

Specific Aims

Multiple myeloma (MM) is an incurable cancer of plasma cells. The bone marrow microenvironment is critical in the pathogenesis and progression of MM and recent improvements in MM survival are due to drugs that target the bone marrow microenvironment¹⁻⁴. Transforming growth factor- β (TGF- β) is a multi-functional growth factor elaborated by MM cells, immune cells, and by bone and bone marrow stromal cells. TGF- β supports progression of MM through its role in stimulation of IL-6 production, Th17/T reg T cell development, angiogenesis, hematopoietic suppression, and its inhibition of terminal osteoblast differentiation and stimulation of osteoclast survival⁵⁻¹¹. These actions contribute to the osteolytic bone disease and immune dysregulation which characterize MM progression and its associated morbidity. *Despite the known importance of TGF- β in myeloma bone disease and immune dysregulation, there are no clinical trials of TGF- β antagonists for MM.* Most TGF- β antagonists broadly target the active ligand, receptors, or downstream kinases and do not distinguish between homeostatic and disease-induced TGF- β , raising the potential for adverse effects (inflammation, carcinogenesis) with chronic use of TGF- β inhibitors. We have developed a unique approach to selectively inhibit disease-related TGF- β in MM through *targeting only the TGF- β which is activated through binding to the extracellular matrix protein, thrombospondin1 (TSP1)*. TSP1 is a secreted and extracellular matrix (ECM) protein, which controls TGF- β activity in disease by binding and activating latent TGF- β ^{12, 13}. TSP1 is increased in the MM microenvironment and it is upregulated by factors associated with MM progression (IGF-1, IL-6, TGF- β)¹⁴⁻²⁰. Our studies show that TSP1 activates latent TGF- β expressed by human and mouse MM cells *in vitro* and importantly, a tetrapeptide antagonist (LSKL) of TSP1-dependent TGF- β activation reduces MM tumor burden, stromal IL-6, and osteolytic bone disease in mouse models of MM in SCID mice. LSKL nearly completely blocks active TGF- β in bone marrow cells of treated MM mice, indicating that the TSP1-TGF- β antagonist peptide is working through targeting TSP1-dependent TGF- β activation in the bone marrow microenvironment. **These data establish that TSP1 is an important regulator of TGF- β activity in MM and suggest that blockade of this pathway represents a novel and selective therapeutic strategy to reduce MM progression and bone disease.** With the goal of developing a “druggable” form of the LSKL peptide for treatment of MM, we have focused on identifying lead molecules with improved metabolic stability and oral availability. We have identified a lead compound (SRI31277) based on LSKL which has dose-dependent *in vivo* activity in a mouse model of MM. SRI31277 has an improved *in vitro* and *in vivo* plasma half-life (10.6 hrs) and oral availability with properties amenable to optimization. SRI31277 will be the basis for further optimization to develop lead drugs with an extended oral T_{max} and increased oral bioavailability. Specifically, the goal of the lead optimization effort is to identify compounds based upon SRI31277 that have an extended T_{max} of 4-6 hours and absolute oral bioavailability of 60 -70%.

This proposal will combine mechanistic studies (Aim 1) with drug development (Aim 2) to achieve our goal of identifying an orally active lead compound for treatment of MM. In Aim 1, we will further determine the role of the TSP1-TGF- β pathway in MM through use of immune competent and TSP1 null models, by comparison of SRI31277 to a global TGF- β inhibitor and by use in drug combinations, and by complementary *in vitro* studies to define this pathway in MM cell cytokine production and osteoblast/osteoclast regulation²¹⁻²³. The key goal of Aim 2 is to identify an orally active derivative of SRI31277 and a back-up peptide mimetic/small molecule suitable for GLP-IND enabling studies.

Specific Aim 1: To determine the mechanisms by which inhibition of TSP1-dependent TGF- β activation reduces MM progression.

