SUMMARY STATEMENT

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Application Number: **2 R44 AI060275-02A1**

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Review Group: **ZRG1 IDM-Q (10)**

Meeting Date: **03/09/2006**
Council: **MAY 2006**
Requested Start: **07/01/2006**

RFA/PA: **PA06-006**
PCC: **M36**

Dual IC(s): **EB**

Project Title: **Identification of E. coli anti-infective rRNA targets**

SRG Action: **Priority Score: 145**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
2	499,539	960,272
3	499,973	961,107
TOTAL	999,512	1,921,379

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

2R44AI060275-02A1 MURPHY, CHERYL

RESUME AND SUMMARY OF DISCUSSION: The overall objective of this project is to develop a ribosome inhibitor that is active against drug resistant strains, unlikely to generate high-level target resistance and be active against a wide range of bacterial pathogens. This study is highly significant and innovative and the research is led by an impressive team, however, it would be important to include an expert in drug discovery. The investigator has done an excellent job in addressing the previous critiques providing very detailed responses and submitting a well described and focused plan. The only weakness is that the investigator did not adequately describe the spectrum of pathogens selected for the study. The Phase I was extremely successful and the progress met exceeded the original expectations. Overall the reviewers have high confidence and enthusiasm for this application.

DESCRIPTION (provided by applicant): Antibiotic resistance is a growing and increasingly serious public health problem. Infectious diseases caused by Escherichia coli and other bacteria are responsible for millions of deaths each year, and much of this mortality is due to the rise of antibiotic resistant organisms. Because antibiotic-resistant infections double the duration of hospital stay, mortality, and morbidity as compared with drug-susceptible infections, economic costs of antibiotic resistance are estimated to be in the billions of dollars. The overall goal of this project is to develop new anti-infectives that are highly effective and refractory to antibiotic resistance using a Combinatorial Genetic Technology (CGT) that allows the identification of new rRNA target sites and the specific nucleotides that are essential for functionality and viability, and RNA Homology Modeling software that allows accurate prediction of mutant RNA structures. Phase I of this project was highly successful. A functional mutation library of E. coli 16S rRNA was constructed and ~5000 viable clones were sequenced. Using CGT, 67 regions of E. coli rRNA that contain nucleotides essential for viability were identified. The 67 functionally important regions include known binding sites for antibiotics, tRNAs, proteins, the large ribosomal subunit and initiation factors. Also included were a number of sites that are clearly essential for ribosome function, but for which no functional role has been identified to date. Some of the individual regions occur near each other in 30S subunit crystal structures and probably contribute to a single functional role. The Phase II specific aims are 1) to select one RNA subdomain as a prioritized target from the four potential targets chosen from the RNA “regions of interest” identified in Phase I; 2) to use CGT to identify every mutation of the target that could lead to drug resistance, and use multidimensional NMR spectroscopy and homology modeling to determine the essential structural components of the target; 3) to screen compound libraries against the wild type target and its viable mutants; and 4) to carry out structural studies of target/hit complexes to allow optimization of hit compounds, and validate the target/compound using in vitro and in vivo assays of antibacterial activity. RiboNovix will complete the work necessary to develop drug candidates from the leads, and will move qualified candidates into pre-clinical development. Anti-infectives developed against the target identified in this study will likely be highly effective against microbial pathogens and resistant to target site mutation, thus resulting in drugs refractory to antibiotic resistance. Antibiotic resistance is a growing and increasingly serious public health problem. Infectious diseases caused by Escherichia coli and other bacteria are responsible for millions of deaths each year, and much of this mortality is due to the rise of antibiotic resistant organisms. The overall goal of this project is to develop new anti-infectives that are highly effective and refractory to antibiotic resistance.

CRITIQUE 1:

Significance: The continued battle against emergence of antimicrobial resistances necessitates novel approaches to drug discovery. In a very successful phase I study, researchers a RiboNovix used a novel Combinatorial Genetic Technology (CGT) for identification of new rRNA target sites that are essential for functionality and viability, and thus unlikely to tolerate mutations. The overall objective of RiboNovix’s research is to develop a ribosome inhibitor that is active against drug resistant strains, unlikely to generate high-level target resistance and be active against a wide range of bacterial pathogens. Such an antimicrobial would be of enormous medical importance and of high commercial value.

