SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **12/23/2010**

Jim Turpin

[redacted]

Application Number: **1 R21 AI094511-01**

Principal Investigator

DEWHURST, STEPHEN PHD

Applicant Organization: UNIVERSITY OF ROCHESTER

Review Group: **ZAI1 RB-A (J1)**
National Institute of Allergy and Infectious Diseases Special Emphasis Panel
Microbicide Innovation Program (MIP VI) (R21/R33)
AIDS - EXP. REV.

Meeting Date: **12/02/2010** ***RFA/PA:*** **AI10-011**

Council: **JAN 2011** ***PCC:*** **A22B**

Requested Start: **03/01/2011**

Dual IC(s): **MH**

Project Title: **The Semen Enhancer of HIV Infection as a Novel Microbicide Target**

SRG Action: **Impact/Priority Score:** [redacted]

Human Subjects: 30-Human subjects involved - Certified, no SRG concerns

Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

[redacted] **3A-Only men, scientifically acceptable**

Minority: **1A-Minorities and non-minorities, scientifically acceptable**

Children: **1A-Both Children and Adults, scientifically acceptable Clinical Research - not NIH-defined Phase III Trial**

Project Year	Direct Costs Requested	Estimated Total Cost
1		[redacted]
2		
3		
4		
5		
TOTAL	[redacted]	

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.

1R21AI094511-01 Dewhurst, S.

RESUME AND SUMMARY OF DISCUSSION: This excellent new R21/R33 application entitled “The Semen Enhancer of HIV Infection as a Novel Microbicide Target” is submitted by the University of Rochester, Rochester, NY, Dr. Stephen Dewhurst, PI, in response to RFA-AI-10-011, “Microbicide Innovation Program (MIP VI) (R21/R33).” Other participating institutions include the University of California, San Diego (Dr. Jerry Yang), [redacted]), and the University of Pittsburgh (Dr. Charlene Dezzutti). The goal of this application is to evaluate amyloid-binding small molecule inhibitors of semen enhancers of viral infection (SEVI) as potential microbicide candidates for blocking sexual transmission of HIV. The objective of the R21 phase is to test whether novel amyloid-binding small molecules inhibit semen-mediated enhancement of HIV infection (Specific Aim 1) and to examine the interaction between novel amyloid-binding small molecules and cells from the female reproductive tract (Specific Aim 2). The R33 phase will continue to improve the efficacy of first generation compounds identified in the R21 phase (Specific Aim 3) and to assess the toxicity and inflammatory effects of lead molecules (Specific Aim 4). Strengths of the application include the significance of targeting a host factor – the Semen Enhancer of Virus Infection (SEVI) – rather than HIV or epithelial barriers. This approach offers the potential advantage of synergism when combined with other microbicides that target HIV or epithelial barriers. The connection with Alzheimer’s disease research and the use of amyloid binding molecules as inhibitors of SEVI is particularly innovative. Sufficient preliminary data support the investigators’ promise of success in spite of an otherwise ambitious research plan. The approach is logical, addressing both efficacy and toxicity. Importantly, the investigators will evaluate any potential toxicity to beneficial lactobacillus species. The investigators are well suited to conduct the proposed work and have some history of collaboration. They are considered innovators in their respective fields and have complementary expertise. The milestones are quantitative with clear go-no-go decision points. The research environment is strong, with adequate space and facilities. The University of Rochester and the University of California, San Diego are committed to research on women’s health. Weaknesses that reduce enthusiasm include skepticism among some reviewers about the significance of targeting SEVI, since there is limited literature to support SEVI’s effects on HIV transmission in vivo. In addition, because SEVI is not required for HIV transmission, inhibition of SEVI may reduce HIV infection, but it may not prevent HIV infection. Some reviewers recommend the addition of an animal model to study the efficacy of SEVI inhibition on HIV transmission. The use of cell lines, cervical explants, and the rabbit vaginal irritation model for toxicity testing is not considered especially innovative. Furthermore, the explant experiments are only using one type of tissue; additional surfaces should be tested in explant systems. Based upon the evaluation of scientific and technical merit, this application received an Overall Impact/Priority score of 30. Supplementary materials received from the applicant were made available to the review committee.