- We will determine the role of TSP1-TGF- β inhibition on T cell subsets (T reg/Th 17), on dendritic cell function, and on angiogenesis in the immunocompetent 5TGM1 syngeneic mouse model and
- the role of stromal TSP1 in the transplantable Vk*MYC model on the TSP1 null background.
- Antagonism of TSP1-dependent TGF- β activation will be compared to global TGF- β antagonism.
- We will determine potential synergy of SRI31277 in combination with current MM therapeutics (dexamethasone, bortezomib) in the 5TGM1 model.
- We will perform *in vitro* studies to identify TSP1 MM cell surface receptors involved in TGF- β activation, the role of TSP1-TGF- β blockade on MM cell IL-6, RANKL, VEGF, and Dkk1 expression and the impact on MM cell-mediated osteoblast/osteoclast formation in co-cultures with bone marrow stromal cells. We will use different human and mouse MM cell lines and primary cells to address MM heterogeneity.

Specific Aim 2: Identify an orally active inhibitor of TSP1-TGF- β activation for the treatment of MM and osteolytic bone disease. The goal is to identify and evaluate an orally active derivative of our current lead compound SRI3127 and a back-up peptide mimetic/small molecule suitable for GLP-IND enabling studies.

The reviewers recognized strengths such as significance, logical specific aims with high quality preliminary data, experienced investigator, excellent environment, and the innovative new approach to multiple myeloma (MM) with the potential for therapeutic application. The major concerns as outlined have been addressed with a new, focused drug development plan, new data from 3 animal studies, and by clarifying TSP1/TGF- β biology.

1. The major weakness was an underdeveloped plan for therapeutic development of LSKL peptide.

Aim 2 is completely revised to focus on identifying an orally active inhibitor of TSP1-TGF- β activation based on our current lead compound SRI31277, which has a plasma $t_{1/2}$ of 10 hrs and oral availability. SRI31277 retains activity with modifications including a D amino acid. We will both develop additional peptide analogues of SRI31277 and also develop small molecule peptide mimetics based on this structure. Leads will be evaluated for toxicity, in vitro ADME, and in vivo pharmacokinetics. Dr. Mark J. Suto, VP Drug Discovery, SRI, is our collaborator on this aim.

2. Lack of in-depth approaches/further evaluation of cytokine responses, impact of blocking TGF-beta on IL-6
New data show that LSKL reduces mouse IL-6 levels *in vivo*. We will use both IL-6 dependent (INA-6) and independent (U266) MM cell lines in Aim1.e.2 to determine the importance of TSP1-activated TGF- β on IL-6 regulation of MM growth, IL-6-dependent signaling (STAT3, Akt, ERK), and *in vitro* bone remodeling.

3. Lack of incorporation of alternative approaches to block TGF-beta activation such as genetic approaches (TSP-1 null) to complement the peptide approach In new Aim 1.b, we will use newly available Vk*MYC mouse MM cells (on a C57B/16 background) (gift of Leif Bergsagel, Mayo) and transplant them into *Thbs1* null mice. The Vk*MYC cells have very low TSP1 expression; this model will discern the role of stromal TSP1 in MM. This remains a high risk study because of loss of TGF- β -independent functions of TSP1 (such as angiogenesis inhibition and a developmental bone phenotype) which could confound results. However, these studies have the potential to identify off-target effects of SRI31277. The caveats are extensively discussed.

4. Lack of consideration of additional readouts for the TGF- β responses. We use the only accepted biological readout of TGF- β activity: we assess Smad activity via phospho-Smad 2 or 3 by IHC or western blot in tissues and TGF- β specific reporters *in vitro*. We also use an ELISA specific for active TGF- β for *in vitro* peptide activity. TGF- β protein or mRNA provides no information on activity. Readouts of cellular effects of TGF- β (IL-6, IGF-1, VEGF, growth, osteoblast/clast differentiation, MM-stromal cell adhesion) are inherent in the studies.

5. Concern regarding efficiency of growing primary myeloma specimens in the animal model and additional markers. This is a valid concern, although reviewers 2 and 3 thought this was a strength. Nonetheless, primary human MM cells could provide important insights into peptide effectiveness in heterogeneous samples. We will only attempt to establish 2-3 primary human cells for use *in vitro* and will instead use currently available human MM lines or primary cells from Dr. K.C. Anderson. MM cells are defined as CD138+ bone marrow cells and this is standard protocol: we will isolate CD138+ CD45- cells to add a second marker.

