

C. NOVYI FOR THE TREATMENT OF SOLID TUMORS IN HUMANS

FIELD OF INVENTION

[0001] The present invention provides, *inter alia*, methods for treating or ameliorating an effect of a solid tumor present in a human, for debulking a solid tumor present in a human, for microscopically precise excising of tumor cells in a human, and for ablating a solid tumor present in a human. Unit doses of *C. novyi* CFUs and kits are also provided.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] The present invention claims benefit to U.S. provisional application serial no. 61/806,497 filed March 29, 2013, the entire contents of which are incorporated by reference.

BACKGROUND OF THE INVENTION

[0003] Strategies that successfully target and destroy human cancers recognize differences between normal and malignant tissues (Dang *et al.*, 2001). Such differences can be found at the molecular level, as is the case with genetic aberrations, or more holistically, as with the physiological aberrations in a tumor.

[0004] It is known that malignant solid tumors are usually composed of a necrotic core and a viable rim. Therapeutic interventions to date have focused on the well-vascularized outer shell of the tumor, but few have targeted the inner hypoxic core (Jain *et al.*, 2001). The inner core of a tumor has unique characteristics that differentiate it from normal tissues. The core

has a poor vascular supply and is therefore deficient in nutrients and oxygen. As a site of active cellular necrosis, the lack of a functional vascular supply limits the clearance of noxious cell breakdown and results in a low pH. Such an environment is not suitable for growth of most human cells but is a rich environment for the growth of certain anaerobic bacteria. More than sixty-years ago, this concept led investigators to inject spores of *Clostridium histolyticus* into tumor-bearing animals (Parker *et al.*, 1947). Remarkably, the bacteria germinated only in the necrotic core of the tumor and liquefied the tumors. In the 1950s and 1960s, spores from *Clostridium butyricum* were injected into patients with a variety of very advanced solid tumor malignancies (Mose, 1967; Mose, 1972). Many patients had significant germination and destruction of large portions of their tumors, but the very poor health and advanced stage of these patients made their clinical management difficult and the absence of complete clinical responses subdued further pursuit of this approach.

[0005] Successful treatment of solid tumors remains an unfulfilled medical goal. Accordingly, there is a need to find treatments for solid tumors. The present invention is directed to meeting this and other needs.

SUMMARY OF THE INVENTION

[0006] One embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0007] Another embodiment of the present invention is a method for debulking a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0008] An additional embodiment of the present invention is a method for debulking a solid tumor present in a human. This method comprises administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores per cycle, each unit dose of *C. novyi* NT being suspended in a pharmaceutically acceptable carrier or solution.

[0009] A further embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor present in a human. This method comprises administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores per cycle, each unit dose of *C. novyi* NT spores being suspended in a pharmaceutically acceptable carrier or solution.

[0010] Another embodiment of the present invention is method for ablating a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution, wherein the tumor is ablated leaving a margin of normal tissue.

[0011] A further embodiment of the present invention is a unit dose of *C. novyi* CFUs. This unit dose comprises about 1×10^3 - 1×10^7 CFUs in a pharmaceutically acceptable carrier or solution, which is effective for treating or ameliorating an effect of a solid tumor present in a human.

[0012] An additional embodiment of the present invention is a kit for treating or ameliorating an effect of a solid tumor present in a human. This kit comprises a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs in a pharmaceutically acceptable carrier or solution and instructions for use of the kit.

[0013] Another embodiment of the present invention is a method for microscopically precise excision of tumor cells in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0014] A further embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor that has metastasized to one or more sites in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising at least about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figures 1A-B show various images of canine osteosarcomas on the right distal radius/ulna of test subjects "Sasha" (Figure 1A) and "Sampson"

(Figure 1B) after radiation treatment and intravenous (IV) *C. novyi* NT injection.

[0016] Figure 2A shows Kaplan-Meier curves showing survival of F433 Fisher rats after orthotopic implantation of a syngeneic glioma cell line (F98). Outer line – *C. novyi*-NT spores injected into tumor 12-15 days after tumor implantation. Inner line – control. Figure 2B shows bioluminescence (Xenogen imaging system) in three representative F433 Fisher rats after orthotopic implantation of F98 glioma cell line. Images acquired on day 0 (pretreatment – day of *C. novyi*-NT spore injection), day 1 after IT injection of *C. novyi*-NT spores, and day 2 after IT injection of *C. novyi*-NT spores. Figure 2C shows luciferase activity (count in millions) on day 0 (pretreatment), day 1 after IT injection of *C. novyi*-NT spores, and day 2 after IT injection of *C. novyi*-NT spores.

[0017] Figures 3A-B and 4A-B show germinated *C. novyi*-NT bacteria within microscopic brain tumor lesions. In these Figures, gram stain showed vegetative *C. novyi*-NT bacteria (white or black arrowheads) localized in tumor (T) and stellate micro-invasion (S), but not in normal brain tissue (Br). Figure 3A is a 100x magnification showing the interface of tumor and normal brain. Figure 3B is a 400x magnification showing the interface of tumor and normal brain. Figure 4A is a 100x magnification showing the interface of normal brain, tumor, and stellate micro-invasion of neoplastic tissue. Figure 4B is a 400x magnification showing *C. novyi*-NT germination in a stellate micro-invasive lesion.

[0018] Figure 5 is a table of summary data for samples sequenced.

[0019] Figure 6 is a table of copy number alterations in canine sarcomas.

[0020] Figures 7A-F are photographic and CT images from dog 11-R01 showing a partial response to *C. novyi*-NT therapy. Images span pre-treatment to day 70 after first IT dose of *C. novyi*-NT spores. Figure 7A shows a pre-treatment image of the peripheral nerve sheath tumor. Figure 7B shows abscess formation on day 3 of the study, with extent confined to tumor. Figure 7C shows medical debridement following spontaneous abscess rupture and discharge of necrotic and purulent material, which allowed healing by second intention. Figure 7D shows that the wound has healed completely by day 70 of the study and 77.6% reduction in tumor longest diameter was noted. Figure 7E is a pre-treatment CT image, taken 4 days before first treatment, which shows extent of tumor (circle) at the intersection of pinna and cranium. Figure 7F is a post-treatment CT image on day 10 of the study showing almost complete de-bulking of tumor.

[0021] Figures 8A-D are photographic and CT images from dog 04-R03 showing a complete response to *C. novyi*-NT therapy. Images span pre-treatment to day 60 after first IT dose of *C. novyi*-NT spores. Figure 8A shows a pre-treatment image of the soft tissue sarcoma. Figure 8B shows a tumor localized abscess formed on day 15 of the study, 1 day after a third dose of *C. novyi*-NT spores. Figure 8C shows that tumor de-bulking was complete by day 27 of the study and healthy granulation tissue had formed. Figure 8D shows that the wound had healed completely by day 60 of the study, and no residual tumor was noted (complete response). Figure 8E is a pre-treatment CT image, taken 5 days before first treatment, showing extent of tumor (circle)

on antebrachium. Figure 8F is a post-treatment CT image on day 62 of the study showing complete loss of tumor mass.

[0022] Figure 9 shows the size of dog 11-R01's tumor from initial IT dosing of *C. novyi* NT spores to completion of the clinical course.

[0023] Figure 10A shows photographic (upper panels) and CT images (lower panels) of a canine soft tissue sarcoma on test subject "Drake" (04-R01) after IT dosing of *C. novyi* NT spores. Circled regions of the CT images indicate tumor location. Figure 10B shows the size of Drake's tumor from initial IT dosing of *C. novyi* NT, through three subsequent doses, to completion of the clinical course.

[0024] Figure 11 shows the size of dog 04-R03's tumor from initial IT dosing of *C. novyi* NT spores, through two subsequent cycles, to completion of the clinical course.

[0025] Figure 12A shows tumor size in eight test subjects (11-R02, 04-R02, 26-R01, 16-R02, 04-R05, 16-R03, 11-R04, and 04-R06) over the clinical course in which four cycles of IT *C. novyi* NT spores were administered. Figure 12B shows tumor size in three test subjects (04-R08, 01-R02, and 10-R02) for which data from a complete clinical course was not available due to necessary amputation or data cutoff.

[0026] Figure 13 shows an injection scheme for tumors treated in the IT study disclosed in Examples 6 and 7.

[0027] Figures 14A-D show CT and MRI images from a human patient. Figure 14A shows a post-treatment CT with contrast on day 3 demonstrating evidence of intra- and extra-medullary air collection. Figure 14B shows a pre-

treatment MRI (T1 with gadolinium contrast) of the right upper humerus showing a contrast enhancing mass involving the soft tissue and possibly adjacent bone. Figure 14C shows a post-treatment MRI on day 4 demonstrating diminished contrast enhancement in the tumor mass compared to baseline. Figure 14D shows a post-treatment MRI on day 29 showing homogenous non-enhancing mass consistent with ongoing necrosis. Tumor is highlighted with arrowheads.

[0028] Figures 15A-D show extensive tumor necrosis in the human patient treated with *C. novyi*-NT spores. Figures 15A and 15B show a pre-treatment tumor biopsy showing viable tumor (leiomyosarcoma) cells, 40x (A) and 100x (B) magnification, respectively. Figures 15C and 15D show a post-treatment tumor biopsy, 4 days after IT injection of *C. novyi*-NT spores, showing extensive necrosis of tumor cells, 40x (A) and 100x (B) magnification, respectively.

[0029] Figures 16A-D show various aspects of the IT injection procedure using a three-tined needle. Figure 16A shows a photograph of the three-tined needle. Figures 16B and 16C show computed tomography (CT) images of the target injection area before and after insertion of the needle. Figure 16D shows a magnified image of the three tines of the needle. Figure 16E shows a CT image with overlaying measurements for determining insertion points of the needle.

DETAILED DESCRIPTION OF THE INVENTION

[0030] One embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of C. novyi colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0031] As used herein, the terms "treat," "treating," "treatment" and grammatical variations thereof mean subjecting an individual subject (e.g., a human patient) to a protocol, regimen, process or remedy, in which it is desired to obtain a physiologic response or outcome in that subject, e.g., a patient. In particular, the methods and compositions of the present invention may be used to slow the development of disease symptoms or delay the onset of the disease or condition, or halt the progression of disease development. However, because every treated subject may not respond to a particular treatment protocol, regimen, process or remedy, treating does not require that the desired physiologic response or outcome be achieved in each and every subject or subject, e.g., patient, population. Accordingly, a given subject or subject, e.g., patient, population may fail to respond or respond inadequately to treatment.

[0032] As used herein, the terms "ameliorate", "ameliorating" and grammatical variations thereof mean to decrease the severity of the symptoms of a disease in a subject.

[0033] As used herein, a "solid tumor" means an abnormal mass of cell growth. Solid tumors may occur anywhere in the body. Solid tumors may be cancerous (malignant) or noncancerous (benign). Examples of solid tumors

according to the present invention include adrenocortical carcinoma, anal tumor/cancer, bladder tumor/cancer, bone tumor/cancer (such as osteosarcoma), brain tumor, breast tumor/cancer, carcinoid tumor, carcinoma, cervical tumor/cancer, colon tumor/cancer, endometrial tumor/cancer, esophageal tumor/cancer, extrahepatic bile duct tumor/cancer, Ewing family of tumors, extracranial germ cell tumor, eye tumor/cancer, gallbladder tumor/cancer, gastric tumor/cancer, germ cell tumor, gestational trophoblastic tumor, head and neck tumor/cancer, hypopharyngeal tumor/cancer, islet cell carcinoma, kidney tumor/cancer, laryngeal tumor/cancer, leiomyosarcoma, leukemia, lip and oral cavity tumor/cancer, liver tumor/cancer (such as hepatocellular carcinoma), lung tumor/cancer, lymphoma, malignant mesothelioma, Merkel cell carcinoma, mycosis fungoides, myelodysplastic syndrome, myeloproliferative disorders, nasopharyngeal tumor/cancer, neuroblastoma, oral tumor/cancer, oropharyngeal tumor/cancer, osteosarcoma, ovarian epithelial tumor/cancer, ovarian germ cell tumor, pancreatic tumor/cancer, paranasal sinus and nasal cavity tumor/cancer, parathyroid tumor/cancer, penile tumor/cancer, pituitary tumor/cancer, plasma cell neoplasm, prostate tumor/cancer, rhabdomyosarcoma, rectal tumor/cancer, renal cell tumor/cancer, transitional cell tumor/cancer of the renal pelvis and ureter, salivary gland tumor/cancer, Sezary syndrome, skin tumors (such as cutaneous t-cell lymphoma, Kaposi's sarcoma, mast cell tumor, and melanoma), small intestine tumor/cancer, soft tissue sarcoma, stomach tumor/cancer, testicular tumor/cancer, thymoma, thyroid tumor/cancer, urethral tumor/cancer, uterine tumor/cancer, vaginal tumor/cancer, vulvar tumor/cancer, and Wilms' tumor. Preferably, the solid

tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, and melanoma. More preferably, the solid tumor is a leiomyosarcoma, such as a retroperitoneal leiomyosarcoma.

[0034] As used herein, a “unit dose” means the amount of a medication administered to a subject, *e.g.*, a human, in a single dose.

[0035] As used herein, “*C. novyi*” means a bacteria belonging to species of *Clostridium novyi* or a bacteria derived therefrom. *Clostridium novyi*, which may be obtained commercially from, *e.g.*, the ATCC (#19402), is a gram-positive anaerobic bacterium. A bacteria derived from *Clostridium novyi* may be made by, *e.g.*, screening native *Clostridium novyi* for clones that possess specific characteristics. Preferred *C. novyi* bacteria are those which are non-toxic or minimally toxic to a subject such as a mammal, *e.g.*, a human. For example, a preferred *C. novyi*, *C. novyi* NT, is a bacteria derived from native *Clostridium novyi* that has lost its single systemic toxin (α -toxin) gene by, *e.g.*, a genetic engineering process or through a selection procedure. *C. novyi* NT may be made, for example, using the procedure disclosed in Dang *et al.*, 2001 and U.S. Patent No. 7,344,710. Thus, the present invention includes *C. novyi* as well as *C. novyi* NT bacteria.

[0036] Pharmacokinetic studies indicate that *C. novyi* NT spores, if injected intravenously, are rapidly cleared from the circulation (greater than 99% spores are cleared within 1 hour) and sequestered within the reticulo-endothelial system. Long-term distribution studies reveal that these spores are eventually eliminated from all tissues within one year. Delivered in spore form (dormant stage), *C. novyi* NT germinates (transitions from the spore to

the vegetative state) when exposed to the hypoxic regions of tumors. Thus, the toxicities of *C. novyi* NT are expected to be greater in tumor-bearing than in healthy patients.

[0037] Healthy mice and rabbits showed no apparent clinical signs (morbidity, mortality, or clinical appearance) of toxicity regardless of treatment dose when injected with *C. novyi* NT intravenously. However, examination of tissues at necropsy revealed both gross and microscopic inflammatory changes that appeared to be treatment-dose dependent. These findings, primarily in the liver, spleen and adrenals, were noted at doses of 5×10^8 spores/kg or greater. Healthy animals receiving lower doses showed no gross or microscopic abnormalities at necropsy. In animals that received high doses, resolution of inflammation was already evident on day 28 and all signs of inflammation were absent in all animals by one year following administration. To determine if *C. novyi* NT spores would germinate in non-tumor hypoxic tissue, studies in elderly mice with atherosclerotic plaques and experimental myocardial infarctions were treated with *C. novyi* NT. There was no evidence of spore localization or germination within these vascular lesions. At the conclusion of the study, no clinical or pathologic abnormalities (other than the pre-existing cardiovascular lesions) were noted in these mice. These studies demonstrated that *C. novyi* NT caused no apparent clinical and minimal pathological toxicity in healthy animals.

[0038] Intravenous (IV) injection of spores into immune-competent tumor-bearing mice leads to lysis of the tumor and an intense inflammatory response. In mice, one of three outcomes is typically observed: One subset (25-35%) of mice are cured (no tumor recurrence after one year of

observation) and develop long-term immunity to the original tumor (Agrawal *et al.*, 2004). Another subset (65-75%) experience complete clinical responses, but undergo a recurrence with re-growth of the original tumor. Finally, the remaining subset (0 to 20%, depending on the experiment) undergoes tumor destruction, but develop significant clinical toxicity 2-5 days after the initiation of therapy. Relatively simple measures, such as hydration, are adequate to reduce this toxicity, often entirely eliminating these signs. Studies in larger animals (rabbits) show the same cure and recurrence rates with *C. novyi* NT therapy, but do not show the life-threatening clinical toxicity observed in a subset of mice. Treatment-related death was observed in tumor-bearing mice, but not in rabbits, treated with *C. novyi* NT spores (Diaz *et al.*, 2005). In these studies toxicity was related to both spore dose and tumor size. In moribund mice, no specific clinical laboratory or pathologic end-organ damage was noted and the only significant finding was hepatosplenomegaly. Cured mice had rare remnant inflammatory changes in the liver and spleen, but were otherwise no different than untreated animals. These studies show that toxicity in tumor-bearing animals can be pronounced (death) in mice with large tumors, but was minimal in larger animals (rabbits), and was manageable in mice with hydration or antibiotics.

[0039] Previous work using *C. novyi* NT spores injected intravenously (1×10^9 spores/ m^2) as a single agent in tumor bearing dogs produced no life threatening toxicities. The dogs were maintained on fluid therapy (2-4 ml/kg/hr) for several days post treatment which may have decreased the toxicity. Unfortunately, there were no measurable tumor responses to the treatment.

[0040] As used herein, “colony forming units” (“CFUs”) mean viable forms of the bacteria which will give rise to an aggregate of cells (or colonies). Such viable forms include vegetative and spore forms, and the present invention includes both forms used separately and in combination. Colony forming unit assays are known in the art. See, e.g., Breed *et al.*, 1916. Media for supporting the growth of *C. novyi* are commercially available, such as Reinforced Clostridial Medium (RCM) from Difco (BD, Franklin Lakes, NJ). As set forth above, the unit dose comprises from about 1×10^3 - 1×10^7 , such as about 1×10^3 - 1×10^4 , about 1×10^4 - 1×10^5 , about 1×10^5 - 1×10^6 , or about 1×10^6 - 1×10^7 , *C. novyi* CFUs.

[0041] In one aspect of this embodiment, the unit dose comprises from about 1×10^6 - 1×10^7 *C. novyi* CFUs. In another aspect of this embodiment, the unit dose comprises about 1×10^4 *C. novyi* CFUs. Surprisingly, the doses disclosed herein for human treatment are unexpectedly lower than would be expected from simply extrapolating from our non-rodent models using 1/6 of the non-rodent highest non-severely toxic does (HNSTD), as is typical for a starting dose therapeutic for oncology indications. See, e.g., Senderowicz, A.M., “Information needed to conduct first-in human oncology trials in the United States: a view from a former FDA medical reviewer.” Clin. Canc. Res., 2010, 16:1719-25.

[0042] Preferably, in the present invention the *C. novyi* is *C. novyi* NT.

[0043] In another aspect of this embodiment, the unit dose comprises about 1×10^6 - 1×10^7 *C. novyi* NT spores. In a further aspect of this embodiment, the unit dose comprises about 1×10^4 *C. novyi* NT spores.

[0044] In an additional aspect of this embodiment, the administering step comprises injecting the unit dose at a single location into the tumor. In another aspect of this embodiment, the administering step comprises injecting the unit dose at multiple unique locations, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or more than 10 unique locations, into the tumor. Preferably, the administering step comprises injecting the unit dose at 1-5 unique locations into the tumor, such as in the configurations shown in Figure 13. In another preferred embodiment, the administering step comprises injecting the unit dose at 5 or more unique locations into the tumor. Multi-site injections may be carried out as disclosed herein, preferably with a multi-tined needle such as Quadra-Fuse® (Rex-Medical, Conshohocken, PA). In the present invention, the administering step, as noted above, includes injections directly into the tumor, but other methods for administering an active agent, such as *C. novyi* or *C. novyi* NT, to a tumor are also contemplated. Such methods include implantation, transdermal delivery, and transmucosal delivery.

[0045] In another aspect of this embodiment, the method further comprises administering a plurality of treatment cycles, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, or more than 30 cycles, to the human, each treatment cycle comprising injecting one unit dose of the *C. novyi* CFUs, such as one unit dose of the *C. novyi* NT spores, into the solid tumor. Preferably, 1-10 treatment cycles are administered. More preferably, 2-4 treatment cycles are administered. The interval between each treatment cycle may be variable. In one preferred embodiment, the interval between each treatment cycle is about 5-100 days. In another preferred embodiment, the interval between each treatment cycle is about 7 days.

[0046] In an additional aspect of this embodiment, the method further comprises administering intravenous (IV) fluids to the human before, during, and/or after each administration of the *C. novyi* CFUs, such as the *C. novyi* NT spores. IV fluids for hydrating the patients are disclosed herein and are well known in the art. Such fluids may be fluids that are isotonic with blood, such as, *e.g.*, a 0.9% sodium chloride solution, or Lactated Ringer's solution.

[0047] In another aspect of this embodiment, the method further comprises providing the human with a first course of antibiotics for a period of time and at a dosage that is effective to treat or alleviate an adverse side effect caused by the *C. novyi* CFUs, such as the *C. novyi* NT spores. In the present invention an adverse side effect (or adverse event, which is used interchangeably with adverse side effect) may include but is not limited to infections (such as those caused by open wounds), vomiting, hematochezia, and fever.

[0048] In one preferred embodiment, the antibiotics are administered for two weeks post *C. novyi* administration. Non-limiting examples of such antibiotics include amoxicillin, clavulanate, metronidazole, and combinations thereof.

[0049] In another preferred embodiment, the method further comprises providing the human with a second course of antibiotics for a period of time and at a dosage that is effective to treat or alleviate an adverse side effect caused by the *C. novyi*. The second course of antibiotics may be initiated after completion of the first course of antibiotics and is carried out for 1-6 months, such as 3 months. Preferably, the antibiotic used in the second

course is doxycycline, but any antibiotic approved by a medical professional may be used.

