TB ALLIANCE
GLOBAL ALLIANCE FOR TB DRUG DEVELOPMENT

Request for Proposal

PRECLINICAL EVALUATION OF NEW DRUG COMBINATIONS AGAINST TUBERCULOSIS

(RFP2006.01)

The Global Alliance for TB Drug Development (the TB Alliance) is promoting a new paradigm for TB drug development that would significantly shorten the time required to develop new, more efficacious regimens for the treatment of tuberculosis. In pursuit of this aim, the TB Alliance is seeking proposals from organizations having the capability to evaluate new drug combinations against tuberculosis in relevant preclinical models, with the goal of moving any promising combinations into clinical development.

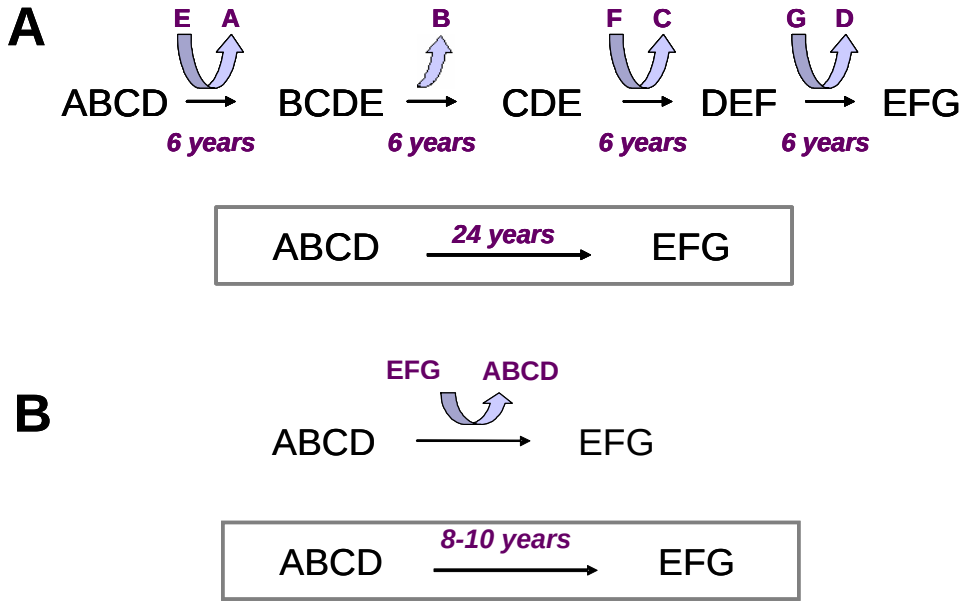
INTRODUCTION

In the past few years, we have witnessed significant progress in TB drug research and development. Currently, there are at least six compounds in various stages of clinical trials. In addition, there are eight or more discovery projects in the lead optimization stage. We anticipate that additional compounds will enter preclinical development within the next two years. These new drug candidates are being optimized and advanced based on their superiority to the current drugs; they tend to be more potent against sub-populations of *M. tuberculosis* living in various microenvironments. The new agents generally have improved pharmacokinetic profiles with longer half-lives and improved target tissue distributions. We believe that a 3-drug combination consisting of new drugs will be adequate to address the problem of drug resistance. With the ultimate goal of developing a fixed-dose-combination (FDC), the concern of resistance development can be further addressed. As some of the new agents appear superior to current drugs, carefully selected 3-drug combinations comprised of new agents will likely provide significant improvement over current therapy in both safety and efficacy. The new combinations could also be designed to address the urgent need for new therapies to treat HIV-TB co-infections, in addition to MDR-TB.

The current approach to TB drug development evaluates new drugs sequentially, one at a time. Each new drug is studied in clinical trials by replacing one of the existing drugs in the standard therapy, a process which takes a minimum of six years to complete. By taking this approach, developing a novel regimen that consists of three new drugs would require more than 24 years; therefore, dramatically improving present treatment using this methodology would most likely require decades (Figure 1, A).

