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(54) **FAS-CHIMERA ADENOVIRUS VECTOR**

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C12N 15/00	(2006.01)
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A61K 35/761	(2015.01)
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(56) **References Cited**

U.S. PATENT DOCUMENTS

3,773,919	A	11/1973	Boswell et al.
7,067,649	B2	6/2006	Harats
7,579,327	B2	8/2009	Harats et al.
7,585,666	B2	9/2009	Harats et al.
7,989,427	B2	8/2011	Harats et al.
8,039,261	B2	10/2011	Harats et al.
8,071,740	B2	12/2011	Harats et al.
8,206,743	B2	6/2012	Harats et al.
8,415,318	B2	4/2013	Harats et al.
8,835,398	B2	9/2014	Harats et al.
8,846,401	B2	9/2014	Harats et al.
8,859,745	B2	10/2014	Harats et al.
8,859,747	B2	10/2014	Harats et al.
2007/0286845	A1	12/2007	Harats et al.
2010/0282634	A1	11/2010	Harats et al.
2010/0298226	A1	11/2010	Breitbart et al.
2011/0201677	A1	8/2011	Harats et al.
2011/0207985	A1	8/2011	Harats et al.
2011/0319479	A1	12/2011	Breitbart et al.
2013/0052165	A1	2/2013	Bangio et al.
2013/0209450	A1	8/2013	Cohen et al.
2013/0272998	A1	10/2013	Harats et al.
2013/0280216	A1	10/2013	Cohen et al.
2013/0280217	A1	10/2013	Cohen et al.
2013/0295053	A1	11/2013	Bangio et al.
2013/0303595	A1	11/2013	Cohen et al.
2014/0010785	A1	1/2014	Cohen et al.
2015/0111957	A1	4/2015	Breitbart et al.

FOREIGN PATENT DOCUMENTS

EP	0058481	A1	8/1982
EP	0133988	A2	3/1985
WO	WO-03033514	A1	4/2003
WO	WO 03093409	A2	11/2003

(Continued)

OTHER PUBLICATIONS

Abboud, C.N. and Lichtman, M.A., "Structure of the Marrow and the Hematopoietic Microenvironment," in *Williams' Hematology,* vol. 6, pp. 29-58, Beutler, E., eds, McGraw-Hill, New York (2001).

Boldin, M.P., et al., "A novel protein that interacts with the death domain of Fas/APO1 contains a sequence motif related to the death domain," The Journal of Biological Chemistry 270(14):7795-7798, The American Society for Biochemistry and Molecular Biology, USA (1995).

Boldin, M.P., et al., "Self-association of the "death domains" of the p55 tumor necrosis factor (TNF) receptor and Fas/APO1 prompts signaling for TNF and Fas/APO1 effects," The Journal of Biological Chemistry 270(1):387-391, The American Society for Biochemistry and Molecular Biology, USA (1995).

(Continued)

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Assistant Examiner — Magdalene Sgagias
(74) *Attorney, Agent, or Firm* — Sterne, Kessler, Goldstein & Fox P.L.L.C.

(57) **ABSTRACT**

The invention provides methods of reducing or decreasing a size of a tumor or eliminating a tumor or inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor in a patient by administering an adenovirus comprising a nucleic acid construct comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter. Also provided is a homogeneous population of an adenovirus comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter and its uses thereof.

23 Claims, 3 Drawing Sheets

(56) **References Cited**

FOREIGN PATENT DOCUMENTS

WO	WO-2006051545	A2	5/2006
WO	WO 2007096882	A2	8/2007
WO	WO-2008015675	A2	2/2008
WO	WO-2011083464	A2	7/2011
WO	WO-2011083466	A1	7/2011
WO	WO-2011086509	A1	7/2011
WO	WO-2012052878	A1	4/2012

OTHER PUBLICATIONS

Chen, Y-H., et al., "Upstream Stimulatory Factors Regulate Aortic Preferentially Expressed Gene-1 Expression in Vascular Smooth Muscle Cells," The Journal of Biological Chemistry 276(50):47658-47663, The American Society for Biochemistry and Molecular Biology, Inc., USA (2001).

Collins, C.J., et al., "Molecular Cloning of the Human Gene for Von Willebrand Factor and Identification of the Transcription Initiation Site," Proceedings of the National Academy of Sciences USA 84(13):4393-4397, National Academy of Sciences, USA (1987).

Collins, T., et al., "Structure and chromosomal location of the gene for endothelial-leukocyte adhesion molecule 1.," The Journal of Biological Chemistry 266(4):2466-2473, The American Society for Biochemistry and Molecular Biology, Inc., USA (1991).

Dennis, M.S., et al., "Albumin Binding as a General Strategy for Improving the Pharmacokinetics of Proteins," The Journal of Biological Chemistry 277(38):35035-35043, The American Society for Biochemistry and Molecular Biology, Inc., USA (2002).

English language Abstract of European Patent Publication No. 0 133 988 A2, European Patent Office, espacenet database—Worldwide, (2014) (listed as document EP0133988A2 on the accompanying form PTO/SB/08A).

Goodman and Gilman's, "The Pharmacological Basis of Therapeutics," Eighth Edition:1202-1263, Pergamon Press, USA (1990).

Harats, D., et al., "Targeting Gene Expression to the Vascular Wall in Transgenic Mice Using the Murine Preproendothelin-1 Promoter," Journal of Clinical Investigation 95(3):1335-1344, The American Society for Clinical Investigation, Inc., USA (1995).

Hoffman, R., et al., "Hematology Basic Principals and Practice," Third Edition:3, Churchill Livingstone, New York (2000).

Horley, K.J., et al., "Molecular Cloning of Murine Intercellular Adhesion Molecule (ICAM-1)," The EMBO Journal 8(10):2889-2896, IRL Press, England (1989).

Horowitz, N.S., et al., "Safety, Efficacy, and Biomarker Exploration in a Phase II Study of Bevacizumab, Oxaliplatin and Gemcitabine in Recurrent Mullerian Carcinoma," Clinical ovarian cancer and other gynecologic malignancies 4(1):26-33, Elsevier Inc., USA (2011).

Iademarco, M.F., et al., "Characterization of the Promoter for Vascular Cell Adhesion Molecule-1 (VCAM-1)," The Journal of Biological Chemistry 267(23):16323-16329, The American Society for Biochemistry and Molecular Biology, Inc., USA (1992).

Jain, R.K., et al., "Biomarkers of Response and Resistance to Antiangiogenic Therapy," Nature Reviews Clinical Oncology 6(6):327-338, Macmillan Publishers Limited, England (2009).

Wold, F., "Posttranslational Protein Modifications: Perspectives and Prospectives," in *Posttranslational Covalent Modification of Proteins,* pp. 1-21, Academic Press, New York, USA (1983).

Joussen, A.M., et al., "Retinal Vascular Disease," Documenta Ophthalmologica 117(3):263-265, Springer-Verlag, UK (2008).

Langer, R., "Controlled release of macromolecules," Chem. Tech 12(2):98-105, Society, USA (1982).

Langer, R., et al., "Biocompatibility of Polymeric Delivery Systems for Macromolecules," Journal of Biomedical Materials Research 15(2):267-277, John Wiley & Sons, Inc., USA (1981).

Layne, M.D., et al., "Characterization of the Mouse Aortic Carboxypeptidase-Like Protein Promoter Reveals Activity in Differentiated and Dedifferentiated Vascular Smooth Muscle Cells," Circulation Research 90(6):728-736, American Heart Association, Inc., USA (2002).

Schalm, O.W., "The Hematopoietic System," in *Schalm's Veterinary Hematology*, 4th edition, pp. 350-387, Lea & Febiger, Philadelphia, USA (1990).

Morishita, K., et al., "A Novel Promoter for Vascular Endothelial Growth Factor Receptor (flt-1) That Confers Endothelial-Specific Gene Expression," The Journal of Biological Chemistry 270(46):27948-27953, The American Society for Biochemistry and Molecular Biology, Inc., USA, (1995).

Newman, P.J., et al.,"PECAM-1 (CD31) Cloning and Relation to Adhesion Molecules of the Immunoglobulin Gene Superfamily," Science 247(4947):1219-1222, American Association for the Advancement of Science, USA (1990).

Oken, M.M., et al., "Toxicity and Response Criteria of the Eastern Cooperative Oncology Group," American Journal of Clinical Oncology 5(6):649-655, Lippincott Williams & Wilkins, USA, (1982).

Picker, L.J. and Siegelman, M.H., "Lymphoid Tissues and Organs," in *Fundamental Immunology*, 4th Edition, pp. 479-531, Paul,W.E.,ed, Lippincott-Raven, Philadelphia, PA, USA (1999).

Rattan, S.I.S., et al., "Protein Synthesis, Posttranslational Modifications, and Aging," Annals of the New York Academy of Sciences 663:48-62, John Wiley & Sons, Inc., USA, (1992).

Rius, C., et al., "Cloning of the Promoter Region of Human Endoglin, the Target Gene for Hereditary Hemorrhagic Telangiectasia Type 1," Blood 92(12):4677-4690, The American Society of Hematology USA (1998).

Ronicke, V., et al., "Characterization of the Endothelium-Specific Murine Vascular Endothelial Growth Factor Receptor-2 (Flk-1) Promoter," Circulation Research 79(2):277-285, American Heart Association, Inc., USA, (1996).

Sato, T.N., et al., "*Tie*-1 and *Tie*-2 Define Another Class of Putative Receptor Tyrosine Kinase Genes Expressed in Early Embryonic Vascular System," Proceedings of the National Academy of Sciences USA 90(20):9355-9358, National Academy of Sciences, USA, (1993).

Seifter, S. and Englard, S., "Analysis for Protein Modifications and Nonprotein Cofactors," Methods in Enzymology 182:626-646, Academic Press, USA, (1990).

Sidman, K.R., et al., "Controlled Release of Macromolecules and Pharmaceuticals from Synthetic Polypeptides Based on Glutamic Acid," Biopolymers 22:547-556, John Wiley & Sons, Inc., USA, (1983).

Sukhatme, V.P., et al., "A Novel Early Growth Response Gene Rapidly Induced by Fibroblast, Epithelial Cell and Lymphocyte Mitogens," Oncogene Research 1(4):343-355, Harwood Academic Publishers GmbH, USA (1987).

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. O19131, Entry Date Jun. 11, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P19438-1, Entry Date Jul. 9, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P19438-2, Entry Date Jul. 9, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P19438-3, Entry Date Jul. 9, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P22934, Entry Date Jun. 11, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P25118, Entry Date Jul. 9, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 1A", Accession No. P50555, Entry Date Apr. 16, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. P25445, Entry Date Jul. 9, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. P25446, Entry Date Jul. 9, 2014.

(56) **References Cited**

OTHER PUBLICATIONS

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. P51867, Entry Date May 14, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. Q63199, Entry Date Jun. 11, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. Q9BDN4, Entry Date Feb. 19, 2014.

UniProtKB Database, "Recommended name: Tumor necrosis factor receptor superfamily member 6", Accession No. Q9BDP2, Entry Date Feb. 19, 2014.

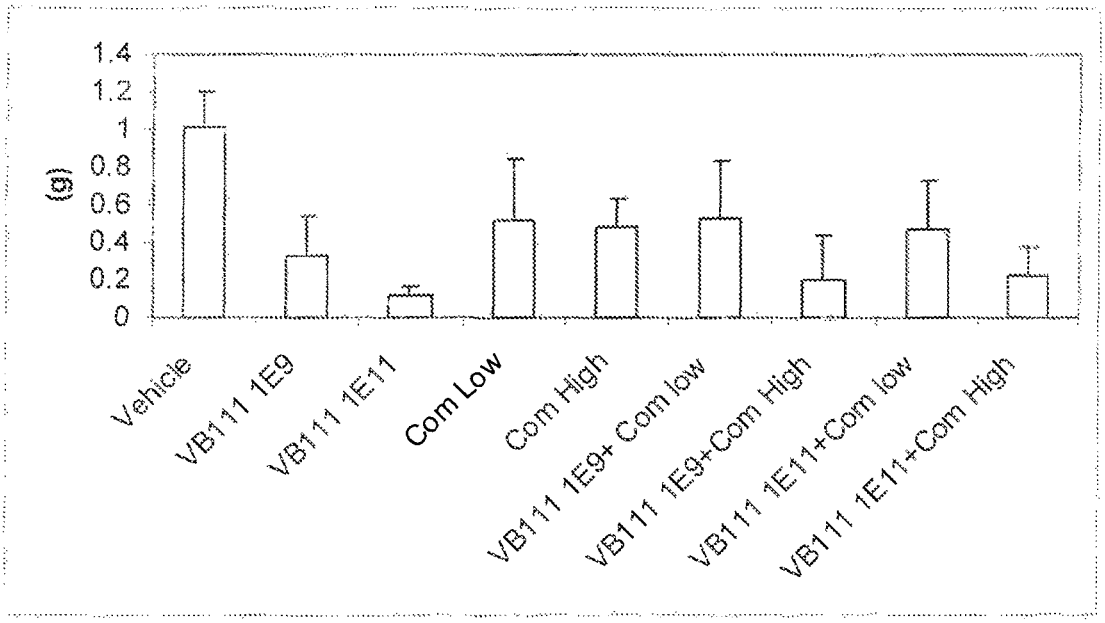
UniProtKB Database, "Submitted name: Tumor necrosis factor rece Varda-Bloom, N., et al., "Tissue-Specific Gene Therapy Directed to Tumor Angiogenesis," Gene Therapy 8:819-827, Nature Publishing Group, England (2001).

Weiss, L. and Geduldig, U., "Barrier Cells: Stromal Regulation of Hematopoiesis and Blood Cell Release in Normal and Stressed Murine Bone Marrow," Blood 78(4):975-990, The American Society of Hematology, USA (1991).

Wickramasinghe, S. N., "Bone marrow," in *Histology for Pathologist*, Chapter 1, pp. 1-31, Sternberg, S.S., ed, Raven Press, New York, USA (1992).

Greenberger, S., et al., "Transcription-controlled gene therapy against tumor angiogenesis," *J. Clin. Invest.* 113:1017-1034, American Society for Clinical Investigation, United States (2004).

International Search Reort for International Application No. PCT/IB2013/003015, Patent Cooperation Treaty, mailed on Sep. 3, 2014.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VB111 1E9
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FIGURE 1

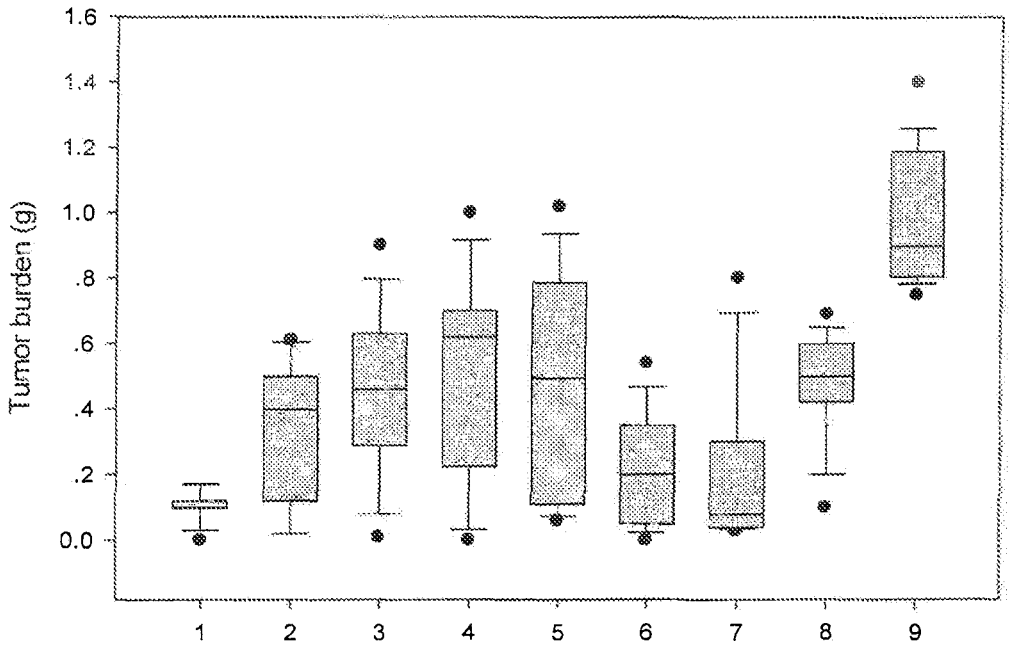

FIGURE 2

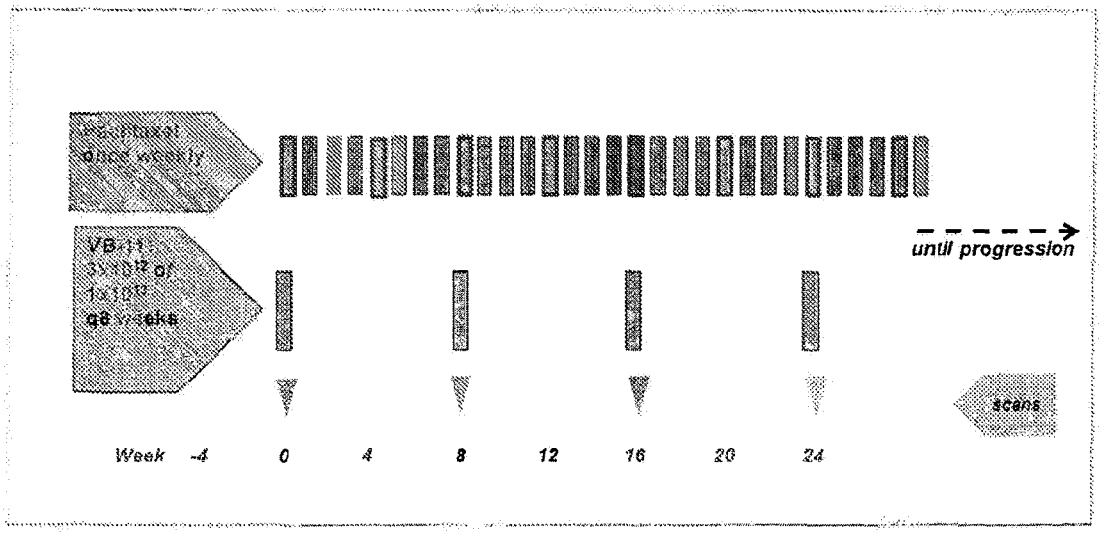

FIGURE 3

FAS-CHIMERA ADENOVIRUS VECTOR

REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of PCT Application No. PCT/IB2013/003015, filed on Oct. 17, 2013, which claims the benefit of U.S. Provisional Patent Application Nos. 61/785,287, filed on Mar. 14, 2013, and 61/715,206, filed on Oct. 17, 2012. The contents of the above applications are all incorporated herein by reference in their entireties.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The contents of this electronically submitted sequence listing in ASCII text file (Name: 3182_0430003_SequenceListing_ST25; Size: 166,097 bytes; and Date of Creation: Sep. 11, 2014), filed herewith, is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

1. Field of the Disclosure

This disclosure relates to cancer biology, immunology and pharmacology. More particularly, it relates to methods of treating diseases or disorders relating to gynecological cancer by administration of a nucleic acid construct expressing a Fas-chimera transgene product or a homogeneous population of the nucleic acid construct.

2. Background Art

Gynecological cancers are clinically aggressive, usually develop in tissues of the female genital tract, and are associated with a poor outcome. These cancers include cancers of the ovaries, uterus, fallopian tubes, and the cervix, and also malignant mixed müllerian tumors (MMMT). In rare instances, MMMTs can also develop in the female peritoneum (lining of the abdominal wall).

Gynecological cancers can be difficult to detect and are often diagnosed when they are at an advanced stage. Ovarian cancer accounts for approximately three percent of cancers in women. While the ninth most common cancer among women, ovarian cancer is the fifth leading cause of cancer-related death among women, and is the deadliest of gynecologic cancers. 2012. Ovarian cancer is sensitive to chemotherapy with a high response rate to platinum and taxane-based therapies. However, in spite of advances in therapeutic design and delivery, cancer recurrence and chemotherapeutic resistance remain obstacles to treatment of these types of cancers. Despite aggressive primary therapy and high initial response rates, most women with advanced ovarian carcinoma will relapse and develop drug-resistant disease. In these advanced disease states, response rates to subsequent chemotherapy are substantially diminished, highlighting the crucial need to develop improved therapeutic agents and strategies.

BRIEF SUMMARY OF THE DISCLOSURE

The present invention is directed to a method of reducing or decreasing a size of a tumor or eliminating or slowing the growth of a tumor in a patient comprising administering to the patient an effective amount of a nucleic acid construct, which comprises a Fas-chimera gene operably linked to an endothelial cell specific promoter, wherein a Fas-chimera gene product encoded by the nucleic acid construct reduces or decreases the size of the tumor or eliminates the tumor in the patient and wherein the tumor is associated with a female gynecological cancer or a metastasis thereof. The invention

also provides a method of inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor comprising administering to a patient having the tumor an effective amount of a nucleic acid construct, which comprises a Fas-chimera gene operably linked to an endothelial cell specific promoter, wherein a Fas-chimera gene product encoded by the nucleic acid construct inhibits, reduces, or decreases the neo-vascularization or angiogenesis in the tumor and wherein the tumor is associated with a female gynecological cancer or a metastasis thereof. In addition, the invention includes a method of treating or preventing a tumor associated with or derived from Müllerian cancer, ovarian cancer, peritoneal cancer, fallopian tube cancer, or uterine papillary serous carcinoma in a patient comprising administering an effective amount of a nucleic acid construct, which comprises a Fas-chimera gene operably linked to an endothelial cell specific promoter, wherein a Fas-chimera gene product encoded by the nucleic acid construct treats or prevents a female gynecological cancer or a metastasis thereof. In one embodiment, the tumor or a metastasis thereof is decreased in size or eliminated after the administration or the growth of the tumor or. In another embodiment, tumor or a metastatis thereof is decreased such that the longest diameter (LD) of the tumor is decreased at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the LD prior to the administration. In other embodiments, the female gynecological cancer is associated with or derived from Müllerian cancer, ovarian cancer, peritoneal cancer, fallopian tube cancer, uterine papillary serous carcinoma, any combinations thereof, or a metastasis thereof.

In some embodiments, the patient has had a prior platinum based therapy. In one example, the patient has recurrent platinum-resistant cancer. In another example, the patient having the recurrent platinum-resistant cancer or the recurrent taxane-resistant cancer has a progressive tumor within six months of completing or receiving a platinum based therapy or a taxane based therapy.

In certain embodiments, the patient does not have a pre-existing antibody against adenovirus or does not develop an antibody against adenovirus.

In other embodiments, the methods of the invention further comprise administering an effective amount of one or more chemotherapeutic agents. The one or more chemotherapeutic agents can be administered prior to, concurrently with, or after the administration of the nucleic acid construct. In a specific embodiment, the chemotherapeutic agent is paclitaxel.

In certain embodiments, the nucleic acid construct for the method of the invention is an adenovirus. In one example, the adenovirus expresses a Fas-chimera gene product comprising an extracellular domain of a TNF Receptor 1 (TNFR1) polypeptide fused to a transmembrane domain and an intracellular domain of a Fas polypeptide. The polynucleotide encoding the Fas-chimera gene product is operably linked to an endothelial cell specific promoter, e.g., a PPE-1-3X promoter. In a particular embodiment, the adenovirus comprises a nucleotide sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19.

One aspect of the invention includes a nucleic acid construct comprising SEQ ID NO: 18. In another aspect, the nucleic acid construct further comprises a nucleotide sequence encoding a Fas-chimera protein. In other embodiments, the invention is a vector comprising SEQ ID NO: 19. The vector can be an adenovirus.

In still other embodiments, the invention is an adenovirus having the European Collection of Cell Cultures (ECACC) deposit designation No. 13021201. The adenovirus can be at

least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In one example, the invention comprises a pharmaceutical composition comprising the adenovirus and a pharmaceutically acceptable carrier, wherein the composition does not contain another type of adenovirus, e.g., adenovirus comprising SEQ ID NO: 20 or SEQ ID NO: 21.

The invention also includes a method of inhibiting, decreasing, or reducing angiogenesis or neo-vascularization in a tissue of a subject in need thereof comprising administering the nucleic acid construct, the vector, the adenovirus, or the composition to the subject. In one embodiment, the tissue comprises a tumor. In another embodiment, the size of the tumor is reduced or decreased after the administration or the growth of the tumor is slowed after the administration. In other embodiments, the tumor is derived from or associated with thyroid cancer, neuroendocrine cancer, glioblastoma, a female gynecological cancer, any combinations thereof or a metastasis thereof.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

FIG. **1** shows the average tumor burden (y axis) among the Lewis Lung Carcinoma mice administered with vehicle, VB111 1E9 (10^9 Virus Particles (VPs) of VB-111), VB111 1E11 (10^{11} VPs of VB-111), ComLow (20 mg/kg Carboplatin+110 mg/kg Alimta), ComHigh (50 mg/kg Carboplatin+30 mg/kg Alimta), VB 111 1E9+ComLow (VB-111 10^9 VP s+20 mg/kg Carboplatin+10 mg/kg Alimta), VB111 1E9+ComHigh (VB-111 10^9 VP+50 mg/kg Carboplatin+30 mg/kg Alimta), VB111 1E11+ComLow (VB-111 10^{11} VP+20 mg/kg Carboplatin+10 mg/kg Alimta), and VB111 1E11+ComHigh (VB-111 10^{11} VP+50 mg/kg Carboplatin+30 mg/kg Alimta) (x axis).

FIG. **2** shows a box plot of the average tumor burden (y axis) among the Lewis Lung Carcinoma mice administered with vehicle (1), VB111 1E9 (10^9 Virus Particles (VPs) of VB-111)(2), VB111 1E11 (10^{11} VPs of VB-111×)(3), ComLow (20 mg/kg Carboplatin+10 mg/kg Alimta)(4), ComHigh (50 mg/kg Carboplatin+30 mg/kg Alimta)(5), VB111 1E9+ComLow (VB-111 10^9 VP s+20 mg/kg Carboplatin+10 mg/kg Alimta)(6), VB111 1E9+ComHigh (VB-111 10^9 VP+50 mg/kg Carboplatin+30 mg/kg Alimta)(7), VB111 1E11+ComLow (VB-111 10^{11} VP+20 mg/kg Carboplatin+10 mg/kg Alimta)(8), and VB111 1E11+ComHigh (VB-111 10^{11} VP+50 mg/kg Carboplatin+30 mg/kg Alimta)(9) (x axis).

FIG. **3** shows a combination therapy regimen of an adenovirus comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter (e.g., VB-111) and paclitaxel. About 3×10^{12} VPs or 1×10^{13} VPs of VB-111 is administered every eight weeks, and paclitaxel is administered once weekly.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications, patents and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

In order to further define this invention, the following terms and definitions are provided.

As used herein, "antibody" means an intact immunoglobulin, or an antigen-binding fragment thereof. Antibodies of this invention can be of any isotype or class (e.g., M, D, G, E and A) or any subclass (e.g., G1-4, A1-2) and can have either a kappa (κ) or lambda (λ) light chain.

The term "effective amount" as used herein refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired result. A desired result can be, for example, reduction or inhibition of neo-vascularization or angiogenesis in vitro or in vivo. An effective amount need not be a complete removal of neo-vascularization or angiogenesis.

As used herein, a "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result may be, e.g., lessening of symptoms, regression or stabilization of tumor size in radiological imaging, prolonged survival, improved mobility, and the like. A therapeutic result need not be a "cure."

As used herein, a "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

The term "polynucleotide" or "nucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construat, e.g., messenger RNA (mRNA) or plasmid DNA (pDNA). In certain embodiments, a polynucleotide comprises a conventional phosphodiester bond or a non-conventional bond (e.g., an amide bond, such as found in peptide nucleic acids (PNA)).

As used herein, a "polynucleotide," "nucleotide," "nucleic acid" can be used interchangeably and contain the nucleotide sequence of the full-length cDNA sequence, including the untranslated 5' and 3' sequences, the coding sequences, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. The polynucleotide can be composed of any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. Polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus,

"polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

In the present invention, a polypeptide can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids (e.g. non-naturally occurring amino acids). The polypeptides of the present invention may be modified by either natural process, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, Proteins—Structure And Molecular Properties, 2nd Ed., T. E. Creighton, W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth Enzymol* 182:626-646 (1990); Rattan et al., *Ann NY Acad Sci* 663:48-62 (1992).)