DESCRIPTION (provided by applicant): Human semen contains cationic amyloid fibrils, termed the "Semen Enhancer of Virus Infection" (SEVI), which strongly enhance HIV-1 infection and may play an important role in viral transmission. Our preliminary data show that amyloid-binding molecules bind to SEVI, and block semen-mediated enhancement of HIV-1 infection. This suggests that (i) SEVI is responsible for semen-mediated enhancement of HIV infection and (ii) SEVI represents a novel microbicide target. We therefore propose to explore a novel, innovative approach to HIV-1 microbicide development, using agents that selectively target SEVI. This high-risk/high-reward approach is fundamentally different from traditional microbicidal strategies that target the virus itself, and is expected to be highly complementary with direct antiviral approaches. Indeed, our long-term goal is to use SEVI-targeting agents in combination with traditional microbicides, to achieve optimal antiviral effectiveness. In the R21 phase, we will test whether novel amyloid-binding small molecules inhibit semen-mediated enhancement of HIV infection. The feasibility of this approach has been established using two amyloid-binding small molecules which contain "shielding" oligo-ethylene glycol (EG) moieties: BTA-EG4 and -EG6. These agents efficiently inhibit SEVI- and semen-mediated enhancement of HIV infection. In Aim 1, we will generate and test novel derivatives of these and other amyloid-binding molecules, including oligovalent molecules that are expected to possess increased

SEVI binding affinity. We will then test their ability to inhibit SEVI- and semen- mediated enhancement of HIV infection using a panel of R5 virus strains (including different clades and transmitted strains). In Aim 2, we will examine the interaction between novel amyloid-binding small molecules and cells from the female reproductive tract. We will evaluate whether our compounds are toxic to human cervicovaginal epithelial cells (HCEC), and we will test whether they inhibit SEVI-enhanced binding of HIV-1 to HCEC and/or SEVI-enhanced trans-infection of PBMC by HCEC exposed to HIV-1 virions. The R33 phase will be undertaken only if well-defined milestones are achieved. In Aim 3, we will use structure-activity relationship (SAR) data to refine our chemical compositions. We will also test whether our lead molecules have efficacy in a cervical explant model for HIV-1 infection, and whether they have a synergistic or additive effect on the ability of other candidate microbicides to inhibit HIV-1 infection in the presence of semen. In the final Aim, we will assess the toxicity and inflammatory effects of the most promising candidate molecules, using beneficial Lactobacillus strains and cervical explants. The R33 phase will culminate with an evaluation of the safety and tolerability of the most promising compound in the rabbit vaginal irritation (RVI) model. The overall goal of these studies is to carefully determine whether small molecules that target SEVI have potential utility as a novel class of microbicides.

PUBLIC HEALTH RELEVANCE: New approaches to prevent the transmission of human immunodeficiency virus type-1 (HIV-1) are urgently needed. This application seeks to develop a new class of microbicidal agents that are targeted not to the virus itself, but to a host protein found in semen that strongly enhances HIV-1 infection. This high risk, high reward approach is fundamentally different from traditional microbicidal strategies that target the virus, and is expected to be highly complementary with direct antiviral approaches.

CRITIQUE 1:

Significance:	3
Investigator(s):	1
Innovation:	2
Approach:	3
Environment:	1

DESCRIPTION (The primary reviewer may enter brief non-evaluative description here): In this application, Dr. Dewhurst and colleagues have put forth four major aims that will evaluate inhibitors of semen enhancers of viral infection (SEVI) as potential microbicide candidates to block sexual transmission of HIV. In the R21 phase of their investigations, they will make derivatives of two existing amyloid-binding small molecules and test their SEVI-binding efficacy, and their ability to inhibit SEVI and semen-mediated enhancement of HIV infection by a broad range of R5 HIV strains. In Specific Aim 2, they will address potential toxicities of their candidate amyloid-binding molecules in endocervical and vaginal cell lines. They will then assess the ability of their candidate microbicide molecules to block binding of HIV to vaginal and endocervical cells, to block HIV transmission to PBMCs in trans across these cells, and to illicit advantageous or harmful cytokine secretion from these genital epithelial cells.

In the R33 portion of the application, the investigators will continue to refine candidate molecules and will retest any new candidates as above. In addition, they will test defined amyloid-binding candidates for stability in vaginal fluids, in semen, and in a delivery placebo gel. They will also use novel toxicity assays and efficacy algorithms to select promising compounds for testing in cervical explant models. The investigators will evaluate whether amyloid-binding molecules can synergize with compounds from other microbicide classes in inhibiting HIV transmission in the presence of semen. Finally the R33 phase of the investigations move toward toxicity testing, including effects on epithelial integrity, toxicity to lactobacilli, effect on cervical explant inflammation, and effects in a rabbit vaginal irritation model.