[0050] In a further aspect of this embodiment, the method further comprises, using a co-treatment protocol by, *e.g.*, administering to the human a therapy selected from the group consisting of chemotherapy, radiation therapy, immunotherapy, and combinations thereof.

[0051] The *C. novyi*, *e.g.*, the *C. novyi* NT spores, and the anti-cancer agent(s) used in the co-treatment therapy may be administered to the human, either simultaneously or at different times, as deemed most appropriate by a physician. If the *C. novyi*, *e.g.*, the *C. novyi* NT spores, and the other anti-cancer agent(s) are administered at different times, for example, by serial administration, then the *C. novyi*, *e.g.*, the *C. novyi* NT spores, may be administered to the human before the other anti-cancer agent. Alternatively, the other anti-cancer agent(s) may be administered to the human before the *C. novyi*, *e.g.*, the *C. novyi* NT spores.

[0052] As used herein, “chemotherapy” means any therapeutic regimen that is compatible with the *C. novyi*, *e.g.*, *C. novyi* NT, treatment of the present invention and that uses cytotoxic and/or cytostatic agents against cancer cells or cells that are associated with or support cancer cells. In a preferred embodiment, the chemotherapy comprises administering to the human an agent selected from the group consisting of an anti-metabolite, a microtubule inhibitor, a DNA damaging agent, an antibiotic, an anti-angiogenesis agent, a vascular disrupting agent, a molecularly targeted agent, and combinations thereof.

[0053] As used herein, an “anti-metabolite” is a substance that reduces or inhibits a cell’s use of a chemical that is part of normal metabolism. Non-limiting examples of anti-metabolite agents or analogs thereof according to the present invention include antifolates, purine inhibitors, pyrimidine inhibitors, and combinations thereof.

[0054] As used herein, an “antifolate” is a substance that alters, reduces, or inhibits the use of folic acid (vitamin B₉) by cells. Non-limiting examples of antifolates include methotrexate (DuraMed Pharmaceuticals, Inc.), pemetrexed (Eli Lilly), pralatrexate (Spectrum Pharmaceuticals), aminopterin (Sigma Aldrich), pharmaceutically acceptable salts thereof, and combinations thereof.

[0055] As used herein, a “purine” is a compound that contains a fused six-membered and a five-membered nitrogen-containing ring. Non-limiting examples of purines that are important for cellular metabolism include adenine, guanine, hypoxanthine, and xanthine. A “purine inhibitor” is a substance that alters, reduces or suppresses the production of a purine or the use of a purine by a cell. Non-limiting examples of purine inhibitors include methotrexate (DuraMed Pharmaceuticals, Inc.), pemetrexed (Eli Lilly), hydroxyurea (Bristol-Myers Squibb), 2-mercaptopurine (Sigma-Aldrich), 6-mercaptopurine (Sigma-Aldrich), fludarabine (Ben Venue Laboratories), clofarabine (Genzyme Corp.), nelarabine (GlaxoSmithKline), pralatrexate (Spectrum Pharmaceuticals), 6-thioguanine (Gate Pharmaceuticals), forodesine (BioCryst Pharmaceuticals), pentostatin (Bedford Laboratories), sapacitabine (Cyclacel Pharmaceuticals, Inc.), aminopterin (Sigma Aldrich),

azathioprine (GlaxoSmithKline), pharmaceutically acceptable salts thereof, and combinations thereof.

[0056] As used herein, a “pyrimidine” is a compound that contains a six-membered nitrogen-containing ring. Non-limiting examples of pyrimidines that are important for cellular metabolism include uracil, thymine, cytosine, and orotic acid. A “pyrimidine inhibitor” is a substance that alters, reduces, or suppresses the production of a pyrimidine or the use of a pyrimidine by the a cell. Non-limiting examples of pyrimidine inhibitors include 5-fluorouracil (Tocris Bioscience), tegafur (LGM Pharma), capecitabine (Xeloda) (Roche), cladribine (LGM Pharma), gemcitabine (Eli Lilly), cytarabine (Bedford Laboratories), decitabine (Eisai Inc.), floxuridine (Bedford Laboratories), 5-azacytidine (Pharmion Pharmaceuticals), doxifluridine (Cayman Chemicals), thiarabine (Access Pharmaceuticals), troxacitabine (SGX Pharmaceuticals), raltitrexed (AstraZeneca), carmofur (Santa Cruz Biotechnology, Inc.), 6-azauracil (MP Biomedicals, LLC), pharmaceutically acceptable salts thereof, and combinations thereof.

[0057] In a preferred aspect of the present invention, the anti-metabolite agent is selected from the group consisting of 5-fluorouracil (Tocris Bioscience), tegafur (LGM Pharma), capecitabine (Xeloda) (Roche), cladribine (LGM Pharma), methotrexate (DuraMed Pharmaceuticals, Inc.), pemetrexed (Eli Lilly), hydroxyurea (Bristol-Myers Squibb), 2-mercaptapurine (Sigma-Aldrich), 6-mercaptapurine (Sigma-Aldrich), fludarabine (Ben Venue Laboratories), gemcitabine (Eli Lilly), clofarabine (Genzyme Corp.), cytarabine (Bedford Laboratories), decitabine (Eisai Inc.), floxuridine (Bedford Laboratories), nelarabine (GlaxoSmithKline), pralatrexate (Spectrum

Pharmaceuticals), 6-thioguanine (Gate Pharmaceuticals), 5-azacytidine (Pharmion Pharmaceuticals), doxifluridine (Cayman Chemicals), forodesine (BioCryst Pharmaceuticals), pentostatin (Bedford Laboratories), sapacitabine (Cyclacel Pharmaceuticals, Inc.), thiarabine (Access Pharmaceuticals), troxacitabine (SGX Pharmaceuticals), raltitrexed (AstraZeneca), aminopterin (Sigma Aldrich), carmofur (Santa Cruz Biotechnology, Inc.), azathioprine (GlaxoSmithKline), 6-azauracil (MP Biomedicals, LLC), pharmaceutically acceptable salts thereof, and combinations thereof.

[0058] As used herein, a “microtubule inhibitor” is a substance that disrupts the functioning of a microtubule, such as the polymerization or the depolymerization of individual microtubule units. In one aspect of the present invention, the microtubule inhibitor may be selected from the group consisting of a microtubule-destabilizing agent, a microtubule-stabilizing agent, and combinations thereof. A microtubule inhibitor of the present invention may also be selected from the group consisting of a taxane, a vinca alkaloid, an epothilone, and combinations thereof. Non-limiting examples of microtubule inhibitors according to the present invention include BT-062 (Biotest), HMN-214 (D. Western Therapeutics), eribulin mesylate (Eisai), vindesine (Eli Lilly), EC-1069 (Endocyte), EC-1456 (Endocyte), EC-531 (Endocyte), vintafolide (Endocyte), 2-methoxyestradiol (EntreMed), GTx-230 (GTx), trastuzumab emtansine (Hoffmann-La Roche), crolibulin (Immune Pharmaceuticals), D1302A-maytansinoid conjugates (ImmunoGen), IMGN-529 (ImmunoGen), lorvotuzumab mertansine (ImmunoGen), SAR-3419 (ImmunoGen), SAR-566658 (ImmunoGen), IMP-03138 (Impact Therapeutics), topotecan/vincristine combinations (LipoCure), BPH-8 (Molecular Discovery

Systems), fosbretabulin tromethamine (OXiGENE), estramustine phosphate sodium (Pfizer), vincristine (Pierre Fabre), vinflunine (Pierre Fabre), vinorelbine (Pierre Fabre), RX-21101 (Rexahn), cabazitaxel (Sanofi), STA-9584 (Synta Pharmaceuticals), vinblastine, epothilone A, patupilone (Novartis), ixabepilone (Bristol-Myers Squibb), Epothilone D (Kosan Biosciences), paclitaxel (Bristol-Myers Squibb), docetaxel (Sanofi-Aventis), HAI abraxane, DJ-927 (Daiichi Sankyo), discodermolide (CAS No: 127943-53-7), eleutherobin (CAS No.: 174545-76-7), pharmaceutically acceptable salts thereof, and combinations thereof.

[0059] DNA damaging agents of the present invention include, but are not limited to, alkylating agents, platinum-based agents, intercalating agents, and inhibitors of DNA replication.

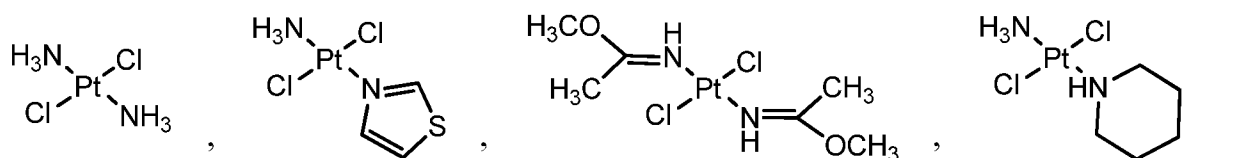
[0060] As used herein, an “alkylating agent” is a substance that adds one or more alkyl groups (C_nH_m , where n and m are integers) to a nucleic acid. In the present invention, an alkylating agent is selected from the group consisting of nitrogen mustards, nitrosoureas, alkyl sulfonates, triazines, ethylenimines, and combinations thereof. Non-limiting examples of nitrogen mustards include mechlorethamine (Lundbeck), chlorambucil (GlaxoSmithKline), cyclophosphamide (Mead Johnson Co.), bendamustine (Astellas), ifosfamide (Baxter International), melphalan (Ligand), melphalan flufenamide (Oncopeptides), and pharmaceutically acceptable salts thereof. Non-limiting examples of nitrosoureas include streptozocin (Teva), carmustine (Eisai), lomustine (Sanofi), and pharmaceutically acceptable salts thereof. Non-limiting examples of alkyl sulfonates include busulfan (Jazz Pharmaceuticals) and pharmaceutically acceptable salts thereof. Non-limiting

examples of triazines include dacarbazine (Bayer), temozolomide (Cancer Research Technology), and pharmaceutically acceptable salts thereof. Non-limiting examples of ethylenimines include thiotepa (Bedford Laboratories), altretamine (MGI Pharma), and pharmaceutically acceptable salts thereof. Other alkylating agents include ProLindac (Access), Ac-225 BC-8 (Actinium Pharmaceuticals), ALF-2111 (Alfact Innovation), trofosfamide (Baxter International), MDX-1203 (Bristol-Myers Squibb), thioureidobutyronitrile (CellCeutix), mitobronitol (Chinoin), mitolactol (Chinoin), nimustine (Daiichi Sankyo), glufosfamide (Eleison Pharmaceuticals), HuMax-TAC and PBD ADC combinations (Genmab), BP-C1 (Meabco), treosulfan (Medac), nifurtimox (Metronomx), improsulfan tosilate (Mitsubishi tanabe Pharma), ranimustine (Mitsubishi tanabe Pharma), ND-01 (NanoCarrier), HH-1 (Nordic Nanovector), 22P1G cells and ifosfamide combinations (Nuvilex), estramustine phosphate (Pfizer), prednimustine (Pfizer), lurbinectedin (PharmaMar), trabectedin (PharmaMar), altretamine (Sanofi), SGN-CD33A (Seattle Genetics), fotemustine (Servier), nedaplatin (Shionogi), heptaplatin (Sk Holdings), apaziquone (Spectrum Pharmaceuticals), SG-2000 (Spirogen), TLK-58747 (Telik), laromustine (Vion Pharmaceuticals), procarbazine (Alkem Laboratories Ltd.), and pharmaceutically acceptable salts thereof.

[0061] As used herein, a “platinum-based agent” is an anti-cancer substance that contains the metal platinum and analogs of such substances. The platinum may be in any oxidation state. Platinum-based agents of the present invention include, but are not limited to, 1,2-diaminocyclohexane (DACH) derivatives, phenanthroimidazole Pt(II) complexes, platinum IV compounds, bi- and tri-nuclear platinum compounds, demethylcantharidin-

integrated platinum complexes, platinum-conjugated compounds, cisplatin nanoparticles and polymer micelles, sterically hindered platinum complexes, oxaliplatin (Debiopharm), satraplatin (Johnson Matthey), BBR3464 (Novuspharma S.p.A.), ZD0473 (Astra Zeneca), cisplatin (Nippon Kayaku), JM-11 (Johnson Matthey), PAD (cis-dichlorobiscyclopentylamine platinum (II)), MBA ((trans-1,2-diaminocyclohexane) bisbromoacetato platinum (II)), PHM ((1,2-Cyclohexanediamine) malonato platinum (II)), SHP ((1,2-Cyclohexanediamine) sulphato platinum (II)), neo-PHM ((trans-R,R-1,2-Cyclohexanediamine) malonato platinum (II)), neo-SHP ((trans-R,R-1,2-Cyclohexanediamine)sulphato platinum (II)), JM-82(Johnson Matthey), PYP ((1,2-Cyclohexanediamine) bispyruvato platinum (II)), PHIC ((1,2-Cyclohexanediamine) isocitrato platinum (II)), TRK-710 ((trans-R,R-1,2-cyclohexanediamine) [3-Acetyl-5-methyl-2,4(3H,5H)-furandionato] platinum (II)), BOP ((1,2-Cyclooctanediamine) bisbromoacetato platinum (II)), JM-40 (Johnson Matthey), enloplatin (UnionPharma), zeniplatin (LGM Pharma), CI-973 (Parke-Davis), lobaplatin (Zentaris AG/Hainan Tianwang International Pharmaceutical), cycloplatam (LGM Pharma), WA2114R (miboplatin/lobaplatin) (Chembest Research Laboratories, Ltd.), heptaplatin (SKI2053R) (SK Chemicals), TNO-6 (spiroplatin) (Haihang Industry Co., Ltd.), ormaplatin (tetraplatin) (LGM Pharma), JM-9 (iproplatin) (Johnson Matthey), BBR3610 (Novuspharma S.p.A.), BBR3005 (Novuspharma S.p.A.), BBR3571 (Novuspharma S.p.A.), BBR3537 (Novuspharma S.p.A.), aroplatin (L-NDDP) (BOC Sciences), Pt-ACRAMTU ($\{[\text{Pt}(\text{en}) \text{Cl}(\text{ACRAMTU-S})](\text{NO}_3)_2$ (en = ethane-1,2-diamine, ACRAMTU=1-[2-(acridin-9-ylamino)ethyl]-1,3-dimethylthiourea)), cisplatin-loaded liposomes (LiPlasomes), SPI-077 (Alza),

lipoplatin (Regulon), lipoxal (Regulon), carboplatin (Johnson Matthey), nedaplatin (Shionogi Seiyaku), miriplatin hydrate (Dainippon Sumitomo Pharma), ormaplatin (LGM Pharma), enloplatin (Lederle Laboratories), CI973 (Parke-Davis), PEGylated cisplatin, PEGylated carboplatin, PEGylated oxaliplatin, transplatin (*trans*-diamminedichloroplatinum(II); mixedZ:*trans*-[PtCl₂{Z-HN=C(OMe)Me}(NH₃)]), CD-37 (estradiol-platinum(II) hybrid molecule), picoplatin (Poniard Pharmaceuticals),



AH44 (Komeda et al., 2006; Harris et al., 2005; Qu et al., 2004), triplatinNC (Harris et al., 2005; Qu et al., 2004), ProLindac (Access), pharmaceutically acceptable salts thereof, and combinations thereof.

[0062] As used herein, an “intercalating agent” includes, but is not limited to, doxorubicin (Adriamycin), daunorubicin, idarubicin, mitoxantrone, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0063] Non-limiting examples of inhibitors of DNA replication include, but are not limited to topoisomerase inhibitors. As used herein, a “topoisomerase inhibitor” is a substance that decreases the expression or the activity of a topoisomerase. The topoisomerase inhibitors according to the present invention may inhibit topoisomerase I, topoisomerase II, or both topoisomerase I and topoisomerase II. Non-limiting examples of topoisomerase I inhibitors according to the present invention include irinotecan (Alchemia), APH-0804 (Aphios), camptothecin (Aphios), cositecan

(BioNumerik), topotecan (GlaxoSmithKline), belotecan hydrochloride (Chon Kun Dang), firtecan pegol (Enzon), HN-30181A (Hanmi), hRS7-SN-38 (Immunomedics), labetuzumab-SN-38 (Immunomedics), etirinotecan pegol (Nektar Therapeutics), NK-012 (Nippon Kayaku), SER-203 (Serina Therapeutics), simmitecan hydrochloride prodrug (Shanghai HaiHe Pharmaceuticals), gimatecan (Sigma-Tau), namitecan (Sigma-Tau), SN-38 (Supratek Pharma), TLC-388 hydrochloride (Taiwan Liposome Company), lamellarin D (PharmaMar), pharmaceutically acceptable salts thereof, and combinations thereof. Non-limiting examples of inhibitors of topoisomerase type II according to the present invention include Adva-27a (Advanomics), zopectarelin doxorubicin (Aeterna Zentaris), valrubicin (Anthra Pharmaceuticals), razoxane (AstraZeneca), doxorubicin (Avena Therapeutics), amsacrine (Bristol-Myers Squibb), etoposide phosphate (Bristol-Myers Squibb), etoposide (Novartis), dexrazoxane (Cancer Research Technology), cytarabine/daunorubicin combination (Celator Pharmaceuticals), CAP7.1 (CellAct Pharma), aldoxorubicin (CytRx), amrubicin hydrochloride (Dainippon Sumitomo Pharma), vosaroxin (Dainippon Sumitomo Pharma), daunorubicin (Gilead Sciences), milatuzumab/doxorubicin combination (Immunomedics), aclarubicin (Kyowa Hakko Kirin), mitoxantrone (Meda), pirarubicin (Meiji), epirubicin (Pfizer), teniposide (Novartis), F-14512 (Pierre Fabre), elliptinium acetate (Sanofi), zorubicin (Sanofi), dexrazoxane (TopoTarget), sobuzoxane (Zenyaku Kogyo), idarubicin (Pfizer), HU-331 (Cayman Chemical), aurintricarboxylic acid (Sigma Aldrich), pharmaceutically acceptable salts thereof, and combinations thereof.

[0064] Chemotherapeutic antibiotics according to the present invention include, but are not limited to, actinomycin, anthracyclines, valrubicin, epirubicin, bleomycin, plicamycin, mitomycin, pharmaceutically acceptable salts thereof, prodrugs, and combinations thereof.

[0065] As used herein, the term “anti-angiogenesis agent” means any compound that prevents or delays nascent blood vessel formation from existing vessels. In the present invention, examples of anti-angiogenesis agents include, but are not limited to, pegaptanib, ranibizumab, bevacizumab (avastin), carboxyamidotriazole, TNP-470, CM101, IFN- α , IL-12, platelet factor 4, suramin, SU5416, thrombospondin, VEGFR antagonists, angiostatic steroids and heparin, cartilage-derived angiogenesis inhibitory factor, matrix metalloproteinase inhibitors, angiostatin, endostatin, 2-methoxyestradiol, tecogalan, prolactin, $\alpha_v\beta_3$ inhibitors, linomide, VEGF-Trap, aminosterols, cortisone, tyrosine kinase inhibitors, anti-angiogenic siRNA, inhibitors of the complement system, vascular disrupting agents, and combinations thereof. Preferably, the anti-angiogenesis agent is bevacizumab.

[0066] VEGFR antagonists of the present invention include, but are not limited to, pazopanib, regorafenib, lenvatinib, sorafenib, sunitinib, axitinib, vandetanib, cabozantinib, vatalanib, semaxanib, ZD6474, SU6668, AG-013736, AZD2171, AEE788, MF1/MC-18F1, DC101/IMC-1C11, ramucirumab, and motesanib. VEGFR antagonists may also include, VEGF inhibitors such as bevacizumab, aflibercept, 2C3, r84, VEGF-Trap, and ranibizumab.

[0067] Angiostatic steroids of the present invention include any steroid that inhibits, attenuates, prevents angiogenesis or neovascularization, or causes regression of pathological vascularization. Angiostatic steroids of the

present invention include those disclosed in European Patent Application Serial No. EP1236471 A2, as well as those 20-substituted steroids disclosed in U.S. Patent Serial No. 4,599,331, those 21-hydroxy steroids disclosed in U.S. Patent Serial No. 4,771,042, those C₁₁-functionalized steroids disclosed in International Application Serial No. WO 1987/02672, 6 α -fluoro-17 α ,21-dihydroxy-16 α -methylpregna-4,9(11)-diene-3,20-dione 21-acetate, 6 α -fluoro-17 α ,21-dihydroxy-16 β -methylpregna-4,9(11)-diene-3,20-dione, 6 α -fluoro-17 α ,21-dihydroxy-16 β -methylpregna-4,9(11)-diene-3,20-dione 21-phosphonoxy and pharmaceutically acceptable salts thereof, hydrocortisone, tetrahydrocortisol, 17 α -hydroxy- progesterone, 11 α -epihydrocortisone, cortexolone, corticosterone, desoxycorticosterone, dexamethasone, cortisone 21-acetate, hydrocortisone 21-phosphate, 17 α -hydroxy-6 α -methylpregn-4-ene-3,20-dione 17-acetate, 6 α -fluoro-17 α ,21-dihydroxy-16 α -methylpregna-4,9(11)-diene-3,20-dione, and Δ 9(11)-etianic esters, all disclosed in International Application Serial No. WO 1990/015816 A1.

[0068] Cartilage-derived angiogenesis inhibitor factors include, but are not limited to, peptide troponin and chondromodulin I.

[0069] Matrix metalloproteinase inhibitors of the present invention include, but are not limited to, succinyl hydroxamates such as marimastat and SC903, sulphonamide hydroxamates such as CGS27023A, phosphinamide hydroxamates, carboxylate inhibitors such as BAY12-9566, thiol inhibitors such as Compound B, aminomethyl benzimidazole analogues, peptides such as regasepin, and tetracyclines such as minocycline.