6. Lack of consideration and concrete research plan to address the issue that activation of TGF-beta is only one of the many factors in MM and the issue of heterogeneity of MM cells as well as their microenvironment.

Yes, TSP1-TGF- β activation is unlikely the sole cause of MM progression. Nonetheless, our data strongly support a key role for TSP1-TGF- β regulation in the CAG-heparanase SCID MM model. The peptide is as effective as dex. The new combination studies in Aim 1.d will examine possible synergistic effects between SRI31277 and currently used MM drugs (dexamethasone, bortezomib) using suboptimal concentrations of SRI31277 and the other drugs. Syngeneic mouse models, cell lines, and primary cells address heterogeneity.

7. Redundancy of activation mechanisms. LSKL-treated mice showed near complete inhibition of Smad phosphorylation in the bone marrow in two models and acLSKLNH2 reduces tumor burden to a similar degree as 1 mg/kg dex, indicating that TSP1-TGF- β activation is a significant pathway in MM. In new Aim 1.c, we will use a TGF- β receptor kinase inhibitor and compare global TGF- β inhibition to TSP1-specific inhibition *in vivo*.

8. Radiographs for effects of peptide on osteoblast activity μ CT data, serum TRAP5b levels, and osteoclast counts support reduced osteoclast activity in peptide-treated mice (new Figures 5,6,8, Table 1). Osteocalcin positive osteoblasts are increased in peptide-treated mice (new Figure 5). Peptide effects on MM cell-bone differentiated MSCs will be tested *in vitro*.

9. If you block TGF- β activation, will Dkk1 still block osteoblasts and will RANKL still stimulate osteoclasts?

Blocking TSP1-TGF- β activation improves bone mass *in vivo* (increased osteoblasts and reduced osteoclasts) in our model using MM cells which express Dkk1. Others showed that TGF- β inhibition increased osteoblast differentiation by stimulating BMP-2 signaling, even though TGF- β inhibition did not affect MM cell inhibition of Wnt signaling. In Aim 1.e.4, we will study LSKL effects on Wnt signaling, Dkk1 levels, and BMP-2. Blocking TGF- β reduces RANKL and we will measure RANKL.

Except for Aim 2, which is entirely new, other major changes are indicated with a line in right margin.

SUMMARY STATEMENT
(Privileged Communication)

Release Date: 03/10/2014

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Application Number: 1 R01 CA175012-01A1

Principal Investigator

MURPHY-ULLRICH, JOANNE E PHD

Applicant Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Review Group: ZRG1 BMCT-C (01)
Center for Scientific Review Special Emphasis Panel
Molecular Targets for Cancer Intervention

Meeting Date: 02/24/2014

RFA/PA: PA13-302

Council: MAY 2014

PCC: F2BP

Requested Start: 07/01/2014

Project Title: The thrombospondin1-TGF-beta axis in multiple myeloma

SRG Action: Impact Score: 26 Percentile: 9

Next Steps: Visit http://grants.nih.gov/grants/next_steps.htm

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

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

Gender: 1A-Both genders, scientifically acceptable

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

Children: 3A-No children included, scientifically acceptable
Clinical Research - not NIH-defined Phase III Trial

Project Year	Direct Costs Requested	Estimated Total Cost
1		
2		
3		
4		
5		
<hr/>		
TOTAL		

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.