The terms "fragment," "variant," "derivative" and "analog" when referring to any polypeptide or polynucleotide of the present invention include any polypeptides or polynucleotides which retain at least some activities, i.e., the ability to function as any naturally-occurring function of the polypeptide or polynucleotide. For example, a "fragment," "variant," "derivative" and "analog" of Tumor necrosis factor Receptor 1 (TNFR1) has some activities of the naturally occurring full-length TNFR1, e.g., the ability to bind to TNFR1 ligand, i.e., TNF-alpha or lymphotoxin. In another example, a "fragment," "variant," "derivative" and "analog" of a FAS polypeptide have some activities of a naturally-occurring full-length FAS polypeptide, e.g., the ability to induce apoptosis. In other examples, a "fragment," "variant," "derivative" and "analog" of an endothelial cell-specific promoter can induce endothelial cell-specific expression of a gene operably linked to the promoter. Additional non-limiting examples of the various fragments, variants, analogues, or derivatives of the TNFR1, FAS polypeptide, and endothelial cell-specific promoters are described below.

In the present invention, a "polypeptide fragment" or "protein fragment" refers to a short amino acid sequence of a polypeptide. Protein or polypeptide fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part of region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising about 5 amino acids, about 10 amino acids, about 15 amino acids, about 20 amino acids, about 30 amino acids, about 40 amino acids, about 50 amino acids, about 60 amino acids, about 70 amino acids, about 80 amino acids, about 90 amino acids, and about 100 amino acids.

A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, if an amino acid in a polypeptide is replaced with another amino acid from the same side chain family, the substitution is considered to be conservative. In another embodiment, a string of amino acids can be conservatively replaced with a structurally similar string that differs in order and/or composition of side chain family members.

The term "percent sequence identity" between two polynucleotide or polypeptide sequences refers to the number of identical matched positions shared by the sequences over a comparison window, taking into account additions or deletions (i.e., gaps) that must be introduced for optimal alignment of the two sequences. A matched position is any position where an identical nucleotide or amino acid is presented in both the target and reference sequence. Gaps presented in the target sequence are not counted since gaps are not nucleotides or amino acids. Likewise, gaps presented in the reference sequence are not counted since target sequence nucleotides or amino acids are counted, not nucleotides or amino acids from the reference sequence.

The percentage of sequence identity is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity. The comparison of sequences and determination of percent sequence identity between two sequences may be accomplished using readily available software both for online use and for download. Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast-.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, e.g., Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at worldwideweb.ebi.ac.uk/Tools/psa.

Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent

sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. Sequence alignments can be derived from multiple sequence alignments. One suitable program to generate multiple sequence alignments is ClustalW2, available from worldwideweb.clustal.org. Another suitable program is MUSCLE, available from worldwideweb.drive5.com/muscle/. ClustalW2 and MUSCLE are alternatively available, e.g., from the EBI.

It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (e.g., crystallographic protein structures), functional data (e.g., location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at worldwideweb.tcoffee-.org, and alternatively available, e.g., from the EBI. It will also be appreciated that the final alignment used to calculated percent sequence identity may be curated either automatically or manually.

As used herein, the terms "linked," "fused," "fusion," "chimeric," and "chimera" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct reading frame of the original ORFs. Thus, the resulting recombinant fusion or chimeric protein is a single protein containing two ore more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments may be physically or spatially separated by, for example, in-frame linker sequence.

The terms "heterologous" and "heterologous moiety" mean that a polynucleotide, polypeptide, or other moiety is derived from a distinct entity from that of the entity to which it is being compared. For instance, a heterologous polypeptide can be synthetic, or derived from a different species, different cell type of an individual, or the same or different type of cell of distinct individuals. In one aspect, a heterologous moiety can be a polypeptide fused to another polypeptide to produce a fusion polypeptide or protein. In another aspect, a heterologous moiety can be a non-polypeptide such as PEG conjugated to a polypeptide or protein.

In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, an RNA or polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product and the translation of such mRNA into polypeptide(s). If the final desired product is biochemical, expression includes the creation of that biochemical and any precursors.

II. Nucleic Acid Constructs Comprising a FAS-Chimera Gene and an Endothelial Cell Specific Promoter

The present invention is related to methods of reducing or decreasing a size of a tumor in a female gynecological cancer by inhibiting, decreasing, or reducing angiogenesis or neovascularization in the tumor comprising administering a nucleic acid construct expressing a FAS-chimera protein. The gene encoding the FAS-chimera protein (or gene product), in the present invention can be linked to an endothelial cell-specific promoter, which directs expression of the FAS-chimera gene product in an endothelial cell. Expression of such a cytotoxic gene product is useful in a situation where excessive neo-vascularization or blood vessel growth is not desirable, e.g., in a tumor.

The present invention also provides a homogeneous population of a nucleic acid construct comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter.

A. FAS-Chimera

A FAS-chimera protein expressed by the nucleic acid construct of the invention comprises at least two "death receptor" polypeptides, each of the polypeptides is derived from a different protein. The first polypeptide of the FAS-chimera protein comprises a ligand binding domain of Tumor Necrosis Factor Receptor 1 (TNFR1). The second polypeptide of the FAS-chimera protein comprises an effector domain of a FAS polypeptide.

The ligand binding domain of TNFR1 can be any domain that binds to a TNFR1 ligand. In one embodiment, the TNFR1 ligand is TNF-α. In another embodiment, the TNFR1 ligand is lymphotoxin-α. The ligand binding domain of TNFR1 can be an extracellular domain of TNFR1 or any fragments, variants, derivatives, or analogues thereof. Non-limiting examples of the TNFR1 ligand binding domain are described below.

The effector domain of a FAS polypeptide useful for the invention comprises any FAS domains that form death-inducing signaling complex (DISC), thereby inducing apoptosis. In one embodiment, an effector domain of a FAS polypeptide comprises an intracellular domain, a trans-membrane domain, or both. Non-limiting examples of FAS polypeptide effector domains are described below.

The TNFR1 and the FAS polypeptide can be linked by a peptide bond or by a linker. The linker connecting the TNFR1 ligand binding domain with the FAS effector domain can be a polypeptide linker or a non-peptide linker. For example, a linker for the FAS-chimera protein can comprise one or more glycine, serine, leucine, or any combinations thereof. In one embodiment, a linker useful for the invention comprises Ser-Leu. In another embodiment, a linker useful for the invention comprises (GGGS)n, (Denise et al. *J. Biol. Chem.* 277:35035-35043 (2002)), wherein n can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more (SEQ ID NO: 27).

1. Tumor Necrosis Factor Receptor 1

The full-length human TNFR1 polypeptide is 455 amino acids in length and is also known as TNF-R1, Tumor necrosis factor receptor type I (TNFRI), TNFR-I, TNFRSF1A, TNFAR, p55, P60, or CD120a. Naturally-occurring human TNFR1 polypeptide is known to bind to TNF-α or homotrimeric lymphotoxin-α. Binding of TNF-α to the extracellular domain leads to homotrimerization of TNFR1, which then interacts specifically with the death domain of Tumor Necro-

sis Factor Receptor Type 1-Associated Death Domain Protein (TRADD). Various TRADD-interacting proteins such as TNF Receptor Associated Factors (TRAFS), Receptor-Interacting Serine/Threonine-Protein Kinase 1 (RIPK1), and Fas-Associated Protein with Death Domain (FADD) are recruited to the complex by their association with TRADD. The complex activates at least two distinct signaling cascades, apoptosis and NF-kappa-B signaling.

A 455 as polypeptide sequence reported as a human TNFR1 polypeptide sequence has the identifier number P19438-1 in the UniProtKB database. This human TNFR1 polypeptide sequence is designated herein as isoform A and SEQ ID NO: 2. SEQ ID NO: 1 is a nucleotide sequence encoding SEQ ID NO: 2. A polypeptide sequence of 108 as was reported as an isoform of the human TNFR1 polypeptide sequence and has the identifier number P19438-2 in the UniProtKB database. The 108 as polypeptide corresponds to amino acids 1 to 108 of isoform A (SEQ ID NO: 2) and is designated herein as isoform B. Another variant of the human TNFR1 polypeptide having 232 as was reported as the identifier number P19438-3 in the UniProtKB database. The 232 as polypeptide corresponds to amino acids 1 to 232 of isoform A (SEQ ID NO: 2) and is designated herein as isoform C. Additional natural variants of human TNFR1 include, but are not limited to, the TNFR1 polypeptide of isoforms A, B, and C comprising one or more mutations selected from the group consisting of H51Q, C59R, C59S, C62G, C62Y, P75L, T79M, C81F, C99S, S115G, C117R, C117Y, R121P, R121Q, P305T, and any combinations thereof. Other known TNFR1 variants include the TNFR1 polypeptide of isoforms A, B, and C comprising L13LILPQ, K255E, S286G, R394L, 412: Missing, GPAA443-446APP, or any combinations thereof.

Table 1 shows the human wild-type TNFR1 amino acid sequence and a nucleotide sequence encoding the wild-type TNFR1.

TABLE 1

TNFR1 Sequences

SEQ ID No.	Sequences
Amino acid sequence of TNFR1 (SEQ ID NO: 2)	MGLSTVPDLLLPLVLLELLVGIYPSGVIGLVPHLGDREKRDSVCPQGKYIHPQNNSICCT KCHKGTYLYNDCPGPGQDTDCRECESGSFTASENHLRHCLSCSKCRKEMGQVEISSCTVD RDTVCGCRKNQYRHYWSENLFQCFNCSLCLNGTVHLSCQEKQNTVCTCHAGFFLRENECV SCSNCKKSLECTKLCLPQIENVKGTEDSGTTVLLPLVIFFGLCLLSLLFIGLMYRYQRWK SKLYSIVCGKSTPEKEGELEGTTTKPLAPNPSFSPTPGFTPTLGFSPVPSSTFTSSSTYT PGDCPNFAAPRREVAPPYQGADPTLATALASDPIPNPLQKWEDSAHKPQSLDTDDPATLY AVVENVPPLRWKEFVRRLGLSDHEIDRLELQNGRCLREAQYSMLATWRRRTPRREATLEL LGRVLRDMDLLGCLEDIEEALCGPAALPPAPSLLR
Nucleotide Sequence encoding TNFR1 (SEQ ID NO: 1)	Atgggcctctccaccgtgcctgacctgctgctgccgctggtgctcctggagctgttggtg Ggaatataccctcaggggttattggactggtccctcacctaggggacagggagaagaga Gatagtgtgtgtccccaaggaaaatatatccaccctcaaaataattcgatttgctgtacc Aagtgccacaaaggaacctacttgtacaatgactgtccaggcccggggcaggatacggac Tgcagggagtgtgagagcggctccttcaccgcttcagaaaaccacctcagacactgcctc Agctgctccaaatgccgaaaggaaatgggtcaggtggagatctcttcttgcacagtggac Cgggacaccgtgtgtggctgcaggaagaaccagtaccggcattattggagtgaaaacctt Ttccagtgcttcaattgcagcctctgcctcaatgggaccgtgcacctctcctgccaggag Aaacagaacaccgtgtgcacctgccatgcaggtttctttctaagagaaaacgagtgtgtc Tcctgtagtaactgtaagaaaagcctggagtgcacgaagttgtgcctaccccagattgag Aatgttaagggcactgaggactcaggcaccacagtgctgttgcccctggtcattttcttt Ggtctttgccttttatccctcctcttcattggtttaatgtatcgctaccaacggtggaag Tccaagctctactccattgtttgtgggaaatcgacacctgaaaaagaggggagcttgaa Ggaactactactaagcccctggccccaaacccaagcttcagtcccactccaggcttcacc Cccaccctgggcttcagtcccgtgcccagttccaccttcacctccagctccacctatacc Cccggtgactgtcccaactttgcggctccccgcagagaggtggcaccaccctatcagggg Gctgaccccatccttgcgacagccctcgcctccgaccccatccccaaccccctt cagaag Tgggaggacagcgcccacaagccacagagcctagacactgatgaccccgcgacgctgtac Gccgtggtggagaacgtgaccccgttgcgctggaaggaattcgtgcggcgcctagggctg Agcgaccacgagatcgatcggctggagctgcagaacgggcgctgcctgcgcgaggcgcaa Tacagcatgctggcgacctggaggcggcgcacgccgccgcgcgaggccacgctggagctg Ctgggacgcgtgctccgcgacatggacctgctgggctgcctggaggacatcgaggaggcg ctttgcggccccgccgccctcccgcccgcgcccagtcttctcaga
Amino acid sequence of a Ligand Binding Domain of TNFR1 (SEQ ID NO: 4)	MGLSTVPDLLLPLVLLELLVGIYPSGVIGLVPHLGDREKRDSVCPQGKYIHPQNNSICCT KCHKGTYLYNDCPGPGQDTDCRECESGSFTASENHLRHCLSCSKCRKEMGQVEISSCTVD RDTVCGCRKNQYRHYWSENLFQCFNCSLCLNGTVHLSCQEKQNTVCTCHAGFFLRENECV SCSNCKKSLECTKLCLP
Nucleotide sequence encoding Ligand Binding Domain of TNFR1 (SEQ ID	atgggcctct ccaccgtgcc tgacctgctg ctgccgctgg tgctcctgga gctgttggtg ggaatatacc cctcaggggt tattggactg gtccctcacc atagggacag ggagaagaga gatagtgtgt gtccccaagg aaaatatatc caccctcaaa ataattcgat ttgctgtacc aagtgccaca aaggaaccta cttgtacaat gactgtccag gcccggggca tgcagggagt ggatacggac gtgagagcgg ctccttcacc gcttcagaaa accacctcag acactgcctc agctgctcca aatgccgaaa ggaaatgggt caggtggaga tctcttcttg cacagtggac cgggacaccg tgtgtggctg caggaagaac cagtaccggc

TABLE 1-continued

TNFR1 Sequences	
SEQ ID No.	Sequences
NO: 3)	attattggag tgaaaacctt ttccagtgct tcaattgcag cctctgcctc aatgggaccg tgcacctctc ctgccaggag aaacagaaca ccgtgtgcac ctgccatgca ggtttctttc taagagaaaa cgagtgtgtc tcctgtagta actgtaagaa aagcctggag tgcacgaagt tgtgcctacc a

The mouse TNFR1 polypeptide sequence and its variants are also reported. The 454 aa mouse TNFR1 polypeptide has the identifier number P25118 in UniProtKB database. TNFR1 polypeptides known in other animals include, but are not limited to, rat (e.g., P22934 in the UniProtKB database), cow (e.g., O19131 in the UniProtKB database), pig (e.g., P50555 in the UniProtKB database), or horse (e.g., D1MH71 in the UniProtKB database).

The full-length TNFR1 can be cleaved into two chains, (1) TNF Receptor Superfamily Member 1A, membrane form (i.e., amino acids 22 to 455 corresponding to full-length TNFR1) and (2) TNF-binding protein 1 (TBPI) (i.e., amino acids 41 to 291 corresponding to full-length TNFR1). The full-length human TNFR1 polypeptide consists of a signal sequence (amino acids 1 to 21 of SEQ ID NO: 2), an extracellular domain (amino acids 22 to 211 of SEQ ID NO: 2), a trans-membrane domain (amino acids 212 to 234 of SEQ ID NO: 2), and a cytoplasmic domain (amino acids 235 to 455 of SEQ ID NO: 2). The TNFR1 extracellular domain comprises four cysteine repeat regions, TNFR-Cys1 (amino acids 43 to 82 corresponding to SEQ ID NO: 2), TNFR-Cys2 (amino acids 83 to 125 corresponding to SEQ ID NO: 2), TNFR-Cys3 (amino acids 126 to 166 corresponding to SEQ ID NO: 2), and TNFR-Cys4 (amino acids 167 to 196 corresponding to SEQ ID NO: 2).

As one of skill in the art will appreciate, the beginning and ending residues of the domains listed above can vary depending upon the computer modeling program used or the method used for determining the domain. As such, various functional domains of TNFR1 may vary from those defined above.

In one embodiment, a ligand binding domain of TNFR1 useful for the FAS-chimera protein comprises, consists essentially of, or consists of an extracellular domain of TNFR1, or any fragment, variant, derivative, or analogue thereof, wherein the extracellular domain of TNFR1, or any fragment, variant, derivative, or analogue thereof binds to TNF-α. In another embodiment, a ligand binding domain of TNFR1 comprises TNFR-Cys1; TNFR-Cys2; TNFR-Cys3; TNFR-Cys4; TNFR-Cys1 and TNFR-Cys2; TNFR-Cys1 and TNFR-Cys3; TNFR-Cys1 and TNFR-Cys4; TNFR-Cys2 and TNFR-Cys3; TNFR-Cys2 and TNFR-Cys4; TNFR-Cys3 and TNFR-Cys4; TNFR-Cys1, TNFR-Cys2, and TNFR-Cys3; TNFR-Cys1, TNFR-Cys2, and TNFR-Cys4; TNFR-Cys2, TNFR-Cys3, and TNFR-Cys4; or TNFR-Cys1, TNFR-Cys2, TNFR-Cys3, and TNFR-Cys4. In other embodiments, a ligand binding domain of TNFR1 in the FAS-chimera protein comprises TNF binding protein I. In yet other embodiments, a TNFR1 ligand binding domain of the FAS-chimera protein comprises, consists essentially of, or consists of an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22 to 190, amino acids 22 to 191, amino acids 22 to 192, amino acids 22 to 193, amino acids 22 to 194, amino acids 22 to 195, amino acids 22 to 196, amino acids 22 to 197, amino acids 22 to 198, amino acids 22 to 199, amino acids 22 to 200, amino acids 22 to 201, amino acids 22 to 202, amino acids 22 to 203, amino acids 22 to 204, amino acids 22 to 205, amino acids 22 to 206, amino acids 22 to 207, amino acids 22 to 208, amino acids 22 to 209, amino acids 22 to 210, or amino acids 22 to 211 of SEQ ID NO: 2, wherein the ligand binding domain binds to a TNFR1 ligand, e.g., TNF-α.

In other embodiments, the ligand binding domain of TNFR1 further comprises a signal peptide. One example of the suitable signal peptides is the signal peptide of TNFR1, e.g., amino acids 1 to 21 of SEQ ID NO: 2. In yet other embodiments, a ligand binding domain of the FAS-chimera gene product comprises, consists essentially of, or consists of an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 1 to 190, amino acids 1 to 191, amino acids 1 to 192, amino acids 1 to 193, amino acids 1 to 194, amino acids 1 to 195, amino acids 1 to 196, amino acids 1 to 197, amino acids 1 to 198, amino acids 1 to 199, amino acids 1 to 200, amino acids 1 to 201, amino acids 1 to 202, amino acids 1 to 203, amino acids 1 to 204, amino acids 1 to 205, amino acids 1 to 206, amino acids 1 to 207, amino acids 1 to 208, amino acids 1 to 209, amino acids 1 to 210, or amino acids 1 to 211 of SEQ ID NO: 2, wherein the ligand binding domain binds to a TNFR1 ligand, e.g., TNF-α. In a specific embodiment, a TNFR1 ligand binding domain of the FAS-chimera protein comprises, consists essentially of, or consists of an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 4, wherein the ligand binding domain binds to a TNFR1 ligand, e.g., TNF-α.

In yet other embodiments, the ligand binding domain of TNFR1 is encoded by a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3.

In still other embodiments, a TNFR1 ligand binding domain of the FAS-chimera protein comprises, consists essentially of, or consists of an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 22 to 108 of SEQ ID NO: 2 (TNFR1 isoform B), amino acids 22 to 232 of SEQ ID NO: 2 (TNFR1 isoform C), or amino acids 44 to 291 of SEQ ID NO: 2 (TBPI), wherein the ligand binding domain binds to a TNFR1 ligand, e.g., TNF-α.

2. FAS Polypeptide

The full-length human FAS polypeptide is 335 amino acids in length and is also known as Tumor Necrosis Factor Receptor Superfamily Member 6, Apo-1 antigen, Apoptosis-mediating surface antigen FAS, FASLG receptor, or CD95. Naturally occurring FAS polypeptide is a receptor for TNFSF6/FASLG. When the FAS polypeptide binds to the FAS ligand (FasL), the interaction between FAS and FasL results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, caspase-8 and caspase-10. In some types of cells (type I), processed caspase-8 directly activates other members of the caspase family, and triggers the execution of apoptosis of the cell. In other types of cells (type II), the FAS-DISC starts a feedback loop that spirals into increasing release of proapoptotic factors from mitochondria and the amplified activation of caspase-8. FAS-mediated apoptosis may have a role in the induction of peripheral tolerance, in the antigen-stimulated suicide of mature cells or both.

A 335 as polypeptide sequence reported as a human FAS polypeptide sequence has the identifier number P25445-1 in the UniProtKB database. This human FAS polypeptide sequence is designated herein as SEQ ID NO: 6. SEQ ID NO: 5 is a nucleotide sequence encoding SEQ ID NO: 6. The nucleotide sequence encoding the FAS polypeptide is also known as APT1, FAS1, or TNFRSF6. The full-length FAS polypeptide contains a signal peptide (amino acids 1 to 25 corresponding to SEQ ID NO: 6), an extracellular domain (amino acids 26 to 173 corresponding to SEQ ID NO: 6), a trans-membrane domain (amino acids 174 to 190 corresponding to SEQ ID NO: 6), and an intracellular (or cytoplasmic) domain (amino acids 191 to 335 corresponding to SEQ ID NO: 6). The intracellular domain contains a death domain (e.g., amino acids 230 to 314 corresponding to SEQ ID NO: 6).

As one of skill in the art will appreciate, the beginning and ending residues of the domains listed above may vary depending upon the computer modeling program used or the method used for determining the domain. As such, various functional domains of FAS may vary from those defined above. Table 2 shows the wild-type human FAS amino acid sequence and a nucleotide sequence encoding the FAS protein.

TABLE 2

FAS Sequences

	Sequences
Amino acid sequence of human FAS protein (SEQ ID NO: 6)	MLGIWTLLPLVLTSVARLSSKSVNAQVTDINSKGLELRKTVTTVETQNLEGLHHDGQFCH KPCPPGERKARDCTVNGDEPDCVPCQEGKEYTDKAHFSSKCRRCRLCDEGHGLEVEINCT RTQNTKCRCKPNFFCNSTVCEHCDPCTKCEHGIIKECTLTSNTKCKEEGSRSNLGWLCLL LLPIPLIVWVKRKEVQKTCRKHRKENQGSHESPTLNPETVAINLSDVDLSKYITTIAGVM TLSQVKGFVRKNGVNEAKIDEIKNDNVQDTAEQKVQLLRNWHQLHGKKEAYDTLIKDLKK ANLCTLAEKIQTIILKDITSDSENSNFRNEIQSLV
Nucleotide Sequence encoding human FAS (SEQ ID NO: 5)	Atgctgggcatctggaccctcctacctctggttcttacgtctgttgctagattatcgtcc Aaaagtgttaatgcccaagtgactgacatcaactccaagggattggaattgaggaagact Gttactacagttgagactcagaacttggaaggcctgcatcatgatggccaatttctgccat Aagccctgtcctccaggtgaaaggaaagctagggactgcacagtcaatggggatgaacca Gactgcgtgccctgccaagaagggaaggagtacacagacaaagcccatttttcttccaaa Tgcagaagatgtagattgtgtgatgaaggacatggcttagaagtggaaataaactgcacc Cggacccagaataccaagtgcagatgtaaaccaaacttttttttgtaactctactgtatgt Gaacactgtgacccttgcaccaaatgtgaacatggaatcatcaaggaatgcacactcacc Agcaacaccaagtgcaaagaggaaggatccagatctaacttggggtggctttgtcttctt Cttttgccaattccactaattgtttgggtgaagagaaaggaagtacagaaaacatgcaga Aagcacagaaaggaaaaccaaggttctcatgaatctccaactttaaatcctgaaacagtg Gcaataaatttatctgatgttgacttgagtaaatatatcaccactattgctggagtcatg Acactaagtcaagttaaaggctttgttcgaaagaatggtgtcaatgaagccaaaatagat Gagatcaagaatgacaatgtccaagacacagcagaacagaaagttcaactgcttcgtaat Tggcatcaacttcatggaaagaaagaagcgtatgacacattgattaaagatctcaaaaaa Gccaatctttgtactcttgcagagaaaattcagactatcatcctcaaggacattactagt Gactcagaaaattcaaacttcagaaatgaaatccaaagcttggtctag
Amino acid sequence of an Effector Domain of FAS (SEQ ID NO: 8)	GSRSNLGWLCLLLLPIPLIVWVKRKEVQKTCRKHRKENQGS HESPTLNPETVAINLSDVDLSKYITTIAGVMTLSQVKGFVR KNGVNEAKIDEIKNDNVQDTAEQKVQLLRNWHQLHGKKEAY DTLIKDLKKANLCTLAEKIQTIILKDITSDSENSNFRNEIQ SLV
Nucleotide sequence encoding an Effector Domain of FAS (SEQ ID NO: 7)	Aggatccagatctaacttggggtggctttgtcttcttcttttgccaattccactaatt Gtttgggtgaagagaaaggaagtacagaaaacatgcagaaagcacagaaaggaaaacc Aaggttctcatgaatctccaaccttaaatcctgaaacagtggcaataaatttatctga Tgttgacttgagtaaatatatcaccactattgctggagtcatgacactaagtcaagtt Aaaggctttgttcgaaagaatggtgtcaatgaagccaaaatagatgagatcaagaatg Acaatgtccaagacacagcagaacagaaagttcaactgcttcgtaattggcatcaact Tcatggaaagaaagaagcgtatgacacattgattaaagatctcaaaaaagccaatctt Tgtactcttgcagagaaaattcagactatcatcctcaaggacattactagtgactcag aaaattcaaacttcagaaatccaaagcttggtctag

The mouse FAS polypeptide sequence and its variants are also reported. The 327 aa mouse FAS polypeptide has the identifier number P25446 in UniProtKB database. FAS polypeptides known in other animals include, but are not limited to, Old World monkey (e.g., Q9BDN4 in the UniProtKB database), Rhesus monkey (e.g., Q9BDP2 in the UniProtKB database), rat (e.g., Q63199 in the UniProtKB database), or cow (e.g., P51867 in the UniProtKB database).

Based on the sequence variation in the FAS polypeptide, a person of ordinary skill in the art can identify sequence variations in the effector domain of the FAS polypeptide. For example, natural variants of the FAS effector domains can include one or more substitutions or mutations of C178R, L180F, P183L, I1184V, T198I, Y232C, T241K, T241P, V249L, R250P, R250Q, G253D, G253S, N255D, A257D, I259R, D260G, D260V, D260Y, I262S, N264K, T270I, T270K, E272G, E272K, L278F, K299N, T305I, I310S, or any combinations thereof.

In one embodiment, an effector domain of the FAS polypeptide useful for the invention comprises a death domain of the FAS polypeptide. In another embodiment, an effector domain of the FAS polypeptide comprises, consists essentially of, or consists of an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 230 to 314 of SEQ ID NO: 6. In other embodiments, an effector domain of the FAS polypeptide comprises an intracellular domain of the FAS polypeptide. In yet other embodiments, an effector domain of the FAS polypeptide comprises an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 185 to 335, amino acids 186 to 335, amino acids 187 to 335, amino acids 188 to 335, amino acids 189 to 335, amino acids 190 to 335, amino acids 191 to 335, amino acids 192 to 335, amino acids 193 to 335, amino acids 194 to 335, amino acids 195 to 335, amino acids 196 to 335, amino acids 197 to 335, amino acids 198 to 335, amino acids 199 to 335 of SEQ ID NO: 6.

In still other embodiments, the effector domain of the FAS polypeptide further comprises a trans-membrane domain of the FAS polypeptide. In yet other embodiments, an effector domain of the FAS polypeptide comprises an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 174 to 335 of SEQ ID NO: 6. In some embodiments, an effector domain of the FAS polypeptide further comprises about ten, about nine, about eight, about seven, about six, about five, about four, about three, about two, or about one amino acid from the C-terminal portion of the FAS extracellular domain. In certain embodiments, an effector domain of the FAS polypeptide comprises an amino acid sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 179 to 335, amino acids 178 to 335, amino acids 177 to 335, amino acids 176 to 335, amino acids 175 to 335, amino acids 174 to 335, amino acids 173 to 335, amino acids 172 to 335, amino acids 171 to 335, amino acids 170 to 335, amino acids 169 to 335, amino acids 168 to 335, amino acids 167 to 335, amino acids 166 to 335, amino acids 165 to 335, amino acids 164 to 335, or amino acids 163 to 335 of SEQ ID NO: 6, wherein the effector domain forms a death-inducing signaling complex (DISC), activates caspase 8, or induces apoptosis.

In some embodiments, an effector domain of the FAS polypeptide comprises, consists essentially of, or consists of an amino acid sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 8, wherein the effector domain forms a death-inducing signaling complex (DISC), activates caspase 8, or induces apoptosis.