[0070] $\alpha_v\beta_3$ inhibitors include, but are not limited to, IS20I, P11 peptide, EMD 85189, and 66203, RGD peptide, RGD mimetics such as S 36578-2,

echistatin, antibodies or antibody fragments against $\alpha_v\beta_3$ integrin such as Vitaxin, which targets the extracellular domain of the dimer, cilengitide, and peptidomimetics such as S247.

[0071] Anti-angiogenic siRNAs include, but are not limited to, siRNAs targeting mRNAs that are upregulated during angiogenesis, optionally PEGylated siRNAs targeting VEGF or VEGFR mRNAs, and siRNAs targeting UPR (unfolded protein response)-IRE1 α , XBP-1, and ATF6 mRNAs. Additionally, it has been shown that siRNAs that are, at minimum, 21 nucleotides in length, regardless of targeting sequence, suppress neovascularization (Kleinman, et al., 2008) and may be included in the anti-angiogenic siRNAs of the present invention.

[0072] Inhibitors of the complement system include, but are not limited to, modified native complement components such as soluble complement receptor type 1, soluble complement receptor type 1 lacking long homologous repeat-A, soluble Complement Receptor Type 1-Sialyl Lewis^x, complement receptor type 2, soluble decay accelerating factor, soluble membrane cofactor protein, soluble CD59, decay accelerating factor-CD59 hybrid, membrane cofactor protein-decay accelerating factor hybrid, C1 inhibitor, and C1q receptor, complement-inhibitory antibodies such as anti-C5 monoclonal antibody and anti-C5 single chain Fv, synthetic inhibitors of complement activation such as antagonistic peptides and analogs targeting C5a receptor, and naturally occurring compounds that block complement activation such as heparin and related glycosaminoglycan compounds. Additional inhibitors of the complement system are disclosed by Makrides (Makrides, 1998).

[0073] As used herein, the term “vascular disrupting agent” means any compound that targets existing vasculature, e.g. tumor vasculature, damages or destroys said vasculature, and/or causes central tumor necrosis. In the present invention, examples of vascular disrupting agents include, but are not limited to, ABT-751 (Abbott), AVE8062 (Aventis), BCN105 (Bionomics), BMXAA (Antisoma), CA-4-P (OxiGene), CA-1-P (OxiGene), CYT997 (Cytopia), MPC-6827 (Myriad Pharmaceuticals), MN-029 (MediciNova), NPI-2358 (Nereus), Oxi4503 (Oxigene), TZT-1027 (Daichi Pharmaceuticals), ZD6126 (AstraZeneca and Angiogene), pharmaceutically acceptable salts thereof, and combinations thereof.

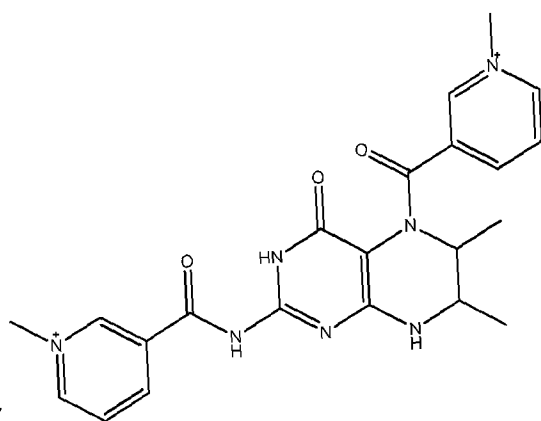
[0074] As used herein, a “molecularly targeted agent” is a substance that interferes with the function of a single molecule or group of molecules, preferably those that are involved in tumor growth and progression, when administered to a subject. Non-limiting examples of molecularly targeted agents of the present invention include signal transduction inhibitors, modulators of gene expression and other cellular functions, immune system modulators, antibody-drug conjugates (ADCs), and combinations thereof.

[0075] As used herein, a “signal transduction inhibitor” is a substance that disrupts communication between cells, such as when an extracellular signaling molecule activates a cell surface receptor. Non-limiting examples of signal transduction inhibitors of the present invention include anaplastic lymphoma kinase (ALK) inhibitors, B-Raf inhibitors, epidermal growth factor inhibitors (EGFRi), ERK inhibitors, Janus kinase inhibitors, MEK inhibitors, mammalian target of rapamycin (mTor) inhibitors, phosphoinositide 3-kinase inhibitors (PI3Ki), and Ras inhibitors.

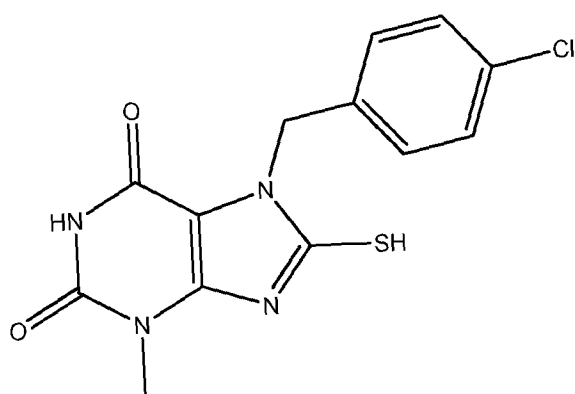
[0076] As used herein, an “anaplastic lymphoma kinase (ALK) inhibitor” is a substance that (i) directly interacts with ALK, *e.g.*, by binding to ALK and (ii) decreases the expression or the activity of ALK. Non-limiting examples of anaplastic lymphoma kinase (ALK) inhibitors of the present invention include crizotinib (Pfizer, New York, NY), CH5424802 (Chugai Pharmaceutical Co., Tokyo, Japan), GSK1838705 (GlaxoSmithKline, United Kingdom), Chugai 13d (Chugai Pharmaceutical Co., Tokyo, Japan), CEP28122 (Teva Pharmaceutical Industries, Ltd., Israel), AP26113 (Ariad Pharmaceuticals, Cambridge, MA), Cephalon 30 (Teva Pharmaceutical Industries, Ltd., Israel), X-396 (Xcovery, Inc., West Palm Beach, FL), Amgen 36 (Amgen Pharmaceuticals, Thousand Oaks, CA), ASP3026 (Astellas Pharma US, Inc., Northbrook, Illinois), and Amgen 49 (Amgen Pharmaceuticals, Thousand Oaks, CA), pharmaceutically acceptable salts thereof, and combinations thereof.

[0077] As used herein, a “B-Raf inhibitor” of the present invention is a substance that (i) directly interacts with B-Raf, *e.g.*, by binding to B-Raf and (ii) decreases the expression or the activity of B-Raf. B-Raf inhibitors may be classified into two types by their respective binding modes. As used herein, “Type 1” B-Raf inhibitors are those inhibitors that target the ATP binding sites of the kinase in its active conformation. “Type 2” B-Raf inhibitors are those inhibitors that preferentially bind to an inactive conformation of the kinase. Non-limiting examples of Type 1 B-Raf inhibitors of the present invention include:

Compound 7

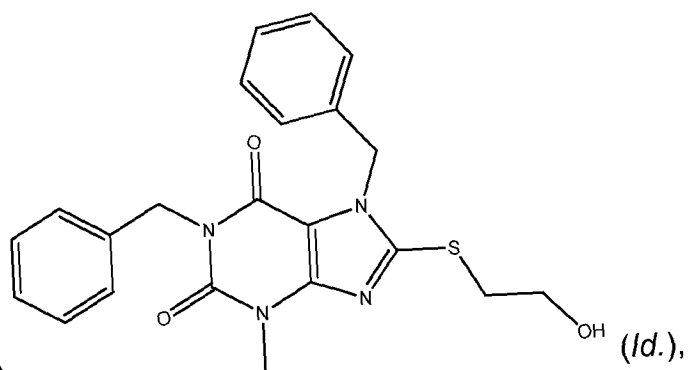
(Li *et al.*, 2010),

Compound 9



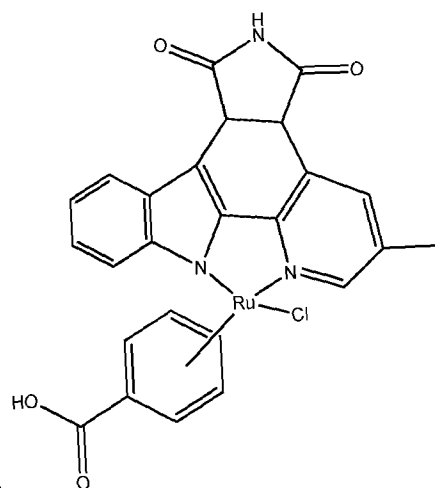
(Id.),

Compound 10

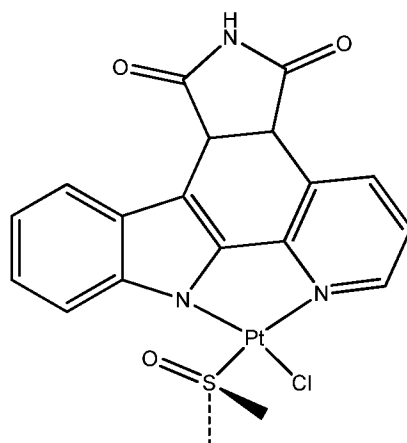


(Id.),

Compound 13



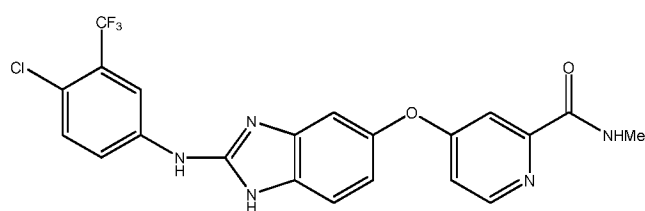
(Id.),



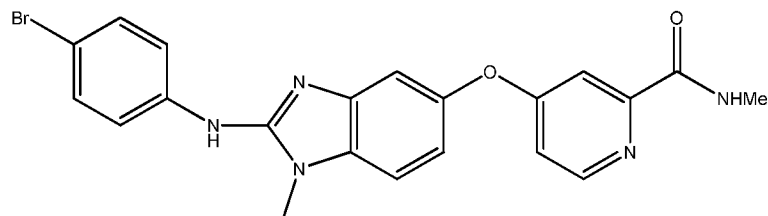
Compound 14 (*Id.*), dabrafenib

(GlaxoSmithKline), GDC-0879 (Genentech), L-779450 B-Raf (Merck), PLX3202 (Plexxikon), PLX4720 (Plexxikon), SB-590885 (GlaxoSmithKline), SB-699393 (GlaxoSmithKline), vemurafenib (Plexxikon), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the type 1 RAF inhibitor is dabrafenib or a pharmaceutically acceptable salt thereof.

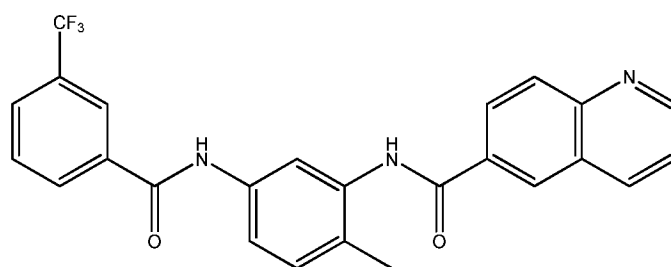
[0078] Non-limiting examples of Type 2 B-Raf inhibitors of the present invention include:



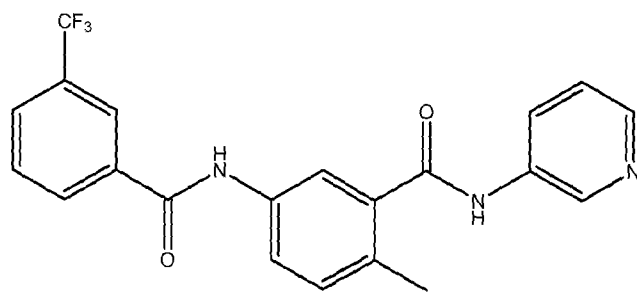
Compound 15 (Li *et al.*, 2010),



Compound 16 (*Id.*),

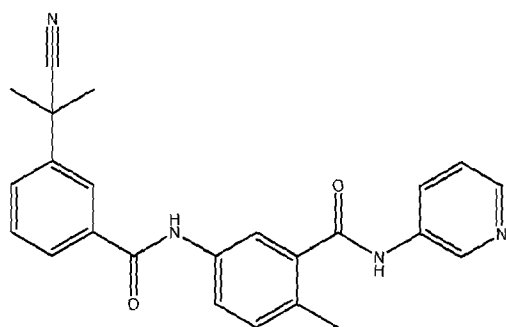


Compound 18 (*Id.*),



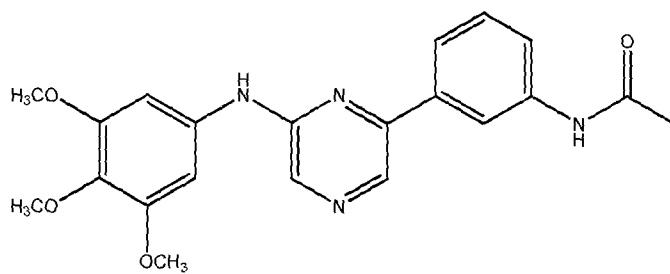
Compound 19

(Id.),



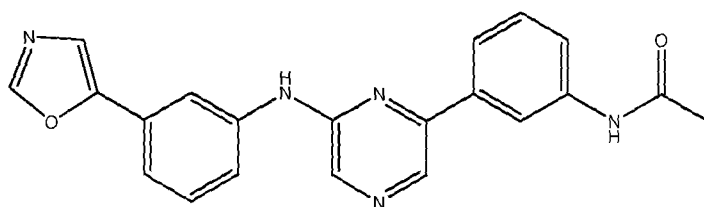
Compound 20

(Id.),



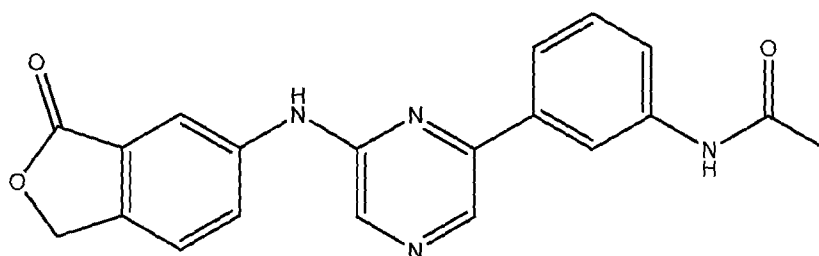
Compound 21

(Id.),

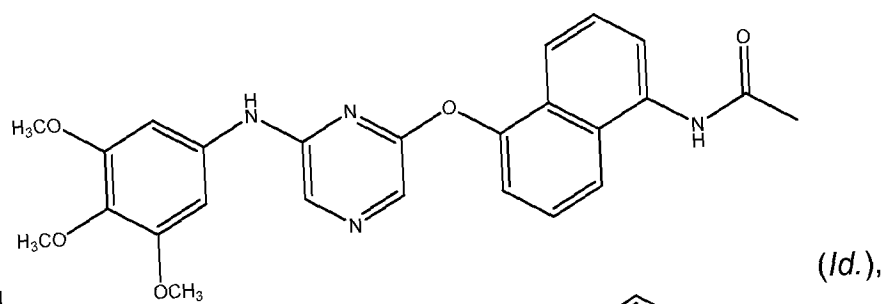


Compound 22

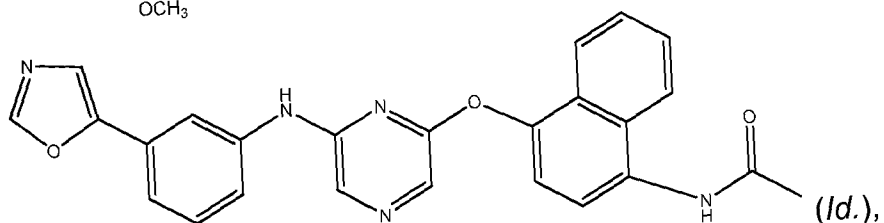
(Id.),

Compound 23
(Id.),

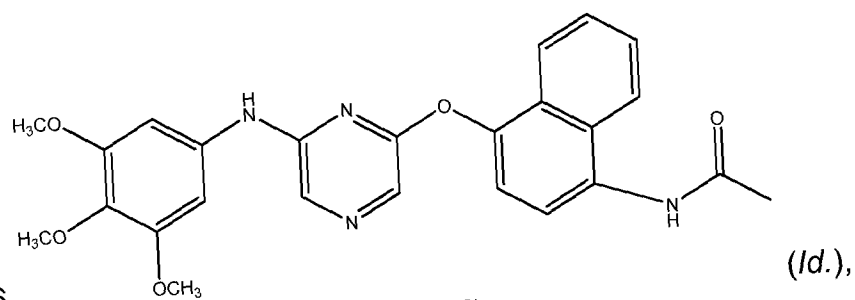
Compound 24



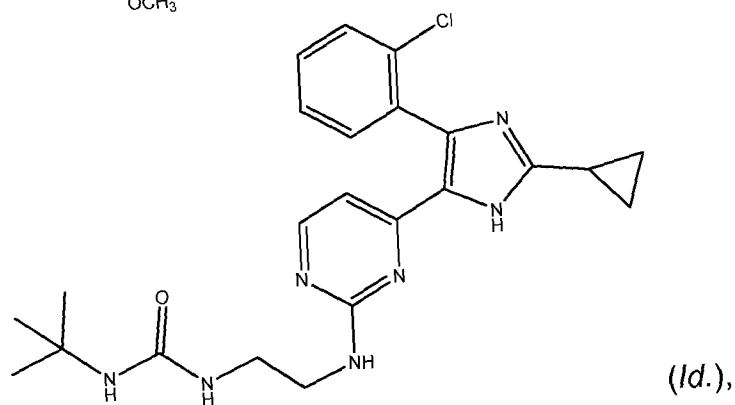
Compound 25



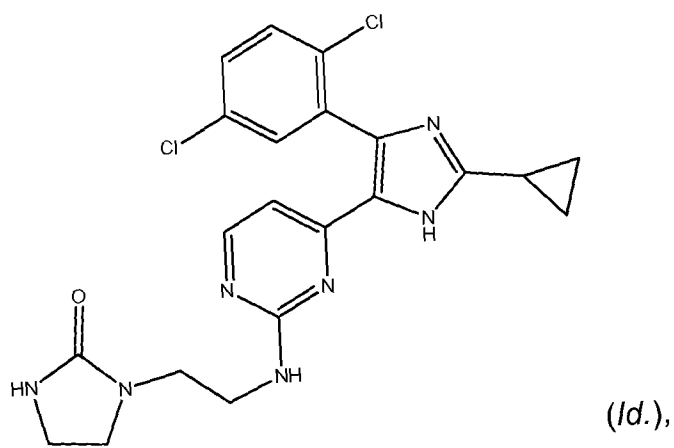
Compound 26

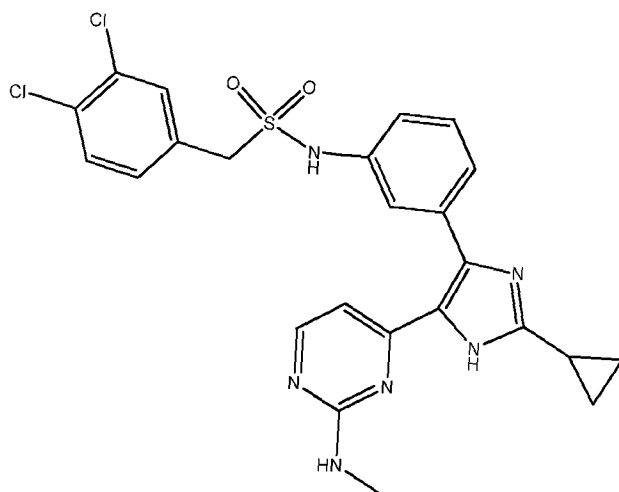


Compound 27



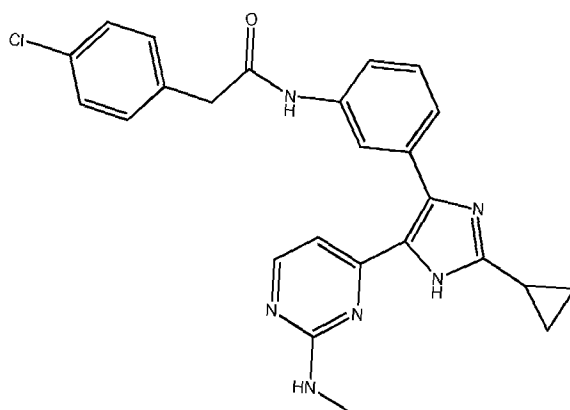
Compound 28





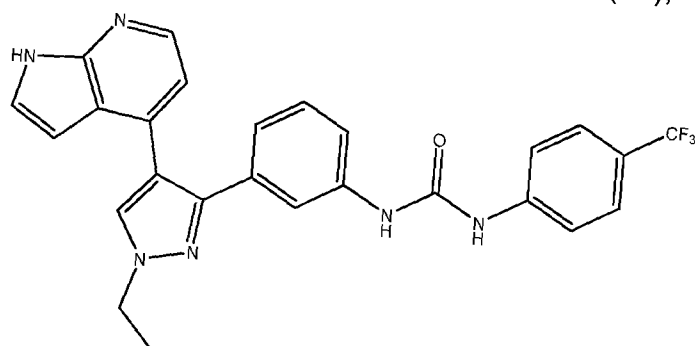
Compound 30

(Id.),



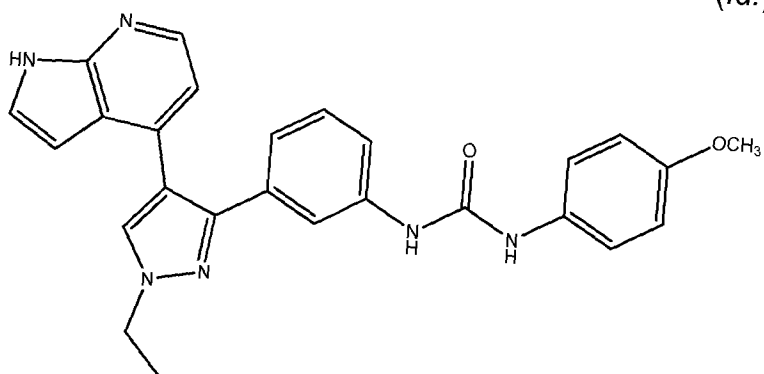
Compound 31

(Id.),



Compound 32

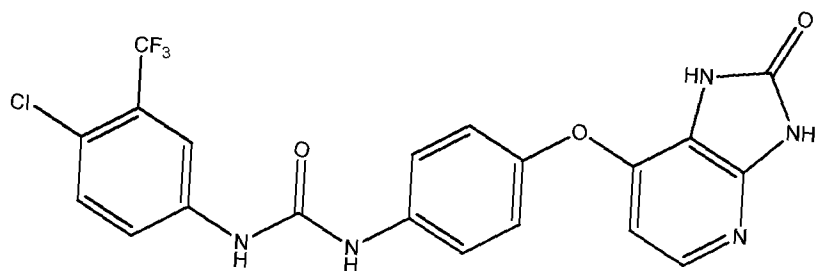
(Id.),



Compound 33

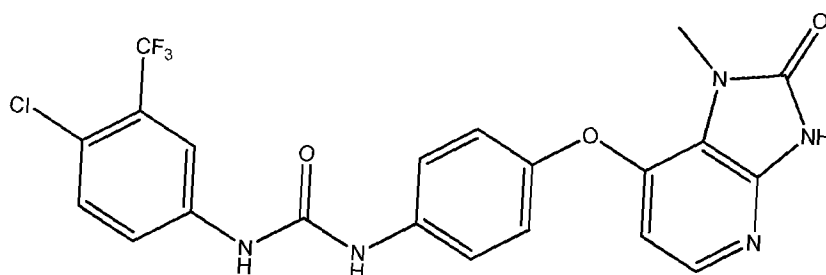
(Id.),

Compound 34

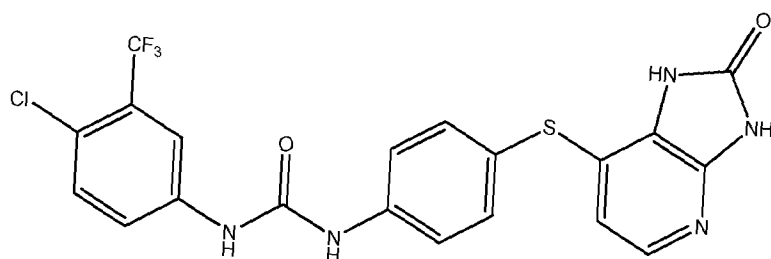


(Id.),

Compound 35
(Id.),

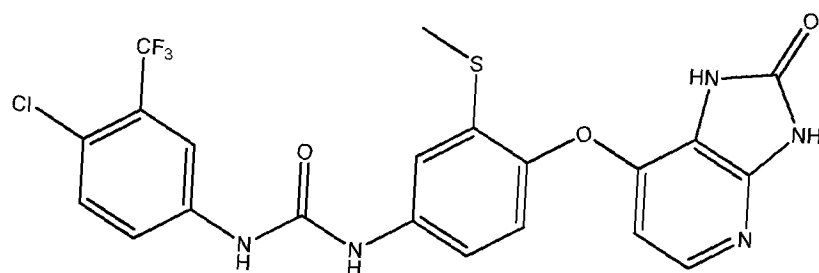


Compound 36



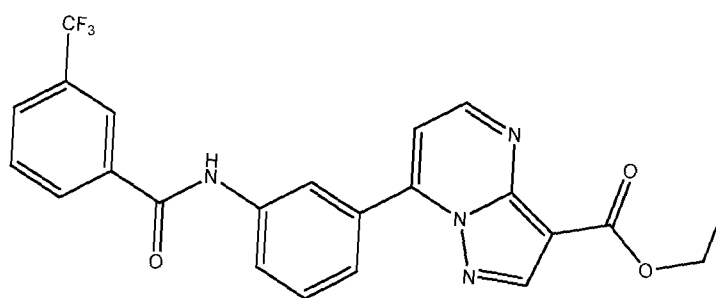
(Id.),

Compound 37

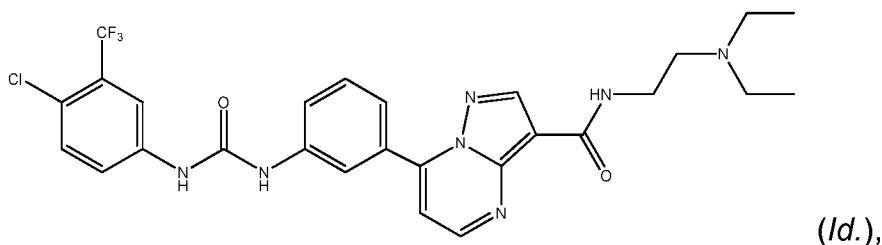
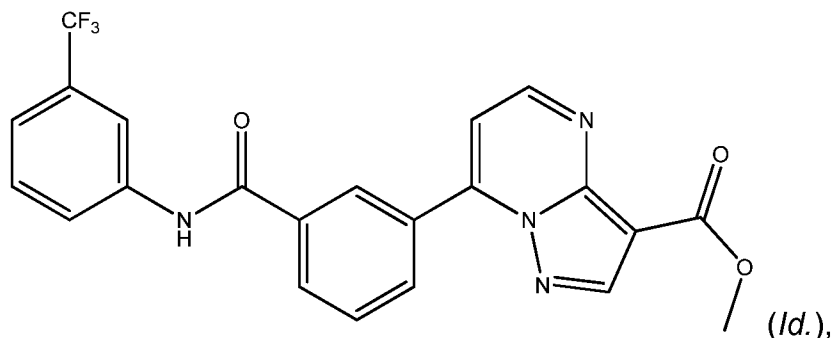


(Id.),

Compound 38



(Id.),



Sorafenib (Onyx Pharmaceuticals), ZM-336372 (AstraZeneca), pharmaceutically acceptable salts thereof, and combinations thereof

[0079] Other B-Raf inhibitors include, without limitation, AAL881 (Novartis); AB-024 (Ambit Biosciences), ARQ-736 (ArQule), ARQ-761 (ArQule), AZ628 (Axon Medchem BV), BeiGene-283 (BeiGene), BIIB-024 (MLN 2480) (Sunesis & Takeda), b raf inhibitor (Sareum), BRAF kinase inhibitor (Selexagen Therapeutics), BRAF siRNA 313 (tacaccagcaagctagatgca) and 253 (cctatcgtagagtcttctg) (Liu et al., 2007), CTT239065 (Institute of Cancer Research), DP-4978 (Deciphera Pharmaceuticals), HM-95573 (Hanmi), GW 5074 (Sigma Aldrich), ISIS 5132 (Novartis), LErafAON (NeoPharm, Inc.), LBT613 (Novartis), LGX 818 (Novartis), pazopanib (GlaxoSmithKline), PLX5568 (Plexxikon), RAF-265 (Novartis), RAF-365 (Novartis), regorafenib (Bayer Healthcare Pharmaceuticals, Inc.), RO 5126766 (Hoffmann-La Roche), TAK 632 (Takeda), TL-241 (Teligene), XL-281 (Exelixis), pharmaceutically acceptable salts thereof, and combinations thereof.

[0080] As used herein, an “EGFR inhibitor” is a substance that (i) directly interacts with EGFR, e.g. by binding to EGFR and (ii) decreases the expression or the activity of EGFR. Non-limiting examples of EGFR inhibitors according to the present invention include (+)-Aeropylsinin-1 (CAS # 28656-91-9), 3-(4-Isopropylbenzylidenyl)-indolin-2-one, ABT-806 (Life Science Pharmaceuticals), AC-480 (Bristol-Myers Squibb), afatinib (Boehringer Ingelheim), AG 1478 (CAS # 153436-53-4), AG 494 (CAS # 133550-35-3), AG 555 (CAS # 133550-34-2), AG 556 (CAS # 133550-41-1), AG 825 (CAS # 149092-50-2), AG-490 (CAS # 134036-52-5), antroquinonol (Golden Biotechnology), AP-26113 (Ariad), ARRY334543 (CAS # 845272-21-1), AST 1306 (CAS # 897383-62-9), AVL-301 (Celgene), AZD8931 (CAS # 848942-61-0), BIBU 1361 (CAS # 793726-84-8), BIBX 1382 (CAS # 196612-93-8), BMS-690514 (Bristol-Myers Squibb), BPIQ-I (CAS # 174709-30-9), Canertinib (Pfizer), cetuximab (Actavis), ciplatinib (Jiangsu Hengrui Medicine), CL-387,785 (Santa Cruz Biotech), compound 56 (CAS # 171745-13-4), CTX-023 (CytomX Therapeutics), CUDC-101 (Curis), dacomitinib (Pfizer), DAPH (CAS # 145915-58-8), daphnetin (Santa Cruz Biotech), dovitinib lactate (Novartis), EGFR Inhibitor (CAS # 879127-07-8), epitinib (Hutchison China MediTech), erbstatin Analog (CAS # 63177-57-1), erlotinib (Astellas), gefitinib (AstraZeneca), GT-MAB 5.2-GEX (Glycotope), GW 583340 (CAS # 388082-81-3), GW2974 (CAS # 202272-68-2), HDS 029 (CAS # 881001-19-0), Hypericin (Santa Cruz Biotech), icotinib hydrochloride (Betapharma), JNJ-26483327 (Johnson & Johnson), JNJ-28871063 (Johnson & Johnson), KD-020 (Kadmon Pharmaceuticals), lapatinib ditosylate (GlaxoSmithKline), Lavendustin A (Sigma), Lavendustin C (Sigma), LY-3016859 (Eli Lilly),

MEHD-7945A (Hoffmann-La Roche), MM-151 (Merrimack), MT-062 (Medisyn Technologies), necitumumab (Eli Lilly), neratinib (Pfizer), nimotuzumab (Center of Molecular Immunology), NT-004 (NewGen Therapeutics), pantumumab (Amgen), PD 153035 (CAS # 153436-54-5), PD 161570 (CAS # 192705-80-9), PD 168393, PD 174265 (CAS # 216163-53-0), pirotinib (Sihuan Pharmaceutical), poziotinib (Hanmi), PP 3 (CAS # 5334-30-5), PR-610 (Proacta), pyrotinib (Jiangsu Hengrui Medicine), RG-13022 (CAS # 136831-48-6), rindopepimut (Celldex Therapeutics), RPI-1 (CAS # 269730-03-2), S-222611 (Shionogi), TAK 285 (CAS # 871026-44-7), TAS-2913 (Taiho), theliatinib (Hutchison China MediTech), Tyrphostin 47 (RG-50864, AG-213) (CAS # 118409-60-2), Tyrphostin 51 (CAS # 122520-90-5), Tyrphostin AG 1478 (CAS # 175178-82-2), Tyrphostin AG 183 (CAS # 126433-07-6), Tyrphostin AG 528 (CAS # 133550-49-9), Tyrphostin AG 99 (CAS # 118409-59-9), Tyrphostin B42 (Santa Cruz Biotech), Tyrphostin B44 (Santa Cruz Biotech), Tyrphostin RG 14620 (CAS # 136831-49-7), vandetanib (AstraZeneca), varlitinib (Array BioPharma), vatalanib (Novartis), WZ 3146 (CAS # 1214265-56-1), WZ 4002 (CAS # 1213269-23-8), WZ8040 (CAS # 1214265-57-2), XL-647 (Exelixis), Z-650 (HEC Pharm), ZM 323881 (CAS # 324077-30-7), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the EGFR inhibitor is selected from the group consisting of panitumumab, erlotinib, pharmaceutically acceptable salts thereof, and combinations thereof.

[0081] As used herein, an “ERK inhibitor” is a substance that (i) directly interacts with ERK, including ERK1 and ERK2, *e.g.*, by binding to ERK and (ii) decreases the expression or the activity of an ERK protein kinase. Therefore,

inhibitors that act upstream of ERK, such as MEK inhibitors and RAF inhibitors, are not ERK inhibitors according to the present invention. Non-limiting examples of ERK inhibitors of the present invention include AEZS-131 (Aeterna Zentaris), AEZS-136 (Aeterna Zentaris), SCH-722984 (Merck & Co.), SCH-772984 (Merck & Co.), SCH-900353 (MK-8353) (Merck & Co.), pharmaceutically acceptable salts thereof, and combinations thereof.

[0082] As used herein, a “Janus kinase inhibitor” is a substance that (i) directly interacts with a Janus kinase, *e.g.*, by binding to a Janus kinase and (ii) decreases the expression or the activity of a Janus kinase. Janus kinases of the present invention include Tyk2, Jak1, Jak2, and Jak3. Non-limiting examples of Janus kinase inhibitors of the present invention include ruxolitinib (Incyte Corporation, Wilmington, DE), baricitinib (Incyte Corporation, Wilmington, DE), tofacitinib (Pfizer, New York, NY), VX-509 (Vertex Pharmaceuticals, Inc., Boston, MA), GLPG0634 (Galapagos NV, Belgium), CEP-33779 (Teva Pharmaceuticals, Israel), pharmaceutically acceptable salts thereof, and combinations thereof

[0083] As used herein, a “MEK inhibitor” is a substance that (i) directly interacts with MEK, *e.g.*, by binding to MEK and (ii) decreases the expression or the activity of MEK. Therefore, inhibitors that act upstream of MEK, such as RAS inhibitors and RAF inhibitors, are not MEK inhibitors according to the present invention. MEK inhibitors may be classified into two types depending on whether the inhibitor competes with ATP. As used herein, a “Type 1” MEK inhibitor is an inhibitor that competes with ATP for binding to MEK. A “Type 2” MEK inhibitor is an inhibitor that does not compete with ATP for binding to MEK. Non-limiting examples of type 1 MEK inhibitors according to the

present invention include bentamapimod (Merck KGaA), L783277 (Merck), RO092210 (Roche), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the type 1 MEK inhibitor is RO092210 (Roche) or a pharmaceutically acceptable salt thereof. Non-limiting examples of type 2 MEK inhibitors according to the present invention include anthrax toxin, lethal factor portion of anthrax toxin, ARRY-142886 (6-(4-bromo-2-chloro-phenylamino)-7-fluoro-3-methyl-3H-benzimidazole-5-carboxylic acid (2-hydroxy-ethoxy)-amide) (Array BioPharma), ARRY-438162 (Array BioPharma), AS-1940477 (Astellas), MEK162 (Array BioPharma), PD 098059 (2-(2'-amino-3'-methoxyphenyl)-oxanaphthalen-4-one), PD 184352 (CI-1040), PD-0325901 (Pfizer), pimasertib (Santhera Pharmaceuticals), refametinib (AstraZeneca), selumetinib (AZD6244) (AstraZeneca), TAK-733 (Takeda), trametinib (Japan Tobacco), U0126 (1,4-diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene) (Sigma), RDEA119 (Ardea Biosciences/Bayer), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the type 2 MEK inhibitor is trametinib or a pharmaceutically acceptable salt thereof. Other MEK inhibitors include, without limitation, antroquinonol (Golden Biotechnology), AS-1940477 (Astellas), AS-703988 (Merck KGaA), BI-847325 (Boehringer Ingelheim), E-6201 (Eisai), GDC-0623 (Hoffmann-La Roche), GDC-0973, RG422, RO4987655, RO5126766, SL327, WX-554 (Wilex), YopJ polypeptide, pharmaceutically acceptable salts thereof, and combinations thereof.

[0084] As used herein, an “mTOR inhibitor” is a substance that (i) directly interacts with mTOR, *e.g.* by binding to mTOR and (ii) decreases the expression or the activity of mTOR. Non-limiting examples of mTOR

inhibitors according to the present invention include zotarolimus (AbbVie), umirolimus (Biosensors), temsirolimus (Pfizer), sirolimus (Pfizer), sirolimus NanoCrystal (Elan Pharmaceutical Technologies), sirolimus TransDerm (TransDerm), sirolimus-PNP (Samyang), everolimus (Novartis), biolimus A9 (Biosensors), ridaforolimus (Ariad), rapamycin, TCD-10023 (Terumo), DE-109 (MacuSight), MS-R001 (MacuSight), MS-R002 (MacuSight), MS-R003 (MacuSight), Perceiva (MacuSight), XL-765 (Exelixis), quinacrine (Cleveland BioLabs), PKI-587 (Pfizer), PF-04691502 (Pfizer), GDC-0980 (Genentech and Piramed), dactolisib (Novartis), CC-223 (Celgene), PWT-33597 (Pathway Therapeutics), P-7170 (Piramal Life Sciences), LY-3023414 (Eli Lilly), INK-128 (Takeda), GDC-0084 (Genentech), DS-7423 (Daiichi Sankyo), DS-3078 (Daiichi Sankyo), CC-115 (Celgene), CBLC-137 (Cleveland BioLabs), AZD-2014 (AstraZeneca), X-480 (Xcovery), X-414 (Xcovery), EC-0371 (Endocyte), VS-5584 (Verastem), PQR-401 (Piquor), PQR-316 (Piquor), PQR-311 (Piquor), PQR-309 (Piquor), PF-06465603 (Pfizer), NV-128 (Novogen), nPT-MTOR (Biotica Technology), BC-210 (Biotica Technology), WAY-600 (Biotica Technology), WYE-354 (Biotica Technology), WYE-687 (Biotica Technology), LOR-220 (Lorus Therapeutics), HMPL-518 (Hutchison China MediTech), GNE-317 (Genentech), EC-0565 (Endocyte), CC-214 (Celgene), and ABTL-0812 (Ability Pharmaceuticals).

[0085] As used herein, a “PI3K inhibitor” is a substance that decreases the expression or the activity of phosphatidylinositol-3 kinases (PI3Ks) or downstream proteins, such as Akt. PI3Ks, when activated, phosphorylate the inositol ring 3'-OH group in inositol phospholipids to generate the second messenger phosphatidylinositol-3,4,5-trisphosphate (PI-3,4,5-P(3)). Akt

interacts with a phospholipid, causing it to translocate to the inner membrane, where it is phosphorylated and activated. Activated Akt modulates the function of numerous substrates involved in the regulation of cell survival, cell cycle progression and cellular growth.

[0086] Non-limiting examples of PI3K inhibitors according to the present invention include A-674563 (CAS # 552325-73-2), AGL 2263, AMG-319 (Amgen, Thousand Oaks, CA), AS-041164 (5-benzo[1,3]dioxol-5-ylmethylene-thiazolidine-2,4-dione), AS-604850 (5-(2,2-Difluorobenzo[1,3]dioxol-5-ylmethylene)-thiazolidine-2,4-dione), AS-605240 (5-quinoxilin-6-methylene-1,3-thiazolidine-2,4-dione), AT7867 (CAS # 857531-00-1), benzimidazole series, Genentech (Roche Holdings Inc., South San Francisco, CA), BML-257 (CAS # 32387-96-5), CAL-120 (Gilead Sciences, Foster City, CA), CAL-129 (Gilead Sciences), CAL-130 (Gilead Sciences), CAL-253 (Gilead Sciences), CAL-263 (Gilead Sciences), CAS # 612847-09-3, CAS # 681281-88-9, CAS # 75747-14-7, CAS # 925681-41-0, CAS # 98510-80-6, CCT128930 (CAS # 885499-61-6), CH5132799 (CAS # 1007207-67-1), CHR-4432 (Chroma Therapeutics, Ltd., Abingdon, UK), FPA 124 (CAS # 902779-59-3), GS-1101 (CAL-101) (Gilead Sciences), GSK 690693 (CAS # 937174-76-0), H-89 (CAS # 127243-85-0), Honokiol, IC87114 (Gilead Science), IPI-145 (Intellikine Inc.), KAR-4139 (Karus Therapeutics, Chilworth, UK), KAR-4141 (Karus Therapeutics), KIN-1 (Karus Therapeutics), KT 5720 (CAS # 108068-98-0), Miltefosine, MK-2206 dihydrochloride (CAS # 1032350-13-2), ML-9 (CAS # 105637-50-1), Naltrindole Hydrochloride, OXY-111A (NormOxys Inc., Brighton, MA), perifosine, PHT-427 (CAS # 1191951-57-1), PI3 kinase delta inhibitor, Merck KGaA (Merck & Co., Whitehouse Station,

NJ), PI3 kinase delta inhibitors, Genentech (Roche Holdings Inc.), PI3 kinase delta inhibitors, Incozen (Incozen Therapeutics, Pvt. Ltd., Hyderabad, India), PI3 kinase delta inhibitors-2, Incozen (Incozen Therapeutics), PI3 kinase inhibitor, Roche-4 (Roche Holdings Inc.), PI3 kinase inhibitors, Roche (Roche Holdings Inc.), PI3 kinase inhibitors, Roche-5 (Roche Holdings Inc.), PI3-alpha/delta inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd., South San Francisco, CA), PI3-delta inhibitors, Cellzome (Cellzome AG, Heidelberg, Germany), PI3-delta inhibitors, Intellikine (Intellikine Inc., La Jolla, CA), PI3-delta inhibitors, Pathway Therapeutics-1 (Pathway Therapeutics Ltd.), PI3-delta inhibitors, Pathway Therapeutics-2 (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Cellzome (Cellzome AG), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Intellikine (Intellikine Inc.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-delta/gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3-gamma inhibitor Evotec (Evotec), PI3-gamma inhibitor, Cellzome (Cellzome AG), PI3-gamma inhibitors, Pathway Therapeutics (Pathway Therapeutics Ltd.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), PI3K delta/gamma inhibitors, Intellikine-1 (Intellikine Inc.), pictilisib (GDC-0941) (Roche Holdings Inc.), PIK-90 (CAS # 677338-12-4), SC-103980 (Pfizer, New York, NY), SF-1126 (Semafore Pharmaceuticals, Indianapolis, IN), SH-5, SH-6, Tetrahydro Curcumin, TG100-115 (Targegen Inc., San Diego, CA), Triciribine, X-339 (Xcovery, West Palm Beach, FL), XL-499 (Evotec, Hamburg, Germany), pharmaceutically acceptable salts thereof,

and combinations thereof. Preferably, the inhibitor of the PI3K/Akt pathway is pictilisib (GDC-0941) or a pharmaceutically acceptable salt thereof.