In a new paradigm, each new compound will be evaluated individually through Phase I and Early Bactericidal Activity (EBA) or other proof-of-concept trials. In parallel, all potential combinations will be evaluated in preclinical models to identify promising new combinations. Based on evaluation of the above data, new regimens will be moved into combination Phase I trials for PK interaction and safety studies, followed by Phase II and III trials (see Figure 2). This new paradigm could dramatically shorten clinical development times required for more efficacious regimens (see Figure 1, B).

Figure 1. Two development paradigms: (A) A conventional approach that requires >24 years to develop a regimen consisting of 3 new drugs; and (B) A new paradigm that requires approximately 8-10 years to replace the current 4-drug regimen with a new 3-drug combination.



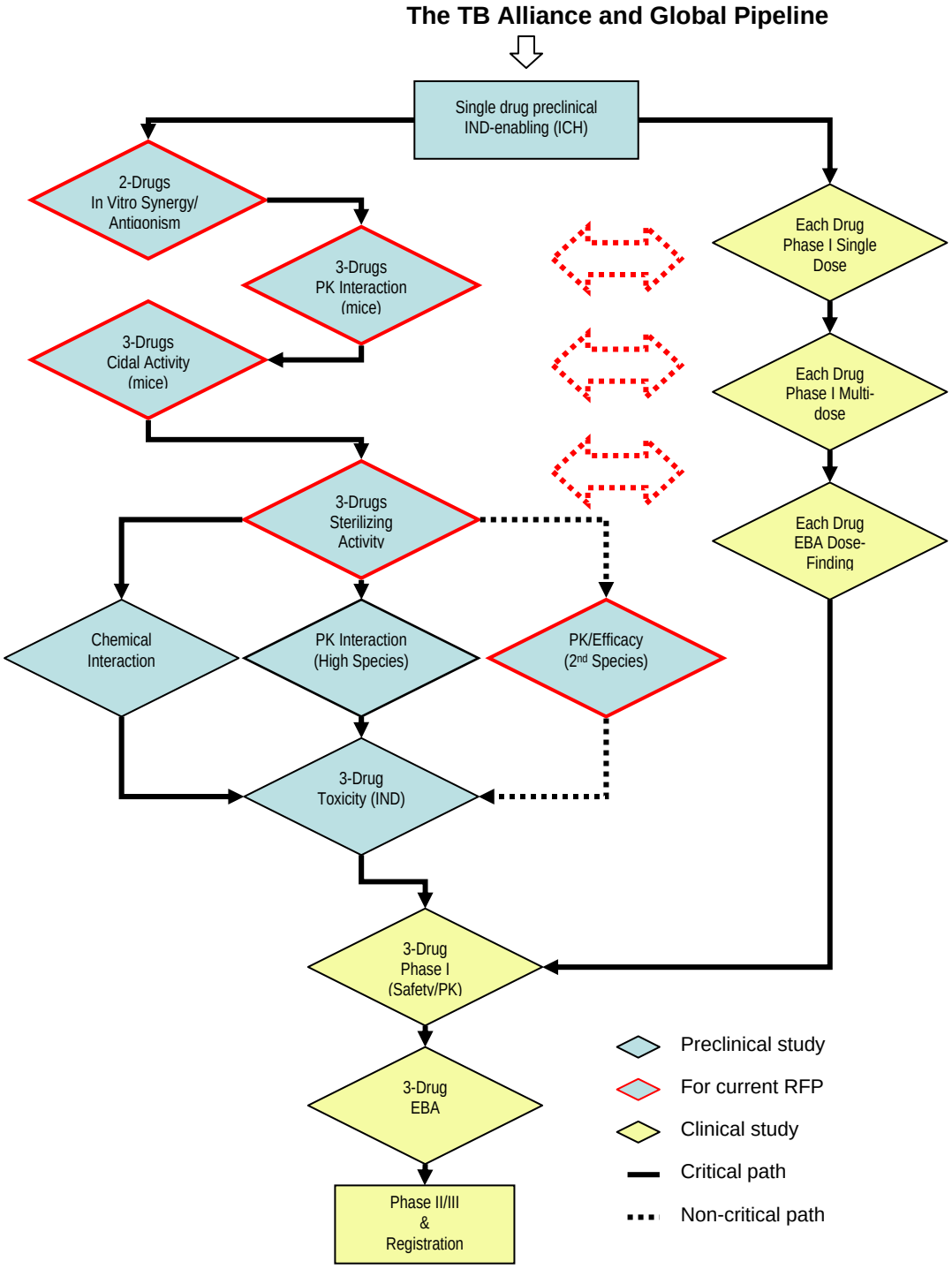
There are two main challenges for moving forward with the new paradigm. First, most sponsors conducting clinical trials have only one new drug at their disposal. Second, regulatory agencies around the world have not yet established clear guidelines for development of combinations consisting of more than one new drug.

