

AD_____

Award Number: W81XWH-05-1-0351

TITLE: Breast Cancer Therapy Using Antibody-Endostatin Fusion
Proteins

PRINCIPAL INVESTIGATOR: Seung-Uon Shin, Ph.D.

CONTRACTING ORGANIZATION: University of Miami
Miami, FL 33136

REPORT DATE: April 2006

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
- ☐ Distribution limited to U.S. Government agencies only;
report contains proprietary information

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 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 01-04-2006		2. REPORT TYPE Annual		3. DATES COVERED 25 Mar 2005 – 24 Mar 2006	
4. TITLE AND SUBTITLE Breast Cancer Therapy Using Antibody-Endostatin Fusion Proteins				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-05-1-0351	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Seung-Uon Shin, Ph.D.				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Miami Miami, FL 33136				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES Original contains colored plates: ALL DTIC reproductions will be in black and white.					
14. ABSTRACT To produce a more effective form of Herceptin and improve efficacy of human endostatin, we have constructed an anti-HER2 IgG3-human endostatin fusion protein by joining human endostatin to the 3' end of humanized anti-HER2 IgG3. The wild type and the mutant type (P125A) of human endostatin were constructed with FLAG tagging to distinguish from the endogenous human endostatin. The anti-HER2 IgG3-huEndo fusion proteins were constructed as wild type and mutant type (P125A) and were stably transfected into B myeloma cells. The secreted huEndo fusion proteins have a molecular weight of 220 kDa for the anti-HER2 IgG3-CH3-huEndo fusion protein and 160 kDa for the anti-HER2 IgG3-Hinge-huEndo fusion protein. The huEndo fusion proteins were faithfully secreted as the fully assembled H2L2 form.					
15. SUBJECT TERMS Immunotherapy, Angiogenesis, Chemotherapy, Antibody, Endostatin, Breast Cancer, HER2/neu					
16. SECURITY CLASSIFICATION OF:			UU	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	9
Conclusions.....	9
References.....	9
Appendices.....	10

BREAST CANCER THERAPY USING ANTIBODY-ENDOSTATIN FUSION PROTEINS

INTRODUCTION

Angiogenesis is critical for growth of primary tumor and for the development of metastasis. Antiangiogenic therapy with agents such as endostatin is under active investigation. Early human trials showed endostatin to be safe, but minimal activity has been observed.¹⁻³ Dosage and schedules may have been suboptimal, and/or late stage disease may not be responsive to recombinant human endostatin. HER2 is overexpressed in 30% of breast cancer and phase II trials of Herceptin demonstrated an 11% response rate in HER2+ patients with metastatic breast disease.⁴⁻⁶ Combining Herceptin with chemotherapy enhances anti-tumor activity resulting in an objective response rate of 60 % or greater in several phase III trials.^{5,7,8} To produce a more effective form of Herceptin and improve the efficacy of endostatin, we have constructed an anti-HER2 IgG3-C_H3-endostatin fusion protein by joining murine endostatin to the 3' end of humanized anti-HER2 IgG3.⁹ Preliminary data using an antibody-murine endostatin fusion protein suggests enhanced effectiveness of anti-HER2 IgG3-endostatin may be due to longer endostatin half-life and the selective targeting of endostatin to tumor by anti-HER2 antibody due to the presence of a fused antiangiogenic factor.⁹

The objective of the proposal is to develop and test novel antibody-fusion proteins with specific ability to deliver antiangiogenic factors to tumors by linking an antiangiogenic factor, human endostatin, with the targeting specificity of an antibody directed against HER2 in order to direct localization of endostatin to the tumor site. Application of the strategy in humans will require careful evaluation of antibody fusion protein antigenicity and might benefit from use of a human endostatin fusion domain. If the antibody-endostatin fusion protein is specifically targeted to the surface of tumor cells, it will be more effective because of retained antibody effector functions, effects on HER2 signaling, and improved ability to inhibit neovascularization in a tumor specific fashion.