1R01CA175012-01A1 MURPHY-ULLRICH, JOANNE

RECOMMENDATE BUDGET MODIFICATION

RESUME AND SUMMARY OF DISCUSSION: The applicant proposes to test the hypothesis thrombospondin1 (TSP1) is acting to increase biologically active TGF- β in multiple myeloma (MM) and that the LSKL peptide represents a selective therapeutic approach to target the "bad" TGF- β which contributes to MM progression. If successful, this study could potentially validate blockade of the TSP1-TGF- β pathway as a treatment for MM and provide the basis for development of the LSKL (four amino acid) peptide as a therapeutic intervention. A number of strengths of the application were discussed including the novel strategy for targeting the interaction between TSP1 and the inactive form of TGF-beta for suppression of MM, the logical experimental design, and the availability of SRI3127 analogues, as well as the well qualified PI along with an excellent research environment. Furthermore, the revised application has improved in response to prior concerns. During the discussion, the reviewers also discussed some of the weaknesses. These weaknesses included the lack of preliminary data regarding the survival of myeloma bearing mice with the treatment either LSKL or SRI3127, the safety or efficacy of available SRI3127 analogues, and the lack of data for supporting the important premise that the blockade of TSP1-dependent activation of TGF-beta would not affect the anti-angiogenic activity or Nitrous Oxide regulation by TSP1. Following the discussion, the review panel concluded that the overall strengths of the proposal outweighed the minor concerns. The review panel remarked that TSP1 is an appropriate target for MM given the nature of rich TSP1 in the tumor microenvironment and the proposed study has potential therapeutic implications. Overall the committee expressed a relatively high level of enthusiasm for this application and rated this application in the outstanding to excellent range.

DESCRIPTION (provided by applicant): Multiple myeloma (MM) is an incurable cancer of plasma cells that is dependent on the bone marrow microenvironment for progression. Transforming growth factor-beta (TGF- β) is a multi-functional growth factor elaborated by MM cells and by cells in the bone marrow microenvironment. TGF- β stimulates MM progression through promotion of catabolic bone remodeling, IL-6 secretion, and Th17 T cell development, leading to osteolytic bone disease and immune dysregulation. Despite the importance of TGF- β in MM, there are no clinical trials of TGF- β antagonists for the treatment of MM. Most TGF- β antagonists broadly target the ligand, receptors, or downstream kinases, which can antagonize homeostatic levels of TGF- β and increase the risk of adverse events. We have developed a novel approach to selectively targeting disease-related TGF- β activity in the MM bone marrow microenvironment through targeting only the TGF- β which is activated through binding to the extracellular matrix protein, thrombospondin1 (TSP1). TSP1 is a secreted and extracellular matrix protein, which controls TGF- β activity in disease by binding and activating latent TGF- β . TSP1 is increased in MM. Our studies show that TSP1 activates latent TGF- β expressed by human and mouse MM cells in vitro and importantly, a tetrapeptide antagonist (LSKL) of TSP1-dependent TGF- β activation reduces MM tumor burden, stromal IL-6, and osteolytic bone disease in mouse models of MM. LSKL nearly completely blocks active TGF- β in bone marrow cells of treated MM mice, indicating that the TSP1-TGF- β antagonist peptide is working through targeting TSP1-dependent TGF- β activation in the bone marrow microenvironment. These data establish that TSP1 is an important regulator of TGF- β activity in MM and suggest that blockade of this pathway represents a novel and selective therapeutic strategy to reduce MM progression and bone disease. Our goal is to develop an orally available, "druggable" form of the LSKL peptide for treatment of MM. We have identified a lead compound (SRI31277) based on LSKL which has dose-dependent in vivo activity in a mouse model of MM, improved pharmacokinetics and oral bioavailability, and which will be the basis for identification and optimization of lead drugs. This proposal will combine mechanistic studies (Aim 1) with drug development efforts (Aim 2) to achieve our goal of identifying an orally active lead compound for treatment of MM. In Aim 1, we will further determine the role of the TSP1-TGF- β pathway in MM through use of immune competent and TSP1 null models, by comparison of SRI31277 to global TGF- β

inhibitors and by use in drug combinations, and by complementary *in vitro* studies to define the TSP1 receptor on MM cells for TGF- β activation and the role of this pathway in MM cell cytokine production and osteoblast/osteoclast regulation. The key goal of Aim 2 is to identify an orally active derivative of SRI31277 and a back-up peptide mimetic/small molecule suitable for GLP-IND enabling studies using both peptide and peptidomimetic/small molecule approaches.

PUBLIC HEALTH RELEVANCE: Multiple myeloma is an incurable cancer of plasma cells involving immune dysfunction and osteolytic bone disease that is dependent on factors, such as the growth factor TGF- β , in the bone marrow microenvironment. In these studies, we will advance pre-clinical development of an antagonist of the thrombospondin1-TGF- β pathway as a therapeutic for treatment of myeloma through performance of mechanistic studies using our current lead antagonist peptide in several pre-clinical models to determine effects on the immune system, the role of stromal TSP1 in myeloma, comparison to global TGF- β inhibitors, and synergy with current myeloma drugs, and through supporting *in vitro* studies. We will pursue our goal of identifying an orally active inhibitor of the TSP1-TGF- β pathway through peptide modification and peptide mimetic approaches to optimize a lead with favorable *in vitro* and *in vivo* activity, pharmacokinetics, and toxicity profiles.