The TB Alliance has initiated discussions with regulatory agencies (including but not limited to the FDA and EMEA) in order to advance the new development paradigm. An open forum with regulatory agencies, research institutions, opinion leaders, and industry groups was held in Washington, DC in December 2005; a follow-up meeting is planned for December 2006 in London.

In addition to two clinical-stage drug candidates (moxifloxacin and PA-824) in its portfolio, the TB Alliance is in discussions with other sponsors with the goal of making all new drugs of potential interest available for the identification of the best possible

combinations. As a result of these discussions, the TB Alliance will be responsible for providing all drug substances to be studied under this RFP.

Figure 2. A draft flowchart for preclinical and clinical development of new combinations. Bactericidal (cidal) activity reflects a drug's ability to reduce the bacterial load within a given period of time. Sterilizing activity reflects a drug's ability to produce a stable cure after drug withdrawal.



The preclinical evaluation and selection of optimal combinations is a key step toward the development of new regimens.

This RFP will focus on the preclinical evaluation and identification of potentially improved 3-drug combinations using relevant *in vitro* and *in vivo* models.

PROJECT DESCRIPTION

1. OBJECTIVES

The objective of this work is to identify 3-drug regimens from a predetermined set of candidates (see below) that can significantly improve TB treatment. The highest priority will be given to new regimens that have the potential to shorten therapy to three months or less, are effective against MDR-TB, and have minimal potential for drug-drug interactions, especially with anti-retroviral therapies.

---

2. SPECIFIC AIMS

There are five specific aims for this project:

Aim 1: Identify potential synergistic and antagonistic effects between any two agents against *M.tb* under both replicating and non-replicating conditions within *in vitro* assays.

Aim 2: Evaluate in mice the potential pharmacokinetic interactions of all 3-drug combinations to be studied for bactericidal activity.

Aim 3: Identify in an appropriate mouse model up to ten promising 3-drug combinations with significantly better bactericidal activity than the current rifampin, pyrazinamide, isoniazid (RHZ) regimen.

Aim 4: Evaluate in an appropriate mouse model the sterilizing activity of the 3-drug combinations identified in Aim 3, and identify up to five combinations that show potential for shortening therapy to three months or less.

Aim 5: Confirm the *in vivo* efficacy of the promising 3-drug combinations in a secondary TB model utilizing a second animal species (such as, but not necessarily limited to, guinea pigs).

---

3. DEVELOPMENT OF DRUG DATABASE

A database generated by the TB Alliance will support the research activities under this RFP, and will be shared with the contractors performing the combination studies.