[0087] As used herein, a “RAS inhibitor” is a substance that (i) directly interacts with RAS, *e.g.*, by binding to RAS and (ii) decreases the expression or the activity of RAS. Non-limiting examples of RAS inhibitors according to the present invention include farnesyl transferase inhibitors (such as, *e.g.*, tipifarnib and lonafarnib), farnesyl group-containing small molecules (such as, *e.g.*, salirasib and TLN-4601), DCAI, as described by Maurer (Maurer, *et al.*, 2012), Kobe0065 and Kobe2602, as described by Shima (Shima, *et al.*, 2013), and HBS 3 (Patgiri, *et al.*, 2011), and AIK-4 (Allinky), pharmaceutically acceptable salts thereof, and combinations thereof.

[0088] As used herein, “gene expression” is a process by which the information from DNA is used in the formation of a polypeptide. A “modulator of gene expression and other cellular functions” is a substance that affects gene expression and other works of a cell. Non-limiting examples of such modulators include hormones, histone deacetylase inhibitors (HDACi), and cyclin-dependent kinase inhibitors (CDKi), and poly ADP ribose polymerase (PARP) inhibitors.

[0089] In the present invention, a “hormone” is a substance released by cells in one part of a body that affects cells in another part of the body. Non-limiting examples of hormones according to the present invention include prostaglandins, leukotrienes, prostacyclin, thromboxane, amylin, antimullerian hormone, adiponectin, adrenocorticotrophic hormone, angiotensinogen, angiotensin, vasopressin, atriopeptin, brain natriuretic peptide, calcitonin, cholecystokinin, corticotropin-releasing hormone, enkephalin, endothelin,

erythropoietin, follicle-stimulating hormone, galanin, gastrin, ghrelin, glucagon, gonadotropin-releasing hormone, growth hormone-releasing hormone, human chorionic gonadotropin, human placental lactogen, growth hormone, inhibin, insulin, somatomedin, leptin, lipotropin, luteinizing hormone, melanocyte stimulating hormone, motilin, orexin, oxytocin, pancreatic polypeptide, parathyroid hormone, prolactin, prolactin releasing hormone, relaxin, renin, secretin, somatostatin, thrombopoietin, thyroid-stimulating hormone, testosterone, dehydroepiandrosterone, androstenedione, dihydrotestosterone, aldosterone, estradiol, estrone, estriol, cortisol, progesterone, calcitriol, and calcidiol.

[0090] Some compounds interfere with the activity of certain hormones or stop the production of certain hormones. Non-limiting examples of hormone-interfering compounds according to the present invention include tamoxifen (Nolvadex®), anastrozole (Arimidex®), letrozole (Femara®), and fulvestrant (Faslodex®). Such compounds are also within the meaning of hormone in the present invention.

[0091] As used herein, an “HDAC inhibitor” is a substance that (i) directly interacts with HDAC, e.g., by binding to HDAC and (ii) decreases the expression or the activity of HDAC. Non-limiting examples of HDAC inhibitors according to the present invention include 4SC-201 (4SC AG), 4SC-202 (Takeda), abexinostat (Celera), AN-1 (Titan Pharmaceuticals, Inc.), Apicidine (Merck & Co., Inc.), AR-42 (Arno Therapeutics), ARQ-700RP (ArQule), Avugane (TopoTarget AS), azelaic-1-hydroxamate-9-anilide (AAHA), belinostat (TopoTarget), butyrate (Enzo Life Sciences, Inc.), CG-1255 (Errant Gene Therapeutics, LLC), CG-1521 (Errant Gene Therapeutics, LLC), CG-

200745 (CrystalGenomics, Inc.), chidamide (Shenzhen Chipscreen Biosciences), CHR-3996 (Chroma Therapeutics), CRA-024781 (Pharmacyclics), CS-3158 (Shenzhen Chipscreen Biosciences), CU-903 (Curis), DAC-60 (Genextra), entinostat (Bayer), hyaluronic acid butyric acid ester (HA-But), IKH-02 (IkerChem), IKH-35 (IkerChem), ITF-2357 (Italfarmaco), ITF-A (Italfarmaco), JNJ-16241199 (Johnson & Johnson), KA-001 (Karus Therapeutics), KAR-3000 (Karus Therapeutics), KD-5150 (Kalypsys), KD-5170 (Kalypsys), KLYP-278 (Kalypsys), KLYP-298 (Kalypsys), KLYP-319 (Kalypsys), KLYP-722 (Kalypsys), m-carboxycinnamic acid bis-hydroxamide (CBHA), MG-2856 (MethylGene), MG-3290 (MethylGene), MG-4230 (MethylGene), MG-4915 (MethylGene), MG-5026 (MethylGene), MGCD-0103 (MethylGene Inc.), mocetinostat (MethylGene), MS-27-275 (Schering AG), NBM-HD-1 (NatureWise), NVP-LAQ824 (Novartis), OCID-4681-S-01 (Orchid Pharmaceuticals), oxamflatin ((2E)-5-[3-[(phenylsulfonyl)aminol phenyl]-pent-2-en-4-ynohydroxamic acid]), panobinostat (Novartis), PCI-34051 (Pharmacyclics), phenylbutyrate (Enzo Life Sciences, Inc.), pivaloyloxymethyl butyrate (AN-9, Titan Pharmaceuticals, Inc.), pivanex (Titan Pharmaceuticals, Inc.), pracinostat (SBIO), PX-117794 (TopoTarget AS), PXD-118490 (LEO-80140) (TopoTarget AS), pyroxamide (suberoyl-3-aminopyridineamide hydroxamic acid), resminostat (Takeda), RG-2833 (RepliGen), ricolinostat (Acetylon), romidepsin (Astellas), SB-1304 (S*BIO), SB-1354 (S*BIO), SB-623 (Merrion Research I Limited), SB-624 (Merrion Research I Limited), SB-639 (Merrion Research I Limited), SB-939 (S*BIO), Scriptaid (N-Hydroxy-1,3-dioxo-1H-benz[de]isoquinoline-2(3H)-hexan amide), SK-7041 (In2Gen/SK Chemical Co.), SK-7068 (In2Gen/SK Chemical Co.),

suberoylanilide hydroxamic acid (SAHA), sulfonamide hydroxamic acid, tributyrin (Sigma Aldrich), trichostatin A (TSA) (Sigma Aldrich), valporic acid (VPA) (Sigma Aldrich), vorinostat (Zolinza), WF-27082B (Fujisawa Pharmaceutical Company, Ltd.), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the HDAC inhibitor is romidepsin, pharmaceutically acceptable salts thereof, and combinations thereof.

[0092] As used herein, “CDK” is a family of protein kinases that regulate the cell cycle. Known CDKs include cdk1, cdk2, ckd3, ckd4, cdk5, cdk6, cdk7, cdk8, cdk9, cdk10, and cdk11. A “CDK inhibitor” is a substance that (i) directly interacts with CDK, e.g. by binding to CDK and (ii) decreases the expression or the activity of CDK. Non-limiting examples of CDK inhibitors according to the present invention include 2-Hydroxyboheminine, 3-ATA, 5-Iodo-Indirubin-3'-monoxime, 9-Cyanopauellone, Aloisine A, Alsterpauellone 2-Cyanoethyl, alvocidib (Sanofi), AM-5992 (Amgen), Aminopurvalanol A, Arcyriaflavin A, AT-7519 (Astex Pharmaceuticals), AZD 5438 (CAS # 602306-29-6), BMS-265246 (CAS # 582315-72-8), BS-181 (CAS # 1092443-52-1), Butyrolactone I (CAS # 87414-49-1), Cdk/Crk Inhibitor (CAS # 784211-09-2), Cdk1/5 Inhibitor (CAS # 40254-90-8), Cdk2 Inhibitor II (CAS # 222035-13-4), Cdk2 Inhibitor IV, NU6140 (CAS # 444723-13-1), Cdk4 Inhibitor (CAS # 546102-60-7), Cdk4 Inhibitor III (CAS # 265312-55-8), Cdk4/6 Inhibitor IV (CAS # 359886-84-3), Cdk9 Inhibitor II (CAS # 140651-18-9), CGP 74514A, CR8, CYC-065 (Cyclacel), dinaciclib (Ligand), (R)-DRF053 dihydrochloride (CAS # 1056016-06-8), Fascaplysin, Flavopiridol, Hygrolidin, Indirubin, LEE-011 (Astex Pharmaceuticals), LY-2835219 (Eli Lilly), milciclib maleate (Nerviano Medical Sciences), MM-D37K (Maxwell Biotech), N9-

Isopropyl-olomoucine, NSC 625987 (CAS # 141992-47-4), NU2058 (CAS # 161058-83-9), NU6102 (CAS # 444722-95-6), Olomoucine, ON-108600 (Onconova), ON-123300 (Onconova), Oxindole I, P-1446-05 (Piramal), P-276-00 (Piramal), palbociclib (Pfizer), PHA-767491 (CAS # 845714-00-3), PHA-793887 (CAS # 718630-59-2), PHA-848125 (CAS # 802539-81-7), Purvalanol A, Purvalanol B, R547 (CAS # 741713-40-6), RO-3306 (CAS # 872573-93-8), Roscovitine, SB-1317 (SBIO), SCH 900776 (CAS # 891494-63-6), SEL-120 (Selvita), seliciclib (Cyclacel), SNS-032 (CAS # 345627-80-7), SU9516 (CAS # 377090-84-1), WHI-P180 (CAS # 211555-08-7), pharmaceutically acceptable salts thereof, and combinations thereof. Preferably, the CDK inhibitor is selected from the group consisting of dinaciclib, palbociclib, pharmaceutically acceptable salts thereof, and combinations thereof.

[0093] As used herein, a “poly ADP ribose polymerase (PARP) inhibitor” is a substance that decreases the expression or activity of poly ADP ribose polymerases (PARPs) or downstream proteins. Non-limiting examples of poly ADP ribose polymerase (PARP) inhibitors of the present invention include PF01367338 (Pfizer, New York, NY), olaparib (AstraZeneca, United Kingdom), iniparib (Sanofi-Aventis, Paris, France), veliparib (Abbott Laboratories, Abbott Park, IL), MK 4827 (Merck, White House Station, NJ), CEP 9722 (Teva Pharmaceuticals, Israel), LT-673 (Biomarin, San Rafael, CA), and BSI 401 (Sanofi-Aventis, Paris, France), pharmaceutically acceptable salts thereof, and combinations thereof.

[0094] In a preferred embodiment, the chemotherapy comprises administering to the human an agent selected from the group consisting of

gemcitabine, taxol, adriamycin, ifosfamide, trabectedin, pazopanib, abraxane, avastin, everolimus, and combinations thereof.

[0095] As used herein, “radiotherapy” means any therapeutic regimen, that is compatible with the C. novyi, *e.g.*, C. novyi NT, treatment of the present invention and in which radiation is delivered to a subject, *e.g.*, a human, for the treatment of cancer. Radiotherapy can be delivered to, *e.g.*, a human subject, by, for example, a machine outside the body (external-beam radiation therapy) or a radioactive material inside the body (brachytherapy, systemic radiation therapy).

[0096] External-beam radiation therapy includes, but is not limited to, 3-dimensional conformal radiation therapy, intensity-modulated radiation therapy, image-guided radiation therapy, tomotherapy, stereotactic radiosurgery, stereotactic body radiation therapy, proton therapy, and other charged particle beam therapies, such as electron beam therapy. External-beam radiation therapies are widely used in cancer treatment and are well known to those of skill in the art.

[0097] Brachytherapy means radiotherapy delivered by being implanted in, or placed on, a subject’s body. Brachytherapy includes, but is not limited to, interstitial brachytherapy, intracavitary brachytherapy, and episcleral brachytherapy. Brachytherapy techniques are also widely used in cancer treatment and are well known to those of skill in the art.

[0098] Systemic radiation therapy means radiotherapy delivered by injection to or ingestion by a subject. One example of systemic radiation therapy is radioiodine therapy. Radioiodine is a radiolabeled iodine molecule

that is safe and effective for use in a subject, such as, *e.g.*, a human. Non-limiting examples of radioiodine according to the present invention may be selected from the group consisting of ^{123}I , ^{124}I , ^{125}I , ^{131}I , and combinations thereof. Preferably, the radioiodine is ^{131}I .

[0099] As used herein, “immunotherapy” means any anti-cancer therapeutic regimen that is compatible with the C. novyi, *e.g.*, C. novyi NT, treatment of the present invention and that uses a substance that alters the immune response by augmenting or reducing the ability of the immune system to produce antibodies or sensitized cells that recognize and react with the antigen that initiated their production. Immunotherapies may be recombinant, synthetic, or natural preparations and include cytokines, corticosteroids, cytotoxic agents, thymosin, and immunoglobulins. Some immunotherapies are naturally present in the body, and certain of these are available in pharmacologic preparations. Examples of immunotherapies include, but are not limited to, granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod and cellular membrane fractions from bacteria, IL-2, IL-7, IL-12, CCL3, CCL26, CXCL7, and synthetic cytosine phosphate-guanosine (CpG).

[0100] In one preferred embodiment, the immunotherapy comprises administering to the human an immune checkpoint inhibitor. As used herein, an “immune checkpoint inhibitor” means a substance that blocks the activity of molecules involved in attenuating the immune response. Such molecules include, for example, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Immune checkpoint inhibitors of the present invention include, but are not limited to, ipilimumab (Bristol-Myers

Squibb), tremelimumab (Pfizer), MDX-1106 (Medarex, Inc.), MK3475 (Merck), CT-011 (CureTech, Ltd.), AMP-224 (Amplimmune), MDX-1105 (Medarex, Inc.), IMP321 (Immutep S.A.), and MGA271 (Macrogenics).

[0101] In an additional aspect of this embodiment, the C. novyi, *e.g.*, C. novyi NT, therapy of the present invention is effective against, *e.g.*, solid tumors that are resistant to a therapy selected from the group consisting of chemotherapy, radiation therapy, immunotherapy, and combinations thereof.

[0102] In another aspect of this embodiment, the solid tumor is refractory to standard therapy or the solid tumor is without an available standard therapy, yet the C. novyi, *e.g.*, C. novyi NT, therapy of the present invention is effective against such a tumor.

[0103] As used herein, “resistant” and “refractory” are used interchangeably. Being “refractory” to a therapy means that the prior therapy or therapies has/have reduced efficacy in, *e.g.*, treating cancer or killing cancer cells, compared to the same subject prior to becoming resistant to the therapy.

[0104] As used herein, the term “standard therapy” means those therapies generally accepted by medical professionals as appropriate for treatment of a particular cancer, preferably a particular solid tumor. Standard therapies may be the same or different for different tumor types. Standard therapies are typically approved by various regulatory agencies, such as, for example, the U.S. Food and Drug Administration.

[0105] In a further aspect of this embodiment, the method induces a potent localized inflammatory response and an adaptive immune response in the human.

[0106] As used herein, an “inflammatory response” is a local response to cellular damage, pathogens, or irritants that may include, but is not limited to, capillary dilation, leukocytic infiltration, swelling, redness, heat, itching, pain, loss of function, and combinations thereof.

[0107] As used herein, an “adaptive immune response” involves B and T cells of a subject’s immune system. Upon exposure to a pathogenic substance, for example, a cancer cell, B cells may produce antibodies against pathogenic antigens on the pathogenic substance, and T cells may become able to target pathogens for eventual destruction. Certain populations of B and T cells, specific for a given antigen, are retained by the immune system and are called upon in the event of subsequent exposure to the pathogenic antigen. An adaptive immune response is thus durable, and provides a host subject’s immune system with the continual ability to recognize and destroy a given pathogenic antigen-presenting pathogen.

[0108] Another embodiment of the present invention is a method for debulking a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi*, preferably *C. novyi* NT, CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0109] As used herein, “debulking” a solid tumor means to reduce the size of or the number of cancer in a solid tumor. Such a procedure is palliative and may be used to enhance the effectiveness of the treatments, including radiation therapy, chemotherapy, or amputation. In this embodiment, solid tumors are as set forth above. Preferably, the solid tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular

carcinoma, breast cancer, pancreatic cancer, and melanoma. More preferably, the solid tumor is a leiomyosarcoma, such as a retroperitoneal leiomyosarcoma.

[0110] An additional embodiment of the present invention is a method for debulking a solid tumor present in a human. This method comprises administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores per cycle, each unit dose of *C. novyi* NT being suspended in a pharmaceutically acceptable carrier or solution. In this embodiment, the types of solid tumors are as set forth above. Preferably, the solid tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, and melanoma.

[0111] A further embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor present in a human. This method comprises administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores per cycle, each unit dose of *C. novyi* NT spores being suspended in a pharmaceutically acceptable carrier or solution. Various types of solid tumors are as set forth above. Preferably, the solid tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, and melanoma.

[0112] Another embodiment of the present invention is method for ablating a solid tumor present in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi*, preferably *C. novyi* NT, CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a

pharmaceutically acceptable carrier or solution, wherein the tumor is ablated leaving a margin of normal tissue.

[0113] As used herein, “ablating” a solid tumor means that the process removes all of the solid tumor. In this process, after carrying out the treatment, a margin of normal tissue is left surrounding the area where the tumor once resided. In this embodiment, the types of solid tumors are as set forth above. Preferably, the solid tumor is a sarcoma. More preferably, the solid tumor is a leiomyosarcoma, such as a retroperitoneal leiomyosarcoma.

[0114] A further embodiment of the present invention is a unit dose of *C. novyi* CFUs. This unit dose comprises about 1×10^3 - 1×10^7 CFUs in a pharmaceutically acceptable carrier or solution, which is effective for treating or ameliorating an effect of a solid tumor present in a human. As set forth above, the *C. novyi* CFUs may be in vegetative and spore forms.

[0115] In one aspect of this embodiment, the *C. novyi* is *C. novyi* NT. Preferably, the unit dose comprises about 1×10^4 - 1×10^7 *C. novyi* NT spores, such as about 1×10^6 - 1×10^7 *C. novyi* NT spores, in a pharmaceutically acceptable carrier or solution. Preferably, the unit dose comprises about 1×10^4 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

[0116] An additional embodiment of the present invention is a kit for treating or ameliorating an effect of a solid tumor present in a human. This kit comprises a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs in a pharmaceutically acceptable carrier or solution and instructions for use of the kit. The kit may be divided into one or more compartments and may have one or more containers for the various reagents. The kit may be further adapted to support storage and shipment of each component.

[0117] In one aspect of this embodiment, the kit further comprises one or more antibiotics, which are effective to treat or alleviate an adverse side effect caused by the *C. novyi* CFUs. The CFUs may be in vegetative or spore forms. Suitable antibiotics are as set forth above. Preferably, the kit further comprises 1-4 unit doses of the *C. novyi* for carrying out 1-4 treatment cycles.

[0118] In another aspect of this embodiment, the *C. novyi* is *C. novyi* NT. Preferably, the unit dose comprises about 1×10^4 - 1×10^7 *C. novyi* NT spores, such as about 1×10^6 - 1×10^7 *C. novyi* NT spores, or about 1×10^4 *C. novyi* NT spores, in a pharmaceutically acceptable carrier or solution. Also preferably, the kit further comprises 1-4 unit doses of the *C. novyi* NT spores for carrying out 1-4 treatment cycles.

[0119] Another embodiment of the present invention is a method for microscopically precise excision of tumor cells in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

[0120] As used herein, "microscopically precise excision" means elimination of a target tissue in a subject, for example, a pathogenic tissue, said elimination being essentially specific, at the cellular level, for the pathogenic tissue while causing minimal or no harm to nearby "healthy" tissue. Elimination of a target tissue may be, but is not limited to, apoptosis, necrosis, and cell lysis. This embodiment may be accomplished by precision delivery of, *e.g.*, the *C. novyi* NT spores of the invention via CT-guided intratumoral injection using, *e.g.*, a multi-pronged delivery device, such as a multi-pronged needle.

[0121] In the present invention, the *C. novyi* spores, such as the *C. novyi* NT spores, are delivered to the subject, *e.g.*, human patient, intratumorally in any medically appropriate manner. For example, *C. novyi* NT spores may be delivered via a single needle used at one or more sites on a tumor. Alternatively, a multi-tined delivery vehicle, such as a multi-tined needle, may be used to deliver, *e.g.*, *C. novyi* NT spores, to a tumor. Delivery of, *e.g.*, the spores may be to the same or multiple depths at one or more sites of the tumor. The selected delivery vehicles may be operated manually or controlled electronically. The delivery vehicles may be positioned and/or repositioned on or within a tumor manually or via a remote controlled device and visualization of the injection site may be augmented using various imaging techniques known in the art, such as CT imaging. Multi-tined delivery vehicles that may be used in the present invention include those disclosed in, *e.g.*, McGuckin, Jr. et al., U.S. Patent Nos. 6,905,480 and 7,331,947, which are incorporated herein by reference.

[0122] A further embodiment of the present invention is a method for treating or ameliorating an effect of a solid tumor that has metastasized to one or more sites in a human. This method comprises administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising at least about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution. Preferably, at least one site of metastasis is distal to the original solid tumor.

[0123] As used herein, "metastasis" and grammatical variations thereof mean the spread of pathogenic cells, *i.e.* tumor cells, from an original, primary region of the body, to a secondary region of the body. Metastasis may be

regional or distal, depending on the distance from the original primary tumor site. Whether a metastasis is regional or distal may be determined by a physician. For example, a breast cancer that has spread to the brain is distal, whereas the spread of breast cancer cells to under arm lymph nodes is regional.