CRITIQUE 1:

Significance:

Investigator(s):

Innovation:

Approach:

Environment:

Overall Impact: This is a revised R01 application from an established investigator who proposes to develop a novel therapy for multiple myeloma targeting increased TSP1-mediated activation of TGF-beta using analogues of compound SRI31277 that has been developed or as a back-up developing analogues of small peptide LSKL to block the TGF-beta effects on cytokine production and disease progression. The revised proposal is improved in response to prior concerns and the proposal has a number of strengths. The strengths include the PI, the investigative team, innovation in targeting TGF-beta activation by TSP1 as a therapeutic strategy to treat myeloma, innovation in the molecules and derivatives to be used, logical experiments and availability of SRI3127 analogues. There remain some weaknesses including the preliminary data that did not include information about the survival of myeloma bearing mice that were exposed to either LSKL or SRI3127. While there were demonstrated effects at a time point on cytokine production or TGF-beta signaling, whether disease control could be sustained or meaningfully extend the lifespan of the mice is unclear. There was no data provided to support the important premise that the blockade of TSP1-dependent activation of TGF-beta would not affect the anti-angiogenic activity or NO regulation by TSP1. No preliminary data regarding safety or efficacy of available SRI3127 analogues was shown. Overall the proposal was felt to have significant merit despite its weaknesses.

1. Significance:

Strengths

- The proposal seeks to develop a novel therapy for multiple myeloma targeting increased TSP1-mediated activation of TGF-beta using a small peptide LSKL to block its effects on cytokine production and disease progression.
- A Lead compound SRI31277 has been developed based on LSKL and demonstrates efficacy in a mouse model as well as oral bioavailability.

- The proposal addresses an unmet need involving the targeting of TGF-beta in multiple myeloma.

Weaknesses

- None

2. Investigator(s):

Strengths

- The PI Dr. Murphy-Ullrich is a Professor of Pathology and Cell Biology at UAB where she also co-directs the BioMatrix Engineering and Regenerative Medicine Center. She has expertise in ECM remodeling by TSP1 including the discovery that it activates TGF-beta in various diseases such as cardiomyopathy and nephropathy. She has numerous publications on TSP1 and TGF-beta and is otherwise well-qualified to lead the proposed work.
- Dr. Weaver adds immunology expertise and Dr. Yang provides basic/translational expertise on multiple myeloma. Dr. Suto is VP at SRI and PI for SRI of NCI's chemical biology consortium.
- The proposal identifies Dr. Ken Anderson at DFCI as an expert in multiple myeloma and its therapy including providing clinical samples and expertise. A letter of support is provided that documents willingness to provide primary myeloma cells.
- Dr. Reddy will provide bone marrow samples at UAB Department of Pathology Bone Marrow Lab that he directs.

Weaknesses

- None

3. Innovation:

Strengths

- There is innovation in the specific activity of TSP1 to activate latent TGF-beta without blocking its anti-angiogenic or NO regulating effects that are important for tumor suppression.
- There is innovation in targeting TGF-beta activation by TSP1 as a therapeutic strategy to treat myeloma.
- The lead compound SRI3127 and peptide blocker LSKL are innovative.

Weaknesses

- None

4. Approach:

Strengths

- Preliminary data demonstrates the TGF-beta inhibitory effect of LSKL peptide including reduced p-Smad 2 both on cells or myeloma *in vivo*, reduced kappa light chain, IL6, and osteoclast number similar to dexamethasone. SRI3127 reduced bone loss and IL6 *in vivo*.
- The proposal has two specific aims addressing mechanism and drug development. Aim 1 will evaluate how inhibition of TSP1-dependent TGF-beta activation reduces myeloma progression through studies of T cell subsets, studies in TSP-null stroma, evaluation of specific blockade of TSP1-dependent TGF-beta activation with general TGF-beta blockade, synergies with other myeloma drugs and cytokine studies. Aim 2 will perform lead optimization of SRI3127 and will also look for peptide mimetic of LSKL.

