USP, Ph.Eur.
		Extragranular		
Hypromellose (Methocel K100 Premium LV)	Control Release Polymer	11.00	110.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.50	15.00	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	10.00	USP-NF, Ph.Eur.
Total unit dose		100.00	1000.0	

Section Number (s)	Section Title(s)	Description of Change (s)

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.

[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.

[c] Removed during processing.

Table 15 Composition of TBI-223 SR Tablets, 900 mg (Prototype 3)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	Active	75.00	900.00	In-House
Lactose monohydrate [b] (Pharmatose 200M)	Diluent	3.90	46.80	USP-NF, Ph.Eur.
Microcrystalline cellulose (Avicel PH 200)	Diluent	2.60	31.20	USP-NF, Ph.Eur.
Hypromellose (Methocel E5 Premium LV)	Binder	5.00	60.00	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	USP. Ph.Eur.
		Extragranular		
Hypromellose (Methocel K100M Premium DC2)	Control Release Polymer	11.00	132.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.50	18.00	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	12.00	USP-NF, Ph.Eur.
Total unit dose		100.00	1200.0	

Section Number (s)	Section Title(s)	Description of Change (s)
		[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used. **[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.** **[c] Removed during processing.** **Container and Closure System** **TBI-223 IR and SR Tablets are packaged as 30-count in 100 cc round high-density polyethylene (HDPE) bottles and closed with aluminum foil lined heat induction seals and 38 mm polypropylene (PP) child resistant closures (CRC).**
Appendix 6	Part 2 of the study	*Add the following:* **Procedures and analyses as outlined in this protocol will be the same, with the following exceptions:** **The dose of TBI-223 will be delivered as follows in Cohort 8:** • **Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 2 – TBI-223 SR Tablets (Prototype 2) - 1800 mg dose (3 x 600 mg tablets), under fed conditions** • **Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed conditions** • **Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions** **In Cohort 9, the subjects from Cohort 8, Arm 4, who received the IR tablet formulation will be brought back following a 7-day washout and administered TBI-223 IR tablets, 2000 mg dose (2 x 1000 mg tablets), under fed conditions.** • **A minimum of 6 subjects per treatment arm in Part 2** • **Part 2 will not be blinded (blinding is not necessary because all subjects will receive the same treatment).** • **Cardiac telemetry and Holter recording will not be performed during Part 2. Subjects will check-in on Day -1 instead of Day -2. With the exception of cardiac telemetry and Holter monitoring, the procedures that were previously scheduled for Day -2 will be performed on Day -1. The Day -1 results will be used to qualify repeat subjects for study participation. If additional subjects are**

Section Number (s)	Section Title(s)	Description of Change (s)
		needed, they will undergo screening to qualify for the study. • **Neurological examinations will be performed at Screening, Day -1, and at 3 and 48 hours postdose.** *Add Table 16, Schedule of Assessments, Part 2*

PROTOCOL SYNOPSIS

<table>
<tr><td colspan="2">Name of Sponsor/Company: Global Alliance for TB Drug Development</td></tr>
<tr><td colspan="2">Name of test product: TBI-223 oral suspension</td></tr>
<tr><td colspan="2">Name of active ingredient: TBI-223</td></tr>
<tr><td colspan="2">Title of study: A Phase 1, Partially-Blinded, Placebo-Controlled, Randomized, Single Ascending Dose (SAD) with a Food Effect Cohort Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of TBI-223 in Healthy Adult Participants</td></tr>
<tr><td colspan="2">Principal Investigator: Cynthia A. Zamora, MD</td></tr>
<tr><td colspan="2">Study Center
Worldwide Clinical Trials Early Phase Services, LLC
2455 NE Loop 410, Suite 150
San Antonio, Texas 78217</td></tr>
<tr><td>Study period (maximum duration, from screening to study exit):
Part 1:
Single ascending dose (SAD) cohort (fasting): 40 days
Food-effect cohort: 50 days
Part 2:
Cohort 8: Single dose cohort of the 4 TBI-223 tablet formulations (Prototypes 1, 2, and 3 Sustained Release [SR] tablet formulations under fed conditions and one Immediate Release [IR] tablet formulation under fasting conditions): Duration 40 days.
Cohort 9: The IR tablet formulation will be evaluated under fed conditions
Single dose: 40 days
The maximum duration of the study will depend upon the final number and timing of each cohort.</td><td>Phase of development: 1</td></tr>
<tr><td>Duration of treatment:
Part 1:
<u>Fasting cohorts</u>: One single dose of TBI-223 oral suspension or placebo for TBI-223 oral suspension
<u>Food-effect cohort</u>: Two single doses of TBI-223 oral suspension or placebo for TBI-223 oral suspension separated by a washout period of at least 7 days</td><td>Number of sites enrolling subjects: 1</td></tr>
</table>

Part 2: **Cohort 8:** Single dose cohort of 4 TBI-223 tablet formulations (Three SR tablet formulations as prototypes 1, 2, and 3 and one IR tablet formulation). The SR tablet formulations will be evaluated under fed conditions and the IR tablet formulation will be evaluated under fasting conditions **Cohort 9:** The IR tablet formulation will be evaluated under fed conditions	

Number of subjects (planned)**:**

Part 1:

Fasting Cohorts: Planned to enroll up to 48 subjects in a total of 6 single ascending dose (SAD) cohorts of 8 subjects each (6 to receive active drug and 2 to receive placebo).

Food-effect Cohort: 1 cohort with 10 subjects (8 active and 2 placebo) who will receive TBI-223 or placebo under both fasting and fed conditions.

Part 2:

Cohort 8: Planned to enroll 24 subjects in a total of 4 arms with 6 subjects in each. Subjects will receive a single dose of 1 of the 3 prototypes of TBI-223 SR tablets under fed conditions (Arms 1-3) or a single dose of TBI-223 IR tablets under fasted conditions (Arm 4).

Cohort 9: Plan to bring back the 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation, following a 7-day washout to receive the IR tablet formulation under fed conditions.

If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).

Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. These decisions on additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach.

Diagnosis and main criteria for inclusion:

Volunteers will be healthy adult male or female, ages 19 to 50 years (inclusive) at screening, with a body mass index (BMI) $\geq$18.5 and $\leq$32.0 kg.m^2 and body weight of no less than 50.0 kg, who do not use tobacco or nicotine-containing products. Females must be of non-childbearing potential or must use an allowable method of birth control.

Objectives:

The primary objective of the study is:

- To evaluate the safety and tolerability of single ascending doses of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations in healthy adult subjects.

The secondary objectives of the study are:

- To determine the pharmacokinetics of TBI-223 and its metabolite M2 after single doses of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations in healthy adult subjects; and
- To compare the rate and extent of absorption of a single dose of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations when administered in healthy adult subjects either after a high-calorie, high-fat meal or in the fasting state.

This is a partially-blinded, placebo-controlled, randomized SAD study with a food effect cohort. The study will be conducted at one study center in the United States.

Safety will be assessed throughout the study for all subjects.

Blood will be collected for pharmacokinetic analysis. Plasma samples will be analyzed for TBI-223 and TBI-223 metabolite (M2).

Part 1:

There will be up to 6 planned dose levels. Based on interim pharmacokinetic data obtained during the dose escalation, a dose cohort will be selected to return for additional dosing after a high-calorie, high-fat meal (food-effect cohort).

Sentinel Cohort: The first cohort will be separated into 2 groups. A sentinel group of 3 subjects (2 active and 1 placebo) will be dosed at least 24 hours before the remaining 5 subjects (4 active and 1 placebo). The remaining cohorts will be dosed in 2 groups of 4 subjects each (3 active and 1 placebo), at least 24 hours apart.

Food-effect Cohort: Based on exposure levels, subjects in one of the dose levels will return after a 7-day minimum washout or 5 half-lives of the drug (whichever is longer) to receive the same dose under fed conditions.

Dose escalation to the next cohort (i.e., dose level) will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort has been demonstrated to permit proceeding to the next cohort.

Part 2:

Cohort 8:

There will be 4 arms each consisting of 6 subjects. Subjects will be assigned to one of the following arms and dose:

- Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 2 – TBI-223 SR Tablets (Prototype 2) - 1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed

conditions

- Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions

Cohort 9:

The 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation will be brought back following a 7-day washout – TBI-223 IR Tablets – 2000 mg (2 x 1000 mg tablets), under fed conditions.

If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).

Additional cohorts may be enrolled if deemed appropriate (e.g. if bioavailability is lower than expected) by the Sponsor to repeat a dose level, to study other dose levels, change proposed cohorts, or to study a different dosage formulation. These decisions regarding changed or additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator and dose escalating committee, has determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach for review and approval.

Test product, dosage and mode of administration:

Part 1:

TBI-223 oral suspension, orally administered

Dose level 1 = single dose of 50 mg TBI-223

Dose level 2 = single dose of 100 mg TBI-223

Dose level 3 = single dose of 300 mg TBI-223

Dose level 3 includes a Cohort 3a with TBI-223 (oral suspension) and the same subjects participated in Cohort 3b with TBI-223 within oral enteric capsules

Dose level 4 = single dose of 600 mg TBI-223

Dose level 5 = single dose of 1200 mg TBI-223

Dose level 6 = single dose of 2000 mg TBI-223

Dose level 7= Single dose of 2600 mg TBI-223

Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or a different dosage formulation.

Part 2:

Cohort 8:

Arm 1 - TBI-223 SR Tablets 600 mg (Prototype 1). Dose of 1800 mg (3 tablets of 600 mg), under fed conditions

Arm 2 - TBI-223 SR Tablets 600 mg (Prototype 2). Dose of 1800 mg (3 tablets of 600 mg), under fed conditions

Arm 3 - TBI-223 SR Tablets 900 mg (Prototype 3). Dose of 1800 mg (2 tablets of 900 mg), under fed conditions

Arm 4 - TBI-223 IR Tablets 1000 mg. Dose of 2000 mg (2 tablets of 1000 mg), under fasting conditions

Cohort 9:

TBI-223 IR Tablets 1000 mg. Dose of 2000 mg (2 tablets of 1000 mg), under fed conditions.

Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or a different dosage formulation.

Control product, dosage and mode of administration:

Part 1 only:

Placebo for TBI-223 oral suspension; orally administered

All levels = Single dose of placebo for TBI-223 oral suspension

Criteria for evaluation:

Safety: Safety assessments will include physical and neurological examinations, vital signs including heart rate and respiratory rate, electrocardiograms (ECGs), cardiac monitoring, adverse events (AEs), and clinical laboratory tests (including hematology, serum chemistry, coagulation, and urinalysis). Female subjects will have blood collected for serum pregnancy testing. Females claiming postmenopausal status will have blood collected to measure follicle stimulating hormone (FSH) levels.

Pharmacokinetics: Final pharmacokinetic calculations will be performed using appropriate software, e.g. Phoenix™ WinNonlin® (Version 8.1 or higher, Certara, L.P. in conjunction with the internet-accessible implementation of Pharsight® Knowledgebase Server™ [PKSO; Version 4.0.4 or higher, Certara, L.P.]) and/or SAS® (Version 9.4 or higher, SAS Institute Inc.). Pharmacokinetic parameters will be calculated using non-compartmental analysis. The following pharmacokinetic parameters will be determined as appropriate for each study part.

AUC_{Extrap} (%)	The percentage of extrapolated AUC to AUC_{inf} based on extrapolation
AUC_{inf}	Area under the concentration-time curve from time-zero extrapolated to infinity; calculated as: $AUC_{\inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$
AUC_{last}	Area under the concentration-time curve from time-zero to the time of the last quantifiable concentration; calculated using the linear trapezoidal rule
C_{last}	The last quantifiable concentration determined directly from individual concentration-time data
CL/F	Apparent total clearance after single administration (TBI-223 only)
C_{max}	Maximum concentration, determined directly from individual concentration-time data
T_{last}	Time of the last quantifiable concentration
T_{max}	Time of the maximum concentration
$T_{1/2}$	The observed terminal half-life, calculated as: $T_{1/2} = \frac{\ln(2)}{\lambda_z}$
Vz/F	Apparent volume of distribution in the terminal phase (TBI-223 only)
λ_z	The observed terminal rate constant; estimated by linear regression through at least 3 data points in the terminal phase of the log concentration-time profile

Pharmacokinetic parameters will be summarized by cohort using descriptive statistics. Dose proportionality will be assessed using the power model approach.

Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters (C_{max}, AUCs) under fed versus fasting conditions using an analysis of variance (ANOVA) approach.

Efficacy: No efficacy evaluations will be performed in this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	analysis of variance
AST	aspartate transaminase
AUC	area under the (plasma concentration vs. time) curve
AUC_{last}	area under the curve from time 0 hours to last quantifiable concentration
AUC_{inf}	area under the curve from time 0 hours to infinity
BID	twice daily
BMI	body mass index
BP	blood pressure
BUN	blood urea nitrogen
bpm	beats per minute
Ca	calcium
CFR	Code of Federal Regulations
CFU	colony-forming unit
CI	confidence interval
Cl^-	chloride
C_{last}	last quantifiable drug concentration
CLIA	Clinical Laboratory Improvement Amendments
cm	centimeter(s)
C_{max}	maximum concentration
CNS	central nervous system
CRF	case report form
CYP	cytochrome P450
DMID	Division of Microbiology and Infectious Disease
ECG	electrocardiogram
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
g	gram(s)
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
HIPAA	Health Insurance Portability and Accountability Act
GLP	Good Laboratory Practice
HIV	human immunodeficiency virus
ICD	informed consent document
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IND	Investigational New Drug
IP	Investigational Product
IRB	institutional review board
ISF	Investigator Site File
IUD	intrauterine device
K^+	potassium
kg	kilogram(s)
K_3-EDTA	ethylenediaminetetraacetic acid
L	liter(s)
lbs	pounds
LDH	lactate dehydrogenase
m	meter(s)
MAO	monoamine oxidase
max.	maximum
MDR-TB	multidrug resistant tuberculosis
mg	milligram(s)
MICs	minimum inhibitory concentrations
min.	minute(s)
mL	milliliter(s)
mmHg	millimeter of mercury
MPS	mitochondrial protein synthesis
msec	millisecond
Mtb	*Mycobacterium tuberculosis*
Na^+	sodium
NOAEL	no-observed-adverse-effect level
OTC	over-the-counter
oz	ounce(s)
PK	pharmacokinetic
PT	prothrombin time
rbc	red blood cell
SAD	Single Ascending Dose
SAE	serious adverse event
SAP	statistical analysis plan
$T_{½}$ or $t_{½}$	terminal elimination half-life
TB	Tuberculosis
T_{last}	time of the last measurable concentration
T_{max}	time to reach C_{max}
Worldwide	Worldwide Clinical Trials Early Phase Services, LLC Worldwide Clinical Trials Early Phase Services/Bioanalytical Sciences, LLC
λ_z	apparent elimination rate constant in terminal phase
°C	degrees Celsius/Centigrade

1 INTRODUCTION

1.1 Background Information

This study will be conducted in accordance with the protocol, International Good Clinical Practice (GCP) Guidelines, the ethical principles that have their origin in the Declaration of Helsinki and the applicable regulatory requirement(s).

A brief overview of available confidential information regarding TBI-223 follows below. Unless noted otherwise, the information in this introduction was provided by TB Alliance.

1.2 Tuberculosis and Rationale for New Medications

Tuberculosis (TB) in humans is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb), which typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB). While incidence rates and mortality for TB have been falling, it remains the ninth leading cause of death worldwide, the world's leading cause of death from a single infectious disease and is responsible for more deaths than human immunodeficiency virus (HIV). In 2016, 6.3 million new cases of TB were reported, with an overall estimated incidence of 10.4 million (World Health Organization [WHO] Global TB Report 2017).

The current TB treatment regimens and treatments for drug-sensitive TB are decades old and are relatively ineffective. The available treatments have a lengthy duration of treatment, can involve multi-drug therapy, many tolerability issues, and require large commitments of resources and infrastructure. High rates of noncompliance are common, which often results in increased mortality, chronic, infectious, and drug-resistant cases. The present TB epidemic and treatment conditions demonstrate the clear need in patients with drug-sensitive or drug-resistant TB for novel drugs and drug regimens that will shorten the current treatment duration and be safe and well tolerated. In addition, new TB drugs and regimens should also be affordable, easy to adopt and implement, suitable for pediatric use and for co-administration with antiretroviral therapy in individuals co-infected with Mtb and HIV. Following the declaration of TB as a global emergency by the WHO in 1993, there has been a resurgence of efforts to develop improved TB therapies and several promising new agents are presently in or approaching clinical evaluation.