Overall Impact:
The proposed investigations led by Dr. Dewhurst are novel and his preliminary data and planned investigations are very promising. The application itself is clear and concise and a pleasure to review. The approach is innovative. If a SEVI-inhibiting microbicide candidate is identified that has limited local toxicities, this compound has the potential to be used to block transmission from male to female and from male to male. Its mechanism of action would predict it may be additive or synergistic when combined with anti-viral or epithelial barrier enhancing approaches.

The application addresses potential pitfalls and alternative approaches well. It includes realistic future directions. The scope of work may be a bit optimistic. There is a limited amount of literature available to indicate that SEVI have dramatic effects on in vivo transmission.

1. Significance:
Strengths:

- The investigation of novel inhibitors of SEVI is a promising approach in microbicide development.
- Targeting enhancers of transmission in the semen should have applicability to both heterosexual and same-sex transmission.
- This novel approach is likely to synergize with microbicide approaches that target the virus or the epithelial barrier.
- Preliminary data are encouraging that a product will be identified that will decrease HIV transmission in vivo and the rigorous toxicity testing will help to avoid untoward effects.

Weaknesses:

- The project is a bit over-ambitious, although the preliminary data are encouraging and may predict success
- There is a limited amount of literature available to indicate that SEVI have dramatic effects on in vivo transmission.

2. Investigators:
Strengths:

- Drs. Dewhurst, Feng, Yang and Dezzutti are well-suited to carry out the proposed investigations. They are true innovators in the field. Their strengths complement each other and synergistic collaboration is likely.
- Inclusion of a company subcontract is appropriate.

Weaknesses:

- No weaknesses are noted.

3. Innovation:
Strengths:

- The concept presented here is highly innovative, targeting a semen-derived enhancer of viral transmission rather than the virus itself or the epithelial barrier across which transmission might occur.
- Unlike other SEVI inhibitors, amyloid-binding molecules are predicted to be more effective and have less bystander effects than those that inhibit SEVI through electrostatic effects. The preliminary data support this and the experiments planned are likely to generate

compounds with improved efficacy when compared with those used to generate the preliminary data.

Weaknesses:

- The use of cell lines and cervical explants and of the rabbit vaginal irritation model for toxicity testing is not particularly innovative.

4. Approach:

Strengths:

- Dr. Dewhurst and colleagues have taken a very logical approach to testing existing SEVI inhibitors and to developing improved compounds.
- The investigators have taken great care to assess both efficacy and potential toxicities of their candidate compounds.
- The models used to test efficacy and toxicities are several and include cell lines from the vagina and endocervix, as well as cervical explants and an in vivo toxicity model. The testing of toxicity to beneficial lactobacillus species is a welcome inclusion.
- Plans for future partnership for an SBIR or STTR are appropriate.

Weaknesses:

- The investigators will likely be using explant cultures that are only representative of a single cervical surface. Whether that be the squamous epithelium, the transformation zone or the endocervix, the other areas should be tested as well. There is relatively good evidence that transmission across the endocervix is likely to occur across the transition zone and/or endocervix, rather than the commonly studied ectocervix.
- The investigation is a bit expansive and will not proceed to the R33 phase if a good candidate is not found. Preliminary data, however, make this possibility less worrisome.

5. Environment:

Strengths:

- The University of Rochester and The University of California San Diego have a long history of strong commitment to women's health research.
- Drs. Dewhurst and Yang have adequate space and facilities to perform the proposed investigations.
- Statistical support is strong.
- The inclusion of the [redacted] collaboration ensures all facilities and techniques are available for the proposed studies.

Weaknesses:

- No weaknesses are noted.

Milestones:

The milestones in this grant are very well presented. They are quantifiable and present defined go-no go decisions. The milestones for the R21 portion of the proposal are reasonable to the time and effort proposed. The R33 milestones are likewise well-defined and future plans are included and follow logically from the planned experiments.

CRITIQUE 2:

Investigator(s):	1
Innovation:	3
Approach:	5
Environment:	1

Overall Impact:
Exploring agents that selectively target SEVI differs from approaches that target the virus. The long-term goal of combining SEVI-targeting agents with traditional microbicides might lead to a useful product. The R21 phase establishes whether this class of molecules will work. The R33 phase will develop more potent agents. The combination of a virologist and synthetic chemist will work well in producing several candidate compounds.

1. Significance:
Strengths:

- There is significance in developing an agent that inhibits HIV infectivity that is enhanced by amyloid fibrils found in semen.
- This is a novel approach that could lead to a new paradigm for microbicides.

Weaknesses:

- The approach to compound development is broad-based.
- There is concern as to whether the approach is relevant in vivo.

2. Investigators:
Strengths:

- The Principal Investigator is an established AIDS investigator.
- The inclusion of an organic synthetic chemist is a strength.
- The inclusion of an established expert in microbicide development is a strength.