- A number of analogues are available for SRI3127 to support progress in AIM2.

Weaknesses

- The preliminary data did not include information about the survival of myeloma bearing mice that were exposed to either LSKL or SRI3127. While there were demonstrated effects at a time point on cytokine production or TGF-beta signaling, whether disease control could be sustained or meaningfully extend the lifespan of the mice is unclear.
- There was no data provided to support the important premise that the blockade of TSP1-dependent activation of TGF-beta would not affect the anti-angiogenic activity or NO regulation by TSP1.
- No preliminary data regarding safety or efficacy of available SRI3127 analogues was shown.

5. Environment:

Strengths

- The environment at UAB is outstanding.

Weaknesses

- None

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Deidentified bone marrow specimens from myeloma patients will be used.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M1A - Minority and Non-minority, Acceptable

C3A - No Children Included, Acceptable

- No concerns with the inclusions.

Vertebrate Animals:

Acceptable

- Animal welfare is protected.

Biohazards:

Not Applicable (No Biohazards)

Resubmission:

- Strengths noted during the prior review included the significance, high quality preliminary data, the investigator and the innovative LSKL peptide that exhibits biological activities to block TSP1 dependent TGF-beta activation, and that the strategy had potential to develop novel therapy for

multiple myeloma. A number of weaknesses were noted including underdevelopment of Aim 2, lack of in depth approaches to investigate cytokine responses, lack of genetic approaches, anticipated difficulties with growing primary myeloma specimens in the animal model, exclusive focus on TGF-beta, and issues of myeloma heterogeneity.

- The PI responded to multiple criticisms by revising aim 2 to focus on lead optimization both for SRI31277 and peptide mimics, further developed the cytokine approaches to add evaluation of IL6 dependence and to look at IL6 dependent signaling as well as *in vivo* bone remodeling, adding studies in TSP1 null mice to evaluate the role of stromal TSP1, incorporating additional TGF-beta responses including phospho-smad 2 and 3, and additional studies to evaluate the proposed strategy in combination with dexamethasone and bortezomib.

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommend as Requested

Recommended budget modifications or possible overlap identified:

- Approximately 380,000 dollars in the form of direct costs are requested each year for 5 years. The requested budget appears to be excessive. Reduce 50% of FTE.

CRITIQUE 2:

Significance:

Investigator(s):

Innovation:

Approach:

Environment:

Overall Impact: This is an amended R01 application submitted from an established investigator. The main goal of this research project is to test the disruption of molecular interaction between thrombospondin 1 (TSP1) and TGF-beta as a potential therapeutic strategy for treatment of multiple myeloma (MM). The rationale is that the interaction between the inactive form of TGF-beta and TSP1 may lead to abnormal activation TGF-beta to promote MM disease development, and a disruption of this interaction by antagonists would abrogate the stimulating effect of TGF-beta on disease progression. This study, if successfully accomplished, would provide valuable information on the role of TGF-beta/TSP1 in multiple myeloma, and may also reveal a possibility to use lead compound SRI31277 or derivatives for potential treatment of MM. Since multiple myeloma is currently incurable, development of new and effective therapeutic strategies is highly important. As such, the proposed

study is in a significant research project. In this resubmitted grant application, the PI has included new data and made substantial revision to improve the research proposal, which is viewed with enthusiasm.

1. Significance:

Strengths

- Targeting the interaction between TSP1 and the inactive form of TGF-beta is a novel strategy to suppress multiple myeloma and has potential therapeutic implications.
- The research team has identified a lead compound (SRI31277) based on LSKL to inhibit TGF-beta/TSP1 activation with improved pharmacokinetic properties and *in vivo* activity in a mouse model of MM. This compound and its derivatives may have a potential for use in treatment of MM.

Weaknesses

- Because there are multiple ways to activate TGF-beta and multiple factors contribute to the development of MM, disruption of TSP1/TGF-beta activation by the proposed strategy might only be partially effective against MM *in vivo*.

