ABSTRACT

The present specification discloses TVEMPs, compositions comprising such TVEMPs and methods of treating cancer in a mammal using such TVEMP compositions.

CLAIMS

1. A method of treating cancer in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, wherein administration of the composition reduces a symptom associated with cancer.

2. The method of Claim 1, wherein the TVEMP comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the targeting domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the targeting domain, the Clostridial toxin translocation domain, 3) the targeting domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the targeting domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.

3. The method of Claim 1 wherein the targeting domain is an interleukin (IL) peptide, vascular endothelial growth factor (VEGF) peptide, an insulin-like growth factor (IGF) peptide, an epidermal growth factor (EGF) peptide, a Transformation Growth Factor-p (TGF(3) peptide, a Bone Morphogenetic Protein (BMP), a Growth and Differentiation Factor (GDF) peptide, an activin peptide, a Fibroblast Growth Factor (FGF) peptide, or a Platelet-Derived Growth Factor (PDGF) peptide.

4. The method of Claim 3, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain is a VEGF-A peptide, a VEGF-B peptide, a VEGF-C peptide, a VEGF-D peptide, or a placenta growth factor (PlGF) peptide.

5. The method of Claim 4, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain comprises amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93.

6. The method of Claim 4, wherein the cancer is a prostate cancer, a renal cell carcinoma, an ovarian cancer, a bladder cancer, a colon cancer, a lymphoma, a rhabdomyosarcoma, a breast cancer, an osteosarcoma, a thyroid tumor, a lung cancer, a non-small cell lung cancer, a melanoma, a pancreatic cancer, an ocular melanoma, a retinoblastoma, an intra-ocular tumor, a leukemia, a Kaposi's sarcoma, a medulloblastoma, a teratocarcinoma, a neuroblastoma, a mesothelioma, an

7. The method of Claim 3, wherein the epidermal growth factor (EGF) peptide targeting domain an EGF, a heparin-binding EGF-like growth factor (HB-EGF), a transforming growth factor-a (TGF-a), an amphiregulin (AR), an epiregulin (EPR), an epigen (EPG), a betacellulin (BTC), a neuregulin-1 (NRG1), a neuregulin-2 (NRG2), a neuregulin-3, (NRG3), or a neuregulin-4 (NRG4).

8. The method of Claim 7, wherein the epidermal growth factor (EGF) peptide targeting domain comprises SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, SEQ ID NO: 102, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, amino acids 353-648 of SEQ ID NO: 105, or SEQ ID NO: 106.

9. The method of Claim 7, wherein the cancer is a lung cancer, a prostate cancer, an ovarian cancer, a bladder cancer, a thyroid cancer, a mixed papillary and follicular thyroid carcinoma, a biliary tract cholangiocarcinoma, a breast cancer, a cervical cancer, a colorectal cancer, a colon cancer, a gastric cancer, an endometrial cancer, an esophageal cancer, a fallopian tube cancer, a gallbladder cancer, a head and neck cancer, a liver cancer, a lung cancer, a myelodysplastic syndrome, a non-small cell lung cancer, an oral cancer, a pancreatic cancer, a peritoneal cavity cancer, a polycythemia vera, a renal cancer, or a skin cancer.

10. The method of Claim 3, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain is a FGF1 peptide, a FGF2 peptide, a FGF3 peptide, a FGF4 peptide, a FGF5 peptide, a FGF6 peptide, a FGF7 peptide, a FGF8 peptide, a FGF9 peptide, a FGF10 peptide, a FGF17 peptide, or a FGF18 peptide.

11. The method of Claim 10, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain comprises amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145.

12. The method of Claim 10, wherein the cancer is an acute myeloblastic leukemia, a chronic lymphocytic leukemia, a breast cancer, an endometrial ovarian cancer, a gastric cancer, a bladder cancer, a colon cancer, a cervical cancer, an epithelial ovarian cancer, a leiomyoma, or a pituitary tumor.

13. The method of Claim 1, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

14. The method of Claim 1, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

15. The method of Claim 1, wherein the exogenous protease cleavage site is a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a Tobacco Vein Mottling Virus cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

Dated this 07/03/2012 /A *A*

HRISHIKES k *RM* CHAUflHURY
OFREMFRY&SAGAR
ATTORNEY FOR THE APPLICANTS

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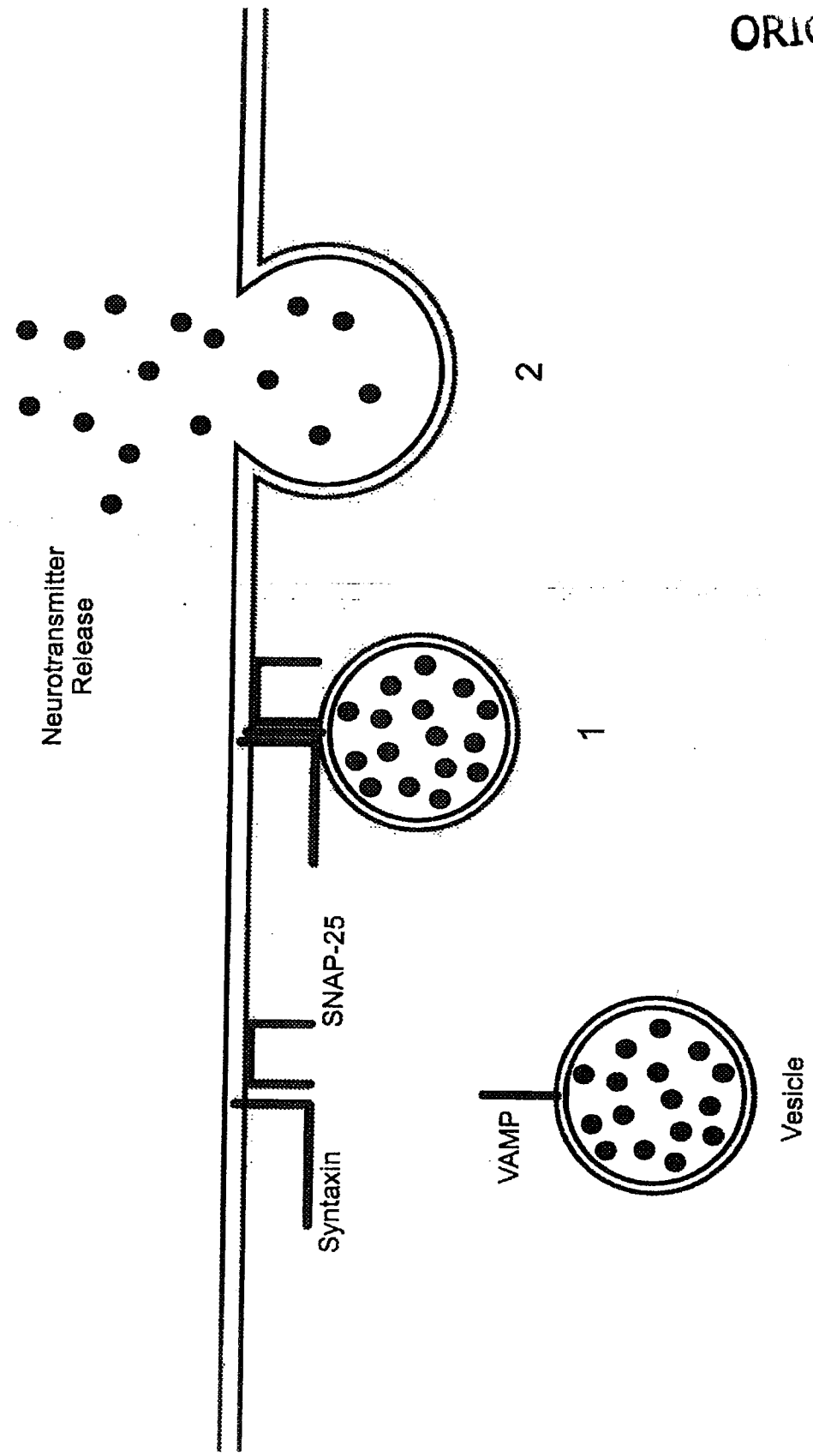

FIG. 1A.

(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

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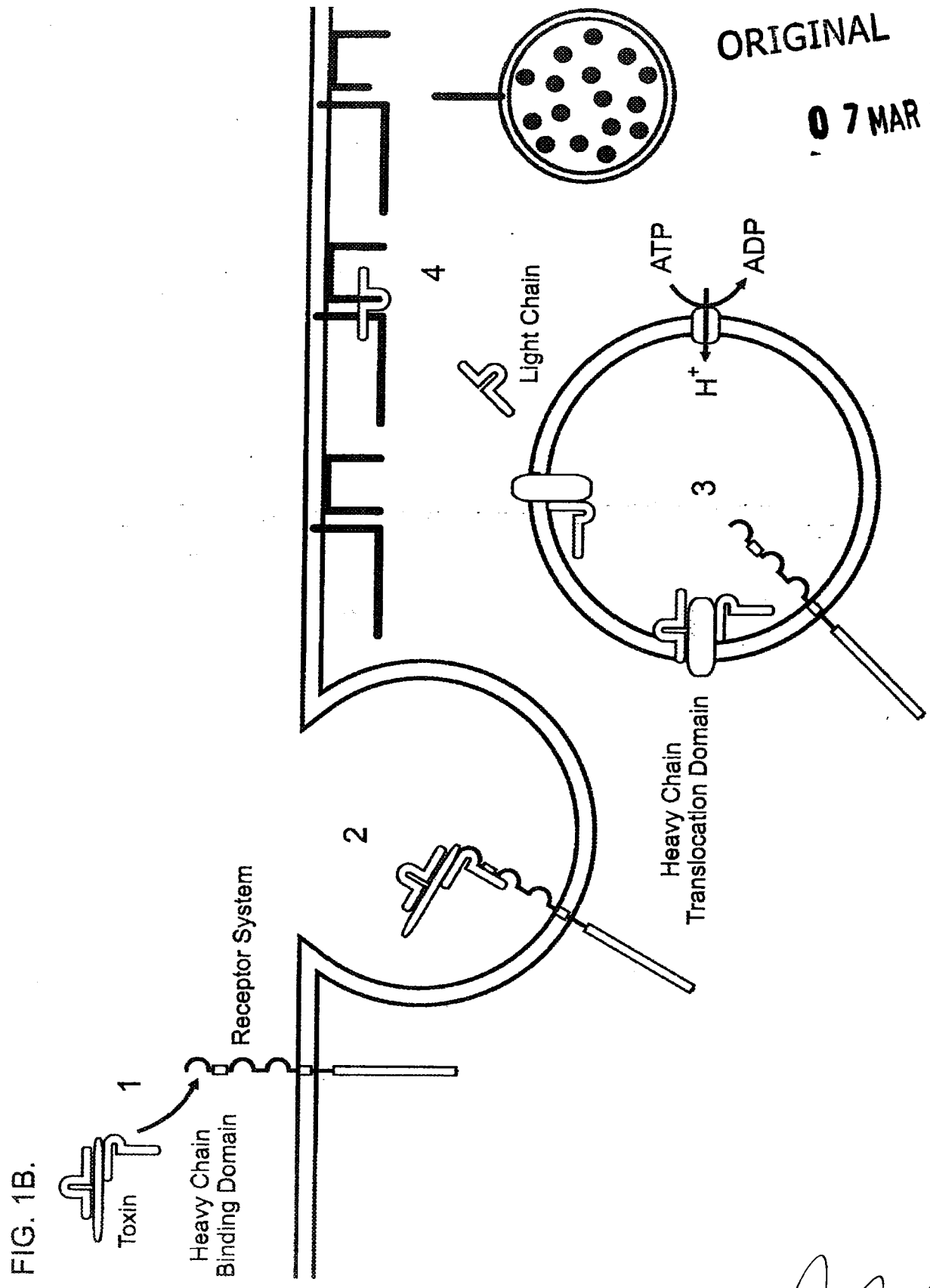

FIG. 1B.

(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

FIG. 2.

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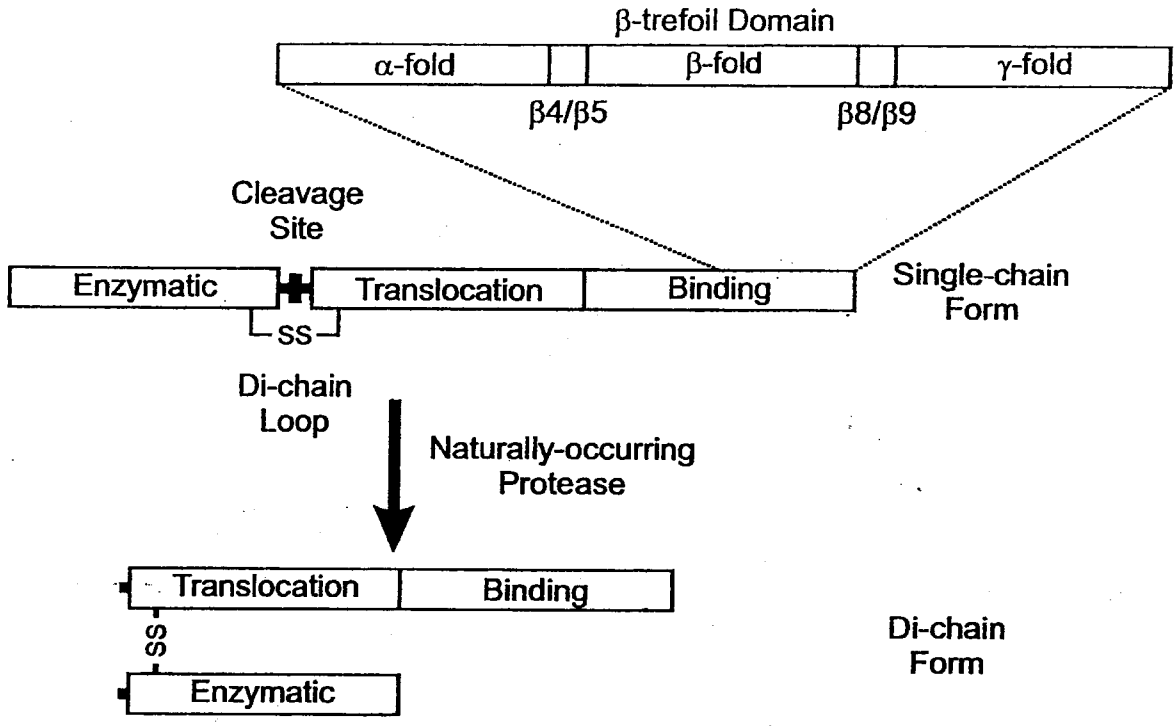

(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

FIG. 3A.

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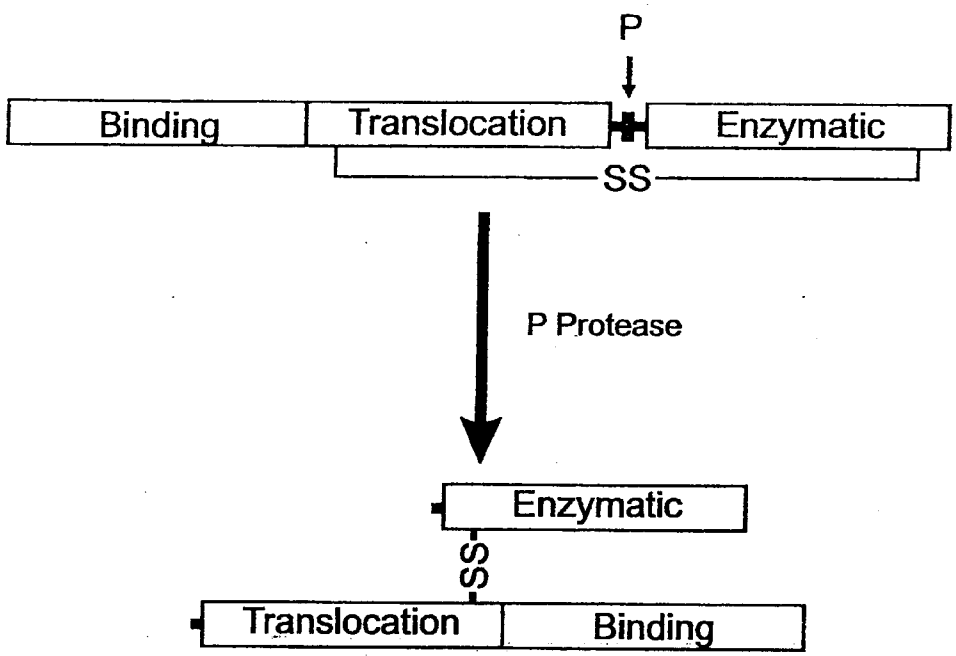
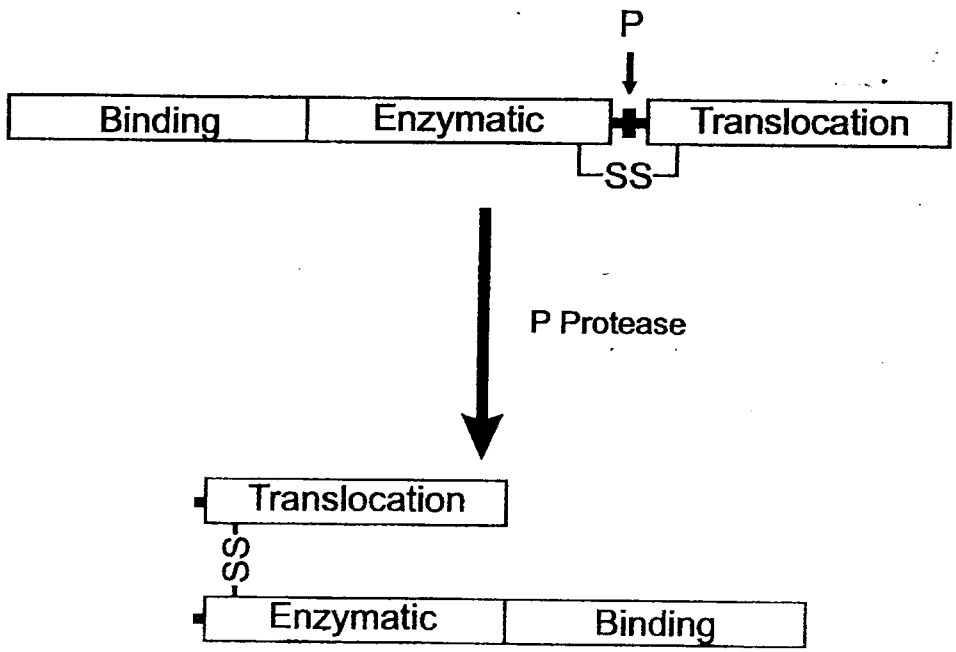


FIG. 3B.



(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

FIG. 4A.

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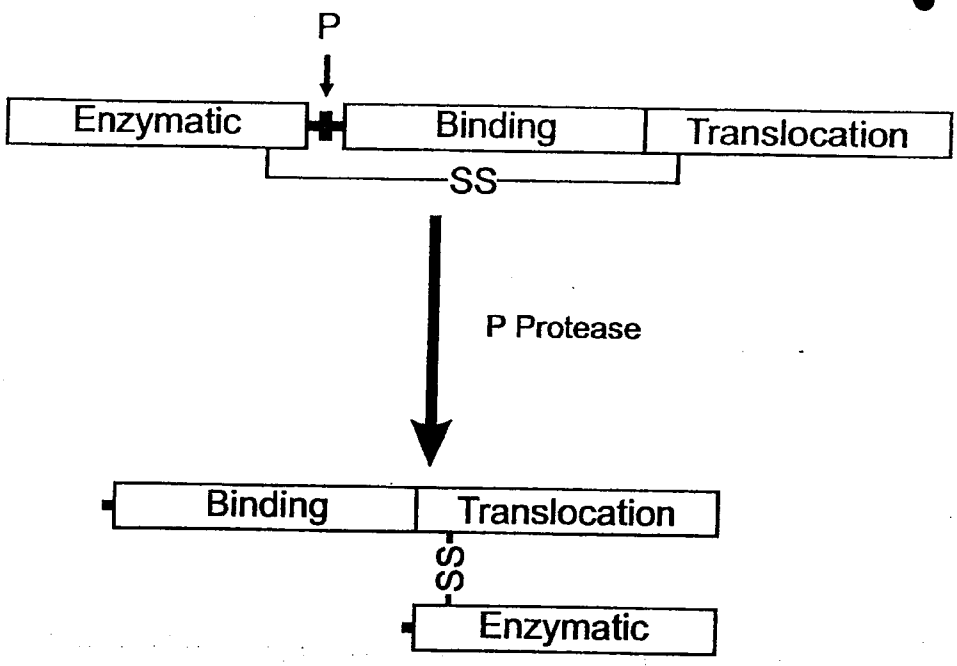
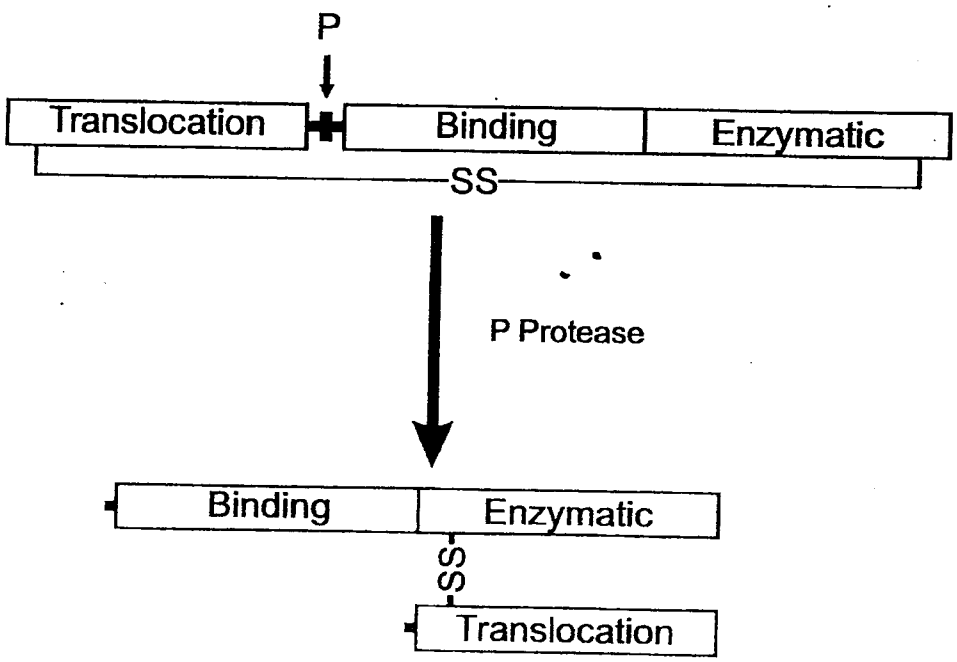


FIG. 4B.



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OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

FIG. 4C.

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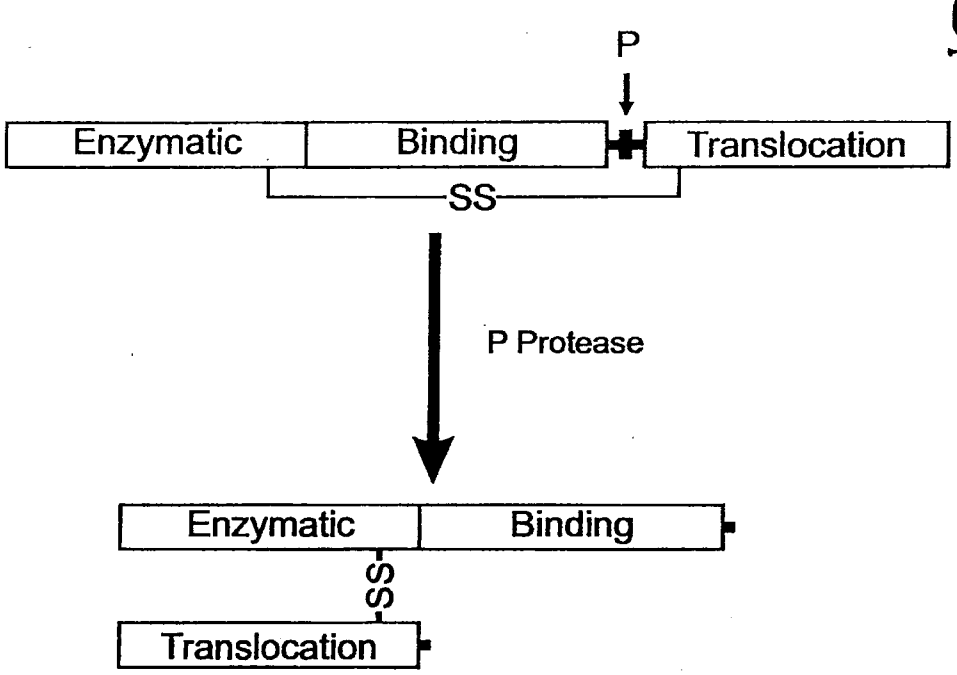
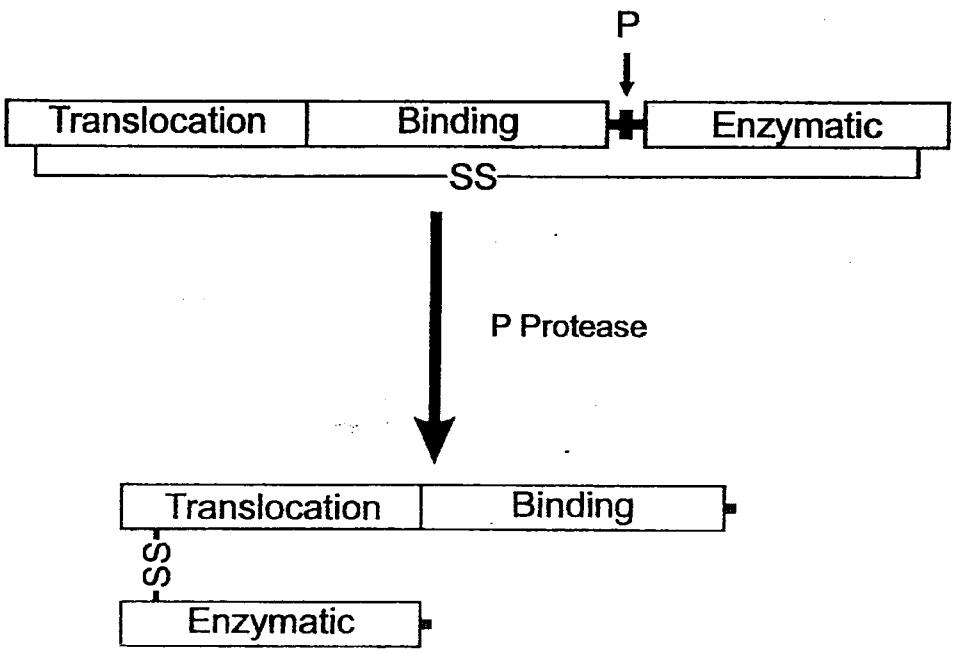


FIG. 4D.



(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

FIG. 5A.

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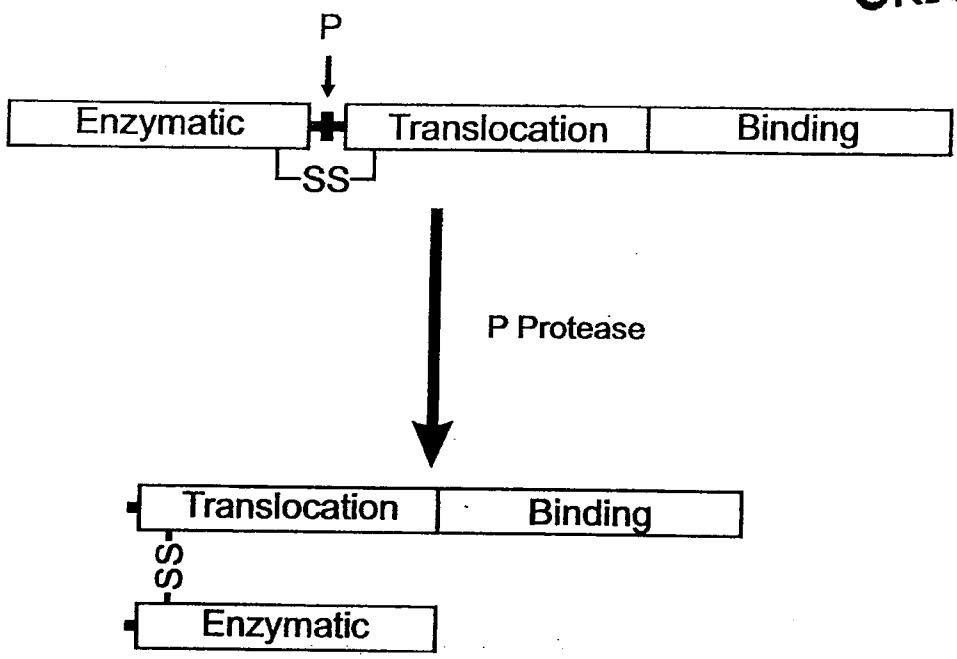
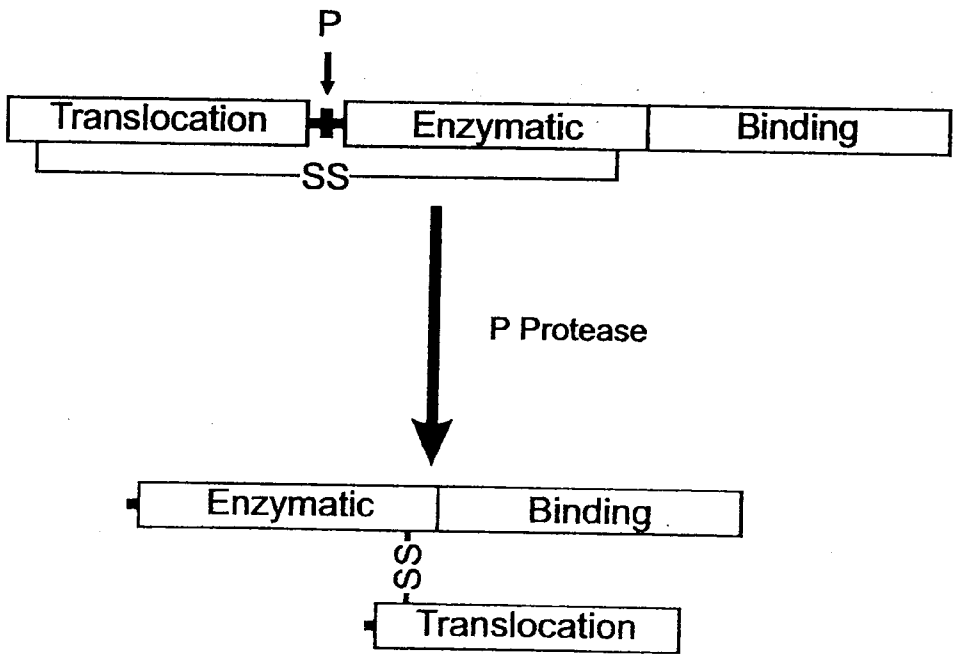


FIG. 5B.



(HRISHIKESH RAY CHAUDHURY)
OF REMFRY & SAGAR
ATTORNEY FOR THE APPLICANTS

[01]This patent application claims priority pursuant to 35 U.S.C. § 119(e) to U. S. Provisional Patent Application Serial No. 61/233,947 filed August 14, 2009, which is hereby incorporated by reference in its entirety.

[02]Cancer is a group of more than 100 diseases in which a group of cells display uncontrolled growth (cell division beyond the normal limits). In most cases, cancer cells form a clump of cells called a tumor, although in some cancers, like leukemia, the cells do not form tumors. Tumors may be malignant or benign. Besides, malignant tumors (or cancers) comprise cells with abnormal genetic material and usually undergo rapid uncontrolled cell growth, invade and destroy adjacent tissue, and sometimes spread to other locations in the body via lymph or blood *(i.e.,* metastasis). Cancer is associated with a high incidence of mortality because if the invasion and metastasis of the cancer cells throughout the body are not stopped, cancer cells will invade vital organs and lead to the dysfunction of the organs and eventual death. The malignant properties of cancers differentiate them from benign tumors, which are usually slow-growing and self-limited, do not invade or metastasize, and as such, are generally not life-threatening. Cancers at the local, regional or distant stage are considered invasive. A very early cancer found in only a few layers of cells, called in situ cancer, is considered non-invasive.

[03]Cancer is a diverse class of diseases which differ widely in their causes and biology. Cancers are caused by a variety of factors working alone or in combination. Some cancers are caused by external factors such as tobacco, diet, certain chemicals, radiation, and viruses. Other cancers are caused by internal factors such as hormones, immune conditions, and inherited genetic mutations. Usually ten or more years pass between exposure to a factor that causes cancer and detectable disease.

[04]Cancers are generally classified by the type of cell that resembles the tumor and, therefore, the tissue presumed to be the origin of the tumor. Carcinomas are malignant tumors derived from epithelial cells. This group represents the most common cancers, including the common forms of breast, prostate, lung and colon cancer. Sarcomas are malignant tumors derived from connective tissue, or mesenchymal cells. Blastomas are usually malignant tumors which resembles an immature or embryonic tissue. Many of these tumors are most common in children. Lymphomas and leukemias are malignancies derived from hematopoietic (blood-forming) cells. Lastly, germ cell tumors are tumors derived from totipotent cells. In adults most often found in the testicle and ovary; in fetuses, babies, and young children most often found on the body midline, particularly at the tip of the tailbone.

[05]Cancer is the second leading cause of death in the U.S., with 1,228,600 new cases and 564,800 deaths estimated for 1998. Over the past 50 years, the death rate from cancer has increased steadily, due mainly to a large rise in lung cancer death rates resulting from smoking. Cancer occurs in people of all ages, but its occurrence increases greatly in people over 45 years of age. However, cancer is the leading cause of death in the United States for people between the ages of 35 and 65 and it is also the

leading cause of non-accidental death among U.S. children under age 15. Men have a higher mortality rate due to cancer than women, and blacks have the highest cancer mortality rate of any major racial group. In the U.S., men have about a 1 in 2 lifetime risk of developing cancer and women have about a 1 in 3 lifetime risk. With the anticipated continued decrease in deaths from heart disease and strokes, cancer will become the overall leading cause of death for the entire American population by the year 2010.

[06]Diagnosis of cancer usually requires a histological examination of a tissue biopsy specimen by a pathologist, although the initial indication of malignancy can be symptoms or radiographic imaging abnormalities. Once diagnosed, cancer is commonly treated by surgery, chemotherapy, radiotherapy, or targeted therapies like immunotherapy, hormonal therapy, or angiogenesis inhibitor therapy. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient (performance status). Furthermore, depending on the type and stage of the cancer, two or more of these types of cancer treatments may be combined at the same time or used after one another. Although complete removal of the cancer without damage to the rest of the body is the goal of treatment, current approaches to treating cancer have met with limited success. With respect to surgery, this is due, in part, to the propensity of individual or small numbers of cancer cells to invade adjacent tissue or metastasis to distant sites, thereby limiting the effectiveness of local surgical treatments. The effectiveness of chemotherapy and radiotherapy is often limited by toxicity to or damage of normal tissues in the body. Although targeted therapies are promising, as implied by their name, these treatments are usually specific for one particular type of cancer. Therefore, compounds and methods that can target all cancer cells, regardless of their location would be highly desirable for the treatment of cancer. In addition, compounds and methods that can target a particular type of cancer for which no current targeted therapy exists would also be highly desirable.

[07]The ability of Clostridial toxins, such as, *e.g.,* Botulinum neurotoxins (BoNTs), BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F and BoNT/G, and Tetanus neurotoxin (TeNT), to inhibit neuronal transmission are being exploited in a wide variety of therapeutic and cosmetic applications, see e.g., William J. Lipham, COSMETIC AND CLINICAL APPLICATIONS OF BOTULINUM TOXIN (Slack, Inc., 2004). Clostridial toxins commercially available as pharmaceutical compositions include, BoNT/A preparations, such as, *e.g.,* BOTOX® (Allergan, Inc., Irvine, CA), DYSPORT®/RELOXIN®, (Beaufour Ipsen, Porton Down, England), NEURONOX® (Medy-Tox, Inc., Ochang-myeon, South Korea) BTX-A (Lanzhou Institute Biological Products, China) and XEOMIN® (Merz Pharmaceuticals, GmbH., Frankfurt, Germany); and BoNT/B preparations, such as, e.g., MYOBLOC™/NEUROBLOC™ (Solstice Neurosciences, Inc. San Francisco, CA). As an example, BOTOX® is currently approved in one or more countries for the following indications: achalasia, adult spasticity, anal fissure, back pain, blepharospasm, bruxism, cervical dystonia, essential tremor, glabellar lines or hyperkinetic facial lines, headache, hemifacial spasm, hyperactivity of bladder, hyperhidrosis, juvenile cerebral palsy, multiple sclerosis, myoclonic disorders, nasal labial lines, spasmodic dysphonia, strabismus and VII nerve disorder.

[08]A Clostridial toxin treatment inhibits neurotransmitter release by disrupting the exocytotic process used to secret the neurotransmitter into the synaptic cleft. This disruption is ultimately accomplished by intracellular delivery of a Clostridial toxin light chain comprising an enzymatic domain where it cleaves a SNARE protein essential for the exocytotic process. There is a great desire by the pharmaceutical industry to expand the use of Clostridial toxin therapies beyond its current myo-relaxant applications to treat other ailments, such a s, *e.g.,* various kinds of sensory nerve-based ailments like chronic pain, neurogenic inflammation and urogentital disorders, as well as non-nerve-based disorders, such as, e.g., pancreatitis and cancer. One approach that is currently being exploited to expand Clostridial toxin-based therapies involves modifying a Clostridial toxin so that the modified toxin has an altered cell targeting capability for a non-Clostridial toxin target cell. This re-targeted capability is achieved by replacing a naturally-occurring targeting domain of a Clostridial toxin with a targeting domain showing a selective binding activity for a non-Clostridial toxin receptor present in a non-Clostridial toxin target cell. Such modifications to a targeting domain result in a modified toxin that is able to selectively bind to a non-Clostridial toxin receptor (target receptor) present on a non-Clostridial toxin target cell (re-targeted). A modified Clostridial toxin with a targeting activity for a non-Clostridial toxin target cell can bind to a receptor present on the non-Clostridial toxin target cell, translocate into the cytoplasm, and exert its proteolytic effect on the SNARE complex of the non-Clostridial toxin target cell. In essence, a Clostridial toxin light chain comprising an enzymatic domain is intracellular^ delivered to any desired cell by selecting the appropriate targeting domain.

[09]The present specification discloses a class of modified Clostridial toxins retargeted to a non-Clostridial toxin receptor called Targeted Vesicular Exocytosis Modulating Proteins (TVEMPs), compositions comprising TVEMPs, and methods for treating an individual suffering from a cancer. A TVEMP is a recombinantly produced protein that comprises a targeting domain, and a translocation domain and enzymatic domain of a Clostridial toxin. The targeting is selected for its ability to bind to a receptor present on a target cancer cell of interest. The Clostridial toxin translocation domain and enzymatic domain serve to deliver the enzymatic domain into the cytoplasm of the target cell where it cleaves its cognate SNARE substrate. SNARE protein cleavage disrupts exocytosis, the process of cellular secretion or excretion in which substances contained in intracellular vesicles are discharged from the cell by fusion of the vesicular membrane with the outer cell membrane. This disruption prevents many fundamental processes of the cell, including, without limitation, insertion of transmembrane proteins including cell-surface receptors and signal transduction proteins; transportation of extracellular matrix proteins into the extracellular space; secretion of proteins including growth factors, angiogenic factors, neurotransmitters, hormones, and any other molecules involved in cellular communication; and expulsion of material including waste products, metabolites, and other unwanted or detrimental molecules. As such, exocytosis disruption severely affects cellular metabolism and ultimately cell viability. Thus a therapeutic molecule that reduces or inhibits exocytosis of a cell decreases the ability of a cell to survive. Based on this premise, the TVEMPs disclosed herein are designed to target cancer cells, where

[010] Thus, aspects of the present invention provide a composition comprising a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. TVEMPs useful for the development of such compositions are described in, e.g., Steward, L.E. et al., *Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells,* U.S. Patent Application No. 11/776,075 (Jul. 11, 2007); Dolly, J.O. et al., *Activatable Clostridial Toxins,* U.S. Patent Application No. 11/829,475 (Jul. 27, 2007); Foster, K.A. et al., *Fusion Proteins,* International Patent Publication WO 2006/059093 (Jun. 8, 2006); and Foster, K.A. et al., *Non-Cytotoxic Protein Conjugates,* International Patent Publication WO 2006/059105 (Jun. 8, 2006), each of which is incorporated by reference in its entirety. A composition comprising a TVEMP can be a pharmaceutical composition. Such a pharmaceutical composition can comprise, in addition to a TVEMP, a pharmaceutical carrier, a pharmaceutical component, or both.