The TB Alliance is currently collecting pertinent information for each existing drug or new drug candidate that will potentially be included in the combination studies. A searchable database will be developed and shared with the contractors awarded the project, and will include as much of the following information as is available:

- Basic chemical and physicochemical information, including chemical structure, molecular formula, solubility, partition coefficient, stability, and protein binding in various animal species.
- Information regarding spectrum, drug target, mechanism of action, and cross-resistance with other agents.
- Human and animal pharmacokinetic/pharmacodynamic parameters, particularly half-life, tissue distribution, metabolic pathway, and active metabolites.
- Optimized human dosage or therapeutic effective level based on animal PK/PD studies.
- Human and animal safety data, target toxicity organs, and potential mechanisms for toxicity.
- Human and animal drug-drug interaction data, particularly P-450 mediated interactions.

The preliminary list of compounds that will be considered for this database is shown in Table 1. This list will be extended to include any new drug candidates that have completed preclinical IND-enabling studies during the course of the project. This database will serve as the basis for selecting compounds to perform Activities 1, 2 and 3.

Table 1. Potential compounds to be included in the TB drug database, including first-line and second-line agents, drugs being used anecdotally against TB, and new drugs in development against TB

Agent	Drug Target	Oral Bioavailability
Rifampin (R)	RNA Polymerase	Yes
Isoniazid (H)	InhA	Yes
Pyrazinamide (Z)	Membrane Potential	Yes
Ethambutol (E)	Cell wall	Yes

Agent	Drug Target	Oral Bioavailability
Clofazimine (C)	Unknown	Yes
Moxifloxacin (M)	DNA Gyrase	Yes
Linezolid (L)	Ribosome	Yes
PA-824 (P)	Unknown	Yes
OPC-67683 (O)	Unknown	Yes
TMC-207 (J)	ATP Synthase	Yes
Thioridazine (T)	Unknown	Yes
LL-3858 (U)	Unknown	Yes

As mentioned earlier, the TB Alliance will be responsible for providing all drug substances to be studied under this RFP.

---

4. MAJOR ACTIVITIES

Activity 1: *In vitro* evaluation of synergistic and antagonistic effects under replicating and non-replicating conditions.

The potential mechanism-based interactions will be evaluated between any two agents from Table 2 over a range of concentrations using the checkerboard format. The suggested concentration range for this study is between 1/4 to 8 times MIC. This study should be performed under both standard replicating conditions and a non-replicating (or slowly replicating) condition. Potential contractors should propose such a non-replicating (or slowly replicating) assay and provide reasonable preliminary data and justifications.

The potential drug pairs to be evaluated in this study are shown in Table 2. Pairs that belong to the same drug class and have cross-resistance, such as moxifloxacin and levofloxacin, should be excluded from the study. For budgetary purposes, please assume that the total number of drugs to be evaluated in this study is 12; there will be 66 potential drug pairs to be evaluated under the two conditions (Table 2).

To explore potential mechanism-based synergistic or antagonistic effects, we have included in this study compounds that have different mechanisms of action. Some compounds, such as clofazimine, are selected for this purpose but may not be suitable candidates for new regimen development, and therefore, not suitable for inclusion in further *in vivo* activities under this RFP.

Table 2. Potential drug pairs for *in vitro* checkerboard studies. One from each target class is selected as a representative in order to identify mechanism-based synergistic and antagonistic effects. The final drug selection will be made by the TB Alliance.

	R	I	Z	E	C	M	L	P	O	J	T	U
R		RI	RZ	RE	RC	RM	RL	RP	RO	RJ	RT	RU
I			IZ	IE	IC	IM	IL	IP	IO	IJ	IT	IU
Z				ZE	ZC	ZM	ZL	ZP	ZO	ZJ	ZT	ZU
E					EC	EM	EL	EP	EO	EJ	ET	EU
C						CM	CL	CP	CO	CJ	CT	CU
M							ML	MP	MO	MJ	MT	MU
L								LP	LO	LJ	LT	LU
P									PO	PJ	PT	PU
O										OJ	OT	OU
J											JT	JU
T												TU
U												

Combinations that contain an antagonistic pair may be excluded from the subsequent *in vivo* studies.