To achieve our goals, we set up three specific aims. I. Design and synthesize two variant antibody-human endostatin (huEndo) fusion proteins (anti-HER2 IgG3-Hinge-huEndo and anti-HER2 IgG3-C_H3-huEndo) directed against HER2, which differ in Fc region and its ability to mediate antibody effector functions (Fig. 1). II. Test the antiangiogenic activity of anti-HER2 antibody-human endostatin fusion protein(s) *in vitro* and *in vivo*. III. Study the antibody-endostatin fusion proteins *in vivo* for effects on tumor growth in animal tumor and/or human xenograft models.



Fig. 1. Schematic diagram of anti-HER2 IgG3-human endostatin fusion proteins. Endostatin domain in orange.

BODY

Specific Aim I. Design and synthesize two variant antibody-human endostatin (huEndo) fusion proteins (anti-HER2 IgG3-Hinge-huEndo and anti-HER2 IgG3-C_H3-huEndo) directed against HER2, which differ in the Fc region and its ability to mediate antibody effector functions.

Task 1. Construction and expression of anti-HER2 IgG3-H-huEndo and anti-HER2 IgG3-C_H3-huEndo fusion proteins (Months 1-6).

- a. Construct anti-HER2 antibody-human endostatin fusion genes.
- b. Express anti-HER2 antibody-human endostatin fusion genes.

Task 2. Produce anti-HER2 H-huEndo and anti-HER2 IgG3-C_H3-huEndo fusion proteins and endostatin (Months 1-24).

- a. Produce anti-HER2 antibody-human endostatin fusion proteins in milligram quantities.
- b. Evaluation of antibody-mediate functions

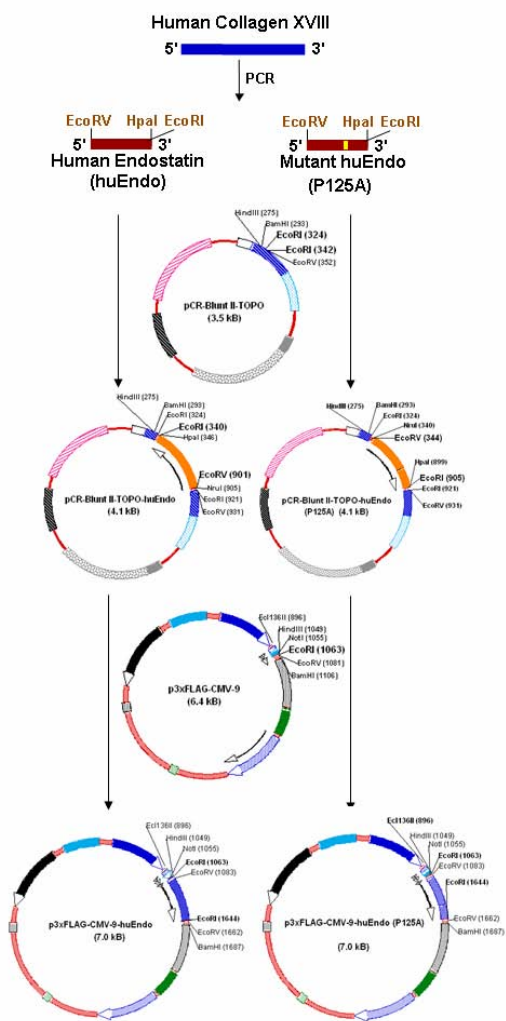
- i. Binding ability to HER2
- ii. Affinity measurement
- iii. Measure immunoreactivity
- c. Fc associated effector function.
 - i. Complement-dependent cytotoxicity (CDC)
 - ii. Antibody-dependent cell-mediated cytotoxicity (ADCC)

Task 3. Determine pharmacokinetics, tumor targeting ability, and tissue biolocalization of endostatin fusion proteins (Months 7-12).

- a. Serum half-life of anti-HER2 antibody-endostatin fusion proteins.
- b. Biodistribution and biolocalization of antibody-endostatin fusion proteins.

Task 4. Analyze antigenicity of the fusion proteins by ELISA (Months 7-12).