Weaknesses:

- No weaknesses are noted.

3. Innovation:
Strengths:

- The non-viral target could lead to developing a new paradigm for treating viral infections.

Weaknesses:

- The Principal Investigator should better address issues of combining the SEVI inhibitors with other agents.

4. Approach:
Strengths:

- The combination of virology and organic chemistry should lead to new compounds.
- Plans to test compounds from Alzheimer's work on amyloids is a strength.
- Plans to use polyvalent drugs is a strength.

Weaknesses:

- In the text of the application, log P's for BTA-EG4 and BTA-EG6 are switched. Is this a typographical error?
- The rationale for small molecules (i.e., Lipinski's Rule) appears to contradict justification for polyvalent compounds.
- Lipinski's Rule applies to the orally administered drugs that cross the blood-brain barrier and not microbicides.

5. Environment:
Strengths:

- The facilities are excellent.

Weaknesses:

- No weaknesses are noted.

CRITIQUE 3:

Significance:	1
Investigator(s):	1
Innovation:	1
Approach:	3
Environment:	1

Overall Impact:
This application is extremely exciting and interesting with the potential to create a substantial shift in the microbicide paradigm. The current observations are solid and support continued study and development of the approach that could yield a new panel of compounds that likely would combine with other lead microbicides for a multi-functional prevention method. The approach is logical and should be accomplished within the allotted funding period. The team is considered an expert group for the work. The application is highly innovative and has high significance. Minor modifications to the research approach are all that would be required to make this an exceptional grant application.

1. Significance:
Strengths:

- Disrupting the seminal fluid enhancement of HIV infection is a significant outcome of novel research avenues including the one described in this application. It would create a significant reduction in transmission events and could help protect females who carry the disproportionate burden of HIV infection and disease.
- The use of compounds being explored as anti-Alzheimer's Disease (AD) therapies is extremely significant and intriguing. The recognition of similar chemical structures (amyloid fibrils) is of great significance and will create the opportunity for field crossing outcomes.
- If successful, the inhibition of SEVI could be used in combination with antiviral microbicides and is less likely to allow for emergence of viral resistance.
- This application represents a truly high risk/high reward project that is based on extremely solid and intriguing preliminary findings. Although the actual contribution of SEVI during natural infections has yet to be quantified, the potential outcome of this work is extremely significant.

Weaknesses:

- The actual contribution of SEVI to HIV transmission is not well quantified and although any reduction is a likely good outcome, the amount of prevention based on SEVI inhibition remains an unanswered question. This is a central issue with this application and the significance of SEVI as a microbicide target. The preliminary data with the two EG shielding compounds supports continued development.

2. Investigators:

Strengths:

- Dr. Dewhurst is the PI on the application and is Professor and Chair of Microbiology and Immunology at the University of Rochester School of Medicine and Dentistry. Co-investigators include Dr. Yang, UCSD, and [redacted], a senior scientist at [redacted]. Dr. Dezzutti, Associate Professor at the University of Pittsburgh, will serve as a consultant.
- Parts of the group have an established collaboration. The project is a direct result of one of the ongoing collaborations.
- Dr. Dewhurst is a productive researcher specializing in HIV infection and pathogenesis in the nervous system. This is complimentary to the work of Drs. Yang (inhibitor compound chemistry), [redacted] (microbicide development and animal testing) and Dezzutti (cervical and rectal cell culture explant models for microbicide testing).
- Descriptions of weekly lab meetings and data exchange plans are included. Virtual lab meetings are also described and this is considered a strength given the geographic distance among the research team sites.

Weaknesses:

- No weaknesses are noted.

3. Innovation:

Strengths:

- The goal of targeting/blocking a host virus interaction by inhibitors of a host target is highly innovative and represents a novel use of known compounds from the family of benzothiazole anilines (BTAs) currently being evaluated as therapies for Alzheimer's Disease because they bind B-amyloid.
- The application brings diverse fields of research (AD research) to the attack against HIV transmission.
- The identification of SEVI specific binding small molecules is innovative and should reduce off-target effects seen with other of the non-specific compounds (e.g. PRO-2000).

Weaknesses:

- Inhibition of the seminal enhancer of HIV infection (SEVI) is innovative but not necessarily novel. It could lead to a substantial means of reducing transmission from the infected male but there is a limited amount of data and what is available indicates SEVI enhances but is not required for HIV infection. At best, an inhibitor of SEVI would reduce but not prevent infection.