[011] Other aspects of the present invention provide a method of treating cancer in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, wherein administration of the composition reduces a symptom associated with cancer. It is envisioned that any TVEMP disclosed herein can be used, including those disclosed in, *e.g.,* Steward, *supra,* (2007); Dolly, *supra,* (2007); Foster, *supra,* WO 2006/059093 (2006); and Foster, *supra,* WO 2006/059105 (Jun. 8, 2006). The disclosed methods provide a safe, inexpensive, out patient-based treatment for the treatment of cancer.

[012] Other aspects of the present invention provide a method of treating cancer in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, wherein administration of the composition reduces a symptom associated with cancer. It is envisioned that any TVEMP disclosed herein can be used, including those disclosed in, e.g., Steward, *supra,* (2007); Dolly, *supra,* (2007); Foster, *supra,* WO 2006/059093 (2006); and Foster, *supra,* WO 2006/059105 (Jun. 8, 2006).

[013] Still other aspects of the present invention provide a use of a TVEMP in the manufacturing a medicament for treating cancer in a mammal in need thereof, wherein the TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain and wherein administration of a therapeutically effective amount of the medicament to the mammal reduces a symptom associated with cancer. It is envisioned that any TVEMP disclosed herein can be used,

[014] Still other aspects of the present invention provide a use of a TVEMP in the treatment of cancer in a mammal in need thereof, the use comprising the step of administering to the mammal a therapeutically effective amount of the TVEMP, wherein the TVEMP comprising a targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain and wherein administration of the TVEMP reduces a symptom associated with cancer. It is envisioned that any TVEMP disclosed herein can be used, including those disclosed in, *e.g.,* Steward, *supra,* (2007); Dolly, *supra,* (2007); Foster, *supra,* WO 2006/059093 (2006); and Foster, *supra,* WO 2006/059105 (Jun. 8, 2006).

BRIEF DESCRIPTION OF THE DRAWINGS

[015] **FIG. 1** shows a schematic of the current paradigm of neurotransmitter release and Clostridial toxin intoxication in a central and peripheral neuron. FIG. 1A shows a schematic for the neurotransmitter release mechanism of a central and peripheral neuron. The release process can be described as comprising two steps: 1) vesicle docking, where the vesicle-bound SNARE protein of a vesicle containing neurotransmitter molecules associates with the membrane-bound SNARE proteins located at the plasma membrane; and 2) neurotransmitter release, where the vesicle fuses with the plasma membrane and the neurotransmitter molecules are exocytosed. FIG. 1B shows a schematic of the intoxication mechanism for tetanus and botulinum toxin activity in a central and peripheral neuron. This intoxication process can be described as comprising four steps: 1) receptor binding, where a Clostridial toxin binds to a Clostridial receptor system and initiates the intoxication process; 2) complex internalization, where after toxin binding, a vesicle containing the toxin/receptor system complex is endocytosed into the cell; 3) light chain translocation, where multiple events are thought to occur, including, e.g., changes in the internal pH of the vesicle, formation of a channel pore comprising the HN domain of the Clostridial toxin heavy chain, separation of the Clostridial toxin light chain from the heavy chain, and release of the active light chain and 4) enzymatic target modification, where the activate light chain of Clostridial toxin proteolytically cleaves its target SNARE substrate, such as, e.g., SNAP-25, VAMP or Syntaxin, thereby preventing vesicle docking and neurotransmitter release.

[016] **FIG. 2** shows the domain organization of naturally-occurring Clostridial toxins. The single-chain form depicts the amino to carboxyl linear organization comprising an enzymatic domain, a translocation domain, and a targeting domain. The di-chain loop region located between the translocation and enzymatic domains is depicted by the double SS bracket. This region comprises an endogenous di-chain loop protease cleavage site that upon proteolytic cleavage with a naturally-occurring protease, such as, e.g., an endogenous Clostridial toxin protease or a naturally-occurring protease produced in the environment, converts the single-chain form of the toxin into the di-chain form. Above the single-chain form, the HCC region of the Clostridial toxin binding domain is depicted. This region comprises the |3-

[017] **FIG. 3** shows TVEMPs with a targeting domain located at the amino terminus. FIG. 3A depicts the single-chain polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising a targeting domain, a translocation domain, a di-chain loop region comprising an exogenous protease cleavage site (P), and an enzymatic domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 3B depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising a targeting domain, an enzymatic domain, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

[018] **FIG. 4** shows TVEMPs with a targeting domain located between the other two domains. FIG. 4A depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising an enzymatic domain, a di-chain loop region comprising an exogenous protease cleavage site (P), a targeting domain, and a translocation domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 4B depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising a translocation domain, a di-chain loop region comprising an exogenous protease cleavage site (P), a targeting domain, and an enzymatic domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 4C depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising an enzymatic domain, a targeting domain, a di-chain loop region comprising an exogenous protease cleavage site (P), and a translocation domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 4D depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising a translocation domain, a targeting domain, a di-chain loop region comprising an exogenous protease cleavage site (P), and an enzymatic domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

[019] **FIG. 5** shows TVEMPs with a targeting domain located at the carboxyl terminus. FIG. 5A depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising an enzymatic domain, a di-chain loop region comprising an exogenous protease cleavage site (P), a translocation domain, and a targeting domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form. FIG. 5B depicts the single polypeptide form of a TVEMP with an amino to carboxyl linear organization comprising a translocation domain, a di-chain loop region comprising an exogenous protease cleavage site (P), an enzymatic domain, and a targeting domain. Upon proteolytic cleavage with a P protease, the single-chain form of the toxin is converted to the di-chain form.

[020] Cancer refers to the uncontrolled growth of cells in a mammalian body, and as such is fundamentally a disease that affects the regulatory mechanism the body uses to control cell growth. In order for a normal cell to transform into a cancer cell, genes which regulate cell growth and differentiation must be altered. Genetic changes can occur at many levels, from gain or loss of entire chromosomes to a mutation affecting a single DNA nucleotide. The vast catalog of cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth: 1) self-sufficiency in growth signals; 2) insensitivity to growth-inhibitory (antigrowth) signals; 3) evasion of programmed cell death (apoptosis); 4) limitless replicative potential; 5) sustained angiogenesis; and 6) tissue invasion and metastasis. Hanahan and Weinberg, The Hallmarks of Cancer, Cell 100(1): 57-70 (2000).

[021] One way cancer cells exhibit self-sufficiency in growth signals is by the expression of oncogenes. Oncogenes may be normal genes which are expressed at inappropriately high levels, or altered genes which have novel properties. In either case, expression of these genes promote the malignant phenotype of cell growth exhibited by cancer cells through a variety of ways. Many can produce secreted factors between cells, like hormones, which encourage mitosis, the effect of which depends on the signal transduction of the receiving tissue or cells. Thus, when a hormone receptor on a recipient cell is stimulated, the signal is conducted from the surface of the cell to the cell nucleus to effect some change in gene transcription regulation at the nuclear level. Some oncogenes are part of the signal transduction system itself, or the signal receptors in cells and tissues themselves, thus controlling the sensitivity to such hormones. Oncogenes often produce mitogens, or are involved in transcription of DNA in protein synthesis, which creates the proteins and enzymes responsible for producing the products and biochemicals cells use and interact with. Mutations in proto-oncogenes, which are the normally quiescent counterparts of oncogenes, can modify their expression and function, increasing the amount or activity of the product protein. When this happens, the proto-oncogenes become oncogenes, and this transition upsets the normal balance of cell cycle regulation in the cell, making uncontrolled growth possible. The chance of cancer cannot be reduced by removing proto-oncogenes from the genome, even if this were possible, as they are critical for growth, repair and homeostasis of the organism. It is only when they become mutated that the signals for growth become excessive. Therefore, therapeutic strategies to inhibit cell growth signals in cancer cells have the potential to provide powerful tools to treat cancers exhibiting self-sufficiency in growth signals due to oncogene expression. Moreover, many cancer cells express growth factor receptors and the ligands that activate those receptors (autocrine loops). In normal tissue one type of cell expresses the growth factor receptor and another type the ligand (paracrine loops) in an effort to maintain homeostasis. Cancer cells by expressing ligand and receptor acquire self-sufficiency for growth.

[022] One way that cancer cells display an insensitivity to growth-inhibitory (antigrowth) signals is by the inhibition of expression of tumor suppressor genes. Tumor suppressor genes are genes which inhibit cell division, survival, or other properties of cancer cells. Tumor suppressor genes are often disabled by cancer-promoting genetic changes. Typically, changes in many genes are required to transform a normal cell into a cancer cell. Generally, tumor suppressors are transcription factors that are activated by cellular stress or DNA damage. Often DNA damage will cause the presence of free-floating genetic material as well as other signs, and will trigger enzymes and pathways which lead to the activation of tumor suppressor genes. The functions of such genes is to arrest the progression of the cell cycle in order to carry out DNA repair, preventing mutations from being passed on to daughter cells. Therefore, therapeutic strategies to inhibit cell division signals in cancer cells have the potential to provide powerful tools to treat cancers displaying insensitivity to growth-inhibitory signals due to the suppression of tumor suppressor gene expression.

[023] One way that cancer cells evade programmed cell death (apoptosis) is by continuous exposure to cell survival signals (antiapoptotic signals). Signals to induce cell survival or cell death are provided by sensors in the plasma membrane (i.e. death receptors) and by intracellular sensors Intracellular sensors monitor the cell's health and in response to detecting abnormalities like DNA damage, oncogene action, survival factor insufficiency, or hypoxia, they activate the death pathway. Therefore, cancer cells should undergo apoptosis as they have DNA damage, activated oncogene, or hypoxia in the center of the tumor. Several types of cancer cells are dependent on survival signals delivered by autocrine loops to counteract apoptotic signals triggered by DNA damage present in these cells. These autocrine loops are established by cancer cells through the expression of growth factor ligands and their cognate receptors. Therefore, therapeutic strategies to inhibit the reception of cell survival signals by cancer cells have the potential to provide powerful tools to treat cancers with overactivation of antiapoptotic signals. In fact, there is evidence in the literature that hormone and/or growth factor withdraw can produce apoptosis in cancer cells as the balance between survival and apoptotic signals is restored.

[024] Another acquired capability of cancer cells is the limitless replicative potential of the tumor cells. Cancer cells overcome the limits of proliferation by maintaining integrity of the telomeres and avoiding the crisis state that results from continue multiplication that erodes the telomeres. Cancer cells overexpress the enzyme telomerase that maintains the size of the telomeres and allow for limitless replicative potential. But another important step is the ability to deliver membrane to the plasma membrane to complete the mitotic process.

[025] As cells proliferate within a tumor they also face other challenges like the limited supply of oxygen and nutrients that would induce apoptosis. So to be able to sustain growth and proliferation the tumor needs to encourage the growth of existing blood vessels as well as the growth of new blood vessels, a process highly regulated in mature tissues. Cancer cells secrete pro-angiogenic factors to activate receptors in endothelial cells. In addition, pro-angiogenic factors sequestered in the extracellular matrix

[026] Finally, tumor cells acquire the capability to invade adjacent tissues and metastasize to distant sites. To accomplish that, tumor cells may first be able to change their adhesion capabilities by altering the expression of adhesion proteins and integrins. More importantly, to be able to migrate cancer cells need to be able to degrade the extracellular matrix that surround them. Cancer cells overexpress matrix degrading proteases either as secreted factors or as membrane anchored proteases and downregulate the expression of protease inhibitors.

[027] As uncontrolled cell growth is the underlying cause of all cancers, compounds and methods that can reduce or prevent this uncontrolled cell growth would be an effective treatment for cancer. The present specification discloses compounds and methods that can reduce or prevent the uncontrolled cell growth displayed by cancer cells. The novel retargeted endopeptidases comprise, in part, a binding domain and an enzymatic domain. The binding domain directs the retargeted endopeptidase to a specific cancer cell type that is expressing the cognate receptor for the binding domain. The endopeptidase activity of the enzymatic domain inhibits exocytosis by cleaving the appropriate target SNARE protein, thereby disrupting exocytosis and delivery of receptors and membrane to the plasma membrane. Preventing exocytosis in cancers cells is therapeutically useful because disruption would, e.g., 1) prevent the release of secreting growth factors by cancer cells which encourage mitosis; or 2) prevent delivery of receptors to the plasma membrane of cancer cells which would interfere with the cancer cell's ability to receive cancer-promoting signals, such as, e.g., receiving a growth stimulating signal or a cell survival signal. The later would be useful in eliminating cancer cells by tilting the balance towards apoptosis of the cancer cells; 3) prevent delivery of membrane to the plasma membrane and thus stopping the process of mitosis that can only occur with a net gain of membrane to produce daughter cells; 4) reduce angiogenesis by inhibiting the release of pro-angiogenic factors by tumor cells or the extracellular matrix; 5) inhibit invasion and metastasis by inhibiting the release of proteases and by interfering with the switch of adhesion proteins and integrins.

[028] Thus, while current cancer therapeutics in the market target only one pathway at a time and are therefore only partially effective and allow cancer cells to acquire resistance to the treatment, A TEVMP-based therapy by means of inhibition of exocytosis, receptor delivery, and membrane delivery, will target several pathways with a single drug delivering a stronger punch to tumor cells and therefore being more effective. Moreover, as normal cells are not proliferating and are not so depending on survival signals they were not be affected by the therapy.

[029] Aspects of the present invention provide, in part, a TVEMP. As used herein, a "TVEMP" means any molecule comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial

[030] Clostridial toxins are each translated as a single chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease (FIG. 1). This cleavage occurs within the discrete di-chain loop region created between two cysteine residues that form a disulfide bridge. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by the single disulfide bond and non-covalent interactions between the two chains. The naturally-occurring protease used to convert the single chain molecule into the di-chain is currently not known. In some serotypes, such as, *e.g.,* BoNT/A, the naturally-occurring protease is produced endogenously by the bacteria serotype and cleavage occurs within the cell before the toxin is release into the environment. However, in other serotypes, such as, *e.g.,* BoNT/E, the bacterial strain appears not to produce an endogenous protease capable of converting the single chain form of the toxin into the di-chain form. In these situations, the toxin is released from the cell as a single-chain toxin which is subsequently converted into the di-chain form by a naturally-occurring protease found in the environment.

[031] Each mature di-chain molecule comprises three functionally distinct domains: 1) an enzymatic domain located in the LC that includes a metalloprotease region containing a zinc-dependent endopeptidase activity which specifically targets core components of the neurotransmitter release apparatus; 2) a translocation domain contained within the amino-terminal half of the HC (H_N) that facilitates release of the LC from intracellular vesicles into the cytoplasm of the target cell; and 3) a binding domain found within the carboxyl-terminal half of the HC (H_c) that determines the binding activity and binding specificity of the toxin to the receptor complex located at the surface of the target cell. D. B. Lacy and R. C. Stevens, Sequence Homology and Structural Analysis of the Clostridial Neurotoxins, J. Mol. Biol. 291: 1091-1104 (1999). The H_c domain comprises two distinct structural features of roughly equal size, separated by an a-helix, designated the H_CN and H_Cc subdomains. Table 1 gives approximate boundary regions for each domain and subdomain found in exemplary Clostridial toxins.

Table 1. Clostridial Toxin Reference Sequences and Regions

Toxin	SEQ ID NO:	LC	Di-Chain Loop	H_N	H_C		
					H_{CN}	α-Linker	H_{CC}
BoNT/A	1	M1/P2-L429	C430-C454	I455-I873	I874-N1080	E1081-Q1091	S1092-L1296
BoNT/B	6	M1/P2-M436	C437-C446	I447-I860	L861-S1067	Q1068-Q1078	S1079-E1291
BoNT/C1	11	M1/P2-F436	C437-C453	R454-I868	N869-D1081	G1082-L1092	Q1093-E1291
BoNT/D	13	M1/T2-V436	C437-C450	I451-I864	N865-S1069	N1069-Q1079	I1080-E1276
BoNT/E	15	M1/P2-F411	C412-C426	I427-I847	K848-D1055	E1056-E1066	P1067-K1252

BoNT/F	18	M1/P2-F428	C429-C445	I446-I865	K866-D1075	K1076-E1086	P1087-E1274
BoNT/G	21	M1/P2-M435	C436-C450	I451-I865	S866-N1075	A1076-Q1086	S1087-E1297
TeNT	22	M1/P2-L438	C439-C467	I468-L881	K882-N1097	P1098-Y1108	L1109-D1315
BaNT	23	M1/P2-L420	C421-C435	I436-I857	I858-D1064	K1065-E1075	P1076-E1268
BuNT	24	M1/P2-F411	C412-C426	I427-I847	K848-D1055	E1056-E1066	P1067-K1251

[032] The binding, translocation, and enzymatic activity of these three functional domains are all necessary for toxicity. While all details of this process are not yet precisely known, the overall cellular intoxication mechanism whereby Clostridial toxins enter a neuron and inhibit neurotransmitter release is similar, regardless of serotype or subtype. Although the applicants have no wish to be limited by the following description, the intoxication mechanism can be described as comprising at least four steps: 1) receptor binding, 2) complex internalization, 3) light chain translocation, and 4) enzymatic target modification (FIG. 3). The process is initiated when the H_c domain of a Clostridial toxin binds to a toxin-specific receptor system located on the plasma membrane surface of a target cell. The binding specificity of a receptor complex is thought to be achieved, in part, by specific combinations of gangliosides and protein receptors that appear to distinctly comprise each Clostridial toxin receptor complex. Once bound, the toxin/receptor complexes are internalized by endocytosis and the internalized vesicles are sorted to specific intracellular routes. The translocation step appears to be triggered by the acidification of the vesicle compartment. This process seems to initiate two important pH-dependent structural rearrangements that increase hydrophobicity and promote formation di-chain form of the toxin. Once activated, light chain endopeptidase of the toxin is released from the intracellular vesicle into the cytosol where it appears to specifically target one of three known core components of the neurotransmitter release apparatus. These core proteins, vesicle-associated membrane protein (VAMP)/synaptobrevin, synaptosomal-associated protein of 25 kDa (SNAP-25) and Syntaxin, are necessary for synaptic vesicle docking and fusion at the nerve terminal and constitute members of the soluble /V-ethylmaleimide-sensitive factor-attachment protein-receptor (SNARE) family. BoNT/A and BoNT/E cleave SNAP-25 in the carboxyl-terminal region, releasing a nine or twenty-six amino acid segment, respectively, and BoNT/C1 also cleaves SNAP-25 near the carboxyl-terminus. The botulinum serotypes BoNT/B, BoNT/D, BoNT/F and BoNT/G, and tetanus toxin, act on the conserved central portion of VAMP, and release the amino-terminal portion of VAMP into the cytosol. BoNT/C1 cleaves syntaxin at a single site near the cytosolic membrane surface. The selective proteolysis of synaptic SNAREs accounts for the block of neurotransmitter release caused by Clostridial toxins *in vivo.* The SNARE protein targets of Clostridial toxins are common to exocytosis in a variety of non-neuronal types; in these cells, as in neurons, light chain peptidase activity inhibits exocytosis, see, e.g., Yann Humeau et al., *How Botulinum and Tetanus Neurotoxins Block Neurotransmitter Release,* 82(5) Biochimie. 427-446 (2000); Kathryn Turton et al., *Botulinum and Tetanus Neurotoxins: Structure, Function and Therapeutic Utility,* 27(11) Trends Biochem. Sci. 552-558. (2002); Giovanna Lalli et al., *The Journey of Tetanus and Botulinum Neurotoxins in Neurons,* 11(9) Trends Microbiol. 431-437, (2003).

[033] Aspects of the present specification provide, in part, a TVEMP comprising a Clostridial toxin enzymatic domain. As used herein, the term "Clostridial toxin enzymatic domain" refers to any Clostridial toxin polypeptide that can execute the enzymatic target modification step of the intoxication process. Thus, a Clostridial toxin enzymatic domain specifically targets a Clostridial toxin substrate and encompasses the proteolytic cleavage of a Clostridial toxin substrate, such as, *e.g.,* SNARE proteins like a SNAP-25 substrate, a VAMP substrate, and a Syntaxin substrate. Non-limiting examples of a Clostridial toxin enzymatic domain include, *e.g.,* a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, and a BuNT enzymatic domain.

[034] A Clostridial toxin enzymatic domain includes, without limitation, naturally occurring Clostridial toxin enzymatic domain variants, such as, e.g., Clostridial toxin enzymatic domain isoforms and Clostridial toxin enzymatic domain subtypes; and non-naturally occurring Clostridial toxin enzymatic domain variants, such as, e.g., conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, active Clostridial toxin enzymatic domain , fragments thereof, or any combination thereof.

[035] As used herein, the term "Clostridial toxin enzymatic domain variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin enzymatic domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin enzymatic domain variants useful to practice disclosed embodiments are variants that execute the enzymatic target modification step of the intoxication process. As non-limiting examples, a BoNT/A enzymatic domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 1/2-429 of SEQ ID NO: 1; a BoNT/B enzymatic domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 6; a BoNT/C1 enzymatic domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 11; a BoNT/D enzymatic domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 1/2-436 of SEQ ID NO: 13; a BoNT/E enzymatic domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 1/2-411 of SEQ ID NO: 15; a BoNT/F enzymatic domain variant will have at least one amino acid difference, such as, e.g., an amino acid

substitution, deletion or addition, as compared to amino acids 1/2-428 of SEQ ID NO: 18; a BoNT/G enzymatic domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 1/2-438 of SEQ ID NO: 21; a TeNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 1/2-438 of SEQ ID NO: 22; a BaNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 1/2-420 of SEQ ID NO: 23; and a BuNT enzymatic domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 1/2-411 of SEQ ID NO: 24.

[036] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin enzymatic domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/AI, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific enzymatic domain subtypes showing about 80% to 95% amino acid identity when compared to the BoNT/A enzymatic domain of SEQ ID NO: 1. As used herein, the term "naturally occurring Clostridial toxin enzymatic domain variant" refers to any Clostridial toxin enzymatic domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin enzymatic domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin enzymatic domain isoforms produced by spontaneous mutation and Clostridial toxin enzymatic domain subtypes. A naturally occurring Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the naturally occurring Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification.

[037] A non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain isoform such as, e.g., a BoNT/A enzymatic domain isoform, a BoNT/B enzymatic domain isoform, a BoNT/C1 enzymatic domain isoform, a BoNT/D enzymatic domain isoform, a BoNT/E enzymatic domain isoform, a BoNT/F enzymatic domain isoform, a BoNT/G enzymatic domain isoform, a TeNT enzymatic domain isoform, a BaNT enzymatic domain isoform, and a BuNT enzymatic domain isoform. Another non-limiting examples of a naturally occurring Clostridial toxin enzymatic domain variant is a Clostridial toxin enzymatic domain subtype such as, e.g., an enzymatic domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, or BoNT/A5; an enzymatic domain from subtype BoNT/B1, B0NT/B2, BoNT/Bbv, or BoNT/Bnp; an enzymatic domain from subtype BoNT/C1-1 or BoNT/C1-2; an enzymatic domain from subtype BoNT/E1, BoNT/E2 and BoNT/E3; an enzymatic domain from subtype BoNT/F1, B0NT/F2, or BoNT/F3; and an enzymatic domain from subtype BuNT-1 or BuNT-2.

[038] As used herein, the term "non-naturally occurring Clostridial toxin enzymatic domain variant" refers to any Clostridial toxin enzymatic domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin enzymatic domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin enzymatic domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin enzymatic domain variants include, *e.g.,* conservative Clostridial toxin enzymatic domain variants, non-conservative Clostridial toxin enzymatic domain variants, Clostridial toxin enzymatic domain chimeric variants, and active Clostridial toxin enzymatic domain fragments.

[039] As used herein, the term "conservative Clostridial toxin enzymatic domain variant" refers to a Clostridial toxin enzymatic domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification. Non-limiting examples of a conservative Clostridial toxin enzymatic domain variant include, *e.g.,* conservative BoNT/A enzymatic domain variants, conservative BoNT/B enzymatic domain variants, conservative BoNT/C1 enzymatic domain variants, conservative BoNT/D enzymatic domain variants, conservative BoNT/E enzymatic domain variants, conservative BoNT/F enzymatic domain variants, conservative BoNT/G enzymatic domain variants, conservative TeNT enzymatic domain variants, conservative BaNT enzymatic domain variants, and conservative BuNT enzymatic domain variants.

[040] As used herein, the term "non-conservative Clostridial toxin enzymatic domain variant" refers to a Clostridial toxin enzymatic domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin enzymatic domain sequence (Table 1). A non-conservative Clostridial toxin enzymatic domain variant can function in substantially the same manner as the reference Clostridial toxin enzymatic domain on which the non-conservative Clostridial toxin enzymatic domain variant is based, and can be substituted for the reference Clostridial toxin enzymatic domain in any aspect of the present specification. Non-limiting examples of a non-conservative Clostridial toxin enzymatic domain variant include, *e.g.,* non-conservative BoNT/A enzymatic domain variants, non-conservative BoNT/B enzymatic domain variants, non-conservative BoNT/C1

enzymatic domain variants, non-conservative BoNT/D enzymatic domain variants, non-conservative BoNT/E enzymatic domain variants, non-conservative BoNT/F enzymatic domain variants, non-conservative BoNT/G enzymatic domain variants, and non-conservative TeNT enzymatic domain variants, non-conservative BaNT enzymatic domain variants, and non-conservative BuNT enzymatic domain variants.

[041] As used herein, the term "active Clostridial toxin enzymatic domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the enzymatic domain can be useful in aspects of the present specification with the proviso that these enzymatic domain fragments can specifically target the core components of the neurotransmitter release apparatus and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The enzymatic domains of Clostridial toxins are approximately 420-460 amino acids in length and comprise an enzymatic domain (Table 1). Research has shown that the entire length of a Clostridial toxin enzymatic domain is not necessary for the enzymatic activity of the enzymatic domain. As a non-limiting example, the first eight amino acids of the BoNT/A enzymatic domain are not required for enzymatic activity. As another non-limiting example, the first eight amino acids of the TeNT enzymatic domain are not required for enzymatic activity. Likewise, the carboxyl-terminus of the enzymatic domain is not necessary for activity. As a non-limiting example, the last 32 amino acids of the BoNT/A enzymatic domain are not required for enzymatic activity. As another non-limiting example, the last 31 amino acids of the TeNT enzymatic domain are not required for enzymatic activity. Thus, aspects of this embodiment include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, *e.g.,* at least 350, 375, 400, 425, or 450 amino acids. Other aspects of this embodiment include Clostridial toxin enzymatic domains comprising an enzymatic domain having a length of, *e.g.,* at most 350, 375, 400, 425, or 450 amino acids.

[042] Any of a variety of sequence alignment methods can be used to determine percent identity, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.,* segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[043] Global methods align sequences from the beginning to the end of the molecule and determine the best alignment by adding up scores of individual residue pairs and by imposing gap penalties. Non-limiting methods include, *e.g.,* CLUSTAL W, see, *e.g.,* Julie D. Thompson et al., *CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice,* 22(22) Nucleic Acids Research 4673-4680 (1994); and iterative refinement, see, *e.g.,* Osamu Gotoh, *Significant Improvement in Accuracy of Multiple Protein Sequence Alignments by Iterative Refinement as Assessed by Reference to Structural Alignments,* 264(4) J. Mol. Biol. 823-838 (1996).

[044] Local methods align sequences by identifying one or more conserved motifs shared by all of the input sequences. Non-limiting methods include, *e.g.,* Match-box, see, *e.g.,* Eric Depiereux and Ernest Feytmans, *Match-Box: A Fundamentally New Algorithm for the Simultaneous Alignment of Several Protein Sequences,* 8(5) CABIOS 501-509 (1992); Gibbs sampling, see, *e.g.,* C. E. Lawrence et al., *Detecting Subtle Sequence Signals: A Gibbs Sampling Strategy for Multiple Alignment,* 262(5131) Science 208-214 (1993); Align-M, see, *e.g.,* Ivo Van Walle et al., *Align-M- A New Algorithm for Multiple Alignment of Highly Divergent Sequences,* 20(9) Bioinformatics,: 1428-1435 (2004).

[045] Hybrid methods combine functional aspects of both global and local alignment methods. Non-limiting methods include, *e.g.,* segment-to-segment comparison, see, *e.g.,* Burkhard Morgenstern et al., *Multiple DNA and Protein Sequence Alignment Based On Segment-To-Segment Comparison,* 93(22) Proc. Natl. Acad. Sci. U.S.A. 12098-12103 (1996); T-Coffee, see, e.g., Cedric Notredame et al., *T-Coffee: A Novel Algorithm for Multiple Sequence Alignment,* 302(1) J. Mol. Biol. 205-217 (2000); MUSCLE, see, e.g., Robert C. Edgar, *MUSCLE: Multiple Sequence Alignment With High Score Accuracy and High Throughput,* 32(5) Nucleic Acids Res. 1792-1797 (2004); and DIALIGN-T, see, e.g., Amarendran R Subramanian et al., *DIALIGN-T: An Improved Algorithm for Segment-Based Multiple Sequence Alignment,* 6(1) BMC Bioinformatics 66 (2005).

[046] The present specification describes various polypeptide variants where one amino acid is substituted for another, such as, e.g., Clostridial toxin enzymatic domain variants, Clostridial toxin translocation domain variants, targeting domain variants, and protease cleavage site variants, A substitution can be assessed by a variety of factors, such as, *e.g.,* the physic properties of the amino acid being substituted (Table 2) or how the original amino acid would tolerate a substitution (Table 3). The selections of which amino acid can be substituted for another amino acid in a polypeptide are known to a person of ordinary skill in the art.

TABLE 2. Amino Acid Properties

Property	Amino Acids
Aliphatic	G, A, I, L, M, P, V
Aromatic	F, H, W, Y
C-beta branched	I, V, T
Hydrophobic	C, F, I, L, M, V, W
Small polar	D, N, P
Small non-polar	A, C, G, S, T
Large polar	E, H, K, Q, R, W, Y
Large non-polar	F, I, L, M, V
Charged	D, E, H, K, R
Uncharged	C, S, T
Negative	D, E
Positive	H, K, R

Acidic	D, E
Basic	K, R
Amide	N, Q

TABLE 3. Amino Acid Substitutions

Amino Acid	Favored Substitution	Neutral Substitutions	Disfavored substitution
A	G, S, T	C, E, I, K, M, L, P, Q, R, V	D, F, H, N, Y, W
C	F, S, Y, W	A, H, I, M, L, T, V	D, E, G, K, N, P, Q, R
D	E, N	G, H, K, P, Q, R, S, T	A, C, I, L,
E	D, K, Q	A, H, N, P, R, S, T	C, F, G, I, L, M, V, W, Y
F	M, L, W, Y	C, I, V	A, D, E, G, H, K, N, P, Q, R, S, T
G	A, S	D, K, N, P, Q, R	C, E, F, H, I, L, M, T, V, W, Y
H	N, Y	C, D, E, K, Q, R, S, T, W	A, F, G, I, L, M, P, V
I	V, L, M	A, C, T, F, Y	D, E, G, H, K, N, P, Q, R, S, W
K	Q, E, R	A, D, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
L	F, I, M, V	A, C, W, Y	D, E, G, H, K, N, P, Q, R, S, T
M	F, I, L, V	A, C, R, Q, K, T, W, Y	D, E, G, H, N, P, S
N	D, H, S	E, G, K, Q, R, T	A, C, F, I, L, M, P, V, W, Y
P	—	A, D, E, G, K, Q, R, S, T	C, F, H, I, L, M, N, V, W, Y
Q	E, K, R	A, D, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
R	K, Q	A, D, E, G, H, M, N, P, S, T	C, F, I, L, V, W, Y
S	A, N, T	C, D, E, G, H, K, P, Q, R, T	F, I, L, M, V, W, Y
T	S	A, C, D, E, H, I, K, M, N, P, Q, R, V	F, G, L, W, Y
V	I, L, M	A, C, F, T, Y	D, E, G, H, K, N, P, Q, R, S, W
W	F, Y	H, L, M	A, C, D, E, G, I, K, N, P, Q, R, S, T, V
Y	F, H, W	C, I, L, M, V	A, D, E, G, K, N, P, Q, R, S, T

Matthew J. Betts and Robert, B. Russell, Amino Acid Properties and Consequences of Substitutions, pp. 289-316, In Bioinformatics for Geneticists, (eds Michael R. Barnes, Ian C. Gray, Wiley, 2003).

[047] Thus, in an embodiment, a TVEMP disclosed herein comprises a Clostridial toxin enzymatic domain. In an aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a naturally occurring Clostridial toxin enzymatic domain variant, such as, *e.g.,* a Clostridial toxin enzymatic domain isoform or a Clostridial toxin enzymatic domain subtype. In another aspect of this embodiment, a Clostridial toxin enzymatic domain comprises a non-naturally occurring Clostridial toxin enzymatic domain variant, such as, e.g., a conservative Clostridial toxin enzymatic domain variant, a non-conservative Clostridial toxin enzymatic domain variant, an active Clostridial toxin enzymatic domain fragment, or any combination thereof.

[048] In another embodiment, a hydrophic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another hydrophic amino acid.

Examples of hydrophic amino acids include, *e.g.,* C, F, I, L, M, V and W. In another aspect of this embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, e.g., A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.,* F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, *e.g.,* F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.,* D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, e.g., A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.,* K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, e.g., D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another small amino acid. Examples of small amino acids include, *e.g.,* A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the Clostridial toxin enzymatic domain can be substituted with another C-beta branching amino acid. Examples of C-beta branching amino acids include, e.g., I, T and V.

[049] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/A enzymatic domain. In an aspect of this embodiment, a BoNT/A enzymatic domain comprises the enzymatic domains of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In other aspects of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises a naturally occurring BoNT/A enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/A isoform or an enzymatic domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises a naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.,* a BoNT/A isoform enzymatic domain or a BoNT/A subtype enzymatic domain. In another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of a naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, e.g., a BoNT/A isoform enzymatic domain or a BoNT/A subtype enzymatic domain. In

still another aspect of this embodiment, a BoNT/A enzymatic domain comprises a non-naturally occurring BoNT/A enzymatic domain variant, such as, *e.g.,* a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, *e.g.,* a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A enzymatic domain comprises amino acids 1/2-429 of a non-naturally occurring BoNT/A enzymatic domain variant of SEQ ID NO: 1, such as, *e.g.,* a conservative BoNT/A enzymatic domain variant, a non-conservative BoNT/A enzymatic domain variant, an active BoNT/A enzymatic domain fragment, or any combination thereof.

[050] In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-429 of SEQ ID NO: 1; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-429 of SEQ ID NO: 1.

[051] In other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5,6, 7, 8, 9, 10,20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In

further other aspects of this embodiment, a BoNT/A enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-429 of SEQ ID NO: 1.

[052] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/B enzymatic domain. In an aspect of this embodiment, a BoNT/B enzymatic domain comprises the enzymatic domains of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In other aspects of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises a naturally occurring BoNT/B enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/B isoform or an enzymatic domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises a naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.,* a BoNT/B isoform enzymatic domain or a BoNT/B subtype enzymatic domain. In another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, such as, *e.g.,* a BoNT/B isoform enzymatic domain or a BoNT/B subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises a non-naturally occurring BoNT/B enzymatic domain variant, such as, e.g., a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO:.8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.,* a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/B enzymatic domain variant of SEQ ID NO: 6, such as, e.g., a conservative BoNT/B enzymatic domain variant, a non-conservative BoNT/B enzymatic domain variant, an active BoNT/B enzymatic domain fragment, or any combination thereof.

[053] In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%,

at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 6; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 6.

[054] In other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/pr substitutions relative to the enzymatic domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In further other aspects of this embodiment, a BoNT/B enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 6.

[055] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/C1 enzymatic domain. In an aspect of this embodiment, a BoNT/C1 enzymatic domain comprises the enzymatic domains of SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 11. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a naturally occurring BoNT/C1 enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/C1 isoform or an enzymatic domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, e.g., a BoNT/C1 isoform enzymatic domain or a BoNT/C1 subtype enzymatic domain. In another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11, such as, e.g., a BoNT/C1 isoform enzymatic domain or a BoNT/C1 subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises a non-naturally occurring BoNT/C1 enzymatic domain variant, such as, e.g., a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1

enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, *e.g.,* a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/C1 enzymatic domain variant of SEQ ID NO: 11, such as, *e.g.,* a conservative BoNT/C1 enzymatic domain variant, a non-conservative BoNT/C1 enzymatic domain variant, an active BoNT/C1 enzymatic domain fragment, or any combination thereof.

[056] In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 11; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 11.

[057] In other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11. In still other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1,2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 11 or SEQ ID NO: 12. In further other aspects of this embodiment, a BoNT/C1 enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 11.

[058] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/D enzymatic domain. In an aspect of this embodiment, a BoNT/D enzymatic domain comprises the enzymatic domains of SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of SEQ ID NO: 13. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises a naturally occurring BoNT/D enzymatic domain variant, such as, *e.g.,* an enzymatic domain from a BoNT/D isoform or an enzymatic domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises a naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, e.g., a BoNT/D isoform enzymatic domain or a BoNT/D subtype enzymatic domain. In another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of a naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13, such as, *e.g.,* a BoNT/D isoform enzymatic domain or a BoNT/D subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises a non-naturally occurring BoNT/D enzymatic domain variant, such as, *e.g.,* a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, e.g., a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D enzymatic domain comprises amino acids 1/2-436 of a non-naturally occurring BoNT/D enzymatic domain variant of SEQ ID NO: 13, such as, e.g., a conservative BoNT/D enzymatic domain variant, a non-conservative BoNT/D enzymatic domain variant, an active BoNT/D enzymatic domain fragment, or any combination thereof.

[059] In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-436 of SEQ ID NO: 13; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-436 of SEQ ID NO: 13.

[060] In other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions,

additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13. In still other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 13 or SEQ ID NO: 14. In further other aspects of this embodiment, a BoNT/D enzymatic domain comprises a polypeptide having, e.g., at least 1,j2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-436 of SEQ ID NO: 13.

[061] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/E enzymatic domain. In an aspect of this embodiment, a BoNT/E enzymatic domain comprises the enzymatic domains of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In other aspects of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1/2-411 of SEQ ID NO: 15. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises a naturally occurring BoNT/E enzymatic domain variant, such as, e.g., an enzymatic domain from a BoNT/E isoform or an enzymatic domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises a naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, e.g., a BoNT/E isoform enzymatic domain or a BoNT/E subtype enzymatic domain. In another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1/2-411 of a naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, such as, e.g., a BoNT/E isoform enzymatic domain or a BoNT/E subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises a non-naturally occurring BoNT/E enzymatic domain variant, such as, e.g., a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, e.g., a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E enzymatic domain comprises amino acids 1/2-411 of a non-naturally occurring BoNT/E enzymatic domain variant of SEQ ID NO: 15, such as, e.g., a conservative BoNT/E enzymatic domain variant, a non-conservative BoNT/E enzymatic domain variant, an active BoNT/E enzymatic domain fragment, or any combination thereof.

[062] In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-411 of SEQ ID NO: 15; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-411 of SEQ ID NO: 15.

[063] In other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15. In still other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In further other aspects of this embodiment, a BoNT/E enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 15.

[064] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/F enzymatic domain. In an aspect of this embodiment, a BoNT/F enzymatic domain comprises the enzymatic domains of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In other aspects of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of SEQ ID NO: 18. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises a naturally occurring BoNT/F enzymatic domain variant, such as, *e.g.,* an enzymatic domain from a BoNT/F isoform or an enzymatic domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises a

naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, e.g., a BoNT/F isoform enzymatic domain or a BoNT/F subtype enzymatic domain. In another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of a naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, such as, *e.g.,* a BoNT/F isoform enzymatic domain or a BoNT/F subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises a non-naturally occurring BoNT/F enzymatic domain variant, such as, *e.g.,* a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, e.g., a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F enzymatic domain comprises amino acids 1/2-428 of a non-naturally occurring BoNT/F enzymatic domain variant of SEQ ID NO: 18, such as, e.g., a conservative BoNT/F enzymatic domain variant, a non-conservative BoNT/F enzymatic domain variant, an active BoNT/F enzymatic domain fragment, or any combination thereof.

[065] In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-428 of SEQ ID NO: 18; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-428 of SEQ ID NO: 18.

[066] In other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18. In still other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or

substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In further other aspects of this embodiment, a BoNT/F enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-428 of SEQ ID NO: 18.

[067] In another embodiment, a Clostridial toxin enzymatic domain comprises a BoNT/G enzymatic domain. In an aspect of this embodiment, a BoNT/G enzymatic domain comprises the enzymatic domains of SEQ ID NO: 21. In other aspects of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises a naturally occurring BoNT/G enzymatic domain variant, such as, *e.g.,* an enzymatic domain from a BoNT/G isoform or an enzymatic domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises a naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.,* a BoNT/G isoform enzymatic domain or a BoNT/G subtype enzymatic domain. In another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of a naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.,* a BoNT/G isoform enzymatic domain or a BoNT/G subtype enzymatic domain. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises a non-naturally occurring BoNT/G enzymatic domain variant, such as, *e.g.,* a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises the enzymatic domain of a non-naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.,* a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G enzymatic domain comprises amino acids 1/2-4435 of a non-naturally occurring BoNT/G enzymatic domain variant of SEQ ID NO: 21, such as, *e.g.,* a conservative BoNT/G enzymatic domain variant, a non-conservative BoNT/G enzymatic domain variant, an active BoNT/G enzymatic domain fragment, or any combination thereof.