Construction and expression of human endostatin and a mutant



Human endostatin (huEndo) gene originated from the human collagen, type XVIII, alpha 1 gene by PCR using primers 5'-CCCCTCGCGATATCACAGCCACCGCGACTTCCAGCCG and 5'-CCCCGAATTTCGTTAACCCTTGGAGGCAGTCATGAAGC. The PCR products were cloned into pCR-Blunt II-TOPO vector and sequenced. After sequencing, clones containing wild-type human endostatin and single-point mutant clones at a position 125 (An alanine residue was substituted for proline at position 125 by site-directed mutagenesis using primer 5'-GGCTCGGACGCCAACGGGCGC; P125A.) were identified. A point mutation in human endostatin at position 125 (proline to alanine) has improved endothelial cell binding and antiangiogenic activity.¹⁰⁻¹² The wild-type (huEndo) and the **mutant** human endostatin (huEndo-P125A) genes were subsequently cloned into p3xFLAG-CMV-9 vector under the control of the human cytomegalovirus promoter, in which preprotrypsin leader sequence precedes the FLAG sequence (Fig. 2).¹³⁻¹⁵ The FLAG-tagged human endostatin will be distinguishable from the endogenous endostatin *in vivo*. p3xFLAG-CMV-9-huEndo and p3xFLAG-CMV-9-huEndo-P125A vectors were stably transfected into COS cells and their expression of endostatin is being investigated now.

Fig. 2. Cloning of human endostatin and a mutant endostatin (P125A). PCR cloned human endostatin genes were subcloned into pBlunt II-TOPO vector, and were subsequently cloned into 3xFLAG-CMV-9 vector to express secreted human endostatin tagged with FLAG.

Construction of anti-HER2 IgG3-human endostatin fusion proteins

The subcloned huEndo and huEndo-P125A genes were ligated in frame to the carboxyl end of the heavy chain constant domain (Hinge or C_H3) of human IgG3 in the vector pAT135 as described previously (Fig. 3)¹⁶ and the endostatin heavy chain constant region was then joined to an anti-HER2 variable region of a recombinant humanized monoclonal antibody 4D5-8 (HER2, trastuzumab; Genentech) in the expression vector (pSV2-his) containing HisD gene for eukaryotic selection (Fig. 4).^{17,18}

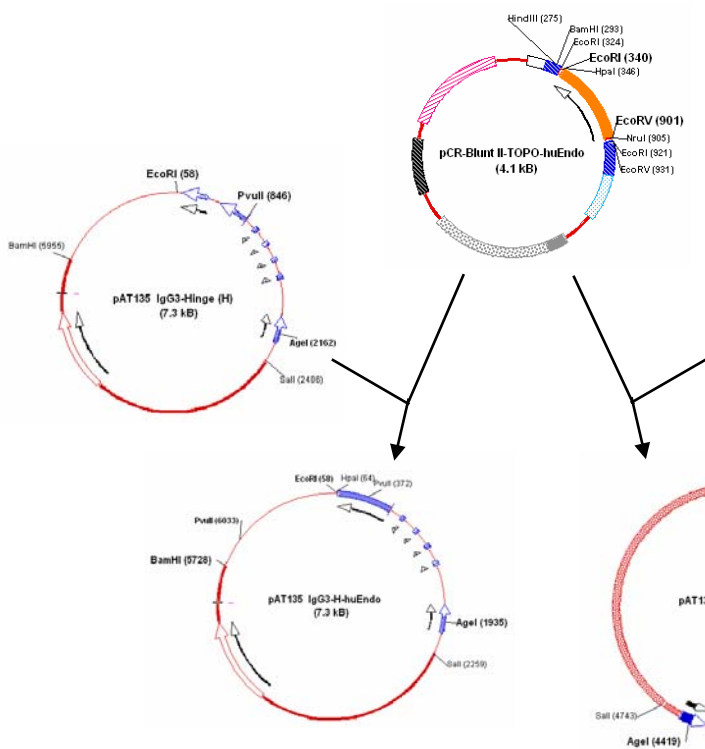


Fig. 3. Schematic diagram of cloning steps of human endostatin into human IgG3 in pAT135 vector.