4. Approach:

Strengths:

- The application is exceptionally well written and effectively designed. The screening paradigm (Figure 11) modified from [redacted]. is logical and should yield a set of prioritized compounds for testing. The workflow plan from Aim 2 forward is logical and well defended with preliminary data or Microbicide Trial Network approval.

- Testing of multiple HIV isolates including MDR strains in the context of heterosexual transmission models is a solid plan to identify effective SEVI inhibitors. Aim 2 studies include three aspects of genital mucosal epithelial cells that are all potential contributors to HIV infection. Importantly, the inclusion of potential impact on cytokine/chemokine release is considered.
- A strong plan for data analyses, including the inclusion of a biostatistician, strengthens the application. Overall, the design is likely to yield a number of high priority compounds that will be screened in the rabbit vaginal irritation model in advance of any additional development. The approach is solid and considered a workable approach to successful identification of lead compounds for future evaluations.

Weaknesses:

- Aim 3 of the R33 phase is complex and dense and may create analysis issues due to its breadth. It could easily have been two distinct aims. If the first portion of the work is to create an additional 900 compounds for screening, why are the synthesis plans included in Aim 1?
- A lack of plans for studying the impact of the compounds on X4 virus isolates is not well justified and reduces enthusiasm.
- The primary efficacy assay in Aim 1 will likely miss good candidates but will allow for higher throughput, inexpensive screening analyses. The lack of quantifiable metrics to this screening system adds to the complexity of the review of later aims. How many is “a subset of compounds” with TI>10, etc.? How many compounds are to be screened initially? What is the predicted ‘hit rate’? More information is provided in Aim 3 but the predicted hit rate and the maximum number of selected compounds is still not provided.
- Although valuable data are presented, the A2En and SiHa cells do not represent ideal tissue types for HIV infection/seminal fluid exposure evaluations. It should also be clearly stated that the A2En cells should never represent the vaginal tract (page 99) given that they are entirely different type of epithelia. As noted in Aim 2B, if the binding of HIV virions to vaginal epithelial cells leads to cytokine release, the ideal cell cultures would be vaginal raft cultures rather than immortalized endothelial or cervical carcinoma cells. Later aims change to using cervical explants. The PI could have selected more similar cell types that are either immortalized or primary in nature without reducing throughput. This is considered a minor concern based on the lead compound(s) evaluation plan.
- While testing of the lead compound(s) against commensal bacteria is important, adding the material to MRS broth will produce data of limited value given that this is not the normal environment for these bacteria. The broth likely will change the behavior of the compounds in ways that are not predictive of anything in vivo. Similarly, the studies proposed in Aims 4B and 4C are redundant and are not described as a screening process. If Aim 4B is to eliminate some compounds prior to testing in Aim 4C, it should be more clearly stated.

5. Environment:

Strengths:

- The environments at each study site are considered strong with appropriate facilities, practices and equipment in place for all proposed work.

Weaknesses:

- No weaknesses are noted.

Milestones:

An extremely strong set of milestones is provided with quantitative endpoints that should clearly provide go/no go decision points. The only negative is that the goals are set relatively low given the strength of

the preliminary data that is included in the application. It is likely that if the R21 is awarded this research team will advance to R33 given the already identified lead compounds with activity.

Vertebrate Animals: Rabbits will be used at the end of the R33 period to test the lead compound's toxicity in the vaginal irritation model. Although standard, this use of vertebrate animals is less successful with IACUCs (approval is noted as pending) and should be refined if this application is funded. Otherwise, the information provided is acceptable.

Budget Recommendations: [redacted] personnel and costs seem more substantial than other aspects of the application.

CRITIQUE 4:

The purpose of developing microbicides that target SEVI-like activity remains weak until the biologic relevance of this activity can be assessed in vivo. Enthusiasm would have been higher if the R33 phase included a proposal to develop an animal model that could demonstrate the enhancing effect of semen when added at physiologically appropriate levels to the viral inoculum.

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:

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE (Code 30) The descriptions of the work at each site involving human subjects is provided and is very well written.

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE (G3A) No women are involved because the human subject involvement consists of semen collection.

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE (M1A)

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE (C1A)

VERTEBRATE ANIMAL (Resume): ACCEPTABLE (Code 30)

BIOHAZARDS COMMENT: ACCEPTABLE Adequate facilities and policies are described for the work with biohazards.

FOREIGN INSTITUTION: NOT APPLICABLE

SELECT AGENTS: NOT APPLICABLE

RESOURCE SHARING PLANS: ACCEPTABLE

BUDGETARY OVERLAP: NOT APPLICABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-10-080 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-10-080.html.

The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.