[068] In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to

amino acids 1/2-4435 of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-4435 of SEQ ID NO: 21.

[069] In other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 21. In further other aspects of this embodiment, a BoNT/G enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-4435 of SEQ ID NO: 21.

[070] In another embodiment, a Clostridial toxin enzymatic domain comprises a TeNT enzymatic domain. In an aspect of this embodiment, a TeNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 22. In other aspects of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of SEQ ID NO: 22. In another aspect of this embodiment, a TeNT enzymatic domain comprises a naturally occurring TeNT enzymatic domain variant, such as, e.g., an enzymatic domain from a TeNT isoform or an enzymatic domain from a TeNT subtype. In another aspect of this embodiment, a TeNT enzymatic domain comprises a naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, e.g., a TeNT isoform enzymatic domain or a TeNT subtype enzymatic domain. In another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of a naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, *e.g.,* a TeNT isoform enzymatic domain or a TeNT subtype enzymatic domain. In still another aspect of this embodiment, a TeNT enzymatic domain comprises a non-naturally occurring TeNT enzymatic domain variant, such as, e.g., a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, e.g., a conservative TeNT enzymatic domain

variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT enzymatic domain comprises amino acids 1/2-438 of a non-naturally occurring TeNT enzymatic domain variant of SEQ ID NO: 22, such as, e.g., a conservative TeNT enzymatic domain variant, a non-conservative TeNT enzymatic domain variant, an active TeNT enzymatic domain fragment, or any combination thereof.

[071] In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-438 of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-438 of SEQ ID NO: 22.

[072] In other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 22. In further other aspects of this embodiment, a TeNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-438 of SEQ ID NO: 22.

[073] In another embodiment, a Clostridial toxin enzymatic domain comprises a BaNT enzymatic domain. In an aspect of this embodiment, a BaNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 23. In other aspects of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of SEQ ID NO: 23. In another aspect of this embodiment, a BaNT enzymatic domain

comprises a naturally occurring BaNT enzymatic domain variant, such as, *e.g.,* an enzymatic domain from a BaNT isoform or an enzymatic domain from a BaNT subtype. In another aspect of this embodiment, a BaNT enzymatic domain comprises a naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.,* a BaNT isoform enzymatic domain or a BaNT subtype enzymatic domain. In another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of a naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, e.g., a BaNT isoform enzymatic domain or a BaNT subtype enzymatic domain. In still another aspect of this embodiment, a BaNT enzymatic domain comprises a non-naturally occurring BaNT enzymatic domain variant, such as, *e.g.,* a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.,* a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT enzymatic domain comprises amino acids 1/2-420 of a non-naturally occurring BaNT enzymatic domain variant of SEQ ID NO: 23, such as, *e.g.,* a conservative BaNT enzymatic domain variant, a non-conservative BaNT enzymatic domain variant, an active BaNT enzymatic domain fragment, or any combination thereof.

[074] In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-420 of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-420 of SEQ ID NO: 23.

[075] In other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid

deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 23. In further other aspects of this embodiment, a BaNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-420 of SEQ ID NO: 23.

[076] In another embodiment, a Clostridial toxin enzymatic domain comprises a BuNT enzymatic domain. In an aspect of this embodiment, a BuNT enzymatic domain comprises the enzymatic domains of SEQ ID NO: 24 or SEQ ID NO: 25. In other aspects of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of SEQ ID NO: 24. In another aspect of this embodiment, a BuNT enzymatic domain comprises a naturally occurring BuNT enzymatic domain variant, such as, e.g., an enzymatic domain from a BuNT isoform or an enzymatic domain from a BuNT subtype. In another aspect of this embodiment, a BuNT enzymatic domain comprises a naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, e.g., a BuNT isoform enzymatic domain or a BuNT subtype enzymatic domain. In another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of a naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24, such as, *e.g.,* a BuNT isoform enzymatic domain or a BuNT subtype enzymatic domain. In still another aspect of this embodiment, a BuNT enzymatic domain comprises a non-naturally occurring BuNT enzymatic domain variant, such as, e.g., a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT enzymatic domain comprises the enzymatic domain of a non-naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, e.g., a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT enzymatic domain comprises amino acids 1/2-411 of a non-naturally occurring BuNT enzymatic domain variant of SEQ ID NO: 24, such as, e.g., a conservative BuNT enzymatic domain variant, a non-conservative BuNT enzymatic domain variant, an active BuNT enzymatic domain fragment, or any combination thereof.

[077] In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most

70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25.

[078] In other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 OR SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the enzymatic domain of SEQ ID NO: 24 or SEQ ID NO: 25. In further other aspects of this embodiment, a BuNT enzymatic domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 1/2-411 of SEQ ID NO: 24 or SEQ ID NO: 25.

[079] The "translocation domain" comprises a portion of a Clostridial neurotoxin heavy chain having a translocation activity. By "translocation" is meant the ability to facilitate the transport of a polypeptide through a vesicular membrane, thereby exposing some or all of the polypeptide to the cytoplasm. In the various botulinum neurotoxins translocation is thought to involve an allosteric conformational change of the heavy chain caused by a decrease in pH within the endosome. This conformational change appears to involve and be mediated by the N terminal half of the heavy chain and to result in the formation of pores in the vesicular membrane; this change permits the movement of the proteolytic light chain from within the endosomal vesicle into the cytoplasm. See e.g., Lacy, et al., *Nature Struct. Biol.* 5:898-902 (October 1998).

[080] The amino acid sequence of the translocation-mediating portion of the botulinum neurotoxin heavy chain is known to those of skill in the art; additionally, those amino acid residues within this portion that are known to be essential for conferring the translocation activity are also known. It would therefore be well within the ability of one of ordinary skill in the art, for example, to employ the naturally occurring N-terminal peptide half of the heavy chain of any of the various *Clostridium tetanus* or *Clostridium botulinum*

neurotoxin subtypes as a translocation domain, or to design an analogous translocation domain by aligning the primary sequences of the N-terminal halves of the various heavy chains and selecting a consensus primary translocation sequence based on conserved amino acid, polarity, steric and hydrophobicity characteristics between the sequences.

[081] Aspects of the present specification provide, in part, a TVEMP comprising a Clostridial toxin translocation domain. As used herein, the term "Clostridial toxin translocation domain" refers to any Clostridial toxin polypeptide that can execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. Thus, a Clostridial toxin translocation domain facilitates the movement of a Clostridial toxin light chain across a membrane and encompasses the movement of a Clostridia! toxin light chain through the membrane an intracellular vesicle into the cytoplasm of a cell. Non-limiting examples of a Clostridial toxin translocation domain include, *e.g.,* a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, and a BuNT translocation domain.

[082] A Clostridial toxin translocation domain includes, without limitation, naturally occurring Clostridial toxin translocation domain variants, such as, e.g., Clostridial toxin translocation domain isoforms and Clostridial toxin translocation domain subtypes; non-naturally occurring Clostridial toxin translocation domain variants, such as, e.g., conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, active Clostridial toxin translocation domain fragments thereof, or any combination thereof.

[083] As used herein, the term "Clostridial toxin translocation domain variant," whether naturally-occurring or non-naturally-occurring, refers to a Clostridial toxin translocation domain that has at least one amino acid change from the corresponding region of the disclosed reference sequences (Table 1) and can be described in percent identity to the corresponding region of that reference sequence. Unless expressly indicated, Clostridial toxin translocation domain variants useful to practice disclosed embodiments are variants that execute the translocation step of the intoxication process that mediates Clostridial toxin light chain translocation. As non-limiting examples, a BoNT/A translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 455-873 of SEQ ID NO: 1; a BoNT/B translocation domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 447-860 of SEQ ID NO: 6; a BoNT/C1 translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 454-868 of SEQ ID NO: 11; a BoNT/D translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 451-864 of SEQ ID NO: 13; a BoNT/E translocation domain variant will have at

least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 427-847 of SEQ ID NO: 15; a BoNT/F translocation domain variant will have at least one amino acid difference, such as, *e.g.,* an amino acid substitution, deletion or addition, as compared to amino acids 446-865 of SEQ ID NO: 18; a BoNT/G translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 451-865 of SEQ ID NO: 21; a TeNT translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 468-881 of SEQ ID NO: 22; a BaNT translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 436-857 of SEQ ID NO: 23; and a BuNT translocation domain variant will have at least one amino acid difference, such as, e.g., an amino acid substitution, deletion or addition, as compared to amino acids 427-847 of SEQ ID NO: 24.

[084] It is recognized by those of skill in the art that within each serotype of Clostridial toxin there can be naturally occurring Clostridial toxin translocation domain variants that differ somewhat in their amino acid sequence, and also in the nucleic acids encoding these proteins. For example, there are presently five BoNT/A subtypes, BoNT/AI, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5, with specific translocation domain subtypes showing about 85-87% amino acid identity when compared to the BoNT/A translocation domain subtype of SEQ ID NO: 1. As used herein, the term "naturally occurring Clostridial toxin translocation domain variant" refers to any Clostridial toxin translocation domain produced by a naturally-occurring process, including, without limitation, Clostridial toxin translocation domain isoforms produced from alternatively-spliced transcripts, Clostridial toxin translocation domain isoforms produced by spontaneous mutation and Clostridial toxin translocation domain subtypes. A naturally occurring Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the naturally occurring Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification.

[085] A non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain isoform such as, e.g., a BoNT/A translocation domain isoform, a BoNT/B translocation domain isoform, a BoNT/C1 translocation domain isoform, a BoNT/D translocation domain isoform, a BoNT/E translocation domain isoform, a BoNT/F translocation domain isoform, a BoNT/G translocation domain isoform, a TeNT translocation domain isoform, a BaNT translocation domain isoform, and a BuNT translocation domain isoform. Another non-limiting examples of a naturally occurring Clostridial toxin translocation domain variant is a Clostridial toxin translocation domain subtype such as, e.g., a translocation domain from subtype BoNT/A1, BoNT/A2, BoNT/A3, BoNT/A4, and BoNT/A5; a translocation domain from subtype BoNT/B1, B0NT/B2, BoNT/B bivalent and BoNT/B nonproteolytic; a translocation domain from subtype BoNT/C1-1 and BoNT/C1-2; a translocation domain

from subtype BoNT/E1, BoNT/E2 and BoNT/E3; a translocation domain from subtype BoNT/F1, BoNT/F2, BoNT/F3; and a translocation domain from subtype BuNT-1 and BuNT-2.

[086] As used herein, the term "non-naturally occurring Clostridial toxin translocation domain variant" refers to any Clostridial toxin translocation domain produced with the aid of human manipulation, including, without limitation, Clostridial toxin translocation domains produced by genetic engineering using random mutagenesis or rational design and Clostridial toxin translocation domains produced by chemical synthesis. Non-limiting examples of non-naturally occurring Clostridial toxin translocation domain variants include, e.g., conservative Clostridial toxin translocation domain variants, non-conservative Clostridial toxin translocation domain variants, and active Clostridial toxin translocation domain fragments.

[087] As used herein, the term "conservative Clostridial toxin translocation domain variant" refers to a Clostridial toxin translocation domain that has at least one amino acid substituted by another amino acid or an amino acid analog that has at least one property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). Examples of properties include, without limitation, similar size, topography, charge, hydrophobicity, hydrophilicity, lipophilicity, covalent-bonding capacity, hydrogen-bonding capacity, a physicochemical property, of the like, or any combination thereof. A conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification. Non-limiting examples of a conservative Clostridial toxin translocation domain variant include, *e.g.,* conservative BoNT/A translocation domain variants, conservative BoNT/B translocation domain variants, conservative BoNT/C1 translocation domain variants, conservative BoNT/D translocation domain variants, conservative BoNT/E translocation domain variants, conservative BoNT/F translocation domain variants, conservative BoNT/G translocation domain variants, conservative TeNT translocation domain variants, conservative BaNT translocation domain variants, and conservative BuNT translocation domain variants.

[088] As used herein, the term "non-conservative Clostridial toxin translocation domain variant" refers to a Clostridial toxin translocation domain in which 1) at least one amino acid is deleted from the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based; 2) at least one amino acid added to the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain is based; or 3) at least one amino acid is substituted by another amino acid or an amino acid analog that does not share any property similar to that of the original amino acid from the reference Clostridial toxin translocation domain sequence (Table 1). A non-conservative Clostridial toxin translocation domain variant can function in substantially the same manner as the reference Clostridial toxin translocation domain on which the non-conservative Clostridial toxin translocation domain variant is based, and can be substituted for the reference Clostridial toxin translocation domain in any aspect of the present specification. Non-

limiting examples of a non-conservative Clostridial toxin translocation domain variant include, e.g., non-conservative BoNT/A translocation domain variants, non-conservative BoNT/B translocation domain variants, non-conservative BoNT/C1 translocation domain variants, non-conservative BoNT/D translocation domain variants, non-conservative BoNT/E translocation domain variants, non-conservative BoNT/F translocation domain variants, non-conservative BoNT/G translocation domain variants, and non-conservative TeNT translocation domain variants, non-conservative BaNT translocation domain variants, and non-conservative BuNT translocation domain variants.

[089] As used herein, the term "active Clostridial toxin translocation domain fragment" refers to any of a variety of Clostridial toxin fragments comprising the translocation domain can be useful in aspects of the present specification with the proviso that these active fragments can facilitate the release of the LC from intracellular vesicles into the cytoplasm of the target cell and thus participate in executing the overall cellular mechanism whereby a Clostridial toxin proteolytically cleaves a substrate. The translocation domains from the heavy chains of Clostridial toxins are approximately 410-430 amino acids in length and comprise a translocation domain (Table 1). Research has shown that the entire length of a translocation domain from a Clostridial toxin heavy chain is not necessary for the translocating activity of the translocation domain. Thus, aspects of this embodiment include a Clostridial toxin translocation domain having a length of, e.g., at least 350, 375, 400, or 425 amino acids. Other aspects of this embodiment include a Clostridial toxin translocation domain having a length of, *e.g.,* at most 350, 375, 400, or 425 amino acids.

[090] Any of a variety of sequence alignment methods can be used to determine percent identity of naturally-occurring Clostridial toxin translocation domain variants and non-naturally-occurring Clostridial toxin translocation domain variants, including, without limitation, global methods, local methods and hybrid methods, such as, *e.g.,* segment approach methods. Protocols to determine percent identity are routine procedures within the scope of one skilled in the art and from the teaching herein.

[091] Thus, in an embodiment, a TVEMP disclosed herein comprises a Clostridial toxin translocation domain. In an aspect of this embodiment, a Clostridial toxin translocation domain comprises a naturally occurring Clostridial toxin translocation domain variant, such as, e.g., a Clostridial toxin translocation domain isoform or a Clostridial toxin translocation domain subtype. In another aspect of this embodiment, a Clostridial toxin translocation domain comprises a non-naturally occurring Clostridial toxin translocation domain variant, such as, *e.g.,* a conservative Clostridial toxin translocation domain variant, a non-conservative Clostridial toxin translocation domain variant, an active Clostridial toxin translocation domain fragment, or any combination thereof.

[092] In another embodiment, a hydrophic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another hydrophic amino acid. Examples of hydrophic amino acids include, *e.g.,* C, F, I, L, M, V and W. In another aspect of this

embodiment, an aliphatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another aliphatic amino acid. Examples of aliphatic amino acids include, *e.g.,* A, I, L, P, and V. In yet another aspect of this embodiment, an aromatic amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another aromatic amino acid. Examples of aromatic amino acids include, *e.g.,* F, H, W and Y. In still another aspect of this embodiment, a stacking amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another stacking amino acid. Examples of stacking amino acids include, e.g., F, H, W and Y. In a further aspect of this embodiment, a polar amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another polar amino acid. Examples of polar amino acids include, *e.g.,* D, E, K, N, Q, and R. In a further aspect of this embodiment, a less polar or indifferent amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another less polar or indifferent amino acid. Examples of less polar or indifferent amino acids include, *e.g.,* A, H, G, P, S, T, and Y. In a yet further aspect of this embodiment, a positive charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another positive charged amino acid. Examples of positive charged amino acids include, *e.g.,* K, R, and H. In a still further aspect of this embodiment, a negative charged amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another negative charged amino acid. Examples of negative charged amino acids include, *e.g.,* D and E. In another aspect of this embodiment, a small amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another small amino acid. Examples of small amino acids include, *e.g.,* A, D, G, N, P, S, and T. In yet another aspect of this embodiment, a C-beta branching amino acid at one particular position in the polypeptide chain of the Clostridial toxin translocation domain can be substituted with another C-beta branching amino acid. Examples of C-peta branching amino acids include, e.g., I, T and V.

[093] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/A translocation domain. In an aspect of this embodiment, a BoNT/A translocation domain comprises the translocation domains of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, orSEQ ID NO: 5. In other aspects of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of SEQ ID NO: 1. In another aspect of this embodiment, a BoNT/A translocation domain comprises a naturally occurring BoNT/A translocation domain variant, such as, e.g., an translocation domain from a BoNT/A isoform or an translocation domain from a BoNT/A subtype. In another aspect of this embodiment, a BoNT/A translocation domain comprises a naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, e.g., a BoNT/A isoform translocation domain or a BoNT/A subtype translocation domain. In another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of a naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, *e.g.,* a BoNT/A isoform translocation domain or a BoNT/A subtype translocation domain. In still another aspect of this

embodiment, a BoNT/A translocation domain comprises a non-naturally occurring BoNT/A translocation domain variant, such as, *e.g.,* a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A translocation domain comprises the translocation domain of a non-naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, such as, e.g., a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/A translocation domain comprises amino acids 455-873 of a non-naturally occurring BoNT/A translocation domain variant of SEQ ID NO: 1, such as, *e.g.,* a conservative BoNT/A translocation domain variant, a non-conservative BoNT/A translocation domain variant, an active BoNT/A translocation domain fragment, or any combination thereof.

[094] In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 455-873 of SEQ ID NO: 1; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 455-873 of SEQ ID NO: 1.

[095] In other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutiqns relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In yet other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1. In still other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID

NO: 4, or SEQ ID NO: 5. In further other aspects of this embodiment, a BoNT/A translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 455-873 of SEQ ID NO: 1.

[096] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/B translocation domain. In an aspect of this embodiment, a BoNT/B translocation domain comprises the translocation domains of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In other aspects of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of SEQ ID NO: 6. In another aspect of this embodiment, a BoNT/B translocation domain comprises a naturally occurring BoNT/B translocation domain variant, such as, *e.g.,* an translocation domain from a BoNT/B isoform or an translocation domain from a BoNT/B subtype. In another aspect of this embodiment, a BoNT/B translocation domain comprises a naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.,* a BoNT/B isoform translocation domain or a BoNT/B subtype translocation domain. In another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of a naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, such as, *e.g.,* a BoNT/B isoform translocation domain or a BoNT/B subtype translocation domain. In still another aspect of this embodiment, a BoNT/B translocation domain comprises a non-naturally occurring BoNT/B translocation domain variant, such as, *e.g.,* a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B translocation domain comprises the translocation domain of a non-naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10, such as, *e.g.,* a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/B translocation domain comprises amino acids 447-860 of a non-naturally occurring BoNT/B translocation domain variant of SEQ ID NO: 6, such as, e.g., a conservative BoNT/B translocation domain variant, a non-conservative BoNT/B translocation domain variant, an active BoNT/B translocation domain fragment, or any combination thereof.

[097] In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%,

at least 90%, or at least 95% to amino acids 447-860 of SEQ ID NO: 6; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 447-860 of SEQ ID NO: 6.

[098] In other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In yet other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6. In still other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10. In further other aspects of this embodiment, a BoNT/B translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 447-860 of SEQ ID NO: 6.

[099] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/C1 translocation domain. In an aspect of this embodiment, a BoNT/C1 translocation domain comprises the translocation domains of SEQ ID NO: 11 or SEQ ID NO: 12. In other aspects of this embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of SEQ ID NO: 11. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises a naturally occurring BoNT/C1 translocation domain variant, such as, e.g., an translocation domain from a BoNT/C1 isoform or an translocation domain from a BoNT/C1 subtype. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises a naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, e.g., a BoNT/C1 isoform translocation domain or a BoNT/C1 subtype translocation domain. In another aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of a naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11, such as, e.g., a BoNT/C1 isoform translocation domain or a BoNT/C1 subtype translocation domain. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises a non-naturally occurring BoNT/C1 translocation domain variant, such as, e.g., a conservative BoNT/C1 translocation

domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises the translocation domain of a non-naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11 or SEQ ID NO: 12, such as, e.g., a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/C1 translocation domain comprises amino acids 454-868 of a non-naturally occurring BoNT/C1 translocation domain variant of SEQ ID NO: 11, such as, *e.g.,* a conservative BoNT/C1 translocation domain variant, a non-conservative BoNT/C1 translocation domain variant, an active BoNT/C1 translocation domain fragment, or any combination thereof.

[0100] In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 454-868 of SEQ ID NO: 11; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 454-868 of SEQ ID NO: 11.

[0101] In other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In yet other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11. In still other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 11 or SEQ ID NO: 12. In further other aspects of this embodiment, a BoNT/C1 translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO: 11; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or

100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 454-868 of SEQ ID NO:11.

[0102] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/D translocation domain. In an aspect of this embodiment, a BoNT/D translocation domain comprises the translocation domains of SEQ ID NO: 13 or SEQ ID NO: 14. In other aspects of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of SEQ ID NO: 13. In another aspect of this embodiment, a BoNT/D translocation domain comprises a naturally occurring BoNT/D translocation domain variant, such as, *e.g.,* an translocation domain from a BoNT/D isoform or an translocation domain from a BoNT/D subtype. In another aspect of this embodiment, a BoNT/D translocation domain comprises a naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, e.g., a BoNT/D isoform translocation domain or a BoNT/D subtype translocation domain. In another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of a naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13, such as, e.g., a BoNT/D isoform translocation domain or a BoNT/D subtype translocation domain. In still another aspect of this embodiment, a BoNT/D translocation domain comprises a non-naturally occurring BoNT/D translocation domain variant, such as, *e.g.,* a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D translocation domain comprises the translocation domain of a non-naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13 or SEQ ID NO: 14, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/D translocation domain comprises amino acids 451-864 of a non-naturally occurring BoNT/D translocation domain variant of SEQ ID NO: 13, such as, e.g., a conservative BoNT/D translocation domain variant, a non-conservative BoNT/D translocation domain variant, an active BoNT/D translocation domain fragment, or any combination thereof.

[0103] In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 451-864 of SEQ ID NO: 13; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 451-864 of SEQ ID NO: 13.

[0104] In other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In yet other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13. In still other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 13 or SEQ ID NO: 14. In further other aspects of this embodiment, a BoNT/D translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-864 of SEQ ID NO: 13.

[0105] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/E translocation domain. In an aspect of this embodiment, a BoNT/E translocation domain comprises the translocation domains of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In other aspects of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of SEQ ID NO: 15. In another aspect of this embodiment, a BoNT/E translocation domain comprises a naturally occurring BoNT/E translocation domain variant, such as, *e.g.,* an translocation domain from a BoNT/E isoform or an translocation domain from a BoNT/E subtype. In another aspect of this embodiment, a BoNT/E translocation domain comprises a naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, e.g., a BoNT/E isoform translocation domain or a BoNT/E subtype translocation domain. In another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of a naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, such as, e.g., a BoNT/E isoform translocation domain or a BoNT/E subtype translocation domain. In still another aspect of this embodiment, a BoNT/E translocation domain comprises a non-naturally occurring BoNT/E translocation domain variant, such as, e.g., a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E translocation domain comprises the translocation domain of a non-naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17, such as, e.g., a

conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/E translocation domain comprises amino acids 427-847 of a non-naturally occurring BoNT/E translocation domain variant of SEQ ID NO: 15, such as, e.g., a conservative BoNT/E translocation domain variant, a non-conservative BoNT/E translocation domain variant, an active BoNT/E translocation domain fragment, or any combination thereof.

[0106] In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 427-847 of SEQ ID NO: 15; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 427-847 of SEQ ID NO: 15.

[0107] In other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In yet other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15. In still other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17. In further other aspects of this embodiment, a BoNT/E translocation domain comprises a polypeptide having, *e.g.;* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 15.

[0108] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/F translocation domain. In an aspect of this embodiment, a BoNT/F translocation domain comprises the translocation domains of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In other aspects of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of SEQ ID NO: 18. In another aspect of this embodiment, a BoNT/F translocation domain comprises a naturally occurring BoNT/F translocation domain variant, such as, *e.g.,* an translocation domain from a BoNT/F isoform or an translocation domain from a BoNT/F subtype. In another aspect of this embodiment, a BoNT/F translocation domain comprises a naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, e.g., a BoNT/F isoform translocation domain or a BoNT/F subtype translocation domain. In another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of a naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, such as, e.g., a BoNT/F isoform translocation domain or a BoNT/F subtype translocation domain. In still another aspect of this embodiment, a BoNT/F translocation domain comprises a non-naturally occurring BoNT/F translocation domain variant, such as, *e.g.,* a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F translocation domain comprises the translocation domain of a non-naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20, such as, e.g., a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/F translocation domain comprises amino acids 446-865 of a non-naturally occurring BoNT/F translocation domain variant of SEQ ID NO: 18, such as, e.g., a conservative BoNT/F translocation domain variant, a non-conservative BoNT/F translocation domain variant, an active BoNT/F translocation domain fragment, or any combination thereof.

[0109] In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 446-865 of SEQ ID NO: 18; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 446-865 of SEQ ID NO: 18.

[0110] In other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous

amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In yet other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18. In still other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 18, SEQ ID NO: 19, or SEQ ID NO: 20. In further other aspects of this embodiment, a BoNT/F translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 446-865 of SEQ ID NO: 18.

[0111] In another embodiment, a Clostridial toxin translocation domain comprises a BoNT/G translocation domain. In an aspect of this embodiment, a BoNT/G translocation domain comprises the translocation domains of SEQ ID NO: 21. In other aspects of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of SEQ ID NO: 21. In another aspect of this embodiment, a BoNT/G translocation domain comprises a naturally occurring BoNT/G translocation domain variant, such as, e.g., an translocation domain from a BoNT/G isoform or an translocation domain from a BoNT/G subtype. In another aspect of this embodiment, a BoNT/G translocation domain comprises a naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, e.g., a BoNT/G isoform translocation domain or a BoNT/G subtype translocation domain. In another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of a naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, e.g., a BoNT/G isoform translocation domain or a BoNT/G subtype translocation domain. In still another aspect of this embodiment, a BoNT/G translocation domain comprises a non-naturally occurring BoNT/G translocation domain variant, such as, e.g., a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G translocation domain comprises the translocation domain of a non-naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, e.g., a conservative BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BoNT/G translocation domain comprises amino acids 451-865 of a non-naturally occurring BoNT/G translocation domain variant of SEQ ID NO: 21, such as, e.g., a conservative

BoNT/G translocation domain variant, a non-conservative BoNT/G translocation domain variant, an active BoNT/G translocation domain fragment, or any combination thereof.

[0112] In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 21. In yet other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 451-865 of SEQ ID NO: 21; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 451-865 of SEQ ID NO: 21.

[0113] In other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21. Iri yet other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21; or at most 1, 2, 3,4, 5,6, 7, 8, 9, 10,20, 30,40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21. In still other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 21. In further other aspects of this embodiment, a BoNT/G translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21; or at most 1, 2, 3,4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 451-865 of SEQ ID NO: 21.

[0114] In another embodiment, a Clostridial toxin translocation domain comprises a TeNT translocation domain. In an aspect of this embodiment, a TeNT translocation domain comprises the translocation domains of SEQ ID NO: 22. In other aspects of this embodiment, a TeNT translocation domain comprises amino acids 468-881 of SEQ ID NO: 22. In another aspect of this embodiment, a TeNT translocation domain comprises a naturally occurring TeNT translocation domain variant, such as, e.g., an translocation domain from a TeNT isoform or an translocation domain from a TeNT subtype. In another aspect of this embodiment, a TeNT translocation domain comprises a naturally occurring TeNT

translocation domain variant of SEQ ID NO: 22, such as, *e.g.,* a TeNT isoform translocation domain or a TeNT subtype translocation domain. In another aspect of this embodiment, a TeNT translocation domain comprises amino acids 468-881 of a naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, *e.g.,* a TeNT isoform translocation domain or a TeNT subtype translocation domain. In still another aspect of this embodiment, a TeNT translocation domain comprises a non-naturally occurring TeNT translocation domain variant, such as, *e.g.,* a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT translocation domain comprises the translocation domain of a non-naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, e.g., a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a TeNT translocation domain comprises amino acids 468-881 of a non-naturally occurring TeNT translocation domain variant of SEQ ID NO: 22, such as, *e.g.,* a conservative TeNT translocation domain variant, a non-conservative TeNT translocation domain variant, an active TeNT translocation domain fragment, or any combination thereof.

[0115] In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 468-881 of SEQ ID NO: 22; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 468-881 of SEQ ID NO: 22.

[0116] In other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22. In yet other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22. In still other aspects of this embodiment, a TeNT translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 22. In further other aspects of this

embodiment, a TeNT translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 468-881 of SEQ ID NO: 22.

[0117] In another embodiment, a Clostridial toxin translocation domain comprises a BaNT translocation domain. In an aspect of this embodiment, a BaNT translocation domain comprises the translocation domains of SEQ ID NO: 23. In other aspects of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of SEQ ID NO: 23. In another aspect of this embodiment, a BaNT translocation domain comprises a naturally occurring BaNT translocation domain variant, such as, *e.g.,* an translocation domain from a BaNT isoform or an translocation domain from a BaNT subtype. In another aspect of this embodiment, a BaNT translocation domain comprises a naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, *e.g.,* a BaNT isoform translocation domain or a BaNT subtype translocation domain. In another aspect of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of a naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, e.g., a BaNT isoform translocation domain or a BaNT subtype translocation domain. In still another aspect of this embodiment, a BaNT translocation domain comprises a non-naturally occurring BaNT translocation domain variant, such as, *e.g.,* a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT translocation domain comprises the translocation domain of a non-naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, e.g., a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BaNT translocation domain comprises amino acids 436-857 of a non-naturally occurring BaNT translocation domain variant of SEQ ID NO: 23, such as, e.g., a conservative BaNT translocation domain variant, a non-conservative BaNT translocation domain variant, an active BaNT translocation domain fragment, or any combination thereof.

[0118] In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 436-857 of SEQ ID NO: 23; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 436-857 of SEQ ID NO: 23.

[0119] In other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23. In yet other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23. In still other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 23. In further other aspects of this embodiment, a BaNT translocation domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 436-857 of SEQ ID NO: 23.

[0120] In another embodiment, a Clostridial toxin translocation domain comprises a BuNT translocation domain. In an aspect of this embodiment, a BuNT translocation domain comprises the translocation domains of SEQ ID NO: 24 or SEQ ID NO: 25. In other aspects of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of SEQ ID NO: 24. In another aspect of this embodiment, a BuNT translocation domain comprises a naturally occurring BuNT translocation domain variant, such as, *e.g.,* a translocation domain from a BuNT isoform or an translocation domain from a BuNT subtype. In another aspect of this embodiment, a BuNT translocation domain comprises a naturally occurring BuNT translocation domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, *e.g.,* a BuNT isoform translocation domain or a BuNT subtype translocation domain. In another aspect of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of a naturally occurring BuNT translocation domain variant of SEQ ID NO: 24, such as, *e.g.,* a BuNT isoform translocation domain or a BuNT subtype translocation domain. In still another aspect of this embodiment, a BuNT translocation domain comprises a non-naturally occurring BuNT translocation domain variant, such as, e.g., a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof. In still another aspect of this embodiment, a BuNT translocation domain comprises the translocation domain of a non-naturally occurring BuNT translocation domain variant of SEQ ID NO: 24 or SEQ ID NO: 25, such as, e.g., a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof. In still another

aspect of this embodiment, a BuNT translocation domain comprises amino acids 427-847 of a non-naturally occurring BuNT translocation domain variant of SEQ ID NO: 24, such as, *e.g.,* a conservative BuNT translocation domain variant, a non-conservative BuNT translocation domain variant, an active BuNT translocation domain fragment, or any combination thereof.

[0121] In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90%, or at most 95% to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0122] In other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, e.g., at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 OR SEQ ID NO: 25. In yet other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.,* at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25. In still other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to the translocation domain of SEQ ID NO: 24 or SEQ ID NO: 25. In further other aspects of this embodiment, a BuNT translocation domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, or 100 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 427-847 of SEQ ID NO: 24 or SEQ ID NO: 25.

[0123] Aspects of the present specification provide, in part, a TVEMP comprising a targeting domain. As used herein, the term "targeting domain" is synonymous with "binding domain", "ligand", or "targeting moiety" and refers to an amino acid sequence region able to preferentially bind to a cell surface marker,

like a receptor, characteristic of the target cell under physiological conditions. The cell surface marker may comprise a polypeptide, a polysaccharide, a lipid, a glycoprotein, a lipoprotein, or may have structural characteristics of more than one of these. As used herein, the term "preferentially interacts" refers to a molecule capable of binding to its target cell surface marker under physiological conditions, or in vitro conditions substantially approximating physiological conditions, to a statistically significantly greater degree relative to other, non-target cell surface marker. With reference to a targeting domain disclosed herein, there is a discriminatory binding of the targeting domain to its cognate receptor relative to other receptors. Examples of binding domains are described in, e.g., Steward, L.E. et al., Modified Clostridial Toxins with Enhanced Translocation Capability and Enhanced Targeting Activity, U.S. Patent Application No. 11/776,043 (Jul. 11, 2007); Steward, L.E. etal., Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Clostridial Toxin Target Cells, U.S. Patent Application No. 11/776,052 (Jul. 11, 2007); and Steward, L.E. et al., Modified Clostridial Toxins with Enhanced Translocation Capabilities and Altered Targeting Activity For Non-Clostridial Toxin Target Cells, U.S. Patent Application No. 11/776,075 (Jul. 11, 2007), each of which is incorporated by reference in its entirety.

[0124] In an embodiment, a binding domain that selectively binds a target receptor has a dissociation equilibrium constant (K_D) that is greater for the target receptor relative to a non-target receptor by, *e.g.,* at least one-fold, at least two-fold, at least three-fold, at least four fold, at least five-fold, at least 10 fold, at least 50 fold, at least 100 fold, at least 1000, at least 10,000, or at least 100,000 fold.

[0125] An example of a targeting domain disclosed herein is an interleukin (IL) peptide binding domain. Non-limiting examples of an interleukin (IL) peptide binding domain include an IL-1, an IL-2, an IL-3, an IL-4, an IL-5, an IL-6, an IL-7, an IL-8, an IL-9, an IL-10, an Il_-11, an IL-32, or an IL-33. Interleukin peptides bind to a family of G-coupled protein receptors. For example, IL-1 and IL-10 bind to IL1R; IL-3, IL-5, and IL-6 bind to IL3R; IL-4 and IL-13 bind to IL4R; IL-6 binds to IL6R; IL-7 binds to IL7R; and IL-8 binds to IL8R.

[0126] Interleukin receptors have been detected on the surface of several different types of cancer cells. For example, IL-3R is expressed in acute myeloid leukemia, IL-4R is expressed in thyroid cancer, and IL-6R, IL-7R , and IL-8R are expressed in colon cancer. See, e.g., L.A. O'Sullivan, et al., Cytokine receptor signaling through the Jak-Stat-Socs pathway in disease, Mol. Immunol. 44(10): 2497-2506 (2007); M.G. Francipane, et al., Suppressor of cytokine signaling 3 sensitizes anaplastic thyroid cancer to standard chemotherapy, Cancer Res. 69(15): 6141-6148 (2009); A.M. Saaf, et al, Parallels between global transcriptional programs of polarizing Caco-2 intestinal epithelial cells in vitro and gene expression programs in normal colon and colon cancer, Mol. Biol. Cell. 18(11): 4245-4260 (2007); A.M. Crawley, et al., lnterleukin-4 downregulates CD127 expression and activity on human thymocytes and mature CD8+ T cells, Eur. J. Immunol. 40(5): 1396-1407 (2010); and IL-8R is expressed in colon cancer. T. Yokoe, et al., Efficient identification of a novel cancer/testis antigen for immunotherapy using three-step microarray

analysis, Cancer Res. 68(4): 1074-1082 (2008), each of which is hereby incorporated by reference in its entirety. As such, a TVEMP comprising an IL peptide targeting domain would be effective in treating cancer, including an acute myeloid leukemia, a thyroid cancer, or a colon cancer.

[0127] Thus, in an embodiment, a targeting domain comprises an IL peptide targeting domain. In aspects of this embodiment, an IL peptide targeting domain comprises an IL-1, an IL-2, an IL-3, an IL-4, an IL-5, an IL-6, an IL-7, an IL-8, an IL-9, an IL-10, an IL-11, an IL-32, or an IL-33. In other aspects of this embodiment, an IL peptide targeting domain comprises SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152. In yet other aspects of this embodiment, an IL peptide targeting domain comprises amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150.

[0128] In other aspects of this embodiment, an IL targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152. In yet other aspects of this embodiment, an IL targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152. In still other aspects of this embodiment, an IL targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID

NO: 84, SEQ **ID NO:** 85, SEQ **ID NO:** 86, SEQ **ID NO: 87,** SEQ **ID NO:** 146, SEQ **ID NO: 147,** SEQ **ID NO:** 148, SEQ **ID NO:** 149, SEQ **ID NO:** 150, SEQ **ID NO:** 151, or SEQ **ID NO:** 152.

[0129] In other aspects of this embodiment, an IL targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150. In yet other aspects of this embodiment, an IL targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150. In still other aspects of this embodiment, an IL targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID

NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, or amino acids 19-142 of SEQ ID NO: 150.

[0130] Another example of a targeting domain disclosed herein is a vascular endothelial growth factor (VEGF) peptide targeting domain. Non-limiting examples of a VEGF peptide targeting domain include a VEGF-A, a VEGF-B, a VEGF-C, a VEGF-D, or a placenta growth factor (PIGF). VEGF peptides bind to a family of G-coupled protein receptors. For example, VEGFA, VEGFB, and VEGFC bind to VEGFR1; VEGFA, VEGFD, VEGFC, and VEGFE bind to VEGFR2; and VEGFA, VEGFC, and VEGFD bind to VEGFR3.