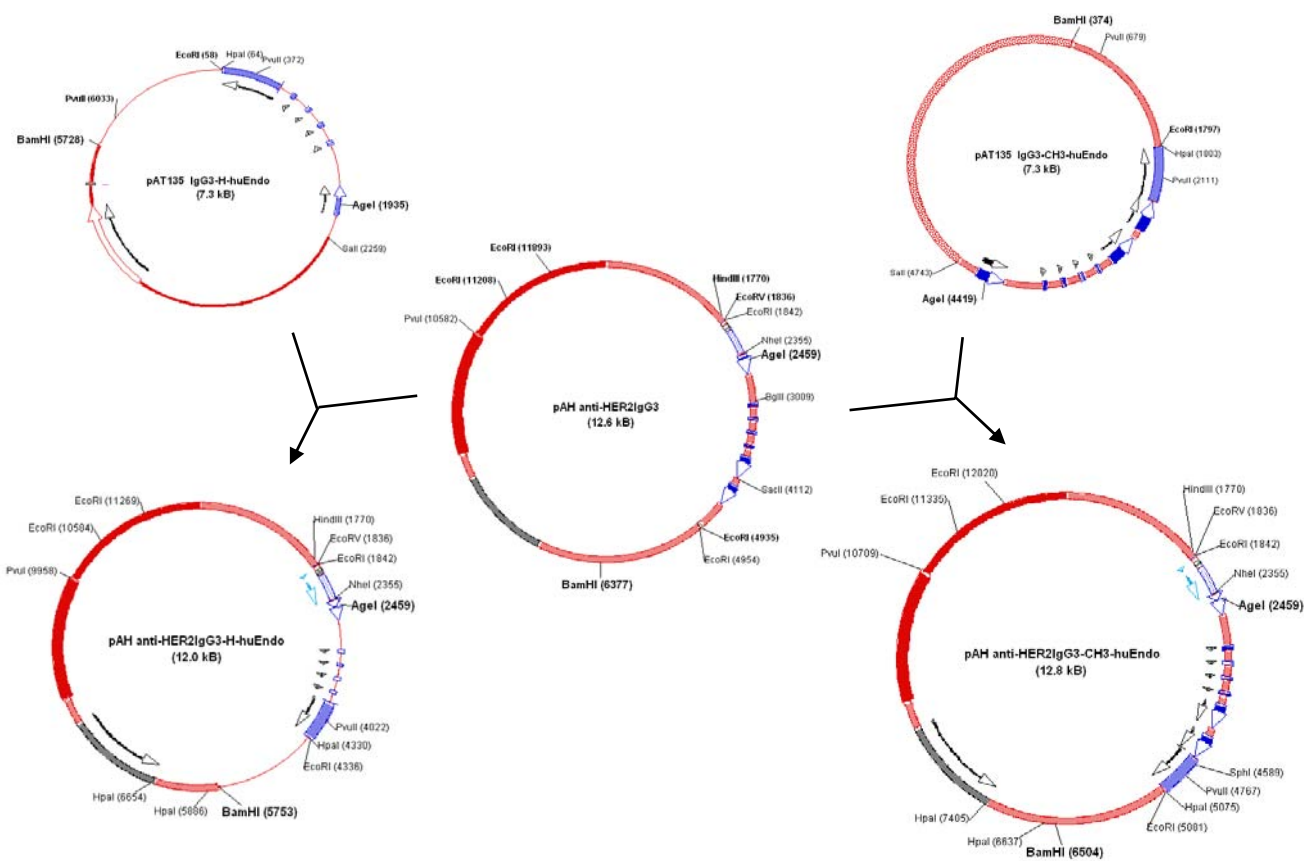


Fig. 4. Schematic diagram of cloning steps of IgG3-huEndo into the expression vector.

Expression of anti-HER2 IgG3-human endostatin fusion proteins

The anti-HER2 IgG3-huEndo fusion protein constructs were stably transfected into SP2/0 or P3X63Ag8.653 myeloma cells stably expressing the anti-HER2 kappa light chain to assemble the entire anti-HER2 IgG3-huEndo fusion proteins as described previously (Fig. 5).¹⁹ The anti-HER2 IgG3-huEndo fusion proteins were biosynthetically labeled with [³⁵S]methionine and analyzed by SDS-PAGE (Fig. 6).¹⁹ Anti-HER2 IgG3-huEndo fusion proteins of the expected molecular weight were secreted as the fully assembled H₂L₂ form (Fig. 6). The endostatin fusion proteins are being purified from culture supernatants using protein A immobilized on Sepharose 4B fast flow.