In other embodiments, an effector domain of the FAS polypeptide is encoded by a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 7.

In one embodiment, the FAS-chimera gene product for the invention comprises, consists essentially of, or consists of an amino acid sequence at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 10, wherein the FAS-chimera gene product induces apoptosis. In another embodiment, the FAS-chimera gene product is encoded by a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 9, wherein the FAS-chimera gene product induces apoptosis.

B. Endothelial Cell-Specific Promoter

The nucleic acid construct comprising a FAS-chimera gene further comprises one or more expression control elements useful for regulating the expression of an operably linked FAS-chimera gene. The expression control elements include, but are not limited to, promoters, secretion signals, and other regulatory elements.

The nucleic acid construct useful for the present invention utilizes an endothelial cell-specific promoter to direct expression of the FAS-chimera protein in an endothelial cell, thereby inducing apoptosis of the endothelial cell.

For the purpose of the present invention, an endothelial cell-specific promoter can contain one or more cis-regulatory elements, which improve the endothelial cell-specificity of the promoters compared to the promoter without the cis-regulatory elements. In one example, the cis-regulatory element comprises an enhancer. In another aspect, the cis-regulatory element comprises a hypoxia response element. In other examples, the cis-regulatory element comprises both an enhancer and a hypoxia response element.

In one embodiment, an enhancer useful for the invention comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 11 or SEQ ID NO: 12 (the complementary sequence of SEQ ID NO: 11), wherein the enhancer improves endothelial cell specificity of a promoter compared to a promoter without the enhancer. The enhancer can further comprise an additional nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 13 or SEQ ID NO: 14 (the complementary sequence of SEQ ID NO: 13).

In another embodiment, an enhancer for the invention comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 13 or SEQ ID NO: 14 (the complementary sequence of SEQ ID NO: 13), wherein the enhancer improves endothelial cell specificity of a promoter compared to a promoter without the enhancer. The enhancer can further comprise an additional nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 11 or SEQ ID NO: 12 (the complementary sequence of SEQ ID NO: 11).

In other embodiments, an enhancer for the invention comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 15 or SEQ ID NO: 16 (the complementary sequence of SEQ ID NO: 15), wherein the enhancer improves endothelial cell specificity of a promoter compared to a promoter without the enhancer. In yet other embodiments, an enhancer for the nucleic acid construct comprises SEQ ID NO: 15 or SEQ ID NO: 16 or any fragments, variants, derivatives, or analogs thereof, wherein the fragments, variants, derivatives, or analogs improve endothelial cell specificity of a promoter compared to a promoter without the enhancer.

In some embodiments, an enhancer for the invention comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 22 or SEQ ID NO: 23, wherein the enhancer improves endothelial cell specificity of a promoter compared to a promoter without the enhancer. In yet other embodiments, an enhancer for the nucleic acid construct comprises SEQ ID

NO: 22 or SEQ ID NO: 23 or any fragments, variants, derivatives, or analogs thereof, wherein the fragments, variants, derivatives, or analogs improve endothelial cell specificity of a promoter compared to a promoter without the enhancer.

In other embodiments, an enhancer for the invention comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 24 or SEQ ID NO: 25, wherein the enhancer improves endothelial cell specificity of a promoter compared to a promoter without the enhancer. In yet other embodiments, an enhancer for the nucleic acid construct comprises SEQ ID NO: 24 or SEQ ID NO: 25 or any fragments, variants, derivatives, or analogs thereof, wherein the fragments, variants, derivatives, or analogs improve endothelial cell specificity of a promoter compared to a promoter without the enhancer.

Table 3 shows various enhancer sequences useful for the invention.

TABLE 3

Endothelial Cell-Specific Enhancer Elements and Promoters

SEQ ID NOs	Sequences
SEQ ID NO: 11	ctggagggtg actttgcttc tggagccagt acttcatact tttcatt
SEQ ID NO: 12	aatgaaaagt atgaagtact ggctccagaa gcaaagtcac cctccag
SEQ ID NO: 13	gtacttcata cttttcattc caatggggtg actttgcttc tgga
SEQ ID NO: 14	tccagaagca aagtcacccc attggaatga aaagtatgaa gtac
SEQ ID NO: 15	3X enhancer element ctccagaagcaaagtcaccccattggaatgaaaagtatgaagtacaatgaaaagtatgaagt actggctccagaagcaaagtcaccctccagaagcaaagtcaccccattggaatgaaaagtat gaagtac
SEQ ID NO: 16	3x enhancer element (Complementary Sequence of SEQ ID NO: 15) gtacttcatacttttcattccaatggggtgactttgcttctggagggtgactttgcttctgg agccagtacttcatacttttcattgtacttcatacttttcattccaatggggtgactttgct tctggag
SEQ ID NO: 17	PPE-1 Promoter gtacgtgtacttctgatcggcgatactagggagataaggatgtgcctgacaaaaccacattg ttgttgttatcattattatttagttttccttccttgctaactcctgacggaatctttctcac ctcaaatgcgaagtactttagtttagaaaagacttggtggaaggggtggtggtggaaaagta gggtgatcttccaaactaatctggttccccgcccgccccagtagctgggattcaagagcgaa gagtggggatcgtccccttgtttgatcagaaagacataaaaggaaaatcaagtgaacaatga tcagccccacctccaccccaccccctgcgcgcgcacaatacaatctatttaattgtacttc atacttttcattccaatggggtgactttgcttctggagaaactcttgattcttgaactctgg ggctggcagctagcaaaaggggaagcgggctgctgctctctgcaggttctgcagcggtctct gtctagtgggtgttttcttttttcttagccctgcccctggattgtcagacggcgggcgtctgc ctctgaagttagccgtgatttcctctagagccgggtcttatctctggctgcacgttgcctgt gggtgactaatcacacaataacattgtttagggctggaatgaagtcagagctgtttaccccc actctataggggttcaatataaaaaggcggcggagaactgtccgagtcagaagcgttcctgc accggcgctgagagcctgacccggtctgctccgctgtccttgcgcgctgcctcccggctgcc cgcgacgctttcgccccagtggaagggccacttgctgcggccgc
SEQ ID NO: 18	PPE-1-3X promoter gtacgtgtacttctgatcggcgatactagggagataaggatgtgcctgacaaaaccacattg ttgttgttatcattattatttagttttccttccttgctaactcctgacggaatctttctcac ctcaaatgcgaagtactttagtttagaaaagacttggtggaaggggtggtggtggaaaagta gggtgatcttccaaactaatctggttccccgcccgccccagtagctgggattcaagagcgaa gagtggggatcgtccccttgtttgatcagaaagacataaaaggaaaatcaagtgaacaatga tcagccccacctccaccccaccccctgcgcgcgcacaatacaatctatttaattgtacttc atacttttcattccaatggggtgactttgcttctggagaaactcttgattcttgaactctgg ggctggcagctagcctccagaagcaaagtcaccccattggaatgaaaagtatgaagtacaat gaaaagtatgaagtactggctccagaagcaaagtcaccctccagaagcaaagtcaccccatt ggaatgaaaagtatgaagtacgctagcaaaaggggaagcgggctgctgctctctgcaggttc tgcagcggtctctgtctagtgggtgttttcttttttcttagccctgcccctggattgtcagac ggcgggcgtctgcctctgaagttagccgtgatttcctctagagccgggtcttatctctggct gcacgttgcctgtgggtgactaatcacacaataacattgtttagggctggaatgaagtcaga gctgtttaccccccactctatagggggttcaatataaaaaggcggcggagaactgtccgagtca gaagcgttcctgcaccggcgctgagagcctgacccggtctgctccgctgtccttgcgcgctg cctcccggctgcccgcgacgctttcgccccagtggaagggccacttgctgcggccgc
SEQ ID NO: 22	ggtgactttg cttctggag
SEQ ID NO: 23	ctccagaagcaaagtcacc

TABLE 3-continued

Endothelial Cell-Specific Enhancer Elements and Promoters	
SEQ ID NOs	Sequences
SEQ ID NO: 24	gtacttcata cttttcatt
SEQ ID NO: 25	aatgaaaagtatgaagtac
SEQ ID NO: 26	Hypoxia Response element gcacgt

An enhancer for the present invention can be linked to a promoter upstream or downstream of the promoter or inserted between the two nucleotides in the promoter. The endothelial cell-specific promoter for the present invention can utilize any promoters known in the art. For example, suitable promoters which can be utilized for the present invention include the endothelial-specific promoters: preproendothelin-1 (PPE-1 promoter), US 2010/0282634, published Nov. 11, 2010; and WO 2011/083464, published Jul. 14, 2011); the PPE-1-3X promoter (U.S. Pat. No. 7,579,327, U.S. Pat. No. 8,071,740, U.S. Pat. No. 8,039,261, US2010/0282634, US 2007/0286845, WO 2011/083464, and WO2011/083466); the TIE-1 (S79347, S79346) and the TIE-2 (U53603) promoters [Sato T N, Proc Natl Acad Sci USA 1993 Oct. 15; 90(20): 9355-8], the Endoglin promoter [Y11653; Rius C, Blood 1998 Dec. 15; 92(12):4677-90], the von Willerbrand factor [AF152417; Collins C J Proc Natl Acad Sci USA 1987 July; 84(13):4393-7], the KDR/flk-1 promoter [X89777, X89776; Ronicke V, Circ Res 1996 August; 79(2):277-85], The FLT-1 promoter [D64016 AJ224863; Morishita K, J Biol Chem 1995 Nov. 17; 270(46):27948-53], the Egr-1 promoter [AJ245926; Sukhatme V P, Oncogene Res 1987 September-October; 1(4):343-55], the E-selectin promoter [Y12462; Collins T J Biol Chem 1991 Feb. 5; 266(4):2466-73], The endothelial adhesion molecules promoters: ICAM-1 [X84737; Horley K J EMBO J 1989 October; 8(10):2889-96], VCAM-1 [M92431; Iademarco M F, J Biol Chem 1992 Aug. 15; 267(23): 16323-9], PECAM-1 [AJ313330 X96849; CD31, Newman P J, Science 1990 Mar. 9; 247(4947): 1219-22], the vascular smooth-muscle-specific elements: CArG box X53154 and aortic carboxypeptidase-like protein (ACLP) promoter [AF332596; Layne M D, Circ Res. 2002; 90: 728-736] and Aortic Preferentially Expressed Gene-1 [Yen-Hsu Chen J. Biol. Chem, Vol. 276, Issue 50, 47658-47663, Dec. 14, 2001], all of which are incorporated herein by reference in their entireties.

In one embodiment, a promoter linked to the endothelial cell-specific enhancer comprises a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of SEQ ID NO: 17, wherein the promoter linked to the enhancer induces endothelial cell-specificity to the gene operably linked to the promoter. In another embodiment, a promoter linked to the endothelial cell-specific enhancer comprises a fragment, a variant, a derivative, or an analog of a wild-type PPE-1 promoter, wherein said fragment, variant, derivative, or analog thereof induces endothelial cell-specificity to the gene operably linked to the promoter. In one example, the endothelial cell-specific enhancer can be inserted between nucleotide residues 442 and 449 corresponding to SEQ ID NO: 17.

In further embodiments, an endothelial cell-specific promoter comprises a hypoxia responsive element. A hypoxia responsive element (HRE) is located on the antisense strand of the endothelin-1 promoter. This element is a hypoxia-inducible factor-1 binding site that is required for positive regulation of the endothelin-1 promoter (of the human, rat and murine gene) by hypoxia. Hypoxia is a potent signal, inducing the expression of several genes including erythropoietin (Epo), VEGF, and various glycolytic enzymes. The core sequence (8 base pairs) is conserved in all genes that respond to hypoxic conditions and the flanking regions are different from other genes. The ET-I hypoxia responsive element is located between the GAT A-2 and the AP-1 binding sites. In one example, a hypoxia response element comprises SEQ ID NO: 26, a fragment, a variant, a derivative, or an analog thereof.

In other embodiments, an endothelial cell-specific promoter useful for the invention comprises, consists essentially of, or consists of a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of SEQ ID NO: 18, wherein the promoter linked to the enhancer induces endothelial cell-specificity to the gene operably linked to the promoter. In another embodiment, an endothelial cell-specific promoter comprises a fragment, a variant, a derivative, or an analog of SEQ ID NO: 18, wherein said fragment, variant, derivative, or analog thereof induces endothelial cell-specificity to the gene operably linked to the promoter.

Additional variations of the endothelial cell-specific promoters can be found at WO2011/083464, WO2011/083466, and WO2012/052423, which are incorporated herein by reference in their entireties.

The present invention also provides a novel promoter sequence comprising a nucleotide sequence SEQ ID NO: 17. In one example, the promoter further comprises an endothelial cell-specific enhancer. In one example, the endothelian cell-specific enhancer comprises SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26 or any fragments, derivatives, variants, or analogs thereof, wherein the fragments, derivatives, variants, or analogs thereof improve endothelial cell-specificity of the promoter compared to a promoter without the enhancer. In another example, the promoter comprises a nucleotide sequence of SEQ ID NO: 18. The invention includes a nucleic acid construct comprising the novel promoter and a heterologous nucleotide sequence. In one embodiment, the heterologous nucleic acid sequence comprises a nucleotide sequence encoding a FAS-chimera protein described herein. In another embodiment, the heterologous nucleotide sequence comprises an adenovirus sequence.

C. Vector

The invention also provides a vector comprising the nucleic acid construct, which comprises a FAS-chimera gene operably linked to an endothelial cell-specific promoter. For the purposes of this invention, numerous vector systems may be employed. For example, various viral gene delivery systems that can be used in the practice of this aspect of the invention include, but are not limited to, an adenoviral vector, an alphavirus vector, an enterovirus vector, a pestivirus vector, a lentiviral vector, a baculoviral vector, a herpesvirus vector, an Epstein Barr viral vector, a papovaviral vector, a poxvirus vector, a vaccinia viral vector, an adeno-associated viral vector and a herpes simplex viral vector.

In another embodiment, a vector comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter is an adenovirus. For example, the adenovirus can be any one or more of human adenovirus species A (serotypes 12, 18, and 31), B (serotypes 3, 7, 11, 14, 16, 21, 34, 35, 50, and 55), C (serotypes 1, 2, 5, 6, and 57), D (8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, and 56), E (serotype 4), F (serotype 40 and 41), or G (serotype 52). In a particular embodiment, the adenovirus for the invention is human adenovirus serotype 5. In some embodiments, the adenovirus useful for gene therapy is a recombinant non-replicating adenovirus, which does not contain an E1 region and an E3 region. In certain embodiments, the adenovirus for the invention is a conditionally replicating adenovirus, which does not contain an E3 region, but contains an E1 region.

D. Biological Deposits

Biological deposits were made with the European Collection of Cell Cultures (ECACC) located at Health Protection Agency Culture Collections, Health Protection Agency, Microbiology Services, Porton Down, Salisbury, SP4 0JG, UK, pursuant to the Budapest Treaty and pursuant to 37 C.F.R. §1.808. Samples of the deposited materials will become available to the public upon grant of a patent. The invention described and claimed herein is not to be limited by the scope of the strain deposited, since the deposited embodiment is intended only as an illustration of the invention.

Strain	ECACC Accession No.	Date Deposited
VB-111	13021201	Feb. 12, 2013

III. Treatment Methods Using Adenovirus Expressing FAS-Chimera Protein

One embodiment of the present invention provides methods for using a nucleic acid construct expressing a FAS chimera protein or an adenovirus comprising the nucleic acid construct. In one aspect, a nucleic acid construct expressing a FAS-chimera protein or an adenovirus comprising the nucleic acid construct is useful for reducing or decreasing a size of a tumor or eliminating a tumor in a subject, wherein the FAS-chimera protein encoded by the nucleic acid construct reduces or decreases the size of the tumor or slows the rate of tumor growth or prevents appearance of new tumor metastatic lesions or eliminates the tumor in the subject and wherein the tumor is associated with or derived from a female gynecological cancer or a metastasis thereof. These effects may be assessed based on radiological diagnostic tests (such as CT scan) and/or tumor markers (such as blood level of CA-125). In another aspect, a nucleic acid construct expressing a FAS-chimera protein or an adenovirus comprising the nucleic acid construct is useful for inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor, wherein a FAS-chimera protein encoded by the nucleic acid construct inhibits, reduces, or decreases the neo-vascularization or angiogenesis in the tumor and wherein the tumor is associated with or derived from a female gynecological cancer or a metastasis thereof. In other aspects, a nucleic acid construct expressing a FAS-chimera protein or an adenovirus comprising the nucleic acid construct is capable of treating or preventing a tumor associated with or derived from a female gynecological cancer or a metastasis thereof in a subject, wherein the FAS-chimera protein encoded by the nucleic acid construct treats or prevents the female gynecological cancer or a metastasis thereof in the subject.

Therefore, in one aspect, the invention provides a method of reducing or decreasing a size of a tumor or a metastasis thereof, eliminating a tumor or a metastasis thereof, or slowing the growth of a tumor or a metastasis thereof in a subject comprising administering to the patient an effective amount of a nucleic acid construct, which comprises a Fas-chimera gene operably linked to an endothelial cell specific promoter, or an adenovirus comprising the nucleic acid construct, wherein a Fas-chimera gene product encoded by the nucleic acid construct reduces or decreases the size of the tumor or a metastasis thereof or eliminates the tumor or a metastasis thereof in the subject and wherein the tumor or a metastasis thereof is associated with or derived from a female gynecological cancer. In another aspect, the invention provides a method of inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor or a metastasis thereof comprising administering to a subject having the tumor or a metastasis thereof an effective amount of a nucleic acid construct, or an adenovirus comprising the nucleic acid construct, which comprises a FAS-chimera gene operably linked to an endothelial cell specific promoter, wherein a FAS-chimera gene product encoded by the nucleic acid construct inhibits, reduces, or decreases the neo-vascularization or angiogenesis in the tumor or a metastasis thereof and wherein the tumor or a metastasis thereof is associated with or derived from a female gynecological cancer. In other aspects, the invention provides a method of treating or preventing a tumor or a metastasis thereof associated with or derived from a female gynecological cancer in a subject comprising administering an effective amount of a nucleic acid construct, which comprises a Fas-chimera gene operably linked to an endothelial cell specific promoter, wherein a Fas-chimera gene product encoded by the nucleic acid construct treats or prevents the female gynecological cancer in the patient. In still other embodiments, the tumor of the female gynecological cancer or a metastasis thereof is decreased in size or eliminated after the administration. In certain embodiments, the size of the tumor or a metastasis thereof is decreased such that the longest diameter (LD) of the tumor is decreased at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% compared to the LD prior to the administration. In some embodiments, the invention includes a method of stabilizing a disease or disorder associated with a female gynecological cancer. For example, the invention includes a method of preventing or slowing further growth of a tumor associated with a female gynecological cancer. In further embodiments, the present invention reduces the volume of malignant peritoneal fluid, e.g., ascites, reduces pain to the subject, prolongs survival of the subject, or any combinations thereof. In other embodiments, the adenovirus of the invention when administered to the subject prolongs the overall survival of the subject. In further embodiments, the adenovirus of the invention when administered to the subject prolongs progression-free survival of the subject.

In one embodiment, the female gynecological cancer can be Müllerian cancer, ovarian cancer, peritoneal cancer, fallopian tube cancer, uterine papillary serous carcinoma, a metastasis thereof, or any combinations thereof. In another embodiment, the female gynecological cancer can be cervical cancer, endometrial cancer, gestational trophoblastic disease, uterine cancer, vulvar cancer, a metastasis thereof, or any combinations thereof. In other embodiments, the female gynecological cancer includes any cancerous growth arising from a gynecological tissue, e.g., uterus, ovary, fallopian tube, cervix, egg cells, the supporting cells, or any combinations thereof. In certain embodiments, the tumor associated with or derived from a female gynecological cancer can be selected from the group consisting of surface epithelial-stromal tumor (adenocarcinoma), papillary serous cystadenocarcinoma, endometrioid tumor, serous cystadenocarcinoma, papillary tumor, mucinous cystadenocarcinoma, clear-cell ovarian tumor, mucinous adenocarcinoma, cystadenocarcinoma, carcinoma, sex cord-stromal tumour, germ cell tumor, teratoma, dysgerminoma, epidermoid (Squamous cell carcinoma), Brenner tumor, a metastasis thereof or any combinations thereof.

Müllerian cancer for the purpose of the present invention includes a malignant mixed Müllerian tumor, also known as malignant mixed mesodermal tumor, MMMT and carcinosarcoma. MMMT is a malignant neoplasm found in the uterus, the ovaries, the fallopian tubes and other parts of the body that contains both carcinomatous (epithelial tissue) and sarcomatous (connective tissue) components. It is divided into two types, homologous (in which the sarcomatous component is made of tissues found in the uterus such as endometrial, fibrous and/or smooth muscle tissues) and a heterologous type (made up of tissues not found in the uterus, such as cartilage, skeletal muscle and/or bone). MMMT account for between two and five percent of all tumors derived from the body of the uterus, and are found predominantly in postmenopausal women with an average age of 66 years.

Ovarian cancer comprises any cancerous growth arising from the ovary. Most (more than 90%) ovarian cancers are classified as "epithelial" and are believed to arise from the surface (epithelium) of the ovary. However, fallopian tubes could also be the source of some ovarian cancers. Since the ovaries and tubes are closely related to each other, it is thought that these fallopian cancer cells can mimic ovarian cancer. Other types may arise from the egg cells (germ cell tumor) or supporting cells. In some embodiments, ovarian cancer is a secondary cancer, the result of metastasis from a primary cancer elsewhere in the body. About 7% of ovarian cancers are due to metastases while the rest are primary cancers. Common primary cancers are breast cancer and gastrointestinal cancer.

Peritoneal cancer or carcinoma is also known as: serous surface papillary carcinoma, primary peritoneal carcinoma, extra-ovarian serous carcinoma, primary serous papillary carcinoma, or psammomacarcinoma. It was historically classified under "carcinoma of unknown primary" (CUP). Primary peritoneal cancer (PPC, or PPCa) is a cancer of the cells lining the peritoneum, or abdominal cavity. Histomorphological and molecular biological characteristics suggest that serous carcinomas, which include ovarian serous carcinoma, uterine serous carcinoma, fallopian tube serous carcinoma, cervical serous carcinoma, and primary peritoneal serous carcinoma really represent one entity.

Primary fallopian tube cancer (PFTC), often just tubal cancer, is a malignant neoplasm that originates from the fallopian tube. Tubal cancer is thought to be a relatively rare primary cancer among women accounting for 1 to 2 percent of all gynecologic cancers.

Uterine serous carcinoma (USC), also known as uterine papillary serous carcinoma (UPSC) and uterine serous adenocarcinoma, is an uncommon form of endometrial cancer that typically arises in postmenopausal women. It is typically diagnosed on endometrial biopsy, prompted by post-menopausal bleeding. Unlike the more common low-grade endometrioid endometrial adenocarcinoma, USC does not develop from endometrial hyperplasia and is not hormone-sensitive. It arises in the setting of endometrial atrophy and is classified as a type II endometrial cancer.

The term "subject" or "individual" or "animal" or "patient" or "mammal," is meant any subject, particularly a mammalian subject, having or being expected to develop at least one tumor associated with or derived from peritoneal cancer or female gynecological cancer. In one embodiment, the subject is a human. In another embodiment, the subject is a cancer patient.

In one embodiment of the invention, the subject is a subject who has had a prior platinum based therapy. Such a prior platinum based therapy includes, but is not limited to, cisplatin, carboplatin, oxaliplatin, nedaplatin, satraplatin, picoplatin, triplatin tetranitrate, or aroplatin. Platinum-based antineoplastic agents cause cross-linking of DNA as monadduct, interstrand crosslinks, intrastrand crosslinks or DNA protein crosslinks. Mostly they act on the adjacent N-7 position of guanine, forming 1, 2 intrastrand crosslink. The resultant crosslinking inhibit DNA repair and/or DNA synthesis in cancer cells. Platinum-based antineoplastic agents are sometimes described as "alkylating-like" due to similar effects as alkylating antineoplastic agents, although they do not have an alkyl group. In certain embodiments, the prior platinum-based therapy is a therapy using cisplatin, also known as cisplatinum or cis-diamminedichloroplatinum(II) (CDDP) (trade name Cisplatin, brand name Platin marketed by Cadila Healthcare according to FDA Orange Book). Cisplatin is administered intravenously as short-term infusion in normal saline for treatment of solid malignancies. It is used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors.

In other embodiments, the subject has had a prior taxane-based therapy. Taxanes are diterpenes produced by the plants of the genus *Taxus* (yews), and are widely used as chemotherapy agents. Taxane can now be synthesized artificially. Taxane agents include, but are not limited to, paclitaxel (TAXOL®) and docetaxel (TAXOTERE®).

In one aspect, taxane can be fused to or bound to a heterologous moiety. Such a heterologous moiety can improve solubility of taxane formulation or reduce toxicity of taxane. For example, taxane can be fused to or bound to albumin: albumin-bound paclitaxel (ABRAXANE®, also called nab-paclitaxel) is an alternative formulation where paclitaxel is bound to albumin nano-particles.

Synthetic approaches to paclitaxel production led to the development of docetaxel. Docetaxel has a similar set of clinical uses to paclitaxel and is marketed under the name of TAXOTERE®.

In another aspect, taxane useful for the present invention includes, but is not limited to, paclitaxel, 10-deacetylbaccatin III, baccatin III, paclitaxel C, and 7-epipaclitaxel in the shells and leaves of hazel plants.

In other embodiments, the subject has had up to three, up to two, or up to one previous line of chemotherapy. The previous line of chemotherapy can be a platinum-based therapy or a

taxane-based therapy. In yet other embodiments, the subject has not had more than 3 prior lines of chemotherapy for recurrent cancer.

In certain embodiments, the subject is a patient who has recurrent platinum-resistant cancer or platinum refractory disease. In some embodiments, the subject is a patient who has recurrent taxane resistant cancer. In one aspect, the recurrent platinum-resistant cancer or the recurrent taxane-resistant cancer has a progressive tumor during the platinum or taxane treatment, within about one months, within about two months, within about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about 11 months, or about 12 months of completing or receiving a platinum based therapy or a taxane based therapy. In a particular embodiment, the recurrent platinum-resistant cancer or the recurrent taxane-resistant cancer has a progressive tumor within about six months of completing or receiving a platinum based therapy or a taxane based therapy. The recurrent platinum-resistant cancer or the recurrent taxane-resistant cancer can be determined by Response Evaluation Criteria In Solid Tumors (RECIST), measurement of one or more tumor markers, e.g., CA-125, physical examination, reassessment or second-look laparotomy, and/or one or more imaging studies (e.g., X-ray, CT or MRI).

RECIST is a set of published rules that define when cancer patients improve ("respond"), stay the same ("stable") or worsen ("progression") during treatments. The original criteria were published in February 2000 by an international collaboration including the European Organization for Research and Treatment of Cancer (EORTC), National Cancer Institute (NCI) of the United States and the National Cancer Institute of Canada Clinical Trials Group. RECIST 1.1, published in January 2009, is an update to the original criteria. The majority of clinical trials evaluating cancer treatments for objective response in solid tumors are using RECIST.

In some embodiments, a subject can exhibit a tumor marker, e.g., CA-125. In one aspect, the CA-125 expression level in the subject is reduced at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 100% after the administration compared to the CA-125 level prior to the administration.

In some embodiments, a subject has received only one platinum-based treatment for recurrent platinum sensitive disease with a subsequence platinum free interval of less than six months.

In other embodiments, a subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0-1. ECOG is scales or criteria used to assess progression of a patient's disease, effects of the disease in daily living of the patient, and determination of appropriate treatment and prognosis. TABLE 4 shows ECOG performance status:

TABLE 4

ECOG PERFORMANCE STATUS*

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

*As published in Am. J. Clin. Oncol.: *Oken, M. M., Creech, R. H., Tormey, D. C., Horton, J., Davis, T. E., McFadden, E. T., Carbone, P. P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5: 649-655, 1982.*

In some embodiments, a subject has a bone marrow function comparable to a subject without the cancer. Bone marrow functions comparable to a subject without the cancer include, but are not limited to, its role as the major hematopoietic organ and a primary lymphoid tissue, and being responsible for the production of blood cells, e.g., erythrocytes, granulocytes, monocytes, lymphocytes and platelets. Detailed description of the bone marrow structure and function is found in Jain, C., 1986b, *Schalm's Veterinary Hematology*, The hematopoietic system (Lea and Febiger, Philadelphia, Pa.), 4, pp 350-387; Weiss and Geduldig, 1991, *Blood* 78:975-90; Wickramasinghe, 1992, in *Histology for Pathologists, Bone marrow*, ed Sternberg S S (Raven Press, New York), pp 1-31; Picker and Siegelman, 1999, in *Fundamental Immunology*, Lymphoid tissues and organs, ed Paul W E (Lippincott-Raven, Philadelphia, Pa.), 4, pp 479-531; Hoffman et al., 2000, *Hematology Basic Principals and Practice* (Churchill Livingstone, New York), 3; Abboud and Lichtman, 2001, in *Williams' Hematology*, Structure of the marrow and the hematopoietic microenvironment, eds Beutler E, Lichtman M A, Coller B S, Kipps T J, Seligsohn U (McGraw-Hill, New York), 6, pp 29-58, each of which is incorporated herein by reference in its entirety.