[0131] VEGF receptors have been detected on the surface of several different types of cancer cells. For example, VEGFR1 is expressed in renal cell carcinomas, ovarian cancer, bladder cancer, colon cancer, lymphomas, rhabdomyosarcomas, breast cancer, osteosarcomas, lung cancer, non-small cell lung cancer, melanomas, pancreatic cancer, ocular melanomas, retinoblastomas, intra-ocular tumors, leukemias, Kaposi's sarcomas, medulloblastomas, teratocarcinomas, neuroblastomas, malignant mesotheliomas, and gliomas. See, e.g., S.P. Gunningham, et al., Vascular endothelial growth factor-B and vascular endothelial growth factor-C expression in renal cell carcinomas: regulation by the von Hippel-Lindau gene and hypoxia, Cancer Res. 61(7): 3206-3211 (2001); C.A. Boocock, et al., Expression of vascular endothelial growth factor and its receptors fit and KDR in ovarian carcinoma, J. Natl. Cancer Inst. 87(7): 506-516 (1995); R. Masood, et al., Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors, Blood, 98(6): 1904-1913 (2001); G.P. Sawiris, et al., Development of a highly specialized cDNA array for the study and diagnosis of epithelial ovarian cancer, Cancer Res. 62(10): 2923-2928 (2002); W. Wu, et al., VEGF receptor expression and signaling in human bladder tumors, Oncogene 22(22): 3361-3370 (2003); F. Fan, et al., Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells, Oncogene 24(16): 2647-2653 (2005); D. P. Lessilie, et al., Vascular endothelial growth factor receptor-1 mediates migration of human colorectal carcinoma cells by activation of Src family kinases, Br. J. Cancer 94(11): 1710-1717 (2006); Y. Aoki and G. Tosato, Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas, Blood 94(12): 4247-4254 (1999); M.F. Gee, et al., Vascular endothelial growth factor acts in an autocrine manner in rhabdomyosarcoma cell lines and can be inhibited with a.ll-trans-retinoic acid, Oncogene 24(54): 8025-8037 (2005); S.U. Mertens-Talcott, et al., The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells, Cancer Res. 67(22): 11001-11011 (2007); R.L. Stephens, et al., Activation of peroxisome proliferator-activated receptor delta stimulates the proliferation of human breast and prostate cancer cell lines, Cancer Res. 64(9): 3162-3170 (2004); J.S. deJong, et al., Expression of growth factors, growth inhibiting factors, and their receptors in invasive breast cancer. I: An inventory in search of autocrine and paracrine loops, J. Pathol. 184: 44-52 (1998); V. Speirs and S.L. Atkin, Production of VEGF and

expression of the VEGF receptors Flt-1 and KDR in primary cultures of epithelial and stromal cells derived from breast tumours, Br. J. Cancer 80(5-6): 898-903 (1999); Y.H. Lee, et al., Cell-retained isoforms of vascular endothelial growth factor (VEGF) are correlated with poor prognosis in osteosarcoma, Eur. J. Cancer 35(7): 1089-1093 (1999); E. Castro-Rivera, et al., Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect, Proc. Natl. Acad. Sci. USA 101(31): 11432-11437 (2004); O. Straume and LA. Akslen, Expresson of vascular endothelial growth factor, its receptors (FLT-1, KDR) and TSP-1 related to microvessel density and patient outcome in vertical growth phase melanomas, Am. J. Pathol. 159: 223-235 (2001); H. Gitay-Goren, et al., Human melanoma cells but not normal melanocytes express vascular endothelial growth factor receptors, Biochem. Biophys. Res. Commun. 190(3): 702-708 (1993); U. Graeven, et al., Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR, J. Cancer Res. Clin. Oncol. 125(11): 621-629 (1999); M. Abdelrahmim, et al., Regulation of vascular endothelial growth factor receptor-1 expression by specificity proteins 1, 3, and 4 in pancreatic cancer cells, Cancer Res. 67(7): 3286-3294 (2007); A.D. Yang, et al., Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in human pancreatic carcinoma cells, Cancer Res. 66(1): 46-51 (2006); J.S. Wey, et al., Vascular endothelial growth factor receptor-1 promotes migration and invasion in pancreatic carcinoma cell lines, Cancer 104(2): 427-438 (2005); P. BUchler, et al., VEGF-RII influences the prognosis of pancreatic cancer, Ann. Surg. 236(6): 738-749 (2002); A.W. Stitt, et al., Expression of vascular endothelial growth factor (VEGF) and its receptors is regulated in eyes with intra-ocular tumours, J. Pathol. 186(3): 306-312 (1998); S. Dias, et al., VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition, Blood 99(7): 2532-2540 (2002); S.A. Kumar, et al., Lysophosphatidic acid receptor expression in chronic lymphocytic leukemia leads to cell survival mediated though vascular endothelial growth factor expression, Leuk. Lymphoma 50(12): 2038-2048 (2009); R. Masood, et al., Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma, Proc. Natl. Acad. Sci. USA 94(3): 979-984 (1997); D. Bagnard, etal., Semaphorin 3A-vascular endothelial growth factor-165 balance mediates migration and apoptosis of neural progenitor cells by the recruitment of shared receptor, J. Neurosci. 21(10): 3332-3341 (2001); G.J. Bauerschmitz, et al., The flt-1 promoter for transcriptional targeting of teratocarcinoma, Cancer Res. 62(5): 1271-1274 (2002); B. Das, et al., A hypoxia-driven vascular endothelial growth factor/Fltl autocrine loop interacts with hypoxia-inducible factor-1 alpha through mitogen-activated protein kinase/extracellular signal-regulated kinase 1/2 pathway in neuroblastoma, Cancer Res. 65(16): 7267-7275 (2005); L. Strizzi, et al., Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma, J. Pathol. 193(4): 468-475 (2001); and R.S. Carroll, et al., KDR activation in astrocytic neoplasms, Cancer 86(7): 1335-1341 (1999).

[0132] As another example, VEGFR2 is expressed in prostate cancer, renal cell carcinomas, ovarian cancer, bladder cancer, rhabdomyosarcomas, breast cancer, osteosarcomas, thyroid tumors, lung cancer, non-small cell lung cancer, melanomas, pancreatic cancer, ocular melanomas, retinoblastomas, intra-ocular tumors, leukemias, Kaposi's sarcomas, malignant mesotheliomas, insulinomas,gastric

adenocarinomas, intestinal tumors, gliomas, astrocytomas, and kidney tumors. See, e.g., R. Masood, et al., Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors, Blood, 98(6): 1904-1913 (2001); J. Li, et al., Upregulation of VEGF-C by androgen depletion: the involvement of IGF-IR-FOXO pathway, Oncogene 24(35): 5510-5520 (2005); S. De, et al., Molecular pathway for cancer metastasis to bone, J. Biol. Chem. 278(40): 39044-39050 (2003); D. Huang, et al., Sunitinib acts primarily on tumor endothelium rather than tumor cells to inhibit the growth of renal cell carcinoma, Cancer Res. 70(3): 1053-1062 (2010); S.P. Gunningham, et al., Vascular endothelial growth factor-B and vascular endothelial growth factor-C expression in renal cell carcinomas: regulation by the von Hippel-Lindau gene and hypoxia, Cancer Res. 61(7): 3206-3211 (2001); C.A. Boocock, et al., Expression of vascular endothelial growth factor and its receptors fit and KDR in ovarian carcinoma, J. Natl. Cancer Inst. 87: 506-516 (1995); W. Wu, et al., VEGF receptor expression and signaling in human bladder tumors, Oncogene 22(22): 3361-3370 (2003); X. Tian et al., Vascular endothelial growth factor: acting as an autocrine growth factor for human gastric adenocarcinoma cell MGC803, Biochem. Biophys. Res. Commun. 286(3): 505-512 (2001); M.F. Gee, et al., Vascular endothelial growth factor acts in an autocrine manner in rhabdomyosarcoma cell lines and can be inhibited with all-trans-retinoic acid, Oncogene 24(54): 8025-8037 (2005); J.S. de Jong, et al., Expression of growth factors, growth inhibiting factors, and their receptors in invasive breast cancer. I: An inventory in search of autocrine and paracrine loops, J. Pathol. 184: 44-52 (1998); V. Spiers and S.L. Atkin, Production of VEGF and expression of the VEGF receptors Flt-1 and KDR in primary cultures of epithelial and stromal cells derived from breast tumours., Br. J. Cancer 80: 898-903 (1999); T.H. Lee, et al., Vascular endothelial growth factor modulates the transendothelial migration of MDA-MB-231 breast cancer cells through regulation of brain microvascular endothelial cell permeability, J. Biol. Chem. 278(7): 5277-5284 (2003); A. Care, et al., HOXB7: a key factor for tumor-associated angiogenic switch, Cancer Res. 61(17): 6532-6539 (2001); Y.H. Lee, et al., Cell-retained isoforms of vascular endothelial growth factor (VEGF) are correlated with poor prognosis in osteosarcoma.Eur. J. Cancer 35(7): 1089-1093 (1999); G. Bunone, et al., Expression of angiogenesis stimulators and inhibitors in human thyroid tumors and correlation with clinical pathological features. Am. J. Pathol. 155: 1967-1976 (1999); E. Castro-Rivera, et al., Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect. Proc. Natl. Acad. Sci. USA 101(31): 11432-11437 (2004); O. Straume, and L.A. Akslen. Expresson of vascular endothelial growth factor, its receptors (FLT-1, KDR) and TSP-1 related to microvessel density and patient outcome in vertical growth phase melanomas.Am. J. Pathol. 159: 223-235 (2001); H. Gitay-Goren, et al., Human melanoma cells but not normal melanocytes express vascular endothelial growth factor receptors.Biochem. Biophys. Res. Commun. 190: 702-708 (1993); U. Graeven, et al., Melanoma-associated expression of vascular endothelial growth factor and its receptors FLT-1 and KDR. J. Cancer Res. Clin. Oncol. 125: 621-629 (1999); K.J. Higgins, et al., Regulation of vascular endothelial growth factor receptor-2 expression in pancreatic cancer cells by Sp proteins.Biochem. Biophys. Res. Commun. 345: 292-301 (2006); P. Buchler, et al., VEGF-RII influences the prognosis of pancreatic cancer.Ann. Surg. 236(6): 738-749 (2002); A.W. Stitt, et al., Expression of vascular endothelial growth factor (VEGF) and its receptors is regulated in eyes with intra-ocular tumours.

J. Pathol. 186: 306-312 (1998); S.A. Kumar, et al., Lysophosphatidic acid receptor expression in chronic lymphocytic leukemia leads to cell survival mediated though vascular endothelial growth factor expression. Leuk. Lymphoma 50(12): 2038-2048 (2009); G. Schuch, et al., In vivo administration of vascular endothelial growth factor (VEGF) and its antagonist, soluble neuropilin-1, predicts a role of VEGF in the progression of acute myeloid leukemia in vivo. Blood 100(13): 4622-4628 (2002); J. LeCouter, et al., Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization. Proc. Natl. Acad. Sci. USA 101(48): 16813-16818 (2004); S. Dias, et al., VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. Blood 99(7): 2532-2540 (2002); R. Masood, et al., Vascular endothelial growth factor/vascular permeability factor is an autocrine growth factor for AIDS-Kaposi sarcoma. Proc. Natl. Acad. Sci. USA 94(3): 979-984 (1997); M.C. Deregivus, et al., HIV-1-Tat protein activates phosphatidylinositol 3-kinase/ AKT-dependent survival pathways in Kaposi's sarcoma cells. J. Biol. Chem. 277(28): 25195-25202 (2002); L. Strizzi, et al., Vascular endothelial growth factor is an autocrine growth factor in human malignant mesothelioma. J. Pathol. 193(4): 468-475 (2001); C. Oberg, et al., Expression of protein tyrosine kinases in islet cells: possible role of the Flk-1 receptor for beta-cell maturation from duct cells. Growth Factors 10(2): 115-126 (1994); C. Blazquez, et al., Cannabinoids inhibit the vascular endothelial growth factor pathway in gliomas. Cancer Res. 64(16): 5617-5623 (2004); R.S. Carroll, etal., KDR activation in astrocytic neoplasms.Cancer 86(7): 1335-1341 (1999); M.M. Valter, et al., Expression of the Ets-1 transcription factor in human astrocytomas is associated with Fms-like tyrosine kinase-1 (Flt-1 Avascular endothelial growth factor receptor-1 synthesis and neoangiogenesis. Cancer Res. 59(21): 5608-5614 (1999); and A.M. Davidoff, et al., rAAV-mediated long-term liver-generated expression of an angiogenesis inhibitor can restrict renal tumor growth in mice. Cancer Res. 62(11): 3077-3083 (2002).

[0133] As yet another example, VEGFR3 is expressed in renal cell carcinomas, lymphomas, rhabdomyosarcomas, breast cancer, thyroid tumors, non-small cell lung cancer, leukemias, Kaposi's sarcomas, and insulinomas. See, e.g., S.P. Gunningham, et al., Vascular endothelial growth factor-B and vascular endothelial growth factor-C expression in renal cell carcinomas: regulation by the von Hippel-Lindau gene and hypoxia. Cancer Res. 61(7): 3206-3211 (2001); S.F. Schoppmann, et al., Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. Am. J. Pathol. 161(3): 947-956 (2002); M.F. Gee, et al., Vascular endothelial growth factor acts in an autocrine manner in rhabdomyosarcoma cell lines and can be inhibited with all-trans-retinoic acid. Oncogene 24(54): 8025-8037 (2005); A.Care, et al., HOXB7: a key factor for tumor-associated angiogenic switch. Cancer Res. 61(17): 6532-6539 (2001); G. Bunone, et al., Expression of angiogenesis stimulators and inhibitors in human thyroid tumors and correlation with clinical pathological features. Am. J. Pathol. 155: 1967-1976 (1999); U. McDermott, et al., Ligand-dependent platelet-derived growth factor receptor (PDGFR)-alpha activation sensitizes rare lung cancer and sarcoma cells to PDGFR kinase inhibitors. Cancer Res. 69(9): 3937-3946 (2009); M.H. Chien, et al., Vascular endothelial growth factor-C (VEGF-C) promotes angiogenesis by induction of COX-2 in leukemic cells via the VEGF-

R3/JNK/AP-1 pathway. Carcinogenesis 30(12): 2005-2013 (2009); P. Blume-Jensen and T. Hunter. Oncogenic kinase signalling. Nature 411(6835): 355-365 (2001); S. Marchio, et al., Vascular endothelial growth factor-C stimulates the migration and proliferation of Kaposi's sarcoma cells. J. Biol. Chem. 274(39): 27617-27622 (1999); and V. Lilla, et al., Differential gene expression in well-regulated and dysregulated pancreatic beta-cell (MIN6) sublines. Endocrinology 144(4): 1368-1379 (2003).

[0134] As such, a TVEMP comprising a VEGF peptide targeting domain would be effective in treating cancer, including a prostate cancer, a renal cell carcinoma, an ovarian cancer, a bladder cancer, a colon cancer, a lymphoma, a rhabdomyosarcoma, a breast cancer, an osteosarcoma, a thyroid tumor, a lung cancer, a non-small cell lung cancer, a melanoma, a pancreatic cancer, an ocular melanoma, a retinoblastoma, an intra-ocular tumor, a leukemia, a Kaposi's sarcoma, a medulloblastoma, a teratocarcinoma, a neuroblastoma, a mesothelioma, an insulinoma, a gastric adenocarinoma, an intestinal tumor, a glioma, an astrocytoma, or a kidney tumor.

[0135] Thus, in an embodiment, a targeting domain comprises a VEGF peptide targeting domain. In aspects of this embodiment, a VEGF peptide targeting domain comprises a VEGF-A, a VEGF-B, a VEGF-C, a VEGF-D, or a PIGF. In aspects of this embodiment, a VEGF peptide targeting domain comprises SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93. In other aspects of this embodiment, a VEGF peptide targeting domain comprises amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93.

[0136] In other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93. In yet other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93. In still other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93; or at most 1, 2, 3, 4, 5, 6, 7, 8,

9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, or SEQ ID NO: 93.

[0137] In other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93. In yet other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93. In still other aspects of this embodiment, a VEGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93.

[0138] Another example of a targeting domain disclosed herein is an insulin-like growth factor (IGF) peptide targeting domain. Non-limiting examples of an IGF peptide targeting domain include an IGF-1 or an IGF-2. IGF peptides bind to a family of protein receptors. For example, IGF-1 and IGF-2 bind to both IGFR1 and IGFR2.

[0139] IGF receptors have been detected on the surface of several different types of cancer cells. For example, IGF1R is expressed in breast cancer, colon cancer, lung cancer, and prostate cancer. See, e.g., G. Thomas, Furin at the cutting edge: from protein traffic to embryogenesis and disease, Nat. Rev. Mol. Cell Biol. 3(10): 753-766 (2002). As another example, IGF2R is expressed in gastric cancer and liver cancer. See, e.g., L. OttinJ, et al., Mutations at coding mononucleotide repeats in gastric cancer with the microsatellite mutator phenotype, Oncogene 16(21): 2767-2772 (1998); and Y.J. Chung, et al., Evidence of genetic progression in human gastric carcinomas with microsatellite instability, Oncogene 15(14): 1719-1726 (1997); and J.J. Mills, et al., Imprinted M6p/Igf2 receptor is mutated in rat liver tumors, Oncogene 16(21): 2797-2802 (1998). As such, a TVEMP comprising an IGF peptide targeting domain would be effective in treating cancer, including a breast cancer, a colon cancer, a lung cancer, a prostate cancer, a gastric cancer or a liver cancer.

[0140] Thus, in an embodiment, a targeting domain comprises an IGF peptide targeting domain. In aspects of this embodiment, an IGF peptide targeting domain comprises an IGF-1 or an IGF-2. In aspects of this embodiment, an IGF peptide targeting domain comprises SEQ ID NO: 94 or SEQ ID NO: 95. In other aspects of this embodiment, an IGF peptide targeting domain comprises amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95.

[0141] In other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 94 or SEQ ID NO: 95. In yet other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95. In still other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 94 or SEQ ID NO: 95.

[0142] In other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95. In yet other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino

acid deletions, additions, and/or substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95. In still other aspects of this embodiment, an IGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95.

[0143] Another example of a targeting domain disclosed herein is an epidermal growth factor (EGF) peptide targeting domain. Non-limiting examples of an EGF peptide targeting domain include an EGF, a heparin-binding EGF-like growth factor (HB-EGF), a transforming growth factor-a (TGF-a), an amphiregulin (AR), an epiregulin (EPR), an epigen (EPG), a betacellulin (BTC), a neuregulin-1 (NRG1), a neuregulin-2 (NRG2), a neuregulin-3, (NRG3), or a neuregulin-4 (NRG4). EGF peptides bind to a family of protein receptors. For example, EGF, LTA4H, TGFA, HBEGF (Heparin-Binding EGF-like growth factor), amphiregulin, epiregulin, and BTC bind to EGFR1; NRG1 and EGF bind to EGFR2; NRG1, NRG2, and BTC bind to EGFR3; NRG1, NRG2, NRG3, EPR, HBEGF, NRG4, BTC, and EPR bind to EGFR4; and TGF-a binds to BMPR1A.

[0144] EGF receptors have been detected on the surface of several different types of cancer cells. For example, EGFR1 is expressed in lung cancer, prostate cancer, ovarian cancer, bladder cancer, thyroid cancer, mixed papillary and follicular thyroid carcinomas. See, e.g., P. Blume-Jensen and T. Hunter, Oncogenic kinase signaling, Nature 411(6835): 355-365 (2001); T. Arao, et al., Small in-frame deletion in the epidermal growth factor receptor as a target for ZD6474, Cancer Res. 64(24): 9101-9104 (2004); H. Ji, et al., Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors, Proc. Natl. Acad. Sci. USA 103(20): 7817-7822 (2006); J. Kim, et al., The phosphoinositide kinase PIKfyve mediates epidermal growth factor receptor trafficking to the nucleus, Cancer Res. 67(19): 9229-9237 (2007); E. Kebebew, et al., Diagnostic and prognostic value of angiogenesis-modulating genes in malignant thyroid neoplasms, Surgery 138(6): 1102-1109 (2005).

[0145] As another example, EGFR2 is expressed in lung cancer, prostate cancer, biliary tract cholangiocarcinomas, breast cancer, cervical cancer, breast cancer, colorectal cancer, gastric cancer, endometrial cancer, esophageal cancer, fallopian tube cancer, gallbladder cancer, head and neck cancer, liver cancer, lung cancer, colorectal cancer, myelodysplastic syndrome, non-small cell lung cancer, oral cancer, ovarian cancer, pancreatic cancer, peritoneal cavity cancer, polycythemia vera, renal cancer, and skin cancer. See, e.g., W. Kassouf, et al., Uncoupling between Epidermal Growth Factor Receptor and Downstream Signals Defines Resistance to the Antiproliferative Effect of Gefitinib in Bladder Cancer

Cells, Cancer Res. 65(22): 10524-10535 (2005); M. Casimiro, et al., ErbB-2 Induces the Cyclin D1 Gene in Prostate Epithelial Cells In vitro and In vivo, Cancer Res. 67(9): 4364-4372 (2007); J. Harder, et al., EGFR and HER2 expression in advanced biliary tract cancer, World J. Gastroenterol. 15(36): 4511-4517 (2009); M. Kobayashi, et al. Protein overexpression and gene amplification of c-erbB-2 in breast carcinomas: a comparative study of immunohistochemistry and fluorescence in situ hybridization of formalin-fixed, paraffin-embedded tissues, Hum. Pathol. 33: 21-28 (2002); D. Xie, et al., Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk, J. Natl. Cancer Inst. 92(5): 412-417 (2000); P.N. Munster, et al., First study of the safety, tolerability, and pharmacokinetics of CP-724,714 in patients with advanced malignant solid HER2-expressing tumors, Clin. Cancer Res. 13(4): 1238-1245 (2007); W. Xia, et al., A model of acquired autoresistance to a potent ErbB2 tyrosine kinase inhibitor and a therapeutic strategy to prevent its onset in breast cancer, Proc. Natl. Acad. Sci. USA 103(20): 7795-7800 (2006); A Wissner, et al., Syntheses and EGFR and HER-2 kinase inhibitory activities of 4-anilinoquinoline-3-carbonitriles: Analogues of three important 4-anilinoquinazolines currently undergoing clinical evaluation as therapeutic antitumor agents, Bioorg. Med. Chem. Lett. 12(20): 2893-2897 (2002); M.D. Sternlicht, et al., How matrix metalloproteinases regulate cell behavior, Annu. Rev. Cell Dev. Biol. 17: 463-516 (2001); J.N Hutchinson, et al., Activation of Akt-1 (PKB-alpha) Can Accelerate ErbB-2-Mediated Mammary Tumorigenesis but Suppresses Tumor Invasion, Cancer Res. 64(9): 3171-3178 (2004); X. Leng, et al., Inhibition of lipocalin 2 impairs breast tumorigenesis and metastasis, Cancer Res. 69(22): 8579-8584 (2009); P.N. Munster, et al. First study of the safety, tolerability, and pharmacokinetics of CP-724,714 in patients with advanced malignant solid HER2-expressing tumors, Clin. Cancer Res. 13(4): 1238-1245 (2007); D. Dankort, et al., Grb2 and She adapter proteins play distinct roles in Neu (ErbB-2)-induced mammary tumorigenesis: implications for human breast cancer, Mol. Cell Biol. 21(5): 1540-1551 (2001); R.S. Muraoka, et al., Increased malignancy of Neu-induced mammary tumors overexpressing active transforming growth factor betal, Mol. Cell Biol. 23(23): 8691-8703 (2003); D.V. Bulavin, et al., Inactivation of the Wip1 phosphatase inhibits mammary tumorigenesis through p38 MAPK-mediated activation of the p16(lnk4a)-p19(Arf) pathway, Nat. Genet. 36(4): 343-350 (2004); X. Ju, et al., Akt1 governs breast cancer progression in vivo, Proc. Natl. Acad. Sci. USA 104(18): 7438-7443 (2007); and H. Ji, et al., Epidermal growth factor receptor variant III mutations in lung tumorigenesis and sensitivity to tyrosine kinase inhibitors, Proc. Natl. Acad. Sci. USA. 103(20): 7817-7822 (2006).

[0146] As yet another example, EGFR3 is expressed in ovarian cancer. See, e.g., K.H. Lu, et al., Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis, Clin. Cancer Res. 10(10): 3291-3300 (2004).

[0147] As still another example, EGFR4 is expressed in prostate cancer, breast cancer, and colon cancer. See, e.g., J.M. Murabito et al. A genome-wide association study of breast and prostate cancer in the NHLBI's Framingham Heart Study, BMC Med. Genet. 8 Suppl 1: S6 (2007); M. Rokavec, et al. A novel polymorphism in the promoter region of ERBB4 is associated with breast and colorectal cancer risk,

Clin. Cancer Res. 13(24): 7506-7514 (2007); and G. Carpenter, ErbB-4: mechanism of action and biology, Exp. Cell Res. 284(1): 66-77 (2003).

[0148] As a further example, BMPR1A is expressed in prostate cancer, biliary tract cancer, ovarian cancer, bone cancer, colon cancer, myelomas, glioblastomas, squamous cell carcinomas, adrenal cortex carcinomas, pancreatic cancer, osteosarcomas. See, e.g., S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells.Oncogene 23(58): 9326-9335 (2004); D.R. Haudenschild, et al., Bone morphogenetic protein (BMP)-6 signaling and BMP antagonist noggin in prostate cancer. Cancer Res. 64(22): 8276-8284 (2004); I.Y. Kim, et al., Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res. 60(11): 2840-2844 (2000); D.E. Hansel, et al., Identification of novel cellular targets in biliary tract cancers using global gene expression technology. Am. J. Pathol. 163(1): 217-229 (2003); T.G. Shepherd and M.W. Nachtigal. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144(8): 3306-3314 (2003); E. Hay, et al., Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in osteoblastic cells. J. Biol. Chem. 279(3): 1650-1658 (2004); W. Jin, et al., TrkC binds to the bone morphogenetic protein type II receptor to suppress bone morphogenetic protein signaling. Cancer Res. 67(20): 9869-9877 (2007); H. Deng, et al., Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. Exp. Cell Res. 313(5): 1033-1044 (2007); T.B. Ro, et al.,Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23(17): 3024-3032 (2004); P. ten Dijke, et al., Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. J. Biol. Chem. 269: 16985-16988 (1994); N. Yamada, et al., Bone morphogenetic protein type IB receptor is progressively expressed in malignant glioma tumours. Br. J. Cancer 73(5): 624-629 (1996); Y. Jin, et al., Overexpression of BMP-2/4, -5 and BMPR-IA associated with malignancy of oral epithelium. Oral Oncol. 37: 225-233 (2001); A.F. Soares, et al., Bone morphogenetic protein-2/4 and bone morphogenetic protein receptor type IA expression in metastatic and nonmetastatic oral squamous cell carcinoma. Am. J. Otolaryngol. 31(4): 266-271 (2010); I.K. Johnsen, et al., Bone morphogenetic proteins 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis. Cancer Res. 69(14): 5784-5792 (2009); J. Kleeff, et al., Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterol. 116(5): 1202-1216 (1999); G. Gobbi, et al., Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. Int. J. Oncology 20(1): 143-147 (2002); and R. Mehdi, et al., Expression of bone morphogenetic protein and its receptors in osteosarcoma and malignant fibrous histiocytoma. Jap. J. Clin. Oncol. 30(6): 272-275 (2000).

[0149] As such, a TVEMP comprising an EGF peptide targeting domain would be effective in treating cancer, including a lung cancer, a prostate cancer, an ovarian cancer, a bladder cancer, a thyroid cancer, a mixed papillary and follicular thyroid carcinoma, a biliary tract cholangiocarcinoma, a breast cancer, a cervical cancer, a colorectal cancer, a colon cancer, a gastric cancer, an endometrial cancer, an esophageal cancer, a fallopian tube cancer, a gallbladder cancer, a head and neck cancer, a liver cancer, a lung cancer, a myelodysplastic syndrome, a non-small cell lung cancer, an oral cancer, a pancreatic cancer, a peritoneal cavity cancer, a polycythemia vera, a renal cancer, or a skin cancer.

[0150] Thus, in an embodiment, a targeting domain comprises an EGF peptide targeting domain. In aspects of this embodiment, an EGF peptide targeting domain comprises an EGF, a HB-EGF, a TGF-a, an AR, an EPR, an EPG, a BTC, a NRG-1, a NRG-2, a NRG-3, or a NRG-4. In aspects of this embodiment, an EGF peptide targeting domain comprises SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106. In other aspects of this embodiment, an EGF peptide targeting domain comprises amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105. In yet another aspect of this embodiment, an EGF peptide targeting domain comprises a NRG-2 isoform like a NRG-2 isoform 1, a NRG-2 isoform 2, a NRG-2 isoform 3, a NRG-2 isoform 4, a NRG-2 isoform 5, or a NRG-2 isoform 6.

[0151] In other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106. In yet other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106. In still other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106; or at most 1,

2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105, or SEQ ID NO: 106.

[0152] In other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105. In yet other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105. In still other aspects of this embodiment, an EGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, or amino acids 353-648 of SEQ ID NO: 105.

[0153] Another example of a targeting domain disclosed herein is a Transformation Growth Factor-B (TGFB) peptide targeting domain. Non-limiting examples of a TGFB peptide targeting domain include a TGF31, a TGFB2, a TGFB3 or a TGFB4. TGF-B peptides bind to a family of protein receptors. For example, TGF-B1, TGF-P2, and TGF-B3 bind to TGFBR1; TGF-B1, TGF-B2, and TGF-B3 bind to TGFBR2; TGF-B1 and TGF-B2 bind to TGFBR3; and TGF-B1 binds to BMPR2. TGFB1 also binds to activin A receptor, type I (ACVR1), activin A receptor, type 2A (ACVR2A), activin A receptor, type 2B (ACVR2B), and activin A receptor, type C (ACVR1C).

[0154] TGF-B receptors have been detected on the surface of several different types of cancer cells. For example, TGFBR1 is expressed in prostate cancer, pheochromocytoma, ovarian cancer, malignant thyroid tumors, colon cancer, lymphomas, stomach cancer, breast cancer, osteosarcomas, fibrosarcomas, hepatomas, papillary thyroid carcinomas, and pancreatic cancer. See, e.g., B.J. Park, et al., Mitogenic conversion of transforming growth factor-betal effect by oncogenic Ha-Ras-induced activation of the mitogen-activated protein kinase signaling pathway in human prostate cancer, Cancer Res. 60(11): 3031-3038 (2000); D.R. Haudenschild, et al., Bone Morphogenetic Protein (BMP)-6 Signaling and BMP Antagonist Noggin in Prostate Cancer, Cancer Res. 64(22): 8276-8284 (2004); M.L. Lamm, et al., A proliferative effect of transforming growth factor-betal on a human prostate cancer cell line, TSU-Prl, Endocrinology 139(2): 787-790 (1998); H.G. Konig, et al., TGF-{beta}1 activates two distinct type I receptors in neurons: implications for neuronal NF-{kappa}B signaling, J. Cell Biol. 168(7): 1077-1086 (2005); R.L. Baldwin, et al., Loss of c-myc Repression Coincides with Ovarian Cancer Resistance to Transforming Growth Factor beta Growth Arrest Independent of Transforming Growth Factor beta/Smad Signaling, Cancer Res. 63(6): 1413-1419 (2003); T. Chen, et al., Transforming growth factor-beta receptor type I gene is frequently mutated in ovarian carcinomas, Cancer Res. 61(12): 4679-4682 (2001); E. Kebebew, et al., Diagnostic and prognostic value of angiogenesis-modulating genes in malignant thyroid neoplasms, Surgery 138(6): 1102-1109 (2005); N. Muller, et al., Smad4 induces the tumor suppressor E-cadherin and P-cadherin in colon carcinoma cells, Oncogene 21(39): 6049-6058 (2002); P. Lagadec, et al., Evidence for control of nitric oxide synthesis by intracellular transforming growth factor-betal in tumor cells. Implications for tumor development, Am. J. Pathol. 154(6): 1867-1876 (1999); P.I. Knaus, et al., A dominant inhibitory mutant of the type II transforming growth factor beta receptor in the malignant progression of a cutaneous T-cell lymphoma, Mol. Cell Biol. 16(7): 3480-3489 (1996); S.H. Kang, et al., Transcriptional repression of the transforming growth factor-beta type I receptor gene by DNA methylation results in the development of TGF-beta resistance in human gastric cancer, Oncogene 18(51): 7280-7286 (1999); V. Katuri, et al., Inactivation of ELF/TGF-beta signaling in human gastrointestinal cancer, Oncogene 24(54): 8012-8024 (2005); S. Fanayan, et al. Signaling through the Smad pathway by insulin-like growth factor-binding protein-3 in breast cancer cells. Relationship to transforming growth factor-beta 1 signaling, J. Biol. Chem. 277(9): 7255-7261 (2002); S. Ammanamanchi, etal. Induction of transforming growth factor-beta receptor type II expression in estrogen receptor-positive breast cancer cells through SP1 activation by 5-aza-2'-deoxycytidine, J. Biol. Chem. 273(26): 16527-16534 (1998); J.A. McEarchern , et al., Invasion and metastasis of a mammary tumor involves TGF-beta signaling, Int. J. Cancer 91(1): 76-82 (2001); D. Rotzer, et al., Type III TGF-beta receptor-independent signalling of TGF-beta2 via TbetaRII-B, an alternatively spliced TGF-beta type II receptor, EMBO J. 20(3): 480-490 (2001); S. Matsuyama, et al., SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells, Cancer Res. 63(22): 7791-7798 (2003); B.A. Hocevar, et al., The adaptor molecule Disabled-2 links the transforming growth factor beta receptors to the Smad pathway, EMBO J. 20(11): 2789-2801 (2001); Birkey et al., X-linked inhibitor of apoptosis protein functions as a cofactor in transforming growth factor-beta signaling, J. Biol. Chem. 276(28): 26542-26549 (2001); K. Giehl, et al., TGFbetal represses proliferation of pancreatic carcinoma cells

which correlates with Smad4-independent inhibition of ERK activation, Oncogene 19(39): 4531-4541 (2000); G. Subramanian, et al., Targeting endogenous transforming growth factor beta receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotypel, Cancer Res. 64(15): 5200-5211 (2004); and N. Jonckheere, et al., A role for human MUC4 mucin gene, the ErbB2 ligand, as a target of TGF-beta in pancreatic carcinogenesis, Oncogene 23(34): 5729-5738 (2004).

[0155] As another example, TGFBR2 is expressed in prostate cancer, ovarian cancer, colon cancer, lymphoma, stomach cancer, breast cancer, osteosarcomas, fibrosarcomas, papillary thyroid carcinomas, myelomas, pancreatic cancer, cervical carcinomas, endometrial adenocarcinomas, melanomas, rhabdomyosarcomas, squamous cell carcinomas, neuroblastomas, and gastric adenocarcinomas. See, e.g., B.J. Park, et al., Mitogenic conversion of transforming growth factor-betal effect by oncogenic Ha-Ras-induced activation of the mitogen-activated protein kinase signaling pathway in human prostate cancer, Cancer Res. 60(11): 3031-3038 (2000); M.L. Lamm, et al., A proliferative effect of transforming growth factor-betal on a human prostate cancer cell line, TSU-PM, Endocrinology 139(2):787-790 (1998); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells, Oncogene 23(58): 9326-9335 (2004); D.J. Taxman, et al., Transcriptional profiling of targets for combination therapy of lung carcinoma with paclitaxel and mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor, Cancer Res. 63(16): 5095-5104 (2003); R.L. Baldwin, et al., Loss of c-myc Repression Coincides with Ovarian Cancer Resistance to Transforming Growth Factor beta Growth Arrest Independent of Transforming Growth Factor beta/Smad Signaling, Cancer Res. 63(6): 1413-1419 (2003); Y. Mori, et al., Instabilotyping reveals unique mutational spectra in microsatellite-unstable gastric cancers, Cancer Res. 62(13): 3641-3645 (2002); S. Takenoshita, et al., Mutation analysis of coding sequences of the entire transforming growth factor beta type II receptor gene in sporadic human colon cancer using genomic DNA and intron primers, Oncogene 14(10): 1255-1258 (1997); P.I. Knaus, et al., A dominant inhibitory mutant of the type II transforming growth factor beta receptor in the malignant progression of a cutaneous T-cell lymphoma, Mol. Cell Biol. 16(7): 3480-3489 (1996); G. Chen, et al., Resistance to TGF-{beta}1 correlates with aberrant expression of TGF-{beta} receptor II in human B-cell lymphoma cell lines. Blood 109(12): 5301-5307 (2007); L. Ottini, et al., Mutations at coding mononucleotide repeats in gastric cancer with the microsatellite mutator phenotype, Oncogene 16(21): 2767-2772 (1998); K. Park, et al., Genetic changes in the transforming growth factor beta (TGF-beta) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-beta, Proc. Natl. Acad. Sci. USA 91(19): 8772-8776 (1994); CD. Liicke, et al., Inhibiting mutations in the transforming growth factor beta type 2 receptor in recurrent human breast cancer, Cancer Res. 61(2): 482-485 (2001); L.Y. Bourguignon, et al., Hyaluronan Promotes Signaling Interaction between CD44 and the Transforming Growth Factor beta Receptor I in Metastatic Breast Tumor Cells, J. Biol. Chem. 277(42): 39703-39712 (2002); C.A. Wilson, et al., HER-2 overexpression differentially alters transforming growth factor-beta responses in luminal versus mesenchymal human breast cancer cells, Breast Cancer Res. 7(6): R1058-R1079 (2005); J.A. McEarchem, et al., Invasion and metastasis of a mammary tumor involves TGF-beta signaling, Int. J.

Cancer 91(1): 76-82 (2001); D. Rotzer, et al., Type III TGF-beta receptor-independent signalling of TGF-beta2 via TbetaRII-B, an alternatively spliced TGF-beta type II receptor, EMBO J. 20(3): 480-490 (2001); B.A. Hocevar, et al., The adaptor molecule Disabled-2 links the transforming growth factor beta receptors to the Smad pathway, EMBO J. 20(11): 2789-2801 (2001); G. Riesco-Eizaguirre, et al., The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer, Cancer Res. 69(21): 8317-8325 (2009); T. Fernandez, et al., Disruption of transforming growth factor beta signaling by a novel ligand-dependent mechanism, J. Exp. Med. 195(10): 1247-1255 (2002); M. Wagner, et al., Transfection of the type I TGF-beta receptor restores TGF-beta responsiveness in pancreatic cancer, Int. J. Cancer 78(2): 255-260 (1998); K. Giehl, et al., TGFbetaI represses proliferation of pancreatic carcinoma cells which correlates with Smad4-independent inhibition of ERK activation, Oncogene 19(39): 4531-4541 (2000); G. Subramanian, et al., Targeting endogenous transforming growth factor beta receptor signaling in SMAD4-deficient human pancreatic carcinoma cells inhibits their invasive phenotypeI, Cancer Res. 64(15): 5200-5211 (2004); A. Villanueva, et al., Disruption of the antiproliferative TGF-beta signaling pathways in human pancreatic cancer cells, Oncogene 17(15): 1969-1978 (1998); N. Kirma, et al., Elevated Expression of the Oncogene c-fms and Its Ligand, the Macrophage Colony-Stimulating Factor-1, in Cervical Cancer and the Role of Transforming Growth Factor-{beta}1 in Inducing c-fms Expression, Cancer Res. 67(5): 1918-1926 (2007); T.V. Parekh, et al., Transforming growth factor beta signaling is disabled early in human endometrial carcinogenesis concomitant with loss of growth inhibition, Cancer Res. 62(10): 2778-2790 (2002); D.J. Taxman, et al., Transcriptional profiling of targets for combination therapy of lung carcinoma with paclitaxel and mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor, Cancer Res. 63(16): 5095-5104 (2003); M. Bouche, et al., TGF-beta autocrine loop regulates cell growth and myogenic differentiation in human rhabdomyosarcoma cells, FASEB J. 14(9): 1147-1158 (2000); M. Reiss, et al., Resistance of human squamous carcinoma cells to transforming growth factor beta 1 is a recessive trait, Proc. Natl. Acad. Sci. USA 90(13): 6280-6284 (1993); K.B. Hahm, et al., Repression of the gene encoding the TGF-beta type II receptor is a major target of the EWS-FLI1 oncoprotein, Nat. Genet. 23(2): 222-227 (1999); Y. Mori, et al., Instabilotyping reveals unique mutational spectra in microsatellite-unstable gastric cancers, Cancer Res. 62(13): 3641-3645 (2002); and Y.J. Chung, et al., Evidence of genetic progression in human gastric carcinomas with microsatellite instability, Oncogene 15(14): 1719-1726 (1997).

[0156] As yet another example, TGFBR3 is expressed in prostate cancer, pheochromocytomas, stomach cancer, breast cancer, adrenocortical cancer, and salivary adenoid cystic carcinoma. See, e.g., D.R. Haudenschild, et al., Bone Morphogenetic Protein (BMP)-6 Signaling and BMP Antagonist Noggin in Prostate Cancer, Cancer Res. 64(22): 8276-8284 (2004); J.M. Cosgaya, et al., Retinoic acid induces secretion of transforming growth factors by PC12 pheochromocytoma cells, Oncogene 14(5): 579-587 (1997); K. Park, el at., Genetic changes in the transforming growth factor beta (TGF-beta) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-beta, Proc. Natl. Acad. Sci. USA 91(19): 8772-8776 (1994); J.A. McEarchern, et al., Invasion and metastasis of a

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[0157] As still another example, BMPR2 is expressed in prostate cancer, ovarian cancer, bone cancer, colon cancer, myelomas, breast cancer, lung carcinomas, adrenal cortex carcinomas, pancreatic cancer, and osteosarcomas. See, e.g., S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); I.Y. Kim, et al., Loss of expression of bone morphogenetic protein receptor type II in human prostate cancer cells. Oncogene 23(46): 7651-7659 (2004); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. Oncogene 23(58): 9326-9335 (2004); I.Y. Kim, et al., Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res. 60(11): 2840-2844 (2000); T.G. Shepherd and M.W. Nachtigal. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144(8): 3306-3314 (2003); Y. Xia, et al., Repulsive guidance molecule RGMa alters utilization of bone morphogenetic protein (BMP) type II receptors by BMP2 and BMP4. J. Biol. Chem. 282(25): 18129-18140 (2007); E. Hay, et al., Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in osteoblastic cells. J. Biol. Chem. 279(3): 1650-1658 (2004); W. Jin, et al., TrkC binds to the bone morphogenetic protein type II receptor to suppress bone morphogenetic protein signaling. Cancer Res. 67(20): 9869-9877 (2007); R. L. Baldwin, et al., Attenuated ALK5 receptor expression in human pancreatic cancer: correlation with resistance to growth inhibition. Int. J. Cancer 67(2): 283-288 (1996); H. Deng, et al., Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. Exp. Cell Res. 313(5): 1033-1044 (2007); T.B. Ro, et al., Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23(17): 3024-3032 (2004); M. Fan, et al., Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and fulvestrant. Cancer Res. 66(24): 11954-11966 (2006); J.A. McEarchem, et al., Invasion and metastasis of a mammary tumor involves TGF-beta signaling.Int. J. Cancer 91(1): 76-82 (2001); V.C. Foletta, et al., Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1. J. Cell Biol. 162(6): 1089-1098 (2003); I.K. Johnsen, et al., Bone morphogenetic proteins 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis. Cancer Res. 69(14): 5784-5792 (2009); J. Kleeff, et al., Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterol. 116(5): 1202-1216 (1999); G. Gobbi, et al., Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. Int. J. Oncology 20(1): 143-147 (2002); R. Mehdi, et al., Expression of bone

morphogenetic protein and its receptors in osteosarcoma and malignant fibrous histiocytoma. Jap. J. Clin. Oncol. 30(6): 272-275 (2000).