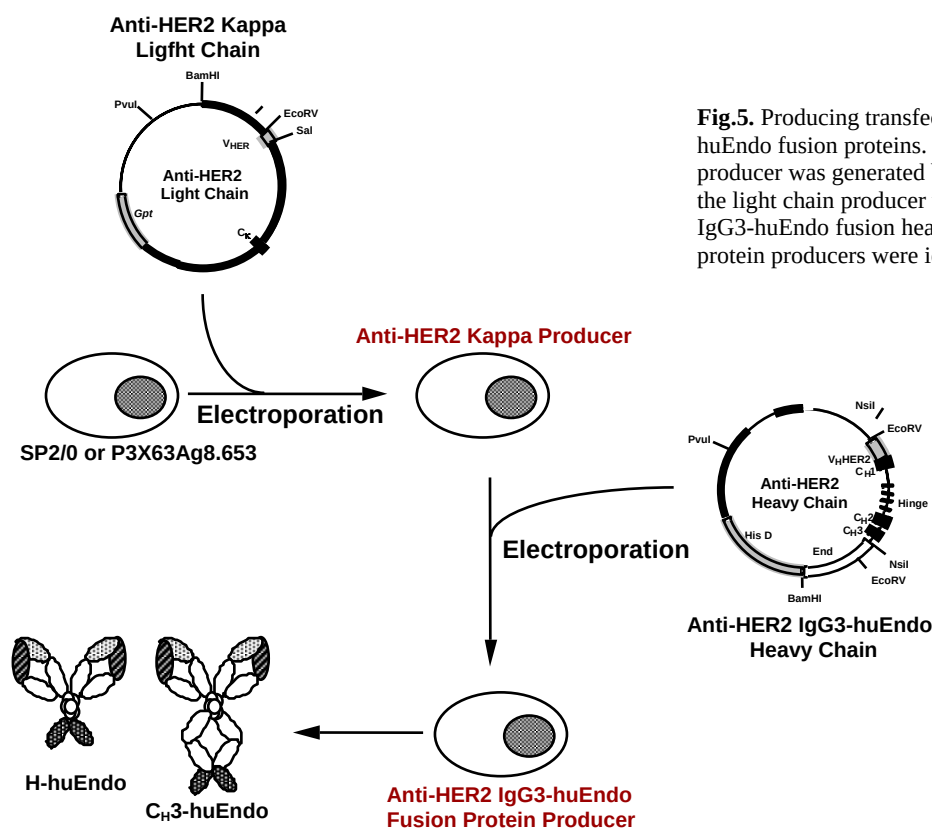


Fig.5. Producing transfectants expressing anti-HER2 IgG3-huEndo fusion proteins. Anti-HER2 kappa light chain producer was generated by electroporation, and subsequently the light chain producer was transfected with anti-HER2 IgG3-huEndo fusion heavy chain gene. The huEndo fusion protein producers were identified through ELISA.

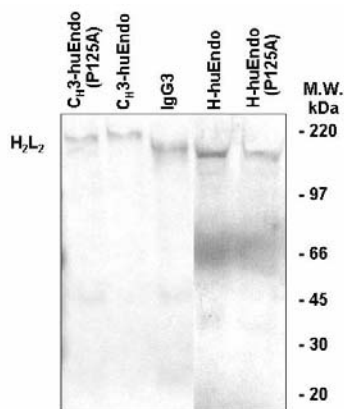


Fig. 6. Immunoprecipitation and SDS-PAGE analysis of anti-HER2 IgG3-huEndo fusion protein. The secreted anti-HER2 IgG3-huEndo fusion proteins biosynthetically labeled with [³⁵S] methionine was immunoprecipitated with Protein A and analyzed under non-reducing condition. Control anti-HER2 IgG3 (IgG3) is included for comparison.

To confirm that the endostatin moiety is present in the anti-HER2 IgG3-huEndo proteins, the purified fusion proteins are being analyzed by Western blotting as described previously.⁹ Antibody functions of the endostatin fusion proteins are under investigation. We will continue to pursue Aim I which was not completed during the first year of the grant.

