

Award Number: W81XWH-11-1-0727

TITLE: Progression of Inflammatory Bowel Disease to Cancer: Is the Patient "Better Off" without Lymphatic Vessels or Nodes (or Angiopoietin 2)?

PRINCIPAL INVESTIGATOR: Marlys H. Witte, MD

CONTRACTING ORGANIZATION: University of Arizona, Tucson, AZ 85724

REPORT DATE: October 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;  
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

| REPORT DOCUMENTATION PAGE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |             |                          |                            | Form Approved<br>OMB No. 0704-0188                  |                                           |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|--------------------------|----------------------------|-----------------------------------------------------|-------------------------------------------|
| Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |             |                          |                            |                                                     |                                           |
| 1. REPORT DATE<br>October 2012                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |             | 2. REPORT TYPE<br>Annual |                            | 3. DATES COVERED<br>30September2011-29September2012 |                                           |
| 4. TITLE AND SUBTITLE<br><br>Progression of Inflammatory Bowel Disease to Cancer: Is the Patient "Better Off" without Lymphatic Vessels or Nodes (or Angiopoietin 2)?                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |             |                          |                            | 5a. CONTRACT NUMBER                                 |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 5b. GRANT NUMBER<br>W81XWH-11-1-0727                |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 5c. PROGRAM ELEMENT NUMBER                          |                                           |
| 6. AUTHOR(S)<br>Dr. Marlys H. Witte, MD<br><br>E-Mail: <a href="mailto:grace@surgery.arizona.edu">grace@surgery.arizona.edu</a>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 5d. PROJECT NUMBER                                  |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 5e. TASK NUMBER                                     |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 5f. WORK UNIT NUMBER                                |                                           |
| 7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)<br><br>University of Arizona<br>Tucson, AZ 85724                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |             |                          |                            | 8. PERFORMING ORGANIZATION REPORT NUMBER            |                                           |
| 9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)<br>U.S. Army Medical Research and Materiel Command<br>Fort Detrick, Maryland 21702-5012                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |             |                          |                            | 10. SPONSOR/MONITOR'S ACRONYM(S)                    |                                           |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          |                            | 11. SPONSOR/MONITOR'S REPORT NUMBER(S)              |                                           |
| 12. DISTRIBUTION / AVAILABILITY STATEMENT<br>Approved for Public Release; Distribution Unlimited                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |             |                          |                            |                                                     |                                           |
| 13. SUPPLEMENTARY NOTES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |             |                          |                            |                                                     |                                           |
| 14. ABSTRACT<br><br>This proposal addresses inflammatory bowel disease (IBD). Multiple factors have been implicated in the progression of ulcerative/granulomatous (Crohn's) colitis to colorectal carcinoma (CRC) in IBD patients and experimental models. Nonetheless, the pathogenic link, interrelationship, and practical clinical application of these various theories of progression have remained elusive. We proposed that a reduced number of functioning lymphatic vessels and impaired lymph drainage (lymphatic vascular insufficiency) in the colon actually protects against progression of inflammatory colitis to CRC. Our primary objective is to determine whether there is a reduced incidence of CRC in mice with lymphatic insufficiency from genetic knockout of angiopoietin2 (Ang2) compared to controls. We have: 1) completed and secured approval of ACURO Appendix; 2) revised dextran sodium sulfate (DSS) dosing in our model; 3) completed mouse cohorts chronically exposed to DSS; 4) added pre-carcinogen azoxymethane (AOM) before chronic DSS; and 5) are completing additional experimental groups, and are continuing analysis, imaging studies, and data analysis. This project has potentially high impact because of the substantial incidence of IBD-CRC progression with associated morbidity and mortality and importance of clarifying interactions of lymphatic functional status and Ang2, identifying potential biomarkers, and developing new imaging, preventive, and treatment approaches to IBD-induced CRC. |             |                          |                            |                                                     |                                           |
| 15. SUBJECT TERMS<br>Inflammatory Bowel Disease (IBD); Colorectal carcinoma (CRC); Dextran sodium sulfate (DSS); Azoxymethane (AOM); Angiopoietin-2 (Ang2) mice.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |             |                          |                            |                                                     |                                           |
| 16. SECURITY CLASSIFICATION OF:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |             |                          | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES                                 | 19a. NAME OF RESPONSIBLE PERSON           |
| a. REPORT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | b. ABSTRACT | c. THIS PAGE             |                            |                                                     | USAMRMC                                   |
| U                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | U           | U                        | UU                         | 6                                                   | 19b. TELEPHONE NUMBER (include area code) |

## Table of Contents

|                                   | <u>Page</u> |
|-----------------------------------|-------------|
| Introduction.....                 | 4           |
| Body.....                         | 4           |
| Key Research Accomplishments..... | 5           |
| Reportable Outcomes.....          | 5           |
| Conclusion.....                   | 5           |
| References.....                   | 6           |
| Appendices.....                   | 6           |

**Interim Annual Report DOD #W81XWH-II-I-0727  
9/30/2011-9/2/2012  
PI Marlys H. Witte, MD, Professor of Surgery  
University of Arizona**

## **Introduction**

This proposal addresses inflammatory bowel disease (IBD). Multiple factors have been implicated in the progression of ulcerative/granulomatous (Crohn's) colitis to colorectal carcinoma (CRC) in patients and in experimental models of IBD. Nonetheless, the pathogenic link, interrelationship, and practical clinical application of these various theories of progression have remained elusive. We proposed that having a reduced number of functioning lymphatic vessels and impaired lymph drainage (lymphatic vascular insufficiency) in the colon actually protects against progression of inflammatory colitis to colon cancer. Our primary objective is to determine whether there is a reduced incidence of colon cancer in mice with lymphatic deficiency from genetic knockout of angiopoietin 2 (Ang2) compared to controls. This project is currently in an extension year to complete project objectives.