[0158] As such, a TVEMP comprising a TGFp peptide targeting domain would be effective in treating cancer, including a prostate cancer, a leukemia, a renal cell carcinoma, a pheochromocytoma, a thyroid tumor, a pituitary cancer, a colon cancer, a lymphoma, a stomach cancer, a breast cancer, an osteosarcoma, a fibrosarcoma, a hepatoma, a hepatocellular carcinoma, a papillary thyroid carcinoma, a myeloma, a pancreatic cancer, a testicular tumor, an ovarian cancer, a cervical carcinoma, an endometrial adenocarcinoma, an endometrioid carcinoma, a melanoma, a rhabdomyosarcoma, a squamous cell carcinoma, a neuroblastoma, an adrenocortical cancer, a salivary adenoid cystic carcinoma, or a gastric adenocarcinoma.

[0159] Thus, in an embodiment, a targeting domain comprises a TGFp peptide targeting domain. In aspects of this embodiment, a TGFp peptide targeting domain comprises a TGFpi, a TGFp2, a TGFP3 or a TGFP4. In aspects of this embodiment, a TGFp peptide targeting domain comprises SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110. In other aspects of this embodiment, a TGFp peptide targeting domain comprises amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110.

[0160] In other aspects of this embodiment, a TGFP targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110. In yet other aspects of this embodiment, a TGFp targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110. In still other aspects of this embodiment, a TGFP targeting domain comprises a polypeptide having, e.g., at least 1, 2,,3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, or SEQ ID NO: 110.

[0161] In other aspects of this embodiment, a TGFp targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 293-390 of SEQ ID NO:

107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110. In yet other aspects of this embodiment, a TGFp targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110. In still other aspects of this embodiment, a TGFp targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110.

[0162] Another example of a targeting domain disclosed herein is a Bone Morphogenetic Protein (BMP) peptide targeting domain. Non-limiting examples of a BMP peptide targeting domain include a BMP2, a BMP3, a BMP4, a BMP5, a BMP6, a BMP7, a BMP8 or a BMP10. BMP peptides bind to a family of protein receptors. For example, BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8, BMP10, and BMP15 bind to BMPR1A; BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8, BMP10, and BMP15 bind to BMPR1B; and BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8, BMP10, and BMP15 bind to BMPR2. In addition, BMP2 and BMP7 bind to ACVR2A.

[0163] BMP receptors have been detected on the surface of several different types of cancer cells. For example, BMPR1A is expressed in prostate cancer, biliary tract cancer, ovarian cancer, bone cancer, colon cancer, myelomas, glioblastomas, squamous cell carcinomas, adrenal cortex carcinomas, pancreatic cancer, osteosarcomas. See, e.g., S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells.Oncogene 23(58): 9326-9335 (2004); D.R. Haudenschild, et al., Bone morphogenetic protein (BMP)-6 signaling and BMP antagonist noggin in prostate cancer. Cancer Res. 64(22): 8276-8284 (2004); I.Y. Kim, et al., Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res. 60(11): 2840-2844 (2000); D.E. Hansel, et al., Identification of novel cellular targets in biliary tract cancers using global gene expression technology. Am. J. Pathol. 163(1): 217-229 (2003); T.G. Shepherd and M.W. Nachtigal. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144(8): 3306-3314 (2003); E. Hay, et al., Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in

osteoblastic cells. J. Biol. Chem. 279(3): 1650-1658 (2004); W. Jin, et al., TrkC binds to the bone morphogenetic protein type II receptor to suppress bone morphogenetic protein signaling. Cancer Res. 67(20): 9869-9877 (2007); H. Deng, et al., Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. Exp. Cell Res. 313(5): 1033-1044 (2007); T.B. Ro, et al.,Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23(17): 3024-3032 (2004); P. ten Dijke, etal., Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. J. Biol. Chem. 269: 16985-16988 (1994); N. Yamada, et al., Bone morphogenetic protein type IB receptor is progressively expressed in malignant glioma tumours. Br. J. Cancer 73(5): 624-629 (1996); Y. Jin, et al., Overexpression of BMP-2/4, -5 and BMPR-IA associated with malignancy of oral epithelium. Oral Oncol. 37: 225-233 (2001); A.F. Soares, et al., Bone morphogenetic protein-2/4 and bone morphogenetic protein receptor type IA expression in metastatic and nonmetastatic oral squamous cell carcinoma. Am. J. Otolaryngol. 31(4): 266-271 (2010); I.K. Johnsen, et al., Bone morphogenetic proteins 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis. Cancer Res. 69(14): 5784-5792 (2009); J. Kleeff, et al., Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterol. 116(5): 1202-1216 (1999); G. Gobbi, et al., Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. Int. J. Oncology 20(1): 143-147 (2002); and R. Mehdi, et al., Expression of bone morphogenetic protein and its receptors in osteosarcoma and malignant fibrous histiocytoma. Jap. J. Clin. Oncol. 30(6): 272-275 (2000).

[0164] As another example, BMPR1B is expressed in prostate cancer, ovarian cancer, bone cancer, colon cancer, myelomas, testicular cancer, breast cancer, glioblastomas, adrenal cortex carcinomas, and osteosarcomas. See, e.g., S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); D.L. Segev, et al., Mullerian-inhibiting substance regulates NF-kappa B signaling in the prostate in vitro and in vivo. Proc. Natl. Acad. Sci. USA 99(1): 239-244 (2002); I.Y. Kim, et al., Loss of expression of bone morphogenetic protein receptor type II in human prostate cancer cells. Oncogene 23(46): 7651-7659 (2004); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. Oncogene 23(58): 9326-9335 (2004); I.Y. Kim, et al., Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res. 60(11): 2840-2844 (2000); T.G. Shepherd and M.W. Nachtigal. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144(8): 3306-3314 (2003); E. Hay, et al., Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in osteoblastic cells. J. Biol. Chem. 279(3): 1650-1658 (2004); W. Jin, et al., TrkC binds to the bone morphogenetic protein type II receptor to suppress bone morphogenetic protein signaling. Cancer Res. 67(20): 9869-9877 (2007); T.B. Ro, et al., Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23(17): 3024-3032 (2004); L. Gouedard, et al.,

Engagement of bone morphogenetic protein type IB receptor and Smadl signaling by anti-MQllerian hormone and its type II receptor. J. Biol. Chem. 275(36): 27973-27978 (2000); V.M. Launch, et al., Mullerian inhibiting substance blocks the protein kinase A-induced expression of cytochrome p450 17alpha-hydroxylase/C(17-20) lyase mRNA in a mouse Leydig cell line independent of cAMP responsive element binding protein phosphorylation. Endocrinology 143(9): 3351-3360 (2002); M.W. Helms, et al., First evidence supporting a potential role for the BMP/SMAD pathway in the progression of oestrogen receptor-positive breast cancer. J. Pathol. 206: 366-376 (2005); P. ten Dijke, et al., Identification of type I receptors for osteogenic protein-1 and bone morphogenetic protein-4. J. Biol. Chem. 269: 16985-16988 (1994); N. Yamada, et al., Bone morphogenetic protein type IB receptor is progressively expressed in malignant glioma tumours. Br. J. Cancer 73(5): 624-629 (1996); I.K. Johnsen, et al., Bone morphogenetic proteins 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis. Cancer Res. 69(14): 5784-5792 (2009); G. Gobbi, et al., Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. Int. J. Oncology 20(1): 143-147 (2002); R. Mehdi, et al., Expression of bone morphogenetic protein and its receptors in osteosarcoma and malignant fibrous histiocytoma. Jap. J.' Clin. Oncol. 30(6): 272-275 (2000).

[0165] As yet another example, BMPR2 is expressed in prostate cancer, ovarian cancer, bone cancer, colon cancer, myelomas, breast cancer, lung carcinomas, adrenal cortex carcinomas, pancreatic cancer, and osteosarcomas. See, e.g., S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); I.Y. Kim, et al., Loss of expression of bone morphogenetic protein receptor type II in human prostate cancer cells. Oncogene 23(46): 7651-7659 (2004); H. Miyazaki, et al., BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells. Oncogene 23(58): 9326-9335 (2004); I.Y. Kim, et al., Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. Cancer Res. 60(11): 2840-2844 (2000); T.G. Shepherd and M.W. Nachtigal. Identification of a putative autocrine bone morphogenetic protein-signaling pathway in human ovarian surface epithelium and ovarian cancer cells. Endocrinology 144(8): 3306-3314 (2003); Y. Xia, et al., Repulsive guidance molecule RGMa alters utilization of bone morphogenetic protein (BMP) type II receptors by BMP2 and BMP4. J. Biol. Chem. 282(25): 18129-18140 (2007); E. Hay, et al., Bone morphogenetic protein receptor IB signaling mediates apoptosis independently of differentiation in osteoblastic cells. J. Biol. Chem. 279(3): 1650-1658 (2004); W. Jin, et al., TrkC binds to the bone morphogenetic protein type II receptor to suppress bone morphogenetic protein signaling. Cancer Res. 67(20): 9869-9877 (2007); R. L. Baldwin, et al., Attenuated ALK5 receptor expression in human pancreatic cancer: correlation with resistance to growth inhibition. Int. J. Cancer 67(2): 283-288 (1996); H. Deng, et al., Bone morphogenetic protein-4 is overexpressed in colonic adenocarcinomas and promotes migration and invasion of HCT116 cells. Exp. Cell Res. 313(5): 1033-1044 (2007); T.B. Ro, et al., Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human myeloma cells. Oncogene 23(17): 3024-3032 (2004); M. Fan, et al., Diverse gene expression and DNA methylation profiles correlate with differential adaptation of breast cancer cells to the antiestrogens tamoxifen and

fulvestrant. Cancer Res. 66(24): 11954-11966 (2006); J.A. McEarchern, et al., Invasion and metastasis of a mammary tumor involves TGF-beta signaling.Int. J. Cancer 91(1): 76-82 (2001); V.C. Foletta, et al., Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1. J. Cell Biol. 162(6): 1089-1098 (2003); I.K. Johnsen, et al., Bone morphogenetic proteins 2 and 5 are down-regulated in adrenocortical carcinoma and modulate adrenal cell proliferation and steroidogenesis. Cancer Res. 69(14): 5784-5792 (2009); J. Kleeff, et al., Bone morphogenetic protein 2 exerts diverse effects on cell growth in vitro and is expressed in human pancreatic cancer in vivo. Gastroenterol. 116(5): 1202-1216 (1999); G. Gobbi, et al., Seven BMPs and all their receptors are simultaneously expressed in osteosarcoma cells. Int. J. Oncology 20(1): 143-147 (2002); R. Mehdi, et al., Expression of bone morphogenetic protein and its receptors in osteosarcoma and malignant fibrous histiocytoma. Jap. J. Clin. Oncol. 30(6): 272-275 (2000).

[0166] As such, a TVEMP comprising a BMP peptide targeting domain would be effective in treating cancer, including a prostate cancer, a leukemia, a biliary tract cancer, an ovarian cancer, a bone cancer, an osteosarcoma, a colon cancer, a myeloma, a testicular cancer, a testicular tumor, a breast cancer, a glioblastoma, a squamous cell carcinoma, a lung carcinoma, an adrenal cortex carcinoma, a pituitary cancer, an endometrioid carcinoma, a hepatoma, a hepatocellular carcinoma, a gastric adenocarcinoma, or a pancreatic cancer.

[0167] Thus, in an embodiment, a targeting domain comprises a BMP peptide targeting domain. In aspects of this embodiment, a BMP peptide targeting domain comprises a BMP2, a BMP3, a BMP4, a BMP5, a BMP6, a BMP7, a BMP8 or a BMP10. In aspects of this embodiment, a BMP peptide targeting domain comprises SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118. In other aspects of this embodiment, a BMP peptide targeting domain comprises amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118.

[0168] In other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118. In yet other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ

ID NO: 117, or SEQ ID NO: 118; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118. Instill other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 111, SEQ ID NO: 112, SEQ ID NO: 113, SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 117, or SEQ ID NO: 118.

[0169] In other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118. In yet other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118. In still other aspects of this embodiment, a BMP targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids

323-424 of SEQ ID NO: 118; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118.

[0170] Another example of a targeting domain disclosed herein is a Growth and Differentiation Factor (GDF) peptide targeting domain. Non-limiting examples of a GDF peptide targeting domain include a GDF1, a GDF2, a GDF3, a GDF5, a GDF6, a GDF7, a GDF8, a GDF10, a GDF11 or a GDF15. GDF peptides bind to the activin protein receptor family in addition to members of the TGFB and BMP family of protein receptors. For example, GDF2 binds to activin A receptor type ll-like 1 (ACVRL1) and activin A receptor, type I (ACVR1), in addition to BMPR2; GDF3 binds to activin A receptor, type IB (ACVR1B) and activin A receptor, type IIB (ACVR2B); GDF5 binds to ACVR1, ACVR1B, ACVR2B, in addition to BMPR1A, BMPR1B, and BMPR2; GDF6 binds to BMPR1A, BMPR1B, and BMPR2; GDF8 binds to ACVR2A and ACVR2B; GDF9 bind to BMPR2; and GDF11 bind to ACVR1B, ACVR1C, activin A receptor, type IIA (ACVR2A), ACVR2B, in addition to TGFBR1.

[0171] GDF receptors have been detected on the surface of several different types of cancer cells. As discussed above, BMPR1A, BMPR1B, BMPR2, and TGFBR1 are expressed in a wide variety of cancer cells. In addition, activin receptors are expressed on the surface of several different types of cancer cells. For example, ACVR1 is expressed in prostate cancer, renal cell carcinomas, leukemias, pituitary cancer, hepatomas, hepatocellular carcinomas, myelomas, and pancreatic cancer, See, e.g., H. Miyazaki, et al. BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells, Oncogene 23(58): 9326-9335 (2004); S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); Q.F. Wang , et al., Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP, Endocrinology 137(12): 5476-5483 (1996); A.C. Dalkin, et al., Activin inhibition of prostate cancer cell growth: selective actions on androgen-responsive LNCaP cells, Endocrinology 137(12): 5230-5235 (1996); S. Naito, et al., Establishment of two human renal cell carcinoma cell lines with different chemosensitivity, Hum. Cell 9(2): 101-108 (1996); J.J. Lebrun and W.W. Vale, Activin and inhibin have antagonistic effects on ligand-dependent heteromerization of the type I and type II activin receptors and human erythroid differentiation, Mol. Cell Biol. 17(3): 1682-1691 (1997); K. Tsuchida, et al. Cloning and characterization of a transmembrane serine kinase that acts as an activin type I receptor, Proc. Natl. Acad. Sci. USA 90(23):11242-11246 (1993); W. Chen, et al. Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins, Endocrinology 141(3): 1263-1272 (2000); K. Wagner, et al., Activin A stimulates vascular endothelial growth factor gene transcription in human hepatocellular carcinoma cells, Gastroenterology 126(7): 1828-1843 (2004); T. Baade Ro, et al., Bone morphogenetic protein-5, -6 and -7 inhibit growth and induce apoptosis in human

myeloma cells, Oncogene 23(17): 3024-3032 (2004); and R.L. Baldwin, et al. Attenuated ALK5 receptor expression in human pancreatic cancer: correlation with resistance to growth inhibition, Int. J. Cancer 67(2): 283-288 (1996).

[0172] As another example, ACVR1B is expressed in prostate cancer, leukemias, testicular tumors, hepatomas, and hepatocellular carcinomas. See, e.g., Q.F. Wang , et al., Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP, Endocrinology 137(12): 5476-5483 (1996); A.C. Dalkin, et al., Activin inhibition of prostate cancer cell growth: selective actions on androgen-responsive LNCaP cells, Endocrinology 137(12): 5230-5235 (1996); S. Naito, et al., Establishment of two human renal cell carcinoma cell lines with different chemosensitivity, Hum. Cell 9(2): 101-108 (1996); J.J. Lebrun and W.W. Vale, Activin and inhibin have antagonistic effects on ligand-dependent heteromerization of the type I and type II activin receptors and human erythroid differentiation, Mol. Cell Biol. 17(3): 1682-1691 (1997); N. Di Simone, et al., Activin regulates betaA-subunit and activin receptor messenger ribonucleic acid and cellular proliferation in activin-responsive testicular tumor cells, Endocrinology 139(3): 1147-1155 (1998); W. Chen, et al. Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins, Endocrinology 141(3): 1263-1272 (2000); and K. Wagner, et al., Activin A stimulates vascular endothelial growth factor gene transcription in human hepatocellular carcinoma cells, Gastroenterology 126(7): 1828-1843 (2004).

[0173] As yet another example, ACVR2A is expressed in prostate cancer, ovarian cancer, leukemias, colon cancer, pituitary cancer, endometrioid carcinomas, testicular tumors, hepatomas, hepatocellular carcinomas, pancreatic cancer, and gastric adenocarcinomas. See, e.g., H. Miyazaki, et al. BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells, Oncogene 23(58): 9326-9335 (2004); S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); Q.F. Wang , et al., Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP, Endocrinology 137(12): 5476-5483 (1996); A.C. Dalkin, et al., Activin inhibition of prostate cancer cell growth: selective actions on androgen-responsive LNCaP cells, Endocrinology 137(12): 5230-5235 (1996); S. Naito, et al., Establishment of two human renal cell carcinoma cell lines with different chemosensitivity, Hum. Cell 9(2): 101-108 (1996); N. Di Simone, et al., Characterization of inhibin/activin subunit, follistatin, and activin type II receptors in human ovarian cancer cell lines: a potential role in autocrine growth regulation, Endocrinology 137(2): 486-494 (1996); T. Minegishi, et al., Expression of gonadotropin and activin receptor messenger ribonucleic acid in human ovarian epithelial neoplasms, Clin. Cancer Res. 6(7): 2764-2770 (2000); J.J. Lebrun and W.W. Vale, Activin and inhibin have antagonistic effects on ligand-dependent heteromerization of the type I and type II activin receptors and human erythroid differentiation, Mol. Cell Biol. 17(3): 1682-1691 (1997); Y. Mori, et al., Instabilotyping reveals unique mutational spectra in microsatellite-unstable gastric cancers, Cancer Res. 62(13): 3641-3645 (2002); K. Tsuchida, et al. Cloning and characterization of a transmembrane serine kinase that acts as an activin type I receptor, Proc. Natl. Acad. Sci. USA 90(23):11242-11246

(1993); N. Di Simone, et al., Activin regulates betaA-subunit and activin receptor messenger ribonucleic acid and cellular proliferation in activin-responsive testicular tumor cells, Endocrinology 139(3): 1147-1155 (1998); W. Chen, et al. Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins, Endocrinology 141(3): 1263-1272 (2000); K. Wagner, et al., Activin A stimulates vascular endothelial growth factor gene transcription in human hepatocellular carcinoma cells, Gastroenterology 126(7): 1828-1843 (2004); and M. Cattaneo, et al., SEL1L affects human pancreatic cancer cell cycle and invasiveness through modulation of PTEN and genes related to cell-matrix interactions, Neoplasia 7(11): 1030-1038 (2005);

[0174] As still another example, ACVR2B is expressed in prostate cancer, ovarian cancer, leukemias, colon cancer, endometrioid carcinomas, testicular tumors, hepatomas, hepatocellular carcinomas, and pancreatic cancer. See, e.g., H. Miyazaki, et al. BMP signals inhibit proliferation and in vivo tumor growth of androgen-insensitive prostate carcinoma cells, Oncogene 23(58): 9326-9335 (2004); S. Yang, et al., Diverse biological effect and Smad signaling of bone morphogenetic protein 7 in prostate tumor cells. Cancer Res. 65(13): 5769-5777 (2005); Q.F. Wang , et al., Activin inhibits basal and androgen-stimulated proliferation and induces apoptosis in the human prostatic cancer cell line, LNCaP, Endocrinology 137(12): 5476-5483 (1996); D.R. Haudenschild, et al., Bone Morphogenetic Protein (BMP)-6 Signaling and BMP Antagonist Noggin in Prostate Cancer, Cancer Res. 64(22): 8276-8284 (2004); N. Di Simone, et al., Characterization of inhibin/activin subunit, follistatin, and activin type II receptors in human ovarian cancer cell lines: a potential role in autocrine growth regulation, Endocrinology 137(2): 486-494 (1996); T. Minegishi, et al., Expression of gonadotropin and activin receptor messenger ribonucleic acid in human ovarian epithelial neoplasms, Clin. Cancer Res. 6(7): 2764-2770 (2000); B.K. Shin, et al., Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function, J. Biol. Chem. 278(9): 7607-7616 (2003); J.J. Lebrun and W.W. Vale, Activin and inhibin have antagonistic effects on ligand-dependent heteromerization of the type I and type II activin receptors and human erythroid differentiation, Mol. Cell Biol. 17(3): 1682-1691 (1997); N. Di Simone, et al., Activin regulates betaA-subunit and activin receptor messenger ribonucleic acid and cellular proliferation in activin-responsive testicular tumor cells, Endocrinology 139(3): 1147-1155 (1998); W. Chen, et al. Activin A-induced HepG2 liver cell apoptosis: involvement of activin receptors and smad proteins, Endocrinology 141(3): 1263-1272 (2000); K. Wagner, et al., Activin A stimulates vascular endothelial growth factor gene transcription in human hepatocellular carcinoma cells, Gastroenterology 126(7): 1828-1843 (2004); and M. Cattaneo, et al., SEL1L affects human pancreatic cancer cell cycle and invasiveness through modulation of PTEN and genes related to cell-matrix interactions, Neoplasia 7(11): 1030-1038 (2005).

[0175] As such, a TVEMP comprising a GDF peptide targeting domain would be effective in treating cancer, including a prostate cancer, a renal cell carcinoma, a pheochromocytoma, a biliary tract cancer, an ovarian cancer, a testicular tumor, a bone cancer, a thyroid tumor, a papillary thyroid carcinoma, a pituitary cancer, an endometrioid carcinoma, a colon cancer, a myeloma, a lymphoma, a leukemia, a testicular cancer, a stomach cancer, a gastric adenocarcinoma, a breast cancer, a glioblastoma, a

fibrosarcoma, a hepatoma, a hepatocellular carcinoma, a squamous cell carcinoma, a lung carcinoma, an adrenal cortex carcinoma, a pancreatic cancer, or an osteosarcoma.

[0176] Thus, in an embodiment, a targeting domain comprises a GDF peptide targeting domain. In aspects of this embodiment, a GDF peptide targeting domain comprises a GDF1, a GDF2, a GDF3, a GDF5, a GDF6, a GDF7, a GDF8, a GDF10, a GDF11 or a GDF15. In aspects of this embodiment, a GDF peptide targeting domain comprises SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128. In other aspects of this embodiment, a GDF peptide targeting domain comprises amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128.

[0177] In other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128. In yet other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128. In still other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, or SEQ ID NO: 128.

[0178] In other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least

95% to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128. In yet other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128. In still other aspects of this embodiment, a GDF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128.

[0179] Another example of a targeting domain disclosed herein is an activin peptide targeting domain. Non-limiting examples of an activin peptide targeting domain include an activin A, an activin B, an activin C, an activin E, an inhibin A, or an inhibin B. Activin peptides bind to the activin family of protein receptors as well as to TGF3 receptor members. For example, activin peptide like activin A, activin B,

activin C, activin E bind to ACVR2A and ACVR2B; inhibin A binds to ACVR1, ACVR1B, ACVR2A, and ACVR2B, in addition to TGFBR3; and inhibin B binds to ACVR1, ACVR1B, ACVR2A, and ACVR2B.

[0180] Activin receptors have been detected on the surface of several different types of cancer cells. As discussed above, ACVR1, ACVR1B, ACVR2A, ACVR2B, and TGFBR3 are expressed in a wide variety of cancer cells. As such, a TVEMP comprising an activin peptide targeting domain would be effective in treating cancer, including a prostate cancer, a renai cell carcinoma, an ovarian cancer, a leukemia, a colon cancer, a pituitary cancer, a pheochromocytoma, a stomach cancer, a breast cancer, an adrenocortical cancer, a salivary adenoid cystic carcinoma, an endometrioid carcinoma, a testicular tumor, a hepatoma, a hepatocellular carcinoma, a myeloma, a pancreatic cancer, or a gastric adenocarcinoma.

[0181] Thus, in an embodiment, a targeting domain comprises an activin peptide targeting domain. In aspects of this embodiment, an activin peptide targeting domain comprises an activin A, an activin B, an activin C, an activin E or an inhibin A. In aspects of this embodiment, an activin peptide targeting domain comprises SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133. In other aspects of this embodiment, an activin peptide targeting domain comprises amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133.

[0182] In other aspects of this embodiment, an activin targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133. In yet other aspects of this embodiment, an activin targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133. In still other aspects of this embodiment, an activin targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133.

[0183] In other aspects of this embodiment, an activin targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133. In yet other aspects of this embodiment, an activin targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133. In still other aspects of this embodiment, an activin targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133.

[0184] Another example of a targeting domain disclosed herein is a Fibroblast Growth Factor (FGF) peptide targeting domain. Non-limiting examples of a FGF peptide targeting domain include a FGF1, a FGF2, a FGF3, a FGF4, a FGF5, a FGF6, a FGF7, a FGF8, a FGF9, a FGF10, a FGF17, and a FGF18. Fibroblast growth factors (FGF) participate in many developmental, differentiation and growth and repair processes of cells through complex combinatorial signaling pathways. Presently, at least 23 ligands (FGF1-23) are known to signal through a family of five transmembrane tyrosine kinase FGF receptors (FGFR1-5). Affinity of FGFRs for their ligands is highly diverse with different affinities for each family member of growth factors, see, e.g., C. J. Powers et al., *Fibroblast growth factors, their receptors and signaling,* 7(3) Endocr. Relat. Cancer. 165-197 (2000). This diversity is achieved in part by the generation of alternatively spliced variants encoding distinct receptor isoforms, see, e.g., Bernhard Reuss & Oliver von Bohlen und Halbach, *Fibroblast growth factors and their receptors in the central nervous system,* 313(2) Cell Tissue Res. 139-157 (2003). The protein region that appears to have the highest influence on ligand binding selectivity is a portion of the IgIII domain, for which isoforms encoded by three

different splice variants have been identified. These three isoforms, designated IgIIIa, IgIIIb and IgIIIc, have relative binding affinities for different FGFR family members. For example, FGF-1, FGF-2, FGF-3, FGF7, FGF-8, FGF9, FGF-10, FGF19, and FGF20 bind to FGFRIIIIb; FGF-1, FGF-2, FGF-4, FGF-5, FGF-6, FGF7, FGF-8, FGF9, FGF-10, FGF-17, FGF19, and FGF20 bind to FGFRIIIIc; FGF-1, FGF-3, FGF-7, and FGF-10 bind to FGFR2IIIb; FGF-1, FGF-2, FGF-4, FGF-5, FGF-6, FGF-8, FGF-9, FGF-17, FGF19, and FGF20bind to FGFR2IIIc; FGF-1 and FGF-9 bind to FGFR3IIIb; FGF-1, FGF-2, FGF-4, FGF7, FGF-8, FGF-9, and FGF23 bind to FGFR3IIIb; FGF1, FGF2, FGF4, FGF5, FGF6, FGF8, FGF9, FGF16, FGF19, FGF20, FGF21, and FGF23 bind to FGFR4; and FGF-1 and FGF-2 bind to FGFR5. Alternative splicing in the FGFR ligand binding domain, designated a and b, generates additional receptor isoforms with novel ligand affinities. Isoforms for IgIIIa, IgIIIb and IgIIIc have been identified for both FGFR1 and FGFR2. Thus far, the IgIIIa isoform of FGFR3 and the IgIIIa and IgIIIb isoforms of FGFR4 and FGFR5 have not been reported.

[0185] FGF receptors have been detected on the surface of several different types of cancer cells. For example, FGFR1 is expressed in acute myeloblasts leukemias, chronic lymphocytic leukemias, and breast cancer. See, e.g., P. Blume-Jensen and T. Hunter. Oncogenic kinase signalling. Nature 411(6835): 355-365 (2001); and Z.Q. Yang, et al., Multiple interacting oncogenes on the 8p11-p12 amplicon in human breast cancer. Cancer Res. 66(24): 11632-11643 (2006).

[0186] As another example, FGFR2 is expressed in breast cancer, endometrial ovarian cancer, and gastric cancer. See, e.g., D. J. Hunter, et al., A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat. Genet. 39(7): 870-874 (2007); D. F. Easton, et al., Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 447(7148): 1087-1093 (2007); S. A. Byron, et al., Inhibition of activated fibroblast growth factor receptor 2 in endometrial cancer cells induces cell death despite PTEN abrogation. Cancer Res. 68(17): 6902-6907 (2008); and J.H. Jang, et al., Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res. 61(9): 3541-3543 (2001).

[0187] As yet another example, FGFR3 is expressed in bladder cancer, colon cancer, and cervical cancer. See, e.g., B.W. van Rhijn, et al., The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. Cancer Res. 61(4): 1265-1268 (2001); J.H. Jang, et al., Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers. Cancer Res. 61(9): 3541-3543 (2001); A.M. Saaf, et al., Parallels between global transcriptional programs of polarizing Caco-2 intestinal epithelial cells in vitro and gene expression programs in normal colon and colon cancer. Mol. Biol. Cell 18(11): 4245-4260(2007); and K. Sibley, etal., Frequency of fibroblast growth factor receptor 3 mutations in sporadic tumours. Oncogene 20(32): 4416-4418 (2001).

[0188] As still another example, FGFR4 is expressed in epithelial ovarian cancer, metastasis, leiomyomas, and pituitary tumors. See, e.g., L. De Cecco, et al., Gene expression profiling of advanced ovarian cancer: characterization of a molecular signature involving fibroblast growth factor 2. Oncogene 23(49): 8171-8183 (2004); N. Seitzer, et al., A single nucleotide change in the mouse genome accelerates breast cancer progression. Cancer Res. 70(2): 802-812 (2010); L. Yu, et al., Differential expression of receptor tyrosine kinases (RTKs) and IGF-I pathway activation in human uterine leiomyomas. Mol. Med. 14(5-6): 264-275 (2008); and S. Ezzat, et al., Targeted expression of a human pituitary tumor-derived isoform of FGF receptor-4 recapitulates pituitary tumorigenesis. J. Clin. Invest. 109(1): 69-78 (2002).

[0189] As such, a TVEMP comprising a FGF peptide targeting domain would be effective in treating cancer, including an acute myeloblastic leukemia, a chronic lymphocytic leukemia, a breast cancer, an endometrial ovarian cancer, a gastric cancer, a bladder cancer, a colon cancer, a cervical cancer, an epithelial ovarian cancer, a leiomyoma, or a pituitary tumor.

[0190] Thus, in an embodiment, a targeting domain comprises a FGF peptide targeting domain. In aspects of this embodiment, a FGF peptide targeting domain comprises a FGF1, a FGF2, a FGF3, a FGF4, a FGF5, a FGF6, a FGF7, a FGF8, a FGF9, a FGF10, a FGF17, and a FGF18. In aspects of this embodiment, a FGF peptide targeting domain comprises SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145. In other aspects of this embodiment, a FGF peptide targeting domain comprises amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145.

[0191] In other aspects of this embodiment, a FGF targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ* ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145. In yet other aspects of this embodiment, a FGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO:

143, SEQIDNO: 144, or SEQ ID NO: 145; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145. In still other aspects of this embodiment, a FGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143, SEQ ID NO: 144, or SEQ ID NO: 145.

[0192] In other aspects of this embodiment, a FGF targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145. In yet other aspects of this embodiment, a FGF targeting domain comprises a polypeptide having, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145. In still other aspects of this embodiment, a FGF targeting

domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177ofSEQIDNO:145.

[0193] Another example of a targeting domain disclosed herein is a Platelet-Derived Growth Factor (PDGF) peptide targeting domain. Non-limiting examples of a PDGF peptide targeting domain include a PDGFa and PDGF(3. PDGFs are mitogenic factors for cells of mesenchymal origin and are characterized by a motif of eight cysteines. PDGFs can exist either as a homodimer or as a heterodimer, where the dimers are connected by disulfide bonds. Studies using knockout mice have shown cellular defects in oligodendrocytes, alveolar smooth muscle cells, and Leydig cells in the testis; knockout mice die either as embryos or shortly after birth. Two splice variants have been identified for this gene. PDGF peptides bind to a family of G-coupled protein receptors. For example, PDGF-AA, PDGF-BB and PDGF-AB bind to PDGFRa; and PDGF-BB and PDGF-AB bind to PDGFRP; and VEGFA, VEGFC, and VEGFD bind to VEGFR3.

[0194] PDGF receptors have been detected on the surface of several different types of cancer cells. For example, PDGFRa is expressed in prostate cancer, non-small cell lung cancer, rhabdomyosarcomas, gastrointestinal stromal tumors, medulloblastomas, glioblastomas, nasopharyngeal carcinomas, fibrosarcomas, basal cell carcinomas, neuroblastomas, astrocytomas, osteosarcomas, breast cancer, testicular tumors, ovarian cancer, melanomas, myelomas, squamous cell carcinomas, and lymphomas.

[0195] As another example, PDGFRS is expressed in prostate cancer, renal cell carcinomas, bladder cancer, glioblastomas, fibrosarcomas, neuroblastomas, astrocytomas, osteosarcomas, ewing's sarcomas, breast cancer, testicular tumors, ovarian cancer, myelomas, leukemias, mesotheliomas, Kaposi sarcomas, and chondrosarcomas.

[0196] As yet another example, PDGFR-like is expressed in myelomas and alveolar basal epithelial carcinomas.

[0197] As such, a TVEMP comprising a PDGF peptide targeting domain would be effective in treating cancer, including a prostate cancer, a renal cell carcinoma, a bladder cancer, a non-small cell lung cancer, a rhabdomyosarcoma, a gastrointestinal stromal tumor, a medulloblastoma, a glioblastoma, a nasopharyngeal carcinoma, a fibrosarcoma, a basal cell carcinoma, a neuroblastoma, an astrocytoma, an osteosarcoma, a Ewing's sarcoma, a breast cancer, a testicular tumor, an ovarian cancer, a melanoma, a myeloma, a squamous cell carcinoma, a lymphoma, a leukemia, a mesothelioma, a Kaposi sarcoma, or a chondrosarcoma.

[0198] Thus, in an embodiment, a targeting domain comprises a PDGF peptide targeting domain. In aspects of this embodiment, a PDGF peptide targeting domain comprises a PDGFa or PDGFp. In aspects of this embodiment, a PDGF peptide targeting domain comprises SEQ ID NO: 153 or SEQ ID NO: 154. In other aspects of this embodiment, a PDGF peptide targeting domain comprises amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154.

[0199] In other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having an amino acid identity of, *e.g.,* at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to SEQ ID NO: 153 or SEQ ID NO: 154; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to SEQ ID NO: 153 or SEQ ID NO: 154. In yet other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 153 or SEQ ID NO: 154; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 153 or SEQ ID NO: 154. In still other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 153 or SEQ ID NO: 154; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to SEQ ID NO: 153 or SEQ ID NO: 154.

[0200] In other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having an amino acid identity of, e.g., at least 70%, at least 75%, at least 80%, at least 85%, at least 90% or at least 95% to amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154; or at most 70%, at most 75%, at most 80%, at most 85%, at most 90% or at most 95% to amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154. In yet other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 non-contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154. In still other aspects of this embodiment, a PDGF targeting domain comprises a polypeptide having, *e.g.,* at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 94-182 of SEQ ID

NO: 153 or amino acids 95-182 of SEQ ID NO: 154; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15 or 20 contiguous amino acid deletions, additions, and/or substitutions relative to amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154.

[0201] Clostridial toxins are each translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease (FIG. 18). This cleavage occurs within the discrete di-chain loop region created between two cysteine residues that form a disulfide bridge. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by the single disulfide bond and non-covalent interactions between the two chains (FIG. 2). To facilitate recombinant production of a TVEMP, an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a TVEMP disclosed herein into the di-chain form. *See, e.g.,* Steward, L.E. et al., *Modified Clostridial Toxins with Enhanced Targeting Capabilities For Endogenous Clostridial Toxin Receptor Systems,* U.S. Patent Publication No. US 2008/0096248 (Apr. 24, 2008); Steward, L.E. et al., *Activatable Clostridial Toxins,* U.S. Patent Publication No. US 2008/0032930 (Feb. 7, 2008); Steward, *supra,* (2007); Dolly, *supra,* (2007); Foster, *supra,* WO 2006/059093 (2006); and Foster, *supra,* WO 2006/059105 (2006), each of which is hereby incorporated by reference in its entirety.

[0202] In is envisioned that any and ail protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form, including, without limitation, endogenous di-chain loop protease cleavage sites and exogenous protease cleavage sites. Thus, in an aspect of the invention, a TVEMP comprises, in part, an endogenous protease cleavage site within a di-chain loop region. In another aspect of the invention, a TVEMP comprises, in part, an exogenous protease cleavage site within a di-chain loop region. As used herein, the term "di-chain loop region" means the amino acid sequence of a Clostridial toxin containing a protease cleavage site used to convert the single-chain form of a Clostridial toxin into the di-chain form. Non-limiting examples of a Clostridial toxin di-chain loop region, include, a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; and a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8 (Table 4).

Table 4. Di-chain Loop Region of Clostridial Toxins

Toxin	SEQ ID NO:	Di-chain Loop Region Containing the Naturally-occurring Protease Cleavage Site
BoNT/A	26	CVRGIITSKTKSLDKGYNK*----ALNDLC
BoNT/B	27	CKSVK*----------APGIC
BoNT/C1	28	CHKAIDGRSLYNK*----------TLDC

BoNT/D	29	CLRLTKNSR*----------DDSTC
BoNT/E	30	CKNIVSVKGIR*----------KSIC
BoNT/F	31	CKSVIPRKGTK*----------APPRLC
BoNT/G	32	CKPVMYKNTGK*----------SEQC
TeNT	33	CKKIIPPTNIRENLYNRTA*SLTDLGGELC
BaNT	34	CKS-IVSKKGTK*----------NSLC
BuNT	35	CKN-IVSVKGIR*----------KSIC

The amino acid sequence displayed are as follows: BoNT/A, residues 430-454 of SEQ ID NO: 1; BoNT/B, residues 437-446 of SEQ ID NO: 2; BoNT/C1, residues 437-453 of SEQ ID NO: 3; BoNT/D, residues 437-450 of SEQ ID NO: 4; BoNT/E, residues 412-426 of SEQ ID NO: 5; BoNT/F, residues 429-445 of SEQ ID NO: 6; BoNT/G, residues 436-450 of SEQ ID NO: 7; TeNT, residues 439-467 of SEQ ID NO: 8; BaNT, residues 421-435 of SEQ ID NO: 9; and BuNT, residues 412-426 of SEQ ID NO: 10. An asterisks (*) indicates the peptide bond that is cleaved by a Clostridial toxin protease.

[0203] As used herein, the term "endogenous di-chain loop protease cleavage site" is synonymous with a "naturally occurring di-chain loop protease cleavage site" and means a naturally occurring protease cleavage site found within the di-chain loop region of a naturally occurring Clostridial toxin and includes, without limitation, naturally occurring Clostridial toxin di-chain loop protease cleavage site variants, such as, *e.g.,* Clostridial toxin di-chain loop protease cleavage site isoforms and Clostridial toxin di-chain loop protease cleavage site subtypes. Non-limiting examples of an endogenous protease cleavage site, include, e.g., a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site and a TeNT di-chain loop protease cleavage site.

[0204] As mentioned above, Clostridial toxins are translated as a single-chain polypeptide of approximately 150 kDa that is subsequently cleaved by proteolytic scission within a disulfide loop by a naturally-occurring protease. This posttranslational processing yields a di-chain molecule comprising an approximately 50 kDa light chain (LC) and an approximately 100 kDa heavy chain (HC) held together by a single disulphide bond and noncovalent interactions. While the identity of the protease is currently unknown, the di-chain loop protease cleavage site for many Clostridial toxins has been determined. In BoNTs, cleavage at K448-A449 converts the single polypeptide form of BoNT/A into the di-chain form; cleavage at K441-A442 converts the single polypeptide form of BoNT/B into the di-chain form; cleavage at K449-T450 converts the single polypeptide form of BoNT/C1 into the di-chain form; cleavage at R445-D446 converts the single polypeptide form of BoNT/D into the di-chain form; cleavage at R422-K423 converts the single polypeptide form of BoNT/E into the di-chain form; cleavage at K439-A440 converts the single polypeptide form of BoNT/F into the di-chain form; and cleavage at K446-S447 converts the single polypeptide form of BoNT/G into the di-chain form. Proteolytic cleavage of the single polypeptide form of TeNT at A457-S458 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BaNT at K431-N432 results in the di-chain form. Proteolytic cleavage of the single polypeptide form of BuNT at R422-K423 results in the di-chain form. Such a di-chain loop protease cleavage site is

operably-linked in-frame to a TVEMP as a fusion protein. However, it should also be noted that additional cleavage sites within the di-chain loop also appear to be cleaved resulting in the generation of a small peptide fragment being lost. As a non-limiting example, BoNT/A single-chain polypeptide cleave ultimately results in the loss of a ten amino acid fragment within the di-chain loop.