Specific Aim II. Test the antiangiogenic activity of anti-HER2 antibody-human endostatin fusion protein(s) *in vitro* and *in vivo*.

- Task 5. Analyze HER2 signaling (Months 4-18).
 - a. Proliferation of tumor cells using ^3H -thymidine.
 - b. MMT assay.
 - c. PI3K/Akt signaling pathway.
 - d. p27/Cdk2 complex formation.
- Task 6. Analyze antiangiogenic activities (Months 8-18).
 - a. Chorioallantoic membrane (CAM) assay.
 - b. Endothelial cell proliferation assay.
 - c. Endothelial cell migration assay.
- Task 7. Analysis of antiangiogenesis (Months 12-24).
 - a. Immunohistochemical staining.
 - b. Confocal microscopical analysis.
- Task 8. Examine VEGF/VEGFR and PDGF/PDGFR expression in tumors (Months 12-24).
 - a. Immunohistochemical staining.
 - b. RT-PCR using TagMan.

We will focus on testing antiangiogenic efficacy of the fusion proteins *in vitro/in vivo* during the second year of the grant.

Specific Aim III. Study the antibody-endostatin fusion proteins *in vivo* for effects on tumor growth in animal tumor and/or human xenograft models.

- Task 9. Anti-tumor activity in human tumor xenografts (Months 18-24)
 - a. Monitoring tumor growth inhibition.
 - b. Analyze blood vessel formation.
- Task 10. Anti-tumor activity in metastatic models (Months 21-27)
 - a. Monitoring mouse survival.
 - b. Analyze blood vessel formation in tissues with metastatic tumors.
- Task 12. Anti-tumor activity in orthotopic metastatic models (Months 24-36)
 - a. Monitoring mouse survival.
 - b. Analyze blood vessel formation in tissues with metastatic tumors.
- Task 13. Combination treatment (Months 24-36)
 - a. PDGF blockade: Imatinib (Months 24-30)
 - i. Monitoring tumor growth inhibition.
 - ii. Analyze blood vessel formation in tumors.
 - b. VEGF blockade: Avastin (Months 24-30)
 - i. Monitoring tumor growth inhibition.
 - ii. Analyze blood vessel formation in tumors.
 - c. Metronomic therapy (Months 27-36)
 - i. Cyclophosphide
 - ii. Taxanes
 - iii. Monitoring tumor growth inhibition
 - iv. Analyze blood vessel formation in tumors

In the third year, we will continue to focus on testing efficacy of the fusion proteins *in vivo* in tumor models. This will include *in vivo* targeting and tumor challenge experiments using the huEndo fusions in the EMT6-HER2 mouse tumor model and/or the SKBR-3 human breast cancer xenograft

model to confirm the tumor regression experiment results and enhanced anti-tumor activity with combination therapy.

KEY RESEARCH ACCOMPLISHMENTS

1. Wild type and mutant type (P125A) of human endostatin have been constructed and expressed.
2. Anti-HER2 IgG3-huEndo fusion proteins (Hinge-huEndo and C_H3-huEndo) of the expected molecular weight were secreted as the fully assembled H₂L₂ form.
3. Mutants (P125A) of anti-HER2 IgG3-huEndo fusion proteins (Hinge-huEndo and C_H3-huEndo) of the expected molecular weight were secreted as the fully assembled H₂L₂ form.

REPORTABLE OUTCOMES

A U.S. patent application (10/858,980) has been filed on June, 2004 (Appendix 1). This patent application covers chimeric antibody molecules and methods of use as an anticancer therapeutic. The results of this grant will support the patent application.

CONCLUSIONS

The wild type and the mutant type (P125A) of human endostatin were constructed with FLAG tagging to distinguish from the endogenous human endostatin. The anti-HER2 IgG3-huEndo fusion proteins were constructed as wild type and mutant type (P125A) and were stably transfected into SP2/0 or P3X63Ag8.653 myeloma cells. The secreted huEndo fusion proteins have a molecular weight of 220 kDa for the anti-HER2 IgG3-C_H3-huEndo fusion protein and 160 kDa for the anti-HER2 IgG3-Hinge-huEndo fusion protein. The huEndo fusion proteins were faithfully secreted as the fully assembled H₂L₂ form.