2. Investigator(s):

Strengths

- Dr. Murphy-Ullrich is a well-established investigator with extensive research experience and expertise in the proposed research area, and is highly qualified to lead the proposed study.

Weaknesses

- None

3. Innovation:

Strengths

- The proposed strategy to suppress the TGF-beta in multiple myeloma by targeting TSP1/TGF-beta interaction is largely based on the original observations by the applicant's group, and has a high degree of originality.
- The newly identified lead compound SRI31227 is novel with potential therapeutic activity against MM.

Weaknesses

- None

4. Approach:

Strengths

- Logical research hypothesis: Activation of TGF-beta promotes MM disease progression, and abrogation of TGF-beta activation by TSP1 is expected to have an inhibitory effect on MM progression.
- Novel approaches: The findings that the binding between TSP1 and the inactive form of TGF-beta results in a non-proteolytic activation and that disruption of this molecular interaction by SRI31227 suppresses TGF-beta activation are innovative.

- The identification of the lead compound SRI31277 with improved pharmacokinetic properties and oral availability is a significant strength.
- The use of MM mouse models and primary MM cells from patients in this study is clinically relevant, and adds strength to the project.

Weaknesses

- Since there are multiple pathways that may lead to activation of TGF-beta, it is possible that disruption of TSP1/TGF-beta interaction by SRI31277 and analogs would only partially inhibit TGF-beta activation, and resistance to such inhibition would eventually develop.
- The specificity and potential off-target effect of SRI31277 should be considered.

5. Environment:

Strengths

- The research environment in the applicant's institution is excellent and appropriate for the proposed study.

Weaknesses

- None

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- This research will involve the use of remnant bone marrow aspirates. The required issues have been addressed.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical Trials)

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M1A - Minority and Non-minority, Acceptable

C3A - No Children Included, Acceptable

- There is no concern regarding human subjects inclusion.

Vertebrate Animals:

Acceptable

- All required issues have been addressed.

Biohazards:

Acceptable

Renewal:

- In this amended grant application, the PI has included new data and made substantial revisions to improve the research proposal.

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

The requested budget appears to be excessive.

CRITIQUE 3:

Significance:

Investigator(s):

Innovation:

Approach:

Environment:

Overall Impact: The application is to explore a novel approach to interfere with the action of TGFbeta in MM. Thrombospondin 1 is present in bone marrow and can activate latent TGFbeta. Preliminary data suggest that TSP1 interaction and activation of TGFbeta in BM stroma and MM cells is responsible for bone remodeling and overall tumor growth, based on addition of an interfering peptide and its derivatives. Because of the peptide-based approach in preliminary data, it is also possible that TSP1 contributes to MM disease phenotypes independent of TGFbeta. The complicated action of TSP1 in MM is supported by the report in reference 16, which suggests that TSP1 is not necessarily detrimental to chemotherapy. This drawback is partly counterbalanced by the proposed genetic analysis of TSP1 and TGFbeta *in vivo*. A detailed characterization of TSP1 in MM is proposed using established pharmacologic approaches, but it seems to be incomplete without subsequent further genetic analysis to determine the impact of each potential TSP1 function (skeletal, immunological, angiogenic effects...) in overall MM disease progression. Based on ongoing results from these studies, it is then proposed to engage in the development of orally bioavailable peptide mimetics that interfere with TSP1 activation of TGF beta. This seems risky given that TGFbeta-independent effects, TSP1 receptors, and other details are not yet defined for TSP1 function in MM. Novelty, investigators, and impact of Aim 1 drive the potential for strong impact, while the second aim risks lesser impact until the specific outcome of Aim 1 studies is achieved.

1. Significance:

Strengths

- TGF-beta targeting in oncology is problematic, in part due to its ability to both promote and suppress growth of transformed cells. An approach to specifically counteract pro-growth TGF-beta would make a significant impact.
- A significant impact of AcLSKLNH2 on tumor burden is demonstrated in a xenograft model.

- PK of SRI31277 by MS demonstrating osmotic pump delivery at relevant concentrations.
- TSP1:TGF beta interaction occurs on cell surfaces, an accessible location for peptide mimetics.