[0205] Thus, in an embodiment, a protease cleavage site comprising an endogenous Clostridial toxin di-chain loop protease cleavage site is used to convert the single-chain toxin into the di-chain form. In aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.,* a BoNT/A di-chain loop protease cleavage site, a BoNT/B di-chain loop protease cleavage site, a BoNT/C1 di-chain loop protease cleavage site, a BoNT/D di-chain loop protease cleavage site, a BoNT/E di-chain loop protease cleavage site, a BoNT/F di-chain loop protease cleavage site, a BoNT/G di-chain loop protease cleavage site, a TeNT di-chain loop protease cleavage site, a BaNT di-chain loop protease cleavage site, or a BuNT di-chain loop protease cleavage site.

[0206] In other aspects of this embodiment, conversion into the di-chain form by proteolytic cleavage occurs from a site comprising, *e.g.,* a di-chain loop region of BoNT/A comprising amino acids 430-454 of SEQ ID NO: 1; a di-chain loop region of BoNT/B comprising amino acids 437-446 of SEQ ID NO: 2; a di-chain loop region of BoNT/C1 comprising amino acids 437-453 of SEQ ID NO: 3; a di-chain loop region of BoNT/D comprising amino acids 437-450 of SEQ ID NO: 4; a di-chain loop region of BoNT/E comprising amino acids 412-426 of SEQ ID NO: 5; a di-chain loop region of BoNT/F comprising amino acids 429-445 of SEQ ID NO: 6; a di-chain loop region of BoNT/G comprising amino acids 436-450 of SEQ ID NO: 7; or a di-chain loop region of TeNT comprising amino acids 439-467 of SEQ ID NO: 8. a di-chain loop region of BaNT comprising amino acids 421-435 of SEQ ID NO: 9; or a di-chain loop region of BuNT comprising amino acids 412-426 of SEQ ID NO: 10.

[0207] It is also envisioned that an exogenous protease cleavage site can be used to convert the single-chain polypeptide form of a TVEMP disclosed herein into the di-chain form. As used herein, the term "exogenous protease cleavage site" is synonymous with a "non-naturally occurring protease cleavage site" or "non-native protease cleavage site" and means a protease cleavage site that is not normally present in a di-chain loop region from a naturally occurring Clostridial toxin, with the proviso that the exogenous protease cleavage site is not a human protease cleavage site or a protease cleavage site that is susceptible to a protease being expressed in the host cell that is expressing a construct encoding an activatable polypeptide disclosed herein. It is envisioned that any and all exogenous protease cleavage sites can be used to convert the single-chain polypeptide form of a Clostridial toxin into the di-chain form are useful to practice aspects of the present invention. Non-limiting examples of exogenous protease cleavage sites include, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus (TEV) protease cleavage site, a

Tobacco Vein Mottling Virus (TVMV) cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

[0208] It is envisioned that an exogenous protease cleavage site of any and all lengths can be useful in aspects of the present invention with the proviso that the exogenous protease cleavage site is capable of being cleaved by its respective protease. Thus, in aspects of this embodiment, an exogenous protease cleavage site can have a length of, e.g., at least 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or at least 60 amino acids; or at most 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or at least 60 amino acids.

[0209] In an embodiment, an exogenous protease cleavage site is located within the di-chain loop of a TVEMP. In aspects of this embodiment, a TVEMP comprises an exogenous protease cleavage site comprises, e.g., a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, a non-human enterokinase protease cleavage site, a Tobacco Etch Virus protease cleavage site, a Tobacco Vein Mottling Virus protease cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, a SUMO/ULP-1 protease cleavage site, and a non-human Caspase 3 cleavage site. In other aspects of this embodiment, an exogenous protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0210] In an aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human enterokinase cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a bovine enterokinase protease cleavage site located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a bovine enterokinase protease cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 36. In still other aspects of this embodiment, a bovine enterokinase protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0211] In another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a TVEMP comprises the consensus sequence E-P5-P4-Y-P2-Q*-G (SEQ ID NO: 377) or E-P5-P4-Y-P2-Q*-S (SEQ ID NO: 38), where P2, P4 and P5 can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Etch Virus protease cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43,

SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47 or SEQ ID NO: 48. In still other aspects of this embodiment, a Tobacco Etch Virus protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0212] In another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site located within the di-chain loop of a TVEMP comprises the consensus sequence P6-P5-V-R-F-Q*-G (SEQ ID NO: 49) or P6-P5-V-R-F-Q*-S (SEQ ID NO: 50), where P5 and P6 can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a Tobacco Vein Mottling Virus protease cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, or SEQ ID NO: 54. In still other aspects of this embodiment, a Tobacco Vein Mottling Virus protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0213] In still another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site located within the di-chain loop of a TVEMP comprises the consensus sequence P5-P4-L-F-Q*-G-P (SEQ ID NO: 55), where P4 is G, A, V, L, I, M, S or T and P5 can any amino acid, with D or E preferred. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a human rhinovirus 3C protease located within the di-chain loop of a TVEMP that can be cleaved by PRESCISSION®. In still other aspects of this embodiment, a human rhinovirus 3C protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0214] In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a TVEMP comprises the consensus sequence P6-P5-P4-P3-H*-Y (SEQ ID NO: 62) or P6-P5-P4-P3-Y-H* (SEQ ID NO: 63), where P3, P4 and P5 and P6 can be any amino acid. In

other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 64, SEQ ID NO: 65, or SEQ ID NO: 66. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a subtilisin cleavage site located within the di-chain loop of a TVEMP that can be cleaved by GENENASE®. In still other aspects of this embodiment, a subtilisin cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0215] In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site comprising multiples of the dipeptide N*G. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a hydroxylamine cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 67, or SEQ ID NO: 68. In still other aspects of this embodiment, a hydroxylamine cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0216] In yet another aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a TVEMP comprising the consensus sequence G-G*-P1'-P2'-P3' (SEQ ID NO: 69), where P1', P2', and P3' can be any amino acid. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a SUMO/ULP-1 protease cleavage site located within the di-chain loop of a TVEMP comprises SEQ ID NO: 70. In still other aspects of this embodiment, a SUMO/ULP-1 protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0217] In an aspect of this embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human Caspase 3 cleavage site is located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a mouse Caspase 3 protease cleavage site located within the di-chain loop of a TVEMP. In other aspects of the embodiment, an exogenous protease cleavage site can comprise, e.g., a non-human Caspase 3 protease cleavage site located within the di-chain loop of a TVEMP comprises the consensus sequence D-P3-P2-D*P1' (SEQ ID NO: 71), where P3 can be any amino acid, with E preferred, P2 can be any amino acid and P1' can any amino acid, with G or S preferred. In other aspects of the embodiment, an exogenous protease cleavage

site can comprise, e.g., a non-human Caspase 3 protease cleavage site located within the di-chain loop of a TVEMP comprising SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, or SEQ ID NO: 77. In still other aspects of this embodiment, a bovine enterokinase protease cleavage site is located within the di-chain loop of, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0218] A di-chain loop region is modified to replace a naturally-occurring di-chain loop protease cleavage site for an exogenous protease cleavage site. In this modification, the naturally-occurring di-chain loop protease cleavage site is made inoperable and thus can not be cleaved by its protease. Only the exogenous protease cleavage site can be cleaved by its corresponding exogenous protease. In this type of modification, the exogenous protease site is operably-linked in-frame to a TVEMP as a fusion protein and the site can be cleaved by its respective exogenous protease. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be a substitution of the sites where the exogenous site is engineered at the position approximating the cleavage site location of the endogenous site. Replacement of an endogenous di-chain loop protease cleavage site with an exogenous protease cleavage site can be an addition of an exogenous site where the exogenous site is engineered at the position different from the cleavage site location of the endogenous site, the endogenous site being engineered to be inoperable. The location and kind of protease cleavage site may be critical because certain targeting domains require a free amino-terminal or carboxyl-terminal amino acid. For example, when a peptide targeting domain is placed between two other domains, e.g., see FIG. 4, a criterion for selection of a protease cleavage site could be whether the protease that cleaves its site leaves a flush cut, exposing the free amino-terminal or carboxyl-terminal of the targeting domain necessary for selective binding of the targeting domain to its receptor.

[0219] A naturally-occurring protease cleavage site can be made inoperable by altering at least one of the two amino acids flanking the peptide bond cleaved by the naturally-occurring di-chain loop protease. More extensive alterations can be made, with the proviso that the two cysteine residues of the di-chain loop region remain intact and the region can still form the disulfide bridge. Non-limiting examples of an amino acid alteration include deletion of an amino acid or replacement of the original amino acid with a different amino acid. Thus, in one embodiment, a naturally-occurring protease cleavage site is made inoperable by altering at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 amino acids including at least one of the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease. In another embodiment, a naturally-occurring protease cleavage site is made inoperable by altering at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 amino acids including at least one of the two amino acids flanking the peptide bond cleaved by a naturally-occurring protease.

[0220] It is understood that a TVEMP disclosed herein can optionally further comprise a flexible region comprising a flexible spacer. A flexible region comprising flexible spacers can be used to adjust the

length of a polypeptide region in order to optimize a characteristic, attribute or property of a polypeptide. As a non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better expose a protease cleavage site thereby facilitating cleavage of that site by a protease. As another non-limiting example, a polypeptide region comprising one or more flexible spacers in tandem can be use to better present a peptide targeting domain, thereby facilitating the binding of that targeting domain to its receptor.

[0221] A flexible space comprising a peptide is at least one amino acid in length and comprises non-charged amino acids with small side-chain R groups, such as, *e.g.,* glycine, alanine, valine, leucine or serine. Thus, in an embodiment a flexible spacer can have a length of, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids; or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids. In still another embodiment, a flexible spacer can be, *e.g.,* between 1-3 amino acids, between 2-4 amino acids, between 3-5 amino acids, between 4-6 amino acids, or between 5-7 amino acids. Non-limiting examples of a flexible spacer include, e.g., a G-spacers such as GGG, GGGG (SEQ ID NO: 78), and GGGGS (SEQ ID NO: 79) or an A-spacers such as AAA, AAAA (SEQ ID NO: 80) and AAAAV (SEQ ID NO: 81). Such a flexible region is operably-linked in-frame to the TVEMP as a fusion protein.

[0222] Thus, in an embodiment, a TVEMP disclosed herein can further comprise a flexible region comprising a flexible spacer. In another embodiment, a TVEMP disclosed herein can further comprise flexible region comprising a plurality of flexible spacers in tandem. In aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1, 2, 3, 4, or 5 G-spacers; or at most 1, 2, 3, 4, or 5 G-spacers. In still other aspects of this embodiment, a flexible region can comprise in tandem, e.g., at least 1, 2, 3, 4, or 5 A-spacers; or at most 1, 2, 3, 4, or 5 A-spacers. In another aspect of this embodiment, a TVEMP can comprise a flexible region comprising one or more copies of the same flexible spacers, one or more copies of different flexible-spacer regions, or any combination thereof.

[0223] In other aspects of this embodiment, a TVEMP comprising a flexible spacer can be, e.g., a modified BoNT/A, a modified BoNT/B, a modified BoNT/C1, a modified BoNT/D, a modified BoNT/E, a modified BoNT/F, a modified BoNT/G, a modified TeNT, a modified BaNT, or a modified BuNT.

[0224] It is envisioned that a TVEMP disclosed herein can comprise a flexible spacer in any and all locations with the proviso that TVEMP is capable of performing the intoxication process. In aspects of this embodiment, a flexible spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and a peptide targeting domain, an enzymatic domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and a peptide targeting domain, an enzymatic domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., an enzymatic domain and a translocation domain, an enzymatic domain and a peptide targeting domain, an enzymatic domain and an exogenous protease cleavage site.

[0225] In other aspects of this embodiment, a flexible spacer is positioned between, e.g., a peptide targeting domain and a translocation domain, a peptide targeting domain and an enzymatic domain, a peptide targeting domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., a peptide targeting domain and a translocation domain, a peptide targeting domain and an enzymatic domain, a peptide targeting domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., a peptide targeting domain and a translocation domain, a peptide targeting domain and an enzymatic domain, a peptide targeting domain and an exogenous protease cleavage site.

[0226] In yet other aspects of this embodiment, a flexible spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and a peptide targeting domain, a translocation domain and an exogenous protease cleavage site. In other aspects of this embodiment, a G-spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and a peptide targeting domain, a translocation domain and an exogenous protease cleavage site. In other aspects of this embodiment, an A-spacer is positioned between, e.g., a translocation domain and an enzymatic domain, a translocation domain and a peptide targeting domain, a translocation domain and an exogenous protease cleavage site.

[0227] It is envisioned that a TVEMP disclosed herein can comprise a peptide targeting domain in any and all locations with the proviso that TVEMP is capable of performing the intoxication process. Non-limiting examples include, locating a peptide targeting domain at the amino terminus of a TVEMP; locating a peptide targeting domain between a Clostridial toxin enzymatic domain and a translocation domain of a TVEMP; and locating a peptide targeting domain at the carboxyl terminus of a TVEMP. Other non-limiting examples include, locating a peptide targeting domain between a Clostridial toxin enzymatic domain and a Clostridial toxin translocation domain of a TVEMP. The enzymatic domain of naturally-occurring Clostridial toxins contains the native start methionine. Thus, in domain organizations where the enzymatic domain is not in the amino-terminal location an amino acid sequence comprising the start methionine should be placed in front of the amino-terminal domain. Likewise, where a peptide targeting domain is in the amino-terminal position, an amino acid sequence comprising a start methionine and a protease cleavage site may be operably-linked in situations in which a peptide targeting domain requires a free amino terminus, see, e.g., Shengwen Li et al., *Degradable Clostridial Toxins,* U.S. Patent Application 11/572,512 (Jan. 23, 2007), which is hereby incorporated by reference in its entirety. In addition, it is known in the art that when adding a polypeptide that is operably-linked to the amino terminus of another polypeptide comprising the start methionine that the original methionine residue can be deleted.

[0228] Thus, in an embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a peptide targeting domain, a translocation domain, an exogenous protease cleavage

site and an enzymatic domain (FIG. 3A). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a peptide targeting domain, a Clostridial toxin translocation domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0229] In another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a peptide targeting domain, an enzymatic domain, an exogenous protease cleavage site, and a translocation domain (FIG. 3B). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a peptide targeting domain, a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

[0230] In yet another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, a peptide targeting domain, and a translocation domain (FIG. 4A). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a peptide targeting domain, and a Clostridial toxin translocation domain.

[0231] In yet another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, a peptide targeting domain, and an enzymatic domain (FIG. 4B). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a peptide targeting domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0232] In another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, a peptide targeting domain, an exogenous protease cleavage site, and a translocation domain (FIG. 4C). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, a peptide targeting domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain.

[0233] In yet another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, a peptide targeting domain, an exogenous protease cleavage site and an enzymatic domain (FIG. 4D). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a peptide targeting domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0234] In still another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising an enzymatic domain, an exogenous protease cleavage site, a translocation

domain, and a peptide targeting domain (FIG. 5A). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin enzymatic domain, an exogenous protease cleavage site, a Clostridial toxin translocation domain, and a peptide targeting domain.

[0235] In still another embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a translocation domain, an exogenous protease cleavage site, an enzymatic domain and a peptide targeting domain, (FIG. 5B). In an aspect of this embodiment, a TVEMP can comprise an amino to carboxyl single polypeptide linear order comprising a Clostridial toxin translocation domain, a peptide targeting domain, an exogenous protease cleavage site and a Clostridial toxin enzymatic domain.

[0236] A composition useful in the invention generally is administered as a pharmaceutical acceptable composition comprising a TVEMP. As used herein, the term "pharmaceutically acceptable" means any molecular entity or composition that does not produce an adverse, allergic or other untoward or unwanted reaction when administered to an individual. As used herein, the term "pharmaceutically acceptable composition" is synonymous with "pharmaceutical composition" and means a therapeutically effective concentration of an active ingredient, such as, *e.g.,* any of the TVEMPs disclosed herein. A pharmaceutical composition comprising a TVEMP is useful for medical and veterinary applications. A pharmaceutical composition may be administered to a patient alone, or in combination with other supplementary active ingredients, agents, drugs or hormones. The pharmaceutical compositions may be manufactured using any of a variety of processes, including, without limitation, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, and lyophilizing. The pharmaceutical composition can take any of a variety of forms including, without limitation, a sterile solution, suspension, emulsion, lyophilizate, tablet, pill, pellet, capsule, powder, syrup, elixir or any other dosage form suitable for administration.

[0237] Aspects of the present invention provide, in part, a composition comprising a TVEMP. It is envisioned that any of the composition disclosed herein can be useful in a method of treating neurogenic inflammation in a mammal in need thereof, with the proviso that the composition prevents or reduces a symptom associated with neurogenic inflammation. Non-limiting examples of compositions comprising a TVEMP include a TVEMP comprising a peptide targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain. It is envisioned that any TVEMP disclosed herein can be used, including those disclosed in, e.g., Steward, *supra,* (2007); Dolly, *supra,* (2007); Foster, *supra,* WO 2006/059093 (2006); Foster, *supra,* WO 2006/059105 (Jun. 8, 2006). It is also understood that the two or more different TVEMPs can be provided as separate compositions or as part of a single composition.

[0238] It is also envisioned that a pharmaceutical composition comprising a TVEMP can optionally include a pharmaceutically acceptable carriers that facilitate processing of an active ingredient into

pharmaceutical^ acceptable compositions. As used herein, the term "pharmacologically acceptable carrier" is synonymous with "pharmacological carrier" and means any carrier that has substantially no long term or permanent detrimental effect when administered and encompasses terms such as "pharmacologically acceptable vehicle, stabilizer, diluent, additive, auxiliary or excipient." Such a carrier generally is mixed with an active compound, or permitted to dilute or enclose the active compound and can be a solid, semi-solid, or liquid agent. It is understood that the active ingredients can be soluble or can be delivered as a suspension in the desired carrier or diluent. Any of a variety of pharmaceutically acceptable carriers can be used including, without limitation, aqueous media such as, e.g., water, saline, glycine, hyaluronic acid and the like; solid carriers such as, *e.g.,* mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like; solvents; dispersion media; coatings; antibacterial and antifungal agents; isotonic and absorption delaying agents; or any other inactive ingredient. Selection of a pharmacologically acceptable carrier can depend on the mode of administration. Except insofar as any pharmacologically acceptable carrier is incompatible with the active ingredient, its use in pharmaceutically acceptable compositions is contemplated. Non-limiting examples of specific uses of such pharmaceutical carriers can be found in PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS (Howard C. Ansel et al., eds., Lippincott Williams & Wilkins Publishers, 7th ed. 1999); REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY (Alfonso R. Gennaro ed., Lippincott, Williams & Wilkins, 20th ed. 2000); GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS (Joel G. Hardman et al., eds., McGraw-Hill Professional, 10th ed. 2001); and HANDBOOK OF PHARMACEUTICAL EXCIPIENTS (Raymond C. Rowe et al., APhA Publications, 4th edition 2003). These protocols are routine procedures and any modifications are well within the scope of one skilled in the art and from the teaching herein.

[0239] It is further envisioned that a pharmaceutical composition disclosed herein can optionally include, without limitation, other pharmaceutically acceptable components (or pharmaceutical components), including, without limitation, buffers, preservatives, tonicity adjusters, salts, antioxidants, osmolality adjusting agents, physiological substances, pharmacological substances, bulking agents, emulsifying agents, wetting agents, sweetening or flavoring agents, and the like. Various buffers and means for adjusting pH can be used to prepare a pharmaceutical composition disclosed herein, provided that the resulting preparation is pharmaceutically acceptable. Such buffers include, without limitation, acetate buffers, citrate buffers, phosphate buffers, neutral buffered saline, phosphate buffered saline and borate buffers. It is understood that acids or bases can be used to adjust the pH of a composition as needed. Pharmaceutically acceptable antioxidants include, without limitation, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene. Useful preservatives include, without limitation, benzalkonium chloride, chlorobutanol, thimerosal, phenylmercuric acetate, phenylmercuric nitrate, a stabilized oxy chloro composition and chelants, such as, *e.g.,* DTPA or DTPA-bisamide, calcium DTPA, and CaNaDTPA-bisamide. Tonicity adjustors useful in a pharmaceutical composition include, without limitation, salts such as, e.g., sodium chloride, potassium chloride, mannitol or glycerin and other pharmaceutically acceptable tonicity adjustor. The pharmaceutical composition may

be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. It is understood that these and other substances known in the art of pharmacology can be included in a pharmaceutical composition.

[0240] In an embodiment, a composition comprising a TVEMP is a pharmaceutical composition comprising a TVEMP. In aspects of this embodiment, a pharmaceutical composition comprising a TVEMP further comprises a pharmacological carrier, a pharmaceutical component, or both a pharmacological carrier and a pharmaceutical component. In other aspects of this embodiment, a pharmaceutical composition comprising a TVEMP further comprises at least one pharmacological carrier, at least one pharmaceutical component, or at least one pharmacological carrier and at least one pharmaceutical component.

[0241] Aspects of the present invention provide, in part, a cancer. As used herein, the term "cancer" means cells exhibiting uncontrolled growth that have a pathophysiology effect. It is envisioned that a TVEMPs, compositions and methods disclosed herein can be useful to treat any cancer comprising cells that express the cognate receptor for the targeting domain present in the TVEMP. For example, a TVEMP comprising an interleukin (IL) targeting domain would be useful in treating cancer cells that express an IL receptor; a TVEMP comprising a vascular endothelial growth factor (VEGF) targeting domain would be useful in treating cancer cells that express a VEGF receptor; a TVEMP comprising an insulin-like growth factor (IGF) targeting domain would be useful in treating cancer cells that express an IGF receptor; a TVEMP comprising an epidermal growth factor (EGF) peptide targeting domain would be useful in treating cancer cells that express an EGF receptor; a TVEMP comprising a Transformation Growth Factor-p (TGFP) peptide targeting domain would be useful in treating cancer cells that express a TGF(3 receptor; a TVEMP comprising a Bone Morphogenetic Protein (BMP) peptide targeting domain would be useful in treating cancer cells that express a BMP receptor; a TVEMP comprising a Growth and Differentiation Factor (GDF) peptide targeting domain would be useful in treating cancer cells that express a GDF receptor; a TVEMP comprising an activin peptide targeting domain would be useful in treating cancer cells that express an activin receptor; a TVEMP comprising a Fibroblast Growth Factor (FGF) peptide targeting domain would be useful in treating cancer cells that express a FGF receptor; and a TVEMP comprising a Platelet-Derived Growth Factor (PDGF) peptide targeting domain would be useful in treating cancer cells that express a PDGF receptor.

[0242] Aspects of the present invention provide, in part, reducing a symptom associated with cancer. In an aspect, the symptom reduced is an increase in the growth rate of cancer cells. In another aspect, the symptom reduced is an increase in the cell division rate of cancer cells. In yet another aspect, the symptom reduced is an increase in the extent of invasion of cancer cells into adjacent tissue or organs. In still another aspect, the symptom reduced is an increase in the extent of metastasis. In a further aspect, the symptom reduced is an increase in angiogenesis. In a yet further aspect, the symptom

reduced is a decrease in apoptosis. In a still further aspect, the symptom reduced is a decrease in cell death or cell necrosis. Thus, a TVEMP treatment will decrease the growth rate of cancer cells, decrease the cell division rate of cancer cells, decrease the extent of invasion of cancer cells into adjacent tissue or organs, decrease the extent of metastasis, decrease angiogenesis, increase apoptosis, and/or increase cell death and/or cell necrosis.

[0243] Aspects of the present invention provide, in part, a mammal. A mammal includes a human, and a human can be a patient. Other aspects of the present invention provide, in part, an individual. An individual includes a human, and a human can be a patient.

[0244] Aspects of the present invention provide, in part, administering a composition comprising a TVEMP. As used herein, the term "administering" means any delivery mechanism that provides a composition comprising a TVEMP to a patient that potentially results in a clinically, therapeutically, or experimentally beneficial result. A TVEMP can be delivered to a patient using a cellular uptake approach where a TVEMP is delivered intracellular or a gene therapy approach where a TVEMP is express derived from precursor RNAs expressed from an expression vectors.

[0245] A composition comprising a TVEMP as disclosed herein can be administered to a mammal using a cellular uptake approach. Administration of a composition comprising a TVEMP using a cellular uptake approach comprise a variety of enteral or parenteral approaches including, without limitation, oral administration in any acceptable form, such as, e.g., tablet, liquid, capsule, powder, or the like; topical administration in any acceptable form, such as, *e.g.,* drops, spray, creams, gels or ointments; intravascular administration in any acceptable form, such as, *e.g.,* intravenous bolus injection, intravenous infusion, intra-arterial bolus injection, intra-arterial infusion and catheter instillation into the vasculature; peri- and intra-tissue administration in any acceptable form, such as, *e.g.,* intraperitoneal injection, intramuscular injection, subcutaneous injection, subcutaneous infusion, intraocular injection, retinal injection, or sub-retinal injection or epidural injection; intravesicular administration in any acceptable form, such as, *e.g.,* catheter instillation; and by placement device, such as, *e.g.,* an implant, a patch, a pellet, a catheter, an osmotic pump, a suppository, a bioerodible delivery system, a non-bioerodible delivery system or another implanted extended or slow release system. An exemplary list of biodegradable polymers and methods of use are described in, e.g., *Handbook of Biodegradable Polymers* (Abraham J. Domb et al., eds., Overseas Publishers Association, 1997).

[0246] A composition comprising a TVEMP can be administered to a mammal by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by ionophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors. Delivery mechanisms for administering a composition comprising a TVEMP to a patient are described in, e.g., Leonid Beigelman et al., *Compositions for the Delivery of Negatively Charged Molecules,* U.S. Patent 6,395,713; and Achim

Aigner, *Delivery Systems for the Direct Application of siRNAs to Induce RNA Interference (RNAi) in vivo,* 2006(716559) J. Biomed. Biotech. 1-15 (2006); *Controlled Drug Delivery: Designing Technologies for the Future* (Kinam Park & Randy J. Mrsny eds., American Chemical Association, 2000); Vernon G. Wong & Mae W. L. Hu, *Methods for Treating Inflammation-mediated Conditions of the Eye,* U.S. Patent No. 6,726,918; David A. Weber et al., *Methods and Apparatus for Delivery of Ocular Implants,* U.S. Patent Publication No. US2004/0054374; Thierry Nivaggioli et al., *Biodegradable Ocular Implant,* U.S. Patent Publication No. US2004/0137059; Patrick M. Hughes et al., *Anti-Angiogenic Sustained Release Intraocular Implants and Related Methods,* U.S. Patent Application 11/364,687; and Patrick M. Hughes et al., *Sustained Release Intraocular Drug Delivery Systems,* U.S. Patent Publication 2006/0182783, each of which is hereby incorporated by reference in its entirety.

[0247] A composition comprising a TVEMP as disclosed herein can also be administered to a patient using a gene therapy approach by expressing a TVEMP within in a cell manifesting a nerve-based etiology that contributes to a cancer. A TVEMP can be expressed from nucleic acid molecules operably-linked to an expression vector, see, e.g., P. D. Good et al., *Expression of Small, Therapeutic RNAs in Human Cell Nuclei,* 4(1) Gene Ther. 45-54 (1997); James D. Thompson, Polymerase Ill-based expression of therapeutic RNAs, U.S. Patent 6,852,535 (Feb. 8, 2005); Maciej Wiznerowicz et al., *Tuning Silence: Conditional Systems for RNA Interference,* 3(9) Nat. Methods 682-688m (2006); Ola Sneve and John J. Rossi, *Expressing Short Hairpin RNAi in vivo,* 3(9) Nat. Methods 689-698 (2006); and Charles X. Li et al., *Delivery of RNA Interference,* 5(18) Cell Cycle 2103-2109 (2006). A person of ordinary skill in the art would realize that any TVEMP can be expressed in eukaryotic cells using an appropriate expression vector.

[0248] Expression vectors capable of expressing a TVEMP can provide persistent or stable expression of the TVEMP in a cell manifesting a nerve-based etiology that contributes to a cancer. Alternatively, expression vectors capable of expressing a TVEMP can provide for transient expression of the TVEMP in a cell manifesting a nerve-based etiology that contributes to a cancer. Such transiently expressing vectors can be repeatedly administered as necessary. A TVEMP-expressing vectors can be administered by a delivery mechanism and route of administration discussed above, by administration to target cells ex-planted from a patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell, see, *e.g.,* Larry A. Couture and Dan T. Stinchcomb, *Anti-gene Therapy: The Use of Ribozymes to Inhibit Gene Function,* 12(12) Trends Genet. 510-515(1996).

[0249] The actual delivery mechanism used to administer a composition comprising a TVEMP to a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of cancer, the location of the cancer, the cause of the cancer, the severity of the cancer, the degree of relief desired, the duration of relief desired, the particular TVEMP used, the rate of excretion of the TVEMP used, the pharmacodynamics of the TVEMP used, the nature of

the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the patient, such as, e.g., age, weight, general health and the like, or any combination thereof.

[0250] In an embodiment, a composition comprising a TVEMP is administered to the site to be treated by injection. In aspects of this embodiment, injection of a composition comprising a TVEMP is by, *e.g.,* intramuscular injection, intraorgan injection, subdermal injection, dermal injection, or injection into any other body area for the effective administration of a composition comprising a TVEMP. In aspects of this embodiment, injection of a composition comprising a TVEMP is a tumor or into the area surrounding the tumor.

[0251] A composition comprising a TVEMP can be administered to a mammal using a variety of routes. Routes of administration suitable for a method of treating a cancer as disclosed herein include both local and systemic administration. Local administration results in significantly more delivery of a composition to a specific location as compared to the entire body of the mammal, whereas, systemic administration results in delivery of a composition to essentially the entire body of the patient. Routes of administration suitable for a method of treating a cancer as disclosed herein also include both central and peripheral administration. Central administration results in delivery of a composition to essentially the central nervous system of the patient and includes, *e.g.,* intrathecal administration, epidural administration as well as a cranial injection or implant. Peripheral administration results in delivery of a composition to essentially any area of a patient outside of the central nervous system and encompasses any route of administration other than direct administration to the spine or brain. The actual route of administration of a composition comprising a TVEMP used in a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of cancer, the location of the cancer, the cause of the cancer, the severity of the cancer, the degree of relief desired, the duration of relief desired, the particular TVEMP used, the rate of excretion of the TVEMP used, the pharmacodynamics of the TVEMP used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the mammal, such as, *e.g.,* age, weight, general health and the like, or any combination thereof.

[0252] In an embodiment, a composition comprising a TVEMP is administered systemically to a mammal. In another embodiment, a composition comprising a TVEMP is administered locally to a mammal. In an aspect of this embodiment, a composition comprising a TVEMP is administered to a tumor of a mammal. In another aspect of this embodiment, a composition comprising a TVEMP is administered to the area surrounding a tumor of a mammal.

[0253] Aspects of the present invention provide, in part, administering a therapeutically effective amount of a composition comprising a TVEMP. As used herein, the term "therapeutically effective amount" is synonymous with "therapeutically effective dose" and when used in reference to treating a cancer means

the minimum dose of a TVEMP necessary to achieve the desired therapeutic effect and includes a dose sufficient to reduce a symptom associated with a cancer. In aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP reduces a symptom associated with a cancer by, e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 100%. In other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP reduces a symptom associated with a cancer by, e.g., at most 10%, at most 20%, at most 30%, at most 40%, at most 50%, at most 60%, at most 70%, at most 80%, at most 90% or at most 100%. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP reduces a symptom associated with a cancer by, e.g., about 10% to about 100%, about 10% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 20% to about 100%, about 20% to about 90%, about 20% to about 80%, about 20% to about 20%, about 20% to about 60%, about 20% to about 50%, about 20% to about 40%, about 30% to about 100%, about 30% to about 90%, about 30% to about 80%, about 30% to about 70%, about 30% to about 60%, or about 30% to about 50%. As used herein, the term "about" when qualifying a value of a stated item, number, percentage, or term refers to a range of plus or minus ten percent of the value of the stated item, percentage, parameter, or term. In still other aspects of this embodiment, a therapeutically effective amount of the TVEMP is the dosage sufficient to inhibit neuronal activity for, e.g., at least one week, at least one month, at least two months, at least three months, at least four months, at least five months, at least six months, at least seven months, at least eight months, at least nine months, at least ten months, at least eleven months, or at least twelve months.

[0254] The actual therapeutically effective amount of a composition comprising a TVEMP to be administered to a mammal can be determined by a person of ordinary skill in the art by taking into account factors, including, without limitation, the type of cancer, the location of the cancer, the cause of the cancer, the severity of the cancer, the degree of relief desired, the duration of relief desired, the particular TVEMP used, the rate of excretion of the TVEMP used, the pharmacodynamics of the TVEMP used, the nature of the other compounds to be included in the composition, the particular route of administration, the particular characteristics, history and risk factors of the patient, such as, e.g., age, weight, general health and the like, or any combination thereof. Additionally, where repeated administration of a composition comprising a TVEMP is used, the actual effect amount of a composition comprising a TVEMP will further depend upon factors, including, without limitation, the frequency of administration, the half-life of the composition comprising a TVEMP, or any combination thereof. In is known by a person of ordinary skill in the art that an effective amount of a composition comprising a TVEMP can be extrapolated from *in vitro* assays and *in vivo* administration studies using animal models prior to administration to humans. Wide variations in the necessary effective amount are to be expected in view of the differing efficiencies of the various routes of administration. For instance, oral administration generally would be expected to require higher dosage levels than administration by intravenous or intravitreal injection. Variations in these dosage levels can be adjusted using standard

empirical routines of optimization, which are well-known to a person of ordinary skill in the art. The precise therapeutically effective dosage levels and patterns are preferably determined by the attending physician in consideration of the above-identified factors.

[0255] As a non-limiting example, when administering a composition comprising a TVEMP to a mammal, a therapeutically effective amount generally is in the range of about 1 fg to about 3.0 mg. In aspects of this embodiment, an effective amount of a composition comprising a TVEMP can be, *e.g.,* about 100 fg to about 3.0 mg, about 100 pg to about 3.0 mg, about 100 ng to about 3.0 mg, or about 100 ug to about 3.0 mg. In other aspects of this embodiment, an effective amount of a composition comprising a TVEMP can be, e.g., about 100 fg to about 750 ug, about 100 pg to about 750 pg, about 100 ng to about 750 pg, or about 1 pg to about 750 pg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP can be, e.g., at least 1 fg, at least 250 fg, at least 500 fg, at least 750 fg, at least 1 pg, at least 250 pg, at least 500 pg, at least 750 pg, at least 1 ng, at least 250 ng, at least 500 ng, at least 750 ng, at least 1 pg, at least 250 pg, at least 500 pg, at least 750 pg, or at least 1 mg. In still other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP can be, *e.g.,* at most 1 fg, at most 250 fg, at most 500 fg, at most 750 fg, at most 1 pg, at most 250 pg, at most 500 pg, at most 750 pg, at most 1 ng, at most 250 ng, at most 500 ng, at most 750 ng, at most 1 pg, at least 250 pg, at most 500 pg, at most 750 pg, or at most 1 mg.

[0256] As another non-limiting example, when administering a composition comprising a TVEMP to a mammal, a therapeutically effective amount generally is in the range of about 0.00001 mg/kg to about 3.0 mg/kg. In aspects of this embodiment, an effective amount of a composition comprising a TVEMP can be, *e.g.,* about 0.0001 mg/kg to about 0.001 mg/kg, about 0.03 mg/kg to about 3.0 mg/kg, about 0.1 mg/kg to about 3.0 mg/kg, or about 0.3 mg/kg to about 3.0 mg/kg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP can be, e.g., at least 0.00001 mg/kg, at least 0.0001 mg/kg, at least 0.001 mg/kg, at least 0.01 mg/kg, at least 0.1 mg/kg, or at least 1 mg/kg. In yet other aspects of this embodiment, a therapeutically effective amount of a composition comprising a TVEMP can be, e.g., at most 0.00001 mg/kg, at most 0.0001 mg/kg, at most 0.001 mg/kg, at most 0.01 mg/kg, at most 0.1 mg/kg, or at most 1 mg/kg.

[0257] Dosing can be single dosage or cumulative (serial dosing), and can be readily determined by one skilled in the art. For instance, treatment of a cancer may comprise a one-time administration of an effective dose of a composition comprising a TVEMP. As a non-limiting example, an effective dose of a composition comprising a TVEMP can be administered once to a patient, *e.g.,* as a single injection or deposition at or near the site exhibiting a symptom of a cancer. Alternatively, treatment of a cancer may comprise multiple administrations of an effective dose of a composition comprising a TVEMP carried out over a range of time periods, such as, e.g., daily, once every few days, weekly, monthly or yearly. As a non-limiting example, a composition comprising a TVEMP can be administered once or twice yearly to a mammal. The timing of administration can vary from mammal to mammal, depending upon such factors

as the severity of a mammal's symptoms. For example, an effective dose of a composition comprising a TVEMP can be administered to a mammal once a month for an indefinite period of time, or until the patient no longer requires therapy. A person of ordinary skill in the art will recognize that the condition of the mammal can be monitored throughout the course of treatment and that the effective amount of a composition comprising a TVEMP that is administered can be adjusted accordingly.

[0258] A composition comprising a TVEMP as disclosed herein can also be administered to a mammal in combination with other therapeutic compounds to increase the overall therapeutic effect of the treatment. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

[0259] Aspects of the present invention can also be described as follows:

1. A method of treating cancer in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, wherein administration of the composition reduces a symptom associated with cancer.

2. A use of a TVEMP in the manufacturing a medicament for treating cancer in a mammal in need thereof, wherein the TVEMP comprises a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain and wherein administration of a therapeutically effective amount of the medicament to the mammal reduces a symptom associated with cancer.

3. A use of a TVEMP for the treatment of cancer in a mammal in need thereof, the use comprising the step of administering to the mammal a therapeutically effective amount of the TVEMP, wherein the TVEMP comprises a targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain and wherein administration of the TVEMP reduces a symptom associated with cancer.

4. A method of treating cancer in a mammal, the method comprising the step of administering to the mammal in need thereof a therapeutically effective amount of a composition including a TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, wherein administration of the composition reduces a symptom associated with cancer.

5. A use of a TVEMP in the manufacturing a medicament for treating cancer in a mammal in need thereof, wherein the TVEMP comprises a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site and wherein

administration of a therapeutically effective amount of the medicament to the mammal reduces a symptom associated with cancer.

6. A use of a TVEMP for the treatment of cancer in a mammal in need thereof, the use comprising the step of administering to the mammal a therapeutically effective amount of the TVEMP, wherein the TVEMP comprises a targeting domain, a Clostridial toxin translocation domain, a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site and wherein administration of the TVEMP reduces a symptom associated with cancer.

7. The method of 1-3, wherein the TVEMP comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the targeting domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the targeting domain, the Clostridial toxin translocation domain, 3) the targeting domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the targeting domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.

8. The method of 4-6, wherein the TVEMP comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the targeting domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the targeting domain, the Clostridial toxin translocation domain, 3) the targeting domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the targeting domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.

9. The method of 1-8, wherein the targeting domain is an interleukin (IL) peptide, vascular endothelial growth factor (VEGF) peptide, an insulin-like growth factor (IGF) peptide, an epidermal growth factor (EGF) peptide, a Transformation Growth Factor-p (TGFB) peptide, a Bone Morphogenetic Protein (BMP), a Growth and Differentiation Factor (GDF) peptide, an activin peptide, a Fibroblast Growth Factor (FGF) peptide, or a Platelet-Derived Growth Factor (PDGF).

10. The method of 9, wherein the interleukin (IL) peptide targeting domain is an IL-1 peptide, an IL-2 peptide, an IL-3 peptide, an IL-4 peptide, an IL-5 peptide, an IL-6 peptide, an IL-7 peptide, an IL-8 peptide, an IL-9 peptide, an IL-10 peptide, an IL-11 peptide, an IL-32 peptide, or an IL-33 peptide.

11. The method of 10, wherein the interleukin (IL) peptide targeting domain comprises amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, amino acids 19-142 of SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152.

12. The method of 10-11, wherein the cancer is an acute myeloid leukemia, a thyroid cancer, or a colon cancer.

13. The method of 9, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain is a VEGF-A peptide, a VEGF-B peptide, a VEGF-C peptide, a VEGF-D peptide, or a placenta growth factor (PIGF) peptide.

14. The method of 13, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain comprises amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93.