The prolonged half-life of anti-HER2 IgG3-huEndo fusion protein and the ability to localize to tumors will increase effective exposure of tumor to endostatin thereby potentiating endostatin activity. The combination of antibody targeting and endostatin may improve efficacy over either antibody or antiangiogenic factor alone.

REFERENCES

1. Eder JP Jr, Supko JG, Clark JW, Puchalski TA, Garcia-Carbonero R, Ryan DP, Shulman LN, Proper J, Kirvan M, Rattner B, Connors S, Keogan MT, Janicek MJ, Fogler WE, Schnipper L, Kinchla N, Sidor C, Phillips E, Folkman J, Kufe DW. Phase I clinical trial of recombinant human endostatin administered as a short intravenous infusion repeated daily. *J Clin Oncol* 2002, 20(18):3772-84.
2. Herbst RS, Hess KR, Tran HT, Tseng JE, Mullani NA, Charnsangavej C, Madden T, Davis DW, McConkey DJ, O'Reilly MS, Ellis LM, Pluda J, Hong WK, Abbruzzese JL. Phase I study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol* 2002, 20(18):3792-803.
3. Thomas JP, Arzoomanian RZ, Alberti D, Marnocha R, Lee F, Friedl A, Tutsch K, Dresen A, Geiger P, Pluda J, Fogler W, Schiller JH, Wilding G. Phase I pharmacokinetic and pharmacodynamic study of recombinant human endostatin in patients with advanced solid tumors. *J Clin Oncol*. 2003, 21(2):223-31.
4. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. *J Clin Oncol* 1996, 14:737-744.
5. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/*neu* monoclonal antibody plus cisplatin in patients with

- HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J Clin Oncol* 1998,16:2659-2671.
6. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/*neu*-overexpressing metastatic breast cancer. *Semin Oncol* 1999, 26(4 Suppl 12):78-83.
 7. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/*neu* overexpressing human breast cancer xenografts. *Cancer Res* 1998, 58:2825-2831.
 8. Burstein HJ, Harris LN, Gelman R, Lester SC, Nunes RA, Kaelin CM, Parker LM, Ellisen LW, Kuter I, Gadd MA, Christian RL, Kennedy PR, Borges VF, Bunnell CA, Younger J, Smith BL, Winer EP. Preoperative therapy with trastuzumab and paclitaxel followed by sequential adjuvant doxorubicin/cyclophosphamide for HER2 overexpressing stage II or III breast cancer: a pilot study. *J Clin Oncol*. 2003, 21(1):46-53.
 9. Cho HM, Rosenblatt JD, Kang YS, Iruela-Arispe ML, Morrison SL, Penichet ML, Kwon YG, Kim TW, Webster KA, Nechustan H, Shin SU. Enhanced inhibition of murine tumor and human breast tumor xenografts using targeted delivery of an antibody-endostatin fusion protein. *Mol Cancer Ther*. 2005, 4(6):956-67.
 10. Calvo A, Yokoyama Y, Smith LE, Ali I, Shih SC, Feldman AL, Libutti SK, Sundaram R, Green JE. Inhibition of the mammary carcinoma angiogenic switch in C3(1)/SV40 transgenic mice by a mutated form of human endostatin. *Int J Cancer*. 2002, 101(3):224-34.
 11. Yokoyama Y, Ramakrishnan S. Improved biological activity of a mutant endostatin containing a single amino-acid substitution. *Br J Cancer*. 2004, 90(8):1627-35.
 12. Subramanian IV, Ghebre R, Ramakrishnan S. Adeno-associated virus-mediated delivery of a mutant endostatin suppresses ovarian carcinoma growth in mice. *Gene Ther*. 2005, 12(1):30-8.
 13. Thomsen DR, Stenberg RM, Goins WF, Stinski MF. Promoter-regulatory region of the major immediate early gene of human cytomegalovirus. *Proc Natl Acad Sci U S A*. 1984, 81(3):659-63.
 14. Chapman BS, Thayer RM, Vincent KA, Haigwood NL. Effect of intron A from human cytomegalovirus (Towne) immediate-early gene on heterologous expression in mammalian cells. *Nucleic Acids Res*. 1991, 19(14):3979-86.
 15. Stevenson BJ, Hagenbuchle O, Wellauer PK. Sequence organisation and transcriptional regulation of the mouse elastase II and trypsin genes. *Nucleic Acids Res*. 1986, 14(21):8307-30.
 16. Shin SU, Wu D, Ramanathan R, Pardridge WM, Morrison SL. Functional and pharmacokinetic properties of antibody-avidin fusion proteins. *J Immunol* 1997, 158:4797-804.
 17. Challita-Eid PM, Penichet ML, Shin SU, et al. A B7.1-antibody fusion protein retains antibody specificity and ability to activate via the T cell costimulatory pathway. *J Immunol* 1998, 160:3419-26.
 18. Coloma MJ, Hastings A, Wims LA, Morrison SL. Novel vectors for the expression of antibody molecules using variable regions generated by polymerase chain reaction. *J Immunol Methods* 1992, 152:89-104.
 19. Shin SU, Morrison SL. Production and properties of chimeric antibody molecules. *Methods Enzymol* 1989, 178:459-76.