[0124] In the present invention, an "effective amount" or a "therapeutically effective amount" of a compound or composition disclosed herein is an amount of such compound or composition that is sufficient to effect beneficial or desired results as described herein when administered to a subject. Effective dosage forms, modes of administration, and dosage amounts are as disclosed herein or as modified by a medical professional. It is understood by those skilled in the art that the dosage amount will vary with the route of administration, the rate of excretion, the duration of the treatment, the identity of any other drugs being administered, the age and size of the patient, and like factors well known in the arts of medicine. In general, a suitable dose of a composition according to the invention will be that amount of the composition, which is the lowest dose effective to produce the desired effect. The effective dose of a composition of the present invention is described above. Further, a composition of the present invention may be administered in conjunction with other treatments.

[0125] The compositions of the invention comprise one or more active ingredients in admixture with one or more pharmaceutically-acceptable carriers and, optionally, one or more other compounds, drugs, ingredients and/or materials. Regardless of the route of administration selected, the agents/compounds of the present invention are formulated into

pharmaceutically-acceptable unit dosage forms by conventional methods known to those of skill in the art. See, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.).

[0126] Pharmaceutically acceptable carriers or solutions are well known in the art (see, *e.g.*, Remington, The Science and Practice of Pharmacy (21st Edition, Lippincott Williams and Wilkins, Philadelphia, PA.) and The National Formulary (American Pharmaceutical Association, Washington, D.C.)) and include sugars (*e.g.*, lactose, sucrose, mannitol, and sorbitol), starches, cellulose preparations, calcium phosphates (*e.g.*, dicalcium phosphate, tricalcium phosphate and calcium hydrogen phosphate), sodium citrate, water, aqueous solutions (*e.g.*, saline, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, lactated Ringer's injection), alcohols (*e.g.*, ethyl alcohol, propyl alcohol, and benzyl alcohol), polyols (*e.g.*, glycerol, propylene glycol, and polyethylene glycol), organic esters (*e.g.*, ethyl oleate and tryglycerides), biodegradable polymers (*e.g.*, polylactide-polyglycolide, poly(orthoesters), and poly(anhydrides)), elastomeric matrices, liposomes, microspheres, oils (*e.g.*, corn, germ, olive, castor, sesame, cottonseed, and groundnut), cocoa butter, waxes (*e.g.*, suppository waxes), paraffins, silicones, talc, silicylate, etc. Each pharmaceutically acceptable carrier or solution used in a unit dose according to the present invention must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Carriers or solutions suitable for a selected dosage form and intended route of administration, *e.g.*, IT, are well known in the art, and

acceptable carriers or solutions for a chosen dosage form and method of administration can be determined using ordinary skill in the art.

[0127] The unit doses of the invention may, optionally, contain additional ingredients and/or materials commonly used in pharmaceutical compositions. These ingredients and materials are well known in the art and include (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (2) binders, such as carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, hydroxypropylmethyl cellulose, sucrose and acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium starch glycolate, cross-linked sodium carboxymethyl cellulose and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, and sodium lauryl sulfate; (10) suspending agents, such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth; (11) buffering agents; (12) excipients, such as lactose, milk sugars, polyethylene glycols, animal and vegetable fats, oils, waxes, paraffins, cocoa butter, starches, tragacanth, cellulose derivatives, polyethylene glycol, silicones, bentonites, silicic acid, talc, salicylate, zinc oxide, aluminum hydroxide, calcium silicates, and polyamide powder; (13) inert diluents, such as water or other solvents; (14) preservatives; (15)

surface-active agents; (16) dispersing agents; (17) control-release or absorption-delaying agents, such as hydroxypropylmethyl cellulose, other polymer matrices, biodegradable polymers, liposomes, microspheres, aluminum monostearate, gelatin, and waxes; (18) opacifying agents; (19) adjuvants; (20) wetting agents; (21) emulsifying and suspending agents; (22), solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan; (23) propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane; (24) antioxidants; (25) agents which render the formulation isotonic with the blood of the intended recipient, such as sugars and sodium chloride; (26) thickening agents; (27) coating materials, such as lecithin; and (28) sweetening, flavoring, coloring, perfuming and preservative agents. Each such ingredient or material must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Ingredients and materials suitable for a selected dosage form and intended route of administration are well known in the art, and acceptable ingredients and materials for a chosen dosage form and method of administration may be determined using ordinary skill in the art.

[0128] Liquid dosage forms include pharmaceutically-acceptable emulsions, microemulsions, liquids, and suspensions. The liquid dosage forms may contain suitable inert diluents commonly used in the art. Besides inert diluents, the oral compositions may also include adjuvants, such as

wetting agents, emulsifying and suspending agents, coloring, and preservative agents. Suspensions may contain suspending agents.

[0129] Dosage forms for the intratumoral administration include solutions, dispersions, suspensions or emulsions, or sterile powders. The active agent(s)/compound(s) may be mixed under sterile conditions with a suitable pharmaceutically-acceptable carrier.

[0130] Unit doses of the present invention may alternatively comprise one or more active agents, e.g., *C. novyi* CFUs or *C. novyi* NT spores in combination with sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain suitable antioxidants, buffers, solutes which render the formulation isotonic with the blood of the intended recipient, or suspending or thickening agents. Proper fluidity can be maintained, for example, by the use of coating materials, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These compositions may also contain suitable adjuvants, such as wetting agents, emulsifying agents and dispersing agents. It may also be desirable to include isotonic agents. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption.

[0131] Intratumorally injectable depot forms may be made by forming microencapsulated matrices of the active ingredient in biodegradable polymers. Depending on the ratio of the active ingredient to polymer, and the nature of the particular polymer employed, the rate of active ingredient release can be controlled. Depot injectable formulations are also prepared by

entrapping the active agent in liposomes or microemulsions which are compatible with body tissue.

[0132] As noted above, the formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a lyophilized condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the type described above.

[0133] The following examples are provided to further illustrate the methods of the present invention. These examples are illustrative only and are not intended to limit the scope of the invention in any way.

EXAMPLES

Example 1

Combined intravenous (IV) dosing of C. novyi NT with radiation

[0134] A study of a single IV dose of C. novyi NT spores in dogs with spontaneous tumors following treatment with external beam radiation was performed.

[0135] The manufacturing and final formulation of C. novyi NT spores was performed by the Johns Hopkins Development laboratory according to the following process. C. novyi NT spores generated according to Dang et al., 2001. were inoculated into a rich sporulation medium and incubated in an anaerobic chamber for 17-19 days at 37°C. Spores were purified by sequential continuous Percoll gradient centrifugation followed by extensive phosphate buffered saline washing. Spores were stored at 2-8°C. Spores

were prepared prior to shipment, suspended in sterile phosphate buffered saline and diluted in 50 ml of 0.9% sodium chloride.

[0136] C. novyi NT spores were reconstituted in a 50 ml saline bag and delivered overnight to the test site. The radiation dose was approximately 54 Gy delivered over 20 fractions: 11 before C. novyi NT IV injection and 9 after injection. C. novyi NT spores were administered as a single injection at a dose of 1×10^9 spores/m², based on body surface area. The transfer of the spores to a syringe occurred on an absorbent pad with an impervious backing. A 22 gauge needle with a 3-way stopcock attached was inserted into the bag. A male portion of a closed chemotherapy system (ONGUARD™, TEVA Medical Ltd.) was attached to a port on the stopcock. The complete contents were withdrawn from the bag into a 60 cubic centimeter (cc) syringe to which was attached a female portion of the closed system. The spores were injected into each subject over 15 minutes through an IV catheter to which was attached the male end of the chemotherapy closed system. The infusion was followed by a 10 cc saline flush. The subject was monitored closely for 6 hours post-infusion as follows: vital signs, blood pressure, and oxygen saturation monitoring every 15 minutes for the first 60 minutes, followed by monitoring every 30 minutes for the next 60 minutes, then every 60 minutes for the next 120 minutes. Subsequent checks were performed every 60 minutes for a total of 6 hours.

[0137] Test subjects were hospitalized for the initial 3 weeks of treatment: 2 weeks for radiation treatments and 1 week following C. novyi NT IV treatment. Subsequent follow-up visits occurred up to 6 months post-

treatment at month 1, 2, 3, and 6. See Tables 1 and 2 for sample treatment schedules.

Table 1
Schedule of spore events

	Screen (Prior to starting radiation therapy)	Day 1 In-Patient Monitoring for 6 Hours Post Infusion	Day 2	Day 3	Day 4	Day 5	Day 8 ± 2 days	Day 15 ± 2 days	Month 1 ± 3 days	Month 2 ± 3 days	Month 3 ± 14 days	Month 6 ± 14 days
Informed Consent	X											
Medical History	X											
Physical Exam	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X
Chest x-Ray	X						X ¹	X ¹	X ¹	X ¹	X ¹	X ¹
Tumor fine needle aspiration (FNA) for culture			X	X	X	X	X					
Abdominal Ultrasound	X						X ¹	X ¹	X ¹	X ¹	X ¹	X ¹
Extremity x-Ray (if indicated)	X						X ¹	X ¹	X ¹	X ¹	X ¹	X ¹
Complete blood count (CBC), Prothrombin time/Partial thromboplastin time (PT/PTT), Chem, Urinalysis	X	X		X		X	X	X	X	X	X	X
Research bloodwork ²	X	X	X	X	X	X	X	X	X	X	X	X
Tumor measurements and photographs		X		X		X	X	X	X	X	X	X
Infuse C. novyi NT spores		X										
Response							X	X	X	X	X	X
Adverse Events (AEs)		X		X		X	X	X	X	X	X	X
Con Meds	X	X		X		X	X	X	X	X	X	X

¹Chest x-ray and additional imaging as clinically indicated

² Research lab work includes plasma, serum, whole blood pellet, and peripheral blood mononuclear cell collection (cells from plasma collection)

Table 2

Calendar of treatments for combined radiation and C. novyi NT (Days)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Radiation Day 1	Rad Day 2	Rad Day 3	Rad Day 4	Rad Day 5		
Rad Day 6	Rad Day 7	Rad Day 8	Rad Day 9	Rad Day 10		
Spore day 1 X Infusion X	Rad Day 11 Spore Day 2 AY	Rad Day 12 Spore Day 3 X	Rad Day 13 Spore Day 4 Y	Rad Day 14 Spore Day 5 X	Spore Day 6	Spore Day 7
Rad Day 15 Spore Day 8 X, Y	Rad Day 16 Spore Day 9	Rad Day 18 B Spore Day 10	Rad Day 19 B Spore Day 11	Rad Day 20 B Spore Day 12	Spore Day 13	Spore Day 14
Spore Day 15 X,Y		Spore Day 30 X,Y		Via CT tumor Re-evaluation 60 days post rad		Spore Day 90 X,Y
Spore Day 180 X,Z						
A Radiation may be interrupted more than one day but will be radiation Day 11 when re-started		B Radiation will be completed one of these days.		X = CBC, Chem Profile, AST, PT/PTT, Research Blood Samples, Adverse Events (AEs), Concomitant Medications, Tumor Measurements, Photos		
Y = Research blood samples				Z =Thoracic metastasis Check and additional Imaging as Indicated Including Abdominal Ultrasound		

[0138] As of September 10, 2012, five dogs were treated in this manner. Of the five, 2 developed an abscess, 1 maintained stable disease, and 2 died or were euthanized. The two test subjects that developed an abscess were photographed throughout treatment as shown in Figures 1A and 1B.

[0139] Figure 1A depicts a canine osteosarcoma located on the right distal radius/ulna over the course of treatment. The test subject, Sasha, exhibited fever and swelling on day 3 and a burst abscess on day 6. Antibiotics were started on day 8 due to the open wound and later, necrotic bone and tissue were removed. Sasha completed 12 of the 19 radiation treatments and, as of September 10, 2012, was healing with stable disease.

[0140] Figure 1B also depicts a canine osteosarcoma located on the right distal radius/ulna over the course of treatment. The test subject, Sampson, exhibited fever and swelling on day 5. On day 6, the abscess was lanced and antibiotics were started. Sampson completed 14 of the 20 radiation treatments and, as of September 10, 2012, was healing with stable disease.

[0141] The other subjects, Chipper, Bailey, and Ruskin, exhibited varying results. Chipper presented with a squamous cell carcinoma of the left mandible. Over the course of treatment, Chipper had swelling at the tumor site and received 20 of 20 radiation treatments. As of September 10, 2012, Chipper had stable disease.

[0142] Another subject, Bailey, presented with a soft tissue sarcoma of the left axillary region. During treatment, Bailey died, having experienced sepsis, acute renal failure, potential disseminated intravascular coagulation, and cardiac arrest. However, necropsy showed all dead tissue inside the tumor, with no tumor cells.

[0143] The remaining subject, Ruskin, presented with an osteosarcoma of the right proximal humerus. During treatment, Ruskin had swelling of the tumor site and

completed 20/20 radiation treatments. However, on day 30, the tumor site was producing large amounts of purulent material and Ruskin was experiencing renal failure. The owner decided to euthanize when renal status did not improve. As of September 10, 2012, necropsy results were still pending.

Example 2

IT-injected *C. novyi*-NT spores specifically target tumor tissue and prolong survival in rats – Methods

Cell lines and tissue culture

[0144] A rat F98 glioma cell line transfected with a luciferase construct via lentivirus was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin.

Rat experiments

[0145] 6 week old female F344 Fisher rats (weight 100-150 grams) were purchased from the National Cancer Institute. For the implantation procedure, female F344 Fisher rats were anesthetized via intraperitoneal (IP) injection of ketamine hydrochloride (75 mg/kg; 100 mg/mL ketamine HCl; Abbot Laboratories), xylazine (7.5 mg/kg; 100 mg/mL Xyla-ject; Phoenix Pharmaceutical, Burlingame, CA), and ethanol (14.25 %) in a sterile NaCl (0.9 %) solution. F98 glioma cells (2×10^4) were stereotactically implanted through a burr hole into the right frontal lobe located 3 mm lateral and 2 mm anterior to the bregma, as described before (Bai, et al., 2011). Tumor size was assessed via a Xenogen instrument with IP injection of 8 mg/rat D-luciferin potassium salt at day 12 after implantation of the tumor cells. Subsequently, 3 million *C. novyi*-NT spores, produced as previously described

(Dang, et al., 2001, Bettgowda, et al., 2006), were stereotactically injected into the intracranial tumor using the same coordinates as described above and the rats were treated with 10 mg/kg/day of IP dexamethasone for the first 2 days. Animals were observed daily for any signs of deterioration, lethargy, neurotoxicity, or pain in accordance with the Johns Hopkins Animal Care and Use Guidelines. If symptoms of distress were present, supportive therapy with hydration and doxycycline (loading dose of 15 mg/kg IP followed by 10 mg/kg every 12 hours as maintenance) was initiated and continued for a 7 day period. If symptoms persisted and/or resulted in debilitation, moribund animals were euthanized. The effectiveness of IT injected *C. novyi*-NT spores was evaluated by Kaplan-Meier survival curves, as well as remaining tumor burden on brain sections. For the latter, brains were collected postmortem, placed in formaldehyde, and embedded in paraffin for additional pathological studies. Gram-stained slides, counter-stained with safranin, and H&E-slides were obtained according to standard procedure guidelines.

Statistical analyses

[0146] Kaplan-Meier survival curves were created and analyzed with a Mantel-Cox test using GraphPad Prism v.5.00 (GraphPad Software, San Diego, CA).

Example 3

IT-injected *C. novyi*-NT spores specifically target tumor tissue and prolong survival in rats – Results

[0147] Complete surgical excision of advanced gliomas is nearly always impossible and these tumors inexorably recur. Though this tumor type generally does not metastasize, there are no highly effective medical therapies available to treat it. Gliomas therefore seemed to represent a tumor type for which local injection of *C. novyi*-NT spores could be therapeutically useful. To evaluate this possibility,

F98 rat glioma cells were orthotopically implanted into 6-week old F433 Fisher rats, resulting in locally invasive tumors that were rapidly fatal (Figure 2A). IT injection of *C. novyi*-NT spores into the tumors of these rats resulted in their germination within 24 hours and a rapid fall in luciferase activity, an indicator of tumor burden, over 24 – 48 hours (Figures 2B and 2C). *C. novyi*-NT germination was evidenced by the appearance of vegetative forms of the bacteria. Strikingly, *C. novyi*-NT precisely localized to the tumor, sparing adjacent normal cells only a few microns away (Figures 3A and 3B). Moreover, these vegetative bacteria could be seen to specifically grow within and concomitantly destroy islands of micro-invasive tumor cells buried within the normal brain parenchyma (Figures 4A and 4B). This bacterial biosurgery led to a significant survival advantage in this extremely aggressive murine model (Figure 2A, P-value < 0.0001).

Example 4

Canine soft tissue sarcomas resemble human tumors – Methods

Genomic DNA isolation for sequencing

[0148] Genomic DNA from dogs participating in the comparative study of IT *C. novyi*-NT spores was extracted from peripheral blood lymphocytes (PBLs) and formalin-fixed, paraffin-embedded tumor tissue using the QIAamp DNA mini kit (QIAGEN, Valencia, CA) according to the manufacturer's protocol.

Sequencing and bioinformatic analysis

[0149] Genomic purification, library construction, exome capture, next generation sequencing, and bioinformatics analyses of tumor and normal samples were performed at Personal Genome Diagnostics (PGDx, Baltimore, MD). In brief, genomic DNA from tumor and normal samples were fragmented and used for Illumina TruSeq library construction (Illumina, San Diego, CA). The exonic regions

were captured in solution using the Agilent Canine All Exon kit according to the manufacturer's instructions (Agilent, Santa Clara, CA). Paired-end sequencing, resulting in 100 bases from each end of the fragments, was performed using a HiSeq 2000 Genome Analyzer (Illumina, San Diego, CA). The tags were aligned to the canine reference sequence (CanFam2.0) using the Eland algorithm of CASAVA 1.7 software (Illumina, San Diego, CA). The chastity filter of the BaseCall software of Illumina was used to select sequence reads for subsequent analysis. The ELAND algorithm of CASAVA 1.7 software (Illumina, San Diego, CA) was then applied to identify point mutations and small insertions and deletions. Known polymorphisms recorded in dbSNP131 (CanFam2.0) were removed from the analysis. Potential somatic mutations were filtered and visually inspected as described previously (Jones, et al., 2010).

Example 5

Canine soft tissue sarcomas resemble human tumors – Results

[0150] Preclinical animal studies of anticancer agents often do not recapitulate the observed effects in people. In dogs, however, clinically used therapeutic agents induce similar toxicities and effects to people (Paoloni, et al., 2008). Studies of investigational therapies in dogs can represent a crucial bridge between preclinical animal studies and human clinical studies. In particular, canine soft tissue sarcomas are an excellent model as they are common in many breeds of dogs and have clinical and histopathologic features remarkably close to those of human soft tissue sarcomas (Paoloni, et al., 2008, Vail, et al., 2000). However, while recent advances in genomics have significantly expanded our knowledge of cancer genetics in people, comparatively little is known about the genetic landscape of canine cancers. Therefore, to determine whether canine tumors were genetically similar to those of

humans, the exome of tumor and matched normal DNA from 11 dogs participating in the comparative study was sequenced (Figure 5). This analysis involved the interrogation of 30,194 nominal genes comprising 32.9 megabases (Mb) of DNA. Ten of the dogs had soft tissue sarcomas (six peripheral nerve sheath tumors) and one had a chondroblastic osteosarcoma. On average, 15.7 gigabases (Gb) (range: 8.1 – 23.3 Gb) of generated sequence were mapped to the genome, and 92.1 % of bases in the targeted regions were covered by at least 10 unique reads in the tumor DNA. Similarly, an average of 16.3 Gb (range: 14.6 – 19.7 Gb) of sequence were mapped to the genome in normal DNA, with 93.6% of targeted bases covered by at least ten unique reads. Average coverage for each targeted base in the tumor was 153-fold (range: 73 – 227-fold) and was 152-fold in the matched normal samples (range: 130 – 178-fold).

[0151] Using stringent analysis criteria, 156 somatic mutations and 28 somatic copy number alterations among the 10 soft tissue sarcomas were identified (Table 3 and Figure 6). The range of somatic mutations was 0 to 95 with a mean of 14 per tumor. Mutation prevalence in the soft tissue sarcomas was low, averaging 0.47 per Mb (range: 0.00 – 2.89 per Mb). Excluding one sample outlier, with 95 somatic alterations, there was a mean prevalence of 0.21 mutations per Mb (range: 0.00 – 0.61 per Mb) (Figure 5), similar to estimates of the mutation rate in human pediatric rhabdoid tumors (Lee, et al., 2012) and other soft tissue sarcomas (Joseph, et al., 2013). The most common type of somatic alteration was a missense mutation, with a preponderance of C to T (45.5%) and G to A transitions (34.0%; Tables 4a and 4b).