In further embodiments, a subject has a hematological function comparable to a subject without the cancer, wherein the indicator of hematological function is selected from the group consisting of:

a. Absolute Neutrophil Count (ANC) is equal to or higher than 1,000/mm3;

b. Platelet (PLT) count is equal to or higher than 100,000/mm3;

c. Prothrombin time (PT) is less than 1.2×Upper Limit of Normal (ULN) seconds;

d. Thromboplastin time (PTT) is less than 1.2×ULN seconds, wherein if PTT is higher than ULN, the patient has a negative lupus anti-coagulant (LAC); and

e. any combinations thereof.

In other embodiments, a subject has an organ function comparable to a subject without the cancer, wherein the organ function is analyzed using common toxicity criteria selected from the group consisting of:

a. less than or equal to grade 1 common toxicity criteria (CTC) neuropathy;

b. no more than 30% of major bone marrow containing areas (e.g. pelvis or lumbar spine) having received prior radiation;

c. less than 2.5× upper limit of normal (ULN) or less than 5×ULN of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), or alkaline phosphatase;

d. less than or equal to 1.5×ULN level of bilirubin;

e. less than or equal to 1.5×ULN level of creatinine;

f. less than 2+ by dip stick of proteinuria at screening or less than 1.0 ratio of urinary protein creatinine; and

g. any combinations thereof.

In still other embodiments, a subject has recovered from acute toxicity from prior treatment, e.g., any chemotherapy, radiotherapy, or biologic therapy. However, the subject may need to recover from grade 1 neuropathy, any grade anemia, or alopecia.

In certain embodiments, a subject has received a prior anti-angiogenic agent. In some embodiments, the subject does not have any prior gastrointestinal (GI) perforation, GI obstruction, or involvement of the bowel on imaging studies. In yet other embodiments, the subject does not have active, untreated psychiatric disease or neurologic symptoms requiring treatment (Grade I sensory neuropathy allowed), does not have presence of untreated central nervous system or brain metastases, does not have any dementia or significantly altered mental status that would prohibit the understanding and/or giving of informed consent, does not have any known hypersensitivity to Cremophor EL, does not have evidence of uncontrolled bacterial, viral or fungal infections, or any combinations thereof. In still other embodiments, the subject is suitable for the present invention if the subject has had a prior paclitaxel reaction, but subsequently tolerated the drug at rechallenge.

A subject suitable for the invention can be identified by measurement of the plasma biomarker or cell surface biomarker for an anti-angiogenic therapy. In one embodiment, a subject suitable for the invention exhibits a plasma biomarker, which includes, but is not limited to vascular endothelial growth factor (VEGF), phosphatidylinositol-glycan biosynthesis class F protein (PIGF), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1), sVEGFR-2, sVEGFR-3, basic fibroblast growth factor (bFGF), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), Von Willebrand Factor (vWF), soluble c-kit (c-kit), stromal derived factor 1α (SDF1α), alpha fetoprotein (AFP), and any combinations thereof. In another embodiment, a subject suitable for the invention exhibits a cell surface biomarker, which includes, but is not limited to, CD31, CD34, CD45, CD133, vascular endothelial growth factor receptor-1 (VEGFR-1), VEGFR-2, or any combinations thereof.

It is known that presence or formation of neutralizing antibodies may hinder efficient gene transfer upon a second administration of virus. In one embodiment, a subject does not have a pre-existing antibody response against adenovirus. In another embodiment, a subject does not develop an antibody response against adenovirus upon administration of the adenovirus.

In some aspects of the invention, a subject does not exhibit lupus anticoagulant (LAC, also known as lupus antibody, LA, or lupus inhibitors). The presence of LAC can be determined by any known methods, e.g., by measuring the LAC by an LAC test or an APLA test. LAC is an immunoglobulin that binds to phospholipids and proteins associated with the cell membrane. Joussen, J., et al., in *Documenta Ophthalmologica,* 2008, Volume 117, Number 3, Pages 263-265, Retinal Vascular Disease, S. J. Ryan (eds), Springer, 2007, 780 p, 1040, ISBN: 978-3-540-29541-9. LAC is a prothrombotic agent; that is, presence of LAC antibodies precipitates the formation of thrombi in vivo. Presence of these antibodies in laboratory tests causes an increase in aPTT. It is speculated that the presence of the antibodies interferes with phospholipids utilized to induce in vitro coagulation. It is thought to interact with platelet membrane phospholipids in vivo, increasing adhesion and aggregation of platelets; thus its in vivo prothrombotic characteristics. Therefore, LAC acts as a coagulation agent in vivo.

IV. Homogeneous Population of Adenovirus Expressing FAS-Chimera

The present invention also provides a homogeneous population of an adenovirus comprising SEQ ID NO: 19 or of an adenovirus having ECACC deposit designation No. 13021201. The term "homogeneous" as used herein refers to a single population of an adenovirus without contamination of heterologous adenoviruses having different sequences. Examples of the heterologous adenovirus include, but are not limited to, the adenovirus comprising a nucleotide sequence which comprises SEQ ID NO: 20 or SEQ ID NO: 21.

The adenovirus comprising a nucleotide sequence which comprises SEQ ID NO: 20 and the adenovirus comprising a nucleotide sequence which comprises SEQ ID NO: 21 were previously disclosed in International Application Nos. PCT/IL2011/00007 and PCT/IL2011/00009, published on Jul. 14, 2011 as WO2011/083464 and WO2011/083466, respectively, which are incorporated herein by reference in their entireties.

The present invention provides an adenovirus comprising SEQ ID NO: 19 (35,208 bps), which includes two nucleotide residues different from SEQ ID NO: 20 (35203 bps) and SEQ ID NO: 21. The two mismatches (i.e., Gly → Ala) in SEQ ID NO: 20 and SEQ ID NO: 21 are at nucleotide residues 501 and 1255. In addition, SEQ ID NO: 19 contains an extra thymidine at nucleotide residue 33624 and four extra base pairs at the 3' end. Moreover, SEQ ID NO: 21 contains an amino acid encoding an extra E1 region.

SEQ ID NO: 19 comprises a nucleotide sequence of an endothelial cell-specific promoter (i.e., PPE-1-3x promoter) at nucleotide residues 458 to 1444 corresponding to SEQ ID NO: 18, and a nucleotide sequence encoding a FAS-chimera protein at nucleotide residues 1469 to 2569 corresponding to SEQ ID NO: 9.

In one embodiment, the present invention is a composition comprising an adenovirus comprising a FAS-chimera gene operably linked to an endothelial cell-specific promoter, wherein the adenovirus does not contain SEQ ID NO: 20 and does not contain SEQ ID NO: 21. In another embodiment, the present invention is a composition comprising an adenovirus comprising SEQ ID NO: 19 or an adenovirus having ECACC deposit designation No. 13021201, wherein the composition does not contain an adenovirus comprising SEQ ID NO: 20 and does not contain an adenovirus comprising SEQ ID NO: 21.

In another embodiment, a composition comprises an adenovirus comprising nucleotide residues 458 to 2569 of SEQ ID NO: 19, wherein the composition does not comprise an adenovirus comprising nucleotide residues 458 to 2569 of SEQ ID NO: 20 or SEQ ID NO: 21.

In other embodiments, a composition of the present invention comprises an adenovirus comprising a nucleic acid sequence at least 80%, 85%, 90%, 95%, 960%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 19, wherein the nucleic acid sequence is not SEQ ID NO: 20 and is not SEQ ID NO: 21 and wherein the composition does not contain an adenovirus comprising SEQ ID NO: 20 and does not contain an adenovirus comprising SEQ ID NO: 21.

In still other embodiments, a composition of the present invention comprising an adenovirus comprising SEQ ID NO: 19 or an adenovirus having ECACC deposit designation No. 13021201, wherein the adenovirus is at least about 51% pure,

at least 55% pure, at least about 60% pure, at least about 70% pure, at least about 80% pure, at least about 90% pure, at least about 95% pure, at least about 96% pure, at least about 97% pure, at least about 98% pure, at least about 99% pure, or about 100% pure. The term "pure" as used herein means a degree of homogeneity, i.e., without heterologous adenoviruses. For example, about 51% pure composition comprising an adenovirus comprising SEQ ID NO: 19 contains about 51% of the adenovirus comprising SEQ ID NO: 19 and about 49% of an adenovirus comprising a heterologous sequence, e.g., SEQ ID NO: 20 or SEQ ID NO: 21. The composition for the invention can contain other ingredients including, but not limited to, a pharmaceutically acceptable carrier, excipient, tonicity modifying agent, or any necessary ingredients for formulation.

In some embodiments, the invention includes a method of isolating or purifying a homogeneous population of the nucleic acid construct. In other embodiments, the invention provides a method of removing heterologous populations of adenovirus, e.g., SEQ ID NO: 20, SEQ ID NO: 21, or both, from a composition comprising an adenovirus comprising SEQ ID NO: 19 or a nucleic acid construct having ECACC deposit designation No. 13021201

Also provided is a method of reducing or decreasing a size of a tumor or slowing the rate of tumor growth eliminating a tumor in a subject comprising administering to the subject an effective amount of a homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus. The invention also includes a method of inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor comprising administering to a subject having the tumor an effective amount of a homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus. Moreover, the invention includes a method of treating or preventing a tumor associated with or derived from cancer in a subject comprising administering an effective amount of the homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus.

The invention also includes a method of reducing or decreasing a size of a tumor or slowing the rate of tumor growth eliminating a tumor in a subject comprising administering to the subject an effective amount of a homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus repeatedly without administering a heterogeneous population of an adenovirus comprising SEQ ID NO: 20 or SEQ ID NO: 21 or a composition comprising the heterogeneous population of the adenovirus. Also included is a method of inhibiting, decreasing, or reducing neo-vascularization or angiogenesis in a tumor comprising administering to a subject having the tumor an effective amount of a homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus repeatedly without administering a heterogeneous population of the adenovirus comprising SEQ ID NO: 20 or SEQ ID NO: 21 or a composition comprising the heterogeneous population of the adenovirus. In addition, the invention includes a method of treating or preventing a tumor associated with or derived from cancer in a subject comprising administering an effective amount of the homogeneous population of the adenovirus or a composition comprising the homogeneous population of the adenovirus repeatedly without administering a heterogeneous population of the adenovirus comprising SEQ ID NO: 20 or SEQ ID NO: 21 or a composition comprising the heterogeneous population of the adenovirus.

The tumor that can be reduced, inhibited, or treated with the homogeneous population of the adenovirus or the composition can be a solid tumor, a primary tumor, or a metastatic tumor. The term "metastatic" or "metastasis" refers to tumor cells that are able to establish secondary tumor lesions in another parts or organ.

In other embodiments, the homogeneous population of the invention when administered to a subject in need thereof prolongs the overall survival of the subject. In further embodiments, the homogeneous population of the invention when administered to a subject in need thereof prolongs progression-free survival of the subject.

A "solid tumor" includes, but is not limited to, sarcoma, melanoma, carcinoma, or other solid tumor cancer. "Sarcoma" refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Sarcomas include, but are not limited to, chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma, fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and telangiectaltic sarcoma.

The term "melanoma" refers to a tumor arising from the melanocytic system of the skin and other organs. Melanomas include, for example, acra-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, metastatic melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

The term "carcinoma" refers to a malignant new growth made up of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases. Exemplary carcinomas include, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, basosquamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare,

lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidernoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, naspharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma viflosum.

Additional cancers that may be inhibited or treated include, for example, Leukemia, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, breast cancer, ovarian cancer, lung cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, small-cell lung tumors, primary brain tumors, stomach cancer, colon cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, papillary thyroid cancer, neuroblastoma, neuroendocrine cancer, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, cervical cancer, endometrial cancer, adrenal cortical cancer, prostate cancer, Müllerian cancer, ovarian cancer, peritoneal cancer, fallopian tube cancer, or uterine papillary serous carcinoma.

V. Pharmaceutical Compositions

Also provided in the invention is a pharmaceutical composition comprising a nucleic acid construct expressing a FAS-chimera protein used in the methods of the invention, an adenovirus comprising the nucleic acid construct, or a homogeneous population of the adenovirus. The pharmaceutical composition can be formulated for administration to mammals, including humans. The pharmaceutical compositions used in the methods of this invention comprise pharmaceutically acceptable carriers, including, e.g., ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. In one embodiment, the composition is formulated by adding saline.

The compositions of the present invention may be administered by any suitable method, e.g., parenterally (e.g., includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques), intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. As described previously, the composition comprising a nucleic acid construct which comprises a FAS-chimera gene or the homogeneous population of the adenovirus expresses the FAS-chimera gene product in an endothelial cell and thereby induces apoptosis of the endothelial cell. Accordingly, the composition can inhibit, reduce, or decrease the size of a tumor or a metastasis thereof by inhibiting neo-vascularization and/or angiogenesis of the tumor endothelial cells. Therefore, in one embodiment, the composition is delivered systemically or locally. For systemic or local delivery, the pharmaceutical formulation containing the nucleic acid construct, the adenovirus, or the homogeneous population of the adenovirus can utilize a mechanical device such as a needle, cannula or surgical instruments.

Sterile injectable forms of the compositions used in the methods of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile, injectable preparation may also be a sterile, injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a suspension in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents which are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

Parenteral formulations may be a single bolus dose, an infusion or a loading bolus dose followed with a maintenance dose. These compositions may be administered at specific fixed or variable intervals, e.g., once a day, or on an "as needed" basis.

Certain pharmaceutical compositions used in the methods of this invention may be orally administered in an acceptable dosage form including, e.g., capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also may be administered by nasal aerosol or inhalation. Such compositions may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

The amount of a nucleic acid construct expressing a FAS-chimera protein, an adenovirus comprising the nucleic acid construct, or a homogeneous population of the adenovirus that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the type of polypeptide used and the particular mode of administration. The composition may be administered as a single dose, multiple doses or over an established period of time in an infusion. Dosage regimens also may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response).

In one embodiment, a composition comprising a nucleic acid construct expressing a FAS-chimera protein, an adenovirus, or a homogeneous population of the adenovirus is infused

on day 1 (i.e., the first dose) and followed by one or more subsequence doses, e.g., every one week cycle, every two week cycle, every three week cycle, every four week cycle, every five week cycle, every six week cycle, every seven week cycle, every eight week cycle, every nine week cycle, every ten week cycle, every 11 week cycle, or every 12 week cycle. In another embodiment, a composition comprising a nucleic acid construct expressing a FAS-chimera protein, an adenovirus, or a homogeneous population of the adenovirus is infused on day 1 (i.e., the first dose) and followed by one or more subsequence doses, e.g., every two weeks cycle, every monthly cycle, every two months cycle, every three months cycle, every four months cycle, every five months cycle or every six months cycle.

The methods of the invention use an "effective amount" or a "therapeutically effective amount" of a composition comprising a nucleic acid construct expressing a FAS-chimera protein, an adenovirus comprising the nucleic acid construct, or a homogeneous population of the adenovirus. Such an effective amount or a therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual. An effective amount or a therapeutically effective amount is also one in which any toxic or detrimental effects are outweighed by the therapeutically beneficial effects.

A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular composition used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

An effective amount of an adenovirus comprising the nucleic acid construct encoding a FAS-chimera or a homogeneous population of an adenovirus comprising the nucleic acid construct encoding a FAS-chimera protein can be any suitable doses. In one embodiment, an effective amount of the adenovirus or the homogeneous population of the adenovirus is at least about 10^9 VPs, at least about 10^{10} VPs, at least about 10^{11} VPs, at least about 10^{12} VPs, or at least about 10^{13} VPs per subject. In another embodiment, an effective amount of the adenovirus or the homogeneous population of the adenovirus is at least about 1×10^{12} VPs, at least about 2×10^{12} VPs, at least about 3×10^{12} VPs, at least about 4×10^{12} VPs, at least about 5×10^{12} VPs, at least about 6×10^{12} VPs, at least about 7×10^{12} VPs, at least about 8×10^{12} VPs, at least about 9×10^{12} VPs, or at least about 1×10^{13} VPs per subject. In other embodiments, an effective amount of the adenovirus or the homogeneous population of the adenovirus is about 10^9 to about 10^{15} VPs, about 10^{10} to about 10^{14} VPs, about 10^{11} to about 10^{13} VPs, or about 10^{12} to about 10^{13} VPs, or about 3×10^{12} to about 10^{13} VPs per subject.

In other aspects, an effective amount of an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is escalated if the first dose does not induce any toxicity. For example, the first dose of the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus can be at least about 1×10^9, about 1×10^{10} VPs, at least about 1×10^{11} VPs, at least about 1×10^{12} VPs, at least about 2×10^{12} VPs, at least about 3×10^{12} VPs, at least about 4×10^{12} VPs, at least about 5×10^{12} VPs, at least about 6×10^{12} VPs, at least about 7×10^{12} VPs, at least about 8×10^{12} VPs, or at least about 9×10^{12} VPs per subject, and the second dose or the subsequence doses of the adenovirus expressing a FAS-chimera protein can be at least about 5×10^{12} VPs, at least about 6×10^{12} VPs, at least about 7×10^{12} VPs, at least about 8×10^{12} VPs, or at least about 9×10^{12} VPs, at least about 1×10^{13} VPs, at least about 2×10^{13} VPs, at least about 3×10^{13} VPs, at least about 4×10^{13} VPs, at least about 5×10^{13} VPs, at least about 6×10^{13} VPs, at least about 7×10^{13} VPs, at least about 8×10^{13} VPs, at least about 9×10^{13} VPs, at least about 1×10^{14} VPs per subject. In a specific example, the first dose of the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is about 3×10^{12} VPs per subject, and the second dose of the adenovirus construct expressing FAS-chimera is about 1×10^{13} VPs per subject. However, an effective amount of the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus can be reduced if a particular dose induces dose limiting toxicity.

A composition comprising the adenovirus or the homogeneous population of the adenovirus can be infused to the subject for about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 60 minutes, about 70 minutes, about 80 minutes, about 90 minutes, about 100 minutes, about 110 minutes, or about 120 minutes. In one embodiment, the nucleic acid construct of the invention is infused intravenously for not more than 60 minutes. In another embodiment, a composition comprising the adenovirus or the homogeneous population of the adenovirus is infused at a rate of 1 mL/minute for doses equal to or less than 3×10^{12} VPs per subject. If the dose is more than 3×10^{12} VPs per subject, the composition comprising the adenovirus or the homogeneous population of the adenovirus is infused at a rate of 1 mL/minute for the first 10 mL and then at a rate of 3 mL/minute for the remainder.

Supplementary active compounds also can be incorporated into the compositions used in the methods of the invention. For example, a nucleic acid construct encoding a FAS-chimera gene product or a homogeneous population of the nucleic acid construct may be coformulated with and/or coadministered with one or more additional therapeutic agents.

The invention encompasses any suitable delivery method for a nucleic acid construct encoding FAS-chimera gene product or a homogeneous population of the nucleic acid construct to a selected target tissue, including bolus injection of an aqueous solution or implantation of a controlled-release system. Use of a controlled-release implant reduces the need for repeat injections.

A nucleic acid construct encoding FAS-chimera gene product, an adenovirus comprising the nucleic acid construct, or a homogeneous population of the adenovirus may be directly infused into the tumor. Various implants for direct tumor infusion of compounds are known and are effective in the delivery of therapeutic compounds to human patients suffering from female gynecological cancer.

The compositions may also comprise a nucleic acid construct encoding FAS-chimera gene product, an adenovirus comprising the nucleic acid construct, or a homogeneous population of the adenovirus dispersed in a biocompatible carrier material that functions as a suitable delivery or support system for the compounds. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles such as suppositories or capsules. Implantable or microcapsular sustained release matrices include polylactides (U.S. Pat. No. 3,773,319; EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., *Biopolymers* 22:547-56 (1985));

poly(2-hydroxyethyl-methacrylate), ethylene vinyl acetate (Langer et al., *J. Biomed. Mater. Res.* 15:167-277 (1981); Langer, *Chem. Tech.* 12:98-105 (1982)) or poly-D-(−)-3hydroxybutyric acid (EP 133,988).

According to the methods of the present invention, administration of the nucleic acid construct, the adenovirus, or the homogeneous population of the adenovirus can be combined with administration of one or more chemotherapeutic agents. The chemotherapeutic agent can be administered prior to, concurrently with, or after administration of the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus.

One or more chemotherapeutic agent that can be co-administered with the adenovirus of the invention include, but are not limited to Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Alimta; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole; Anthramycin; Asparaginase; Asperlin; Azacitidine; Azetepa; Azotomycin; Batimastat; Benzodepa; Bicalutamide; Bisantrene Hydrochloride; Bisnafide Dimesylate; Bevacizumab, Bizelesin; Bleomycin Sulfate; Brequinar Sodium; Bropirimine; Busulfan; Cactinomycin; Calusterone; Caracemide; Carbetimer; Carboplatin; Carmustine; Carubicin Hydrochloride; Carzelesin; Cedefingol; Chlorambucil; Cirolemycin; Cisplatin; Cladribine; Crisnatol Mesylate; Cyclophosphamide; Cytarabine; Dacarbazine; Dactinomycin; Daunorubicin Hydrochloride; Decitabine; Dexormaplatin; Dezaguanine; Dezaguanine Mesylate; Diaziquone; Docetaxel; Doxorubicin; Doxorubicin Hydrochloride; Droloxifene; Droloxifene Citrate; Dromostanolone Propionate; Duazomycin; Edatrexate; Eflornithine Hydrochloride; Elsamitrucin; Enloplatin; Enpromate; Epipropidine; Epirubicin Hydrochloride; Erbulozole; Esorubicin Hydrochloride; Estramustine; Estramustine Phosphate Sodium; Etanidazole; Etoposide; Etoposide Phosphate; Etoprine; Fadrozole Hydrochloride; Fazarabine; Fenretinide; Floxuridine; Fludarabine Phosphate; Fluorouracil; Fluorocitabine; Fosquidone; Fostriecin Sodium; Gemcitabine; Gemcitabine Hydrochloride; Hydroxyurea; Idarubicin Hydrochloride; Ifosfamide; Ilmofosine; Interferon Alfa-2a; Interferon Alfa-2b; Interferon Alfa-n1; Interferon Alfa-n3; Interferon Beta-I a; Interferon Gamma-I b; Iproplatin; Irinotecan Hydrochloride; Lanreotide Acetate; Letrozole; Leuprolide Acetate; Liarozole Hydrochloride; Lometrexol Sodium; Lomustine; Losoxantrone Hydrochloride; Masoprocol; Maytansine; Mechlorethamine Hydrochloride; Megestrol Acetate; Melengestrol Acetate; Melphalan; Menogaril; Mercaptopurine; Methotrexate; Methotrexate Sodium; Metoprine; Meturedepa; Mitindomide; Mitocarcin; Mitocromin; Mitogillin; Mitomalcin; Mitomycin; Mitosper; Mitotane; Mitoxantrone Hydrochloride; Mycophenolic Acid; Nocodazole; Nogalamycin; Ormaplatin; Oxisuran; pazotinib; Paclitaxel; Pegaspargase; Peliomycin; Pentamustine; Peplomycin Sulfate; Perfosfamide; Pipobroman; Piposulfan; Piroxantrone Hydrochloride; Plicamycin; Plomestane; Porfimer Sodium; Porfiromycin; Prednimustine; Procarbazine Hydrochloride; Puromycin; Puromycin Hydrochloride; Pyrazofurin; Riboprine; Rogletimide; Safingol; Safingol Hydrochloride; Semustine; Simtrazene; Sorafinib; Sparfosate Sodium; Sparsomycin; Spirogermanium Hydrochloride; Spiromustine; Spiroplatin; Streptonigrin; Streptozocin; Sulofenur, Sunitinib; Talisomycin; Taxol; Tecogalan Sodium; Tegafur; Teloxantrone Hydrochloride; Temoporfin; Teniposide; Teroxirone; Testolactone; Thiamiprine; Thioguanine; Thiotepa; Tiazofuirin; Tirapazamine; Topotecan Hydrochloride; Toremifene Citrate; Trestolone Acetate; Triciribine Phosphate; Trimetrexate; Trimetrexate Glucuronate; Triptorelin; Tubulozole Hydrochloride; Uracil Mustard; Uredepa; Vapreotide; Verteporfin; Vinblastine Sulfate; Vincristine Sulfate; Vindesine; Vindesine Sulfate; Vinepidine Sulfate; Vinglycinate Sulfate; Vinleurosine Sulfate; Vinorelbine Tartrate; Vinrosidine Sulfate; Vinzolidine Sulfate; Vorozole; Zeniplatin; Zinostatin; or Zorubicin Hydrochloride. Additional antineoplastic agents include those disclosed in Chapter 52, Antineoplastic Agents (Paul Calabresi and Bruce A. Chabner), and the introduction thereto, 1202-1263, of Goodman and Gilman's "The Pharmacological Basis of Therapeutics", Eighth Edition, 1990, McGraw-Hill, Inc.

In some embodiments, a subject administered with an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is concurrently treated with radiotherapy. In other embodiments, a subject administered with an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is concurrently treated with two or more chemotherapeutic agents. In certain embodiments, a subject administered with an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is concurrently treated with a chemotherapeutic agent and radiotherapy.

In the combination therapy aspect of the invention, the chemotherapeutic agent can be paclitaxel. In one aspect, an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus can be administered concurrently with paclitaxel. In another aspect, an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is administered before or after administration of paclitaxel. In other embodiments, paclitaxel is administered at least 30 minutes, at least about one hour, at least about two hours, at least about three hours, at least about four hours, at least about five hours, at least about six hours, at least about seven hours, at least about eight hours, at least about nine hours, at least about ten hours, at least about 11 hours, at least about 12 hours, at least about 13 hours, at least about 14 hours, at least about 19 hours, at least about 20 hours, at least about 21 hours, at least about 22 hours, at least about 23 hours, at least about 24 hours, at least about one day, at least about 36 hours, at least about 2 days, at least about 60 hours, or at least about 3 days prior to the administration of an adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus.

The chemotherapeutic agent can also be administered by any suitable methods, e.g., parenterally, intraventricularly, orally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, intraperitoneal, intracranial injection or infusion techniques.

An effective amount of the chemotherapeutic agent is available in the art. In one aspect, for example, an effective amount of paclitaxel can be at least about 10 mg/m^2, at least about 20 mg/m^2, at least about 30 mg/m^2, at least about 40 mg/m^2, at least about 50 mg/m^2, at least about 60 mg/m^2, at least about 70 mg/m^2, at least about 80 mg/m^2, at least about 90 mg/m^2, at least about 100 mg/m^2, or at least about 110 mg/m^2. In another aspect, an effective amount of paclitaxel is from about 10 mg/m^2 to about 200 mg/m^2, from about 20 mg/m^2 to about 150 mg/m^2, from about 30 mg/m^2 to about 100 mg/m^2, or from 40 mg/m^2 to about 80 mg/m^2. In other aspects, an effective amount of paclitaxel is about 10 mg/m^2, about 20 mg/m^2, about 30 mg/m^2, about 40 mg/m^2, about 50 mg/m^2, about 60 mg/m^2, about 70 mg/m^2, about 80 mg/m^2, about 90 mg/m^2, or about 100 mg/m^2.

In certain aspects for the paclitaxel administration, paclitaxel is infused for at least 10 minutes, at least 20 minutes, at least 30 minutes, at least 40 minutes, at least 50 minutes, at least 60 minutes, at least 70 minutes, at least 80 minutes, at least 90 minutes, at least 100 minutes, at least 110 minutes, at least 120 minutes, at least 150 minutes, at least 180 minutes, at least 210 minutes, at least 240 minutes, at least 270 minutes, or at least 300 minutes. In a specific example, paclitaxel is infused for at least one hour. The infusion methods for paclitaxel can be used any methods known in the art. For example, paclitaxel can be administered through an in-line filter with a microporous membrane not greater than 0.22 microns over three hours.

In some aspects, paclitaxel is infused on day 1 (the same day that the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is administered) (i.e., the first dose) and followed by one or more subsequence doses, e.g., every three days, every four days, every five days, every six days, every seven days, every eight days, every nine days, every ten days, every 11 days, every 12 days, every 13 days, or every 14 days infusion. In a specific example, paclitaxel is administered on day 1 (i.e., the first dose) and followed by 8 day (i.e., the second dose), 15 day (i.e., the third dose) and 22 day (i.e., the fourth dose) schedule every 28 days.