Weaknesses

- Reference 16 reports that thrombospondin levels were increased in patients who responded well to conventional therapy, as opposed to those with poor responses. Moreover, thrombospondin levels were further elevated after successful therapy. These results challenge the rationale for interfering with thrombospondin function.

2. Investigator(s):

Strengths

- Strong track record of expertise in the activity of TGFbeta and thrombospondin.

Weaknesses

- None

3. Innovation:

Strengths

- Novel approach to counteract TGFbeta oncogenic effects.
- Novel compounds to be developed and tested.
- Use of Vk*myc MM model.

Weaknesses

- None

4. Approach:

Strengths

- Use of Vk*MyC MM model, which is considered to resemble human disease.
- Analysis of both TGF beta-dependent and independent effects of TSP1 *in vivo*.
- Comparison of global TGFbeta inactivation to TSP1 inactivation *in vivo* using TGF- β receptor II kinase inhibitor SD-208 and SRI31277 in the 5TGM1 model.
- Statistical (isobologram) analysis for synergism between SRI31277 and MM chemotherapeutics performed by core.
- Analysis of cytokine production in response to SRI31277 in primary human MM samples.

Weaknesses

- A long list of tumor:host interactions will be tested in the MM 5TGM1 syngeneic model. As various effects of SRI31277 are measured, it will be important to follow up on one or more of these effects with genetic analysis to determine which mediate tumor suppression. There is little plan for prioritizing or following up on the initial characterization.
- It is pointed out in Aim1 that TSP1 does not directly activate latent TGF beta in MM conditioned medium, suggesting an important interaction with cell surface receptors. This interaction will need to be well defined to improve the probability of success in the drug development aim of the proposal, as the ability to activate TGFbeta in MM medium is assay 2 in the drug development procedure.

- Similarly Aim 1 entertains the concept of TGF beta independent activities of thrombospondin. Depending on the outcome of these experiments, drug development may need to focus on additional or alternative approaches.

5. Environment:

Strengths

- The environment is supportive for the accomplishment of Aim 1 studies.

Weaknesses

- None

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Risks are low as patient material will be collected as part of standard of care.

Data and Safety Monitoring Plan (Applicable for Clinical Trials Only):

Not Applicable (No Clinical trials)

Inclusion of Women, Minorities and Children:

G1A - Both Genders, Acceptable

M1A - Minority and Non-minority, Acceptable

C3A - No Children Included, Acceptable

- Exclusion of children is justified as MM is rare in this population.

Vertebrate Animals:

Acceptable

- A detailed description illustrates excellent preparation for the experiments.

Biohazards:

Acceptable

Resubmission:

- The resubmission offers a revision of Aim 2 with a substantial addition of genetic analysis in Aim 1. The changes are strengths but do not completely address the potential that the major activity of TSP1 in MM is mediated by TGF beta activation.

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Recommend to reduce the subcontract as the subcontract work is half of the overall budget but does not appear to be half of the work based on the description in the research plan.

Additional Comments to Applicant:

- Some data panels were too small to be clearly visible.

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

PROTECTION OF HUMAN SUBJECTS (Resume): ACCEPTABLE

The four points related to human subject protection were adequately addressed. No concerns were raised by the review panel.

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

Both genders included in the study and the plan was considered appropriate. No concerns were raised.

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

The inclusion plan for minorities' participation was considered appropriate. No concerns were raised.

INCLUSION OF CHILDREN PLAN (Resume): ACCEPTABLE

Children were excluded because they are unlikely to be affected by the disease. No concerns were raised.

VERTEBRATE ANIMAL (Resume): ACCEPTABLE

The five points under the animal welfare section were described adequately. No concerns were noted by the review panel.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was modified.

The requested budget appears to be excessive. The committee recommended reducing the efforts of the technician by 50%. In addition, the subcontract budget is not well justified as the subcontract work is half of the overall budget but does not appear to be half of the work based on the description in the research plan. Therefore, the committee recommended reducing the subcontract budget by \$50,000 per year.

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. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

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CENTER FOR SCIENTIFIC REVIEW
Molecular Targets for Cancer Intervention
ZRG1 BMCT-C (01) S
February 24, 2014

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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.