Table 3 Somatic Alterations in Canine Sarcomas

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
04-R03	STS	CCDC61	coiled-coil domain containing 61	ENSCAFT0000006986	chr1_112524782-112524782_C_T	NA	Substitution	Splice site donor	CCCTANC TGGG	0.41
		FAM83B	family with sequence similarity 83, member B	ENSCAFT0000003643	chr12_25277449-25277449_G_T	68V>F	Substitution	Nonsynonymous coding	AAAACNT CCAG	0.39
		Novel Gene	uncharacterized protein	ENSCAFT0000006899	chr23_3005035-3005035_T_A	32N>I	Substitution	Nonsynonymous coding	GGTCANT ATTA	0.34
		Novel Gene	uncharacterized protein	ENSCAFT00000028936	chr20_55267898-55267898_C_T	323R>X	Substitution	Nonsense	AGGAGNG ACGC	0.17
		NUP210	nucleoporin 210kDa	ENSCAFT00000007053	chr20_6644043-6644043_G_T	1627P>T	Substitution	Nonsynonymous coding	GCCCGN GATGG	0.38
		PLMN	Plasminogen Plasmin heavy chain A Plasmin light chain B	ENSCAFT00000001179	chr1_52549843-52549843_C_T	598G>E	Substitution	Nonsynonymous coding	CGCACNC ACCT	0.28
		UFSP2	UFM1-specific peptidase 2	ENSCAFT00000012105	chr16_48180970-48180970_T_G	271L>R	Substitution	Nonsynonymous coding	TTACCNC AATC	0.61
		ZNFX1	zinc finger, NFX1-type containing 1	ENSCAFT00000018115	chr24_38909185-38909185_T_G	1195I>L	Substitution	Nonsynonymous coding	AACAANG TCAT	0.34
16-R03	STS	ANKRD11	ankyrin repeat domain 11	ENSCAFT00000031567	chr5_67220009-67220009_G_A	NA	Substitution	Splice site donor	CCGTGNT GAGT	0.19
		TMEM132B	transmembrane protein 132B	ENSCAFT00000011029	chr26_7467030-7467030_C_T	198G>D	Substitution	Nonsynonymous coding	ACAAGNC GGCC	0.18
16-R02	STS	CAPN6	calpain 6	ENSCAFT00000028872	chrX_87423838-87423838_C_T	433R>H	Substitution	Nonsynonymous coding	ATCTGCG GTTC	0.45

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		CNGB3	cyclic nucleotide-gated cation channel beta-3	ENSCAFT00000014134	chr29_35801978-35801978_G_A	451R>X	Substitution	Nonsense	GATTCGG AAGT	0.22
		Novel gene	uncharacterized protein	ENSCAFT00000035928	chr4_69847894-69847894_C_G	352Y>X	Substitution	Nonsense	ACCTACT TTGA	0.11
		PLAC8L1	PLAC8-like 1	ENSCAFT00000010364	chr2_43368179-43368179_C_T	99C>Y	Substitution	Nonsynonymous coding	TGTCACA CTCA	0.2
11-R04	STS	AIDA	axin interactor, dorsalization associated	ENSCAFT00000021486	chr38_19939874-19939874_A_G	258F>S	Substitution	Nonsynonymous coding	AAGCANA GCAC	0.25
		BRWD3	bromodomain and WD repeat domain containing 3	ENSCAFT00000027493	chrX_65189965-65189965_A_C	275S>A	Substitution	Nonsynonymous coding	AGTTGNT GGAC	0.7
		Novel gene	uncharacterized protein	ENSCAFT00000027037	chrX_58551749-58551749_A_G	104K>R	Substitution	Nonsynonymous coding	CCTGANG AATT	0.17
11-R02	STS-PNST	AFAP1L1	actin filament associated protein 1-like 1	ENSCAFT00000029078	chr4_62838379-62838379_G_A	425S>F	Substitution	Nonsynonymous coding	TCTTGNA GAAG	0.25
		ATP7B	copper-transporting ATPase 2	ENSCAFT00000006859	chr22_3134952-3134952_A_C	288K>Q	Substitution	Nonsynonymous coding	ACCCANA GATG	0.2
		C11orf63	chromosome 11 open reading frame 63	ENSCAFT00000018556	chr5_14445155-14445155_A_G	55S>P	Substitution	Nonsynonymous coding	CTGGGNC TTAC	0.18
		FIP1L1	FIP1 like 1 (S. cerevisiae)	ENSCAFT00000003220	chr13_48967897-48967897_C	NA	Deletion	Frameshift	AGGTANA GCAG	0.4
		KRT23	keratin 23 (histone deacetylase	ENSCAFT00000025377	chr9_25094298-25094298_A_T	389K>M	Substitution	Nonsynonymous coding	ATCGANG TCAA	0.25

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
			inducible)							
		MLL3	myeloid/lymphoid or mixed-lineage leukemia 3	ENSCAFT0000007959	chr16_18937990-18937992_TGC_	3177QQ>Q	Deletion	In-frame deletion	GCTGTNGCTGC	0.11
		MUC5AC	mucin 5B, oligomeric mucus/gel-forming	ENSCAFT00000015796	chr18_48561759-48561759_G_A	3305G>S	Substitution	Nonsynonymous coding	AGACANGCCCC	0.12
		Novel gene	uncharacterized protein	ENSCAFT00000036128	chr14_61936959-61936959_T	NA	Insertion	Frameshift	CGGTCNCCAG	0.16
		OR52N1	olfactory receptor, family 52, subfamily N, member 1	ENSCAFT00000010210	chr21_32133356-32133356_C_T	239A>T	Substitution	Nonsynonymous coding	GAAGGNC TTCT	0.28
		PREX1	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	ENSCAFT00000017540	chr24_38467733-38467733_C_T	96R>H	Substitution	Nonsynonymous coding	AGGCGNGCACA	0.29
		PRPF39	PRP39 pre-mRNA processing factor 39 homolog	ENSCAFT00000022300	chr8_25550886-25550886_T_	NA	Deletion	Frameshift	GAAGANTTTGG	0.24
		Q6W6S1	uncharacterized protein	ENSCAFT00000030697	chr9_50634661-50634661_A_T	310S>T	Substitution	Nonsynonymous coding	TTTGGNTTTAT	0.27
		TENM2	teneurin transmembrane protein 2	ENSCAFT00000027184	chr4_46714792-46714792_C_T	364R>H	Substitution	Nonsynonymous coding	TTCGGNGGCGG	0.21

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		ZNF641	zinc finger protein 641	ENSCAFT00000014313	chr27_9390690-9390690_C_T	363P>S	Substitution	Nonsynonymous coding	CCCCCNCAGTG	0.26
11-R01	STS-PNST	ACTN2	actinin, alpha 2	ENSCAFT00000017321	chr4_6385028-6385028_C_T	90G>E	Substitution	Nonsynonymous coding	TTTTTNCTCGG	0.24
		GPR139	G protein-coupled receptor 139	ENSCAFT00000028634	chr6_28316728-28316728_C_T	132P>L	Substitution	Nonsynonymous coding	CCACCNGCTCA	0.27
		KCNJ16	potassium inwardly-rectifying channel, subfamily J, member 16	ENSCAFT00000017085	chr9_19566120-19566120_G_T	5G>C	Substitution	Nonsynonymous coding	ATTACNGCAGC	0.26
		KCNJ5	potassium inwardly-rectifying channel, subfamily J, member 5	ENSCAFT00000016271	chr5_8746471-8746471_C_G	116G>R	Substitution	Nonsynonymous coding	ATCACNC CGGA	0.32
04-R08	STS-PNST	A1ILJ0	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 precursor	ENSCAFT00000036554	chr8_66432888-66432888_C_T	194D>N	Substitution	Nonsynonymous coding	GACATNCTCTA	0.42
		AASS	aminoadipate-semialdehyde synthase	ENSCAFT00000005673	chr14_62956632-62956632_C_T	66G>S	Substitution	Nonsynonymous coding	AATGCNACCAG	0.62
		ABCB10	ATP-binding cassette, sub-	ENSCAFT00000019279	chr4_12734254-12734254_C_T	495R>C	Substitution	Nonsynonymous coding	CAGCTNGCCCA	0.47

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
			family B (MDR/TAP), member 10							
		ACTL9	actin-like 9	ENSCAFT00000029470	chr20_56179685-56179685_G_A	363P>S	Substitution	Nonsynonymous coding	GGGGGN CAGGC	0.37
		ADAM7	ADAM metallopeptidase domain 7	ENSCAFT00000014408	chr25_35952270-35952270_C_T	473E>K	Substitution	Nonsynonymous coding	CACTTNA GGAA	0.31
		ADCYAP1R1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	ENSCAFT00000005018	chr14_46708954-46708954_C_T	448S>F	Substitution	Nonsynonymous coding	GGGCTNC TTCC	0.63
		ALDH7A1	aldehyde dehydrogenase 7 family, member A1	ENSCAFT00000000904	chr11_18836811-18836811_G_A	523T>I	Substitution	Nonsynonymous coding	TGATANT ACTA	0.3
		ANKLE1	ankyrin repeat and LEM domain containing 1	ENSCAFT00000024464	chr20_48444251-48444251_G_A	74Q>X	Substitution	Nonsense	CTCCTNG TCTC	0.27
		ARMC9	armadillo repeat containing 9	ENSCAFT00000017508	chr25_46161506-46161506_C_T	296T>I	Substitution	Nonsynonymous coding	TTCAANC ATGT	0.29
		ASPM	Abnormal spindle-like microcephaly-associated protein homolog	ENSCAFT00000018114	chr7_8578487-8578487_C_T	1156L>F	Substitution	Nonsynonymous coding	CATTTNTT TGC	0.2
		ATP13A1	ATPase type 13A1	ENSCAFT00000022481	chr20_46627633-46627633_C_T	633S>F	Substitution	Nonsynonymous coding	AATGTNC GTGC	0.2

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		ATP2B3	ATPase, Ca ⁺⁺ transporting, plasma membrane 3	ENSCAFT00000030531	chrX_124404772-124404772_C_T	22P>L	Substitution	Nonsynonymous coding	GGCCCNC CATG	0.19
		B6EY10	tryptophan 5-hydroxylase 1	ENSCAFT00000014485	chr21_43753174-43753174_C_T	98R>Q	Substitution	Nonsynonymous coding	ATTTTNG GGAC	0.47
		BCAR1	breast cancer anti-estrogen resistance 1	ENSCAFT00000031962	chr5_78491554-78491554_C_T	150P>S	Substitution	Nonsynonymous coding	AGATGNC CCAT	0.28
		BOD1L1	biorientation of chromosomes in cell division 1-like 1	ENSCAFT00000024431	chr3_69317598-69317598_C_T	2128P>S	Substitution	Nonsynonymous coding	AACTCNC TGCG	0.29
		BRDT	bromodomain, testis-specific	ENSCAFT00000032118	chr6_59977191-59977191_C_T	874E>K	Substitution	Nonsynonymous coding	ATTTTNTT GAA	0.5
		BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	ENSCAFT00000008397	chr17_25386278-25386278_G_T	372Q>H	Substitution	Nonsynonymous coding	AACCANC CTTC	0.36
		C11orf80	chromosome 11 open reading frame 80	ENSCAFT00000019460	chr18_53566794-53566794_G_A	206P>L	Substitution	Nonsynonymous coding	TCAGANG CAGA	0.45
		C1orf168	chromosome 1 open reading frame 168	ENSCAFT00000030112	chr5_55715053-55715053_C_T	219T>I	Substitution	Nonsynonymous coding	AGAAANC CCTC	0.26
		C6orf211	chromosome 6 open reading frame 211	ENSCAFT00000000674	chr1_44848305-44848305_C_T	38R>X	Substitution	Nonsense	TGCATNG ACAT	0.32

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		CABP2	calcium binding protein 2	ENSCAFT00000018054	chr18_52987478-52987478_G_A	67G>E	Substitution	Nonsynonymous coding	AGTGGNG CCGG	0.35
		CEP250	centrosomal protein 250kDa	ENSCAFT00000012850	chr24_27405113-27405113_C_T	550L>F	Substitution	Nonsynonymous coding	TCATTNTT CGG	0.6
		CSMD1	CUB and Sushi multiple domains 1	ENSCAFT00000013885	chr16_58244318-58244318_G_A	1551S>F	Substitution	Nonsynonymous coding	TCTGGNA ATGG	0.48
		CSMD2	CUB and Sushi multiple domains 2	ENSCAFT00000005882	chr15_11028241-11028241_C_T	728S>L	Substitution	Nonsynonymous coding	GACTTNG CCCA	0.18
		DCDC2	doublecortin domain containing 2	ENSCAFT00000016283	chr35_25388917-25388917_C_T	192G>E	Substitution	Nonsynonymous coding	GTTTTNC TTCT	0.54
		DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	ENSCAFT00000011678	chr24_25068698-25068698_C_T	61S>F	Substitution	Nonsynonymous coding	ATTGTNC AAGA	0.26
		EMR2	EGF-like module-containing mucin-like hormone receptor-like 2 precursor	ENSCAFT00000025982	chr20_50969425-50969425_C_T	75S>N	Substitution	Nonsynonymous coding	GGCTGNT GAAG	0.43
		EXOC3L1	exocyst complex component 3-like 1	ENSCAFT00000032455	chr5_85189666-85189666_G_A	539R>K	Substitution	Nonsynonymous coding	GGTGANA GTCC	0.46
		FCRLB	Fc receptor-like A	ENSCAFT00000020702	chr38_23962108-23962108_C_A	21A>S	Substitution	Nonsynonymous coding	GGCTGNC CAGA	0.14

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		FLRT1	fibronectin leucine rich transmembrane protein 1	ENSCAFT00000023385	chr18_55953743-55953743_C_T	616G>D	Substitution	Nonsynonymous coding	CGGGGN CCCGG	0.31
		FMR1	fragile X mental retardation 1	ENSCAFT00000030311	chrX_119344462-119344462_G_A	331E>K	Substitution	Nonsynonymous coding	CCAAGNA AATT	0.24
		FMR1	fragile X mental retardation 1	ENSCAFT00000030311	chrX_119344481-119344481_C_T	337S>F	Substitution	Nonsynonymous coding	AAATTNC CTAC	0.2
		FSCN3	fascin homolog 3, actin-bundling protein, testicular (Strongylocentrotus purpuratus)	ENSCAFT00000002697	chr14_11685668-11685668_G_A	310R>C	Substitution	Nonsynonymous coding	TGCACNA AGCT	0.48
		FUT9	Alpha-(1,3)-fucosyltransferase	ENSCAFT00000005507	chr12_57775088-57775088_G_A	331E>K	Substitution	Nonsynonymous coding	TTTGGNA ATCA	0.28
		FXD3	FXD domain containing ion transport regulator 3	ENSCAFT00000011413	chr1_120363321-120363321_C_T	NA	Substitution	Splice site donor	TCTCANC ATAG	0.88
		GPR126	G protein-coupled receptor 126	ENSCAFT00000000457	chr1_37098753-37098753_C_T	415S>F	Substitution	Nonsynonymous coding	AATTTNC ATAG	0.24
		GPR128	G protein-coupled receptor 128	ENSCAFT00000014844	chr33_10191962-10191962_C_T	34R>W	Substitution	Nonsynonymous coding	AAGGANG GAGG	0.33
		GPR82	G protein-coupled receptor 82	ENSCAFT00000022877	chrX_36056596-36056596_C_T	213S>L	Substitution	Nonsynonymous coding	ATTTT NAT TTT	0.32

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		GRM6	glutamate receptor, metabotropic 6	ENSCAFT0000000509	chr11_5596380-5596380_C_T	523P>L	Substitution	Nonsynonymous coding	CCTCCNC TGTG	0.53
		GSX1	GS homeobox 1	ENSCAFT00000010870	chr25_14841844-14841844_C_T	NA	Substitution	Splice site acceptor	GCTGTNT GGAG	0.36
		GTF2I	general transcription factor Ili	ENSCAFT00000038018	chr6_8807549-8807549_G_A	145Q>X	Substitution	Nonsense	AGACTNA TCTC	0.43
		HDAC8	histone deacetylase 8	ENSCAFT00000027174	chrX_59408793-59408793_G_A	359S>F	Substitution	Nonsynonymous coding	GGGAANA GAAG	0.71
		HECTD4	HECT domain containing E3 ubiquitin protein ligase 4	ENSCAFT00000014076	chr26_12845851-12845851_C_T	541R>Q	Substitution	Nonsynonymous coding	CTTCCNG CTTG	0.38
		K1C10	keratin, type I cytoskeletal 10	ENSCAFT00000025391	chr9_25194405-25194405_G_A	316E>K	Substitution	Nonsynonymous coding	AATACNA ACAA	0.3
		KCNG3	potassium voltage-gated channel, subfamily G, member 3	ENSCAFT00000035514	chr17_37144629-37144629_G_A	366S>F	Substitution	Nonsynonymous coding	TGTTGNA TGTT	0.43
		KIF25	kinesin family member 25	ENSCAFT00000001345	chr1_58634208-58634208_G_A	509E>K	Substitution	Nonsynonymous coding	TGTCGNA GCGC	0.33
		LAMB2	laminin, beta 2 (laminin S)	ENSCAFT00000018765	chr20_43058275-43058275_C_T	1054P>L	Substitution	Nonsynonymous coding	GTGCCNG TCCA	0.38
		LIMK1	LIM domain kinase 1	ENSCAFT00000019799	chr6_9274167-9274167_G_A	222R>W	Substitution	Nonsynonymous coding	GATCCNG TCTC	0.6
		LY9	lymphocyte antigen 9	ENSCAFT00000020056	chr38_24536297-24536297_C_T	263E>K	Substitution	Nonsynonymous coding	CGACTNC CCCA	0.58
		MBD5	methyl-CpG binding domain	ENSCAFT00000008917	chr19_53239621-53239621_C_T	1189P>L	Substitution	Nonsynonymous coding	TGGTCNA GCTA	0.32

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
			protein 5							
		MLF1	myeloid leukemia factor 1	ENSCAFT00000014162	chr23_54989572-54989572_C_T	164A>V	Substitution	Nonsynonymous coding	CCGAGNT CATG	0.33
		NELL1	NEL-like 1 (chicken)	ENSCAFT00000015919	chr21_46027895-46027895_G_A	105E>K	Substitution	Nonsynonymous coding	CTGTCNA ATGT	0.24
		NF1	neurofibromin 1	ENSCAFT00000029545	chr9_44834512-44834512_G_A	1933P>S	Substitution	Nonsynonymous coding	CCACGNA GTCA	0.48
		Novel gene	Uncharacterized protein	ENSCAFT00000021819	chr27_39478508-39478508_G_A	1291E>K	Substitution	Nonsynonymous coding	GTTCTNA ACTA	0.36
		Novel gene	Uncharacterized protein	ENSCAFT00000004310	chr1_106460436-106460436_G_A	314E>K	Substitution	Nonsynonymous coding	GGGAGNA GAAA	0.47
		Novel gene	Uncharacterized protein	ENSCAFT00000028222	chr6_27157711-27157711_C_T	319M>I	Substitution	Nonsynonymous coding	AAAATNA TGCA	0.39
		Novel gene	Uncharacterized protein	ENSCAFT00000027418	chr8_56643270-56643270_G_A	395R>C	Substitution	Nonsynonymous coding	TAAACNA TCAG	0.38
		Novel gene	Uncharacterized protein	ENSCAFT00000012946	chr25_30547894-30547894_G_A	397D>N	Substitution	Nonsynonymous coding	GGCATNA TGGC	0.31

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		Novel gene	Uncharacterized protein	ENSCAFT00000030235	chrX_115997637-115997637_C_T	6E>K	Substitution	Nonsynonymous coding	CAATTNG CCAG	0.41
		Novel gene	Uncharacterized protein	ENSCAFT00000024549	chr6_14378075-14378075_G_A	734S>F	Substitution	Nonsynonymous coding	TTTTGNA AATT	0.36
		Novel gene	Uncharacterized protein	ENSCAFT00000009040	chr1_116977163-116977163_C_A	56E>X	Substitution	Nonsense	CACTTNG GAGC	0.17
		NTN5	netrin 5	ENSCAFT00000006331	chr1_110537423-110537423_G_A	259W>X	Substitution	Nonsense	CTTCTNG AGGG	0.17
		NUP210 L	nucleoporin 210kDa-like	ENSCAFT00000027524	chr7_46057921-46057921_C_T	287P>S	Substitution	Nonsynonymous coding	GATTTNC TCTG	0.25
		NVL	nuclear VCP-like	ENSCAFT00000025949	chr7_43088033-43088033_C_T	783S>L	Substitution	Nonsynonymous coding	CTACTNG TGAG	0.16
		OLFM4	olfactomedin 4	ENSCAFT00000038323	chr22_13020301-13020301_G_C	245Q>H	Substitution	Nonsynonymous coding	GTTCANC TCAA	0.26
		OR11H4	olfactory receptor, family 11, subfamily H, member 4	ENSCAFT00000008634	chr15_20603710-20603710_G_A	352M>I	Substitution	Nonsynonymous coding	GACATNA AATT	0.33

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		OR11L1	olfactory receptor, family 11, subfamily L, member 1	ENSCAFT00000039246	chr14_4576143-4576143_C_T	164S>F	Substitution	Nonsynonymous coding	GATTTCNCAAGT	0.25
		PEPB	pepsin B precursor	ENSCAFT00000031388	chr6_43778633-43778633_G_A	367D>N	Substitution	Nonsynonymous coding	TGGGANA TGTC	0.14
		PHKA2	phosphorylase kinase, alpha 2 (liver)	ENSCAFT00000020564	chrX_14879295-14879295_C_T	NA	Substitution	Splice site donor	ACTTANT TTAT	0.46
		PKHD1	polycystic kidney and hepatic disease 1 (autosomal recessive)	ENSCAFT00000003416	chr12_22675987-22675987_G_A	1323S>L	Substitution	Nonsynonymous coding	TCACTNAGTTG	0.38
		PRDM2	PR domain containing 2, with ZNF domain	ENSCAFT00000025940	chr2_86311966-86311966_G_A	1366P>S	Substitution	Nonsynonymous coding	GGACGNCAGCG	0.31
		PTPRO	protein tyrosine phosphatase, receptor type, O	ENSCAFT00000020369	chr27_34189070-34189070_C_T	309E>K	Substitution	Nonsynonymous coding	TTTTTCNCGTCT	0.57
		PTPRZ1	protein tyrosine phosphatase, receptor-type, Z polypeptide 1	ENSCAFT00000005646	chr14_62891929-62891929_T_C	1733L>P	Substitution	Nonsynonymous coding	TAAACNTGCAC	0.11
		Q28302	Uncharacterized protein	ENSCAFT00000035111	chr20_54398781-54398781_C_T	202L>F	Substitution	Nonsynonymous coding	AACTCNTCAAC	0.34