TBI-223 like linezolid (Zyvox®), is a novel oxazolidinone antimicrobial that inhibits the growth of Mtb by blocking microbial translation and, thereby, protein synthesis. Linezolid, which is licensed for the treatment of complicated skin infections and hospital-acquired pneumonia (at an adult dose of 600 mg given twice daily [BID] for up to 28 days), has been used to treat difficult cases of multiple drug-resistant and extensively drug-resistant TB with apparent clinical benefit. Since bacterial protein

synthesis is not currently targeted by any of the drugs in the first-line standard of care for treatment of TB (isoniazid, rifampin, pyrazinamide, ethambutol), TBI-223, like linezolid, has no known pre-existing resistance. Therefore, TBI-223 should be effective against multi-drug resistant and extensively drug-resistant forms of TB (MDR-TB and XDR-TB) as well.

The standard dose of linezolid for a multitude of indications is 400 mg or 600 mg BID. Doses of linezolid varying from 300 mg to 1200 mg per day were used to treat pulmonary TB in combination with other TB drugs in novel regimes over periods of up to 20 months with impressive improved outcomes - e.g. in the Nix-TB trial (A Phase 3 Study Assessing the Safety and Efficacy of Bedaquiline Plus PA-824 (Pretomanid) Plus Linezolid in Subjects with Drug Resistant Pulmonary Tuberculosis: NCT02333799), but treatments can be impacted with periods of non-compliance and adverse events (AEs), especially neuropathic and myelosuppressive effects. These AEs are often ameliorated if the dose is reduced or if the drug is discontinued for several weeks and then resumed at a lower dose. Although manageable there is a clear need to develop effective and tolerable oxazolidinones with a better toxicology profile.

TBI-223 is a preclinical drug candidate from TB Alliance's efforts to develop a safer oxazolidinone with the potential to deliver efficacy similar to linezolid, without the characteristic bone marrow and neuropathic toxicities of the class. It emerged from a scaffold expansion effort based on the linezolid and sutezolid structures. Mammalian mitochondrial protein synthesis (MPS) inhibition is presumed to be the reason for the side effects associated with long-term administration of linezolid, such as anemia, thrombocytopenia, and peripheral neuropathy (Migliori et al., 2009). TBI-223 was tested in an MPS inhibition assay and found to have a half maximal inhibitory concentration (IC_{50}) of 126 μM (46 μg/mL). This concentration exceeds the expected maximum clinical TBI-223 concentration by 2-fold and suggests a lower risk of MPS inhibition. In preclinical studies, TBI-223 showed low minimum inhibitory concentrations (MICs) against Mtb in vitro and efficacy against mouse models of TB as a single agent and in combination with bedaquiline and pretomanid. TBI-223 did not show significant myelosuppression in 28-day Good Laboratory Practice (GLP) toxicity studies in rats and dogs.

Current data suggest that TBI-223 could contribute to a new TB treatment, consistent with the high-priority target product profile for a novel and universal TB drug regimen. A comprehensive nonclinical pharmacology, pharmacokinetic, toxicology, and safety program have been completed to support the Investigational New Drug Application and clinical development of TBI-223.The first clinical trial will be a randomized, Single Ascending Dose Study with a Food Effect Cohort. The objectives are to evaluate the safety, tolerability, and pharmacokinetics, and to explore the potential maximum tolerable dose of TBI-223 in healthy men and women.

1.3 Preclinical Studies and Toxicity Studies

To support Investigational New Drug (IND) filing, a nonclinical development plan has been executed based upon the ICH M3(R2) guidance document, "Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals," January 2010.

A series of in vitro and in vivo studies to evaluate the efficacy of TBI-223 were carried out. TBI-223 was evaluated against a large, diverse panel of Mtb clinical isolates, consisting of 96 isolates representative of all identified genetic groups of Mtb, broad geography and diverse drug-susceptibility profiles. The panel included MDR-TB and XDR-TB strains. The MIC of TBI-223 was found to be 1.28 μg/mL for 90% of the strains tested with a range of 0.04 to 5. Like linezolid, TBI-223 demonstrated a mild bactericidal activity against acute mouse infections of TB as a single agent, good activity against chronic mouse infections, including additive activity within the Nix-like drug combination of bedaquiline, an ATP synthase inhibitor, and pretomanid, a nitroimidazole. This combination efficacy demonstrated a strong potential for TBI-223 to replace linezolid in the Nix trial regimen and deliver a universal treatment shortening regimen that is not confined to the XDR-TB population.

A comprehensive battery of in vitro and in vivo safety pharmacology assessments was conducted to characterize the effects of TBI-223 on the function of the nervous, respiratory, and cardiovascular systems. The battery included non-GLP studies of several cardiac ion channels with TBI-223, a GLP human Ether-à-go-go-Related Gene (hERG) assay with TBI-223, a standalone GLP single-dose cardiovascular study. Nervous system and respiratory assessments were conducted in rats administered as single doses of TBI-223.

The pharmacokinetics and oral bioavailability of TBI-223 were evaluated in mice, rats, dogs and monkeys given single or repeated daily doses of TBI-223.

In support of planned human studies, the following toxicology studies have been completed with TBI-223:

- Non-GLP, 4-week, repeat-dose, oral toxicity study in mice.
- Non-GLP, 2- and 4-week, repeat-dose, oral toxicity studies in rats.
- GLP-compliant, 4-week, repeat-dose, oral toxicity study with recovery in rats.
- Non-GLP, 5-day and 2-week, repeat-dose, oral toxicity studies in beagle dogs.
- GLP-compliant, 4-week, repeat-dose, oral toxicity study with recovery in beagle dogs.
- Standard battery of GLP-compliant in vitro and in vivo genetic toxicology studies.

Dogs tolerated doses up to 100 mg/kg/day (50 mg/kg BID) for 28 days but showed dose- and duration-related nervous system effects at repeated doses ≥200 mg/kg/day. There were no correlating histopathology findings in the nervous system in these studies. It is unclear whether the nervous system effects are related to the maximum concentration (C_{max}) or the area under the (plasma concentration vs. time) curve (AUC) of TBI-223.

- In a 5-day study, dogs developed prolonged tremors and 2 of 4 dogs were found dead after being given 400 mg/kg/day for 3 days. These dogs had previously been administered 200 mg/kg/day of TBI-223 without clinical signs.
- During the first 4 days of dosing of a 14-day DRF study, tremors and emesis were observed in dogs administered 200 mg/kg/day, and ataxia, prostration, and convulsions were observed in dogs administered 400 mg/kg/day. These dogs were given a 2-day washout period and then dosed at 75 mg/kg/day BID or 150 mg/kg daily (QD). Reversible tremors were observed after 8 to 10 days of dosing at 150 mg/kg/day (QD or 75 mg/kg BID). In the pivotal 28-day dog GLP study, 2 dogs given 200 mg/kg/day (100 mg/kg BID) had continuous tremors after 12 days of dosing.

In electrocardiogram (ECG) evaluation during the pivotal 28-day dog toxicity study, QT prolongation was observed. The findings are detailed below and correlated with similar findings in the GLP cardiovascular safety study in dogs, and likely related to inhibition of the hERG channel observed in the GLP hERG study.

- In dogs administered 200 mg/kg/day (100 mg/kg BID) doses of TBI-223, QT prolongation was observed in the GLP repeat dose toxicology study. The mean QTc interval at 2 to 4 hours post the first daily dose on Day 25 of the dosing phase was longer in males by 23 msec (10%) and in females by 27 msec (11%) administered 200 mg/kg/day compared with the respective controls. Mean uncorrected QT interval was also prolonged (up to 42 msec; 22%). The QT and QTc intervals returned to baseline during the recovery phase.

In addition to the above adverse effects, rats had nonadverse abdominal distension reported in repeat dose studies with TBI-223, which was associated with food-filled stomach in rats even though they were fasted overnight prior to necropsy.

1.4 Adverse Effects, Warnings, and Precautions

TBI-223 has not been tested in humans, therefore its potential adverse effects are unknown. The following adverse reactions were noted in more than 1% of adult subjects in a study of different drug in the oxazolidinone class, linezolid (marketed as Zyvox®): headache, diarrhea, nausea, vomiting, dizziness, rash, anemia, taste alteration, vaginal and oral candidiasis, abnormal liver functions tests, fungal infection, tongue discoloration, and abdominal pain.[1]

The Zyvox® label carries warnings and precautions for myelosuppression, peripheral and optic neuropathy, serotonin syndrome, *Clostridium difficile* associated diarrhea, elevated blood pressure in patients with hypertension, lactic acidosis, convulsions, hypoglycemia, and development of drug-resistant bacteria. [1]

Given the key safety findings in the nonclinical safety studies for the cardiovascular system (prolonged QTc) and the nervous (CNS) system (tremors), we will implement the following monitoring procedures.

Cardiovascular Monitoring

Cardiovascular monitoring will be conducted and include the following at both protocol-defined time points and whenever deemed necessary by the study investigator. Subjects treated with TBI-223 will have their ECGs evaluated prior to treatment and during treatment. During treatment, the timing of ECGs will be guided by pharmacokinetic (PK) data so that collection occurs around the estimated T_{max}.

ECGs will be interpreted and signed and dated by the Principal Investigator or designee. The ECGs will be classified as normal, having a clinically insignificant abnormality, or having a clinically significant abnormality. All clinically significant abnormalities will be recorded as AEs. In addition, ECG parameters of ventricular rate, PQ or PR interval, QRS duration, and QT interval (corrected and uncorrected) will be noted on the case report form (CRF). If a patient's corrected QT interval (QTcF) exceeds 500 msec while on treatment or there is a change from baseline exceeding 60 msec, TBI-223 administration should be stopped, and the patient monitored with ECGs and/or telemetry until the QTcF returns to pre-treatment levels.
Study subjects will also be monitored with Holter monitoring. Holter monitoring will begin approximately 24 hours prior to and continue until 48 hours after dosing.

Central Nervous System Monitoring

A detailed neurological exam will be conducted and include the following at both protocol-defined time points and whenever deemed necessary by the study investigator:

- Mental status – assessment of orientation, speech, and memory
- Cranial nerves – assessment of cranial nerves II-XII, excluding fundoscopic examination
- Motor system – brief assessment of tone and strength
- Sensory system – brief survey for light touch and temperature of the face, neck, arms, trunk, and legs
- Reflexes – assessment of deep tendon reflexes and plantar responses (Babinski sign)

- Coordination – assessment of upper and lower extremities, including assessment for tremor
- Gait – assessment of tandem gait

1.5 Overview and Dose Rationale

A two-compartment PK model with non-linear clearance was built to project TBI-223 human PK parameters from animal PK parameters.

The projected human efficacious doses range from 800 to1200 mg, with a predicted C_{max} of 15.7-24.9 µg/mL and predicted ($AUC_{0\text{-}24hr}$) of 101 to 202 µg.hr/mL.

TBI-223, as a single agent at 100 mg/kg, demonstrated moderate activity against acute murine TB in BALB/c mice, and in a C3HeB/FeJ mouse model of TB. When combined with the TB drug bedaquiline and the investigational TB drug pretomanid, TBI-223 at 100 mg/kg demonstrated bactericidal and sterilizing activity against murine TB in BALB/c mice that was similar to that of linezolid at the same dose (100 mg/kg). The PK exposure at 100 mg/kg was 179 µg•hr/mL, with T>MIC at 61% (or 14.6 hr/day) in the female BALB/c mice.

A dose fractionation study of TBI-223 in a BALB/c mouse model of TB showed that the efficacy drivers were AUC ($R^2 = 0.92$) and T>MIC ($R^2 = 0.83$). The study indicated that a 2 Log lung colony-forming unit (CFU) reduction can be achieved with total a weekly AUC of 868 µg.hr/mL, or daily $AUC_{0\text{-}24hr}$ of 124 µg.hr/mL, with T>MIC of 34% (T>MIC at 8.2 hours over 24 hours).

Comparing to the projected human exposure at 1200 mg based on nonlinear two-compartment model, the human $AUC_{0\text{-}24hr}$ (205.2 µg.hr/mL) is 1.7-fold the mouse efficacious exposure (124 µg.hr/mL) for a 2 Log CFU reduction (Table 1). The $AUC_{0\text{-}24hr}$ at 202 µg.hr/mL could result in 2.8 Log CFU reduction. The predicted T>MIC at 1200 mg was 62.9% which is 1.8-fold the mouse efficacious T>MIC (34%) for 2 Log CFU reduction. At T>MIC of 62.9%, the calculated Log CFU reduction would be 3.4 based on the dose fraction study.

Comparing to linezolid clinical efficacious doses at 600 mg and 1200 mg daily dose, the projected TBI-223 exposure $AUC_{0\text{-}24hr}$ at 1200 mg is 2-fold that of linezolid exposure at 600 mg daily dose and 0.7-fold of linezolid exposure at 1200 mg daily dose.

Table 1 Comparison of Projected TBI-223 Efficacious Exposure and T>MIC at 1200 mg to the Efficacious Exposures in Mouse Efficacy Studies

	Dose or Log CFU reduction	$AUC_{0\text{-}24hr}$ (µg.hr/mL)	T>MIC (%)
TBI-223 Projected human efficacy dose	1200 mg QD	202	62.5
TBI-223 Mouse efficacy studies	100 mg/kg	179	61
TBI-223 Dose fraction study	2 Log CFU reduction	124	34
	AUC for 2.8 Log CFU reduction, or T>MIC for 3.7 Log CFU reduction	202	62.5
Linezolid efficacious dose	600 mg, human	104	91
	1200 mg, human	300	100

Starting dose for the SAD

A single dose of 50 mg appears to be an appropriate starting dose for the single ascending dose SAD study for the following reasons:

- The proposed 33-fold safety margin to the human equivalent dose (HED) derived from the no-observed-adverse-effect level (NOAEL, 100 mg/kg) in the 28-day dog toxicology study is based on twice daily dosing (50 mg/kg BID) compared to once daily dosing proposed in humans. The daily dosing frequency was changed from QD to BID in the animal studies to increase tolerability of TBI-223 when orally administered to dogs.
- The more relevant NOAEL at 50 mg/kg QD (HED 27 mg/kg) in the repeat-dose 28-day dog toxicology study provides a 17-fold safety margin to a first-in-human (FIH) starting dose of 100 mg QD. However, in the nonclinical safety pharmacology cardiovascular study in dogs, a trend of increasing QTc prolongation was observed in dogs after a single oral administration of TBI-223 at 50 and 100 mg/kg (max increase 11 to14 msec from baseline, 12 msec from control 2 hours post-dose) at 1 to 13 hours post-dose, and a statistically significant increase in QTc interval was detected at 200 mg/kg (mean increase 22 msec from baseline and control 2 to 5 hours post-dose, and 21 to 25 hours post-dose).
- Significant toxicity was observed in the 28-day dog toxicology study at a dose of 200 mg/kg/day (100 mg/kg BID) including intermittent and continuous tremors, ataxia, convulsions in several animals, QTc prolongation (10 to 11% baseline), decreased body weight, and vomitus. It should be noted that the dosing interval between twice daily dosing was not provided, and the clinical observations were

performed at 1 and 12 hours post-dose, and not 2 to 4 hours at C_{max}/T_{max} when tremors may have a greatest chance of occurring and being detected.

- In the 14-day dog toxicology study with orally administered TBI-223, dose reduction from 200 mg/kg daily to 150 mg/kg (75 mg/kg BID) on Day 7 continued to cause tremors on Days 8 to 10 of dosing. This may be a result of drug accumulation noted with repeated dosing with TBI-223. Cage-side observations more appropriately timed at 3 to 4 hours post-dose and 6 to 9 hours post-dose in this 14-day study may have provided a greater opportunity of detecting tremors in animals. Had the clinical observations been performed at similar times in the 28-day dog toxicology study, perhaps tremors might have been observed at 100 mg/kg/day (50 mg/kg BID), reportedly the NOAEL in this study. No tremors were observed at 75 mg/kg/day QD in dogs in the 14-day study.

In the first cohort of this Phase 1 study, the proposed starting dose of 50 mg once daily (QD) is 66-fold lower than the HED of 3243 mg/day, derived from the lowest NOAEL, which was 50 mg/kg BID (100 mg/kg/day) in a 28-day dog GLP toxicity study. The proposed starting dose is 17-fold less than the HED of a single 50 mg/kg dose in dogs.

The predicted C_{max} and $AUC_{0\text{-}24hr}$ at the proposed first in human dose of 50 mg once daily are 0.711 µg/mL and 2.5 µg.hr/mL, which are 20.4- and 86-fold less than the NOAEL in the dog toxicity study, respectively. The proposed starting doses for this first-in-human SAD study are well below the predicted NOAEL. The following dose escalations for each cohort receiving a single dose are proposed: 50 mg, 100 mg, 300 mg, 600 mg, 1200 mg, and 2000 mg. These proposed doses (cohorts) could change (e.g. if bioavailability is lower than expected or for potential emerging safety reasons).