15. The method of 13-14, wherein the cancer is a prostate cancer, a renal cell carcinoma, an ovarian cancer, a bladder cancer, a colon cancer, a lymphoma, a rhabdomyosarcoma, a breast cancer, an osteosarcoma, a thyroid tumor, a lung cancer, a non-small cell lung cancer, a melanoma, a pancreatic cancer, an Ocular melanoma, a retinoblastoma, an intra-ocular tumor, a leukemia, a Kaposi's sarcoma, a medulloblastoma, a teratocarcinoma, a neuroblastoma, a mesothelioma, an insulinoma, a gastric adenocarinoma, an intestinal tumor, a glioma, an astrocytoma, or a kidney tumor.

16. The method of 9, wherein the insulin-like growth factor (IGF) peptide targeting domain is an IGF-1 peptide or an IGF-2 peptide.

17. The method of 16, wherein the insulin-like growth factor (IGF) peptide targeting domain comprises amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180 of SEQ ID NO: 95.

18. The method of 1.6-17, wherein the cancer is a breast cancer, a colon cancer, a lung cancer, a prostate cancer, a gastric cancer or a liver cancer.

19. The method of 9, wherein the epidermal growth factor (EGF) peptide targeting domain an EGF, a heparin-binding EGF-like growth factor (HB-EGF), a transforming growth factor-a (TGF-a), an amphiregulin (AR), an epiregulin (EPR), an epigen (EPG), a betacellulin (BTC), a neuregulin-1 (NRG1), a neuregulin-2 (NRG2), a neuregulin-3, (NRG3), or a neuregulin-4 (NRG4).

20. The method of 19, wherein the epidermal growth factor (EGF) peptide targeting domain comprises SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, SEQ ID NO: 102, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, amino acids 353-648 of SEQ ID NO: 105, or SEQ ID NO: 106.

21. The method of 19-20, wherein the cancer is a lung cancer, a prostate cancer, an ovarian cancer, a bladder cancer, a thyroid cancer, a mixed papillary and follicular thyroid carcinoma, a biliary tract cholangiocarcinoma, a breast cancer, a cervical cancer, a colorectal cancer, a colon cancer, a gastric cancer, an endometrial cancer, an esophageal cancer, a fallopian tube cancer, a gallbladder cancer, a head and neck cancer, a liver cancer, a lung cancer, a myelodysplastic syndrome, a non-small cell lung cancer, an oral cancer, a pancreatic cancer, a peritoneal cavity cancer, a polycythemia vera, a renal cancer, or a skin cancer.

22. The method of 9, wherein the Transformation Growth Factor-P (TGFP) peptide targeting domain is a TGFP1 peptide, a TGFP2 peptide, a TGFP3 peptide, or a TGFP4 peptide.

23. The method of 22, wherein the Transformation Growth Factor-p (TGFP) peptide targeting domain comprises amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110.

24. The method of 22-23, wherein the cancer is a prostate cancer, a leukemia, a renal cell carcinoma, a pheochromocytoma, a thyroid tumor, a pituitary cancer, a colon cancer, a lymphoma, a stomach cancer, a breast cancer, an osteosarcoma, a fibrosarcoma, a hepatoma, a hepatocellular carcinoma, a papillary thyroid carcinoma, a myeloma, a pancreatic cancer, a testicular tumor, an ovarian cancer, a cervical carcinoma, an endometrial adenocarcinoma, an endometrioid carcinoma, a melanoma, a rhabdomyosarcoma, a squamous cell carcinoma, a neuroblastoma, an adrenocortical cancer, a salivary adenoid cystic carcinoma, or a gastric adenocarcinoma.

25. The method of 9, wherein the Bone Morphogenetic Protein (BMP) peptide targeting domain is a BMP2 peptide, a BMP3 peptide, a BMP4 peptide, a BMP5 peptide, a BMP6 peptide, a BMP7 peptide, a BMP8 peptide, or a BMP10 peptide.

26. The method of 25, wherein the Bone Morphogenetic Protein (BMP) peptide targeting domain comprises amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118.

27. The method of 25-26, wherein the cancer is a prostate cancer, a leukemia, a biliary tract cancer, an ovarian cancer, a bone cancer, an osteosarcoma, a colon cancer, a myeloma, a testicular cancer, a testicular tumor, a breast cancer, a glioblastoma, a squamous cell carcinoma, a lung carcinoma, an adrenal cortex carcinoma, a pituitary cancer, an endometrioid carcinoma, a hepatoma, a hepatocellular carcinoma, a gastric adenocarcinoma, or a pancreatic cancer.

28. The method of 9, wherein the Growth and Differentiation Factor (GDF) peptide targeting domain is a GDF1 peptide, a GDF2 peptide, a GDF3 peptide, a GDF5 peptide, a GDF6 peptide, a GDF7 peptide, a GDF8 peptide, a GDF10 peptide, a GDF11 peptide, or a GDF15 peptide.

29. The method of 28, wherein the Growth and Differentiation Factor (GDF) peptide targeting domain comprises amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128.

30. The method of 28-29, wherein the cancer is a prostate cancer, a renal cell carcinoma, a pheochromocytoma, a biliary tract cancer, an ovarian cancer, a testicular tumor, a bone cancer, a thyroid tumor, a papillary thyroid carcinoma, a pituitary cancer, an endometrioid carcinoma, a colon cancer, a myeloma, a lymphoma, a leukemia, a testicular cancer, a stomach cancer, a gastric adenocarcinoma, a breast cancer, a glioblastoma, a fibrosarcoma, a hepatoma, a hepatocellular carcinoma, a squamous cell carcinoma, a lung carcinoma, an adrenal cortex carcinoma, a pancreatic cancer, or an osteosarcoma.

31. The method of 9, wherein the activin peptide targeting domain is an activin A peptide, an activin B peptide, an activin C peptide, an activin E peptide, or an inhibin A peptide.

32. The method of 31, wherein the activin peptide targeting domain comprises amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133.

33. The method of 31-32, wherein the cancer is a prostate cancer, a renal cell carcinoma, an ovarian cancer, a leukemia, a colon cancer, a pituitary cancer, a pheochromocytoma, a stomach cancer, a breast cancer, an adrenocortical cancer, a salivary adenoid cystic carcinoma, an endometrioid carcinoma, a testicular tumor, a hepatoma, a hepatocellular carcinoma, a myeloma, a pancreatic cancer, or a gastric adenocarcinoma.

34. The method of 9, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain is a FGF1 peptide, a FGF2 peptide, a FGF3 peptide, a FGF4 peptide, a FGF5 peptide, a FGF6 peptide, a FGF7 peptide, a FGF8 peptide, a FGF9 peptide, a FGF10 peptide, a FGF17 peptide, or a FGF18 peptide.

35. The method of 34, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain comprises amino acids 29-151 of SEQ ID NO! 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145.

36. The method of 34-35, wherein the cancer is an acute myeloblastic leukemia, a chronic lymphocytic leukemia, a breast cancer, an endometrial ovarian cancer, a gastric cancer, a bladder cancer, a colon cancer, a cervical cancer, an epithelial ovarian cancer, a leiomyoma, or a pituitary tumor.

37. The method of 9, wherein the Platelet-Derived Growth Factor (PDGF) peptide targeting domain is a PDGFa peptide or a PDGFS peptide.

38. The method of 34, wherein the Platelet-Derived Growth Factor (PDGF) peptide targeting domain comprises amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154.

39. The method of 34-35, wherein the cancer is a prostate cancer, a renal cell carcinoma, a bladder cancer, a non-small cell lung cancer, a rhabdomyosarcoma, a gastrointestinal stromal tumor, a medulloblastoma, a glioblastoma, a nasopharyngeal carcinoma, a fibrosarcoma, a basal cell carcinoma, a neuroblastoma, an astrocytoma, an osteosarcoma, a Ewing's sarcoma, a breast cancer, a testicular tumor, an ovarian cancer, a melanoma, a myeloma, a squamous cell carcinoma, a lymphoma, a leukemia, a mesothelioma, a Kaposi sarcoma, or a chondrosarcoma.

40. The method of 1-39, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

41. The method of 1-39, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

42. The method of 4-6 and 8, wherein the exogenous protease cleavage site is a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a Tobacco Vein Mottling Virus cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

43. A TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, wherein administration of the composition reduces a symptom associated with cancer.

44. A TVEMP comprising a targeting domain, a Clostridial toxin translocation domain and a Clostridial toxin enzymatic domain, and an exogenous protease cleavage site, wherein administration of the composition reduces a symptom associated with cancer.

45. The TVEMP of 43, wherein the TVEMP comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the targeting domain, 2) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the targeting domain, the Clostridial toxin translocation domain, 3) the targeting domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the targeting domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.

46. The TVEMP of 44, wherein the TVEMP comprises a linear amino-to-carboxyl single polypeptide order of 1) the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, the targeting domain, 2) the Clostridial toxin enzymatic domain, the

exogenous protease cleavage site, the targeting domain, the Clostridial toxin translocation domain, 3) the targeting domain, the Clostridial toxin translocation domain, the exogenous protease cleavage site and the Clostridial toxin enzymatic domain, 4) the targeting domain, the Clostridial toxin enzymatic domain, the exogenous protease cleavage site, the Clostridial toxin translocation domain, 5) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the Clostridial toxin enzymatic domain and the targeting domain, or 6) the Clostridial toxin translocation domain, the exogenous protease cleavage site, the targeting domain and the Clostridial toxin enzymatic domain.

47. The TVEMP of 43-46, wherein the targeting domain is an interleukin (IL) peptide, vascular endothelial growth factor (VEGF) peptide, an insulin-like growth factor (IGF) peptide, an epidermal growth factor (EGF) peptide, a Transformation Growth Factor-S (TGF(3) peptide, a Bone Morphogenetic Protein (BMP), a Growth and Differentiation Factor (GDF) peptide, an activin peptide, or a Fibroblast Growth Factor (FGF) peptide.

48. The TVEMP of 47, wherein the interleukin (IL) peptide targeting domain is an IL-1 peptide, an IL-2 peptide, an IL-3 peptide, an IL-4 peptide, an IL-5 peptide, an IL-6 peptide, an IL-7 peptide, an IL-8 peptide, an IL-9 peptide, an IL-10 peptide, an IL-11 peptide, an IL-32 peptide, or an IL-33 peptide.

49. The TVEMP of 48, wherein the interleukin (IL) peptide targeting domain comprises comprises amino acids 123-265 of SEQ ID NO: 82, amino acids 21-153 of SEQ ID NO: 83, amino acids 57-210 of SEQ ID NO: 84, amino acids 21-99 or amino acids 31-94 of SEQ ID NO: 85, amino acids 37-173 or amino acids 19-178 of SEQ ID NO: 86, amino acids 37-199 of SEQ ID NO: 87, amino acids 20-137 of SEQ ID NO: 146, amino acids 25-153 of SEQ ID NO: 147, amino acids 24-131 of SEQ ID NO: 148, amino acids 27-173 of SEQ ID NO: 149, amino acids 19-142 of SEQ ID NO: 150, SEQ ID NO: 151, or SEQ ID NO: 152.

50. The TVEMP of 47, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain is a VEGF-A peptide, a VEGF-B peptide, a VEGF-C peptide, a VEGF-D peptide, or a placenta growth factor (PIGF) peptide.

51. The TVEMP of 50, wherein the vascular endothelial growth factor (VEGF) peptide targeting domain comprises amino acids 50-133 of SEQ ID NO: 88, amino acids 45-127 of SEQ ID NO: 89, amino acids 129-214 of SEQ ID NO: 90, amino acids 109-194 of SEQ ID NO: 91, amino acids 46-163, amino acids 49-162, amino acids 168-345, amino acids 244-306, or amino acids 248-340 of SEQ ID NO: 92, or amino acids 50-131 or amino acids 132-203 of SEQ ID NO: 93.

52. The TVEMP of 47, wherein the insulin-like growth factor (IGF) peptide targeting domain is an IGF-1 peptide or an IGF-2 peptide.

53. The TVEMP of 52, wherein the insulin-like growth factor (IGF) peptide targeting domain comprises amino acids 52-109 or amino acids 49-118 of SEQ ID NO: 94, or amino acids 31-84 or amino acids 25-180ofSEQIDNO:95.

54. The TVEMP of 47, wherein the epidermal growth factor (EGF) peptide targeting domain an EGF, a heparin-binding EGF-like growth factor (HB-EGF), a transforming growth factor-a (TGF-a), an amphiregulin (AR), an epiregulin (EPR), an epigen (EPG), a betacellulin (BTC), a neuregulin-1 (NRG1), a neuregulin-2 (NRG2), a neuregulin-3, (NRG3), or a neuregulin-4 (NRG4).

55. The TVEMP of 54, wherein the epidermal growth factor (EGF) peptide targeting domain comprises SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, amino acids 101-251 or amino acids 107-251 of SEQ ID NO: 99, amino acids 63-108 of SEQ ID NO: 100, amino acids 23-154 of SEQ ID NO: 101, SEQ ID NO: 102, amino acids 235-630 of SEQ ID NO: 103, amino acids 398-718 of SEQ ID NO: 104, amino acids 353-648 of SEQ ID NO: 105, or SEQ ID NO: 106.

56. The TVEMP of 47, wherein the Transformation Growth Factor-p (TGFp) peptide targeting domain is a TGFB1 peptide, a TGF62 peptide, a TGFP3 peptide, or a TGFP4 peptide.

57. The TVEMP of 56, wherein the Transformation Growth Factor-p (TGFP) peptide targeting domain comprises amino acids 293-390 of SEQ ID NO: 107, amino acids 317-414 of SEQ ID NO: 108, amino acids 315-412 of SEQ ID NO: 109, or amino acids 276-373 of SEQ ID NO: 110.

58. The TVEMP of 47, wherein the Bone Morphogenetic Protein (BMP) peptide targeting domain is a BMP2 peptide, a BMP3 peptide, a BMP4 peptide, a BMP5 peptide, a BMP6 peptide, a BMP7 peptide, a BMP8 peptide, or a BMP10 peptide.

59. The TVEMP of 58, wherein the Bone Morphogenetic Protein (BMP) peptide targeting domain comprises amino acids 296-396 of SEQ ID NO: 111, acids 370-472 of SEQ ID NO: 112, amino acids 309-409 of SEQ ID NO: 113, amino acids 353-454 or amino acids 323-454 of SEQ ID NO: 114, amino acids 412-513 or amino acids 374-513 of SEQ ID NO: 115, amino acids 330-431 or amino acids 293-431 of SEQ ID NO: 116, amino acids 301-402 of SEQ ID NO: 117, or amino acids 323-424 of SEQ ID NO: 118.

60. The TVEMP of 47, wherein the Growth and Differentiation Factor (GDF) peptide targeting domain is a GDF1 peptide, a GDF2 peptide, a GDF3 peptide, a GDF5 peptide, a GDF6 peptide, a GDF7 peptide, a GDF8 peptide, a GDF10 peptide, a GDF11 peptide, or a GDF15 peptide.

61. The TVEMP of 60, wherein the Growth and Differentiation Factor (GDF) peptide targeting domain comprises amino acids 267-372 of SEQ ID NO: 119, amino acids 327-429 of SEQ ID NO: 120, amino

acids 264-364 of SEQ ID NO: 121, amino acids 400-501 of SEQ ID NO: 122, amino acids 354-455 of SEQ ID NO: 123, amino acids 352-450 of SEQ ID NO: 124, amino acids 281-375 of SEQ ID NO: 125, amino acids 376-478 of SEQ ID NO: 126, amino acids 313-407 of SEQ ID NO: 127, or amino acids 211-308 of SEQ ID NO: 128.

62. The TVEMP of 47, wherein the activin peptide targeting domain is an activin A peptide, an activin B peptide, an activin C peptide, an activin E peptide, or an inhibin A peptide.

63. The TVEMP of 62, wherein the activin peptide targeting domain comprises amino acids 321-426 of SEQ ID NO: 129, amino acids 303-406 of SEQ ID NO: 130, amino acids 247-352 or amino acids 237-352 of SEQ ID NO: 131, amino acids 247-350 of SEQ ID NO: 132, or amino acids 262-366 or amino acids 233-366 of SEQ ID NO: 133.

64. The TVEMP of 47, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain is a FGF1 peptide, a FGF2 peptide, a FGF3 peptide, a FGF4 peptide, a FGF5 peptide, a FGF6 peptide, a FGF7 peptide, a FGF8 peptide, a FGF9 peptide, a FGF10 peptide, a FGF17 peptide, or a FGF18 peptide.

65. The TVEMP of 64, wherein the Fibroblast Growth Factor (FGF) peptide targeting domain comprises amino acids 29-151 of SEQ ID NO: 134, amino acids 30-152 of SEQ ID NO: 135, amino acids 46-181 of SEQ ID NO: 136, amino acids 84-206 of SEQ ID NO: 137, amino acids 91-219 of SEQ ID NO: 138, amino acids 38-198 of SEQ ID NO: 139, amino acids 67-191 of SEQ ID NO: 140, amino acids 43-167 of SEQ ID NO: 141, amino acids 64-191 of SEQ ID NO: 142, amino acids 80-204 of SEQ ID NO: 143, amino acids 55-178 of SEQ ID NO: 144, or amino acids 55-177 of SEQ ID NO: 145.

66. The TVEMP of 47, wherein the Platelet-Derived Growth Factor (PDGF) peptide targeting domain is a PDGFa peptide or a PDGFB peptide.

67. The TVEMP of 66, wherein the Platelet-Derived Growth Factor (PDGF) peptide targeting domain comprises amino amino acids 94-182 of SEQ ID NO: 153 or amino acids 95-182 of SEQ ID NO: 154

68. The TVEMP of 43-67, wherein the Clostridial toxin translocation domain is a BoNT/A translocation domain, a BoNT/B translocation domain, a BoNT/C1 translocation domain, a BoNT/D translocation domain, a BoNT/E translocation domain, a BoNT/F translocation domain, a BoNT/G translocation domain, a TeNT translocation domain, a BaNT translocation domain, or a BuNT translocation domain.

69. The TVEMP of 43-67, wherein the Clostridial toxin enzymatic domain is a BoNT/A enzymatic domain, a BoNT/B enzymatic domain, a BoNT/C1 enzymatic domain, a BoNT/D enzymatic domain, a BoNT/E

enzymatic domain, a BoNT/F enzymatic domain, a BoNT/G enzymatic domain, a TeNT enzymatic domain, a BaNT enzymatic domain, or a BuNT enzymatic domain.

70. The TVEMP of 44 and 46, wherein the exogenous protease cleavage site is a plant papain cleavage site, an insect papain cleavage site, a crustacian papain cleavage site, an enterokinase cleavage site, a human rhinovirus 3C protease cleavage site, a human enterovirus 3C protease cleavage site, a tobacco etch virus protease cleavage site, a Tobacco Vein Mottling Virus cleavage site, a subtilisin cleavage site, a hydroxylamine cleavage site, or a Caspase 3 cleavage site.

71. A composition comprising a TVEMP of 40-65.

72. The composition of 71, wherein the composition is a pharmaceutical composition.

73. The composition of 72, wherein the pharmaceutical composition comprisies a pharmaceutical carrier, pharmaceutical excipient, or any combination thereof.

EXAMPLES

[0260] The following examples illustrate representative embodiments now contemplated, but should not be construed to limit the disclosed TVEMPs, compositions including TVEMPs, and methods of treating cancer using such compositions.

Example 1
Light Chain Assays

[0261] This example illustrates how to screen cancer cells in order to determine which Clostridial toxin light chain had an effect sufficient to provide a therapeutic benefit in a cancer treatment.

[0262] To identify which Clostridial toxin light chain or active fragment thereof was useful in making a TVEMP for treating a cancer using a method disclosed herein, a Clostridial toxin light chain cleavage assay was conducted. These assays address two fundamental issues. First, the light chains of the various botulinum neurotoxin serotypes cleave different SNARE substrates. In addition, some cells may only express SNAP-23 which is not cleavable by naturally-occurring botulinum neurotoxins. These cells would not be sensitive to LC/A, but may be sensitive to LC/B and LC/C1 if they express synaptobrevin-2 (VAMP-2) and/or Syntaxin, respectively. Second, this transfection assay allows the examination of the cellular effects of the light chains on cancer cells in a way that is independent of receptor binding and translocation into the cell. Taken together, this assay allows the examination of the effects of cleaving SNARE proteins on a variety of cancer cell lines encompassing several types of human cancers.

[0263] Mammalian expression constructs encoding a fusion protein comprising a green fluorescent protein (GFP) linked to a light chain of different botulinum neurotoxin serotypes were made using standard procedures. These expression constructs were designated 1) pQBI25/GFP, a construct expressing GFP of SEQ ID NO: 155 encoded by the polynucleotide of SEQ ID NO: 1564; 2) pQBI25/GFP-LC/A, a construct expressing GFP-LC/A fusion protein of SEQ ID NO: 157 encoded by the polynucleotide of SEQ ID NO: 158; 3) pQBI/GFP-LC/B, a construct expressing GFP-LC/B fusion protein of SEQ ID NO: 159 encoded by the polynucleotide of SEQ ID NO: 160; 4) pQBI/GFP-LC/C1, a construct expressing GFP-LC/C1 fusion protein of SEQ ID NO: 161 encoded by the polynucleotide of SEQ ID NO: 162; and 5) pQBI/GFP-LC/E, a construct expressing GFP-LC/E fusion protein of SEQ ID NO: 163 encoded by the polynucleotide of SEQ ID NO: 164. The light chains for these particular botulinum toxin serotypes were selected because overall, the light chains cleave one of the three predominant SNARE proteins SNAP-25, VAMP, or Syntaxin.

[0264] To culture cells, an appropriate density of cells were plated into the wells of 6-well tissue culture plates containing 3 mL of an appropriate medium (Table 5). The cells were grown in a 37 °C incubator under 5% carbon dioxide until cells reached the appropriate density (about 1 x 10^6 cells). A 500 uL transfection solution was prepared by adding 250 uL of OPTI-MEM Reduced Serum Medium containing 10 uL of LipofectAmine 2000 (Invitrogen Inc., Carlsbad, CA), incubated at room temperature for 5 minutes, to 250 uL of OPTI-MEM Reduced Serum Medium containing 5 ug of the desired mammalian expression construct. This transfection mixture was incubated at room temperature for approximately 25 minutes. The growth media was replaced with fresh unsupplemented serum-free media and the 500 uL transfection solution was added to the cells. The cells were then incubated in a 37 ^{C}C incubator under 5% carbon dioxide for approximately 8 hours. The transfection media was replaced with fresh unsupplemented serum-free media and the cells then incubated in a 37 °C incubator under 5% carbon dioxide for approximately 48 hours. After this incubation, the cells were washed by aspirating the media and rinsing each well with 3 mL of 1 x PBS.

Table 5. Cell Lines and Media

Cell Line	Origin	Source	Serum Growth Media Composition
RT4	Human urinary bladder transitional cell carcinoma	ATCC HTB-2	McCoy's 5a media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
P19	Mouse embryonic carcinoma	ATCC CRL-1825	Alpha Minimal Essential Medium media with 7.5 % bovine calf serum, 2.5% fetal bovine calf serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
NCI H69	Human small lung carcinoma	ATCC HTB-119	RPMI-1640 media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
NCI H82	Human small lung carcinoma	ATCC HTB-175	RPMI-1640 media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin

DU-145	Human prostate carcinoma derived from brain	ATCC HTB-81	Eagle's Minimum Essential Medium with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
T24	Human urinary bladder transitional cell carcinoma	ATCC HTB-4	McCoy's 5a media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
J82	Human urinary bladder transitional cell carcinoma	ATCC HTB-1	Eagle's Minimum Essential Medium with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
HIT-T15	Syrian Golden Hamster, pancreatic islet of Langerhans beta cells	ATCC CRL-1777	Eagle's Minimum Essential Medium (low glucose) with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin

[0265] The cells were first analyzed using fluorescent microscopy for the expression of GFP, which also indicated the simultaneous expression of the attached light chain. To detect the expression and subcellular localization of the GFP-LC fusion proteins, the cells were examined by confocal microscopy. Cells from the cell lines RT4, P19, NCI H69, NCI H82, DU145, T24, and J82, transfected and washed as described above, were fixed with 4% paraformaldehyde. The fixed cells were imaged with a confocal microscope using a 488 nm excitation laser and an emission path of 510-530 nm. The data shows that each cell type was successfully transfected and, that except the small cell lung cancer cell lines NCI H69 and NCI H82, cells from each cell line expressed both GFP and the GFP-light chain fusion proteins (Table 6).

Table 6. Expression of Mammalian Constructs in Cells

Cell Line	Origin	Expression				
		GFP	GFP-LC/A	GFP-LC/B	GFP-LC/C1	GFP-LC/E
RT4	Bladder carcinoma	+	+	+	+	+
P19	Embryonic carcinoma	+	+	+	+	+
NCI H69	Small Cell Lung carcinoma	–	–	–	–	–
NCI H82	Small Cell Lung carcinoma	–	–	–	–	–
DU145	Prostate carcinoma	+	+	+	+	+
T24	Bladder carcinoma	+	+	+	+	+
J82	Bladder carcinoma	+	+	+	+	+

[0266] In order for cancer cells to be sensitive to the endoproteolytic cleavage, the target SNARE protein must be endogenously expressed and accessible to the light chain cleavage. To detect the presence of cleaved SNARE products a Western blot analysis was performed. Cells from the cell lines RT4, P19, NCI H69, NCI H82, DU145, T24, and J82, transfected and washed as described above, were lysed, by adding

200 (JL of 2 x SDS-PAGE Loading Buffer to each well, and the lysates were transferred to tubes and heated to 95 °C for 5 minutes. A 12 uL of each sample was separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen Inc., Carlsbad, CA) under denaturing, reducing conditions. Separated peptides were transferred from the gel onto nitrocellulose membranes by Western blotting using an electrophoretic tank transfer apparatus. The membranes were blocked by incubation, at room temperature, for 1 hour with gentle agitation, in a Blocking Solution containing Tris-Buffered Saline (TBS) (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% Bovine Serum Albumin (BSA), and 5% nonfat dry milk. Blocked membranes were incubated at 4 °C over night in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% BSA, and either 1) a 1:5,000 dilution of S9684 a-SNAP-25 rabbit polyclonal antiserum as the primary antibody (Sigma, St. Louis, MO); 2) a 1:5,000 dilution of sc17836 a-Syntaxin-1 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); or 3) a 1:5,000 dilution of sc69706 a-VAMP-2 mouse polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibody probed blots were washed three times for 5 minutes each time in TBS, polyoxyethylene (20) sorbitan monolaureate. Washed membranes were incubated at room temperature for 1 hour in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% BSA containing either 1) a 1:5,000 dilution of 81-6720 goat polyclonal a-mouse immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Invitrogen, Inc., Carlsbad, CA) as a secondary antibody; or 2) a 1:5,000 dilution of 81-6120 goat polyclonal a-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Invitrogen, Inc., Carlsbad, CA) as a secondary antibody. Secondary antibody-probed blots were washed three times for 5 minutes each time in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate. Signal detection of the labeled SNARE products were visualized using the ECL Plus™ Western Blot Detection System, a chemiluminescence-based detection system, (GE Healthcare-Amersham, Piscataway, NJ). The membranes were imaged and the percent of cleaved SNARE product were quantified with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (GE Healthcare-Amersham, Piscataway, NJ). The data shows that SNAP-25 and VAMP-2 were expressed in some cell types, while Syntaxin was expressed in each cell type tested (Table 7).

Table 7. Presence of SNARE in Cells

Cell Line	Origin	SNARE Presence in Cells		
		SNAP-25	VAMP-2	Syntaxin-1
RT4	Bladder carcinoma	–	+	+
P19	Embryonic carcinoma	+	–	+
NCI H69	Small cell Lung carcinoma	ND	ND	ND
NCI H82	Small cell Lung carcinoma	ND	ND	ND

DU145	Prostate carcinoma	+	+	+
T24	Bladder carcinoma	–	+	+
J82	Bladder carcinoma	+	–	+

[0267] In addition, the data shows that 1) BoNT/A light chain was able to cleave SNAP-25 present in cells from a P19 embryonic carcinoma cell line, a DU145 prostate carcinoma cell line, and a J82 urinary bladder carcinoma cell line (Table 8); 2) BoNT/E light chain was able to cleave SNAP-25 present in cells from a P19 embryonic carcinoma cell line and a J82 urinary bladder carcinoma cell line (Table 8); 3) BoNT/B light chain was unable to cleave VAMP-2 in all cell lines tested (Table 8); and 4) BoNT/C1 light chain was able to cleave Syntaxin-1 present in cells from a T24 urinary bladder carcinoma cell line (Table 8). These results indicate that treatment of cancer cells with the appropriate Clostridial toxin light chain will cleave one of three SNARE proteins to inhibit exocytosis. This inhibition will prevent the release of growth factors, angiogenic factors, and anti-apoptotic survival factors necessary for cancer cell growth and survival.

Table 8. Cleavage of SNARE by Light Chain

Cell Line	Origin	SNARE Cleavage by Light Chain			
		SNAP-25		VAMP-2	Syntaxin-1
		LC/A	LC/E	LC/B	LC/C1
RT4	Bladder carcinoma	–	–	–	–
P19	Embryonic carcinoma	+	+	–	–
NCI H69	Small Cell Lung carcinoma	ND	ND	ND	ND
NCI H82	Small Cell Lung carcinoma	ND	ND	ND	ND
DU145	Prostate carcinoma	+	–	–	–
T24	Bladder carcinoma	–	–	–	+
J82	Bladder carcinoma	+	+	–	–

[0268] To further test whether SNARE cleavage disrupts exocytosis, an insulin release assay was performed. HIT-T15 cells release insulin when placed in high concentration of glucose. It has also been shown these cells express SNAP-25, and that SNAP-25 is an integral component of the SNARE complex needed for insulin release. HIT-T15 cells, transfected and washed as described above, were placed in DMEM media containing either 1) 5.6 mM glucose for basal insulin release (low glucose); or 2) 25.2 mM glucose for evoked insulin release (high glucose). Cells were incubated in a 37 °C incubator under 5% carbon dioxide for approximately 1 hour to allow for insulin release. The incubated media was collected

and the amount of insulin released was determined using an insulin ELISA kit. The assay was performed according to the manufacturer's instructions (APLCO Diagnostics, Salem, NH). Exocytosis was expressed as the amount of insulin released per 1 x 10^6 cells per hour.

[0269] The data shows that HIT-T15 cells transfected with GFP-LC/A, GFP-LC/B, and GFP-LC/E released less insulin than untransfected cells or cells transfected with GFP (Table 9). In addition, the basal insulin released in media containing a low glucose concentration (5.6 mM) remained unchanged between the transfected cells. The data indicate that BoNT/A, BoNT/B and BoNT/E light chains inhibited the release of insulin by cleaving SNAP-25 or VAMP-2 in HIT-T15 cells.

Table 9. Insulin Release from HIT-H15 Cells

Construct	5.6 mM Glucose (Low)	25.2 mM Glucose (High)
Untransfected Control	6:5 +/- 0.1	9.9 +/- 2.9
GFP	4.3 +/- 0.7	10.8+/-2.1
GFP-LCA	3.2 +/- 0.4	4.5 +/- 0.6
GFP-LCB	3.4 +/- 0.2	5.5 +/- 0.9
GFP-LCE	4.2+/-0.7	4.4+/- 1.0

[0270] The botulinum toxin light chain activity may also inhibit the trafficking of proteins to and from the plasma membrane. To test whether SNARE cleavage disrupts delivery and localization of receptors to the plasma membrane, the presence or absence of cell membrane proteins was determined in cells transfected with botulinum toxin light chains. Cells from the cell lines DU145 and J82, transfected and washed as described above, were treated with 2 mM NHS-LC-Biotin (Thermo Scientific, Rockford, IL) at 4 °C for 2 hours. The cells were then treated with 250 mM Tris-HCI (pH 7.5) for 30 minutes at 4 °C, and then washed three times in TBS. Membranes proteins were isolated using the Membrane Protein extraction kit (Calbiochem, San Diego, CA) according to the manufacturer's instructions. The biotinylated proteins were precipitated with immobilized-avidin (Thermo Scientific, Rockford, IL). After three washes with TBS, the samples were suspended in 50 uL 2x SDS-PAGE loading buffer and separated by MOPS polyacrylamide gel electrophoresis using NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen Inc., Carlsbad, CA) under denaturing, reducing conditions. The gel was washed and fixed in 10% methanol and 7% acetic acid for 30 minutes. The wash solution was removed and the gel incubated in SYPRO® Ruby protein gel stain solution (Bio-Rad Laboratories, Hercules, CA) for 3 hours to overnight at room temperature. The stained gel was destained in 10% methanol and 7% acetic acid for 30 minutes. Chemiluminescence from the destained gel was visualized with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (GE Healthcare-Amersham, Piscataway, NJ). The data show that treatment with a BoNT/A light chain inhibits the trafficking of proteins to and from the plasma membrane, which would necessarily affect the population of receptors located on the surface of the cell. This disrupted trafficking may cause the cancer cells to become more sensitive to apoptotic factors and less sensitive to growth signals and angiogenic factors.

[0271] By establishing the SNARE cleavage effects by the light chains, and which light chains cleaved which SNARE proteins in each cell line, TVEMPs were subsequently designed in a manner that targeted the TVEMP to receptors that were overexpressed or uniquely expressed in cancers cells in order to deliver the catalytic light chain.

Example 2
Presence of Receptor and Target in Cancer Cells

[0272] This example illustrates how to determine the presence of a cognate receptor that can bind with the targeting moiety of a TVEMP disclosed herein as well as the presence of the target SNARE protein of the enzymatic domain of a TVEMP disclosed herein.

[0273] In order for a TVEMP to be an effective agent for the methods of treating cancer disclosed herein, the cancer cells must express the appropriate receptor that can bind with the targeting moiety of a TVEMP as well as the appropriate SNARE protein that can be cleaved by the enzymatic domain of the TVEMP.

[0274] To culture cells, an appropriate density of cells were plated into the wells of 96-well tissue culture plates containing 100 uL of an appropriate medium (Table 10), but without serum, and with or without 25 ug/mL of GT1b (Alexis Biochemicals, San Diego, CA). Cells were plated and incubated in a 37 °C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest (approximately 3 days). The media was aspirated from each well and replaced with 100 uL of fresh media containing various concentrations of the botulinum toxin or TVEMP being tested in order to generate a full dose-response. The assay was done in triplicate. After 24 hrs treatment, the cells were washed, incubated for an additional two days without toxin or TVEMP to allow for the cleavage of the SNARE substrate. After this incubation, the cells were washed by aspirating the media and rinsing each well with 3 mL of 1 x PBS. The cells were harvested by lysing in freshly prepared Lysis Buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% , 4-octylphenol polyethoxylate) at 4°C for 30 minutes ;with constant agitation. Lysed cells were centrifuged at 4000 rpm for 20 min at 4°C to eliminate debris using a bench-top centrifuge. The total protein concentrations of the cell lysates were measured by Bradford assay.

Table 10. Cell Lines and Media

Cell Line	Origin	Source	Serum Growth Media Composition
RT4	Human urinary bladder transitional cell carcinoma	ATCC HTB-2	McCoy's 5a media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
P19	Mouse embryonic carcinoma	ATCC CRL-1825	Alpha Minimal Essential Medium media with 7.5 % bovine calf serum, 2.5% fetal bovine calf serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin

NCI H69	Human small lung carcinoma	ATCC HTB-119	RPMI-1640 media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
NCI H82	Human small lung carcinoma	ATCC HTB-175	RPMI-1640 media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
DU-145	Human prostate carcinoma derived from brain	ATCC HTB-81	Eagle's Minimum Essential Medium with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
PC-3	Human prostate carcinoma derived from brain	ATCC CRL-1435	F-12K media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
LNCaP clone FGC	Human prostate carcinoma derived from brain	ATCC CRL-1740	RPMI-1640 Eagle's with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
RWPE-1	Human prostate	ATCC CRL-11609	Dulbecco's Minimum Essential Medium with 10% Fetal Bovine Serum, 2 mM GlutaMAX™ I with 0.1 mM Non-Essential Amino-Acids, 10 mM HEPES, 1 mM Sodium Pyruvate, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
T24	Human urinary bladder transitional cell carcinoma	ATCC HTB-4	McCoy's 5a media with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
J82	Human urinary bladder transitional cell carcinoma	ATCC HTB-1	Eagle's Minimum Essential Medium with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
MCF-7	Human breast carcinoma	ATCC HTB-22	Dulbecco's Minimum Essential Medium with 10% Fetal Bovine Serum, 2 mM GlutaMAX™ I with 0.1 mM Non-Essential Amino-Acids, 10 mM HEPES, 1 mM Sodium Pyruvate, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
SiMa	Human neuroblastoma	DSMZ ACC 164	RPMI 1640 with 10% Fetal Bovine Serum, 0.1 mM Non-Essential Amino-Acids, 10 mM HEPES, 1 mM Sodium Pyruvate, 100 U/mL Penicillin, and 100 µg/mL Streptomycin,
266.6	Mouse pancreatic	ATCC CRL -2151	Dulbecco's Minimum Essential Medium with 10% Fetal Bovine Serum, 2 mM GlutaMAX™ I with 0.1 mM Non-Essential Amino-Acids, 10 mM HEPES, 1 mM Sodium Pyruvate, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
HIT-T15	Hamster pancreatic islet of Langerhans beta cells	ATCC CRL-1777	Eagle's Minimum Essential Medium (low glucose) with 10 % fetal bovine serum, 100 U/mL Penicillin, and 100 µg/mL Streptomycin
HUVEC	Human Umbilical Vein Endothelial Cells	Cell Applications, Inc., San Diego, CA, Cat. No. 200-05n	Endothelial Cell Growth Medium (Cell Applications, Inc., San Diego, CA, Cat. No. 211-500)

[0275] To determine whether a cancer cell expresses the appropriate receptor and target SNARE protein, a Western blot analysis can be performed.

[0276] In one experiment, cells from the cell lines RT4, P19, NCI H69, NCI H82, DU-145, T24, J82, LNCaP, and PC-3, transfected and washed as described above, were harvested by adding 40 uL of 2 x SDS-PAGE Loading Buffer (Invitrogen, Inc., Carlsbad, CA) and heating the plate to 95 °C for 5 min. A 12 uL of the harvested sample was separated by MOPS polyacrylamide gel electrophoresis under denaturing, reducing conditions using 1) CRITERION® 12% Bis-Tris precast polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA), when separating the SNAP-25_{197} cleavage product; 2) NuPAGE® 12% Bis-Tris precast polyacrylamide gels (Invitrogen Inc., Carlsbad, CA), when separating both the uncleaved SNAP-25_{20}6 substrate and the SNAP-25_{197} cleavage product; or 3) NuPAGE® Novex 4-12% Bis-Tris precast polyacrylamide gels (Invitrogen Inc., Carlsbad, CA), when separating all other proteins. Separated peptides were transferred from the gel onto nitrocellulose membranes by Western blotting using a electrophoretic tank transfer apparatus. The membranes were blocked by incubation at room temperature for 1 hour with gentle agitation in a Blocking Solution containing Tris-Buffered Saline (TBS) (25 mM 2-amino-2-hydroxymethyl-1,3-propanediol hydrochloric acid (Tris-HCl)(pH 7.4), 137 mM sodium chloride, 2.7 mM potassium chloride), 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% Bovine Serum Albumin (BSA), and 5% nonfat dry milk. Blocked membranes were incubated at 4 °C overnight in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% BSA, and either 1) a 1:5,000 dilution of S9684 a-SNAP-25 rabbit polyclonal antiserum as the primary antibody (Sigma, St. Louis, MO); 2) a 1:5,000 dilution of sc123 a-Syntaxin-1 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 3) a 1:5,000 dilution of sc13992 a-VAMP-1/2/3 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 4) a 1:5,000 dilution of sc50371 a-SNAP-23 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 5) a 1:5,000 dilution of sc28955 a-SVC2 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 6) a 1:5,000 dilution of sc123 a-FGFR3 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 7) a 1:5,000 dilution of sc9112 a-KOR1 rabbit polyclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA); 8) a 1:5,000 dilution of H00004987-D01P a-OPRL1 rabbit polyclonal antiserum as the primary antibody (Novus Biologicals, Littleton, CO); and 9) a 1:5,000 dilution of sc47778 a-p-actin mouse monoclonal antiserum as the primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Primary antibody probed blots were washed three times for 5 minutes each time in TBS, polyoxyethylene (20) sorbitan monolaureate. Washed membranes were incubated at room temperature for 1 hour in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate, 2% BSA containing either 1) a 1:5,000 dilution of 81-6720 goat polyclonal a-mouse immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Invitrogen, Inc., Carlsbad, CA) as a secondary antibody; or 2) a 1:5,000 dilution of 81-6120 goat polyclonal a-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Invitrogen, Inc., Carlsbad, CA) as a secondary antibody. Secondary antibody-probed blots were washed three times for 5 minutes each time in TBS, 0.1% polyoxyethylene (20) sorbitan monolaureate. Signal detection of the labeled SNARE products were visualized using the ECL Plus™ Western Blot Detection System, a chemiluminescence-based detection

system (GE Healthcare-Amersham, Piscataway, NJ). The membranes were imaged and the percent of cleaved SNARE product was quantified with a Typhoon 9410 Variable Mode Imager and Imager Analysis software (GE Healthcare-Amersham, Piscataway, NJ). The data shows that this approach can identify the receptors and SNARE proteins present in the cells comprising each cell line (Table 11).

