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TITLE: Novel Listeria Vectors Secreting Gut Flora-Altering Agents to Prevent Colon Cancer and Treat Colitis

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14. ABSTRACT

In the prior period we obtained needed IACUC and biosafety approvals, and developed the backbone *Listeria* vector needed to produce the B7-H1 scFv and B7-H1-expressing *Listeria* vectors for follow up work. We demonstrated the significance of host B7-H1 in preventing/mitigating colitis and also colon cancer, suggesting that a B7-H1-expressing vector could be useful for clinical uses in colitis and cancer prevention. We generating a full PD-L1 KO cell line using CRISPR to optimize library screening for anti-B7-H1 scFv. We collected useful data on immune and signaling events in colon during DSS-induced colitis to guide studies of colon tissue explants.

15. SUBJECT TERMS

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Table of contents

Among known factors contributing to cancer, advancing age is the leading risk[1]. Colorectal cancer (herein "colon cancer" for simplicity) is a top-three cancer killer in the USA, and its risk rises with age. SEER data (http://seer.cancer.gov/statfacts/html/colorect.html) show that peak colorectal cancer incidence in the USA is at 65-84 years of age. The significant age risk for colorectal cancer is extensively validated in the USA and worldwide in industrial countries (reviewed in 2,3). Few studies have addressed specific mechanisms underlying this age-specific increased risk. Further, treatments for advanced-stage colon cancers are toxic and poorly-tolerated by the aged hosts most at risk. If we could understand age-associated factors contributing to increasing colon cancer risk, we could develop strategies to reduce its age-associated increased incidence, and develop effective, age-specific therapies. Work proposed here to prevent increased colon cancer risk with age by reducing age-related gut dysbiosis could lead to effective prevention strategies that will reduce societal, financial and personal costs of colon cancer. Further, much work here relates to age-related cancer in general. Thus, findings could also apply to risks for developing other age-associated cancers, representing the majority of cancers. Finally, inflammatory bowel disease also arises through gut dysbiosis and thus concepts and strategies identified here could help prevent or treat inflammatory bowel disease, which confers a specific risk for colon cancer.

This proposal addresses the PRMRP research topic areas of colorectal cancer and *Listeria* vaccines for cancer, and addresses the PRMRP focus area of gaps in cancer prevention. The proposal objectives are to: 1) generate recombinant *Listeria* bacteria that alter colon epithelium B7-H1 expression, ii) test the capacity of these bacteria to reduce gut dysbiosis and iii) test the capacity of these bacteria to mitigate age-related colitis and related colon cancer risk. Effects will be tested in both young and aged mice in well-established models of colitis and related colon cancer. Immune, metabolic, metagenomic and signaling data will identify mechanisms of reduced colitis and colon cancer. We will validate effects in fresh human colon epithelium biopsy explants *ex vivo*.

Our overarching hypothesis is that we can control age-related gut dysbiosis and risk for colorectal cancer by therapeutic reduction of colon epithelial B7-H1 signaling. We will use validated and safe *Listeria* bacterial vectors that readily infect colon epithelium to reduce B7-H1 through secreted anti-B7-H1 scFv.

Rationale. It is well established that disturbances in gut flora (dysbiosis) contribute to colon cancer[4-6]. Mechanisms are obscure but dysbiosis-driven alterations of metabolism, colon epithelial integrity and immunity are likely. Mechanisms regulating gut flora and dysbiosis are likewise obscure. The immune co-signaling molecule B7-H1 regulates colon inflammation by regulating gut dysbiosis that increases colitis risk in mice[7]. We will use well-validated mouse models of colitis and related colon cancer to test if therapeutic manipulation of colon epithelium B7-H1 can mitigate gut dysbiosis that contributes to age-related gut inflammation and to age-

related colon cancer risk, using recombinant *Listeria* to mediate B7-H1 effects for reasons set forth below.