For all the reasons noted above, a starting dose of 50 mg in the SAD would likely provide a reasonable margin of safety to any adverse -cardiovascular or CNS- effects observed with TBI-223 during administration to dogs.

Dose escalation will be guided by emerging safety and pharmacokinetic data so that predicted median C_{max} of a cohort can exceed 20.2 µg/mL (the NOAEL plasma C_{max} in the 28-day GLP dog toxicity study) only if, after data review, the prior dose was considered safe and well tolerated. Human plasma M2 metabolite exposure will be measured at each dose level, and the M2 exposure for next dose level will be predicted for safety analysis by comparing to M2 exposures in dogs. However, the planned doses could change if the generated bioavailability is lower than expected. Therefore, additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study other dose levels or a different dosage formulation. These decisions regarding planned additional cohorts will not take place until the Sponsor, in conjunction with the Principal Investigator and dose-escalating committee, has

determined that adequate safety, tolerability, and pharmacokinetics from the previous cohort have been demonstrated to permit proceeding to the next cohort. The Institutional Review Board (IRB) should be immediately notified of this revised approach for review and approval. The new approved dose for Cohort 6 will be 2000 mg.

2 OBJECTIVE

The primary objective of the study is:

- To evaluate the safety and tolerability of single doses of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations in healthy adult subjects.

The secondary objectives of the study are:

- To determine the pharmacokinetics of TBI-223 and its metabolite M2 after single doses of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations in healthy adult subjects; and
- To compare the rate and extent of absorption of a single dose of TBI-223 oral suspension, TBI-223 oral enteric capsules, and TBI-223 tablet formulations when administered in healthy adult subjects either after a high-calorie, high-fat meal or in the fasting state.

3 STUDY DESIGN SUMMARY

Part 1:

Up to 48 subjects in 6 dosing cohorts are planned to be enrolled; within each cohort, 6 subjects will be assigned to active treatment and 2 to placebo.

Each subject will participate in 1 dose level. Based on interim pharmacokinetic data obtained during the dose escalation, a dose cohort will be selected to return for additional dosing after a high-calorie, high-fat meal (food-effect cohort).

Each cohort will be dosed in 2 groups in order to monitor subjects for adverse experiences, in particular, convulsions (in the dog toxicity studies, tremors were observed at C_{max} or plasma concentration of ≥82µg/mL, and convulsions were observed at plasma concentration or C_{max} of ≥ 158µg/mL).

In the first cohort, a sentinel group of 3 subjects (2 active and 1 placebo) will be dosed at least 24 hours before the remaining 5 subjects (4 active and 1 placebo).

The remaining cohorts will be dosed in 2 groups of 4 subjects each (3 active and 1 placebo), at least 24 hours apart.

Safety will be assessed throughout the study for all subjects. Safety assessments will include physical and neurological examinations, vital signs, ECGs, cardiac monitoring, AEs, and clinical laboratory tests (including hematology, serum chemistry, coagulation, and urinalysis).

Blood and urine will be collected for clinical laboratory evaluations.

Female subjects will have blood collected for serum pregnancy testing. Postmenopausal females will have blood collected to measure follicle-stimulating hormone (FSH) levels.

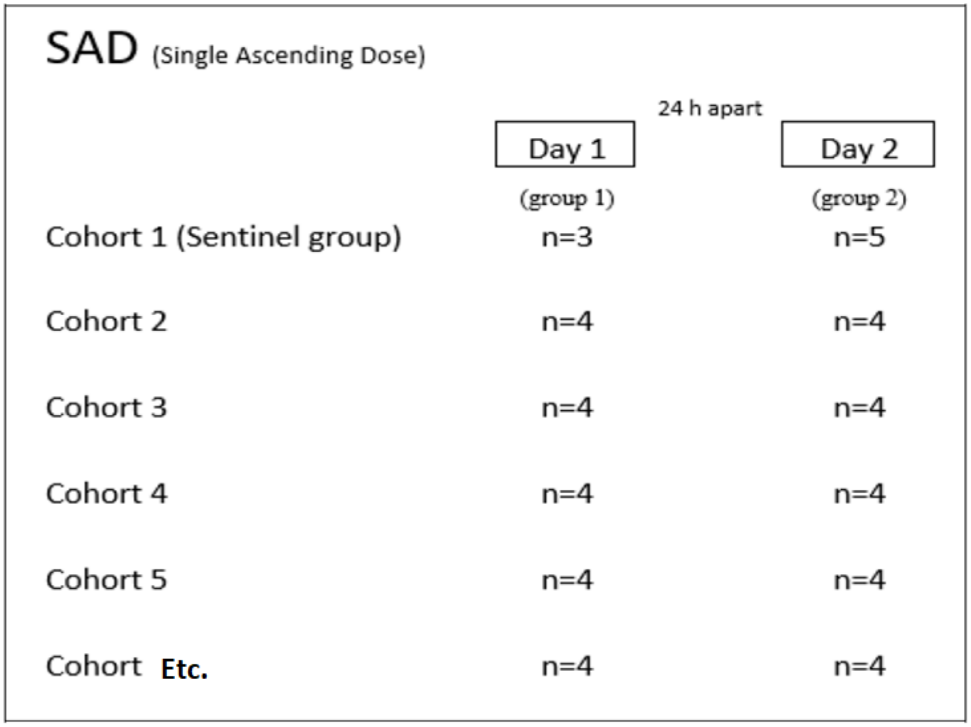
Blood will be collected for pharmacokinetic analysis.

Dose escalation to the next cohort (i.e., dose level) will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined that adequate safety, tolerability and pharmacokinetic data from the previous cohorts have been demonstrated to permit proceeding to the next cohort.

The Principal Investigator, in conjunction with the Sponsor may collect additional blood if necessary, for repeat laboratory or safety evaluations including AE follow up.

An overview of the dosing groups in Part 1 is presented in Figure 1.

Figure 1 Dosing Groups



Based on the interim pharmacokinetics for the dose escalation decisions, 10 subjects will be selected to participate in the **food-effect cohort**. These subjects will receive TBI-223 or placebo under fasting conditions in the first study period. They will return after a washout of at least 7 days or 5 half-lives (whichever is longer) of their fasting dose to receive the same dose under fed conditions.[3]

Additional cohorts (8 subjects per cohort) may be enrolled if it is deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or dosage formulation. The IRB will notified of this revised approach.

Note: In the event that a dose level is repeated to study a different dosage formulation, procedures and analyses will be conducted as outlined in Appendix 5.

Interim pharmacokinetic analyses will be performed for the dose escalation decisions, to select the dose for the food-effect cohort, and to reconsider the timing, reducing, or adding of sampling time points as the study progresses.

Blood samples will be obtained prior to study drug administration on Day 1 and at serial timepoints through 72 hours after study drug administration. Plasma pharmacokinetic samples will be analyzed for TBI-223 and M2 using validated analytical methods. Appropriate pharmacokinetic parameters will be calculated for each formulation using non-compartmental methods.

An unblinded pharmacist will be responsible for dispensing the study treatment and to ensure that study personnel are blinded.

Except for during the fed period of the food-effect cohort, TBI-223 or matching placebo dose will be administered orally with 200 mL of water after a minimum 10-hour overnight fasting, and food will be given approximately 4 hours after each dose level.

Table 2 presents the actual dose cohorts for Part 1 of the study.

Table 2 Actual Dose Cohorts for Part 1

Cohort[a] (Group[b])	Dose[c]	
1 (sentinel group)	Single dose of 50 mg TBI-223 (n=2)	Placebo (n=1)
1 (remainder of cohort)	Single dose of 50 mg TBI-223 (n=4)	Placebo (n=1)
2 (Group 1)	Single dose of 100 mg TBI-223 (n=3)	Placebo (n=1)
2 (Group 2)	Single dose of 100 mg TBI-223 (n=3)	Placebo (n=1)
3a (Group 1)	Single dose of 300 mg TBI-223 (n=3)	Placebo (n=1)
3a(Group 2)	Single dose of 300 mg TBI-223 (n=3)	Placebo (n=1)
3b (Capsule cohort)	Single dose of 300 mg (TBI-223 in oral enteric capsules) (n=4)	N/A
4 (Group 1)	Single dose of 600 mg TBI-223 (n=3)	Placebo (n=1)
4 (Group 2)	Single dose of 600 mg TBI-223 (n=3)	Placebo (n=1)
5 (Group 1)	Single dose of 1200 mg TBI-223 (n=3)	Placebo (n=1)
5 (Group 2)	Single dose of 1200 mg TBI-223 (n=3)	Placebo (n=1)
6 (Group 1)	Single dose of 2000 mg TBI-223 (n=3)	Placebo (n=1)
6 (Group 2)	Single dose of 2000 mg TBI-223 (n=3)	Placebo (n=1)
7 (Group 1)	Single dose of 2600 mg TBI-223 (n=3)	Placebo (n=1)
7 (Group 2)	Single dose of 2600 mg TBI-223 (n=3)	Placebo (n=1)

mg = milligram; n = number of subjects.

a) Additional cohorts may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level or another dosage formulation.
b) Dosing between Groups 1 and 2 to occur at least 24 hours apart.
c) Subjects will be dosed under fasted conditions. One of the dose cohorts will be selected to return for an additional dose under fed conditions.

Part 2:

Cohort 8: A minimum of 24 subjects in 4 dosing arms are planned to be enrolled; within each arm, 6 subjects will be assigned to receive a single dose treatment. Subjects will be assigned to one of the following arms and dose:

- Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 2 – TBI-223 SR Tablets (Prototype 2) -1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed conditions
- Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions

Procedures and analyses will be conducted as outlined in Appendix 6.

Cohort 9: The 6 subjects from Cohort 8, Arm 4, who received the IR tablet formulation fasted will return after a 7-day washout and receive a single dose of TBI-223 IR Tablets, 2000 mg dose (2 x 1000 mg tablets), under fed conditions.

If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).

Procedures and analyses will be conducted as outlined in Appendix 6.

3.1 Part 1 Dose Escalation

Each of the fasting cohorts will be dosed in 2 groups. The first cohort will start with a sentinel group. The decisions on continuing of dosing Group 2 (at least 24 hours after dosing of Group 1) will not take place until the Sponsor, in conjunction with the Principal Investigator, has determined adequate safety and tolerability.

Dose Escalation Review

A review period will take place after the dosing of all subjects in each cohort in order to evaluate and interpret all pharmacokinetic and safety data collected.

The review of all pertinent safety/tolerability data will include physical and neurological examinations, ECGs, vital signs, clinical laboratory tests, cardiac monitoring, and AEs or serious adverse events (SAEs) through Day 4. Also, data from the current dose cohort and all previous cohorts will be considered.

The Sponsor and Investigator will determine if there are sufficient data available from all subjects to make a dose escalation decision. Any AEs of grade ≥2 toxicities (per Division of Microbiology and Infectious Disease [DMID] toxicity table; Appendix 3) must be considered in the decision to escalate to the next dose level. ECG data will be analyzed in a blinded fashion and unblinded by ECG vendor in order to be presented by treatment group in aggregate (reviewers will not be unblinded by subject). Pharmacokinetic analysis will be completed only on the active treatment subjects and will be blinded to subject. Blinding will also extend to others who are intimately involved with the study except for the designated unblinded pharmacy staff and in cases of medical emergencies.

Upon review of relevant cohort data, the Sponsor, in conjunction with the Investigator may decide to:

1: Escalate the dose as planned

2: If concerns arise from pharmacokinetic and safety data that do not warrant ceasing escalation:

   a: evaluate an intermediate dose level prior to the next planned dose level;

OR

b: repeat a given dose level in a new cohort of subjects

c: Repeat a dose level using a different dosage formulation.

3. Add a cohort or increase the dose of the next cohort if the bioavailability is lower than expected in the previous cohort and there were no safety concerns.
4. Halt the study.

The next dose in the escalating phase of the SAD study will be guided by emerging safety and PK data. After each cohort, Bayesian linear models will be fitted to the data from all available cohorts with log-transformed C_{max} as the response variable and log-transformed dose as the predictor variable. Different prior distributions on the coefficient of log-transformed dose, representing different assumptions about dose proportionality, will be considered. Predictive distributions will be generated for C_{max} at doses to be considered for the next level of escalation. These predictive distributions, together with emerging safety data, will be assessed by the clinical team to reach a decision on the next dose. Key features of the predictive distributions to be evaluated are the median C_{max} and the number of subjects out of those receiving TBI-223 in the next cohort expected to have C_{max} exceed 20.2 μg/mL. (the NOAEL plasma C_{max} in the 28-day GLP dog toxicity study).

3.2 Stopping Rules for Cohorts

If any of the cohort stopping rules are met, cohort dosing must be stopped until review of the data by the Sponsor and the Investigator.

Rules for stopping dosing of a cohort include the following:

1. Two or more subjects experience drug-related SAEs;
2. Two or more subjects experience grade 2 or higher (per DMID toxicity table; Appendix 3) central nervous system (CNS) AEs considered related to drug.
3. Two or more subjects develop drug-related QTcF prolongation greater than or equal to 500 msec or a change from baseline greater than 60 msec, after completing repeat testing.
4. Two or more subjects experience drug-related Grade 3 or Grade 4 cardiac rhythm cardiac disturbances.

4 IDENTITY OF INVESTIGATIONAL PRODUCT

The Investigational Medicinal Product (IMP) will be supplied as TBI-223 25 mg/mL oral suspension or matching placebo.

Refer to Appendix 2 for details regarding the description and composition of TBI-223.

Table 3 Identity of Investigational Product

Part 1	
Test Product:	TBI-223 oral suspension 25 mg/mL
	Manufactured for Global Alliance for TB Drug Development
Control Product:	Placebo for TBI-223 oral suspension
	Manufactured for Global Alliance for TB Drug Development
Test Product: Cohort 3b	TBI-223 Oral Enteric Capsules, 150 mg
	Manufactured for Global Alliance for TB Drug Development
Part 2	
Test Products: Cohort 8 (Arms 1-4)	TBI-223 SR Tablets 600 mg (Prototype 1)
	Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited
	TBI-223 SR Tablets 600 mg (Prototype 2)
	Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited
	TBI-223 SR Tablets 900 mg (Prototype 3)
	Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited
	TBI-223 IR Tablets 1000 mg
	Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited
Test Products: Cohort 9	TBI-223 IR Tablets 1000 mg
	Manufactured for Global Alliance for TB Drug Development by Piramal Enterprise Limited

5 SUBJECT SELECTION

5.1 Inclusion Criteria

All volunteers must satisfy the following criteria to be considered for study participation:

1. Understands study procedures and voluntarily provides written informed consent prior to the start of any study-specific procedures.
2. Is a healthy adult male or a healthy adult female, 19 to 50 years of age (inclusive) at the time of screening.

3. Has a body mass index (BMI) ≥18.5 and ≤32.0 (kg/m^2) and a body weight of no less than 50.0 kg.
4. Is medically healthy with no clinically significant screening results, as determined by the Principal Investigator (e.g., laboratory profiles are normal up to and including Grade 1 per DMID toxicity tables; Appendix 3), medical history, vital signs, ECG, or physical/neurological examination findings. Note: If exclusionary lab criteria are met, values may be confirmed by repeat evaluation.
5. Has not used tobacco- or nicotine-containing products (including smoking cessation products), for a minimum of 6 months before dosing.
6. If female of non-childbearing potential, she has undergone one of the following sterilization procedures at least 6 months before dosing:
    - Hysteroscopic sterilization;
    - Bilateral tubal ligation or bilateral salpingectomy;
    - Hysterectomy; or
    - Bilateral oophorectomy;
    - Or is postmenopausal with amenorrhea for at least 1 year before the first dose with serum follicle-stimulating hormone (FSH) levels consistent with postmenopausal status (i.e., greater than 40 mIU/mL) at screening.

    Or, if female of childbearing potential, must agree to use an allowable form of birth control from screening until 14 days after study completion. The following are allowed birth control methods for this study:

    - Vasectomized partner (at least 6 months before dosing);
    - Non-surgical permanent sterilization (e.g., Essure® procedure) at least 3 months before dosing;
    - Double barrier method (e.g., diaphragm with spermicide; condoms with spermicide);
    - Intrauterine device (IUD);
    - Abstinence (and must agree to use a double barrier method if they become sexually active during the study);
    - Implanted or intrauterine hormonal contraceptives in use for at least 6 consecutive months before study dosing; and/or
    - Oral, patch, or injected contraceptives, or vaginal hormonal device (i.e. NuvaRing®), in use for at least 3 consecutive months before study dosing.

7. If a non-vasectomized male (or male vasectomized less than 120 days prior to study start) he must agree to the following during study participation and for 90 days after the last administration of study drug:
   - Use a condom with spermicide while engaging in sexual activity or be sexually abstinent
   - Not donate sperm during this time.