Activity 2: Evaluation of potential pharmacokinetic interactions of all potential 3-drug combinations that meet certain defined criteria in mice.

We tentatively propose that the following ten agents be considered in this Activity based on various factors including safety and efficacy, as well as their mechanisms of action. This list will be modified and updated when more information regarding these agents and new agents becomes available. The final decision of which agents to use in Activity 2 will be made by the TB Alliance in consultation with the PI(s).

1. Rifampin (R)
2. Isoniazid (H)
3. Pyrazinamide (Z)

4. Ethambutol (E)
5. Moxifloxacin (M)
6. Linezolid (L)
7. PA-824 (P)
8. OPC-67683 (O)
9. TMC-207 (J)
10. LL-3858 (U)

For budgetary purposes, assume eight of the above compounds will be selected for combination studies. There are a total of 56 potential 3-drug combinations based on eight compounds.

The potential 3-drug combinations will be triaged based on the following criteria:

- Any two agents that belong to the same drug class and have known cross resistance.
- Any two agents that show an antagonistic effect.
- Any two agents that have known chemical interactions.
- Any two agents that have significantly unmatched PK in humans.
- Any two agents that go through the same metabolic pathway and have potential for drug-drug interactions.
- Any two agents that exhibit the same toxicity or negatively target the same organs.

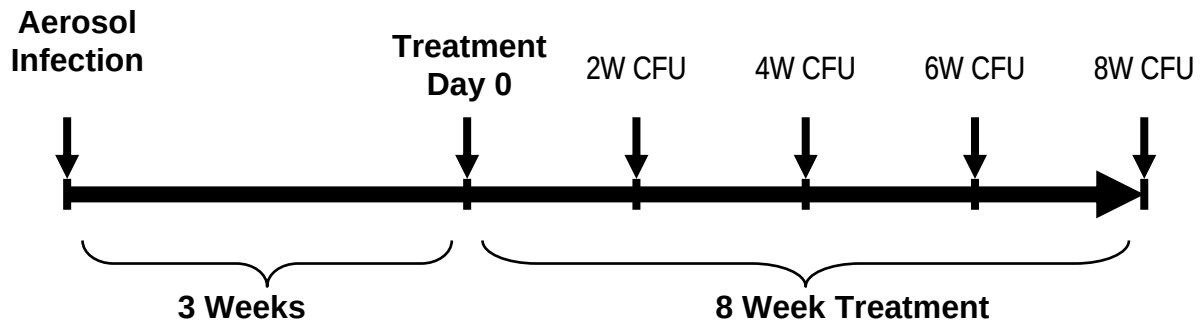
After the triaging process, we anticipate that approximately 75% of the combinations (approximately 42 in number) will be selected by the TB Alliance for further evaluation. These combinations will be evaluated for potential PK interactions in mice by administering the three drugs together and comparing the results with baseline PK obtained by administering the drugs individually. This will be a single dose study; for known drugs, the dose will be selected based on the human dose, and for new drugs, the dose will be selected based on the projected human effective dose. Any combinations that show significant PK interactions will be removed from further development. The remaining combinations will be further prioritized based on whether they will be suitable for QD dosing and free of interactions with antiretrovirals (ARVs).

Activity 3: *In vivo* evaluation in an appropriate mouse model of potential 3-drug combinations for bactericidal activity and pre-selection of candidate combinations.

The combinations prioritized in Activity 2 will be evaluated in a mouse model for bactericidal activity against *M.tb*. A potential testing protocol is illustrated in Figure 3. Investigators may propose alternative protocols based on their experience. A strong scientific and technical rationale should be provided for the proposed protocol. For

budgetary purposes, please assume that 30 potential 3-drug combinations will be evaluated in this model.