In yet other aspects, an effective amount of a chemotherapeutic agent to be coadministered together with the adenovirus expressing a FAS-chimera protein or a homogeneous population of the adenovirus is escalated if the first dose does not induce any toxicity. For example, the first dose of paclitaxel can be about 10 to about 70 mg/m^2, about 20 to about 60 mg/m^2, about 30 to about 50 mg/m^2, or about 40 mg/m^2, and the second dose or any subsequent doses can be escalated by about 10 mg/m^2, about 20 mg/m^2, about 30 mg/m^2, about 40 mg/m^2, about 50 mg/m^2, about 60 mg/m^2, about 70 mg/m^2, or about 80 mg/m^2. In one embodiment, the first dose of paclitaxel is about 40 mg/m^2, and the second dose of paclitaxel is about 80 mg/m^2. In another embodiment, the first dose of paclitaxel is about 40 mg/m^2, the second dose of paclitaxel is about 80 mg/m^2, and the third dose of paclitaxel is about 80 mg/m^2. In other embodiments, the first dose of paclitaxel is about 40 mg/m^2, the second dose of paclitaxel is about 80 mg/m^2, the second dose of paclitaxel is about 80 mg/m^2, and the fourth dose of paclitaxel is about 80 mg/m^2.

However, an effective amount of paclitaxel can be reduced if the first dose induces a dose limiting toxicity to the subject. The dose limiting toxicity can be determined by any known methods: for example, (1) absolute neutrophil count (ANC) of $<0.5\times10^9$/L lasting for ≥4 days or an absolute neutrophil count$<0.5\times10^9$/L with sepsis, or grade 3-4 fever (>100.2° F.) which is not readily controlled with anti-pyretic medication; (2) platelet count$<10\times10^9$/i for any duration; (3) any other drug-related non hepatic and non-hematologic grade≥3 toxicity, or any combinations thereof. This does not include grade≥3 nausea or vomiting that can be controlled medically (if nausea and/or vomiting cannot be controlled medically and occurs during the first cycle, it will be considered a DLT) or grade≥3 hypokalemia, hyponatremia, hypophosphatemia, hypomagnesemia, and hypocalcemia if they can be easily corrected, are clinically asymptomatic, and not accompanied by medically significant complications (e.g., ECG changes).

In one embodiment, the first dose of paclitaxel in day 1 is 40 mg/m^2, and if the subject exhibits no dose limiting toxicity, the second dose at day 8 and the subsequent doses at day 15, day 22, and day 28 are escalated to 80 mg/m^2. In another embodiment, if ANC/drug in a subject is ≥1,000/mm^3, the subject is administered with a full dose of paclitaxel, e.g., 80 mg/m^2. If ANC/drug in the subject is <1,000/mm^3, the paclitaxel administration is stopped. If there is a further incidence of ANC<1,000/mm^3, the paclitaxel dose can be reduced to 60 mg/m^2. In other embodiments, if a subject has the platelets/drug of ≥100,000/mm^3 or has the first incidence of <100,000/mm^3 and ≥75,000/mm3, the subject is administered with a full dose of paclitaxel, e.g., 80 mg/m^2. If a subject shows the platelets/drug of <75,000/mm^3, the paclitaxel administration is stopped. If the subject shows a repeat incidence of platelets/drug of <100,000/mm^3 and >75,000/mm^3, the paclitaxel dose is reduced to 69 mg/m^2. In some embodiments, if a subject shows the first incidence of neuropathy grade>2, the paclitaxel dose is reduced from the full dose, e.g., about 80 mg/m^2, to about 60 mg/m^2. If the subject exhibits the second incidence (or shows persistence despite the dose reduction) of neuropathy grade>2, the paclitaxel dose is reduced to 40 mg/m^2. If the subject exhibits the third incidence (or persistence despite dose reduction), the paclitaxel administration is discontinued.

Paclitaxel is not known to cause hepatic toxicity; however, its elimination is delayed in patients with severe hepatic dysfunction. Therefore, the dose of paclitaxel in subject with hepatic dysfunction can be modified according to the following table.

TABLE 5

Paclitaxel Dose Modification for Subjects with Hepatic Dysfunction.

Serum AST, or ALT	Paclitaxel
<3x ULN	80 mg/m2
≥3x ULN	60 mg/m2
≥5x ULN	Hold drug

Subjects with elevated bilirubin>1.5 mg/dl may not receive paclitaxel until the abnormal laboratory values improve to ≤grade 1. Treatment can be resumed after recovery with paclitaxel at one dose level lower, per the above table. The subjects exhibiting paclitaxel toxicity that lasts more than 2 weeks can discontinue the drug.

In certain embodiments, a subject is administered with an immunosuppressant agent prior to, concomitantly with, or after administration of one or more chemotherapeutic agent, e.g., paclitaxel. In one embodiment, the immunosuppressant agent useful for administering to the subject is selected from the group consisting of H_2-receptor antagonists, cimetidine, ranitidine, famotidine, corticosteroids, dexamethasone, cyclosporine, diphenhydramine, and any combinations thereof.

In further embodiments, methods of the invention further comprise administering one or more anti-angiogenetic agents prior to, concurrently with, or after the administration of a nucleic acid construct expressing a FAS chimera protein.

In some embodiments, a subject for the invention is administered with an anti-emetic agent. Examples of suitable anti-emetic agents include, but are not limited to, 5-HT3 receptor antagonists (e.g., dolasetron, granisetron, ondansetro, tropisetron, palonosetron, or mirtazapine), dopamine antagonists (e.g., domperidone, olanzapine, droperidol, haloperidol, chlorpromazine, promethazine, prochlorperazine, metoclopramide, alizapride, or prochlorperazine, compazine, stemzine, buccastem, stemetil, or phenotil), NK1 receptor antagonist (e.g., aprepitant or casopitant), antihistamines (H1 histamine receptor antagonists) (e.g, cyclizine, diphenhydramine, dimenhydrinate, doxylamine, meclozine, promethazine, or hydroxyzine), cannabinoids (e.g., cannabis, dronabinol, nabilone, the JWH series, or Sativex),

benzodiazepines (e.g., midazolam or lorazepam), anticholinergics (e.g., hyoscine), steroids (e.g., dexamethasone), trimethobenzamide, ginger, emetrol, propofol, peppermint, muscimol, or ajwain), or any combinations thereof. In other embodiments, the suitable anti-emetic agents are dexamethasone, PRN Ativan, kytril, compazine, perphenazine, zofran, perphenazine, or any combinations thereof.

In other embodiments, antipyretic agents is administered prior to, concurrently with, or after the administration of the nucleic acid construct encoding a FAS-chimera protein. Examples of the antipyretic agents include, but are not limited to NSAIDs (e.g., ibuprofen, naproxen, ketoprofen, and nimesulide), aspirin, acetaminophen, metamizole, nabumetone, phenazone, quinine, or any combinations thereof.

The methods of the present invention further comprise determining disease progression in the subject before or after receiving the nucleic acid construct expressing a FAS-chimera protein or the chemotherapeutic agent. In one embodiment, disease progression is determined by measuring size of the tumor. In another embodiment, disease progression is determined by measuring expression of a tumor antigen, e.g., CA-125. In order to determine the disease progression, the subject's blood and urine are collected prior to the infusion, at the end of the infusion, about three hours after the infusion, and/or about six hours after the infusion of the nucleic acid construct expressing a FAS-chimera gene product. In other embodiments, vital signs for the subject are recorded at 0 minutes-15 minutes (just prior to dosing), 30 minutes±15 minutes after start of dosing 60 minutes±15 minutes after start of dosing, four hours±15 minutes post start of dosing, six hours±15 minutes post start of dosing, and/or on the occasion of any adverse events. The vital signs to be measured include, but are not limited to systolic and diastolic blood pressure, peripheral heart rate, body temperature, respiration rate, or any combinations thereof. In still other embodiments, the subject is measured for hematology (e.g., complete blood count with differential, INR and activated PTT); coagulation (e.g., PTT level); biodistribution of the nucleic acid construct expressing FAS-chimera; expression of angiogenic and inflammatory biomarkers (e.g., VEGF, P1GF, sVEGFR1, bFGF, IL-1β, IL-6, IL-8, TNF-α, sVEGFR2, SDF1α, CD31, CD34, VEGFR2, vWF, any combinations thereof); expression of tumor marker, e.g., CA-125, or any combinations thereof.

The subjects suitable for the methods of the invention are managed in the usual fashion for fever and neutropenia. The subjects need to recover from fever and active infectious issues prior to resuming therapy. In some embodiments, the subjects who are not on growth factors have Neupogen or Neulasta added to their next cycle. The subjects on Neupogen 5 ug/kg/day can have their dose escalated to 10 ug/kg on the same schedule.

EXAMPLES

Example 1

Construction and Cloning of the Viral Vector

The vector was constructed using a backbone containing most of the genome of adenovirus type 5, as well as partial homology to an adaptor plasmid, which enables recombination.

The E1 early transcriptional unit was deleted from the backbone plasmid, and further modified by deleting the pWE25 and the Amp resistance selection marker site.

The adaptor plasmid, containing sequences of the Ad5, CMV promoter, MCS, and SV40 polyA was modified to delete deleting the CMV promoter, and the PPE-1 promoter and Fas-c fragment were inserted by restriction digestion. The modified PPE-1 promoter (PPE-1-3X, SEQ ID NO: 18) and the Fas-chimera transgene (Fas-c, SEQ ID NO: 9) were utilized for construction of the adenoviral vector. The PPE-1-(3X)-Fas-c element (2115 bp) was constructed from the PPE-1-(3X)-luc element. This element contains the 1.4 kb of the murine preproendothelin PPE-1-(3X) promoter, the Luciferase gene, the SV40 polyA site and the first intron of the murine ET-1 gene, originated from the pEL8 plasmid (8848 bp) used by Harats et al (Harats D. et al., JCI, 1995). The PPE-3-Luc cassette was extracted from the pEL8 plasmid using the BamHI restriction enzyme. The Luciferase gene was substituted by the Fas-c gene [composed of the extra cellular and intra membranal domains of the human TNF-R1 (Tumor Necrosis Factor Receptor 1, SEQ ID NO: 4) and of the Fas (p55) intracellular domain (SEQ ID NO: 8) (Boldin et al, JBC, 1995)] to obtain the PPE-1-3X-Fas-c cassette.

PPE-1 (3x)-Fas-c Plasmid—The cassette was further introduced into the backbone plasmid by restriction digestion, resulting with the PPE-1 (3x)-Fas-c plasmid.

Adaptor-PPE-1(3x)-Fas-c Plasmid—The PPE-1-3x-Fas-c element was extracted from the first generation construct PPE-1-3x-Fas-c plasmid, and was amplified with designated PCR primers introducing SnaB1 and EcoR1 restriction sites at the 5'-and-3'-end respectively. These sites were used to clone the PPE-Fas-c fragment into the adaptor plasmid digested with SnaB1 and EcoR1, resulting in the adaptor-PPE-1-3x-Fas-c used for transfection of the host cells (for example, PER.C6 cells).

Example 2

Efficacy and Safety of VB-111 in Mice with Metastatic Lewis Lung Carcinoma (LLC)

Summary

In this study, LLC model mice were treated with VB-111 (10^9 or 10^{11} virus particles (VPs)), Carboplatin (20 or 50 mg/kg) and Alimta (10 or 30 mg/kg) or with a combinations of these small molecules and the adenovector.

VB-111 treatment with both doses resulted in 100% survival. Administration of low dosage of chemotherapy resulted in a mildly lowered survival rate (94.1%) which was similar to that seen with vehicle administration (93.8%). However, administration of high dosage of chemotherapy resulted in the lowest percentage of survival (50%). Combination treatments decreased survival compared to administration of VB-111 alone (64%-93%).

Organ weights (heart, kidneys and brain) were mostly similar among the different groups. Some differences between groups were seen in liver, spleen and testes weights, mainly higher weights for the single VB-111 higher dose, or any of the combinations with this dose.

No significant differences were observed in liver function compared to vehicle treatment. Generally, combination therapy did not result in higher toxicities than the single therapies from which they are assembled.

The combination of VB-111 10^9+high chemo is significantly more effective than high chemo treatment alone. Furthermore, the combined treatment improves the average and median tumor burden of VB-111 10^9 treatment alone (1.5 and 5 fold for average and median, respectively) although not statistically significant. This effect may exhibit statistical significance when applying the treatment on larger groups.

The combination of VB-111 10^9+high chemo resembles the high tumor burden reduction obtained with VB-111 10^{11}, as there is no significant difference between these two groups. This significant resemblance to VB-111 10^{11} is not obtained with treatment with VB-111 10^9 alone.

The combination of VB111 10^9+high chemo therapy may enable reducing VB-111 dose while preserving its high efficacy and improving the low efficacy of Carboplatin and Alimta chemotherapy alone.

Introduction

Lewis Lung Carcinoma (LLC) is a widely-used mouse model for metastasis (Varda-Bloom et al., 2001). This study is to identify the potential synergism of combined treatments of VB-111 with established chemotherapies (e.g. Carboplatin and Alimta): To assess the efficacy of single dose VB-111 (10^9 or 10^{11} VPs/mouse) as sole treatment or in combination with repeat intra peritoneal treatments of Carboplatin (20 or 50 mg/kg) and Alimta (10 or 30 mg/kg) and to assess and characterize the safety and tolerability of VB-111 as single treatment and in combination with the mentioned above chemotherapy.

The study design is shown in TABLE 6.

TABLE 6

Study Design

STUDY GROUPS and BASIC TIMELINE	9 groups of treatment are planned as below:			
		Treatment	Dose (vp/mice)	Number of Animals
	Group 1	VB-111	10^{11}	15
	Group 2	VB-111	10^9	15
	Group 3	VB-111	10^{11}	15
		Carboplatin +	20 mg/kg +	
		Alimta	10 mg/kg	
	Group 4	VB-111	10^9	13
		Carboplatin +	20 mg/kg +	
		Alimta	10 mg/kg	
	Group 5	Carboplatin +	20 mg/kg +	16
		Alimta	10 mg/kg	
	Group 6	VB-111	10^{11}	15
		Carboplatin +	50 mg/kg +	
		Alimta	30 mg/kg	
	Group 7	VB-111	10^9	14
		Carboplatin +	50 m/kg +	
		Alimta	30 mg/kg	
	Group 8	Carboplatin +	50 mg/kg +	14
		Alimta	30 mg/kg	
	Group 9	Vehicle	—	16
	Each animal received a single injection of either VB-111 dose or vehicle in a randomized fashion on day 0 ± 1, 5 days after primary tumor amputation. 2 doses of Carboplatin treatment were given on days 5-6, 10 doses of Alimta treatment were given from day 5. The animals were evaluated for safety and efficacy throughout the study and until sacrifice day, which took place on the same day on which the 5th control animal (Group 9) died.			
STUDY DURATION	Approximately 1 month from time 0			
ANIMALS	133 male C57B16 mice (11-13 weeks old), which were expected to have developed metastases in the lung, following injection of D122 Lewis Lung Carcinoma cells to the left footpad and resection of the primary tumor (amputation of the distal segment of the limb).			
TEST DRUG AND FORMULATION	VB-111: Adenovirus 5, E1 deleted, partial E3 deleted, with the PPE-1(3x) promoter, containing the transgene fas-chimera, formulated in the vehicle (see below). Vehicle: PBS 10% glycerol. ALIMTA: Pemetrexed, 500 mg powder. Carboplatin: "EBEWE" 10 mg/ml.			
DOSAGE	VB-111 treatment was administered by injection to the mouse tail vein, in a total volume of 100 µl, 20 or 50 mg/kg/Carboplatin was administered in two doses on days 5-6, 0.1 ml IP, and 10 doses of 10 or 30 mg/kg/Alimta were administered from day 5, 0.1 ml IP.			
SAFETY EVALUATIONS	The general health of all animals was followed on a daily basis throughout the study. Weight of the animals and clinical signs were recorded prior to the beginning of the study, after resection of the primary tumor, on day 0 (day of dosing), and then on a weekly basis until the end of the study. Laboratory assessment included blood chemistry (for liver function) of samples taken from 5 mice/group at the end of the study. Major organs (liver, spleen, heart, kidney, lung, gonads and brain) were evaluated upon animal sacrifice (5 mice/group).			

TABLE 6-continued

Study Design	
EFFICACY EVALUATIONS	The effect of treatment was evaluated upon the death of each animal, by lung weight and tumor burden.
STATISTICS	Treatment groups were compared using one way ANOVA for comparison of Organ weights, Tumor burden and Liver function parameters. To isolate the group or groups that differ from the others, Dunn's multiple comparison procedure followed (GPT day 16 GOT day 16 & tumor burden). Body weights of each group on day 0 were compared with those on sacrifice day (Paired t-test). For Efficacy, groups were analyzed using one way ANOVA. In cases where normality test failed, Dunn's method was applied. Additionally, Mann-Whitney test was performed for individual comparisons between two groups.

Materials and Methods

Test and Reference Materials

Name: VB-111 (PPE-fas).

Chemical Name: Not specified

Active components: Adenovirus 5, E1 deleted, E3 partially deleted, with the PPE-1(3x) promoter, containing the fas-chimera transgene

Vehicle: PBS and glycerol

Supplied by: VBL

Physical state: Liquid

Storage conditions: ≤−65° C., in cryogenic vials

Item preparation: Vial is to be thawed on day of treatment and mixed by inversions

Name: Carboplatin

Chemical Name: Ebewe

Vehicle: Water for Injection

Supplied by: Ebewe

Physical state: Liquid (450 mg/45 ml)

Storage conditions: RT

Name: Alimta

Chemical Name: Pemetrexed

Vehicle: Water for Injection

Supplied by: Lilly

Physical state: powder

Storage conditions: RT

Control Item:

Name: PBS 10% glycerol

Supplied by: VBL

Physical state: Liquid

Storage conditions: ≤−65° C., in cryogenic vial.

Item preparation: Vial is to be thawed on day of treatment and kept on ice for not more than 30 minutes.

D122 Lewis Lung Carcinoma cells were thawed. On day one of cell injection, cells were collected and suspended to a final concentration of 5×10^5/50 μl and injected to the left foot pad. Tumor diameter was measured using a caliper five days after injection. It was subsequently measured weekly until it reached 5 mm, and then daily until amputation at 7 mm (defined as day −5). Following sacrifice, brain, heart, liver, spleen, kidneys and testes were collected, weighed and evaluated according to the following parameters: colour and texture of the intact organ, existence of lesions or any evidence of metastasis in the organ internally (after slicing). Laboratory analyses of liver functions (GOT and GPT levels in blood) included samples taken from 5 mice/group on day 5±1 and at the end of the study (day 16).

All animal procedures were approved by the "Animal Care and Use Committee" of Sheba Medical Center, Tel-Hashomer. The study was a placebo-controlled, blinded study. C57B16 mice received Lewis Lung Carcinoma cells (D122) by a subcutaneous injection to the left foot pad. When the tumor tissue reached a diameter of 7 mm (approximately 3 weeks after injection of cells), the foot-pad with the primary tumor was resected under anesthesia. This day was defined as day −5. Day 0 is the day of first dosing, 5 days after primary tumor resection. The mice were randomized to the various treatment groups on day 0, as described in the table below. The tested adenoviral vector or the control substance, were injected to the tail vein in a total volume of 100 μl per mouse. Carboplatin and Alimta were administrated IP in a total volume of 100 μl per mouse per daily dose.

The animals were followed for safety and efficacy (as listed in the schedule of evaluations below) throughout the study and until sacrifice time. The death of each animal during the study was recorded and an attempt was made to identify the cause of death. The day of sacrifice for each mouse was set as follows: when the 5th of the control mice (PBS 10% glycerol—Group 9) dies of metastasis, the number of days that passed from day 0 (the first IV administration of vehicle to that mouse) was determined. That day number was set as the day of sacrifice for all surviving mice (i.e., if the 5th control mouse died on its day 16, every mouse was sacrificed when it reaches its own day 16).

The evaluation of the effects was scheduled as shown in TABLE 7.

TABLE 7

Schedule of Evaluation							
		Study day number					
Parameter	Method	<−5	−5 Primary tumor resection	0	5 ± 1	Weekly thereafter ±1	End
Primary tumor width	Caliper	Starting 5 days after D122 injection once every 5 days until					

TABLE 7-continued

Schedule of Evaluation

Parameter	Method	Study day number <−5	−5 Primary tumor resection	0	5 ± 1	Weekly thereafter ±1	End
Body weight	—	✓	reaches 5 mm, then daily After resection baseline weight w/o foot	✓		✓	✓
Clinical signs	See below*	✓	✓	✓	✓	✓	✓
Blood chemistry (liver functions)**	SM 30-20-06				✓		✓
General health	SOP 10-25-01	✓	✓	✓	✓	✓	✓
Lung weight	Weight						✓
Major organs Assesment**	Visual & Staining of abnormal tissues						✓

*Skin, Fur, Eyes, Mucous membranes, Breathing, Neural control, Tremors, Salivation, Diarrhea, Somatomotor activity, Lethargy, Convulsions, Abnormal behavior patterns, Sleep, Coma.
**From 5 mice per group

Results

In mice groups treated with 10^9 and 10^{11} VPs VB-111 alone, all animals survived to day 16. Combination treatment revealed decrease in survival, while the lowest percentage of survival was seen in mice treated with high dose of chemotherapy as shown in TABLE 8.

TABLE 8

Mortality by Day 16.

Group	Total (n)	Survived (n)	Survival (%)
Vehicle	16	15	93.8%
VB-111 10^9 VP	15	15	100%
VB-111 10^{11} VP	15	15	100%
20 mg/kg Carboplatin + 10 mg/kg Alimta	17	16	94.1%
50 mg/kg Carboplatin + 30 mg/kg Alimta	14	7	50%
VB-111 10^9 VP + 20 mg/kg Carboplatin + 10 mg/kg Alimta	13	11	84.6%
VB-111 10^9 VP + 50 mg/kg Carboplatin + 30 mg/kg Alimta	14	9	64.3%
VB-111 10^{11} VP + 20 mg/kg Carboplatin + 10 mg/kg Alimta	15	12	80%
VB-111 10^{11} VP + 50 mg/kg Carboplatin + 30 mg/kg Alimta	15	14	93.3%

For evaluation of efficacy, lungs were weighed on the day of sacrifice. The mass of normal lungs is ~0.2 g. Tumor burden is defined as lungs mass minus 0.2 g, The average and median tumor burden is shown in TABLE 9.

TABLE 9

Average and Median Tumor Burden

Group number	treatment	Average Tumor burden (g) ± SD	Median (g)
1	VB111 10^{11}	0.10 ± 0.04	0.10
2	VB111 10^9	0.34 ± 0.21	0.40
8	high chemo	0.48 ± 0.15	0.50
5	low chemo	0.49 ± 0.34	0.50
6	VB111 10^{11} + high chemo	0.22 ± 0.16	0.20

TABLE 9-continued

Average and Median Tumor Burden

Group number	treatment	Average Tumor burden (g) ± SD	Median (g)
7	VB111 10^9 + high chemo	0.20 ± 0.24	0.08
3	VB111 10^{11} + low chemo	0.47 ± 0.26	0.46
4	VB111 10^9 + low chem	0.53 ± 0.30	0.62
9	Vehicle	1.00 ± 0.20	0.90

All treatments resulted with lower tumor burden compared to vehicle treatment as shown in FIG. **1**. FIG. **1** shows that treatment with E11, E9+high chemo and E11+high chemo all showed lower average tumor burden than other treatment groups.

Several statistical comparisons for efficacy were performed. When one way ANOVA method was used, there is a statistically significant higher tumor burden for the Vehicle treatment ($p=<0.001$) than for all other treatments except for the combination of VB E9+low chemo. Tumor burden was significantly lower for VB-111 E11 treatment compared to both chemo treatments and to both combination treatments with low chemo. When Mann-Whitney with individual comparisons between two groups was used, tumor burden in VB-111 E11 is significantly lower than in all groups except for E9+high chemo. E9+high chemo is significantly lower than all groups except for E11, E9 and E11+high chemo. FIG. **2** shows the box plot of the data.

Conclusion

VB-111 treatment in both doses resulted in 100% survival. Administration of low dosage of chemotherapy resulted in a mildly lowered survival rate (94.1%) which was similar to that seen with vehicle administration (93.8%). However, administration of high dosage of chemotherapy resulted in the lowest percentage of survival (50%). Combination treatments decreased survival compared to administration of VB-111 alone (64%-93%).

Organ weights (heart, kidneys and brain) were mostly not different among the different groups. Some differences between groups were seen in liver, spleen and testes weights, mainly higher weights for the single VB-111 higher dose, or combination with this dose.

No significant differences in liver function compared to vehicle treatment were observed. Generally, combination therapy did not result in higher toxicities than for the single therapies from which they are assembled.

The combination of VB111 10^9+high chemo treatment significantly improves the effect compared to high chemo treatment alone. Furthermore, it improves the average and median tumor burden compared to VB111 10^9 treatment alone (1.5 and 5 fold for average and median, respectively) although not statistically significant. This effect may exhibit statistical significance when applying the treatment on larger groups.

The combination of VB111 10^9+high chemo resembles the tumor burden reduction effect obtained with E11, as there is no significant difference between these two groups (can be seen in the box plot and the Mann-Whitney statistical comparison). This significant resemblance to VB111 10^{11} is not obtained by treatment with VB111 10^9 alone.

The combination of VB111 10^9+high chemo therapy may enable reducing VB-111 dose while preserving its high efficacy and improving the low efficacy of Carboplatin and Alimta chemotherapy alone.

Both survival rate and weight loss in the VB111 10^9+high chemo treatment group were better than for the high chemotherapy single treatment: survival rate—64% and 50%, respectively, weight loss—9.7% and 11.6%, respectively. No differences between these two groups were seen for any of the organ weights tested.

Example 3

Administration of Ad5PPE-1-3X-Fas Chimera in Combination with Paclitaxel

This will be a prospective, open label, dose escalating, multi-center (2 centers), Phase I/II study to determine the safety and efficacy of administration of AD5PPE-1-3X-Fas-chimera (VB-111) in the clinical setting, outcomes such as toxicity, adverse effects, antibody titer, biodistribution, disease progression and disease recurrence and survival will be monitored in subjects with solid primary and metastatic tumors (such as recurrent epithelial ovarian cancer, fallopian, primary peritoneal, MMMT and papillary serous müllerian tumors) receiving intravenous infusion of a range of doses of the Ad5PPE-1-3X-Fas chimera adenovirus vector in combination with paclitaxel.

The effect of VB-111 on the development of antibodies to the adenoviral vector, tumor response, and angiogenic biomarkers will also be evaluated.

Materials and Experimental Methods

Study Objectives

The research hypothesis is that VB-111 plus weekly paclitaxel will be associated with acceptable toxicity and response rate or clinical benefit sufficient to warrant future evaluation.

Primary Objectives

1. Define toxicities of a limited number of doses of combination VB-111 and weekly paclitaxel spanning anticipated effective doses.

2. Explore efficacy (RECIST response, CA-125 response and progression-free survival (PFS)) in an expanded cohort of the optimally tolerated dose of combination VB-111 and weekly paclitaxel.

Secondary Objective

Explore predictive markers of toxicity and response.

Overview of Study Design and Evaluation

This open-label, single center, Phase I/II trial combines VB-111 infused on day 1 every 2nd 28-day cycle with weekly paclitaxel 40-80 mg/m^2 infused over 1 hour on a 1, 8, 15 and 22 day schedule every 28 days in order to ascertain whether or not this combination can improve response rates compared to historical controls in this pretreated population. Patients may continue therapy with benefit and change to have scheduled breaks in paclitaxel dosing to avoid excess neuropathy but no interruption of VB-111 infusions. The trial is designed in a 2-stage Simon's design, based on ≥2 responses in 10 patients, further enrollment will proceed.

Subject Population:

Subjects with recurrent epithelial ovarian cancer, fallopian cancer, primary peritoneal, MMMT, and papillary serous müllerian tumors will be enrolled.

Number of Patients Planned to be Enrolled

Based on the study design it is estimated that 2 to 42 patients with advanced cancer are needed (max. 6 per cohort during dose escalation (=18 patients), and up to 29 patients on the MTD dose level to allow evaluation of the correlative/special studies as well as to confirm safety.