   In the event the sexual partner is surgically sterile or postmenopausal, use of a condom with spermicide is not necessary. None of the birth control restrictions listed above are required for vasectomized males whose procedure was performed more than 120 days before study start.
8. Is willing to answer inclusion and exclusion criteria questionnaire at check-in.
9. Is able to comply with the protocol and the assessments therein, including all restrictions.
10. Is willing and able to remain in the study unit for the entire duration of the assigned confinement period(s), return for outpatient visit(s), and receive a phone call for follow-up questioning about AEs.
11. If enrolled in the food-effect cohort, is willing and able to consume the entire high-calorie, high-fat breakfast meal in the timeframe required.

5.2 Exclusion Criteria

Volunteers will be excluded from study participation for any of the following:

1. History or presence of clinically significant cardiovascular, pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine, immunologic, dermatologic, neurological (including epilepsy), oncologic, or psychiatric disease or any other condition that, in the opinion of the Investigator, would jeopardize the safety of the subject or the validity of the study results.
2. Evidence on physical exam and targeted neurologic exam of specific findings such as resting or intention tremor, dysmetria, nystagmus or ataxia, or abnormal deep tendon reflexes (either zero or hyper-reflexia).
3. History of any illness that, in the opinion of the Investigator, might confound the results of the study or pose an additional risk to the subject by their participation in the study.
4. Surgery within the past 90 days prior to dosing as determined by the Investigator to be clinically relevant, or any history of cholecystectomy.
5. History or presence of alcoholism or drug abuse within the past 2 years as determined by the Investigator to be clinically relevant.

6. History of sensitivity or contraindication to use of linezolid, sulfa drugs, or any study investigational products.
7. Participation in another clinical trial within 30 days prior to dosing.
8. Female subjects who are pregnant or lactating.
9. Positive result on a urine drug/alcohol/cotinine screen at Baseline or check-in.
10. Seated blood pressure is less than 90/40 mmHg or greater than 140/90 mmHg at screening. Out-of-range vital signs may be repeated once for confirmation.
11. Seated heart rate is lower than 40 bpm or higher than 99 bpm at screening. Out-of-range vital signs may be repeated once for confirmation.
12. Any clinically significant ECG abnormality at Screening (as deemed by decision of the Investigator and the Sponsor's Medical Monitor).

    NOTE: The following may be considered not clinically significant without consulting the Sponsor's Medical Monitor:

    - Mild first degree A-V block (P-R interval <0.23 sec)
    - Right or left axis deviation
    - Incomplete right bundle branch block
    - Isolated left anterior fascicular block (left anterior hemiblock) in younger athletic subjects
    - Early repolarization
    - Tall T waves
    - RSR in V1/V2 consistent with right ventricular conduction delay (with acceptable QRS)
    - Sinus rhythm or sinus bradycardia with sinus arrhythmia
    - Minimal or moderate voltage criteria for left ventricular hypertrophy (LVH).
13. QTcF interval >450 msec for males or >470 msec for females at screening, Day -1, or Day 1 (pre-dose), or history of prolonged QT syndrome. For the triplicate ECGs taken at screening and on Day -1, the average QTcF interval of the 3 ECG recordings will be used to determine qualification.
14. Family history of long-QT syndrome or sudden death without a preceding diagnosis of a condition that could be causative of sudden death (such as known coronary artery disease, congestive heart failure, or terminal cancer).
15. History of 1 or any combination of, the following:

- Seizures or seizure disorders, other than childhood febrile seizures
- Brain surgery
- History of head injury in the last 5 years
- Any serious disorder of the CNS or related neurological system, particularly one that may lower the seizure threshold.

16. Lactose intolerant.
17. History or presence of allergic or adverse response to Listerine breath strips or aspartame.

Specific Treatments

18. Use of any prescription medication within 14 days prior to dosing.
19. Use of any of the following medications within 30 days before the first dose of study drug or during the study drug treatment period: monoamine oxidase (MAO) inhibitors (phenelzine, tranylcypromine), tricyclic antidepressants (amitriptyline, nortriptyline, protriptyline, doxepin, amoxapine, etc.), antipsychotics such as chlorpromazine and buspirone, serotonin re-uptake inhibitors (fluoxetine, paroxetine, sertraline, etc.), bupropion, agents known to prolong the QTc interval (erythromycin, clarithromycin, astemizole, type Ia [quinidine, procainamide, disopyramide] and III [amiodarone, sotalol] anti-arrhythmics, carbamazepine, sulfonylureas, and meperidine).
20. Use of any over-the-counter (OTC) medication, including herbal products and vitamins, within 7 days prior to dosing, except acetaminophen. Up to 3 grams per day of acetaminophen is allowed at the discretion of the Investigator prior to dosing.
21. Use of any drugs or substances known to be significant inhibitors of cytochrome P450 (CYP) enzymes and/or significant inhibitors or substrates of P-glycoprotein (P-gp) and/or organic anion transporting polypeptides (OATP) within 14 days prior to the first dose of study drug.
22. Use of any drugs or substances known to be inducers of CYP enzymes and/or Pgp, including St. John's Wort, within 30 days prior to the first dose of study drug.
23. Use of any drugs or substance known to lower the seizure threshold.

Laboratory Abnormalities

24. Serum magnesium, potassium, or calcium laboratory values outside of the normal range at screening. If exclusionary lab criteria are met, values may be confirmed by repeat evaluation.
25. Positive results at screening for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies (HCV).

5.3 Medication and Activity Restrictions

1. Subject must not donate blood from 56 days or plasma from 7 days prior to the first dose of study medication until after the follow-up phone call. It is recommended that blood/plasma donations not be made for at least 30 days after discharge from the clinic.
2. Subject must not use tobacco- or nicotine-containing products (including smoking cessation products) from 6 months prior to the first dose of study medication until after the follow-up phone call.
3. Subjects must not consume alcohol from 72 hours prior to the first dose of study medication until after discharge from the clinic.
4. Subject must not consume beverages or foods that contain grapefruit or Mandarin oranges from 10 days before the first dose of study medication, or poppy seeds, broccoli, Brussels sprouts, pomegranate, star fruit, char-grilled meat, or caffeine/xanthine from 48 hours before the first dose of study medication, until after discharge from the clinic. Subjects will be instructed not to consume any of the above products; however, allowance for an isolated single incidental consumption may be evaluated and approved by the study Investigator based on the potential for interaction with the study drug.
5. Subject should avoid large quantities of foods or beverages with high tyramine content 48 hours prior to the first dose of study medication until discharge from the clinic. Quantities of tyramine consumed should be less than 100 mg per meal. Foods high in tyramine content include those that may have undergone protein changes by aging, fermentation, pickling, or smoking to improve flavor, such as aged cheeses (0 to 15 mg tyramine per ounce); fermented or air-dried meats (0.1 to 8 mg tyramine per ounce); sauerkraut (8 mg tyramine per 8 ounces); and soy sauce (5 mg tyramine per 1 teaspoon). The tyramine content of any protein-rich food may be increased if stored for long periods or improperly refrigerated.
6. Subject must not engage in strenuous exercise from 48 hours prior to the first dose of study medication until after discharge from the clinic.

7. Subject must be willing to remove any artificial nails (e.g., acrylic, gel) or fingernail polish and not use such products for the duration of the study.
8. Subject must not take any adrenergic/serotonergic agonists, such as pseudoephedrine and phenylpropanolamine (frequently found in cold and cough remedies), within 7 days before the first dose of study drug or during the study drug treatment period.

5.4 Screening

The informed consent documents (ICDs) will be discussed with each potential participant, and each individual will sign an ICD for the study prior to any study-specific procedures being performed.

Each potential study participant will have the following assessments by the Investigator or designee within 28 days prior to study start:

- Demographic data, including sex, age, race, ethnicity, body weight (kg), height (cm), BMI (kg/m^2), and smoking habits.
- Medical history
- Serology tests for HIV, hepatitis B and C
- Clinical laboratory tests (hematology, coagulation, chemistry, and urinalysis)
- Serum pregnancy (all female subjects)
- FSH test (female subjects claiming post-menopausal status)
- Urine test for drugs of abuse, alcohol, and cotinine
- Physical and neurological examinations
- Triplicate 12-lead safety ECGs
- Vital signs (blood pressure, pulse rate, respiration rate, temperature, and pulse oximetry)
- Concomitant medication review

Only medically healthy subjects with clinically acceptable laboratory profiles and ECGs within the defined parameters who fulfill all other inclusion criteria and meet none of the exclusion criteria outlined in the previous sections will be enrolled in the study.

Subjects will be assigned numbers in an ascending order, based on successful completion of the screening process.

6 STUDY PROCEDURES

6.1 Subject Assignment

For Part 1, up to 50 subjects are planned to be enrolled in the study. Additional subjects may be enrolled if deemed appropriate by the Sponsor to repeat a dose level or to study another dose level. For Part 2, Cohort 8, 24 subjects will be enrolled. For Part 2,

Cohort 9, the 6 subjects from Cohort 8 who received the IR tablet formulation fasted will return after a 7-day washout and receive the IR tablet formulation under fed conditions. If more than 2 subjects of these 6 subjects from Cohort 8 drop-out, they will be replaced by heathy volunteers (a minimum of 4 subjects are needed to complete Cohort 9).

The number of subjects selected for the study was based on the adequate number considered to provide sufficient safety data. This study has not been formally powered.

For Part 1, each subject will receive an assigned treatment (active or placebo) based on the randomization schedule prepared by the unblinded statistician. The unblinded pharmacy staff will ensure compliance with the randomization schedule.

Note: the repeat cohort using a different dosage formulation in Part 1 and subjects in Part 2 will be non-randomized and unblinded; see Appendix 5 and Appendix 6.

6.2 Blinding

All subjects and clinical staff (except for the unblinded pharmacy staff) will be blinded to treatment. An unblinded pharmacy staff will be required at the Clinical Site to comply with the study's randomization and blinding requirements. At the Clinical Site, prior to study initiation, the Principal Investigator will be responsible for designating a qualified pharmacy staff to serve as the unblinded pharmacy staff in the study. Unblinded pharmacy staff may dose subjects but may not participate in any subject assessments.

Throughout the study, the designated unblinded pharmacy staff will be responsible for all drug accountability issues, including preparing, labeling, dispensing, and dosing study drug according to the randomization code provided, yet remain independent of all subject assessments. The pharmacy staff will follow the Standard Operating Procedures and Work Instructions related to pharmacy services and protocol-specific requirements.

Randomization codes will be provided to the unblinded pharmacy staff. Confirmation of receipt of the randomization code will be required by the Sponsor. The unblinded pharmacy staff will be responsible for maintaining the blind, consistent with protocol design, throughout the study. All documentation is to be filed in the Pharmacy Manual. Access to this manual by study personnel will be restricted to the unblinded pharmacy staff.

The subjects, Principal Investigator, and all other study personnel involved with subject assessments will remain blinded to the actual treatment assignments of the subjects. The Principal Investigator will be ultimately responsible for ensuring that the integrity of the blind is maintained throughout the study and will be required to notify the Sponsor in the event of any breaking of the blind for any reason.

Note: The blinding rules and procedures described in the protocol do not apply to the unblinded repeat cohort using a different dosage formulation; see Appendix 5.

6.2.1 Clinical Research Staff

All observers who evaluate any reported AE, laboratory abnormalities, ECGs, and changes in the ECGs will be blinded as to what treatment sequence each subject is assigned.

6.2.2 Study Subjects

All subjects will be blinded as to which treatment they are receiving at any dose.

6.2.3 Bioanalytical Laboratory

All samples will be sent to the bioanalytical laboratory for analysis. The bioanalytical laboratory will be unblinded and only run the analysis on active treatment subjects.

6.2.4 Pharmacokinetic Analysis

Pharmacokinetic analysis will be completed only on the active treatment subjects and will be blinded by subject for interim assessments.

6.2.5 Cardiac Vendor

Electrocardiogram data will be analyzed in a blinded fashion and then unblinded by the cardiac vendor in order to be presented by treatment group in aggregate (reviewers will remain blinded by subject).

6.2.6 Unblinding Procedures

Unblinding of cohort data will occur after completion of the final subject study visit and follow-up telephone call for the cohort.

The treatment assignment should be unblinded at the clinic only in the case of an emergency, when knowledge of the study drug assignment is absolutely necessary for the clinical management or welfare of the subject. Breaking of the blind at the clinic under any other circumstances will be considered a protocol violation.

The Investigator is strongly encouraged to contact the Sponsor before unblinding the study drug assignment prior to the scheduled assessment of tolerance and safety data. If the blind is broken for any reason, the Investigator must notify the Sponsor within 1 day, and an SAE form must be completed, if appropriate. In addition, the Investigator will record the date and reason for revealing the blinded study drug assignment for that subject in the source documents and appropriate CRF page(s).

6.3 Check-In Procedures

Subjects will check into the clinic on Day -2 for Part 1 and Day -1 for Part 2.

At check-in, all subjects will be evaluated to confirm they continue to meet all the inclusion criteria (Section 5.1) and none of the exclusion criteria (Section 5.2).

Subjects will undergo the following assessments during the confinement period prior to dosing:

- Physical and neurological examinations
- Weight
- Vital signs (temperature, respirations, blood pressure, pulse, and pulse oximetry)
- Triplicate 12-lead ECG
- Clinical laboratory tests
- Urine drug, alcohol, and cotinine screens – results must be negative for the subject to continue study participation
- Serum pregnancy test (all female subjects) – results must be negative for the subject to continue study participation
- Concomitant medication review
- AE review
- Cardiac telemetry (Part 1 only)

6.4 Confinement

Part 1 fasting cohorts, and Part 2, Cohort 8, Arm 4 only: Subjects will be admitted to the research center on Day -2 (Part 1) or Day -1 (Part 2) and remain confined until after completion of the 48-hour procedures on Day 3. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) and receive a follow up phone call on Day 11 (+1 day).

Part 1 food-effect cohort, Part 2, Cohort 8, Arms 1-3 and Cohort 9: Subjects will be admitted to the research center on Day -2 of Period 1 and Period 2 (Part 1) or Day -1 (Part 2) and remain confined until after completion of the 48-hour procedures on Day 3 of each study period. Subjects will return for a follow-up visit approximately 72 hours after dosing (Day 4) in each study period. Subjects will receive a follow up phone call on Day 11 (+1 day) of Period 2.

Please note that Cohort 9 is the same 6 subjects that were in Cohort 8, Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8, Arm 4, subjects fasted.

6.5 Fasting/Meals/Beverages

6.5.1 Fasting/Meals[3]

Part 1 fasted cohorts and Part 2, Cohort 8, Arm 4 only Optional meals (lunch, snack, and dinner) may be served the day of check-in. All subjects will then be required to fast for at least 10 hours before dosing. The subjects will fast for 4 hours after dosing. Standard meals will be provided at appropriate times after dosing.

Part 1 food-effect cohort, Part 2, Cohort 8, Arms 1-3 and Cohort 9: Optional meals (lunch, snack, and dinner) may be served the day of check-in.

In the fasting study period, all subjects will be required to fast overnight for at least 10 hours before dosing.

In the fed study period of Part 1 and Part 2 (Cohort 8, Arms 1-3 and Cohort 9) subjects will be required to fast for at least 10 hours overnight before consuming a required FDA standard high-fat, high-calorie breakfast. Subjects will receive the required FDA standard high-fat, high-calorie breakfast to begin 30 minutes prior to scheduled administration of the dose and to end (last bite taken) within 5 minutes prior to dosing. The subjects will fast for 4 hours thereafter. The following high-fat (approximately 50% of total caloric content of the meal), high-calorie (approximately 1000 calories) breakfast will be ingested 30 minutes prior to administration of the drug:

2 eggs fried in butter
2 strips of bacon
2 slices of toast with butter
4 ounces of hash brown potatoes
8 ounces of whole milk

This meal contains approximately 150 protein calories, 250 carbohydrate calories, and 500-600 fat calories. An equivalent meal may be substituted with documentation of the menu and caloric contents with Sponsor approval prior to administration of the meal.

Subjects will fast for 4 hours after dosing. Standard meals will be provided at appropriate times after dosing.

Please note that Part 2, Cohort 9 is the same 6 subjects that were in Cohort 8 Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8 Arm 4, subjects fasted.

6.5.2 Beverages

Each dose of TBI-223 oral suspension and placebo for TBI-223 oral suspension will be administered orally followed by approximately 200 mL of room temperature water.

Except for the room temperature water provided with the study treatment, no water may be consumed for 1 hour prior to each dose through 1 hour after each dose.

At other times, subjects will be encouraged to drink water ad libitum.

6.6 Drug Administration

6.6.1 Administration of oral care strips

Listerine® oral care strips (or similar product) will be used to blind subjects to treatment (TBI-223 Oral Suspension or placebo for TBI-223 oral suspension). One strip will be added to the subject's tongue immediately before administration of the study treatment. Another strip will be placed on the subject's tongue immediately after the subject has swallowed the study treatment and 200 mL of water. Research staff will place the strip on the midsection of the subject's tongue. The subject should keep the strip on the

tongue while it dissolves but may swallow when they feel the need to. A mouth check will be performed, using a flashlight and tongue depressor, after each administration of Listerine oral care strip to ensure that all of the film has been dissolved.