Figure 3. Evaluation in a mouse model of bactericidal activity of potential 3-drug combinations. RHZ will be used as the positive control. Dose selection should be based on therapeutic dose in humans for marketed drugs, or efficacious dose in mice for experimental drugs. Final dose selection will be decided by the project team. Lung CFU will be used to evaluate drug efficacy.

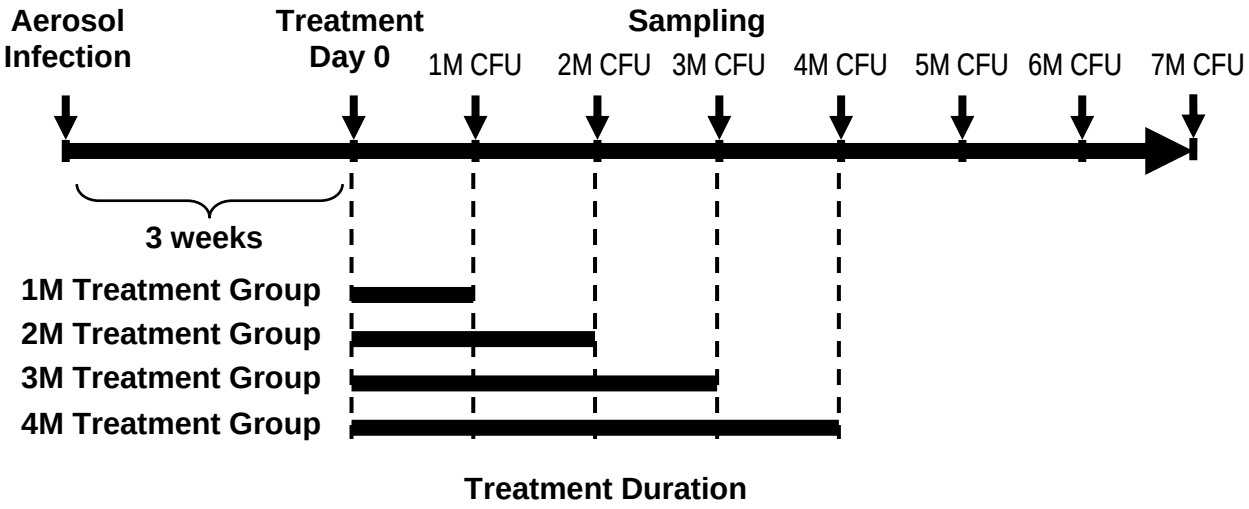


Combinations that demonstrate better efficacy than the RHZ regimen will be selected by the TB Alliance in consultation with the PI(s) for advancement to the next stage. We anticipate that between five and ten combinations will be selected in this process.

Activity 4: *In vivo* evaluation of the pre-selected candidates for sterilizing activity in a mouse model of treatment shortening, and selection of final regimen candidates for preclinical development.

The 3-drug combinations selected in Activity 3 will advance to this study. The objective is to evaluate in a mouse model the treatment duration required to produce a stable cure without relapse as compared to the RHZ control. The experimental design for this study should therefore include several different drug treatment groups for each combination. Each group should be treated for a fixed duration of between one and four months, followed by three months of observation for relapse. A potential experimental design for this study is illustrated in Figure 4 as an example. The potential contractor may propose an alternative design, but a strong scientific and technical rationale for the proposed protocol should be provided. For budgetary purposes, assume ten 3-drug combinations will be evaluated in this model.

Figure 4. Evaluation of the sterilizing activity and treatment duration required to produce a stable cure for the selected combinations. As a prototype model, 2RHZ+4RH will be used as a positive control. Dose selection should be based on either therapeutic dose for marketed drugs, or efficacious dose in mice for experimental drugs. Final dose selection will be approved by the TB Alliance. The mice should be observed for 3 months after drug treatment for relapse. Lung CFU will be evaluated every month for up to 3 months after completing the treatment.