APPENDICES

Appendix 1 has been attached.

SUPPORTING DATA

Not applicable.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)			ATTORNEY DOCKET NUMBER																										
As a below named inventor, I hereby declare that: My residence, mailing address and citizenship are as stated below next to my name. I believe that I am the original and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: the specification of which (check only one item below): ☐ is attached hereto. ☐ was filed as U.S. Patent Application Serial Number _____ on _____, as amended on _____ (if applicable). ☐ was filed as a PCT international application number _____ on _____, as amended on _____ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application. I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or (f), or §365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the applications on which priority is claimed: <table border="1" style="width: 100%; border-collapse: collapse;"><thead><tr><th style="width: 25%;">Prior Foreign Application Number(s)</th><th style="width: 15%;">Country</th><th style="width: 20%;">Foreign Filing Date (MM/DD/YYYY)</th><th style="width: 15%;">Priority Not Claimed</th><th style="width: 25%;">Certified Copy Attached?</th></tr></thead><tbody><tr><td> </td><td> </td><td> </td><td style="text-align: center;">☐</td><td style="text-align: center;">☐ YES ☐ NO</td></tr><tr><td> </td><td> </td><td> </td><td style="text-align: center;">☐</td><td style="text-align: center;">☐ YES ☐ NO</td></tr><tr><td> </td><td> </td><td> </td><td style="text-align: center;">☐</td><td style="text-align: center;">☐ YES ☐ NO</td></tr><tr><td> </td><td> </td><td> </td><td style="text-align: center;">☐</td><td style="text-align: center;">☐ YES ☐ NO</td></tr></tbody></table> ☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:					Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?				☐	☐ YES ☐ NO				☐	☐ YES ☐ NO				☐	☐ YES ☐ NO				☐	☐ YES ☐ NO
Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?																									
			☐	☐ YES ☐ NO																									
			☐	☐ YES ☐ NO																									
			☐	☐ YES ☐ NO																									
			☐	☐ YES ☐ NO																									

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Applications)				ATTORNEY DOCKET NUMBER	
I hereby claim the benefit under 35 U.S.C. Sections 120 or 119(e) of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:					
PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:					
U.S. APPLICATIONS				STATUS (Check One)	
U.S. APPLICATION NUMBER	U.S. FILING DATE		PATENTED	ABANDONED	PENDING
PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NUMBER	PCT FILING DATE	U.S. SERIAL NUMBERS			
POWER OF ATTORNEY: As a named inventor, I hereby appoint registered patent practitioners associated with Customer Number 30448 to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith.					
Send Correspondence to: *Customer Number 30448*			Direct Telephone Calls to:		
201	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP	
	MAILING ADDRESS	MAILING ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP	
	MAILING ADDRESS	MAILING ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP	
	MAILING ADDRESS	MAILING ADDRESS	CITY	STATE & ZIP CODE/COUNTRY	
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.					
SIGNATURE OF INVENTOR 201		SIGNATURE OF INVENTOR 202		SIGNATURE OF INVENTOR 203	
DATE		DATE		DATE	