6.6.2 Administration of TBI-223 Oral Suspension or Placebo for TBI-223 Oral Suspension

Note: Detailed instructions for preparing and dispensing TBI-223 will be provided in a separate pharmacy manual. Any instructions in the pharmacy manual shall supersede those presented in this protocol.

TBI-223 Oral Suspension or placebo for TBI-223 oral suspension will be transferred and stored in Pyrex glass reagent bottles with a polypropylene (PP) screw cap without a liner. These bottles will be used for mixing, sampling, and storage prior to administration.

The required doses of the drug product will be dispensed using commercially available oral syringes of suitable capacity. The suspension will be dispensed just prior to dosing and not stored in the oral syringes.

Each dose of TBI-223 will be administered orally followed by approximately 200 mL of room temperature water. A mouth check will be performed immediately after dose to ensure that the medication has been appropriately swallowed.

Subjects will remain seated, except as otherwise required for study procedures or personal needs, for the first 4 hours after dosing. Should the need to move about occur during the first 4 hours after dosing on these days, subjects may be escorted to such procedures or activities by research personnel as deemed medically necessary. Subjects will not be allowed to lie down, except as directed by clinical staff secondary to AEs, for the first 4 hours after dosing.

6.6.3 Administration of TBI-223 Sustained Release and Immediate Release Tablets

For Part 2, Cohort 8 and Cohort 9, each subject will receive the oral dose of the assigned study treatment (TBI-223 SR tablet formulations [Prototypes 1, 2, or 3] or TBI-223 IR tablet formulation) with approximately 240 mL (8 fl. oz.) of room temperature water. Subjects must swallow the study medication intact. The medication should NOT be crushed or chewed. A hand and mouth check will be performed immediately after dose to ensure that the medication has been appropriately swallowed.

The subjects will remain seated, except as otherwise required for study procedures or personal needs, for the first 4 hours after dosing. Should the need to move about occur during the first 4 hours after each dose, subjects may be escorted to such procedures or activities by research personnel as deemed medically necessary. Subjects will not be allowed to lie down, except as directed by clinical staff secondary to AEs, for the first 4 hours after dosing.

6.7 Blood Sampling, Processing and Shipment

Blood samples will be collected as detailed in Appendix 1.

Refer to the Schedule of Events for specific blood collection time points. The Principal Investigator, in conjunction with the Sponsor, may collect additional blood if necessary for repeat laboratory or safety evaluations including AE follow-up.

6.7.1 Part 1 fasting cohorts, and Part 2, Cohort 8, Arm 4 only

A total of 80 mL (20 x 4 mL samples) will be collected for pharmacokinetic analysis from each subject. Approximately 51.5 mL of blood will be collected for the clinical laboratory evaluations and coagulation testing. The total volume of blood collected from each subject will not exceed approximately 131.5 mL.

Table 4 Total Volume of Blood to be Collected for Testing: Fasting Cohorts

Reason for Collection	Number of Samples	Volume per Sample (mL)	Total Volume (mL)
Clinical labs at screening	1	12.5	12.5
Clinical labs during study (check-in, 24 and 72 hours/end-of-study)	3	8.5	25.5
Coagulation	5	2.7	13.5
Pharmacokinetic analysis	20	4.0	80.0
		Total	131.5

6.7.2 Part 1 food-effect cohort, Part 2, Cohort 8, Arms 1-3 only, and Cohort 9

A total of 160 mL (40 x 4 mL samples) will be collected for pharmacokinetic analysis from each subject. Approximately 90.5 mL of blood will be collected for the clinical laboratory evaluations and coagulation testing. The total volume of blood collected from each subject will not exceed approximately 250.5 mL.

Please note that Part 2, Cohort 9 is the same 6 subjects that were in Cohort 8 Arm 4 (IR tablet formulation) under fed conditions. In Cohort 8 Arm 4, subjects fasted.

Table 5 Total Volume of Blood to be Collected for Testing: Food-effect Cohorts

Reason for Collection	Number of Samples	Volume per Sample (mL)	Total Volume (mL)
Clinical labs at screening	1	12.5	12.5
Clinical labs during study (check-in, 24 and 72 hours/end-of-study)	6	8.5	51.0
Coagulation	10	2.7	27.0
Pharmacokinetic analysis	40 (20 in each study period)	4.0	160.0
		Total	250.5

6.7.3 Pharmacokinetic Sampling Time Windows

Blood samples collected within the time windows listed below will not be considered deviations. Note: For the food-effect cohort, time windows are relative to dosing in each study period.

Table 6 Acceptable Pharmacokinetic Sampling Time Windows

Investigation and examination	Allowable Time Window		
	Postdose*		
	≤24 hours	>24 hours to ≤48 hours	>48 hours
Plasma sample collection for pharmacokinetic assessment	± 2 minutes	± 5 minutes	± 10 minutes

For the food-effect cohort, time windows are relative to dosing in each study period.

6.8 End-of-Study Procedures

On the day of study discharge (Day 4), the following procedures will be conducted:

- Physical and neurological examinations
- Vital signs measurements (blood pressure, pulse, temperature, respirations, and pulse oximetry)
- Blood collection for hematology, chemistry, coagulation
- Urine collection for urinalysis
- Concomitant medication review
- AE assessment

Subjects will receive a follow up phone call (Day 11 [+1 day].

For the food-effect cohort, the timing of these assessments is relative to study period 2.

6.9 Safety Monitoring and Procedures

6.9.1 Adverse Events

Subjects will be instructed to inform the study physician and/or research personnel of any AEs that occur at any time during the study. Subjects will be monitored for AEs from the first dose through the end-of-study visit.

Refer to Section 7 for details regarding AE reporting.

6.9.2 Clinical Laboratory Evaluations

Clinical laboratory evaluations will be performed at the times noted on the appropriate events schedule (Table 9), A Clinical Laboratory Improvement Amendments (CLIA) certified laboratory will perform all clinical laboratory tests for this study. If exclusionary lab criteria are met, values may be confirmed by repeat evaluation.

- Hematology: hemoglobin, hematocrit, total and differential leukocyte count, red blood cell count (RBC), and platelet count.
- Serum Chemistry: albumin, blood urea nitrogen (BUN), creatinine, total bilirubin, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), sodium (Na+), potassium (K+), chloride (Cl-), lactate dehydrogenase (LDH), calcium (Ca), uric acid, glucose, gamma-glutamyltransferase (GGT), and magnesium.
- Serology: hepatitis B surface antigen, hepatitis C antibody, and HIV.
- Coagulation: prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR).
- Urinalysis - The following will be evaluated by an automated or manual urine "dipstick" method: pH, specific gravity, protein, glucose, ketones, bilirubin, blood, nitrite, leukocyte esterase, and urobilinogen. If protein, occult blood, nitrite, or leukocyte esterase values are out of range, a microscopic examination will be performed.
- Urine Drug, Cotinine, and Alcohol Screens: drugs of abuse (amphetamines, benzodiazepines, barbiturates, cannabinoids, cocaine, opiates), alcohol, and cotinine.
- Pregnancy test (all female subjects).
- Follicle-stimulating hormone (female subjects claiming post-menopausal status).

6.9.3 Vital Signs

Vital signs (blood pressure, pulse rate, and respiration rate, pulse oximetry, and temperature) will be measured performed at the times noted on the appropriate events schedule (Table 9).

NOTE: Blood pressure and pulse rate will be noted and collected at the same time.

For purposes of qualifying any given subject for study participation, out-of-range vital signs may be repeated once.

Predose vital signs will be assessed by the Principal Investigator or designee (e.g., a medically qualified Sub-Investigator) prior to each study drug administration. The Principal Investigator or designee will verify the eligibility of each subject with out-of-range vital signs and document approval prior to dosing.

Additional vital signs measurements may be performed as deemed medically necessary by research personnel.

Blood pressure and heart rate should be measured after subjects are in a seated position for at least 2 minutes and then again after standing for at least 1 minute, except when they are supine or semi-reclined because of study procedures and/or AEs, or as deemed necessary by the Investigator.

6.9.4 Physical Examinations

Physical examinations, including height and weight measurements, will be conducted at the times noted on the appropriate events schedule (Table 9).

6.9.5 Neurological Examinations

Detailed neurological examinations will be conducted at the times noted on the appropriate events schedule (Table 9) and at unscheduled time points as deemed necessary by the Investigator. Each neurological exam will assess the following:

- Mental Status – orientation, speech, and memory
- Cranial Nerves – excluding fundoscopic examination
- Muscle tone and strength
- Sensory System – brief survey for light touch and temperature of the face, neck, arms, trunk, and legs
- Reflexes –deep tendon reflexes and plantar responses (Babinski sign)
- Coordination – upper and lower extremities, including tremor
- Gait – tandem gait

6.9.6 12-Lead Electrocardiograms

Safety 12-lead ECGs will be recorded and printed for on-site review by the Principal Investigator or designee at the times noted on the appropriate events schedule (Table 9). The interpretation of these ECGs will be noted, but not the ECG intervals, unless as part of an AE (e.g. 1st degree AV block with PR interval of 240 msec).

All safety ECGs will be performed after the subject has been in supine position for a minimum of 5 minutes.

6.9.7 Cardiac Holter Monitoring, and Cardiodynamic Assessment

In order to detect potential changes in ECG parameters, study subjects will undergo cardiac Holter monitoring. Holter monitoring will begin approximately 24 hours before dosing and continue for at least 48 hours after dosing.

On Day -2 and Day -1, subjects should fast until lunch to avoid circadian fluctuations.

In addition, replicate ECGs will be obtained after dosing, specifically around the estimated T_{max} (which is estimated to be around 3 hours after dosing; although the timing may be altered based on generated phase 1 pharmacokinetic data). ECG data will be analyzed focusing in particular on changes of the QT segment although other ECG parameters will be analyzed, as specified in the Statistical Analysis Plan (SAP).

Continuous 12-lead ECGs (Holter) will be recorded for 24 hours prior to dose and for at least 48 hours post dose. The ECG waveforms will be stored for analysis as appropriate after the completion of the study. Replicate 12-lead ECGs will be extracted prior to dosing and serially after dosing by the central ECG laboratory at the time points shown in Table 9. At each time point for ECG extraction, subjects should be resting in the supine position for at least 10 minutes.

NOTE: When ECG extractions coincide with safety ECGs, vital signs assessment and blood draws, procedures should be carried out in said order.

Details regarding the equipment and methods to be used for continuous Holter extraction and analysis are provided in Appendix 4.

Part 2 of the study will not have cardiac Holter monitoring.

6.9.8 Other Safety Measures

Medical emergency personnel trained in advanced cardiac life support will be on site to monitor subjects during the confinement period in the research center. Emergency medical equipment including but not limited to intubation equipment and pulse oximetry shall be maintained on site to administer appropriate medical care should it be required.

Procedures will be completed as specified in this protocol unless contraindicated due to a reported AE.

7 ADVERSE EVENTS

The Investigator or a suitably medically qualified designee are responsible for eliciting adverse events by observing and questioning the subject and recording all adverse events observed by him/her or reported by the subject during the trial.

7.1 Definitions

7.1.1 Adverse Event

Any untoward medical occurrence in a clinical investigation participant, temporally associated with the use of a trial treatment whether or not considered related to trial treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a trial treatment, whether or not related to the trial treatment.

7.1.2 Serious Adverse Event

Any untoward medical occurrence that at any dose:

- Results in death;
- Is life threatening (any event in which the participant was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe);
- Requires inpatient hospitalization or prolongation of existing hospitalization; In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE;
- Results in persistent or significant disability/incapacity; the term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza,

and accidental trauma (e.g., sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption;

- Is a congenital anomaly/birth defect; or
- Is a medically important event.

Note: Medical and scientific judgment should be exercised in deciding which is a medically important event that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the participant or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse. A "suspected transmission of infectious agent by a medicinal product" is also considered a serious adverse event under the SAE criterion "Other medically important condition".

7.2 Attribution/Causality

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product).

- The investigator is obligated to assess the relationship between trial treatment and each occurrence of each AE/SAE.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to trial treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to the Sponsor/designee. However, it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor/designee.
- The investigator may change his/her opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.

Table 7 Adverse Event Attribution/Causality Ratings

Relatedness Rating	Definition
Not Related	An adverse event, which is not related to the use of the drug.
Unlikely	An adverse event for which an alternative explanation is more likely, e.g., concomitant drug(s) or concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.
Possible	An adverse event, which might be due to the use of the drug. An alternative explanation, e.g., concomitant drug(s) or concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
Probable	An adverse event, which might be due to the use of the drug. The relationship in time is suggestive, e.g., confirmed by dechallenge. An alternative explanation is less likely, e.g., concomitant drug(s) or concomitant disease(s).
Certain	An adverse event, which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation, e.g., concomitant drug(s) or concomitant disease(s).

Table 8 Definitions for Adverse Event Severity Gradings

Grade	Severity Rating	Definition
GRADE 1	Mild	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required.
GRADE 2	Moderate	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required.
GRADE 3	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.
GRADE 4	Potentially Life-Threatening	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

See Appendix 3 for full DMID Toxicity Tables. The above ratings should be used to estimate the grade for abnormalities NOT found elsewhere in the Toxicity Tables. Lab results within the testing facility's normal range will not be considered AEs when referenced to the DMID assessment/grading scale (Appendix 3).

Laboratory abnormalities and cardiovascular findings of hypertension or hypotension Grade 2 or above on the DMID toxicity tables will be considered AEs.

7.3 Reporting

7.3.1 Adverse Event

Adverse Events will be collected by the Investigator or qualified designee(s) from the time a subject signs the Informed Consent Form through the follow-up phone calls (Day 11+1 day). Any AE (serious or non-serious) observed by the Investigator (or a suitably medically qualified designee) or reported by the subject will be recorded on the Adverse Event Case Report Form. The Investigator will review each AE and assess its relationship to drug treatment based on all available information at the time of the completion of the CRF. The following information will be recorded for each Adverse Event reported:

- Diagnosis of the AE, if possible. In the case where an overall diagnosis cannot be made, each specific sign and/or symptom will be recorded as individual AEs;
- Date of onset;
- Stop date (with duration, if applicable);
- Severity;
- Action taken with IMP;
- Other action taken;
- Outcome;
- Relationship to IMP;
- Occurrence;
- Seriousness

7.4 Serious Adverse Event Reporting

The Investigator or designee will notify the appropriate Sponsor contact immediately after the SAE detection, observation, or report of occurrence (regardless of the relationship to test article). The Sponsor contact information for SAE reporting is provided below:

PPD PVG (pharmacovigilance service provider for TB Alliance)
Email: rtpsafety@ppdi.com
Safety Hotline: +1 888 483 7729
Safety Fax Number +1 888 529 3580

And

Paul Bruinenberg MD, MBA
Global Alliance for TB Drug Development
40 Wall Street, 24th Floor
New York, NY 10005, United States of America
Telephone: +1 646.616.8629

Mobile: +1 201.312.0988
Facsimile: +1 212.227.7541
Email: Paul.Bruinenberg@tballiance.org

These SAE reports must contain the following information:

A. Study name/number (for EU also the EudraCT number)
B. Study drug
C. Investigator details (name, phone, fax, e-mail)
D. Subject number
E. Subject demographics
F. Clinical event:
    1) Description
    2) Date of onset
    3) Treatment (drug, dose, dosage form)
    4) Adverse event relationship to study drug
    5) Action taken regarding study drug in direct relationship to the AE
G. If the AE was fatal or life-threatening
H. If applicable, cause of death (whether or not the death was related to study drug)
I. If applicable, autopsy findings (if available)

Any new SAE that occurs within one month after the study period and is considered to be possibly related to the Investigational Product (IP) should be recorded and reported immediately to the Sponsor.

The person responsible for the study shall take care that the study has been carried out in accordance with pharmacovigilance local regulations.

All serious event reporting will adhere to U.S. Code of Federal Regulations (21 CFR Part 312.32) for IND drugs and 21 CFR 314.80 for marketed drugs (15-day alerts). The Institutional Review Board (IRB) will be notified of the alert reports per Food and Drug Administration (FDA) regulations.

All AEs, including SAEs, will be followed to resolution when possible. All AEs and treatment administered will be recorded on the case report form (CRF).

The Sponsor will be responsible for processing and reporting any SAEs (and their relevant updates) to the FDA or other applicable regulatory agency.

7.5 Follow up of Adverse Events

All AEs will be followed until:

- Satisfactory clinical resolution or stabilization; or
- End of the follow-up period; and
- All queries on these AEs have been resolved.