Approach: In a successful 12 month phase I study, 67 rRNA regions were identified that contain nucleotides essential for viability and function. These regions include known binding sites for antibiotics, tRNAs, proteins, the large ribosomal subunit, initiation factors, as well as other regions of unknown, but essential functions. The proposed Phase II studies are now focusing on four target RNAs, which will be evaluated for correct folding and same natural conformation that they would occupy in the complete ribosome. The choice of targets is well described and rationalized. Saturation mutagenesis, followed by CGT and molecular modeling will then be used to identify viable and functional mutants. These mutant variants will allow identification of compounds that are active against all possible sequence variants and are thus unlikely to elicit emergence of target mutations. The choice of various libraries – phage display, natural and synthetic compound, as well as libraries enriched for RNA binders - will ensure a high likelihood of success for identifying hits. These will then be tested in bacterial and prokaryotic *in vitro* translation assays. This is a great way to assess specificity for the bacterial ribosome and demonstrate activity on solvent accessible sites. Leads emerging from the screens and *in vitro* tests will then be tested for *in vivo* antibacterial activity against various pathogens.

While these are all significant strengths of the application, few weaknesses remain. First, although assessments of antibacterial activity, spectrum and selectivity are an integral part of lead selection in a Phase II study, the descriptions are cursory at best. The selection of pathogens used for these studies is suggestive, but not explained and rationalized. Although it is mentioned that compounds with poor *in vivo* activity will be modified by established medicinal chemistry techniques to increase cell wall permeability, no explanation will be given on how and when this will be done. Second, an evaluation of sequence conservation of targets among important pathogens should have been included as an indicator of the likelihood of finding broad-spectrum inhibitors.

Innovation: The proposed approaches to validate and screen rRNA targets are highly innovative. They are superior to traditional screening approaches because the likelihood of success in finding translation inhibitors that are highly efficacious, but at the same time unlikely to select for target mutations, is very high.

Investigators: For this Phase II study, the principal investigator Dr. Murphy has established an excellent team. Although a vast array of expertise will be required, these are mostly represented: CGT and ribosomal genetics (Dr. Cunnigham); structure modeling (Dr. SantaLucia); small molecule-RNA interactions (Dr. Chow, Dr. X and Dr. Y), chemistry (Dr. X
and Dr. Z) and support with screening of compound libraries (Dr. Chiang and Dr. Y). The only perceived weak link in the team is the absence of a dedicated microbiologist who would ensure that assessments of antibacterial activity, spectrum and selectivity can be completed in a timely manner. Dr. McNeil may be able to assist with these studies, but he is a cellular biologist by training and his tasks are too many already.

Environment: RiboNovix currently leases _ sft. of space and adequate equipment from another company in Lexington, MA, but apparently has options for expanding into an adjacent _sft. laboratory. It is not clear where the proposed structural chemistry hire would be housed and whether the appropriate equipment would be available. The facilities at Wayne State University and _ _ are very well equipped for execution of their share of the studies.

Progress in Phase I: The goals of the 12 month phase I study were completed and the results exceeded the original expectations. A functional mutation library of *E. coli* 16S rRNA was constructed and ~5000 viable clones were sequenced. This allowed identification of 67 regions required for ribosome function and viability, and led to identification of the four targets that form the starting point and basis for this Phase II application. The success of the phase I application instills confidence that the aims of the Phase II studies are technically feasible and can be completed with a high likelihood of success.

Response to Previous Review: For the most part, the principal investigator presents an excellent and very detailed response to the previous review.

Commercialization Plan: Given the early stage of discovery that this project is currently in, the commercialization plan more than adequately addresses the seven required points. The plan reflects an excellent knowledge of the field, competition and what it will take to secure long-term funding. It is recognized that the commercialization potential for this type of translation inhibitor is high, although the road to commercialization will still be very long.