The combinations that produce a stable cure without relapse within the shortest time during the three-month observation period will be prioritized. We anticipate identifying or prioritizing three to five combinations that can significantly shorten treatment duration.

Activity 5: Confirmation of *in vivo* efficacy of the final combinations from Activity 4 in a secondary TB animal model using a different animal species.

The combinations identified in Activity 4 will be evaluated in a secondary animal model for efficacy against TB in conjunction with PK interaction studies in the same species. Applicants are encouraged to propose a secondary animal model for this purpose, and to provide a strong rationale and supporting data for the proposed model. The selection of the animal species and protocol for this study should be based on both predictability and feasibility considerations. For budgetary purposes, please assume five combinations will be evaluated in this model.

The results of this study will be used to prioritize combinations for further preclinical development.

---

5. TIMELINE AND MILESTONES

This project is expected to require three years. As an example, the initiation and completion times for each activity are illustrated in Table 3. Due to the dynamic nature

of the drug portfolio, compounds may be removed and added to the drug list during the course of the project; this will have significant impact on the timeline.

Table 3. A sample timeline for major activities

Activities	Year 1				Year 2				Year 3			
	1Q	2Q	3Q	4Q	1Q	2Q	3Q	4Q	1Q	2Q	3Q	4Q
1. *In vitro* Study: Synergistic/Antagonistic												
2. Pharmacokinetic Interactions (Mice)												
3. *In vivo* Combination: Cidal Activity (Mice)												
4. *In vivo* Combination: Sterilizing Activity (Mice)												
5. *In vivo* Combination: PK/Efficacy (2nd Species)												
Other Preclinical PK/PD and Toxicity												

An interim report will be required within one month after completion of each activity, and a final report will be due by the end of the project. Detailed reporting requirements will be defined in the final contract between the TB Alliance and the contractor(s).

---

6. APPLICATION PROCEDURE AND DEADLINE

Investigators are encouraged to submit a Letter of Intent (LOI) based on their area of expertise and the facilities available to them. Proposals encompassing either the entire project, or any one or more of the following segments, will be accepted:

Activity 1: *In vitro* synergistic/antagonistic studies.

Activities 2, 3 and 4: *In vivo* PK interactions, cidal and sterilizing activities in mouse models.

Activity 5: *In vivo* PK interaction and efficacy in a non-murine species.

Proposals may involve a number of partners contributing particular skills and/or facilities; therefore, the proposed plan must clearly delineate the contribution to be made by each partner; the relationship of each partner to the other(s); and a work plan

that includes a plan for efficient and effective communication among the partners. Proposals that are from or include the private sector (*e.g.,* pharmaceutical or biotechnology companies) are encouraged. The program of activities must define a progression strategy with clear go/no-go decision points, and set out milestones and timelines.

Selected proposals may be funded either in whole or in part.

Detailed information, including Letter of Intent and Application forms, is available on the TB Alliance website, http://www.tballiance.org. You are encouraged to submit questions or seek clarification on the scope of the application by contacting the TB Alliance at **RFP@tballiance.org.**

LOIs must arrive at the TB Alliance as e-mail attachments or by mail no later than close of business October 6, 2006. Contractors who have submitted acceptable LOIs will be invited to submit full proposals by November 30, 2006. Full proposals must be received by the TB Alliance no later than January 31, 2007.

Both the original completed and signed application, and an electronic copy of the completed application, must be submitted to the TB Alliance. Complete information is essential for consideration of proposals. Please ensure that all required signatures have been obtained, and that any additional documentation is appended to the original proposal. Also, please make sure that all partners submit official letters of support, and that these letters are attached to the application. Proposals may be submitted to the TB Alliance electronically; the original hard-copy proposal should be sent to the TB Alliance by mail or by courier.

E-mail: *RFP@tballiance.org*

Ms. Marie Messina
The Global Alliance for TB Drug Development
80 Broad Street, 31st Floor
New York, New York 10004
USA