Certain long-term AEs cannot be followed until resolution within the setting of this protocol. In these cases, follow-up will be the responsibility of the treating physician. However, this will have to be agreed upon with the Sponsor Medical Monitor.

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Sponsor or representative to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. Investigator should contact Sponsor/designee to discuss appropriate medical follow-up if consultation required.

If a participant dies during participation in the trial or during a recognized follow-up period, the investigator will provide Sponsor/representative with a copy of any post-mortem findings including histopathology.

New or updated information on an SAE will be recorded in the originally completed CRF and submitted to Sponsor within 24 hours of the information becoming known per SAE reporting guidelines.

7.6 Post-Trial Serious Adverse Events

Any new SAEs reported by the subject to the Investigator that occur up to 30 days after last contact and are determined by the Principal Investigator to be possible, probable or very likely related to the use of the IMP, will be reported to the Sponsor, IRB and FDA on an expedited basis as required in accordance with local requirements and ICH guidelines for GCP.

7.7 Clinical Laboratory Adverse Events

Changes in the results of the Clinical Laboratory assessment results which the Investigator feels are clinically significant will be reported as AEs. It is the Investigators' responsibility to review the results of all laboratory tests as they become available. This review must be documented by the Investigators' dated signature on the laboratory report. For each abnormal laboratory test result, the Investigator needs to ascertain and document if this is a clinically significant change from baseline for that individual participant. This determination, however, does not necessarily need to be made the first time an abnormal value is observed. The Investigator may repeat the laboratory test or request additional tests to verify the results of the original laboratory

tests. If this laboratory value is determined by the Investigator to be a clinically significant change from baseline for that participant, it is considered to be an AE.

7.8 Drug Interaction

If the Investigator becomes aware that the subject has experienced a drug interaction which has resulted in an adverse event, it will be recorded as an AE.

7.9 Pregnancy

The Investigator will immediately notify the Sponsor of any pregnancy that is discovered during the clinical trial. Pregnancy forms will be completed for all pregnancies reported during the study or in the 30 days after completion of the IMP. In addition, the Investigator will report to the Sponsor follow up information regarding the outcome of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for 6 months.

If pregnancy is suspected while the subject is receiving IMP, the IMP will be withheld immediately until the result of the pregnancy test is known. If pregnancy is confirmed, the IMP will be permanently discontinued in an appropriate manner and the subject withdrawn from the trial. Protocol-required procedures for trial discontinuation and follow up will be performed unless contraindicated by the pregnancy.

If the Investigator becomes aware the female partner of a male subject becomes pregnant during the study or in the 30 days after the completion of IMP, consent will be requested from the female partner for collection of information on her pregnancy history and for information on the current pregnancy and birth.

7.10 Monitoring and Safety for Specific Toxicities

During preclinical testing, issues with increased heart rate and lowering of blood pressure were detected. Therefore, heart rate and blood pressure changes should be monitored carefully. When abnormalities are detected both blood pressure and pulse rate should be captured simultaneously

8 GENERAL CONSIDERATIONS

8.1 Basic Principles

This research will be carried out in accordance with the protocol, the International Council for Harmonisation (ICH), Guideline for Good Clinical Practice: Consolidated Guidance (E6), and applicable regulatory requirements(s) including clinical research guidelines established by the Basic Principles defined in the U.S. 21 CFR Parts 50, 56, and 312 and the principles enunciated in the Declaration of Helsinki (revised version Fortaleza 2013).

8.2 Institutional Review Board

This protocol will be reviewed by an appropriate IRB and study enrollment will not commence until the Board has approved the protocol or a modification thereof. The Board is constituted and operates in accordance with the principles and requirements described in 21 CFR Part 56.

8.3 Informed Consent

Written informed consent will be obtained from each subject prior to performing any baseline study-specific evaluations. The ICD is prepared by the Investigator or designee, subject to review and approval by the Sponsor, and forwarded to a qualified IRB for final review and approval. The IRB-approved document must contain, at minimum, the eight basic elements of informed consent. Only the most recently IRB-approved ICD must be used to consent prospective study subjects. One copy of the signed and dated ICD will be given to the subject and the original retained by the Investigator/site.

8.4 Indications for Subject Withdrawal

Subjects will be free to withdraw at any time for any reason, or they may be withdrawn if necessary, to protect their health and safety or the integrity of the study data.

The final report will include reasons for withdrawals. In the event of an early termination, subjects will undergo the procedures described in Section 6.8.

Subjects who experience emesis may be replaced if the emesis could potentially impact drug absorption and therefore the pharmacokinetic data. Cases of emesis will be evaluated by the Sponsor and Principal Investigator to determine if subject replacement is needed.

For Part 1, replacement subjects will be numbered 17XX where XX is 10 plus the original subject's last 2 assigned numbers (e.g. if subject 1702 needs to be replaced due to emesis, the replacement subject number would be 1712). Replacement subjects will receive the same dose allocation of study drug (active or placebo) and they will all be blinded aside from the pharmacist assigning the drug.

8.5 Termination of the Study

The Principal Investigator reserves the right to terminate the study in the interest of subject safety and welfare. The Sponsor reserves the right to terminate the study at any time for administrative reasons.

8.6 Documentation

All documents pertaining to the study, including a copy of the approved protocol, copy of the ICD and Health Insurance Portability and Accountability Act (HIPAA) documents, completed CRFs (where applicable), drug accountability and retention records, and other study related documents will be retained in the permanent archives of the study site. These will be available for inspection at any time by the Sponsor or the FDA. Per 21 CFR 312, record retention for this study is required for a period of two years following the date on which this study agent is approved by the FDA for the marketing purposes that were the subject of this investigation; or, if no application is to be filed or if the application is not approved for such indication, until two years following the date on which the entire study is completed, terminated, or discontinued, and the FDA is notified.

Subject records will be kept private except when ordered by law. The following individuals will have access to study subject records: Principal Investigator and designees, study Sponsor, monitors, and auditors, the FDA, other government offices, and the IRB.

8.7 Trial Monitoring

Sponsor personnel (or designees) will be responsible for monitoring the study to ensure compliance with the protocol and GCP. Compliance may be verified by one or more of the following methods: on-site visits, frequent communication with the Investigator, and/or review of CRFs and source documents. The Investigator agrees to permit such monitoring as well as audits or reviews by regulatory authorities and the IRB.

8.8 Reimbursement, Indemnity, and Insurance

Reimbursement, indemnity, and insurance shall be addressed in a separate agreement on terms agreed upon by the parties.

9 PHARMACOKINETIC ANALYSIS

9.1 Analytical Methodology

Plasma samples will be analyzed for TBI-223 and M2 using validated assays. Plasma samples from subjects who receive placebo for TBI-223 oral suspension will not be analyzed.

9.2 Pharmacokinetic Analysis

Final pharmacokinetic calculations will be performed using appropriate software, e.g. Phoenix™ WinNonlin® (Version 8.1 or higher, Certara, L.P. in conjunction with the internet-accessible implementation of Pharsight® Knowledgebase Server™ [PKSO;

Version 4.0.4 or higher, Certara, L.P.]) and/or SAS® (Version 9.4 or higher, SAS Institute Inc.).

Pharmacokinetic parameters will be calculated using non-compartmental analysis. The following pharmacokinetic parameters will be determined as appropriate as detailed below.

AUC_{Extrap} (%)	The percentage of AUC_{inf} based on extrapolation
AUC_{inf}	Area under the concentration-time curve from time-zero extrapolated to infinity; calculated as: $AUC_{\inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$
AUC_{last}	Area under the concentration-time curve from time-zero to the time of the last quantifiable concentration; calculated using the linear trapezoidal rule
C_{last}	The last quantifiable concentration determined directly from individual concentration-time data
CL/F	Apparent total clearance after single administration (TBI-223 only)
C_{max}	Maximum concentration, determined directly from individual concentration-time data
T_{last}	Time of the last quantifiable concentration
T_{max}	Time of the maximum concentration
$T_{1/2}$	The observed terminal half-life, calculated as: $T_{1/2} = \frac{\ln(2)}{\lambda_z}$
Vz/F	Apparent volume of distribution in the terminal phase (TBI-223 only)
λ_z	The observed terminal rate constant; estimated by linear regression through at least 3 data points in the terminal phase of the log concentration-time profile

Additional pharmacokinetic parameters may be calculated if deemed appropriate.

Pharmacokinetic parameters will be summarized by cohort using descriptive statistics. Dose proportionality will be assessed using the power model approach.

Food Effect: The effect of food will be assessed comparing pharmacokinetic parameters (C_{max}, AUCs) under fed versus fasting conditions using an analysis of variance (ANOVA) approach.

9.3 Statistical Analysis

Final statistical analyses will be performed using appropriate software, e.g. Phoenix™ WinNonlin® (Version 8.1 or higher, Certara, L.P. in conjunction with the internet-accessible implementation of Pharsight® Knowledgebase Server™ [PKSO; Version 4.0.4 or higher, Certara, L.P.]) and SAS® (Version 9.4 or higher, SAS Institute Inc.).

Pharmacokinetic parameters will be summarized by cohort using descriptive statistics. Summary statistics will also be presented by gender within each cohort. Dose proportionality for exposure parameters (C_{max}, AUCs) will be assessed using a power model approach.

Food-effect cohort: The effect of food will be assessed comparing pharmacokinetic parameters (C_{max}, AUCs) under fed versus fasting conditions using an analysis of variance (ANOVA) approach.[3]

10 FACILITIES

CLINICAL TRIAL SITE

Worldwide Clinical Trials Early Phase Services, LLC
2455 N.E. Loop 410, Suite 150
San Antonio, Texas 78217
Telephone: 210.635.1500
Fax: 210.635.1646

CLINICAL LABORATORIES

Worldwide Clinical Trials Early Phase Services, LLC
2455 N.E. Loop 410, Suite 150
San Antonio, Texas 78217
Telephone: 210.635.1500
Fax: 210.635.1646

ANALYTICAL LABORATORY

Alliance Pharma, Inc.
Project Manager: Michelle Black
Contact for Sample Shipment: Anna Cucinotta
17 Lee Boulevard
Malvern, PA 19355
Phone: 610.296.3152
Fax: 610.296.3153
Email: samples@alliancepharmaco.com
Web: www.alliancepharmaco.com

CARDIAC VENDOR

iCardiac Technologies, Inc.
150 Allens Creek Road
Rochester, New York 14618
Phone: 585.295.7610
Fax: 585.285.4130

11 DRUG SUPPLIES

Global Alliance for TB Drug Development will supply sufficient a quantity of the study drug, TBI-223 for preparation of TBI-223 oral suspension. Study drug will be shipped to Worldwide Clinical Trials Early Phase Services, LLC pursuant to site standard operating procedures. Upon receipt of the study drug, the study drug will be inventoried and stored in an environmentally controlled and secure, limited access area. The suspending vehicle and other supplies will be procured, inventoried, and stored appropriately by Worldwide Clinical Trials Early Phase Services, LLC pursuant to site standard operating procedures.

The lot numbers of the study drug, vehicle, and other supplies along with the expiration dates (where available) will be recorded and copies of the Certificate of Analysis (where available) will be maintained on file. Records will be maintained of the receipt and dispensation of the drugs supplied.

Samples will be collected from each batch of test product (TBI-223 Oral Suspension) and stored frozen until the clinical study report is issued. At the conclusion of the study, any unused study drug will be returned to the Sponsor or destroyed by the site pursuant to written authorization by the Sponsor and applicable federal and state regulations.

Global Alliance for TB Drug Development will supply sufficient quantity of the study drug, TBI-223 SR tablet formulations (Prototypes 1, 2, and 3) and TBI-223 IR tablet formulation for Part 2 of the study.

Study drug formulations will be shipped to Worldwide Clinical Trials Early Phase Services, LLC pursuant to site standard operating procedures. Upon receipt of the study drug products, the supplies will be inventoried and stored in an environmentally controlled and secure, limited access area. The lot numbers of the drugs along with the expiration dates (where available) will be recorded and copies of the Certificate of Analysis (where available) will be maintained on file. Records will be maintained of the receipt and dispensation of the drugs supplied. At the conclusion of the study, any unused study drug will be returned to the sponsor or destroyed by the site pursuant to written authorization by the sponsor and applicable federal and state regulations.

12 ADMINISTRATIVE ISSUES

The Investigator is referred to the Investigator Brochure, or information provided during the study initiation visit, information provided by the study monitor, and ICH Guidelines for Good Clinical Practice for information regarding the study drug, details, or general considerations to be followed during the course of this study.

13 EVENTS SCHEDULE

Table 9 Schedule of Assessments, Part 1

	SCR	CONFINEMENT																				FU	
STUDY DAY	-28 to -2	-2 CI	-1	DAY 1													DAY 2					3	4	11 (+1)[a]
				STUDY HOUR																				
EVENT				0	0.5	1	1.5	2	3	4	5	6	7	8	12	16[b]	20	24	30	36	42	48	72	N/A
Informed consent and medical history	X																							
Height	X																							
Weight	X	X																						
Physical and neurological examinations[c]	X	X		X					X						X			X				X	X	
Vital signs (blood pressure, pulse, temperature, respiration rate, and pulse oximetry) [d]	X	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	
12-lead safety ECGs[e]	X	X	X[f]	X		X		X	X	X		X		X	X	X	X	X	X	X		X		
HIV/hepatitis B, C	X																							
Clinical laboratory tests (hematology, chemistry, and urinalysis)	X	X																X					X	
Coagulation tests	X	X													X			X					X	

	SCR	CONFINEMENT																				FU
STUDY DAY	-28 to -2	-2 CI	-1	DAY 1													DAY 2					3	4	11 (+1)[a]
				STUDY HOUR																				
EVENT				0	0.5	1	1.5	2	3	4	5	6	7	8	12	16[b]	20	24	30	36	42	48	72	N/A
Urine drug, alcohol, cotinine screen	X	X																						
Serum pregnancy (females only)	X	X																						
FSH (post-menopausal females only)	X																							
Concomitant medication review	X	<----------X---------->																						
Check-in questions		X																						
Adverse events[g]	X	X		X		X		X	X	X		X		X	X	X	X	X	X	X	X	X	X	X
High-fat, high-calorie breakfast (food-effect cohort only)[h]				X																				
Breath strip application[i]				X																				
Dose[j]				X																				
PK blood collection[k]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Cardiac telemetry 12-lead Holter monitoring[l]			<----------X---------->																					
ECG extractions from Holter recording[m]			X	X*	X	X		X	X	X	X	X		X	X	X	X	X	X	X		*X*		

CI = check-in; ECG = electrocardiogram; FSH = follicle-stimulating hormone; FU = follow up; HIV = human immunodeficiency virus; N/A = not applicable; PK = pharmacokinetic; SCR = screening; T and R = temperature and respirations

a. For the food-effect cohort, the timing of the Day 11 (+1) follow up phone call will be relative to Period 2 dosing.
b. The 16-hour time point following dosing on Day 1 will either be on Day 1 or Day 2, depending upon time of dosing on Day 1.
c. A detailed neurological exam will be conducted by a licensed physician or suitably medically qualified designee at both scheduled and unscheduled time points as deemed necessary by the Investigator. The neurological examination is planned to be performed around the estimated T_{max} (currently estimated at 3 hours post dosing; this time could change based on data generated during the study). Timepoints can be altered based on generated PK data.
d. Vitals signs will be measured at screening, check-in, and while subjects are in confinement. Vitals signs will be measured within 90 minutes prior to dosing and within 15 minutes of the remaining defined time points in flowchart. Both blood pressure and heart rate (pulse) should be captured simultaneously. Blood pressure and heart rate should be measured after subjects are in a seated position for at least 2 minutes and then again after standing for 1 minute, except when they are supine or semi-reclined because of study procedures and/or AEs, or as deemed necessary by the Investigator.
e. 12-lead safety ECGs will be printed and reviewed on-site by the Principal Investigator or designee. Triplicate ECGs will be completed at screening, Day -2, Day -1, and at 48 hours only. All other time points will be single ECG readings within 15 minutes at time points within flowchart. The predose ECG should be performed prior to the pre-dose blood draw. If a subject experiences a post-dosing QTcF >500 msec or a change-from-baseline QTcF >60 msec, after repeat testing, additional ECGs should be recorded until normalization or return to baseline.
f. The first ECG may be done within 2 hours prior to dosing and must be completed 20 minutes prior to the pre-dose blood draw. All safety ECGs on Day 1 and Day 2 will be extracted from a continuous 12 lead Holter recording at discrete timepoints as noted in above flow chart and Day -1 will be extracted at the same time as vital sign measurements.
g. Subjects will be monitored for treatment-emergent AEs from the time of dosing and throughout the study via safety assessments, observation, and subject reporting. A specific inquiry regarding AEs will be conducted prior to dosing and at time points determined by the Investigator postdose and upon return to clinic for subsequent visits. The inquiry to collect AEs using non-leading questions on Day 11 (±1 day) will be done via a phone call.
h. In the fed treatment period of the food-effect cohort, a high-fat, high-calorie breakfast meal will be given 30 minutes before treatment administration.
i. A breath strip will be added to the subject's tongue before and after dose administration in order to blind the subject to treatment.
j. TBI-223 oral suspension and placebo for TBI-223 oral suspension will be administered orally followed by approximately 200 mL of water.
k. Samples collected for plasma pharmacokinetic analysis will be processed per the instructions in Appendix 1.
l. Cardiac telemetry will begin at least 24 hours before and end at least 48 hours after dosing.
m. Replicate 12-lead ECGs will be extracted from a continuous Holter recording at discrete timepoints as noted on Day 1 in above flow chart for cardiodynamic assessment.