KEYWORDS
Colon cancer, inflammation, PD-L1, prevention

ACCOMPLISHMENTS

Specific Aim 1 Generate recombinant *Listeria* that produce anti-B7-H1 scFv in colon epithelial cells	**Timeline**	**Site 1**
Major Task 1 Regulatory approvals	Months	
Subtask 1 IACUC approval Done	1-3	Dr. Curiel
Subtask 2 Biosafety approval Done	1-3	Dr. Curiel
Subtask 3 IRB approval Delayed due to regulatory issues, but issues are resolved and we expect approval within 6 weeks	1-3	Dr. Curiel
Milestone(s) Achieved: IACUC and biosafety approvals done and IRB approvals in final processes	3	Dr. Curiel
Major Task 2 Generate recombinant *Listeria* strains	1-6	Dr. Dube
Subtask 1 generate LH1169 inlA^m strains Done and validate as per the proposal.	1-3	Dr. Dube
Subtask 2 screen scFv library for anti-B7-H1 Completion delayed due to problem generating suitable screening cell lines (see Problems), but now in progress	1-4	Drs. Curiel/Dube
Subtask 3 generate LH1169 and LH1169 inlA^m expressing anti-B7-H1 scFv Completion delayed due to difficulty in generating suitable screening cell lines (see Problems), but now in progress	3-8	Dr. Dube
Subtask 4 generate LH1169 and LH1169 inlA^m strains expressing B7-H1 Completion delayed due to difficulty in cloning full length B7-H1 (see Problems), but is in progress	1-4	Dr. Dube
Milestone(s) Achieved: First generation of recombinant *Listeria* strains constructed. Suitable screening lines for scFv screen constructed.	8	Dr. Dube
Major Task 3 Characterize recombinant *Listeria* strains *in vitro*	6-9	Dr. Dube
Subtask 1 characterize B7-H1 scFv expressing bacterial strains Delayed due to delay in scFv library screen as above	6-9	Drs. Curiel/Dube
Subtask 1 characterize B7-H1 over expressing strains Delayed due to difficulty cloning the B7-H1 gene to produce these bacterial strains as above .	6-9	Dr. Dube
Milestone(s) Achieved: Delayed	9	Dr. Dube
Specific Aim 2 Test the hypothesis that *Listeria*-mediated alterations in colon epithelium B7-H1		

expression alter factors affecting colitis and related colon cancer risk		
Major Task 1 Test the *in vitro* effects of recombinant *Listeria*	9-18	Dr. Curiel
Subtask 1 evaluate colon epithelial effects in mouse and human colon explants Start delayed, but overall progress is not delayed. In the meanwhile, we examined epithelial effects of dextran sodium sulfate (DSS)-induced colitis *in vivo* in wild type versus B7-H1 KO mice (which represent mice treated with recombinant scFv vectors). Epithelial damage was significantly more severe in B7-H1 KO versus wild type mice after DSS, suggesting that a vector expressing B7-H1 could be protective. We also developed Western blot techniques to test MAPK signals and proteasome effects in colitis which allows testing of explants when available, and points to ERK and IL-18 as initial targets to assess for vector effects.	6-18	Drs. Curiel/ Dube
Subtask 2 evaluate immune effects in colon explants from mice and humans Start delayed, but overall progress is not delayed. In the meanwhile, we used Luminex kits to test immune effects of colon during colitis and found that IL-1, IL-6, IL-17 and IL-18 were increased during colitis. We found that γδ T cells but not conventional T cells were increased by flow cytometry. These provide important leads for explant studies.	6-24	Drs. Curiel/Dube
Subtask 3 generate additional *Listeria* or *E. coli* constructs as needed Not started as it is not yet required, and may not be required.	10-20	Dr. Dube
Milestone(s) Achieved: We have demonstrated the importance of B7-H1 signals in colon during colitis. We have demonstrated that the B7-H1-expressing vector could be better protective against colitis versus the scFv vector based on B7-H1 KO data.	18	Drs. Curiel/Dube
Specific Aim 3 Test the hypothesis that B7-H1-altering *Listeria* reduce colitis and colon cancer risk	12-24	
Major Task 1 Test B7-H1 modulation in models of colitis and colon cancer	12-24	Dr. Curiel
Subtask 1 Test recombinant *Listeria* strains in the DSS colitis model Scheduled to start in year 2. In the meanwhile, we found that B7-H1 KO mice have increased colitis (see above).	12-24	Dr. Curiel
Subtask 2 Test recombinant *Listeria* strains in the DSS/AOM colorectal cancer model Scheduled to start in year 2. In the meanwhile, we found that B7-H1 KO mice have and increased colon cancer after AOM/DSS versus wild type, validating this model to test vector effects. These data also suggest that the B7-H1-	12-24	Dr. Curiel

expressing vector could be beneficial to prevent colon cancer.		
Subtask 3 evaluate impact of B7-H1 modulating recombinant bacteria on microbiome and metabolism We will start when the vectors above are available.	9-24	Dr. Curiel
Milestone(s) Achieved: We demonstrated that B7-H1 is protective in AOM/DSS colon cancer, validating the model for these studies. We have demonstrated that the B7-H1-expressing vector could be better protective against colon cancer versus the scFv vector based on B7-H1 KO data.	24	Dr. Curiel

What opportunities for training and professional development has the project provided?
Nothing to Report

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
We will finish screening the scFv library to develop scFv for vectors, and test other approaches (see Problems) to express B7-H1 in the bacterial vectors. When vectors are available, we will perform remaining tasks that require them as described above in the SOW. We will get the IRB in place to get the required human samples needed for human work as planned in the SOW.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?
We have shown that B7-H1 protects from colitis and colon cancer in mouse models. These data suggest that the B7-H1 expressing vector could be more beneficial versus the scFv vector in colitis and/or cancer protection, but will assess both approaches and vectors as planned.