## **Body**

Task 1: Existing Institutional Animal Care and Use Committee protocol was modified and specific approved for all aspects of this project in conjunction with the DOD ACURO Appendix was obtained. Subsequent renewal application was approved by IACUC on 8/16/12 (until 8/16/2015) and renewal was identical (without any changes) for this project protocol.

Task 2: The inflammatory colitis to colorectal tumors/cancer model was implemented in angiopoietin-deficient and wildtype control mice:

Initially, groups of wildtype mice were chronically administered dextran sodium sulfate (DSS) in an on-off regimen to produce inflammatory colitis and assess colonic tumor formation. Because of variability in the potency of the DSS lots and heightened mortality observed at our previously tolerated regimen of 3% DSS, it was necessary to scale down the dose stepwise to 2%, then 1-1/2% to achieve comparable clinical efficacy, acceptable tolerability, and low mortality.

At the 1-1/2% DSS on-off regimen, whereas fully-developed clinical symptoms and lab findings of inflammatory colitis were noted as previously (at a substantially higher % DSS), no colonic tumors were noted grossly in wildtype (+/+) mice or in the smaller number of Ang2-haploinsufficient (+/-) and knockout (-/-) mice that survived for the 9 week period of observation. Detailed microscopic and serologic examinations are pending.

In 3 successive randomized subsets of mice, addition of a single priming dose (12mg/Kg) of carcinogen azoxymethane (AOM) followed by the 1-1/2% DSS on-off regimen for 8 weeks led not only to fully-developed clinical symptoms, lab findings, and gross pathologic changes of inflammatory colitis (e.g. colon shortening and thickening) but also gross colonic tumors visible protruding from the mucosa into the lumen, largely confined to the distal portion of the colon, in all +/+, +/-, and -/- mice. Number, size, and extent of these tumors varied greatly, with a trend to smaller tumor load in the Ang-2 deficient mice. However, for statistical significance, these findings require confirmation in a larger number of mice (particularly expanding numbers of Ang-2 deficient mice which exhibited disproportionately higher mortality during the experimental

regimen) along with refined, standardized quantization of surface tumor volume. Moreover, detailed microscopic analysis has not been completed, which will better define the colonic tumor stages, depth and invasion for comparison. Serologic examination, specifically measurement of Ang-2 serum levels in batched blood, is also pending.

Task 3: Detailed tissue and imaging analysis of the neoplastic changes as well as serial blood marker analysis for the completed experimental groups is underway (see Task 2).

For completed experimental groups, tissues have been harvested at sacrifice, gross and dissecting microscope examination carried out, and predetermined colon segments identified spanning from the proximal to distal colon, preserved and processed appropriately for histologic and immunohistochemical examination.

Blood has also been collected, and sera prepared and frozen for batch ELISA analysis of Ang-2 as a tumor biomarker.

The potential of advanced non-invasive, dynamic ultrasound imaging has been explored in individual +/- mice using the Vevo ultrasound machine, and technical issues with colonic ultrasound imaging have been identified, e.g. tissue depth and gas in the colon; Approaches to lymphatic ultrasound imaging are also being examined. Alternative or supplementary use of serial endoscopy using ultra-high resolution optical coherence tomography, particularly suitable to the distal colon, where the tumors are largely confined, is also being evaluated.

Task 4: Project data analysis is ongoing.

## **Key Research Accomplishments**

- 1) Completed and secured approval of ACURO Appendix
- 2) Revised dextran sodium sulfate (DSS) dosing in our model
- 3) Completed mouse cohorts with chronic exposure to DSS
- 4) Added pre-carcinogen azoxymethane (AOM) to the chronic protocol
- 5) Are completing additional experimental groups, have undertaken tissue analysis and imaging studies, and data analysis is ongoing.

## **Reportable Outcomes**

Work in this project is still ongoing to complete research objectives and there are no reportable outcomes in this first Annual Report. Data analysis and reporting of Outcomes will follow in the extension year and be included in the final report.

## **Conclusion**

This project is in a no-cost extension year to complete objectives. Encouraging results in the use of the model and determination of functional model parameters have been obtained for which data analysis is ongoing to determine significance and for reporting. Reportable Outcomes are anticipated during the extension year.

## References

These references are supplied as background for the project (1,2) and the chronic DSS mouse model (4) with our modification to use the Angiopoietin2 knockout mice (4) with lymphatic deficiency (5) to explore the role of the lymphatic system in IBD.

1. Abraham C, Cho JH: Inflammatory bowel disease. *N Engl J Med.* 361(21):2066-2078, 2009.
2. Colombel JF, Watson AJM, Neurath MF: The 10 remaining mysteries of inflammatory bowel disease. *Gut.* 57:429-433, 2008.
3. Gale N, Thurston G, Hackett SF, Renard R, Wang Q, McClain J, Martin C, Witte C, Witte M, Suri C, Campochiaro P, Wiegand S, Yancopoulos G: Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter is rescued by angiopoietin-1. *Develop Cell.* 3:411-423, 2002.
4. Okaysu I, Yamada M, Mikami T, Yoshida T, Kanno J, Ohkusa T: Dysplasia and carcinoma development in a repeated dextran sulfate sodium-induced colitis model. *J Gastroent Hep.* 17:1078-1083, 2002.
5. Yoffey JM, Courtice, FC (eds): *Lymphatics, Lymph and the Lymphomyeloid Complex.* Academic Press, London, 942 p, 1970.

## Appendices

None

## Supporting Data

None