Overall Evaluation: This is an excellent application by an accomplished research team which has the potential of identifying novel inhibitors against a proven bacterial target. A novel selection scheme minimizes the emergence of target mutations, which are commonly observed with other ribosome inhibitors. Despite a few weaknesses, including poorly described assessments of antibacterial activity, spectrum and selectivity, as well as medicinal chemistry attempts aimed at overcoming potentially poor *in vivo* activities, the enthusiasm for this application remains very high.

Vertebrate Animals: No animals will be used in this study.

Biohazards: Most of the experiments will be conducted with *E. coli* strains. However, *in vivo* assessments of antibacterial activity, spectrum and selectivity will involve several BSL-2 pathogens and although it is mentioned that a laminar flow hood is available it is unclear whether RiboNovix's facilities are properly certified and equipped to handle such pathogens.

Budget: No concerns.

CRITIQUE 2:

Significance: This is an application exploiting the conserved regions of ribosomal RNA to develop new drugs to combat a broad spectrum of diseases. The need for new anti-bacterial compounds, given drug resistance and emerging drug resistance is not only warranted but critical. The study is based on exploiting regions of 16S rRNA that are unlikely to be mutable, thus generating antibacterial compounds that would have decreased likelihood of resistance development. Clearly the need for such drugs is present and the utility to the medical community would be broad.

Approach: The approach is logical and straightforward. This phase ll application is attractive with respect to its design and potential products, and is strengthened immensely by the phase I data. Multiple immutable sites were identified in phase I which provide ample targets for the Phase II application. There are a number of anti-bacterial drugs currently on the market which exploit different aspects of ribosomes for effectiveness. This application is unique in its use of superb modeling systems to identify conserved regions of 16S rRNA that for functional reasons cannot be altered. The approach could be broadened for further exploitation. Milestones are well described and the specific aims are clear and rational.

Innovation: This is a highly innovative application. Its innovation is based on conserved ribosomal sequences. Exploitation of these sequences could lead to new anti-bacterial drugs refractory to resistance. Drug resistance is a significant or more likely the highest antibiotic related priority to date. The production of new anti-bacterial compounds refractory to drug resistance would be a major advance in the repertoire of antimicrobials. Directing this approach towards other organisms including eukaryotic infective agents could make a significant positive worldwide health impact.

Investigators: Dr. Murphy the principal investigator is well qualified to direct the proposed work. Additionally, she appears well organized and directed, having assembled an impressive team of individuals in the appropriate areas of needed expertise.

Environment: RiboNovix has leased appropriate laboratory facilities to complete the majority of the proposed work on site. This had been a significant criticism in the last submission and has been adequately dealt with. Appropriate collaborators and consultants are available.

Progress in Phase I: Progress in phase one has been significant and more rapid and successful than originally anticipated.

Response to Previous Review: The response to previous reviews is more than adequate. The Principal Investigator has responded to all of the comments made by previous reviewers. Inclusive are 1: compound library description (which all three previous reviewers criticized) 2: Information and clarification on screening and structural characterization methodology, and inclusion of *in vivo* studies 3: compound screening descriptions 4: assessment of antibacterial activity 5: Commercialization plan 6: Ribonovix laboratory facilities. All criticisms have been dealt with in a more than adequate manner.

Commercialization Plan: The commercialization plan is sound.

Overall Evaluation: This is a well written, very detailed application. The depth and detail of the application is laudatory and reflects a mastery of knowledge regarding antibiotic development and commercialization. The data from phase I are impressive, and patents have been or are being applied for. Given the previous weaknesses have been addressed in a thorough manner, enthusiasm for this application is high.

Biohazards: No concerns

Budget: The budget remains high for a Phase II SBIR but is justified given the limited resources currently available to RiboNovix.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

MEETING ROSTER

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CENTER FOR SCIENTIFIC REVIEW
ZRG1 IDM-Q (10)
March 09, 2006 - March 10, 2006

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.