Selection of Patients

Phase I/II design to evaluate the response rates defined by RECIST criteria and CA-125 (Gynecological Cancer Intergroup (GCIG) criteria) and to describe the safety profile and characterize adverse events and toxicities. The Phase I study will enroll in a 3+3 design to 3 dose levels. The Phase II study will recruit up to 10 patients in the first stage, including participants in Dose Levels 2 and 3 who get full dose chemotherapy. If there are at least two responses, enrollment will continue with an additional 19 patients in an expansion cohort to a maximum of 29 participants. The efficacy analysis will be to estimate the response rate. Ongoing safety review will be conducted and if there are 2 or more Grade≥3 GI perforations in the first stage, or 3 at any time the study will be closed.

Criteria for Subject's Inclusion in the Treatment Group are:

1. Patients aged>18.

2. Histologically confirmed epithelial ovarian, peritoneal, or fallopian tube cancer, and uterine papillary serous carcinomas (UPSC), and gynecologic MMMTs.

3. Up to 3 previous lines of chemotherapy for metastatic disease are allowed.

4. Patients must have had prior platinum or platinum based therapy.

5. Eastern Cooperative Oncology Group (ECOG) status 0-1.

6. Platinum resistant or refractory disease defined as progressive disease by imaging or CA-125 within 6 months of completing or while receiving a platinum and taxane containing regimen (Primary OR secondary—i.e., patients can have received only one platinum based treatment for recurrent platinum sensitive disease with a subsequent platinum free interval<6 months).

7. Measurable or evaluable disease is required using RECIST or CA-125 (to standardized eligibility if some patients are eligible by both RECIST and CA-125, evaluation by RECIST takes precedent).

8. Adequate bone marrow function.

9. Adequate hematological functions:

i. ANC≥1000/mm^3

ii. PLT≥100,000/mm^3

iii. PT and PTT (seconds)<1.2×ULN (subjects with PTT>ULN must have a negative LAC)

10. Adequate organ function:

a. CTC neuropathy less than or equal to grade 1.

b. Prior radiation must not have included ≥30% of major bone marrow containing areas (pelvis, lumbar spine)

c. SGOT/SGPT/Alkaline Phosphatase≤2.5×ULN or <5×ULN for documented liver metastases

d. Bilirubin≤1.5×ULN

e. Creatinine≤1.5×ULN

f. Proteinuria<2+ by dip stick at screening or UPC (Urinary protein creatinine ratio<1.0).

11. Must have recovered from acute toxicity from prior treatment including radiation therapy, chemotherapy, biologic therapy with the exception of grade 1 neuropathy or any grade anemia and alopecia.

12. Prior treatment with an anti-angiogenic agent is NOT an exclusion criterion.

13. No prior GI perforation, or GI obstruction or involvement of the bowel on imaging

14. No active, untreated psychiatric disease or neurologic symptoms requiring treatment (Grade I sensory neuropathy allowed). No presence of untreated central nervous system or brain metastases. No dementia or significantly altered mental status that would prohibit the understanding and/or giving of informed consent.

15. No patients with known hypersensitivity to Cremophor® EL. However, participants are eligible if they have had a prior paclitaxel reaction, but subsequently tolerated the drug at rechallenge.

16. No evidence of uncontrolled bacterial, viral or fungal infections.

17. No patients receiving other investigational therapy for the past 30 days before dosing.

18. Must be competent to give informed consent.

Criteria for Subject's Exclusion from the Treatment Group Will be:

1. More than 3 prior lines of chemotherapy for recurrent cancer.

2. No active malignancy, other than superficial basal cell and superficial squamous cell, or carcinoma in situ of the cervix within last 2 years. Patient diagnosed with a concurrent müllerian tumor (typically endometrial cancer) are NOT excluded. Patients with a low risk (localized, non-inflammatory) breast cancer diagnosed within 2 years and treated with curative intent are NOT excluded.

3. Inability to comply with study and/or follow-up procedures.

4. Life expectancy of less than 3 months.

5. Common Toxicity Criteria (CTC) Grade 1 or greater neuropathy (motor or sensory) from comorbidity other than prior taxane exposure, such as diabetes.

6. Although rarely applicable, sexually active women of childbearing potential must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. All women of childbearing potential MUST have a negative pregnancy test within 14 days prior to first receiving investigational product. If the pregnancy test is positive, the patient must not receive investigational product and must not be enrolled in the study.

7. Inadequately controlled hypertension.

8. Prior history of hypertensive crisis or hypertensive encephalopathy.

9. New York Heart Association (NYHA) Grade II or greater congestive heart failure.

10. History of myocardial infarction or unstable angina within 6 months prior to study Day 1.

11. History of stroke or transient ischemic attack within 6 months prior to Day 1.

12. Known CNS disease, except for treated brain metastasis: Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 3 months prior to Day 1 will be excluded.

13. Significant vascular disease (e.g., aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day 1.

14. History of hemoptysis (>½ teaspoon of bright red blood per episode) within 1 month prior to Day 1.

15. Evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation).

16. Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 1 or anticipation of need for major surgical procedure during the course of the study.

17. Core biopsy or other minor surgical procedure, excluding placement of a vascular access device, within 7 days prior to Day 1.

18. History of abdominal fistula or gastrointestinal perforation within 6 months prior to Day 1.

19. Current signs and symptoms of bowel obstruction.

20. Current dependency on IV hydration or total parenteral nutrition (TPN).

21. Serious, non-healing wound, active ulcer, or untreated bone fracture.

22. Patients who received anti-angiogenic therapy within the previous 4 weeks for a TKI or 6 weeks for antibody or peptibody based therapy.

23. Patients with an ongoing requirement for an immunosuppressive treatment, including the use of cyclosporine, or with a history of chronic use of any such medication within the last 4 weeks before enrollment. A stable dose (e.g. started at least 2 weeks prior to dosing) of corticosteroids is allowed.

Composition: AD5-PPE-L-3X-Fas-Chimera

Ad5-PPE-1-3X-fas-chimera (SEQ ID NO: 19) is a vascular disruptive gene therapeutic, consisting of a non-replicating adenovirus vector (Ad5, E1 deleted) which contains a modified murine pre-proendothelin promoter (PPE-1-3x, SEQ ID NO:18) and a fas-chimera transgene [Fas and human tumor necrosis factor (TNF) receptor] (SEQ ID NO: 9). It is formulated as a sterile vector solution and supplied frozen (below −65° C.), in single use vials. Each vial contains 1.1 mL of vector solution at a specific viral titer.

Efficacy and Pharmacodynamics Objectives:

Treatment Plan

Patients will be treated with Ad5-PPE-1-3X-fas-chimera in combination with once-weekly paclitaxel. The cycle length will be 28 days. Patients will receive paclitaxel as a 60-minute IV infusion weekly (Days 1, 8, 15, and 22). Ad5-PPE-1-3X-fas-chimera will be administered as an IV infusion on Day 1 of odd cycles starting with Cycle 1 and occurring every 2 cycles thereafter. The treatment plan is shown in FIG. **3**. Dose levels for Phase I are shown below in TABLE 10.

TABLE 10

Dose Levels For Phase I[1] component:			
Dose Level	n	VB-111 × Q2 cycles[2]	Paclitaxel IV Q7d
1[3]	3-6	3×10^{12} VPs	40 mg/m^2
2	3-6[4]	3×10^{12} VPs	80 mg/m^2
3	3-6	1×10^{13} VPs	80 mg/m^2
Expansion cohort	29	MTD	MTD

Footnotes:
[1]The crossover to Phase II will occur after the maximum tolerated dose (MTD) has been defined or Dose Level 3 has been completed.
[2]Please note that total VB-111 viral particle dose for each Dose Level is different than shown for subjects who are <50 kg.
[3]Within subject dose escalation is planned for Dose Level 1. For participants enrolled to Dose Level 1 at Cycle 2 Day 1 the dose of paclitaxel may be escalated to 80 mg/m^2 given that this is the standard dose used clinically. If 2 participants experience dose limiting toxicities (DLTs) at Dose Level 1, the study will close without identifying an MTD.
[4]These numbers may reflect the inclusion of: (a) participants directly enrolled to Dose Level 2 and (b) Dose Level 1 participants who have been dose-escalated to Dose Level 2 at Cycle 2 Day 1 for evaluation of DLTs during Cycle 3.

Participants in the Phase I cohort will therefore be enrolled using a modified 3+3 design as shown in TABLE 11 and as described below.

Dose Escalation from Dose Level 1 to Dose Level 2: If 2 participants experience DLTs at Dose Level 1, the study will close without identifying an MTD. In Dose Level 1 there is a planned within-subject participant dose escalation to 80 mg/m2 paclitaxel at Cycle 2 Day 1, and these participants can then be counted towards the evaluation of participants enrolled to Dose Level 2, even though the concurrent VB-111 and paclitaxel dosing occurred at Cycle 3 in Dose Level 1 (i.e. both participants enrolling at Dose Level 2 and participants receiving Cycle 3 on Dose Level 1 with a paclitaxel dose escalation to 80 mg/m^2 will be evaluated such that at Dose Level 2 there may be 2 to 12 enrollees with up to 6 of them initially enrolled to Dose Level 1 and then dose escalated). If there is no DLT at Dose Level 2 after 3 participants have been evaluated (either as new enrollees to Dose Level 2 or as dose-escalating paclitaxel in Dose Level 1) the study will enroll to Dose Level 3. This plan only applies to the within subject dose escalation for paclitaxel (to minimize the number of participants getting a 40 mg/m^2 dose) and there is no within subject dose escalation of VB-111.

Dose Escalation from Dose Level 2 to Dose Level 3: Once 3 participants have been enrolled at Dose Level 2 and observed for 14 days following initial administration with VB-111 without DLT, subjects may be enrolled into Dose Level 3 (1×10^{13} VPs), as at least 6 participants will have been dosed at 3×10^{12} VPs in Dose Levels 1 and 2. The 2nd dose of VB-111 on Dose Level 3 may only be given after at least 2 patients have received a dose of 3×10^{12} VPs without DLT. Additionally, DLT monitoring will be performed simultaneously in parallel VB-111 Phase I/II studies. Safety findings from these studies will be shared with this team. If any DLT occurs at Dose Level 3, participants may continue on study and will receive further dosing with Dose Level 2 VB-111 (3×10^{12} VPs).

TABLE 11

Dose Escalation Decision Rules	
Number of Patients with DLT at a given Dose Level	Escalation Decision Rule
0 out of 3**	Proceed to next dose level: **Within subject dose escalation is not permitted unless it is anticipated that 3 participants will have been fully evaluated for DLTs over 28 days on Dose Level 1. If three participants enroll within 4 weeks of initial dosing on the study and do not experience DLTs, Dose Level 2 will open and enroll 3 participants concurrent to the three participants dose-escalating to Dose Level 2 at Week 5 (Cycle 2 Day 1) on study. So, it is therefore possible, if accrual is slow, that participants in Dose Level 1 will not be allowed to dose escalate at Week 5 (Cycle 2 Day 1) until the evaluation of DLTs on Dose Level 1 has been complete, and that this will be further delayed if DLTs are experienced in this cohort.
1 out of 3	Enter 3 additional patients: If 0 of these 3 additional participants experiences a DLT, proceed to the next dose level. If 1 or more of these participants experiences a DLT, then dose escalation is stopped and the dose below this dose is the MTD. If the extra participants were entered at Dose Level 1 the study will close without identifying an MTD.
≥2	Dose escalation will be stopped and the dose below this dose is the MTD. If 2 participants experience DLTs at Dose Level 1, the study will close without identifying an MTD.
≤1 out of 6 at highest administered dose	This is the MTD and recommended Phase II dose in this population.

Given the novel nature of the agent, new participants will be enrolled sequentially with at least 2 days between each new person starting on study. Multiple new study participants may not be dosed on the same day. A completion of one 28-day cycle of treatment will be the basis for determining the MTD and DLT's on each of the dosing cohorts. Toxicity grade will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4 (available as a downloadable file on the internet: ctep.info.nih.gov).

Definition of Dose Limiting Toxicity (DLT)

Assessment of potential DLTs will occur during the first cycle (Cycle 1) and also during the third cycle for DL1 participants.

Toxicities will be assessed by the CTCAE, version 4.0. Dose-limiting toxicity is defined as follows:

1. Absolute neutrophil count of $<0.5\times10^9$/L lasting for ≥4 days or an absolute neutrophil count$<0.5\times10^9$/L with sepsis, or grade 3-4 fever (>100.2° F.) which is not readily controlled with anti-pyretic medication.

2. Platelet count$<10\times10^9$/L for any duration.

3. Any other drug-related non hepatic and non-hematologic grade≥3 toxicity. This does not include grade≥3 nausea or vomiting that can be controlled medically (if nausea and/or vomiting cannot be controlled medically and occurs during the first cycle, it will be considered a DLT) or grade≥3 hypokalemia, hyponatremia, hypophosphatemia, hypomagnesemia, and hypocalcemia if they can be easily corrected, are clinically asymptomatic, and not accompanied by medically significant complications (e.g., ECG changes).

Events of Grade 3-4 fever that occur within 24 hours post-dosing with VB-111 shall not be considered DLT if they

respond to symptomatic therapy. Patients with either unacceptable toxicities and/or progression at any time point will be removed from the study.

Study Procedures

Pre-Treatment Evaluation:

A Clinician (MD or NP) will evaluate patients meeting the eligibility criteria. A history, physical examination and recording of the weight and vital signs and ECG recording will be performed within 14 days before commencing the first cycle of treatment. Investigations to establish baseline measurements, where applicable, should be done within 28 days before commencing the first cycle of treatment.

Prior to any study-dosing, on each cycle's Day 1, the eligibility of the subjects must be reconfirmed.

The following evaluations should be done within 3 days of D1 (Except for cycle 1, when blood tests can be checked within 14 days of day 1):

1. Clinical evaluation: Medical History, Physical exam, Vital Signs (VS) and check for risk of bleeding. At other times VS evaluation can be performed per institutional standard of care.

2. Hematology: complete blood count with differential, INR and activated PTT.

3. Coagulation: In case of partial thromboplastin time (PTT) prolongation above upper limit of normal (ULN), blood should be drawn for lupus anticoagulant (LAC). Patients with prolonged aPTT should not receive VB-111 until aPTT normalization. In patients who tested positive for lupus anticoagulant (LAC), a negative test is required prior to repeat dose of VB-111. For a persistent positive LAC or antiphospholipid antibodies (APLA) test, the test must be repeated within 12 weeks from the initial positive test.

4. Comprehensive metabolic panel: including electrolytes, liver function tests (LFTS), blood urea nitrogen (BUN)/creatinine (Cr), calcium, and magnesium.

5. Urine: collected for routine analysis.

6. VB-111 specific labs: Blood will be drawn for:

(i) Biodistribution: VB-111 Adenovirus DNA levels and transgene expression determination.

(ii) Biomarkers: Biomarkers for anti-angiogenic therapy will be tested in peripheral blood samples obtained from all patients enrolled in this study. Plasma analysis will be carried out for circulating angiogenic and inflammatory biomarkers VEGF, P1GF, sVEGFR1, bFGF, IL-1β, IL-6, IL-8, and TNF-α (using multiplex ELISA plates from Meso-Scale Discovery) and sVEGFR2 and SDF1α (using R&D Systems kits). Blood-circulating cells will be enumerated in fresh samples using a standard flow cytometry protocol. Archival tissue will be evaluated for CD31, CD34, VEGFR2, and vWF.

(iii) Tumor marker: CA-125

(iv) Antibodies: Levels of antibodies to AD-5 virus (including neutralizing antibodies).

Study Treatment Infusion:

ANC must be ≥1,000/mm^3 and Platelet count≥100,000/mm^3 prior to study treatment infusion (paclitaxel or VB-111).

Paclitaxel Infusion:

Anti-emetic therapy: Anti-emetic therapy may include Dexamethasone 10 mg i.v. prn, Ativan 0.5-2.0 mg i.v., and/or Compazine 10 mg p.o., or Perphenazine 4 mg p.o., as per institutional standards.

The administration sequence will be paclitaxel followed by VB-111.

Paclitaxel will be administered weekly at an initial dose of 40-80 mg/m^2 through an in-line filter with a microporous membrane not greater than 0.22 microns over 60 minutes.

All patients must receive premedication before the administration of paclitaxel in order to prevent severe hypersensitivity reactions. Patients will take oral dexamethasone 20 mg the night before treatment and 20 mg the morning of treatment for the first dose, and then if paclitaxel is well tolerated, for week 2 no oral, and only IV premedication, and the dose may be weaned off and no longer used after week 3. A typical premedication regimen consists of the following given 30-60 minutes prior to paclitaxel: 10-20 mg intravenous (IV) dexamethasone, 50 mg IV diphenhydramine (or its equivalent), and 300 mg cimetidine or 50 mg IV ranitidine. Famotidine 20 mg IV can be substituted as an alternative per local formulary. The dexamethasone dose may be increased at the investigator's discretion if a patient experiences a hypersensitivity reaction when given paclitaxel.

Day 1 of Odd Cycles:

In addition to the above mentioned procedures, on D1 of odd cycles, the following procedures will be performed.

Tumor Measurements:

Prior to D1 of cycle 1 (up to 1 month prior is allowed) and then in every odd cycle (e.g. e.g. cycles 1, 3, 5 etc=every 8 weeks) until disease progression, tumor measures with CT will be performed on the chest, abdomen, and pelvis. The assessment will be performed prior to drug administration, up to 3 days before dosing.

Study Treatment Infusion: VB-111

VB-111 will be administered on Day 1 of each odd cycle until disease progression.

VB-111 should be administered after paclitaxel. This is based on the paradigm that the investigative agent should be given last as a safety precaution. There is no anticipation that there will be sequence dependent alteration in pharmacology of the two agents. Although this is anticipated to be immediately (within 1 hour) after paclitaxel it may be administered later (within 24 hours) if clinically indicated.

Patients with prolonged aPTT should not receive VB-111 until aPTT normalization. In patients who tested positive for LAC and/or APLA, a negative test is required prior to repeat dose of VB-111. While test remains positive, further VB-111 should not be administered. For a persistent positive LAC or APLA test, the test must be repeated within 12 weeks from the initial positive test.

Antipyretic Treatment: 1000 mg of acetaminophen shall be administered prior to VB-111 dosing and PRN acetaminophen post-dosing. In patients who develop a grade 3 fever, or at investigator's discretion, i.v. dexamethasone 10 mg may be administered 10 minutes prior to dosing in subsequent VB-111 doses.

VB-111 preparation and infusion: Upon completing the infusion of paclitaxel, VB-111 will be administered as a single dose of 3×10^{12} VPs (Dose Levels 1-2) or 1×10^{13} VPs (Dose Level 3) to patients who are fasting or have had only a lighter blander meal. Please note: fasting is suggested to avoid vomiting if the patient has significant chills rather than the usual low-grade and self-limiting fever. It is not an absolute requirement. The final solution for administration should be administrated not more than 60 minutes after preparation. A regular meal will be allowed 0.5 hour after dosing. A single intravenous infusion of diluted VB-111 should be administered at the following rates:

1. For dose 3×10^{12} VPs: 1 mL/minute
2. For dose 1×10^{13} VPs: 1 mL/minute for the first 10 mL, and then 3 mL/minute for the remainder of the infusion.

Observation Post-VB-111 Administration

Study participants should be observed in the clinic or infusion area for the first 8 hours after the first administration of study drug, and subsequently as clinically indicated.

VB-111 Distribution Labs

Blood and urine samples will be collected for VB-111 adenovirus DNA levels expression determinations at the following time points:

Blood:

1. 0 (Prior to dosing)
2. At the end of infusion
3. 3±0.5 hours
4. 6±0.5 hours

Vital Signs:

Vital signs (systolic and diastolic blood pressure, peripheral heart rate, body temperature, respiration rate) will be recorded:

1. 0 minutes (just prior to dosing) (−15 minute range)
2. 30 minutes after start of dosing (+/−15 minute range)
3. 60 minutes after start of dosing (+/−15 minute range)
4. 4 hours post start of dosing (+/−15 minute range)
5. 6 hours post start of dosing (+/−15 minute range)
6. On the occasion of any adverse event

Days 8, 15, and 22 of Each Cycle

Each patient will be required to return to the clinic at a fasting state, for the following evaluations:

1. Vital signs: supine systolic and diastolic blood pressure, peripheral heart rate, body temperature, respiration rate.
2. Hematology: complete blood count with differential, INR and activated PTT.
3. Coagulation: if necessary to follow up on abnormal PTT levels.
4. Urine: collected for routine analysis

Blood labs will be drawn on day 8 of cycles 1-6. Blood will be drawn for:

1. Biodistribution: VB-111 Adenovirus DNA levels and transgene expression determination (every 2nd cycle following VB-111 infusion)
2. Biomarkers: Plasma analysis will be carried out for circulating angiogenic and inflammatory biomarkers VEGF, P1GF, sVEGFR1, bFGF, IL-1β, IL-6, IL-8, and TNF-α (using multiplex ELISA plates from Meso-Scale Discovery) and sVEGFR2 and SDF1α (using R&D Systems kits). Blood-circulating cells will be enumerated in fresh samples using a standard flow cytometry protocol. Archival tissue will be evaluated for CD31, CD34, VEGFR2, and vWF.
3. Tumor marker: CA-125
4. Antibodies: Levels of antibodies to the virus (including neutralizing antibodies)

NOTE: Comprehensive metabolic panel including electrolytes, LFTS, BUN/Cr, calcium, and magnesium is only required on D1, with only LFTs on Days 1, 8, 15, 22.

Paclitaxel will be administered as detailed above. ANC must be (>1,000/mm^3 and platelet count>100,000/mm^3

Anti-emetic therapy: Anti-emetic therapy may include Kytril 750 μg i.v. or 1 mg p.o, or Zofran 10 mg i.v. or 8 mg p.o., with Dexamethasone 10 mg i.v. as well as prn Ativan 0.5-2.0 mg i.v., and/or Compazine 10 mg p.o., or Perphenazine 4 mg p.o. as per institutional standards.

Study Completion Visit

Subjects will be dosed with the combination therapy (paclitaxel and VB-111) according to the cycle schedule described above until disease progression.

Following the final dose with VB-111, each patient will be required to return to the clinic for a final follow up visit, with the same clinical evaluation and laboratory samples drawn routinely and in response to a clinically significant event which will be documented as unscheduled laboratory evaluations. Adverse event and concomitant medications should be recorded in the same fashion as earlier in the study. The subject will undergo Physical Examination that will be carried out by the treating clinician (MD or NP). An ECG will be performed.

Patients who discontinued the study or experience disease progression will be followed up by clinic or telephone contact to evaluate for survival. However, following disease progression, data describing the CA125 and RECIST will be maintained in the clinical research files.

Additional Procedures

Adverse Events

Full supportive measures should be employed for all patients with any adverse event. All adverse events occurring following drug administration will be documented in the case report forms (CRFs), together with the intensity, the therapeutic measures applied, the outcome and an evaluation of the relationship to the investigational drug. Related adverse events will be followed through resolution. Unrelated adverse events will be followed through resolution or end of study.

Common side effects include nausea, vomiting, and loss of appetite. The patient may also experience constipation, loose stools or diarrhea. It is important to increase fluid intake if diarrhea occurs. If this becomes severe, the patient may have to be hospitalized and receive intravenous fluids. The administration of any investigational product involves a general risk of side effects. Since VB-111 is an investigational product, not all of the potential side effects in humans are known. VB-111 may cause all, some, or none of the side effects listed below. Side effects are undesirable medical conditions or a worsening of a pre-existing medical condition that may occur while you are in a study. In addition to the possible adverse effects listed below unexpected or uncommon side effects, which could be serious or life threatening, may occur when VB-111 is given alone or when it is combined with other medications. One of the purposes of this study is to investigate the possible side effects of VB-111. The study doctor will monitor the patient closely during this study and discuss with the patient any questions regarding risks, discomforts, and adverse effects.

Risks Associated with VB-111

Likely (More than a 50% chance that this will happen)

Flu-like symptoms such as fever, muscle aches, fatigue, chills,

Nausea

Vomiting

Occasional (Between a 1-10% chance that this will happen)

Constipation

Abnormally high levels of enzymes produced by the liver meaning that your liver is not functioning properly and can cause fatigue and jaundice (yellowing of the skin and eyes). Although this is usually mild and reversible, this can be serious or life threatening

Increase in the size of the spleen usually doesn't cause any symptoms but may cause stomach pain if the spleen ruptures, and could be life threatening.

Increase in the making of new blood cells in the bone marrow that may result in increased blood cell counts but without an increased risk of leukemia

An allergic reaction at the site where an injection (shot) was given, which may cause some redness and swelling

Bleeding

Abnormally prolonged coagulation tests (PTT) and development of certain types of antibodies (antiphospholipid antibodies) that may interfere with the normal blood clotting. This may result in thrombosis (blood clots) or bleeding, which may require treatment and may be serious or life threatening.

The ability for your wounds to heal might be affected. This can lead to infections and may require hospitalization.
High blood pressure
Excess protein in the urine. This is usually an asymptomatic lab finding but, if excessive, may cause fluid retention, such as swelling of the legs.
Headache
Loss of appetite and weight loss (anorexia)
Decreased levels of sodium in the blood, which can cause confusion, seizures, fatigue and low levels of consciousness.
Excess sugar in the blood, if severe may require hospitalization and urgent treatment.
Increased sweating (hyperhidrosis)
Rare (Less than a 1% chance that this will happen)
Low number of red blood cells that can causes tiredness and shortness of breath. This may require a blood transfusion.
Low number of platelets, which may cause bleeding and bruising. Bleeding may be serious or life threatening and may required a blood transfusion.
Mild increase in white blood cells (may increase the risk of infection)
Severe bleeding
The function of the kidneys may deteriorate (Acute Renal Failure) which may require treatment and may be serious or life threatening.
Cerebral Edema may develop in patients with tumor involving the brain (for example, brain metastasis)
Diarrhea
Cardiac (heart) and blood vessel complications: heart attack, angina (chest pain), or blood clots (thrombosis) that could occlude blood supply to vital organs and result in stroke or damage to other organs.
Congestive Cardiac Failure—The heart is not able to pump blood properly, which can cause weakness and tiredness, fluid retention, and fluid build-up in the lungs, which can cause shortness of breath. This may be serious or life threatening
Risks Associated with Paclitaxel
Likely (Chance of more than 50% that this will happen)
Mild to severe allergic reaction which may be life-threatening
Numbness and pain of the hands and feet that sometimes worsens with additional treatment and may not disappear after the drug is stopped
Hair loss
Muscle and joint aches
Fatigue
Frequent (Chance of 10-50% that this will happen)
Nausea and/or vomiting
Diarrhea
Sores in the mouth or throat
Lightheadedness
Headaches
Liver damage
Skin irritation and swelling if the drug leaks from the vein into which it is being injected into the surrounding skin
Taste changes
Irritation of the skin at a site of previous radiation
Rash
Occasional (Chance of 1-10% that this will happen)
Inflammation of the colon which may cause a change in your bowel movements
Inflammation of the pancreas which may cause abdominal pain that only lasts for a short time
A sensation of flashing lights or spots
Kidney damage
Increased blood level of a form of fat called triglyceride (hypertriglyceridemia)
A slowing of the heart rate
Irregular heartbeats
Rare (Chance of less than 1% that this will happen)
Liver failure
Seizures
Confusion; mood changes

Risks Associated with Biopsies:

Biopsies are normally performed under the guidance of an imaging technique. Each procedure requires a separate consent prior to the biopsy. The risks may include:

Pain and discomfort. The amount of pain and discomfort will vary, depending on the location of the biopsy site. These risks can be discussed with the study doctor.
Minor bleeding at the biopsy site.
Tenderness at the biopsy site.
Scarring at the biopsy site.
Rarely, an infection at the biopsy site.

Uncommonly, complications from biopsies can be life threatening. As with any interventional procedure, other potentially serious complications from bleeding or organ damage may occur. These might require additional surgical intervention.

Risks Associated with Radiological Scans and X-Rays:

While the patient is in the research study, CT scans may be used to evaluate the disease. The frequency of these exams is standard care. In the long term, over many years, there is a very low risk of developing a new cancer as a result of the radiological evaluation and treatment for the cancer. Certain types of drugs or combinations of these drugs with radiation may further slightly increase the risk of developing a new cancer.

There is a small risk with using the contrast agent that is injected into a vein during the scan. It may worsen kidney function in people who already have decreased kidney function. Therefore, kidney function will be closely monitored during participation in this study. If there is any change in the patient's kidney function, the patient may have to be removed from the study.

Uncommonly, some people have allergic reactions (such as hives and itching) to the contrast agent. Serious reactions (for example, drop in blood pressure, difficulty breathing or severe allergic reaction and death) are rare.

Reproductive Risks:

The drugs used in this research study may affect a fetus. While participating in this research study, the patient should not become pregnant, and should not nurse a baby. The patient should let the doctor know immediately if she becomes pregnant. Counseling about preventing pregnancy for either male or female study participants will be provided.