What was the impact on other disciplines?
As B7-H1 can alter colitis and colon events can affect systemic immunity, these data suggest that our approaches could improve immunity in other areas. For example, it was recently reported that gut bacteria (which are altered by B7-H1) can alter the efficacy of cancer immunotherapy. Our vectors could alter immunotherapy as well, as a result.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change
Our gastroenterology collaborator, Mike Krier, was transferred to San Diego as is no longer able to participate. He was not a funded investigator so there is no change to the budget. We have established collaborations with our institutional gastroenterologists who will now provide the needed colon biopsy to derive colon explants. IRB approval for them to participate is in progress. Neither collaborator is funded on this proposal and simply provides materials, so there is no material change to personnel or budget.

We found that screening the scFv library against a B7-H1^{+} cell line was insufficient, so we used shRNA to knock down B7-H1 in the B16F10 line for screening, but this was also inefficient. We then used CRISPR/Cas9 to effect a total KO of B7-H1 in the B16F10 cell line, which we validated by qPCR, sequencing and Western blot. We are now able to screen the library for binding scFv as planned, with a delay, but with reagents now in hand so that we can proceed and finish by the end of the funding period.

We have had technical difficulties with the amplification and cloning of B7-H1 from our mouse cDNA library. We have also had difficulty with our transfer vector integrating properly on another project and anticipate we will have a similar problem with B7-H1. The solutions we are working on are: 1) get a different transfer vector. This has proven to be somewhat difficult but we are trying a colleague that has been helpful in the past. 2) Alternatively, we will have to either use a different integration vector or build a new one. We have requested new integration vectors from a colleague as a back-up. 3) We will try the cDNA library one last time with PCR using a new aliquot of it. If we still cannot amplify B7-H1, we will order a synthetic gene.

Changes that had a significant impact on expenditures
We were unable to generate initial scFv vectors or clone B7-H1 on schedule and could thus not do characterizations of corresponding vectors in year 1. However, we did additional and useful work on host B7-H1 effects on colitis, colon cancer and immunity instead. We have had to generate additional cell lines for the scFv screening (see Problems) and do much more library screening and cloning that initially planned, but as we did not incur costs of vector characterizations, these effects are a budgetary wash.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
None.

Significant changes in use or care of human subjects
We will now recruit human subjects from UTHSCSA instead of BAMMC and use UTHSCSA instead of BAMMC collaborators to procure materials.

Significant changes in use or care of vertebrate animals
Nothing to report.

Significant changes in use of biohazards and/or select agents
Nothing to report.

PRODUCTS
Nothing to report

PARTICIPANTS and other collaborating organizations
What individuals have worked on the project?
Name: Tyler Curiel
Project role: PI
Research identifier: TCURIEL
Nearest person month worked: 1
Contribution to project: Provided overall supervision, interpreted data, provided troubleshooting guidance, managed budget, wrote progress report

Name: Peter Dube
Project role: co-investigator
Research identifier: Dube01
Nearest person month worked: 1
Contribution to project: provided expertise on library screening and vector construction, helped interpret data, helped write progress report

Name: Angele Cantwell
Project role: Research Scientist
Research identifier: None
Nearest person month worked: 2
Contribution to project: did hands on *in vitro* work for library screening and vector construction, helped interpret and graph data

Name: Vincent Hurez
Project role: senior scientist
Research identifier: None
Nearest person month worked: 1
Contribution to project: developed and performed flow cytometry data, supervised Ms. Chen and provided technical guidance, helped design and perform experiments

Name: Wanjiao (May) Chen
Project role: scientist
Research identifier: None
Nearest person month worked: 6
Contribution to project: maintained mice, gave treatments, monitored for toxicities and tumor growth, helped stock lab supplies, helped process tissues for *ex vivo* work

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report.

What other organizations were involved as partners?
In the initial proposal, we were going to get patient samples from BAMCC, but our contact person there was transferred, so we no longer will use this site for tissue collections.

Special reporting requirements
None.

Appendices
None.