*Three timepoints before dosing: -45, -30, and -15 minutes.

NOTE: When ECG extractions coincide with safety ECGs, vital signs assessments, and/or blood draws, then procedures should be carried out in said order.

14 REFERENCES

1. Prescription Monograph for Linezolid
2. FDA Guidance for Industry, Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers
3. FDA Food-Effect Guidance [https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070241.pdf].

APPENDIX 1 PHARMACOKINETIC SAMPLE PROCESSING

A. Collection and Processing

A more detailed description of plasma sample preparation requirements may be provided by the analytical laboratory. If such a description is provided, the method of sample preparation provided by the laboratory shall supersede those provided in this protocol and appropriate documentation shall be placed in the Investigator Site File (ISF).

Processing Instructions	
1	Samples will be collected via direct venipuncture at the time points delineated in the appropriate Events Schedule (Section 13).
2	Blood will be drawn into pre-chilled 4 mL evacuated tubes containing K_3-EDTA and immediately placed on wet ice and then processed to plasma within 60 minutes.
3	Samples will be centrifuged at 1500 g at approximately 4°C (± 10 degrees) for 10 minutes. After centrifugation, two aliquots of plasma (the first containing at least 0.5 mL and the second containing the remainder of the plasma) will be removed and placed in appropriately labeled 5 mL polypropylene vials.
4	The aliquots will be immediately placed on dry ice. Within 90 minutes of collection, the aliquots will be stored in a freezer set at -20°C ± 10°C and remain in the freezer until transferred for analysis.
5	Samples will be transferred at the agreed upon intervals.
6	The aliquots will be transferred on dry ice to the Alliance Pharma, Inc. bioanalytical laboratory for the determination of plasma concentrations of TBI-223 and TBI-223 M2, using a validated procedure at Alliance Pharma.
7	Blood sampling, plasma processing, and storage time will be documented in the study records.

<u>Labeling of aliquot tubes</u>

Labels will contain at least the following information:

a) Study number

b) Subject number

c) Period or dosing phase; sampling time (relative to dosing)

d) Aliquot letter (A or B)

Shipment

1. The samples will be transferred to the analytical laboratory after completion of the study or at mutually agreed upon time points during the clinical conduct of the study. The second set of samples will be shipped after the bioanalytical laboratory confirms receipt of the first set of samples.
2. Samples will be packaged into cryoboxes and sorted by subject and time point.
3. Prior to shipment, the samples will be appropriately packed in a cooler containing dry ice. Sufficient dry ice will be added to ensure that the samples will remain frozen for at least 72 hours.
4. An electronic manifest will be provided in advance. The shipment will be accompanied by documentation containing the following information: name of the study drug product, protocol number, number of subjects, and number of samples included in the shipment. Expected samples that are not present will be identified.
5. All frozen pharmacokinetic samples will be transferred with accompanying documentation to:

   Alliance Pharma, Inc.
   17 Lee Blvd
   Malvern, PA 19355
   Telephone: 610.296.3152
   Fax: 610.296.3153
   Contact name: Anna Cucinotta
   Contact e-mail: samplesa@alliancepharmaco.com

APPENDIX 2 DESCRIPTION AND COMPOSITION OF TEST PRODUCT

Oral Suspension

Description of the Dosage Form

TBI-223 Oral Suspension is a compounded preparation at 25 mg/mL TBI-223 in Ora-Blend®. The suspension is compounded at the clinical study site with TBI-223 and Ora-Blend®, a commercially available flavored suspending vehicle.

Placebo for TBI-223 Oral Suspension is Ora-Blend®, a commercially available flavored suspending vehicle (without TBI-223). The placebo suspension, 100% Ora-Blend®, is purchased commercially by the clinical study site.

The required doses of the drug product are dispensed using commercially available oral syringes of suitable capacity.

Composition of the Drug Product

The quantitative composition, function and quality of each ingredient in the drug product (TBI-223 Oral Suspension, 25 mg/mL) is described in the table below.

Qualitative and Quantitative Composition of the TBI-223 Oral Suspension, 25 mg/mL

Ingredient	Function	Quality Standard	Quantity per Dose (50 mg)	Quantity per Dose (100 mg)	Quantity per Dose (300 mg)	Quantity per Dose (600 mg)	Quantity per Dose (1200 mg)	Quantity per Dose (1400 mg)
TBI-223	Drug Substance	In-house	50 mg	100 mg	300 mg	600 mg	1200 mg	1400 mg
Ora-Blend®[1]	Flavored Oral Suspending Vehicle	Commercially available	q.s. to 2 mL	q.s to 4 mL	q.s to 12 mL	q.s. to 24 mL	q.s. to 48 mL	q.s to 56 mL
Total Volume			2 mL	4 mL	12 mL	24 mL	48 mL	56 mL

[1]Purified water, sucrose, glycerin, sorbitol, flavoring, microcrystalline cellulose, carboxymethylcellulose sodium, xanthan gum, carrageenan, calcium sulfate, trisodium phosphate, citric acid and sodium phosphate as buffers, dimethicone antifoam emulsion. Preserved with methylparaben and potassium sorbate.

Container and Closure System

TBI-223 drug substance is shipped to the clinical study site in amber colored wide mouth glass bottles with a polypropylene screw cap without a liner.

TBI-223 Oral Suspension is transferred and stored in Pyrex glass reagent bottles with a polypropylene (PP) screw cap without a liner. These bottles are used for mixing, sampling and storage prior to administration.

Enteric Capsules

Description of the Dosage Form

TBI-223 Oral Enteric Capsules contain 150 mg TBI-223 in Vcaps® Enteric capsules. The capsules are filled at the clinical study site by manually filling TBI-223 into Vcaps® Enteric capsules which are commercially available empty capsule shells.

Composition of the Drug Product

The quantitative composition, function and quality of each ingredient in the drug product (TBI-223 Oral Enteric Capsules, 150 mg) is provided in Table 10.

Table 10 Qualitative and Quantitative Composition of the TBI-223 Oral Enteric Capsules, 150 mg

Ingredient	Function	Quality Standard	Quantity per 300 mg Dose
TBI-223	Drug Substance	In-house	300 mg
Vcaps® Enteric Capsules[1], Off-white, Opaque, Size 00	Capsule shell	Commercially available	2 capsules
Total Quantity			300 mg in 2 capsules

[1]Capsule shell composition: titanium dioxide 2% in Hypromellose-Hypromellose AS.

Container and Closure System

TBI-223 Oral Enteric Capsules are stored in white HDPE bottles with PP CRC with a liner. These bottles are used for storage prior to administration.

Tablets

Description of the Dosage Form

TBI-223 tablets are supplied as immediate release (IR) or sustained release (SR). The description of the tablets and strengths are presented in Table 11.

Table 11 Strength, Tablet Weight and Appearance of TBI-223 IR and SR Tablets

Tablet Strength	Tablet Weight	Tablet Appearance
TBI-223 Immediate Release (IR) Tablets		
600 mg	750 mg	White to off-white, capsule shaped tablets, debossed with "TBA" on one side and "22" on the other
1000 mg	1250 mg	White to off-white, oval shaped tablets, debossed with "TBA" on one side and "23" on the other
TBI-223 Sustained Release (SR) Tablets		
600 mg (Prototype 1)	1000 mg	White to off-white, capsule shaped tablets, debossed with "TBA" on one side and "11" on the other
600 mg (Prototype 2)	1000 mg	White to off-white, capsule shaped tablets, debossed with "TBA" on one side and "SR" on the other
900 mg (Prototype 3)	1200 mg	White to off-white, oval shaped tablets, debossed with "TBA" on one side and "900" on the other

Composition of the Drug Product

The composition of TBI-223 IR tablets, 600 mg and 1000 mg is presented in Table 12. The composition of TBI-223 SR Tablets, 600 mg (Prototype 1), 600 mg (Prototype 2) and 900 mg (prototype 3) is presented in Table 13, Table 14 and Table 15, respectively.

Table 12 Composition of TBI-223 IR Tablets, 600 mg and 1000 mg

Component	Function	Percentage (% w/w)	600 mg	1000 mg	Reference to Standard
			mg/tablet		
		Intragranular			
TBI-223 [a]	Active	80.00	600.00	1000.00	In-House
Microcrystalline cellulose (Avicel PH 302) [b]	Diluent	10.00	75.00	125.00	USP-NF, Ph.Eur.
Hypromellose (Methocel E5 Premium LV)	Binder	3.00	22.50	37.50	USP-NF, Ph.Eur.
Crospovidone (Kollidon CL)	Disintegrant	3.00	22.50	37.50	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	q.s	USP, Ph.Eur.
		Extragranular			
Crospovidone (Kollidon CL)	Disintegrant	2.00	15.00	25.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.00	7.50	12.50	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	7.50	12.50	USP-NF, Ph.Eur.
Total unit dose		**100.00**	**750.0**	**1250.0**	

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.
[b] The quantity of microcrystalline cellulose may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.
[c] Removed during processing.

Table 13 Composition of TBI-223 SR Tablets, 600 mg (Prototype 1)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	Active	60.00	600.00	In-House
Lactose monohydrate [b] (Pharmatose 200M)	Diluent	9.90	99.00	USP-NF, Ph.Eur.
Microcrystalline cellulose (Avicel PH 200)	Diluent	6.60	66.00	USP-NF, Ph.Eur.
Hypromellose (Methocel E5 Premium LV)	Binder	5.00	50.00	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	USP, Ph.Eur.

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
		Extragranular		
Hypromellose (Methocel K100M Premium DC2)	Control Release Polymer	16.00	160.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.50	15.00	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	10.00	USP-NF, Ph.Eur.
Total unit dose		**100.00**	**1000.0**	

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.
[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.
[c] Removed during processing.

Table 14 Composition of TBI-223 SR Tablets, 600 mg (Prototype 2)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	Active	60.00	600.00	In-House
Lactose monohydrate [b] (Pharmatose 200M)	Diluent	12.90	129.00	USP-NF, Ph.Eur.
Microcrystalline cellulose (Avicel PH 200)	Diluent	8.60	86.00	USP-NF, Ph.Eur.
Hypromellose (Methocel K4M Premium CR)	Control Release Polymer	5.00	50.00	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	USP, Ph.Eur.
		Extragranular		
Hypromellose (Methocel K100 Premium LV)	Control Release Polymer	11.00	110.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.50	15.00	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	10.00	USP-NF, Ph.Eur.
Total unit dose		**100.00**	**1000.0**	

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.
[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.
[c] Removed during processing.

Table 15 Composition of TBI-223 SR Tablets, 900 mg (Prototype 3)

Component	Function	Percentage (% w/w)	mg/tablet	Reference to Standard
		Intragranular		
TBI-223 [a]	Active	75.00	900.00	In-House
Lactose monohydrate [b] (Pharmatose 200M)	Diluent	3.90	46.80	USP-NF, Ph.Eur.
Microcrystalline cellulose (Avicel PH 200)	Diluent	2.60	31.20	USP-NF, Ph.Eur.
Hypromellose (Methocel E5 Premium LV)	Binder	5.00	60.00	USP-NF, Ph.Eur.
Purified water [c]	Granulating solvent	q.s	q.s	USP. Ph.Eur.
		Extragranular		
Hypromellose (Methocel K100M Premium DC2)	Control Release Polymer	11.00	132.00	USP-NF, Ph.Eur.
Colloidal silicon dioxide (Aerosil 200 Pharma)	Glidant	1.50	18.00	USP-NF, Ph.Eur.
Magnesium stearate (Ligamed MF-2-V)	Lubricant	1.00	12.00	USP-NF, Ph.Eur.
Total unit dose		**100.00**	**1200.0**	

[a] The molecular weight of TBI-223 is 365.4 g/mol. The actual quantity may be adjusted based on the assay value (anhydrous basis) and water content of the drug substance lot used.
[b] The quantity of lactose monohydrate may be adjusted to compensate for the actual amount of TBI-223 and to maintain a constant tablet weight.
[c] Removed during processing.

Container and Closure System

TBI-223 IR and SR Tablets are packaged as 30-count in 100 cc round high-density polyethylene (HDPE) bottles and closed with aluminum foil lined heat induction seals and 38 mm polypropylene (PP) child resistant closures (CRC).

APPENDIX 3 DIVISION OF MICROBIOLOGY AND INFECTIOUS DISEASE TOXICITY TABLE

Division of Microbiology and Infectious Disease (DMID) Toxicity Table

Source: U.S. National Institute of Allergy and Infectious Diseases, DMID, November 2007 (Draft)

ABBREVIATIONS: Abbreviations utilized in the Table:

ULN = Upper Limit of Normal	LLN = Lower Limit of Normal
R_x = Therapy	Req = Required
Mod = Moderate	IV = Intravenous
ADL = Activities of Daily Living	Dec = Decreased

ESTIMATING SEVERITY GRADE

For abnormalities NOT found elsewhere in the Toxicity Tables use the scale below to estimate grade of severity:

Grade	Severity Rating	Definition
GRADE 1	Mild	Transient or mild discomfort (< 48 hours); no medical intervention/therapy required.
GRADE 2	Moderate	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required.
GRADE 3	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.
GRADE 4	Potentially Life-threatening	Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

SERIOUS OR LIFE-THREATENING AEs

ANY clinical event deemed by the clinician to be serious or life-threatening should be considered a grade 4 event. Clinical events considered to be serious or life-threatening include, but are not limited to: seizures, coma, tetany, diabetic ketoacidosis, disseminated intravascular coagulation, diffuse petechiae, paralysis, acute psychosis, severe depression.

COMMENTS REGARDING THE USE OF THESE TABLES

- Standardized and commonly used toxicity tables (Division of AIDS, NCI's Common Toxicity Criteria (CTC), and World Health Organization (WHO)) have been adapted for use by the Division of Microbiology and Infectious Diseases (DMID) and modified to better meet the needs of Patients in DMID trials.
- For parameters not included in the following Toxicity Tables, sites should refer to the "Guide For Estimating Severity Grade" located above.

- Criteria are generally grouped by body system.
- Some protocols may have additional protocol specific grading criteria, which will supersede the use of these tables for specified criteria.

HEMATOLOGY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hemoglobin	9.5 - 10.5 gm/dL	8.0 - 9.4gm/dL	6.5 - 7.9 gm/dL	< 6.5 gm/dL
Absolute Neutrophil Count	1000-1500/mm^3	750-999/mm^3	500-749/mm^3	<500/mm^3
Platelets	75,000-99,999/mm^3	50,000-74,999/mm^3	20,000-49,999/mm^3	<20,000/mm^3
WBCs	11,000-13,000/ mm^3	13,000-15,000 /mm^3	15,000-30,000/mm^3	>30,000 or <1,000 /mm^3
% Polymorphonuclear Leucocytes + Band Cells	> 80%	90 – 95%	>95%	----------
Abnormal Fibrinogen	Low: 100-200 mg/dL High: 400-600 mg/dL	Low: <100 mg/dL High: >600 mg/dL	Low: < 50 mg/dL ----------	Fibrinogen associated with gross bleeding or with disseminated coagulation
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Methemoglobin	5.0 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20.0 %

CHEMISTRIES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or abnormal sodium *with* mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement therapy or hospitalization required	< 2.0 mEq/L or abnormal potassium *with* paresis, ileus or life-threatening arrhythmia

CHEMISTRIES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or abnormal potassium *with* life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or abnormal glucose *with* mental status changes or coma
Hyperglycemia (non-fasting and no prior diabetes)	116 - 160 mg/dL	161- 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or abnormal glucose *with* ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or abnormal calcium *with* life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL or abnormal calcium *with* life threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or abnormal magnesium *with* life-threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 -1.9 mg/dL or replacement Rx required	1.0 -1.4 mg/dL intensive therapy or hospitalization required	< 1.0 mg/dL or abnormal phosphate *with* life-threatening arrhythmia
Hyperbilirubinemia (when accompanied by any increase in other liver function test)	1.1 - <1.25 x ULN	1.25 - <1.5 x ULN	1.5 – 1.75 x ULN	> 1.75 x ULN
Hyperbilirubinemia (when other liver function are in the normal range)	1.1 - <1.5 x ULN	1.5 - <2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Hyperuricemia (uric acid)	7.5 – 10.0 mg/dL	10.1 – 12.0 mg/dL	12.1 – 15.0 mg/dL	>15.0 mg/dL
Creatinine	1.1 - 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or dialysis required

ENZYMES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
AST (SGOT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
ALT (SGPT)	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN

ENZYMES				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
GGT	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Alkaline Phosphatase	1.1 - <2.0 x ULN	2.0 – <3.0 x ULN	3.0 – 8.0 x ULN	> 8 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
Lipase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN

URINALYSIS				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Proteinuria	1+ or 200 mg - 1 gm loss/day	2-3+ or 1- 2 gm loss/day	4+ or 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria*	microscopic only <10 rbc/hpf	gross, no clots >10 rbc/hpf	gross, with or without clots, OR red blood cell casts	obstructive or required transfusion

*Assessment does not apply if a subject is on menses.