Concomitant Medications

There is no restriction on concomitant medication, besides the drugs listed in the exclusion criteria. However, VB-111 should not be mixed with other drugs. All concomitant medication administered during the study will be documented from baseline until a 1 month follow-up visit following the final dose. Routine drugs will be recorded with product name, indication, dosage, units, frequency, start and stop dates. Any continuing concomitant medication will be recorded as such, and does not need to reappear unless a stop date is noted prior to study end.

Antiemetic Regimens

It is anticipated that nausea should be a mild side effect. The following representative antiemetic regimens are suggested: Dexamethasone 4-8 mg PO, or Lorazepam 0.5-1.0

mg, or Prochlorperazine 10 mg PO, or Metoclopramide 10-20 mg, 30 minutes prior to administration of chemotherapy.

Aspirin

Low-dose aspirin (≤325 mg/d) may be commenced or continued in subjects at higher risk for arterial thromboembolic disease. Subjects developing signs of arterial ischemia or bleeding on study should be evaluated for drug discontinuation.

Anti-Pyretic Treatment

VB-111 administration is associated with self-limited fever usually lasting 24 hours post dosing, in some cases a low grade fever may extend for up to 2-3 days post dosing. 1000 mg of acetaminophen shall be administered 1-2 hours prior to VB-111 dosing and PRN acetaminophen post-dosing. In patients who develop a grade 3 fever, or at investigator's discretion, IV Dexamethasone 10 mg may be administered 10 minutes prior to dosing in subsequent VB-111 doses.

Other Laboratory Analyses

Laboratory samples drawn in response to a clinically significant event will be documented as unscheduled laboratory evaluations. In the event of clinically relevant abnormal laboratory values, the tests will be followed-up until the values have returned to within normal range and/or an adequate explanation of the abnormality is found. All such laboratory investigations will be performed at the study site, except for distribution assessments, which will be sent to an Independent Central Laboratory. Should any of these results require confirmation, re-testing will be performed in the same hospital laboratory where possible. Laboratory accreditation certificates and normal reference ranges must be provided for each hospital laboratory. If safety labs are drawn at local laboratories, all efforts should be made to obtain lab accreditation certificates and normal reference ranges for these labs.

ECG will be performed at screening visit and every 6 months or at early withdrawal visit.

Study Duration

Each patient will be administered with paclitaxel weekly and VB-111 every second cycle. Patients will participate in this study until disease progression and then only their files will be made available for collection of CA125 and RECIST details.

Patients can decide to withdraw from study at any time. Patients who withdraw should still be contacted and questioned about adverse effects (AEs). All AEs, irrespective of relatedness to drug or disease will be documented in the patient's record and CRF. Based on the toxicity profile from pre-clinical studies completed so far, as well as the specificity of expression of the transgene, one year is considered adequate time to identify any longer term toxicities that may emerge after treatment. Patients who have withdrawn or experience disease progression should be contacted approximately every 2 months for survival data.

Dose Limiting Toxicity (Cycle #1, 2 and 3 Only)

Dose limiting toxicities (DLTs) will be defined in the first cycle of the protocol and all patients triggering the DLT definitions during these cycles will be removed from protocol. No dose reductions will be allowed during cycle 1. DLT is defined as >4 days of grade IV neutropenia (ANC<500/mm^3), fever and neutropenia (defined as a temperature>100.2° F. and ANC<500/mm^3), grade IV thrombocytopenia (platelets<25,000/mm^3) and grade≥III non-hematological toxicity, excluding nausea and grade III vomiting.

Events of Grade 3-4 fever that occur within 24 hours post-dosing with VB-111 shall not be considered DLT if they respond to symptomatic therapy.

Given that paclitaxel 80 mg/m^2 is the clinically accepted dose for the weekly schedule, there is no plan to escalate above this dose.

Dose Modifications—Paclitaxel

No dose modification may be made during the first cycle of the dose escalation phase of the study.

In patients with symptomatic neuropathy a 'break week' is allowed after discussion with the study PI. This is anticipated to allow a dosing schedule of 3 out of 4 weeks, or 7 out of 8 weeks if on alternate cycles. If dose density cannot be maintained at one of these schedules the patient should be removed from study. If a dose modification is made to the Paclitaxel Schedule, the VB-111 dosing will therefore not need to be modified to follow the Paclitaxel cycles.

Participants may stay on study with interruptions in dosing for up to 3 weeks to allow recovery of toxicity, or social commitments.

Note: After day 28, once a patient is dose reduced, dose escalation is not permitted and the patient should continue on this dose for all subsequent cycles.

Dose adjustments should be made according to the system showing the greatest toxicity graded by the Common Terminology Criteria for Adverse Events (CTCAE-4).

In general dose modifications for hematologic toxicity will be based on blood counts obtained within 72 hours of the time of retreatment unless specifically stated otherwise. Dosing of paclitaxel will be determined based on ANC (Table 12), platelet count (Table 13), and neuropathy (Table 14).

TABLE 12

Neutropenia Pre-Weekly Dose

ANC/Drug	Paclitaxel
≥1000/mm^3	Full dose
<1000/mm^3	Hold drug
Further incidence if ANC <1000/mm^3	Dose reduce to 60 mg/m^2

TABLE 13

Thrombocytopenia Pre-Weekly Dose

Platelets/Drug	Paclitaxel
≥100,000/mm^3 or first incidence of <100,000/mm^3 and >75,000/mm^3	Full dose
<75,000/mm^3	Hold drug
Repeat incidence of platelets <100,000/mm^3 and >75,000/mm^3	Dose reduce to 60 g/m^2

TABLE 14

Sensory Neuropathy Dose Reduction Schema

Neuropathy Grade >2	
First incidence	1st dose reduction - Paclitaxel 60 mg/m^2
Second incidence (or persistence despite dose reduction)	2nd dose reduction - Paclitaxel 40 mg/m^2
Third incidence (or persistence despite dose reduction)	Off study

Once paclitaxel dose is reduced, it should not be re-escalated.

Management of Prolonged Myelosuppression

If platelets or neutrophils have failed to recover by day 28, an additional 7 days will be allowed for hematologic recovery. Patients with incomplete recovery (ANC<1,000/mm^3 or Platelets<100,000/mm^3) at 35 days will be removed from protocol.

Management of Fever and Neutropenia

Patients will be managed in the usual fashion for fever and neutropenia. Patients will need to recover from fever and active infectious issues prior to resuming therapy. Patients who are not on growth factors will have Neupogen or Neulasta added to their next cycle. Patients on Neupogen 5 ug/kg/day will have their dose escalated to 10 ug/kg on the same schedule.

Additional Toxicities and Dose Adjustments

Other toxicities are not anticipated but are possible on this trial. Patients developing drug associated grade III or IV non-hematological or non-mucosal toxicities that do not resolve or improve to grade I within 7 days of the next cycle will be removed from protocol. Patients developing serious grade III or IV non-hematological/non mucosal toxicities that resolve or improve to grade I by within one week of the start of the next cycle may continue on trial if the patient is responding to therapy and the patient toxicities have been reviewed with the principal investigator.

Dose Reductions for Abnormal Liver Function Tests are as Follows:

Paclitaxel is not known to cause hepatic toxicity; however, its elimination is delayed in patients with severe hepatic dysfunction. Therefore, the dose of paclitaxel in patients with hepatic dysfunction should be modified according to Table 15.

TABLE 15

Paclitaxel Dosing Based on Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Levels

Serum AST, or ALT	Paclitaxel
<3x ULN	80 mg/m^2
≥3x ULN	60 mg/m^2
≥5x ULN	Hold drug

Patients with elevated bilirubin>1.5 mg/dl should not receive paclitaxel until the abnormal laboratory values improve to <grade 1. Treatment should be resumed after recovery with paclitaxel at one dose level lower, per the above table, and toxicity that lasts more than 2 weeks, will removed from study. Once a patient is dose reduced, dose escalation is not permitted and the patient should continue on this dose for all subsequent cycles.

Patients with prolonged aPTT should not receive VB-111 until aPTT normalization. In patients who tested positive for lupus anticoagulant (LAC) and/or antiphospholipid antibodies (APLA), a negative test is required prior to repeat dose of VB-111. While test remains positive, further VB-111 should not be administered. For a persistent positive LAC or APLA test, the test must be repeated within 12 weeks from the initial positive test.

Patients developing serious toxicities thought secondary to their ovarian carcinoma (i.e. bowel obstruction) will also be removed from protocol. In the unusual circumstance where a reversible grade 3-4 toxicity is secondary to carcinoma and the patient is felt to be benefiting from chemotherapy the treating physician should review with the study P.I. for potential continuation on the protocol once the toxicity has resolved.

The next cycle of treatment can only commence if all non-hematological toxicity has resolved to grade I or better.

Up to two dose delays, each of a week are allowed. If there is persistent toxicity the patient will be taken off study.

Participant Safety

A number of measures will be taken to ensure the safety of patients participating in this trial. These measures will be addressed through exclusion criteria and routine monitoring as follows.

Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study. Safety evaluations will consist of medical interviews, recording of adverse events, physical examinations, blood pressure, and laboratory measurements (performed by local laboratories). Patients will be evaluated for adverse events (all grades), serious adverse events, and adverse events requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study. Patients discontinued from the treatment phase of the study for any reason will be evaluated ~30 days (28-42 days) after the decision to discontinue treatment.

If patients on treatment require elective major surgery, it is recommended that therapy be held for 2-4 weeks prior to the surgical procedure.

Re-Treatment Criteria

Dose adjustments at the start of a cycle should be based on nonhematologic toxicity or blood counts at that time. Patients should not begin a new cycle of treatment unless the absolute neutrophil count is >1,000 cells/mm^3, the platelet count is >100,000 cells/mm^3, and nonhematologic toxicities have improved to grade 1 (mild) or resolved.

Patients may be delayed for up to three weeks before re-treatment. If toxicity recovery takes more than three weeks they should be removed from study.

Treatment can continue in the setting of grade 2 fatigue, diarrhea, alopecia and nausea, if the toxicity: benefit trade-off is felt to be beneficial to participant and clinician.

Paclitaxel Formulation, Supply, Storage and Stability

Paclitaxel (NSC #673089)

Paclitaxel 40-80 mg/m2 will be infused IV over 1 hour day 1, 8, 15 and 22 with a cycle being 28 days. Drug will be dosed to a maximum body surface area (BSA) of 2.0 m^2, and BSA will be calculated from that day's weight.

Formulation

Paclitaxel is supplied as a 6 mg/mL non-aqueous solution in multi dose vials containing 30 mg/5 mL, 100 mg/16.7 mL, or 300 mg/50 mL of paclitaxel. In addition to 6 mg of paclitaxel, each mL of sterile non-pyrogenic solution contains 527 mg of purified Cremophor® EL (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.

Storage

Unopened vials of paclitaxel are stable to the date indicated on the package when stored between 20 to 25° C. (68 to 77° F.). Protect from light.

Preparation

Paclitaxel will be diluted prior to infusion. Paclitaxel will be diluted in 0.9% Sodium Chloride for Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. The solutions are physically and chemically stable for up to 27 hours at ambient temperature (approximately 25° C./77° F.) and room lighting conditions. In order to minimize patient exposure to the plasticizer DEHP, which may be leached from PVC infusion bags or sets, diluted paclitaxel solutions should be stored in bottles (glass, polypropylene) or plastic (polypro-

pylene, polyolefin) bags and administered through polyethylene-lined administration sets.

Administration

Paclitaxel will be administered through an inline filter with a microporous membrane not greater than 0.22 microns. Use of filter devices such as IVEX-2® or IVEX-HP®, which incorporate short inlet and outlet PVC-coated tubing, has not resulted in significant leaching of DEHP.

Premedication

All patients will be premedicated with corticosteroids, diphenhydramine, and H2 antagonists prior to the first paclitaxel administration in order to prevent severe hypersensitivity reactions. Patients who experience severe hypersensitivity reactions to drug may need to repeat the premedication and to be rechallenged with a dilute solution and slow infusion. For severe hypersensitivity reactions to paclitaxel do not have to proceed with are challenge. When the drug is well tolerated, or tolerance to cemophor is established, the oral dexamethasone may be progressively withdrawn. Docetaxel may not be substituted.

Adverse Effects: Consult the package insert for the most current and complete information.

Supplier: Commercially available both from Bristol-Myers Squibb Oncology as well as generic manufacturers. Consult the American Hospital Formulary Service Drug Information guide, Facts and Comparisons, or the package insert for additional information.

VB-111

Description

Study drug vials will be supplied for each subject dose in labeled 1.8 ml cryovials (polypropylene). Each cryovial contains a volume of 1.1 ml (10^{12} vp/ml).

Formulation

VB-111 is a formulated as a sterile vector solution. The solution is supplied frozen (below 65° C.), in single use, plastic screw vials. Each vial contains 1.1 mL of vector at a viral titer of 10^{12} vp/ml and vehicle (10% glycerol in Phosphate Buffered Saline). The vector solution should be thawed and maintained at 2-8° C. during dilution and handling.

Stability and Storage

Stability studies of VB-111 are ongoing and to date support a shelf-life of 30 months below 65° C. Open and/or diluted vials SHOULD NOT BE RE-USED. VB-111 vials should be stored in closed vials frozen (below 65° C.), protected from light.

Preparation

VB-111 will be prepared as shown in Table 16.

The entire process of drug preparation shall be carried out at room temperature in the BSC type II room. After thawing, the drug may be maintained up to 3 hours in ice water during preparations.

The drug is diluted in room temperature saline.

The preparation of the drug and drug injection shall be completed in the shortest time possible, not to exceed 1 hour.

The pharmacist preparing the drug shall verify that the information on the container is appropriate for the study and for the subject: product name, concentration, batch number.

Place volume needed of saline (brought to room temperature) in a sterile plastic tube. Thaw the vials of VB-111 solution by rubbing between the gloved hands. Immediately, pull 1 ml of VB-111 from each of the cryo vial intended for the specific subject. Use a new syringe for each 1 ml of VB-111. Add VB-111 to the plastic tube containing the saline solution prepared in advance. Mix the diluted drug by swirling the contents by hand. Determine the volume to be applied according to the patient's weight and draw the required volume for injection into the syringe for administration (see Table 16). After completing the preparation, perform a reconciliation process: check that the correct number of source vials was used and that the volume left in the tubes is approximately as expected and complete the drug accountability log. After preparation of the drug solution, clean the drug formulation area in the pharmacy according to the pharmacy procedures.

Infusion

1. A single intravenous infusion of the diluted VB-111 should be administered via a syringe pump according to the instructions below:

   a. Dose Levels 1-2: 3×100^{12} VPs (15 or 10.5 ml depending on the subject's weight) should be administered at a rate of 1 ml/minute

   b. Dose Level 3: 1×10^{13} VPs (50 or 35 ml depending on the subject's weight)

      i. Infusion should be administered at the following rate:

         1. 1 ml/minute for the first 10 minutes

         2. 3 ml/minute for the remaining volume of infusion

A regular meal may be provided to the subject 30 minutes after completion of dosing.

Subject Discontinuation

Subjects who meet the following criteria should be discontinued from study treatment:

1. Grade 3 neuropathy lasing>7 days
2. Grade 4 hypertension or Grade 3 hypertension not controlled with medication
3. Nephrotic syndrome

TABLE 16

VB-111 Preparation

Dose	Concentration in vial (VP/ml)	Volume of VB 111 in tube	# Vials of VB 111	Take this volume of VB-111 (ml)	Syringe type for VB-111	Volume of saline	Syringe type for saline	Total volume prepared	Volume to inject Subject Weight ≥50 Kg	Volume to inject Subject Weight <50 Kg
3×10^{12} VPs Dose Levels 1 + 2	10^{12}	1.1 ml	3	3 ml 3 × 1 ml	3 ml	12 ml (2 × 6 ml)	2 × 10 ml	15 ml	Entire volume (15 ml)	10.5 ml
1×10^{13} VPs Dose Level 3	10^{12}	1.1 ml	10	10 × 1 ml	10 ml	40 ml	*	50 ml	Entire volume (50 ml)	35 ml

* The pharmacy can either use a sterile empty bag and individually add 40 ml NS + 10 ml VB-111 to the bag, or the pharmacy can use a 50 ml bag of NS and remove the excess volume then add the VB-111. Either way is an acceptable pharmacy practice.

4. Grade≥2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
5. Any grade arterial thromboembolic event
6. Grade 4 congestive heart failure
7. Gastrointestinal perforation
8. Any grade fistula
9. Any grade bowel obstruction.
10. Wound dehiscence requiring medical or surgical intervention
11. Unwillingness or inability of subject to comply with study requirements
12. Determination by the investigator that it is no longer safe for the subject to continue therapy
13. All Grade 4 events thought to be related to study treatment by the investigator

Contraindications

Study medication is contraindicated in patients who have a known, prior, severe (NCI CTC Grade 3/Grade 4) history of hypersensitivity reaction to a drug formulated in Cremophor® EL (polyoxyethylated castor oil).

Clinical Tests and Procedures

A series of clinical tests and procedures will be performed at specified intervals throughout the study according to Table 17.

TABLE 17

Clinical Tests and Procedures

Assessment	Screening Baseline[a]	Treatment: D1 of each cycle	Treatment: Days 8, 15, 22	Follow-Up: Study Completion[i]
Inc./exclusion criteria	X			
Informed Consent	X			
Medical history; vital signs (incl. BP, HR, weight)[#]	X	X	X	X
Toxicity		X	X	X
Physical examination	X	X		X
Performance status	X	X		X
CBC dif.[h]	X	X	X	X
Comprehensive metabolic panel[b]	X	X	Just LFTs	X
Urinalysis[d]	X	X	X	X
Coagulation[e]	X	X		X
EKG	X			X
Biodistribution		X* (Odd cycles only)	X*	X
Tumor response[f]	X	q2 cycles[c]		
Ad-5 Antibodies		X* (Odd cycles only)	X^	X
Correlative Science/ Biomarkers[g]	X	Cycle 1, 2, and then odd cycles only	Cycle 1 Day 8 for Cycles 2-6	
Optional tumor biopsy[h]				X[b]
CA-125	X	X		X
Paclitaxel dose		X	X	
VB-111 dose		Odd cycles only		

*Samples to be collected on VB-111 dosing days (every other cycle) prior to dosing, end of infusion, 6 hours post dosing and on Day 8 of that cycle (Odd cycles).

^Samples to be collected prior to dosing # Vital signs will be monitored on VB-111 dosing days prior to dosing, and 30 minutes, 60 minutes, 4 hours and 6 hours post dosing

[a]All physical examinations, blood tests, and urinalysis must be performed within 14 days prior to registration. Radiological assessment of tumors should be performed within 4 weeks prior to registration.

[b]Bloods can be drawn within 3 days of D1, 8, 15, 22 of all subsequent cycles.

CBC dif.: CBC with differential and platelets; Comprehensive metabolic panel including electrolytes, LFTs, BUN/Cr, calcium, and magnesium. Only LFTs and a CBC dif are required days 8, 15, 22.

[c]It is anticipated that tumor response will be evaluated 2 cycles.

[d]If there is new, or increased proteinuria, a 24 hr urine may be required. +2 dipstick requires a 24-hour collection but +3 dipstick requires holding study drug and a 24-hour collection. Urinalysis will be obtained on D1, D8, D15, D22, and as clinically indicated, as proteinuria has not been a feature of dosing with VB-111.

[e]Bloods can be drawn within 3 days of D1 of each cycle for PT, PTT. In case of PTT prolongation above ULN, blood should be drawn for lupus anticoagulant (LAC) and for anti-phospholipid antibody (IgG and IgM for beta-2-GP-1 and anticardiolipin).

[f]Tumor assessment can be by institutional standards such as tumor response assessments: CT, MRI, etc.

NOTE: The tumor assessment is scheduled to occur at regular intervals (every 8 weeks), which will be typically coinciding with every 2 cycles, but at a fixed interval which will allow cleaner evaluation of PFS, the primary end point.

[g]Specific biomarkers will be collected prior to dosing on Cycle 1 day 1 and on days 8, 15, 22 of Cycle 1, Cycle 2 day 1, Cycle 3 day 1 prior to dosing and every 2 cycles thereafter until disease progression.

[h]Tissue acquisition is not a mandated part of the study, but optional with a budget to cover non-clinically indicated biopsy or paracentesis to procure samples. There is no specified time for this to be done. See TISSUE COLLECTION section below.

[i]Study Completion data should be completed within one week of 30 days from last dose of study medication. Survival data may then be collected by phone.

Efficacy and Safety Assessments

Recist Criteria

Tumor response will be assessed using RECIST 1.1 criteria at baseline and every other cycle thereafter i.e. after cycles 2, 4, 6, etc. If patients continue with paclitaxel formal evaluation will continue. Independent evaluation of imaging will be performed by TumorMetrics.

Tumor Measurement

Measurable disease: the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable Lesions

Lesions that can be accurately measured in at least one dimension with the longest diameter>2.0 cm. With a spiral CT scan, the lesion must be >1.0 cm in at least one dimension, and for lymphnodes, the shortest diameter must be >1.5 cm.

Non-Measurable Lesions

All other lesions, including small lesions (longest diameter<2.0 cm with conventional techniques or <1.0 cm with spiral CT scans) and other non-measurable lesions. These include: bone lesions; leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitis cutis/pulmonis; abdominal masses that are not confirmed and followed by imaging techniques; and cystic lesions.

All measurements should be recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph

nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

Baseline Documentation of Target and Non-Target Lesions

All measurable lesions up to a maximum of 5 lesions representative of each involved organ should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present" or "absent."

Response Criteria

Evaluation of target lesions:

Complete response (R)—disappearance of all target lesions.

Partial response (PR)—at least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progression (PD)—at least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable disease (SD)—neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of non-target lesions:

Complete response (CR)—disappearance of all non-target lesions and normalization of tumor marker level.

Non-complete response (non-CR)/non-progression (non-PD)—persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits.

Progressive disease (PD)—appearance of one or more new lesions. Unequivocal progression of existing non-target lesions. Although a clear progression of non-target lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by a review panel (or study chair/primary investigator).

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria as shown in Table 18.

TABLE 18

Overall Patient Responses Based on Measurement of Target, Non-Target, and New Lesions

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time will be reported as "clinical deterioration." Every effort will be made to document the objective progression even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

Confirmation: To be assigned a status of partial response (PR) or complete response (CR), changes in tumor measurements must be confirmed by repeat studies that should be performed no less than 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 6-8 weeks.

Rustin criteria: If only CA125 is evaluable (Elevated over 35 U/ml), response will be defined per the GCIG rather than the Rustin criteria.

GCIG CA125 response definition: A response according to CA125 has occurred if there is at least a 50% reduction in CA125 levels from a pretreatment sample. The response must be confirmed and maintained for at least 28 days. Patients can be evaluated according to CA 125 only if they have a pretreatment sample that is at least twice the upper limit of normal and within 2 weeks prior to starting treatment.

Progression according to CA125 has occurred if there is a confirmed (documented on two occasions) rise in CA125 or a previously normal CA125 rises to ≥2×ULN documented on two occasions.

Expected Adverse Events

VB-111 caused minimal toxicity in preclinical toxicology studies in mice. Mild anemia, mild thrombocytopenia, mild leukocytosis, splenomegaly, and bone marrow hyperplasia were observed. Transient liver enzyme elevations with no correlation with clinical pathology were also observed. The administration of adenovirus vectors systemically has been well tolerated. Flu-like symptoms (fever, fatigue, rigors, nausea, and/or vomiting) are the most common adverse events. Asymptomatic prolongation of aPTT and positive LAC were observed in several patients participating in Phase I/II trials. Additionally, a single case of severe diarrhea was reported in a Phase II patient. The majority of intravenously injected adeno VPs are sequestered by the liver, which in turn causes an inflammatory response characterized by acute transaminitis and vascular damage. The major adverse effects of anti-angiogenic agents have been wound healing disorders, bleeding, thromboembolic, and cardiovascular events, hypertension and proteinuria.

Correlative Studies

Distribution

For distribution assessment, blood samples will be collected from all patients according to Appendix I. Testing of these samples for Adenovirus and VB-111 transgene level determination will be conducted at the maximal tolerated dose group or the highest dose cohort. Distribution will be assessed by determination of levels of viral DNA and transgene by Q-PCR and Q-RT-PCR respectively in the blood, at predetermined time points following dosing. Samples will be collected for all patients at all pre-defined time points. Testing will be conducted in samples from patients starting at the highest does and will continue to the lower doses. Samples found with non-detectable levels of viral DNA following dosing will not be tested for levels of the transgene and will not be evaluated for later time points.

Antibodies

Serum samples will be collected for analysis of levels of antibodies (total IgG and neutralizing antibodies) to the adenovirus.

Angiogenic Biomarkers

There are no biomarkers to date for antiangiogenic therapy, but several biomarker candidates have been identified [Jain 2009]. These will be tested in peripheral blood samples obtained from all patients enrolled in this study. Plasma analysis will be carried out for circulating angiogenic and inflammatory biomarkers VEGF, P1GF, sVEGFR1, bFGF, IL-1β, IL-6, IL-8, and TNF-α (using multiplex ELISA plates from Meso-Scale Discovery) and sVEGFR2 and SDF1α (using R&D Systems kits), with samples run in duplicate, using established protocols [Horowitz Clinical Ovarian Cancer 2011 in press]. Blood-circulating cells will be enumerated in fresh samples using a standard flow cytometry protocol. The quantitative analysis endpoint was the change in the fraction of circulating CD34brightCD45dim CPCs or VEGFR2+ CD45+ monocytic cells among blood mononuclear cells after treatment, as previously described [Horowitz Clinical Ovarian Cancer 2011 in press]. Archival tissue will be evaluated for CD31, CD34, VEGFR2, and vWF.

Tissue Collection

Tissue acquisition is not a mandated part of the study, but optional with a budget to cover non-clinically indicated biopsy or paracentesis to procure samples. There is no specified time for this to be done. The optimal time is considered to be one to two months after first dose of VB-111. It is anticipated that 20-50% of participants may have suitable areas for safe biopsy or ascites amenable to paracentesis.

Description of Statistical Methods

The MTD of VB-111 will be determined using a standard "3+3" design. That is, 3 patients will initially be treated at a particular dose level. If more than one patient experiences DITT accrual will stop and the next lower dose level will be accepted as the MTD. If no DLT occurs, accrual to the next higher dose level will begin. If one DLT occurs, 3 additional patients will be entered at that dose level. VB-111 will be escalated to the next higher level if none of these 3 patients experiences DLT, however, if one or more patients experience such an event, accrual will stop and the MTD will be defined to be the next lower dose level. With this escalation scheme, Table 19 gives the probability of escalation to the next higher dose level for a variety of hypothesized underlying toxicity rates.

TABLE 19

Probability of Escalation of VB-111 Dose Based on DLT

	True Rate of DLT						
	10%	20%	30%	40%	50%	60%	70%
Probability of Escalation	0.91	0.71	0.49	0.31	0.17	0.08	0.03

As can be seen from TABLE 19, there is a >71% chance of escalating the combination if the underlying risk of DLT is <20% and a >91% chance of escalation if the underlying risk is <10%. In contrast, there is at most a 17% chance of escalation if the underlying DLT risk is >50% and <8% chance if the risk is >60%.

The assessment of objective response will primarily be descriptive. In general, the overall response rate and corresponding 95% confidence interval will be calculated.

Phase II aspect of the design will evaluate the response rates defined by RECIST criteria or CA125 (GCIG not Rustin criteria) and to describe the safety profile and characterize adverse events and toxicities. Thirty percent will be chosen as the target response rate based on reported responses of combination chemo-antiangiogenic agents in patients with recurrent ovarian cancer, typically in the 20-25% range.

The design will be two-stage optimal design in that an initial 10 patients will be enrolled during Stage 1. This will include participants who commence at a paclitaxel dose of 80 mg/m2 on the Phase I (i.e., participants in Dose Levels 2 and 3). With a range of two to six patients enrolled from DL-2 and 3 cohorts of the Phase I part of the study, a further eight to four participants will be enrolled to the Phase II first stage before an interim analysis of efficacy. If one or no response is observed, then the trial will stop recruitment. Otherwise, if there are two or more responses, then 19 additional participants will be enrolled (i.e. possible maximum total=29) to the Phase II (Total for the whole study will then be 2-18 for Phase I overlapping with 0-29 for the Phase II). If there are five or fewer responses, then no further investigation of this therapy is warranted.

If the true RR≤10%, the chance of ending the trial during Stage 1 is at least 74%. If the true RR is ≥30%, the chance that the trial will be stopped in Stage 1 is ≤15%. The power of final analysis is 80% to reject H0: RR≤10% in favor of H1: RR≥30% at a target type-1 error rate of 5%.