Table 11. Expression of Receptors and SNARE Proteins In Cells

Cell Line	Expression							
	SNAP-25	SNAP-23	VAMP-2	Syntaxin-1	FGFR3	SV2C	OPRL-1	KOR-1
RT4	+	–	+	+	+	+	ND	+
P19	+	–	–	+	+	–	ND	+
NCI H69	+	–	+	+	+	–	ND	+
NCI H82	+	–	+	+	+	–	ND	+
DU-145	++	+	++	++	+++	ND	ND	+
PC-3	–	++	+/–	++	+++	ND	ND	+
LNCaP clone FGC	+	+	+	+	+++	+++	++	+
T24	–	++	+	+	++	++	++	+
J82	++	+/–	++	+	+++	++	++	+

ND, not determined

[0277] Once cell lines comprising cells including the appropriate receptor and SNARE proteins were identified, the ability of a botulinum toxin or TVEMP to intoxicate these cells can be determined by detecting the presence of cleaved SNARE products using Western blot analysis. An appropriate density of cells from each cell line to be tested are plated into the wells of 96-well tissue culture plates containing 100 uL of an appropriate medium (Table 7) with or without 25 ug/mL of GT1b (Alexis Biochemicals, San Diego, CA). Cells are plated and incubated in a 37 ^{C}C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest (approximately 3 days). The media is aspirated from each well and is replaced with 100 uL of fresh media containing various concentrations of the botulinum toxin or TVEMP being tested sufficient to generate a full dose-response. The assay is done in triplicate. After 24 hrs treatment, the cells are washed, incubated for an additional two days without toxin or TVEMP to allow for the cleavage of the SNARE substrate. After this incubation, the cells are washed by aspirating the media and rinsing each well with 3 mL of 1 x PBS. The cells are harvested by lysing in freshly prepared Lysis Buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% , 4-octylphenol polyethoxylate) at 4°C for 30 minutes with constant agitation. Lysed cells are centrifuged at 4000 rpm for 20 min at 4°C to eliminate debris using a bench-top centrifuge. The protein concentrations of cell lysates are measured by Bradford assay. Samples of the cell lysates are analyzed by Western blot analysis as described above.

[0278] In one experiment, differentiated cells from the cell lines LNCaP, J82, and MCF-7, transfected as described above. The media was aspirated from each well and the differentiated cells were treated by replacing with fresh media containing either 1) 0 (untreated sample), 0.12 nM, 0.36 nM, 1.1 nM, 3.3 nM,

10 nM, 30 nM, and 90 nM of a BoNT/A; 2) 0 (untreated sample), and 50 nM of a BoNT/A; 3) 0 (untreated sample), 0.12 nM, 0.36 nM, 1.1 nM, 3.3 nM, 10 nM, 30 nM, and 90 nM of a TVEMP designated Noci-LH_N/A; or 4) 0 (untreated sample), and 166 nM of a TVEMP designated Noci-LHN/A. After 1)3-15 hours; 2) 6 hours or 3) 24 hours treatment, the cells were washed, incubated for an additional 16 hours without toxin or TVEMP to allow for the cleavage of the SNAP-25 substrate. After this incubation, the cells were washed and harvested as described above. The presence of cleaved SNAP-25 product was detected using Western blot analysis as described above using a 1:5,000 dilution of S9684 a-SNAP-25 rabbit polyclonal antiserum as the primary antibody (Sigma, St. Louis, MO) as the primary antibody and a 1:5,000 dilution of 81-6120 goat polyclonal a-rabbit immunoglobulin G, heavy and light chains (IgG, H+L) antibody conjugated to horseradish peroxidase (Invitrogen, Inc., Carlsbad, CA) as a secondary antibody. These results are shown in Table 12.

Table 12. Cleavage of SNARE Substrate

Cell Line	Lowest Concentration and Earliest Time for Cleavage Detection	
	BoNT/A	Noci-LH_N/A
LNCaP	50 nM at 9 hours	166 nM at 9 hours
J82	50 nM at 3 hours 1.1 nM at 24 hours	166 nM at 3 hours
MCF-7	1.1 nM at 6 hours	ND

ND, not determined

[0279] Taken together, the data shows that 1) BoNT/A was able to cleave SNAP-25 present in cells from a LNCaP prostate carcinoma cell line, a J82 urinary bladder carcinoma cell line, and a MCF-7 breast carcinoma cell line (Table 9); 2) Noci-LH_N/A was able to cleave SNAP-25 present in cells from a LNCaP prostate carcinoma cell line and a J82 urinary bladder carcinoma cell line (Table 9). These results indicate that treatment of cancer cells with the appropriate Clostridial toxin light chain will cleave one of three SNARE proteins to inhibit exocytosis. This inhibition will prevent the release of growth factors, angiogenic factors, and anti-apoptotic survival factors necessary for cancer cell growth and survival. Lastly, these experiments illustrate the validity of the general concept that intracellular delivery of a botulinum light chain into cancer cells results in cleavage of the appropriate SNARE protein not only by transfecting light chain constructs, but also by using the endogenous signal transduction pathway for the targeting domain.

Example 3

Effects of Light Chain Delivery on Angiogenesis

[0280] This example illustrates that treatment with a botulinum toxin or TVEMP will affect angiogenesis to a degree sufficient to provide a therapeutic benefit in a cancer treatment.

[0281] The blockade of exocytosis resulting from a treatment with botulinum toxin or TVEMP based on LHN/A-G will likely prevent the release of angiogenic factors, including, e.g., Vascular endothelial growth factor (VEGF), Fibroblast Growth Factor-1 (FGF1) and FGF2. Preventing the release of these angiogenic factors will reduce, or altogether inhibit, angiogenesis in the area where the toxin or TVEMP is administered. To test whether such a treatment reduces or inhibits angiogenesis, four different assays were performed: a VEGF release assay, a cell migration assay, an in vitro blood vessel formation assay, and a human angiogenesis protein array assay.

[0282] VEGF is known to be a potent mitogen for vascular endothelial cells and an inducer of physiological and pathological angiogenesis. To validate the potential for a botulinum toxin or TVEMP in inhibiting angiogenesis, the ability of a toxin or TVEMP to inhibit release of VEGF from a cell was assessed. To conduct a VEGF release assay, about 600,000 cells from a SiMa cell line were plated into the wells of 6-well collagen IV tissue culture plates containing 3 mL of a serum-free medium containing Minimum Essential Medium, 2 mM GlutaMAX™ I with Earle's salts, 1 x B27 supplement, 1 x N2 supplement, 0.1 mM Non-Essential Amino Acids, 10 mM HEPES and 25 ug/mL GT1b. These cells were incubated in a 37 °C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest and neurite extension (approximately 3 days). The media from the differentiated cells was aspirated from each well and replaced with fresh media containing either 0.77 mg/mL of a BoNT/A or 1 mg/mL of a Noci-LH_N/A TVEMP. As a control, cells were treated with media alone in parallel. After treatment the media was removed and replaced with fresh differentiation media. A 60 uL aliquot of media was removed from each well and replaced with 100 uL differentiation media 1 day, 2 days, 3 days, and 4 days after the addition of fresh differentiation media. The removed media was stored at -20 °C until needed. After the last sample was removed, the cells were trypsinized and the number of cells in each well was counted.

[0283] The presence of VEGF in the collected samples was detected using a K151BMB-1 VEGF tissue culture assay (Meso Scale Discovery, Gaithersburg, MD). A MULTI-ARRAY® 96-well Small Spot Plate VEGF plate was blocked with 150 uL Blocking Buffer (PBS with 0.05% polyoxyethylene (20) sorbitan monolaureate, 2% ECL Blocking reagent (GE Healthcare-Amersham, Piscataway, NJ), and 1% goat serum (Rockland Immunochemicals, Gilbertsville, PA) and shaken at 600 rpm for one hour. The blocking buffer was discharged and 25 uL of each sample was added to each well of the VEGF plate and the plate was incubated at 4 °C for 2 hours. The plate was washed three times with 200 uL PBS-T (PBS plus 0.05% Tween-20) and then 25 ul of SULFO-TAG a-hVEGF mouse monoclonal antibody 5 ug/mL in 2% antibody buffer (PBS plus 0.05 % polyoxyethylene (20) sorbitan monolaureate, and 2% ECL Blocking reagent (GE Healthcare-Amersham, Piscataway, NJ) added and incubated on a shaker at 600 rpm at RT for 1 hour. Plates were washed three times with PBS-T and then 150 uL Read Buffer (MSD, Cat# R92TC-1) were added per well. Plates were read in a SECTOR™ Imager 6000 Image Reader (Meso Scale Discovery, Gaithersburg, MD). The data was then exported.into Microsoft Office Excel 2007. The amount

of VEGF detected was normalized to the number of cells present in the well and the percent VEGF release value was calculated using the control as the 100% value.

[0284] The data shows that treatment with BoNT/A inhibits VEGF release by about 50 % in SiMa cells (Table 13). Although the addition of Noci-LH_N/A TVEMP did not appear to inhibit VEGF release, this result could be due to the lower potency of Noci-LH_N/A TVEMP compared to BoNT/A in SiMa cells. The EC_{50} of BoNT/A in differentiated SiMa cells is less than about 0.5 nM, while the EC_{50} of Noci-LH_N/A TVEMP is more than 30 nM. As such, the lack of effect of Noci-LH_N/A TVEMP in SiMa cells is simply due to the low amount of OPRL-1 receptor present in these cells. This lack of effect corroborates the concept that cells expressing low levels of the targeted receptor will not be affected by botulinum toxin or TVEMP treatment (i.e. normal cells surrounding tumors over-expressing a receptor of interest). In addition, the finding that the addition of IL-6, a known transcriptional regulator of VEGF, had no effect on VEGF release is consistent with reports that the addition of exogenous IL-6 does not affect VEGF secretion.

Table 13. VEGF Release Assay

Time Point	VEGF Release		
	Control	BoNT/A	Noci-LH_N/A TVEMP
Day 1	100%	69%	119%
Day 2	100%	57%	123%
Day 3	100%	53%	125%
Day 4	100%	57%	104%

[0285] Since VEGF is an inducer of migration, a compound that affects the release of VEGF should effect migration as well. Moreover, inhibition of exocytosis by a compound will also inhibit the release of additional factors involved in cell migration. To determine whether a botulinum toxin or TVEMP treatment could reduce or inhibit cell migration, a cell migration assay (Essen Bioscience, Ann Arbor, MI) was performed according to the manufacturer's instructions. On day 1, DU-145 cells were plated at 25,000 cells per well in a 96-well Essen ImageLock plate in growth media. On day 2 the cells were treated with either 10 nM BoNT/A, 40 nM Noci-LH_N/A TVEMP, or 90 nM Gal-LH_N/A TVEMP in growth media. As a positive control for inhibition of migration, cells were treated with 0.11 uM, 0.33 uM, or 1 uM Cytochalasin-D. As a negative control, cells were treated with media alone. On day 3, after the cells had reached 100 % confluence, the cells were washed with media and then a 96-pin WoundMaker (Essen Bioscience, Ann Arbor, MI) was used to simultaneously create wounds in all the wells. After cell wounding, the media was removed and the cells were washed two times with 150 uL Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} and then 100 ul_ of media was added. The plate was then placed in an INCUCYTE™ scanner (Essen Bioscience, Ann Arbor, MI) and images were taken every 1 hour for 45 consecutive hours. The data was analyzed as relative wound density versus time using the INCUCYTE™ Cell Migration software. Relative wound density is designed to be zero at time zero, and 100% when the cell density inside the wound is the same as the cell density outside the initial wound.

[0286] The results are presented in Table 14. The results showed that cells pre-treated with either Noci-LH_N/A TVEMP or Gal-LH_N/A TVEMP migrated slightly slower than cells treated with media alone. The result showed that treatment with Noci-LH_N/A TVEMP or Gal-LH_N/A TVEMP resulted in a significant reduction in cell migration after 24 hours, about 10 % reduction when compared to cells treated with media alone. Cells treated with BoNT/A did not exhibit an affect on cell migration. The cells treated with Cytochalasin-D did not migrate. When the same experiment was performed with PC-3 cells, that do not contain SNAP-25, rather than a reduction, an increase in migration was observed (data not shown), suggesting that initially, likely via activation of their ligand receptors, BoNT/A, , Noci-LH_N/A TVEMP, and Gal-LH_N/A TVEMP function to increase migration. But after cleavage of SNAP-25 migration is reduced. As such, a longer exposure to a botulinum toxin and/or TVEMP will most likely result in more dramatic reduction in migration of such treated cells.

Table 14. Cell Migration Assay

Treatment	Relative Wound Density at 24 Hours	
	Mean	Percent Relative to Media
Media Control	78.2 ± 2.4	100%
BoNT/A	78.6 ± 1.1	101%
Noci-LH_N/A TVEMP	71.5 ± 3.3	91%
Gal-LH_N/A TVEMP	69.5 ± 4.4	89%
Cytochalasin-D	3.3 ± 0.2	4%

[0287] Angiogenesis involves multiple steps; to achieve new blood vessel formation, endothelial cells must first escape their stable location by breaking through the basement membrane. Once this is achieved, endothelial cells migrate towards an angiogenic stimulus that might be released from cancer cells, or wound-associated macrophages. In addition, endothelial cells proliferate to provide the necessary number of cells for making a new vessel. Subsequent to this proliferation, the new outgrowth of endothelial cells needs to reorganize into a three-dimensionally tubular structure. To determine whether a botulinum toxin or TVEMP treatment could reduce or inhibit blood vessel formation, an in vitro Endothelial Tube Formation assay (Cell Biolabs, Inc., San Diego, CA) was performed according to the manufacturer's instructions. Human Umbilical Vein Endothelial Cells (HUVECs) were grown to 80% confluence in T-75 culture flasks until confluent. Cells were harvested and then plated at 500,000 cells per well for HUVECs in a 6-well plate for 24 hours. After incubation, cells were either kept untreated or treated with 2 nM or 5 nM of BoNT/A or 6 nM or 25 nM of Noci-LH_N/A TVEMP for 24 hours. As a positive control for inhibition, cells were treated with a collagenase inhibitor. As a negative control for inhibition, cells were treated with media alone. The cells were then harvested again and plated at 35,000 cells per well onto the ECM gel prepared from murine Engelbreth-Holm-Swan (EHS) tumor cells, which contain multiple angiogenic stimulating factors, such as, *e.g.,* laminin, type IV collagen, heparan sulfate proteoglycans, entactin and growth factors such as FGF2 and TGF-ps. The cells were incubated for 3-4 hours on the ECM gels and then inspected under a microscope and photographed, either before or after staining with Calcein AM.

[0288] A Endothelial Tube Formation assay was also modified to use cells from a tumor cell line. In this modified assay, cells from a LNCaP, PC-3, DU-145, T24, and J82 cell lines were grown to 80% confluence in T-75 culture flasks. Cells were then harvested and plated at 400,000 cell per well in a 6-well plate containing 3 mL of an appropriate medium (Table 10), but with 1% serum. Cells were incubated in a 37 °C incubator under 5% carbon dioxide for 3 days. After incubation, cells were either kept untreated or treated with 20 nM of BoNT/A or 40 nM of Noci-LH_N/A TVEMP for 24 hours. The cells were then harvested, plated on ECM gel plates and inspected as described above.

[0289] The results show that in HUVEC, DU145 and J82 cells, and to a lesser degree in T24 and LNCaP cells, tubes formed on ECM plates treated with media alone, whereas treatment with a collagenase inhibitor prevented the formation of tubes (Table 15). No tubes formed in PC-3 cells. BoNT/A and Noci-LH_N/A TVEMP treatment of cells from a LNCaP prostate carcinoma cell line and a J82 bladder carcinoma cell line inhibited the formation of tubes. BoNT/A and Noci-LH_N/A TVEMP treatment had no effect on tube formation from HUVEC cultures. This inhibition of tube formation maybe due to inhibition of migration, delivery of receptors and other proteins to the membrane (motility factors and their receptors), adhesion molecules that interact with the matrix or other cells, and/or secretion of proteases.

Table 15. Endothelial Tube Formation Assay

Cell Line	Inhibition of Endothelial Tube Formation			
	Media	Collagenase Inhibitor	BoNT/A	Noci-LH_N/A
LNCaP	No	Yes	Yes	Yes
PC-3	—	—	—	—
DU-145	No	ND	ND	ND
T24	No	ND	ND	ND
J82	No	Yes	Yes	Yes
HUVEC	No	ND	No	No

ND, not determined

[0290] To conduct a human angiogenesis protein array screen, cells from a DU-145 prostate cancer cell line were plated in a 100 mm^2 plate containing Eagle's Minimum Essential Medium with 1% charcoal stripped FBS, 100 U/mL Penicillin, and 100 ug/mL Streptomycin. Cells were grown to a density of 5 x 10^6 cells by incubating in a 37 °C incubator under 5% carbon dioxide overnight. After this incubation, the cells were washed by aspirating the media and rinsing the plate with 10 mL of 1 x PBS. The washed cells were treated by replacing with fresh media containing 50 nM BoNT/A. For comparison, cells treated with media alone were run in parallel. After 24 hour treatment, the cells were washed, and harvested by lysing in freshly prepared Lysis Buffer (50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% , 4-octylphenol polyethoxylate) on ice for 30 minutes with constant gentle agitation. Lysed cells were centrifuged at 14,000 g for 5 minutes at 4°C to eliminate debris. The protein concentrations of cell lysates were measured by Bradford assay. To perform an assay, an array was incubated with 250 uL of each cell lysate containing 500 ug of protein. Array images were captured by scanning the blots with a

Typhoon 9410 Imager and quantitation of array was performed with Image Quant TL V2005. Fold increased was determined by dividing signal from untreated over treated sample.

[0291] The results show that the majority of the 35 angiogenesis-related proteins detected were up-regulated in the cells treated with BoNT/A, compared to the untreated control (Table 16). Proteins that increased in expression were involved in promoting angiogenesis except for two proteins that are anti-angiogenic (endostatin and angiostatin). There was increased presence of GDNF, PDGF-AA, and FGF1 that promote cell proliferation, differentiation, cell growth and development. Proteins that promote or initiate angiogenesis were; Coagulation Factor III, EG-VEGF, Angiopoetin-1, Angiopoetin-2, and PD-ECGF. Expressions in proteins involved in glucose metabolism were; DPPIV, IGFBP-1, IGFBP-2, and IGFBP-3. Proteins that enhance cell-cell adhesion were also up-regulated; MIP-1, MMP-9, Endothelin-1, Platelet Factor 4 and TGF-P1. The most significant increase was observed for Endocrine gland-derived vascular endothelial growth factor (EG-VEGF), which was almost 100-fold increased. The increase of these proteins in cell lysates may reflect their accumulation in the cytoplasm since exocytosis has been inhibited and the cells cannot release them to the media.

Table 16. Human Angiogenesis Array In DU145 Cell line

Analyte	Mean Pixels Density		Fold Increased	Function
	Untreated	Treated		
External Control	65451	68877	1.1	—
Internal Control	50052	59543	1.2	—
Coagulation Factor III/TF	12736	26726	2.1	Promotes angiogenesis
GDNF	156	428	2.7	Promotes survival and differentiation
MIP-1 alpha	153	535	3.5	Chemotaxis
CXCL 16	3465	2352	0.7	Cytokine
GM-CSF	5001	1457	0.3	Cytokine
Serpin E1	677	2214	3.3	Inhibit proteases
Activin A	552	1672	3.0	Regulate morphogenesis in prostate
DPPIV	3790	8923	2.4	Glucose metabolism
HB-EGF	8990	6717	0.7	Cell proliferation
MMP-9	2454	5050	2.1	Breakdown extracellular matrix
Serpin F1	743	882	1.2	Inhibit proteases
TIMP-1	95918	86280	0.9	Anti-angiogenic
Angiogenin	6022	5468	0.9	Promotes angiogenesis
EG-VEGF	15	1368	88.3	Promotes angiogenesis
IGFBP-1	122	1147	9.4	Insulin growth factor protein
Pentraxin 3	119	732	6.2	Involved in complement-mediated clearance of apoptotic cells
TIMP-4	152	845	5.6	Matrix metalloproteinases inhibitor
Angiopoietin-1	137	807	5.9	Promotes angiogenesis
IGFBP-2	2379	8330	3.5	Insulin growth factor protein
PD-ECGF	942	12924	13.7	Promotes angiogenesis
Thrombospondin-1	2138	12359	5.8	Anti-angiogenic
Angiopoietin-2	129	1985	15.3	Antagonist of angiopoietin 1

Endostatin/Collagen XVIII	2388	6800	2.8	Anti-angiogenic
IGFBP-3	1145	11329	9.9	Insulin like promotes cell survivor
PDGF-AA	202	908	4.5	Regulates cell proliferation, cellular differentiation, cell growth, development
Angiostatin/Plasminogen	142	893	6.3	Anti-angiogenic
Endothelin-1	581	5828	10.0	Vascular homeostasis
uPA	30656	57108	1.9	Serine protease
Amphiregulin	33908	20736	0.6	Interacts with the EGF/TGF-alpha receptor to promote the growth
FGF1	1189	1875	1.6	Promotes proliferation & differentiation
IL-8	45837	19261	0.4	Angiogenic factor
FGF2	28018	23513	0.8	Promotes proliferation & differentiation
LAP/TGF-β1	360	1914	5.3	Increases extracellular matrix production
Platelet Factor 4	456	819	1.8	Cytokine
VEGF	33513	31434	0.9	Affects permeability

[0292] Taken together, the experiments described in this Example show an overall decrease in angiogenic potential after treatment with botulinum toxin of TVEMP together with an observed increase in intracellular angiogenic proteins. This could be due to either activation of receptors for botulinum toxin or TVEMP that promotes angiogenesis and/or accumulation of vesicular proteins due to blockage of exocytosis after cleavage of SNARE proteins.

Example 4
Effects of Light Chain Delivery on Apoptosis

[0293] This example illustrates that treatment with a botulinum toxin or TVEMP will affect apoptosis to a degree sufficient to provide a therapeutic benefit in a cancer treatment.

[0294] The blockade of exocytosis resulting from a treatment with botulinum toxin or TVEMP based on LHN/A-G will likely result in decreased metabolic activity and decreased cell viability. As such, cancer cells with inhibited exocytosis capability due to a toxin or TVEMP effect will have a reduced ability to survive. To test whether such a treatment causes decreased cancer cell viability, three different assays were performed: a cell viability and metabolism assay, a Caspase-3/8 activity assay, and a human apoptotic protein array assay.

[0295] To determine whether a botulinum toxin or TVEMP treatment could decrease cancer cell viability, a CELLTITER 96® AQueous One Solution Cell Proliferation Assay cell metabolic activity assay (Promega Corp., Madison, WI) was performed according to the manufacturer's instructions. This assay is a colorimetric assay containing a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] that is reduced by NADPH or NADH in metabolically active cells. The reduced MTS is a colored formazan product that can be

measured at an absorbance of 490nm. An appropriate density of cells from the cell lines MCF-7, SiMa, PC-12, 266.6, RWPE-1, and N2a, were plated into the wells of 96-well tissue culture plates containing 100 uL of an appropriate medium (Table 7), but without serum, and with or without 25 ug/mL of GT1b (Alexis Biochemicals, San Diego, CA). Cells were plated and incubated in a 37 °C incubator under 5% carbon dioxide until the cells differentiated, as assessed by standard and routine morphological criteria, such as growth arrest (approximately 3 days). The media was aspirated from each well and the differentiated cells were treated by replacing with fresh media containing 0 (untreated sample), 0.3125 nM, 1.25 nM, and 20 nM of a BoNT/A. After 24 hrs treatment, the cells were washed by aspirating the media and rinsing each well with 100 uL of 1 x PBS. After washing, 100 uL of MTS solution was added to each well, incubated for 2 hours, and then the absorbance at 490nm recorded with a 96-well plate reader. The quantity of formazan product as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture. A similar design can be employed to examine the effects of a TVEMP on cell viability.

[0296] The results show that a BoNT/A treatment decreased the metabolic activity in the cancerous cell lines tested (Table 17).

Table 17. Cell Metabolic Activity Assay

Cell Line	BoNT/A Concentration			
	0 nM	0.3125 nM	1.25 nM	20 nM
MCF-7	1.60	1.45	1.41	1.30
SiMa	1.68	1.40	1.07	0.33
PC-12	1.68	1.66	1.45	1.15
266.6	1.10	1.05	1.02	0.82
RWPE-1	0.99	1.01	0.89	0.67
N2a	1.63	1.50	1.43	1.28

[0297] To further demonstrate that a botulinum toxin or TVEMP treatment could decrease cancer cell viability, a CELLTITER GLO® Luminescent Cell Viability Assay (Promega Corp., Madison, WI) was performed according to the manufacturer's instructions. In this assay, cell viability is quantified on the bases of the presence of ATP, which signals the presence of metabolically active cells. A decreased in ATP content corresponds to less metabolically active cells. Ceils from the cell lines LNCaP, J82, T24, and DU-145 were differentiated as described above. The media was aspirated from each well and the differentiated cells were treated by replacing with fresh media containing either 1) 0 (untreated sample), 25 nM, and 50 nM of a BoNT/A; or 2) 0 (untreated sample), 250 nM, and 500 nM of a Noci-LH_N/A TVEMP. After 24 hrs treatment, the cells were washed by aspirating the media and rinsing each well with 100 uL of 1 x PBS. After washing, 100 uL of CELLTITER GLO® reagent was added to each well. After ten minutes incubation at room temperature, the sample luminescence was measured using a SpectraMAX L luminescence reader (Molecular Devices, Sunnyvale, CA). Assays were performed in triplicate and cell viability was noted every day for four or five days.

[0298] The data shows that decreased viability was observed in cells from both a DU-145 prostate carcinoma cell line and a J82 bladder carcinoma cell line after BoNT/A treatments (Table 18) or Noci-LH_N/A TVEMP treatments (Table 19).

Table 18. Cell Viability Assay for BoNT/A

Time	BoNT/A Concentration							
	DU-145				J82			
	0 nM	25 nM	0 nM	50 nM	0 nM	25 nM	0 nM	50 nM
Day 1	3356	3291 (0.385)	404219	301228 (0.325)	3077	2853 (0.223)	543436	318900 (0.398)
Day 2	2360	2433 (0.433)	649139	394645 (0.174)	5211	4646 (0.016)	741025	493817 (0.129)
Day 4	ND	ND	1277552	809182 (0.058)	ND	ND	1242627	649797 (0.010)
Day 5	4823	2325 (0.0001)	ND	ND	7384	4262 (0.0001)	ND	ND

P value indicating significant difference relative to non-treated control is listed in parenthesis.
ND, not determined

Table 19. Cell Viability Assay for Noci-LH_N/A TVEMP

Time	Noci-LH_N/A TVEMP Concentration							
	DU-145				J82			
	0 nM	250 nM	0 nM	500 nM	0 nM	250 nM	0 nM	500 nM
Day 1	3356	3630 (0.087)	404219	408023 (0.959)	3077	3189 (0.223)	543436	406420 (0.103)
Day 2	2360	2379 (0.876)	649139	622596 (0.802)	5211	4639 (0.015)	741025	677236 (0.581)
Day 4			1277552	1030346 (0.171)			1242627	854124 (0.020)
Day 5	4823	3595 (0.0003)			7384	6349 (0.009)		

P value indicating significant difference relative to non-treated control is listed in parenthesis.
ND, not determined

[0299] To determine whether a botulinum toxin or TVEMP treatment decreased cancer cell viability by an apoptotic process, the activity of Caspase-3/8 was measured in cell treated with BoNT/A. Cells from the cell lines LNCaP, J82, and T24 were differentiated as described above. The media was aspirated from each well and the differentiated cells were treated by replacing with fresh media containing either 1) 0 (untreated sample), 0.5 nM, 5 nM, and 50 nM of a BoNT/A; or 2) 0 (untreated sample), 1.6 nM, 16 nM, and 166 nM of a Noci-LH_N/A TVEMP. After 24 hrs treatment, the cells were washed by aspirating the media and rinsing each well with 100 uL of 1 x PBS To measure cellular caspase 9 activity, 50 uL of CASPASE-GLO® 9 (Promega, Corp., Madison, WI) reagent was added to the culture media of each well. After 30 minute incubation at 37 °C, the luminescence of each sample was measured using a Spectramax

L luminometer (Molecular Devices, Sunnyvale, CA). T24 does not express SNAP-25 and should not be sensitive to treatment with BoNT/A or Noci-LH_N/A TVEMP.

[0300] The data shows that an effect on Caspase 3/8 activity was most prevalent in LNCaP cell after exposure to BoNT/A, indicating that LNCaP cell line viability decreases with BoNT/A treatment (Table 20). These data are supported by the cell viability assays measuring the number of live and dead cells in populations treated with BoNT/A (Table 18). Although cells from a J82 cell line did not show significant differences in Caspase 3/8 activity, this cell line did contain a higher amount of dead cells after BoNT/A or Noci-LH_N/A TVEMP treatments (Table 19). The reason for the observation of no caspase activity in J82 cells could be due to at least two possibilities: 1) the timing of BoNT/A treatment to detect Caspase 3/8activity is different for J82 and LNCaP (e.g., Caspase 3/8activation may had occur earlier in J82 cells); or 2) the cell death pathway for J82 is independent of Caspase 3/8.

Table 20. Caspase 3/8 Activity Assay

Cell Line	BoNT/A Concentration				Noci-LH_N/A TVEMP			
	0 nM	0.5 nM	5 nM	50 nM	0 nM	1.6 nM	16 nM	166 nM
LNCaP	270	283	239	572	218	232	233	263
T24	656	612	634	646	637	602	623	617
J82	235	146	256	194	132	133	103	98

[0301] To test whether cell death of cells treated with a botulinum toxin or TVEMP was directed by a process independent of Caspase 3/8 pathway, cells were assayed for the presence of cleaved nuclear poly (ADP-ribose) polymerase (PARP). PARP is a 116 kDa nuclear poly (ADP-ribose) polymerase and appears to be involved in DNA repair in response to environmental stress. This protein can be cleaved by many ICE-like caspases in vitro and is one of the main cleavage targets of Caspase-3 in vivo. In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis. To determine whether changes in cell viability are due to cells undergoing apoptosis, cells from the cell lines DU-145 and J82 were differentiated as described above. The media was aspirated from each well and the differentiated cells were treated by replacing with fresh media containing either 1) 0 (untreated sample) and 50 nM of a BoNT/A; or 2) 0 (untreated sample) and 500 nM of a Noci-LH_N/A TVEMP. After 48 hrs treatment, the cells were washed, harvested and Western blot analysis performed as described in Example 1, except an a-PARP antibodies were used as the primary antibody. Cells from both cell lines showed an increased of cleaved PARP after 2 days of Noci-LH_N/A TVEMP treatment. However, the presence of cleaved PARP was minimal in cells from both cell lines treated with a Bo NT/A.

[0302] To conduct a human apoptosis protein array screen, cells from a DU-145 prostate cancer cell line were treated with a BoNT/A, harvested, and assayed as described above in Example 3. The results

show that after treatment of cells from the DU-145 cell line with 50 nM BonT/A for 24 hours, most of apoptosis-related proteins remained unchanged when compared to control. There were only 10 apoptotic-related proteins where expression decreased from 1.5-fold to 2.4-fold (Table 21). A decreased in expression was noted in three anti-apoptotic proteins (Livin, survivin, and BCL-x), two cell cycle related proteins (Claspin and P27), antioxidant related protein (PON2), chaperone protein (clusterin) and two pro-apoptotic related proteins (Bax and Cytochrome C).

Table 21. Human Apoptosis Array in DU-145 Cell line

	Mean Pixel density			
Analyte	**Untreated**	**Treated**	**Fold Decrease**	**Function**
Livin	644.1	469.7	1.7	Anti-apoptotic
Cytochrome c	3423	1889	1.9	Pro-apoptotic
XIAP	10099	10045	1.0	Anti-apoptotic
HTRA2/Omi	7542	9368	0.8	IAP antagonist
Clusterin	1139	816	1.6	Chaperones misfolded proteins
TNF rRI/TNFRSF1A	2036	1467	1.5	Activates NFkB
HSP70	7058	9669	0.7	Stress response chaperone
Claspin	6630	3390	2.0	Cell cycle check point
Survivin	8717	3739	2.4	Anti-apoptotic
HSP60	945	855	1.2	Stress response chaperone
cIAP-2	2862	3156	0.9	Inhibitor of Apoptosis (IAP)
SMAC/Diablo	8379	7132	1.2	Promotes caspase activation by interaction with IAP proteins
HSP27	5716	5683	1.0	Stress response chaperone
cIAP-1	16916	15297	1.1	Inhibitor of Apoptosis (IAP)
Phospho-Rad17	1646	999	1.8	cell cycle check point
HO-2/HMOX2	8930	8934	1.0	Microsomal enzyme
Catalase	18742	18710	1.0	Prevent cell damage from oxidative stress
p53	19134	22007	0.9	Induces apoptosis
HO-1/HMOX1/HSP32	9878	11333	0.9	Microsomal enzyme
Cleaved Caspase-3	715	614	1.3	Downstream mediator of apoptotis
p53	8623	11225	0.8	Induces apoptosis
HIF-1 alpha	6832	6703	1.0	Binds to hypoxia response elements
Pro-Caspase-3	36318	42668	0.9	Downstream mediator of apoptotis
p53	20019	24725	0.8	Induces apoptosis
Fas/TNFSF6	34978	35878	1.0	Induces apoptosis
Bcl-x	571	445	1.6	Anti-apoptotic
p27	1293	852	1.7	Cell cycle check point
FADD	9996	8647	1.2	Induces apoptosis
Bcl-2	967	1427	0.7	Anti-apoptotic
p21	1062	1029	1.1	Blocks cell cycle
TRAIL R2/DR5	25985	21477	1.2	Induces apoptosis

Bax	2097	1436	1.6	Apoptotic activator
PON2	2611	1784	1.5	Antioxidant enzyme
TRAIL R1	28443	20518	1.4	Induces apoptosis
Bad	5097	5932	0.9	Pro-apoptotic

[0303] Taken together, the experiments described in this Example show that treatment with a BoNT/A or TVEMP results in decreased metabolic activity and decreased cells viability. Events related to apoptosis were identified following light chain delivery into cancer cells, Caspase 3/8 activity was observed after treatment with BoNT/A in LNCaP cells as well as increased cleavage of PARP, the main substrate for Caspase 3 was observed after treatment with Noci-LH_N/A TVEMP in the DU-145 and J82 cells, showing that cells are pushed towards apoptosis after treatment with a BoNT/A or a TVEMP. Overall, the amounts of proteins involved with apoptosis in the cell lysates did not change after treatment with BoNT/A. Most of the pro-apoptotic and anti-apoptotic proteins exert their function by translocating from the cytoplasm to the mitochondria without changes in total protein amount. The small changes detected may be a short term response of the tumor cells to the inhibition of exocytosis and the interference with the input from the autocrine or paracrine loops that the cancer cell needs to survive. Eventually these cells will be pushed into apoptosis due to the lack of survival signals.

Example 5
Treatment of Cancer

[0304] The following examples are provided by way of describing specific embodiments without intending to limit the scope of the invention in any way.

[0305] A physician examines a 62 year old woman who complains of a lump in her left breast and diagnoses her with breast cancer. The woman is treated by local administration a composition comprising a TVEMP as disclosed herein in the vicinity of the affected area. The patient's condition is monitored and after about 1-7 days after treatment, the physician notes that the growth of the malignant tumor has slowed down. At one and three month check-ups, the physician determines that the size of the tumor has become smaller. This reduction in tumor size indicates successful treatment with the composition comprising a TVEMP. In addition, a systemic administration of a composition comprising a TVEMP as disclosed herein could also be used to administer a disclosed TVEMP to treat the breast cancer.

[0306] A physician examines a 58 year old man who complains of difficulty in urinating and diagnoses him with prostate cancer. The man is treated systemically by intravenous administration a composition comprising a TVEMP as disclosed herein. The patient's condition is monitored and after about 1-7 days after treatment, the physician determines that the size of the prostate has become smaller. At one and three month check-ups, the physician determines that the size of the prostate has returned to its normal size and that serum PSA levels are within the normal range. This reduction in tumor size and/or reduces

serum PSA levels indicates successful treatment with the composition comprising a TVEMP. In addition, a local administration of a composition comprising a TVEMP as disclosed herein could also be used to administer a disclosed TVEMP to treat the prostate cancer.

[0307] A physician examines a 67 year old man who complains of wheezing when he breathes and diagnoses him with lung cancer. The man is treated systemically by intravenous administration a composition comprising a TVEMP as disclosed herein. The patient's condition is monitored and after about 1-7 days after treatment, the physician notes that the growth of the malignant tumor has slowed down. At one and three month check-ups, the man indicates that his breathing has returned to normal and the physician determines that the size of the tumor has become smaller. The normal breathing and/or the reduction in tumor size indicate successful treatment with the composition comprising a TVEMP. In addition, systemic administration could also be used to administer a disclosed TVEMP to treat cancer. In addition, administration by inhalation could also be used to administer a disclosed TVEMP to treat the lung cancer.

[0308] A physician examines a **33** year old woman who complains of pelvic pain and diagnoses her with bladder cancer. The woman is treated by local administration a composition comprising a TVEMP as disclosed herein in the vicinity of the affected area. The patient's condition is monitored and after about 1-7 days after treatment, the physician notes that the growth of the malignant tumor has slowed down. At one and three month check-ups, the woman indicates that the pelvic pain has subsided and the physician determines that the size of the tumor has become smaller. The reduced pain and/or the reduction in tumor size indicate successful treatment with the composition comprising a TVEMP. In addition, a systemic administration of a composition comprising a TVEMP as disclosed herein could also be used to administer a disclosed TVEMP to treat the bladder cancer.

[0309] A physician examines a 73 year old woman who complains of abdominal pain and diagnoses her with colon cancer. The woman is treated by systemically by intravenous administration of a composition comprising a TVEMP as disclosed herein. The patient's condition is monitored and after about 1-7 days after treatment, and the physician notes that the growth of the malignant tumor has slowed down. At one and three month check-ups, the woman indicates that the abdominal pain has subsided and the physician determines that the size of the tumor has become smaller. The reduced pain and/or the reduction in tumor size indicate successful treatment with the composition comprising a TVEMP. In addition, a local administration of a composition comprising a TVEMP as disclosed herein could also be used to administer a disclosed TVEMP to treat the colon cancer.

[0310] A physician examines a 37 year old man who complains of headaches and dizziness and diagnoses him with a neuroblastoma. The man is treated by intracranial administration a composition comprising a TVEMP as disclosed herein in the vicinity of the affected area. The patient's condition is monitored and after about 1-7 days after treatment, the physician determines that the size of the

malignant tumor has become smaller. At one and three month check-ups, the man indicates that he no longer suffers form headaches and dizziness and the physician determines that the neuroblastoma is gone. The disappearance of headache, dizziness and/or the neuroblastoma indicates successful treatment with the composition comprising a TVEMP.

[0311] A physician examines a 46 year old man who complains of painful skin moles and discoloration and diagnoses him with a melanoma. The man is treated by topical administration of a composition comprising a TVEMP as disclosed herein. The patient's condition is monitored and after about 1-7 days after treatment, the physician determines that the size of the skin moles has reduced slightly and the skin is not as discolored as before. At one and three month check-ups, the man indicates that he no longer suffers any pain and the physician determines that the skin moles and discoloration has disappeared. The reduced pain and/or the disappearance of the skin moles indicate successful treatment with the composition comprising a TVEMP. In addition, a systemic administration of a composition comprising a TVEMP as disclosed herein could also be used to administer a disclosed TVEMP to treat the bladder cancer.

[0312] In closing, it is to be understood that although aspects of the present specification have been described with reference to the various embodiments, one skilled in the art will readily appreciate that the specific examples disclosed are only illustrative of the principles of the subject matter disclosed herein. Therefore, it should be understood that the disclosed subject matter is in no way limited to a particular methodology, protocol, and/or reagent, etc., described herein. As such, various modifications or changes to or alternative configurations of the disclosed subject matter can be made in accordance with the teachings herein without departing from the spirit of the present specification. Lastly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Accordingly, the present invention is not limited to that precisely as shown and described.

[0313] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0314] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any

combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0315] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." As used herein, the term "about" means that the item, parameter or term so qualified encompasses a range of plus or minus ten percent above and below the value of the stated item, parameter or term. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0316] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0317] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term "consisting of excludes any element, step, or ingredient not specified in the claims. The transition term "consisting essentially of limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[0318] All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for the purpose of describing and disclosing, for example, the compositions and methodologies described in such publications that might be used in connection with the present invention. These publications are provided solely for their disclosure prior to the filing date of the present application. Nothing in this regard should be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. All statements as to the date or representation as to the contents of these documents is based on the information available to the applicants and does not constitute any admission as to the correctness of the dates or contents of these documents.