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; symptomatic Rx required	unstable dysrythmia; hospitalization and treatment required
Hypertension	transient increase > 20 mm/Hg; no treatment[1]	recurrent, chronic increase > 20mm/Hg. /treatment required	acute treatment required; outpatient treatment or hospitalization possible	end organ damage or hospitalization required
Hypotension	transient orthostatic hypotension with heart rate increased by <20 beat/min or decreased by <10 mm Hg systolic BP, No treatment required[1]	symptoms due to orthostatic hypotension or BP decreased by <20 mm Hg systolic; correctable with oral fluid treatment	requires IV fluids; no hospitalization required	mean arterial pressure <60mm/ Hg or end organ damage or shock; requires hospitalization and vasopressor treatment

CARDIOVASCULAR				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no treatment	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

1) For protocol TBI-223-CL-001, defined as increase from baseline in predose vital signs.

RESPIRATORY				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Cough	Transient - no treatment	persistent cough; treatment responsive	Paroxysmal cough; uncontrolled with treatment	----------
Bronchospasm, Acute	transient; no treatment; 70% - 80% FEV_1 of peak flow	requires treatment; normalizes with bronchodilator; FEV_1 50% - 70% (of peak flow)	no normalization with bronchodilator;FEV_1 25% - 50% of peak flow; or retractions present	cyanosis: FEV_1 < 25% of peak flow or intubation necessary
Dyspnea	dyspnea on exertion	dyspnea with normal activity	dyspnea at rest	dyspnea requiring Oxygen therapy

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Nausea	mild or transient; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	no significant intake; requires IV fluids	hospitalization required;
Vomiting	1 episode in 24 hours	2-5 episodes in 24 hours	>6 episodes in 24 hours or needing IV fluids	physiologic consequences requiring hospitalization or requiring parenteral nutrition
Constipation	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Diarrhea	mild or transient; 3-4 loose stools/day or mild diarrhea	moderate or persistent; 5-7 loose stools/day or	>7 loose stools/day or bloody diarrhea; or orthostatic	hypotensive shock or physiologic consequences

GASTROINTESTINAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
	last < 1 week	diarrhea lasting >1 week	hypotension or electrolyte imbalance or >2L IV fluids required	requiring hospitalization
Oral Discomfort/Dysphagia	mild discomfort; no difficulty swallowing	some limits on eating/drinking	eating/talking very limited; unable to swallow solid foods	unable to drink fluids; requires IV fluids

NEUROLOGICAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Psychiatric	mild anxiety or depression	moderate anxiety or depression; therapy required; change in normal routine	severe mood changes requiring therapy; or suicidal ideation; or aggressive ideation	acute psychosis requiring hospitalization; or suicidal gesture/attempt or hallucinations
Muscle Strength	Subjective weakness no objective symptoms/ signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis
Paresthesia (burning, tingling, etc.)	mild discomfort; no treatment required	moderate discomfort; non-narcotic analgesia required	severe discomfort; or narcotic analgesia required with symptomatic improvement	incapacitating; or not responsive to narcotic analgesia
Neuro-sensory	mild impairment in sensation (decreased sensation, e.g., vibratory, pinprick, hot/cold in great toes) in focal area or symmetrical distribution; or change in taste, smell, vision and/or hearing	moderate impairment (mod decreased sensation, e.g., vibratory, pinprick, hot/cold to ankles) and/or joint position or mild impairment that is not symmetrical	severe impairment (decreased or loss of sensation to knees or wrists) or loss of sensation of at least mod degree in multiple different body areas (i.e., upper and lower extremities)	sensory loss involves limbs and trunk; paralysis; or seizures

MUSCULOSKELETAL				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Arthralgia (joint pain)	mild pain not interfering with function	moderate pain, analgesics and/or pain interfering with function but not with activities of daily living	severe pain; pain and/or analgesics interfering with activities of daily living	disabling pain
Arthritis	mild pain with inflammation, erythema or joint swelling – but not interfering with function	moderate pain with inflammation, erythema or joint swelling – interfering with function, but not with activities of daily living	severe pain with inflammation, erythema or joint swelling –and interfering with activities of daily living	permanent and/or disabling joint destruction
Myalgia	myalgia with no limitation of activity	muscle tenderness (at other than injection site) or with moderate impairment of activity	severe muscle tenderness with marked impairment of activity	frank myonecrosis

SKIN				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation or moist desquamation or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery
Induration	< 15mm	15-30 mm	>30mm	
Erythema	< 15mm	15-30 mm	>30mm	
Edema	< 15mm	15-30 mm	>30mm	
Rash at Injection Site	< 15mm	15-30 mm	>30mm	
Pruritus	slight itching at injection site	moderate itching at injection extremity	itching over entire body	

SYSTEMIC				
	Grade 1	**Grade 2**	**Grade 3**	**Grade 4**
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Headache	mild, no treatment required	transient, moderate; treatment required	severe; responds to initial narcotic therapy	intractable; requires repeated narcotic therapy
Fever: oral	37.7 - 38.5 C or 100.0 - 101.5 F	38.6 - 39.5 C or 101.6 - 102.9 F	39.6 - 40.5 C or 103 - 105 F	> 40 C or > 105 F
Fatigue	normal activity reduced < 48 hours	normal activity decreased 25- 50% > 48 hours	normal activity decreased > 50% can't work	unable to care for self

APPENDIX 4 HOLTER RECORDING AND ANALYSIS

Continuous 12-lead ECGs (Holters) will be recorded using the Mortara Surveyor system.

The continuous 12-lead digital ECG data will be stored and transmitted to iCardiac using the appropriate medium (e.g., file transfer protocol [FTP], disc, or flash drive). ECGs to be used in the analyses will be selected by pre-determined time points as defined in the Schedule of Assessments and Procedures, and will be read centrally by iCardiac Technologies, Inc.

The following principals will be followed in iCardiac's core laboratory:

- ECG analysts are blinded to the subject, visit and treatment allocation
- Baseline and on-treatment ECGs for a particular subject will be over-read on the same lead and will be analyzed by the same reader.
- The primary analysis lead is lead II. If lead II is not analyzable, then primary lead of analysis will be changed to another lead for the entire subject data set.

The following is a brief description of ECG analysis methods utilized by iCardiac' core laboratory.

TQT Plus ECG Extraction Technique

Ten 14-second digital 12-lead ECG tracings will be extracted from the continuous Holter recordings using the "TQT Plus method", a computer-assisted and statistical process utilized by iCardiac Technologies. The method enables extraction of ECGs with the lowest HR variability and noise within the protocol-specified extraction time window (e.g., the HR and QT changes from beat-to-beat in the range of <10%). At each protocol-specified timepoint, 10 ECG replicates will be extracted from a 5-minute "ECG window" (typically, the last 5 minutes of the 15-minute period when the subject is maintained in a supine or semi-recumbent quiet position).

High-Precision QT Analysis

High-precision QT analysis will be performed on all analyzable (non-artifact) beats in the 10 ECG replicates. Statistical quality control procedures are used to review and assess all beats and identify "high" and "low" confidence beats using several criteria, including:

- QT or QTc values exceeding or below certain thresholds (biologically unlikely).
- RR values exceeding or below certain thresholds (biologically unlikely).
- Rapid changes in QT, QTc or RR from beat to beat.

Measurements of all primary ECG parameters (QT, QTc, RR) in all recorded beats of all replicates that are deemed "high confidence" is performed using COMPAS software. All low confidence beats are reviewed manually and adjudicated using pass-fail criteria. The final QC assessment is performed by a cardiologist. The beats found acceptable by manual review are included in the analysis. The median QT, QTc, and RR value from each extracted replicate is

calculated, and then the mean of all available medians from a nominal timepoint is used as the subject's reportable value at that timepoint.

Categorical T-wave morphology analysis and the measurement of PR and QRS intervals will be performed manually in 3 of the 10 ECG replicates at each timepoint. Each fiducial point (onset of P-wave, onset of Q-wave, offset of S-wave, and offset of T-wave) is electronically marked.

T-wave morphology categories (assessed manually)

Category	Description
Normal T-wave	Any T-wave not meeting any criterion below
Flat T-waves	T amplitude < 1 mm (either positive or negative) including flat isoelectric line
Notched T-wave (+)	Presence of notch(es) of at least 0.05 mV amplitude on ascending or descending arm of the positive T-wave
Biphasic	T-wave that contains a second component with an opposite phase that is at least 0.1 mV deep (both positive and negative/positive and polyphasic T-waves included)
Normal T-wave (-)	T amplitude that is negative, without biphasic T-wave or notches
Notched T-wave (-)	Presence of notch(es) of at least 0.05 mV amplitude on descending or ascending arm of the negative T-wave

In addition to the T-wave categorical analysis, the presence of abnormal U-waves is noted.

APPENDIX 5 REPEAT COHORT USING DIFFERENT DOSAGE FORMULATION

Procedures and analyses as outlined in this protocol will be the same, with the following exceptions:

- The dose of TBI-223 will be delivered in enteric capsules.
- All subjects will receive active treatment.
- A minimum of 6 subjects is needed. Ideally, all 8 subjects who participated in the original cohort will participate in the re-dose cohort. If less than 6 subjects from the original cohort agree to participate, additional subjects will be screened as needed in order to dose a minimum of 6 subjects.
- Repeat subjects will be assigned a new subject number so as not to break the blinding of the original cohort.
- The repeat cohort will not be blinded (blinding is not necessary because all subjects will receive the same treatment).
- Cardiac telemetry and Holter recording will not be performed during the re-dose cohort.
- Subjects will check-in on Day -1 instead of Day -2. With the exception of cardiac telemetry and Holter monitoring, the procedures that were previously scheduled for Day -2 will be performed on Day -1. The Day -1 results will be used to qualify repeat subjects for study participation. If additional subjects are needed, they will undergo screening to qualify for the study.
- Neurological examinations will be performed at Screening, Day -1, and at 3 and 48 hours postdose.

APPENDIX 6 PART 2 OF THE STUDY

Procedures and analyses as outlined in this protocol will be the same, with the following exceptions:

The dose of TBI-223 will be delivered as follows in Cohort 8:

- Arm 1 – TBI-223 SR Tablets (Prototype 1) - 1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 2 – TBI-223 SR Tablets (Prototype 2) - 1800 mg dose (3 x 600 mg tablets), under fed conditions
- Arm 3 – TBI-223 SR Tablets (Prototype 3) - 1800 mg dose (2 x 900 mg tablets), under fed conditions
- Arm 4 – TBI-223 IR Tablets - 2000 mg dose (2 x 1000 mg tablets), under fasting conditions

In Cohort 9, the subjects from Cohort 8, Arm 4, who received the IR tablet formulation will be brought back following a 7-day washout and administered TBI-223 IR tablets, 2000 mg dose (2 x 1000 mg tablets), under fed conditions.

- A minimum of 6 subjects per treatment arm in Part 2.
- Part 2 will not be blinded (blinding is not necessary because all subjects will receive the same treatment).
- Cardiac telemetry and Holter recording will not be performed during Part 2. Subjects will check-in on Day -1 instead of Day -2. With the exception of cardiac telemetry and Holter monitoring, the procedures that were previously scheduled for Day -2 will be performed on Day -1. The Day -1 results will be used to qualify repeat subjects for study participation. If additional subjects are needed, they will undergo screening to qualify for the study.
- Neurological examinations will be performed at Screening, Day -1, and at 3 and 48 hours postdose.

Table 16 Schedule of Assessments, Part 2

	SCR	CONFINEMENT																			FU	
STUDY DAY	-28 to -2	-1 CI	DAY 1												DAY 2						3	4	11 (+1)
			STUDY HOUR																				
EVENT			0	0.5	1	1.5	2	3	4	5	6	7	8	12	16[a]	20	24	30	36	42	48	72	N/A
Informed consent and medical history	X																						
Height	X																						
Weight	X	X																					
Physical and neurological examinations[b]	X	X	X					X									X				X	X	
Vital signs (blood pressure, pulse, temperature, respiration rate, and pulse oximetry) [c]	X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	
12-lead safety ECGs[d]	X	X	X[e]		X		X	X	X		X		X	X	X	X	X	X	X		X		
HIV/hepatitis B, C	X																						
Clinical laboratory tests (hematology, chemistry, and urinalysis)	X	X															X					X	
Coagulation tests	X	X												X			X					X	
Urine drug, alcohol, cotinine screen	X	X																					

	SCR	CONFINEMENT																			FU	
STUDY DAY	-28 to -2	-1 CI	DAY 1												DAY 2						3	4	11 (+1)
			STUDY HOUR																				
EVENT			0	0.5	1	1.5	2	3	4	5	6	7	8	12	16[a]	20	24	30	36	42	48	72	N/A
Serum pregnancy (females only)	X	X																					
FSH (post-menopausal females only)	X																						
Concomitant medication review	X	<----------X---------->																					
Check-in questions		X																					
Adverse events[f]	X	X	X		X		X	X	X		X		X	X	X	X	X	X	X	X	X	X	X
High-fat, high-calorie breakfast[g]			X																				
Dose			X																				
PK blood collection[h]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

CI = check-in; ECG = electrocardiogram; FSH = follicle-stimulating hormone; FU = follow up; HIV = human immunodeficiency virus; N/A = not applicable; PK = pharmacokinetic; SCR = screening; T and R = temperature and respirations

a. The 16-hour time point following dosing on Day 1 will either be on Day 1 or Day 2, depending upon time of dosing on Day 1.

b. A detailed neurological exam will be conducted by a licensed physician or suitably medically qualified designee at both scheduled and unscheduled time points as deemed necessary by the Investigator. The neurological examination is planned to be performed around the estimated T_{max} (currently estimated at 3 hours post dosing; this time could change based on data generated during the study). Timepoints can be altered based on generated PK data.

c. Vitals signs will be measured at screening, check-in, and while subjects are in confinement. Vitals signs will be measured within 90 minutes prior to dosing and within 15 minutes of the remaining defined time points in flowchart. Both blood pressure and heart rate (pulse) should be captured simultaneously. Blood pressure and heart rate should be measured after subjects are in a seated position for at least 2 minutes and then again after standing for 1 minute, except when they are supine or semi-reclined because of study procedures and/or adverse events, or as deemed necessary by the Investigator.

d. 12-lead safety ECGs will be printed and reviewed on-site by the Principal Investigator or designee. Triplicate ECGs will be completed at screening, Day -2, Day -1, and at 48 hours only. All other time points will be single ECG readings within 15 minutes at time points within flowchart. The predose ECG should be performed prior to the pre-dose blood draw. If a subject experiences a post-dosing QTcF >500 msec or a change-from-baseline QTcF >60 msec, after repeat testing, additional ECGs should be recorded until normalization or return to baseline.

e. The first ECG may be done within 2 hours prior to dosing and must be completed 20 minutes prior to the pre-dose blood draw. All safety ECGs on Day 1 and Day 2 will be extracted from a continuous 12 lead Holter recording at discrete timepoints as noted in above flow chart and Day -1 will be extracted at the same time as vital sign measurements.

f. Subjects will be monitored for treatment-emergent adverse events from the time of dosing and throughout the study via safety assessments, observation, and subject reporting. A specific inquiry regarding adverse events will be conducted prior to dosing and at time points determined by the Investigator postdose and upon return to clinic for subsequent visits. The inquiry to collect adverse events using non-leading questions on Day 11 (±1 day) will be done via a phone call.

g. In Part 2, Cohort 8, Arms 1-3 and Cohort 9, a high-fat, high-calorie breakfast meal will be given 30 minutes before treatment administration.

h. Samples collected for plasma pharmacokinetic analysis will be processed per the instructions in Appendix 1.

*Three timepoints before dosing: -45, -30, and -15 minutes.

NOTE: When ECG extractions coincide with safety ECGs, vital signs assessments, and/or blood draws, then procedures should be carried out in said order.