The trial will be terminated if more than 2 Grade IV GI perforations are observed during Stage I. If there are 3 GI perforations at any time, then this trial will be terminated. Assuming a true GI perforations rate of 4% the probability of observing more than 2 events during Stage I is 6.2%, and the probability to observe more than 3 events from the entire trial is 2.7%.

The quantitative and semi-quantitative data such as IHC data will be considered preliminary pilot data, using descriptive statistics and non-parametric analyses, attempting to explore correlations, acknowledging that these sample sizes lack sufficient power to draw definitive conclusions, but the advantage of using the same set of analyses in multiple studies will enable us to evaluate the potentially most useful predictors of efficacy and toxicity.

Biomarkers

Primarily non-parametric methods (e.g. Wilcoxon signed-rank or rank sum test) will be used to assess the impact of VB-111 on CECs, and correlative/predictive measures, all

tests of statistical significance will be two-sided and no adjustment will be made for multiple comparisons.

Level of Significance

Confidence intervals will be calculated at the (two-sided) 95% level of confidence.

Laboratory Testing

Angiogenesis Biomarkers Analyses (Local)

Plasma analysis will be carried out for circulating angiogenic and inflammatory biomarkers VEGF, P1GF, sVEGFR1, bFGF, IL-1β, IL-6, IL-8, and TNF-α (using multiplex ELISA plates from Meso-Scale Discovery) and sVEGFR2 and SDF1α (using R&D Systems kits. Blood-circulating cells will be enumerated in fresh samples using a standard flow cytometry protocol. Archival tissue will be evaluated for CD31, CD34, VEGFR2, and vWF. Tumor marker: CA-125. Ten cc total plasma for each time-point, in EDTA tubes at room temperature, will be assayed.

Samples shall be collected at the following time points:

1. At Baseline

2. At Cycle 1: on Days 1 prior to study drug dosing, and days 8, 15, and 22

3. At Cycle 2 Day 1 prior to study drug dosing

4. Every 2 cycles thereafter beginning with Cycle 3, prior to study drug dosing, until disease progression

Antibody Testing

Titers of antibodies to the Ad-5 virus including IgG and neutralizing antibodies shall be collected for analysis. Samples shall be collected at the following time points:

1. At Each Cycle: on Days 1, 8, 15, and 22 prior to study drug dosing;

2. At the Study Completion Visit.

Blood shall be collected and prepared in the following manner:

1. 6 ml of blood shall be collected in tubes with no anticoagulant.

2. Samples shall be left at room temperature for 1 hour, and then stored at 2-8° C. overnight to permit clot retraction.

3. Blood shall be centrifuged the next day.

4. Approximately 2 ml serum shall be extracted and split into 4 aliquot tubes (0.5 ml each, total of 2 ml each in a Nalgene 100 1.5 ml) and stored at −20° C. until shipping for analysis.

Biodistribution Testing

The following tests will be performed:

1. Blood biodistribution: Viral DNA and Transgene expression

2. Urine biodistribution: Viral DNA

Whole blood sample will be collected for Biodistribution at the following time points:

1. On Day 1 & 8 of each odd cycle, prior to study drug dosing.

2. On VB-111 dosing days:

a. Prior to infusion (Same as Day 1 sample)

b. At end of infusion

c. 3±0.5 hours

d. 6±0.5 hours

e. At the Study Completion Visit.

Blood samples will be prepared in the following manner:

1. Blood from each time point will be collected in 4 tubes (0.75 mL/tube) containing EDTA.

a. 2 tubes for the analysis of viral DNA

b. 2 tubes for the analysis of transgene expression

2. The tubes should be labeled with the subject numbers, initials, date and time of sample collection and stored in a freezer at or below −70° C.

Urine samples will be prepared in the following manner:

1. Two urine samples of 1-2 ml will be collected from the total collection volume saved

2. Urine samples and total collection volumes will be stored frozen until further analyses.

Results

VB-111 was administered to six patients, each having fallopian tube or epithelial ovarian cancer for a duration of at least 1 year prior to study entry, at a dose of 3×10^{12} VPs in combination with paclitaxel (40 mg or 80 mg) per subject as shown in FIG. **3**. The patients did not exhibit any serious adverse events that were related to VB-111. There were no dose limiting toxicities observed.

SEQUENCE LISTING

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His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
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Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
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Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
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Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
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Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
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Gly Glu Leu Glu Gly Thr Thr Thr Lys Pro Leu Ala Pro Asn Pro Ser
            260                 265                 270

Phe Ser Pro Thr Pro Gly Phe Thr Pro Thr Leu Gly Phe Ser Pro Val
        275                 280                 285

Pro Ser Ser Thr Phe Thr Ser Ser Ser Thr Tyr Thr Pro Gly Asp Cys
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Pro Asn Phe Ala Ala Pro Arg Arg Glu Val Ala Pro Pro Tyr Gln Gly
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Ala Asp Pro Ile Leu Ala Thr Ala Leu Ala Ser Asp Pro Ile Pro Asn
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Pro Leu Gln Lys Trp Glu Asp Ser Ala His Lys Pro Gln Ser Leu Asp
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Thr Asp Asp Pro Ala Thr Leu Tyr Ala Val Val Glu Asn Val Pro Pro
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Leu Arg Trp Lys Glu Phe Val Arg Arg Leu Gly Leu Ser Asp His Glu
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Ile Asp Arg Leu Glu Leu Gln Asn Gly Arg Cys Leu Arg Glu Ala Gln
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Thr Leu Glu Leu Leu Gly Arg Val Leu Arg Asp Met Asp Leu Leu Gly
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agcaacacca agtgcaaaga ggaaggatcc agatctaact tggggtggct ttgtcttctt 540

cttttgccaa ttccactaat tgtttgggtg aagagaaagg aagtacagaa aacatgcaga 600

aagcacagaa aggaaaacca aggttctcat gaatctccaa ctttaaatcc tgaaacagtg 660

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gcaataaatt tatctgatgt tgacttgagt aaatatatca ccactattgc tggagtcatg 720

acactaagtc aagttaaagg ctttgttcga aagaatggtg tcaatgaagc caaaatagat 780

gagatcaaga atgacaatgt ccaagacaca gcagaacaga aagttcaact gcttcgtaat 840

tggcatcaac ttcatggaaa gaaagaagcg tatgacacat tgattaaaga tctcaaaaaa 900

gccaatcttt gtactcttgc agagaaaatt cagactatca tcctcaagga cattactagt 960

gactcagaaa attcaaactt cagaaatgaa atccaaagct tggtctag 1008

<210> SEQ ID NO 6
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: full-length FAS

<400> SEQUENCE: 6

Met Leu Gly Ile Trp Thr Leu Leu Pro Leu Val Leu Thr Ser Val Ala
1 5 10 15

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser
20 25 30

Lys Gly Leu Glu Leu Arg Lys Thr Val Thr Thr Val Glu Thr Gln Asn
35 40 45

Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys Pro Cys Pro
50 55 60

Pro Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro
65 70 75 80

Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr Thr Asp Lys Ala His
85 90 95

Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His Gly
100 105 110

Leu Glu Val Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg
115 120 125

Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp
130 135 140

Pro Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr
145 150 155 160

Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg Ser Asn Leu Gly Trp
165 170 175

Leu Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg
180 185 190

Lys Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly
195 200 205

Ser His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu
210 215 220

Ser Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met
225 230 235 240

Thr Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu
245 250 255

Ala Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu
260 265 270

Gln Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys
275 280 285

Glu Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys
290 295 300

```
Thr Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser
305                 310                 315                 320

Asp Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
                325                 330                 335


<210> SEQ ID NO 7
<211> LENGTH: 505
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Effector Domain of FAS

<400> SEQUENCE: 7

aggatccaga tctaacttgg ggtggctttg tcttcttctt ttgccaattc cactaattgt     60

ttgggtgaag agaaaggaag tacagaaaac atgcagaaag cacagaaagg aaaaccaagg    120

ttctcatgaa tctccaacct taaatcctga aacagtggca ataaatttat ctgatgttga    180

cttgagtaaa tatatcacca ctattgctgg agtcatgaca ctaagtcaag ttaaaggctt    240

tgttcgaaag aatggtgtca atgaagccaa aatagatgag atcaagaatg acaatgtcca    300

agacacagca gaacagaaag ttcaactgct tcgtaattgg catcaacttc atggaaagaa    360

agaagcgtat gacacattga ttaaagatct caaaaaagcc aatctttgta ctcttgcaga    420

gaaaattcag actatcatcc tcaaggacat tactagtgac tcagaaaatt caaacttcag    480

aaatgaaatc caaagcttgg tctag                                          505


<210> SEQ ID NO 8
<211> LENGTH: 167
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Effector Domain of FAS

<400> SEQUENCE: 8

Gly Ser Arg Ser Asn Leu Gly Trp Leu Cys Leu Leu Leu Leu Pro Ile
1               5                   10                  15

Pro Leu Ile Val Trp Val Lys Arg Lys Glu Val Gln Lys Thr Cys Arg
            20                  25                  30

Lys His Arg Lys Glu Asn Gln Gly Ser His Glu Ser Pro Thr Leu Asn
        35                  40                  45

Pro Glu Thr Val Ala Ile Asn Leu Ser Asp Val Asp Leu Ser Lys Tyr
    50                  55                  60

Ile Thr Thr Ile Ala Gly Val Met Thr Leu Ser Gln Val Lys Gly Phe
65                  70                  75                  80

Val Arg Lys Asn Gly Val Asn Glu Ala Lys Ile Asp Glu Ile Lys Asn
                85                  90                  95

Asp Asn Val Gln Asp Thr Ala Glu Gln Lys Val Gln Leu Leu Arg Asn
            100                 105                 110

Trp His Gln Leu His Gly Lys Lys Glu Ala Tyr Asp Thr Leu Ile Lys
        115                 120                 125

Asp Leu Lys Lys Ala Asn Leu Cys Thr Leu Ala Glu Lys Ile Gln Thr
    130                 135                 140

Ile Ile Leu Lys Asp Ile Thr Ser Asp Ser Glu Asn Ser Asn Phe Arg
145                 150                 155                 160

Asn Glu Ile Gln Ser Leu Val
                165


<210> SEQ ID NO 9
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<211> LENGTH: 1101
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: FAS-chimera

<400> SEQUENCE: 9

atgggcctct ccaccgtgcc tgacctgctg ctgccgctgg tgctcctgga gctgttggtg 60

ggaatatacc cctcaggggt tattggactg gtccctcacc taggggacag ggagaagaga 120

gatagtgtgt gtccccaagg aaaatatatc caccctcaaa ataattcgat ttgctgtacc 180

aagtgccaca aaggaaccta cttgtacaat gactgtccag gcccggggca ggatacggac 240

tgcagggagt gtgagagcgg ctccttcacc gcttcagaaa accacctcag acactgcctc 300

agctgctcca aatgccgaaa ggaaatgggt caggtggaga tctcttcttg cacagtggac 360

cgggacaccg tgtgtggctg caggaagaac cagtaccggc attattggag tgaaaacctt 420

ttccagtgct tcaattgcag cctctgcctc aatgggaccg tgcacctctc ctgccaggag 480

aaacagaaca ccgtgtgcac ctgccatgca ggtttctttc taagagaaaa cgagtgtgtc 540

tcctgtagta actgtaagaa aagcctggag tgcacgaagt tgtgcctacc aagcttagga 600

tccagatcta acttggggtg gctttgtctt cttcttttgc caattccact aattgtttgg 660

gtgaagagaa aggaagtaca gaaaacatgc agaaagcaca gaaaggaaaa ccaaggttct 720

catgaatctc caaccttaaa tcctgaaaca gtggcaataa atttatctga tgttgacttg 780

agtaaatata tcaccactat tgctggagtc atgacactaa gtcaagttaa aggctttgtt 840

cgaaagaatg gtgtcaatga agccaaaata gatgagatca agaatgacaa tgtccaagac 900

acagcagaac agaaagttca actgcttcgt aattggcatc aacttcatgg aaagaaagaa 960

gcgtatgaca cattgattaa agatctcaaa aaagccaatc tttgtactct tgcagagaaa 1020

attcagacta tcatcctcaa ggacattact agtgactcag aaaattcaaa cttcagaaat 1080

gaaatccaaa gcttggtcta g 1101

<210> SEQ ID NO 10
<211> LENGTH: 366
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: FAS-chimera

<400> SEQUENCE: 10

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

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Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180                 185                 190

Lys Leu Cys Leu Pro Ser Leu Gly Ser Arg Ser Asn Leu Gly Trp Leu
        195                 200                 205

Cys Leu Leu Leu Leu Pro Ile Pro Leu Ile Val Trp Val Lys Arg Lys
    210                 215                 220

Glu Val Gln Lys Thr Cys Arg Lys His Arg Lys Glu Asn Gln Gly Ser
225                 230                 235                 240

His Glu Ser Pro Thr Leu Asn Pro Glu Thr Val Ala Ile Asn Leu Ser
                245                 250                 255

Asp Val Asp Leu Ser Lys Tyr Ile Thr Thr Ile Ala Gly Val Met Thr
            260                 265                 270

Leu Ser Gln Val Lys Gly Phe Val Arg Lys Asn Gly Val Asn Glu Ala
        275                 280                 285

Lys Ile Asp Glu Ile Lys Asn Asp Asn Val Gln Asp Thr Ala Glu Gln
    290                 295                 300

Lys Val Gln Leu Leu Arg Asn Trp His Gln Leu His Gly Lys Lys Glu
305                 310                 315                 320

Ala Tyr Asp Thr Leu Ile Lys Asp Leu Lys Lys Ala Asn Leu Cys Thr
                325                 330                 335

Leu Ala Glu Lys Ile Gln Thr Ile Ile Leu Lys Asp Ile Thr Ser Asp
            340                 345                 350

Ser Glu Asn Ser Asn Phe Arg Asn Glu Ile Gln Ser Leu Val
        355                 360                 365


<210> SEQ ID NO 11
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific Enhancer Elements

<400> SEQUENCE: 11

ctggagggtg actttgcttc tggagccagt acttcatact tttcatt                  47


<210> SEQ ID NO 12
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific Enhancer

<400> SEQUENCE: 12

aatgaaaagt atgaagtact ggctccagaa gcaaagtcac cctccag                  47


<210> SEQ ID NO 13
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific Enhancer

<400> SEQUENCE: 13
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gtacttcata cttttcattc caatggggtg actttgcttc tgga 44

<210> SEQ ID NO 14
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific Enhancer Element

<400> SEQUENCE: 14

tccagaagca aagtcacccc attggaatga aaagtatgaa gtac 44

<210> SEQ ID NO 15
<211> LENGTH: 131
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific 3X Enhancer Element

<400> SEQUENCE: 15

ctccagaagc aaagtcaccc cattggaatg aaaagtatga agtacaatga aaagtatgaa 60

gtactggctc cagaagcaaa gtcaccctcc agaagcaaag tcaccccatt ggaatgaaaa 120

gtatgaagta c 131

<210> SEQ ID NO 16
<211> LENGTH: 131
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific 3X Enhancer Element

<400> SEQUENCE: 16

gtacttcata cttttcattc caatggggtg actttgcttc tggagggtga ctttgcttct 60

ggagccagta cttcatactt ttcattgtac ttcatacttt tcattccaat ggggtgactt 120

tgcttctgga g 131

<210> SEQ ID NO 17
<211> LENGTH: 850
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific PPE-1 Promoter

<400> SEQUENCE: 17

gtacgtgtac ttctgatcgg cgatactagg gagataagga tgtgcctgac aaaaccacat 60

tgttgttgtt atcattatta tttagttttc cttccttgct aactcctgac ggaatctttc 120

tcacctcaaa tgcgaagtac tttagtttag aaaagacttg gtggaagggg tggtggtgga 180

aaagtagggt gatcttccaa actaatctgg ttccccgccc gccccagtag ctgggattca 240

agagcgaaga gtggggatcg tccccttgtt tgatcagaaa gacataaaag gaaaatcaag 300

tgaacaatga tcagccccac ctccacccca ccccccctgcg cgcgcacaat acaatctatt 360

taattgtact tcatactttt cattccaatg gggtgacttt gcttctggag aaactcttga 420

ttcttgaact ctggggctgg cagctagcaa aaggggaagc gggctgctgc tctctgcagg 480

ttctgcagcg gtctctgtct agtgggtgtt ttctttttct tagccctgcc cctggattgt 540

cagacggcgg gcgtctgcct ctgaagttag ccgtgatttc ctctagagcc gggtcttatc 600

tctggctgca cgttgcctgt gggtgactaa tcacacaata acattgttta gggctggaat 660

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gaagtcagag ctgtttaccc ccactctata ggggttcaat ataaaaaggc ggcggagaac 720

tgtccgagtc agaagcgttc ctgcaccggc gctgagagcc tgacccggtc tgctccgctg 780

tccttgcgcg ctgcctcccg gctgcccgcg acgctttcgc cccagtggaa gggccacttg 840

ctgcggccgc 850

<210> SEQ ID NO 18
<211> LENGTH: 987
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Endothelial Cell-Specific PPE-1 3X Promoter

<400> SEQUENCE: 18

gtacgtgtac ttctgatcgg cgatactagg gagataagga tgtgcctgac aaaaccacat 60

tgttgttgtt atcattatta tttagttttc cttccttgct aactcctgac ggaatctttc 120

tcacctcaaa tgcgaagtac tttagtttag aaaagacttg gtggaagggg tggtggtgga 180

aaagtagggt gatcttccaa actaatctgg ttccccgccc gccccagtag ctgggattca 240

agagcgaaga gtggggatcg tccccttgtt tgatcagaaa gacataaaag gaaaatcaag 300

tgaacaatga tcagccccac ctccacccca ccccccctgcg cgcgcacaat acaatctatt 360

taattgtact tcatactttt cattccaatg gggtgacttt gcttctggag aaactcttga 420

ttcttgaact ctggggctgg cagctagcct ccagaagcaa agtcacccca ttggaatgaa 480

aagtatgaag tacaatgaaa agtatgaagt actggctcca gaagcaaagt caccctccag 540

aagcaaagtc accccattgg aatgaaaagt atgaagtacg ctagcaaaag gggaagcggg 600

ctgctgctct ctgcaggttc tgcagcggtc tctgtctagt gggtgttttc tttttcttag 660

ccctgccccc ggattgtcag acggcgggcg tctgcctctg aagttagccg tgatttcctc 720

tagagccggg tcttatctct ggctgcacgt tgcctgtggg tgactaatca cacaataaca 780

ttgtttaggg ctggaatgaa gtcagagctg tttaccccca ctctataggg gttcaatata 840

aaaaggcggc ggagaactgt ccgagtcaga agcgttcctg caccggcgct gagagcctga 900

cccggtctgc tccgctgtcc ttgcgcgctg cctcccggct gcccgcgacg ctttcgcccc 960

agtggaaggg ccacttgctg cggccgc 987

<210> SEQ ID NO 19
<211> LENGTH: 35207
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VB-111 entire construct

<400> SEQUENCE: 19

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ttgtgacgtg gcgcggggcg tgggaacggg gcgggtgacg tagtagtgtg gcggaagtgt 120

gatgttgcaa gtgtggcgga acacatgtaa gcgacggatg tggcaaaagt gacgtttttg 180

gtgtgcgccg gtgtacacag gaagtgacaa ttttcgcgcg gttttaggcg gatgttgtag 240

taaatttggg cgtaaccgag taagatttgg ccattttcgc gggaaaactg aataagagga 300

agtgaaatct gaataatttt gtgttactca tagcgcgtaa tatttgtcta gggccgcggg 360

gactttgacc gtttacgtgg agactcgccc aggtgttttt ctcaggtgtt ttccgcgttc 420

cgggtcaaag ttggcgtttt attattatag tcagtacgta cgtgtacttc tgatcggcga 480

tactagggag ataaggatgt gcctgacaaa accacattgt tgttgttatc attattattt 540

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agttttcctt ccttgctaac tcctgacgga atctttctca cctcaaatgc gaagtacttt 600

agtttagaaa agacttggtg gaaggggtgg tggtggaaaa gtagggtgat cttccaaact 660

aatctggttc cccgcccgcc ccagtagctg ggattcaaga gcgaagagtg gggatcgtcc 720

ccttgtttga tcagaaagac ataaaaggaa aatcaagtga acaatgatca gccccacctc 780

caccccaccc ccctgcgcgc gcacaataca atctatttaa ttgtacttca tacttttcat 840

tccaatgggg tgactttgct tctggagaaa ctcttgattc ttgaactctg gggctggcag 900

ctagcctcca gaagcaaagt caccccattg gaatgaaaag tatgaagtac aatgaaaagt 960

atgaagtact ggctccagaa gcaaagtcac cctccagaag caaagtcacc ccattggaat 1020

gaaaagtatg aagtacgcta gcaaaagggg aagcgggctg ctgctctctg caggttctgc 1080

agcggtctct gtctagtggg tgttttcttt ttcttagccc tgcccctgga ttgtcagacg 1140

gcgggcgtct gcctctgaag ttagccgtga tttcctctag agccgggtct tatctctggc 1200

tgcacgttgc ctgtgggtga ctaatcacac aataacattg tttagggctg gaatgaagtc 1260

agagctgttt acccccactc tataggggtt caatataaaa aggcggcgga gaactgtccg 1320

agtcagaagc gttcctgcac cggcgctgag agcctgaccc ggtctgctcc gctgtccttg 1380

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ccgctaattc tgcagatcgg gatccggcat gggcctctcc accgtgcctg acctgctgct 1500

gccgctggtg ctcctggagc tgttggtggg aatatacccc tcaggggtta ttggactggt 1560

ccctcaccta ggggacaggg agaagagaga tagtgtgtgt ccccaaggaa aatatatcca 1620

ccctcaaaat aattcgattt gctgtaccaa gtgccacaaa ggaacctact tgtacaatga 1680

ctgtccaggc ccggggcagg atacggactg cagggagtgt gagagcggct ccttcaccgc 1740

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ggtggagatc tcttcttgca cagtggaccg ggacaccgtg tgtggctgca ggaagaacca 1860

gtaccggcat tattggagtg aaaacctttt ccagtgcttc aattgcagcc tctgcctcaa 1920

tgggaccgtg cacctctcct gccaggagaa acagaacacc gtgtgcacct gccatgcagg 1980

tttctttcta agagaaaacg agtgtgtctc ctgtagtaac tgtaagaaaa gcctggagtg 2040

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tcttttgcca attccactaa ttgtttgggt gaagagaaag gaagtacaga aaacatgcag 2160

aaagcacaga aaggaaaacc aaggttctca tgaatctcca accttaaatc ctgaaacagt 2220

ggcaataaat ttatctgatg ttgacttgag taaatatatc accactattg ctggagtcat 2280

gacactaagt caagttaaag gctttgttcg aaagaatggt gtcaatgaag ccaaaatagа 2340

tgagatcaag aatgacaatg tccaagacac agcagaacag aaagttcaac tgcttcgtaa 2400

ttggcatcaa cttcatggaa agaaagaagc gtatgacaca ttgattaaag atctcaaaaa 2460

agccaatctt tgtactcttg cagagaaaat tcagactatc atcctcaagg acattactag 2520

tgactcagaa aattcaaact tcagaaatga aatccaaagc ttggtctagc tcgagcatgc 2580

atctaggcgg ccgcatggca gaaattcgcg aattcgctag cgttaacgga tcctctagac 2640

gagatccgaa cttgtttatt gcagcttata atggttacaa ataaagcaat agcatcacaa 2700

atttcacaaa taaagcattt ttttcactgc attctagttg tggtttgtcc aaactcatca 2760

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agaatatata aggtgggggt cttatgtagt tttgtatctg ttttgcagca gccgccgccg 2880

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tgcccgcaaa ctctactacc ttgacctacg agaccgtgtc tggaacgccg ttggagactg 3060

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tgacggctct tttggcacaa ttggattctt tgacccggga acttaatgtc gtttctcagc 3240

agctgttgga tctgcgccag caggtttctg ccctgaaggc ttcctcccct cccaatgcgg 3300

tttaaaacat aaataaaaaa ccagactctg tttggatttg gatcaagcaa gtgtcttgct 3360

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gggatgggtg catacgtggg gatatgagat gcatcttgga ctgtattttt aggttggcta 3720

tgttcccagc catatccctc cggggattca tgttgtgcag aaccaccagc acagtgtatc 3780

cggtgcactt gggaaatttg tcatgtagct tagaaggaaa tgcgtggaag aacttggaga 3840

cgcccttgtg acctccaaga ttttccatgc attcgtccat aatgatggca atgggcccac 3900

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tgagatcgtc ataggccatt tttacaaagc gcgggcggag ggtgccagac tgcggtataa 4020

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gggagatcag ctgggaagaa agcaggttcc tgagcagctg cgacttaccg cagccggtgg 4200

gcccgtaaat cacacctatt accggctgca actggtagtt aagagagctg cagctgccgt 4260

catccctgag cagggggcc acttcgttaa gcatgtccct gactcgcatg ttttccctga 4320

ccaaatccgc cagaaggcgc tcgccgccca gcgatagcag ttcttgcaag gaagcaaagt 4380

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35 40

What is claimed is:

1. A vector comprising the sequence set forth in nucleotides 1 to 35207 of SEQ ID NO: 19.

2. A pharmaceutical composition comprising the vector of claim **1** and a pharmaceutically acceptable carrier.

3. The pharmaceutical composition of claim **2**, wherein the pharmaceutically acceptable carrier is selected from ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene block polymers, polyethylene glycol and wool fat.

4. The pharmaceutical composition of claim **2**, wherein the composition is formulated in phosphate-buffered saline (PBS) and 10% glycerol.

5. A method of producing the vector of claim **1**, the method comprising transducing a host cell with the vector and expressing the vector in the host cell.

6. An isolated mammalian cell transfected with the vector of claim **1**.

7. The pharmaceutical composition of claim **2**, wherein the composition is formulated for administration to a mammal.

8. The pharmaceutical composition of claim **7**, wherein the mammal is a human.

9. The pharmaceutical composition of claim **2**, wherein the composition is formulated for parenteral administration.

10. The pharmaceutical composition of claim **9**, wherein the parenteral administration is intravenous.

11. The pharmaceutical composition of claim **2**, wherein the composition is coformulated with one or more additional therapeutic agents.

12. The pharmaceutical composition of claim **2**, wherein the vector of the composition is at least about 60% pure, at least about 70% pure, at least about 80% pure, at least about 90% pure, at least about 95% pure, at least about 96% pure, at least about 97% pure, at least about 98% pure, at least about 99% pure, or about 100% pure.

13. The pharmaceutical composition of claim **2**, wherein the composition is formulated by adding saline.

14. The pharmaceutical composition of claim **13**, wherein the composition is formulated in a fixed unit dose.

15. The pharmaceutical composition of claim **14**, wherein the unit dose is 1×10^{12} virus particles per milliliter.

16. The pharmaceutical composition of claim **4**, wherein the composition is formulated in a fixed unit dose.

17. The pharmaceutical composition of claim **16**, wherein the unit dose is 1×10^{12} virus particles per milliliter.

18. The pharmaceutical composition of claim **2**, wherein the composition is formulated as an aqueous suspension.

19. The pharmaceutical composition of claim **4**, wherein the composition is formulated as a single bolus dose, an infusion, or a loading bolus dose.

20. The pharmaceutical composition of claim **13**, wherein the composition is formulated as a single bolus dose, an infusion, or a loading bolus dose.

21. The vector of claim **1**, wherein the vector is at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure.

22. A pharmaceutical composition comprising the vector of claim **21**.

23. The isolated mammalian cell of claim **6**, wherein the mammalian cell is a PER.C6 cell.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,200,056 B2
APPLICATION NO. : 14/527667
DATED : December 1, 2015
INVENTOR(S) : Breitbart et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the claims

In column 183 lines 17 and 18, claim 1, please replace "A vector comprising the sequence set forth in nucleotides 1 to 35207 of SEQ ID NO: 19." with --An adenovirus expression vector comprising the nucleotide sequence set forth in SEQ ID NO: 19, wherein the vector comprises a Fas-chimera transgene comprising the nucleotide sequence set forth in SEQ ID NO: 9, which is operably linked to a promoter comprising the nucleotide sequence set forth in SEQ ID NO: 18.--

In column 183 lines 38-40, claim 5, please replace "A method of producing the vector of claim 1, the method comprising transducing a host cell with the vector and expressing the vector in the host cell." with --A method of producing the adenovirus expression vector of claim 1, the method comprising transfecting a host cell with the adenovirus expression vector and replicating the adenovirus in the host cell.--

Signed and Sealed this
Nineteenth Day of April, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office