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		Q38IV3	Multidrug resistance protein 3	ENSCAFT00000027259	chr9_29903253-29903253_G_A	761R>Q	Substitution	Nonsynonymous coding	CCAGCNA CAGC	0.47
		Q8HYR2	Uncharacterized protein	ENSCAFT00000019633	chr27_29388021-29388021_A_T	166I>F	Substitution	Nonsynonymous coding	GAAATNT TATA	0.59
		RCC2	regulator of chromosome condensation 2	ENSCAFT00000024961	chr2_83776440-83776440_C_T	309P>L	Substitution	Nonsynonymous coding	GGTCCNC CGGC	0.46
		RP1	oxygen-regulated protein 1	ENSCAFT00000011204	chr29_9140829-9140829_G_A	1861E>K	Substitution	Nonsynonymous coding	AATCANA AAGA	0.3
		RTKN2	rhotekin 2	ENSCAFT00000020670	chr4_17382177-17382177_G_A	602S>L	Substitution	Nonsynonymous coding	GCCATNA TCTG	0.29
		SAMD7	sterile alpha motif domain containing 7	ENSCAFT00000023423	chr34_37539386-37539386_G_A	369R>Q	Substitution	Nonsynonymous coding	TCTTCNA AGCA	0.29
		SLAF1	Signaling lymphocytic activation molecule	ENSCAFT00000019982	chr38_24663637-24663637_C_T	233S>L	Substitution	Nonsynonymous coding	GTCTTNG GGTG	0.53
		SLC47A2	solute carrier family 47, member 2	ENSCAFT00000036298	chr5_43495248-43495248_C_T	83S>F	Substitution	Nonsynonymous coding	AGTTTNC ATAG	0.38
		SULT4A1	sulfotransferase family 4A, member 1	ENSCAFT00000035674	chr10_24862764-24862764_G_A	72M>I	Substitution	Nonsynonymous coding	TTGATNA ACAT	0.26

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		TAF7L	TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 50kDa	ENSCAFT00000027954	chrX_78291782-78291782_C_T	366E>K	Substitution	Nonsynonymous coding	CTTTTNAT AAT	0.41
		TBC1D15	TBC1 domain family, member 15	ENSCAFT00000000735	chr10_16382190-16382190_C_T	176S>F	Substitution	Nonsynonymous coding	TGACTNT CTTG	0.3
		TLR1	toll-like receptor 1 precursor	ENSCAFT00000037196	chr3_76368607-76368607_G_A	234W>X	Substitution	Nonsense	GGATGNT CTTA	0.3
		TMEM74	transmembrane protein 74	ENSCAFT00000001114	chr13_12451185-12451185_G_A	61R>C	Substitution	Nonsynonymous coding	AGGGCNA AGTT	0.34
		TOM1	target of myb1 (chicken)	ENSCAFT00000002700	chr10_31874137-31874137_A_C	50V>G	Substitution	Nonsynonymous coding	GCATCNC CTC A	0.36
		TRIM58	tripartite motif containing 58	ENSCAFT00000001915	chr14_4533386-4533386_G_C	455T>R	Substitution	Nonsynonymous coding	CGTTTNT TACA	0.23
		TRIM66	tripartite motif containing 66	ENSCAFT00000011106	chr21_35253035-35253035_G_A	662L>F	Substitution	Nonsynonymous coding	TGGGANA GGCG	0.43
		TTN	titin	ENSCAFT00000022319	chr36_25212813-25212813_C_T	25277E>K	Substitution	Nonsynonymous coding	ACTTTNTT TAA	0.31

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		TTN	titin	ENSCAFT00000022319	chr36_25208898-25208898_G_A	26582P>S	Substitution	Nonsynonymous coding	GACCGNTTCGC	0.36
		TTN	titin	ENSCAFT00000022319	chr36_25207752-25207752_C_T	26964E>K	Substitution	Nonsynonymous coding	GTTTTNTGCAT	0.32
		TTN	titin	ENSCAFT00000022319	chr36_25363681-25363681_C_T	6209E>K	Substitution	Nonsynonymous coding	GTTCTNGTGAC	0.32
		USP45	ubiquitin specific peptidase 45	ENSCAFT00000005638	chr12_60682412-60682412_G_A	232P>S	Substitution	Nonsynonymous coding	GGGAGNA AAAA	0.43
04-R04	OSA _c	ASTN1	astrotactin 1	ENSCAFT00000022524	chr7_25651338-25651338_C_T	762A>V	Substitution	Nonsynonymous coding	TGTGGNC TTGT	0.26
		ASXL3	additional sex combs like 3 (Drosophila)	ENSCAFT00000028551	chr7_59080331-59080331_G_A	1100P>L	Substitution	Nonsynonymous coding	CGGCCN GAGGC	0.33
		FRMPD4	FERM and PDZ domain containing 4	ENSCAFT00000018460	chrX_9178376-9178376_G_A	1180A>T	Substitution	Nonsynonymous coding	TGGACNC GGGC	0.17
		MC4R	melanocortin receptor 4	ENSCAFT00000000145	chr1_19140979-19140979_G_A	47V>I	Substitution	Nonsynonymous coding	TCTTCNT CTCC	0.33
		MGAM	maltase-glucoamylase (alpha-glucosidase)	ENSCAFT00000006194	chr16_10143723-10143723_T_	NA	Deletion	Frameshift	GGGTGNT TTTT	0.24

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		NFATC1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	ENSCAFT0000000013	chr1_4124943-4124943_A_G	8V>A	Substitution	Nonsynonymous coding	AAAGGNC TGGA	0.4
		NFE2L3	nuclear factor (erythroid-derived 2)-like 3	ENSCAFT00000038557	chr14_42452261-42452264_GATG —	NA	Deletion	Frameshift	AAGATNA TGTA	0.3
		TP53	cellular tumor antigen p53	ENSCAFT00000026465	chr5_35558664-35558664_A_G	260F>S	Substitution	Nonsynonymous coding	CCTCANAGCTG	0.54
		PLEKHB1	pleckstrin homology domain containing, family B (evectins) member 1	ENSCAFT00000009009	chr21_27601782-27601782_C_T	142R>H	Substitution	Nonsynonymous coding	CTCGGNG GCTC	0.43
		PTPN14	protein tyrosine phosphatase, non-receptor type 14	ENSCAFT00000019934	chr7_15317710-15317710_C_T	911G>R	Substitution	Nonsynonymous coding	CATTCNCTCTT	0.12
		RBBP6	retinoblastoma binding protein 6	ENSCAFT00000027846	chr6_24499626-24499626_T_C	1730K>R	Substitution	Nonsynonymous coding	TCTTTNT GCTG	0.3
		TDRD6	tudor domain containing 6	ENSCAFT00000003223	chr12_17857549-17857549_G_A	1517W>X	Substitution	Nonsense	AACTGNT ATAA	0.49

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
04-R02		TEX15	testis expressed 15	ENSCAFT00000010405	chr16_36456696-36456696_G_T	1265V>F	Substitution	Nonsynonymous coding	TTTCANTT TTG	0.58
		TRAP1	TNF receptor-associated protein 1	ENSCAFT00000030584	chr6_40616562-40616562_C_A	42A>D	Substitution	Nonsynonymous coding	TCCAGNC CAGT	0.3
	STS-PNST	KIAA1217	uncharacterized protein	ENSCAFT00000006799	chr2_11859851-11859851_G_A	356A>V	Substitution	Nonsynonymous coding	GAGAGNC GGGG	0.45
		MFSD2B	major facilitator superfamily domain containing 2B	ENSCAFT00000006341	chr17_21486565-21486565_C_T	494R>C	Substitution	Nonsynonymous coding	GTGCANG TGGG	0.42
		Novel gene	uncharacterized protein	ENSCAFT00000030447	chrX_123930541-123930541_G_C	327R>P	Substitution	Nonsynonymous coding	AGGGCNC CCCG	0.14
		SLC16A2	solute carrier family 16, member 2 (thyroid hormone transporter)	ENSCAFT00000027229	chrX_60903455-60903455_G_A	72A>T	Substitution	Nonsynonymous coding	CCTTCNC CTTT	0.4
		TEP1	telomerase-associated protein 1	ENSCAFT00000008693	chr15_20729329-20729329_G_A	1900L>F	Substitution	Nonsynonymous coding	CAGGANG CCCC	0.42

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		XPNPEP2	X-prolyl aminopeptidase (aminopeptidase P) 2, membrane-bound	ENSCAFT00000029688	chrX_104033303-104033303_C_T	502R>X	Substitution	Nonsense	CAGGGN GAATG	0.25
01-R02	STS-PNST	ACD	adrenocortical dysplasia homolog (mouse)	ENSCAFT00000032411	chr5_84799806-84799806_C_A	388P>H	Substitution	Nonsynonymous coding	TGGCCNC CTGC	0.13
		ADAMTS5	ADAM metalloproteinase with thrombospondin type 1 motif, 5	ENSCAFT00000013627	chr31_25306205-25306205_G_A	226H>Y	Substitution	Nonsynonymous coding	CTGATNC TGCC	0.13
		ADRB2	beta-2 adrenergic receptor	ENSCAFT00000029135	chr4_63253706-63253706_C_T	76C>Y	Substitution	Nonsynonymous coding	CAGCANA GGCC	0.12
		ATP7B	copper-transporting ATPase 2	ENSCAFT00000006859	chr22_3160667-3160667_G_A	1119V>M	Substitution	Nonsynonymous coding	TGGGCNT GGCC	0.2
		CDK14	cyclin-dependent kinase 14	ENSCAFT00000003009	chr14_19522937-19522937_C_T	102R>W	Substitution	Nonsynonymous coding	TCAGGNG GCAC	0.2
		IER5L	immediate early response 5-like	ENSCAFT00000031805	chr9_57855189-57855189_G_A	20S>N	Substitution	Nonsynonymous coding	CCACANC TCCC	0.16
		IRS1	insulin receptor substrate 1	ENSCAFT00000016522	chr25_42687032-42687032_C_T	139S>N	Substitution	Nonsynonymous coding	CCGAGNT GCCG	0.11

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		JAG1	jagged 1	ENSCAFT0000009074	chr24_14655994-14655994_G_A	93S>N	Substitution	Nonsynonymous coding	CTGTANC TTCG	0.11
		JUNB	jun B proto-oncogene	ENSCAFT00000027182	chr20_52362490-52362490_G_A	77S>L	Substitution	Nonsynonymous coding	GCTCCNA TGAG	0.14
		LMNA	lamin A/C	ENSCAFT00000026695	chr7_44690367-44690367_G_A	64T>I	Substitution	Nonsynonymous coding	ACTCGNT GATG	0.15
		MADCAM1	mucosal addressin cell adhesion molecule 1 precursor	ENSCAFT00000031356	chr20_61126306-61126306_G_	NA	Deletion	Frameshift	AAAGTNG GGGG	0.27
		MEFV	Mediterranean fever	ENSCAFT00000037775	chr6_41024970-41024970_C_A	673N>K	Substitution	Nonsynonymous coding	GGAAANA AGAC	0.26
		Novel Gene	Aldehyde dehydrogenase	ENSCAFT00000017771	chr18_52833141-52833141_A_G	250V>A	Substitution	Nonsynonymous coding	ACAGGNC GTAG	0.11
		NRM	nurim (nuclear envelope membrane protein)	ENSCAFT00000000694	chr12_3488483-3488483_C_T	524S>N	Substitution	Nonsynonymous coding	GGCAGNT GCGG	0.11
		PIM1	proto-oncogene serine/threonine -protein kinase pim-1	ENSCAFT00000002258	chr12_9213964-9213964_G_A	73G>D	Substitution	Nonsynonymous coding	CCCCGNC TCCT	0.22

Case ID	Tumor Type	Gene Symbol	Gene Description	Transcript Accession	Nucleotide (genomic) Position of Mutation	Amino Acid (protein) Position of Mutation	Mutation Type	Consequence	Sequence Context (Position of Mutation Indicated by "N")	% Mutant Reads
		PIM1	proto-oncogene serine/threonine-protein kinase pim-1	ENSCAFT0000002258	chr12_9214807-9214807_C_T	250H>Y	Substitution	Nonsynonymous coding	ACTGCNACAAC	0.22
		PIM1	proto-oncogene serine/threonine-protein kinase pim-1	ENSCAFT0000002258	chr12_9214750-9214750_C_T	231Q>X	Substitution	Nonsense	CCCTGNA GGAG	0.2
		PTCH1	Patched-like protein 1	ENSCAFT0000001978	chr1_74305255-74305255_G_A	73A>T	Substitution	Nonsynonymous coding	GGAAANC TACT	0.16
		TRPS1	trichorhinophalangeal syndrome I	ENSCAFT0000001274	chr13_18226051-18226051_C_T	530S>N	Substitution	Nonsynonymous coding	CATGANT GTCC	0.13
		ZFP36L1	zinc finger protein 36, C3H type-like 1	ENSCAFT00000026141	chr8_45703888-45703888_C_T	14S>N	Substitution	Nonsynonymous coding	CTTCGNT CAAG	0.13

STS - soft tissue sarcoma; STS-PNST - soft tissue sarcoma, peripheral nerve sheath tumor; OSA_c - chondroblastic osteosarcoma.

Table 4a

Types of somatic changes observed across canine soft tissue sarcomas

Type	Subtype	Number of alterations	Percentage of alterations (%)
Substitutions	Nonsense	11	6
	Missense (non-synonymous)	135	73
	Splice site acceptor	1	1
	Splice site donor	4	2
Subtotal		151	82
INDELs	Deletion	4	2
	Insertion	1	1
Subtotal		5	3
CNAs	Deletion	0	0
	Amplification	28	15
Subtotal		28	15
Total		184	100

INDELs – insertions and deletions; CNAs – copy number alterations

Table 4b

Type of somatic mutations across canine soft tissue sarcomas

Type of somatic alteration	Number	Percentage
1 bp deletion	3	1.9
3 bp deletion	1	0.6
1 bp deletion	1	0.6
A:T>C:G	3	1.9
A:T>G:C	4	2.6
A:T>T:A	3	1.9
C:G>A:T	4	2.6
C:G>G:C	2	1.3
C:G>T:A	71	45.5
G:C>A:T	53	34.0

Type of somatic alteration	Number	Percentage
G:C>C:G	3	1.9
G:C>T:A	4	2.6
T:A>A:T	1	0.6
T:A>C:G	1	0.6
T:A>G:C	2	1.3
Total	156	100

Amplifications and deletions were less common, with an average of three per tumor (range: of 0 – 17) (Figure 5). Seven of the 10 soft tissue sarcomas harbored no amplifications or deletions. The chondroblastic osteosarcoma exome was similar to those of the soft tissue sarcomas, with 14 somatic mutations and four amplifications (Table 3 and Figure 6).

[0152] Single base substitutions were identified in four tumor suppressor genes that are frequently mutated in human tumors (*NF1*, *MLL3*, *TP53*, and *PTCH1*). Additionally, *MDM4*, an oncogene that has been shown to be amplified but not point-mutated in human cancers was found to be amplified (but not point-mutated) in one canine tumor (Lee, et al., 2012, Barretina, et al., 2010, Chmielecki, et al., 2013, Vogelstein, et al., 2013). The only genes mutated in more than one tumor were *ATP7B* (missense mutations in two tumors) and *AIG1* (amplified in two tumors). Interestingly, mutations in *ATP7B* were also found in a human liposarcomas (Joseph, et al., 2013). Twenty-two of the 184 somatic mutations in canine tumors occurred in genes previously shown to be mutated in human soft tissue sarcomas (Table 5).

Table 5
Genes mutated in both human and canine cancers

Gene	Number of somatic alterations	Type of alteration	Number of samples	Human driver gene or mutated in human soft tissue sarcoma
ANKRD11	1	SBS (splice site)	1	Joseph <i>et al.</i> , 2013
ATP7B	2	SBS (missense)	2	Joseph <i>et al.</i> , 2013
BRDT	1	SBS (missense)	1	Chemielecki <i>et al.</i> , 2013
BRWD3	1	SBS (missense)	1	Joseph <i>et al.</i> , 2013
CSMD2	1	SBS (missense)	1	Joseph <i>et al.</i> , 2013
FCRLB	1	SBS (missense)	1	Lee <i>et al.</i> , 2012
IRS1	1	SBS (missense)	1	Barretina <i>et al.</i> , 2010
LIMK1	1	SBS (missense)	1	Lee <i>et al.</i> , 2012
MBD5	1	SBS (missense)	1	Lee <i>et al.</i> , 2012
MLL3	1	Deletion	1	Vogelstein <i>et al.</i> , 2013
NF1	1	SBS (missense)	1	Barretina <i>et al.</i> , 2010
PKHD1	1	SBS (missense)	1	Lee <i>et al.</i> , 2012
PTCH1	1	SBS (missense)	1	Vogelstein <i>et al.</i> , 2013
PTPRZ1	1	SBS (missense)	1	Chemielecki <i>et al.</i> , 2013
RP1	1	SBS (missense)	1	Chemielecki <i>et al.</i> , 2013
TTN	4	SBS (missense)	1	Chemielecki <i>et al.</i> , 2013
MDM4	1	Amplification	1	Vogelstein <i>et al.</i> , 2013
CNTN2	1	Amplification	1	Chemielecki <i>et al.</i> , 2013

Larger studies of soft tissue sarcomas in both species will be required to determine whether these represent driver mutations that signify important, conserved tumorigenic pathways. Regardless, the genetic landscapes of canine tumors were similar to those of humans in terms of the numbers of genetic alterations and

spectrum of mutations. Specifically, they exclude the possibility that the canine tumors have a very large number of mutations which might make them more likely to mount an immune response than analogous tumor types in humans.

Example 6

Intratumoral (IT) administration of C. novyi NT – Study 1 Methods

[0153] To investigate the safety and efficacy of the method of the present invention, a comparative study in 16 dogs with spontaneously occurring solid tumors was performed (Table 6).

Table 6
Patient Characteristics

Case ID	Sex ^a	Breed	Age (years)	Body Weight (kg)	Tumor type ^b	Grade ^c	Location	Longest diameter ^d (mm)	Previous treatment	# of IT <i>C. novyi</i> -NT treatments
01-R02	FN	Border collie	14.3	21.7	STS-PNST	II	Left flank	43	None	4
04-R01	MN	Golden retriever	7.9	34.0	STS-PNST	II	Right maxilla	15	Surgical	4
04-R02	MI	Golden retriever	12.0	38.8	STS-PNST	I	Right lateral metacarpus	46	Surgical	4
04-R03	MN	Boxer	9.6	29.4	STS	I	Left medial antebrachium	56	None	3 ^{TR}
04-R04	FN	St. Bernard	11.7	31.0	OSA _c	III	Right proximal humerus	ND	Surgical	1 ^{AE}
04-R05	MN	Shetland sheepdog	14.0	13.4	STS	III	Right cranial antebrachium	45	Surgical & <i>C. novyi</i> -NT spores IV	4
04-R06	FN	Labrador retriever	11.6	24.3	MCT	III	Right hindlimb digit III	23	None	4
04-R08	FN	Shepherd	7.2	28.9	STS-PNST	I	Right medial hindlimb paw	65	Surgical	3 ^{PD}
10-R01	MN	Golden retriever	13.7	33.6	OMM	III	Left mandible	27	Surgical	2 ^{AE}

Case ID	Sex ^a	Breed	Age (years)	Body Weight (kg)	Tumor type ^b	Grade ^c	Location	Longest diameter ^d (mm)	Previous treatment	# of IT C. <i>novyi</i> -NT treatments
10-R02	MN	Pit bull terrier	10.0	43.6	STS	I	Right flank	53	Surgical	4
11-R01	MN	Maltese	11.1	8.1	STS-PNST	II	Left pinna	28	Surgical	1 ^{TR}
11-R02	FN	Labrador retriever	12.2	30.3	STS-PNST	II	Left stifle	43	None	3 ^{IV}
11-R04	MN	Husky	10.3	44.3	STS	I	Right forelimb paw	29	None	4
16-R02	MN	Labrador retriever	9.8	36.8	STS	I	Left lateral thigh	91	Surgical	4
16-R03	FN	Shepherd	10.8	20.8	STS	I	Left forelimb paw	53	Surgical	4
26-R01	MN	Labrador retriever	7.9	30.8	STS	II	Right forelimb paw	24	None	4

^aFN - female neutered; MN - male neutered; MI - male intact. ^bSTS - soft tissue sarcoma; STS - PNST - peripheral nerve sheath tumor; OSA_c - chondroblastic osteosarcoma; MCT - mast cell tumor; OMM - oral malignant melanoma. ^cGrading based on published criteria (Dennis et al., 2011, Patnaik et al., 1984, Smedley et al., 2011, Sabattini et al., 2014): I - low grade; II - intermediate grade; III - high grade; NA - not assessed. ^dLongest diameter at time of first C. *novyi* -NT administration (day 0). ND - unmeasurable due to location. ^e04-R05 - previous C. *novyi* -NT therapy with a single IV injection of 1x10⁷ spores/m² 437 days prior to the first IT administration of C. *novyi*-NT spores. ^fReason for number of treatments less than 4 given in superscript: TR - tumor response; AE - adverse event; PD - progressive disease; IV - 4th dose given intravenously.

[0154] Dogs were enrolled at multiple sites participating in the Animal Clinical Investigation oncology network (ACI, Washington, DC) and written informed consent was obtained from owner(s) prior to enrollment. Treatment, management, and study evaluations were overseen by board-certified veterinary oncologists. Enrollment was offered to client-owned dogs with spontaneous solid tumors, with a preference for soft-tissue sarcomas that had failed standard therapy or whose owner(s) had declined such therapy. Participation was restricted to tumor bearing dogs with a target lesion having a longest diameter between 1 and 7 centimeters. Dogs with tumors located in areas where abscess development would be catastrophic (e.g., nasal tumors that extended into the brain or significant pulmonary metastatic disease) were excluded from the study.

[0155] Dogs with evidence of an active bacterial infection requiring systemic antibiotic therapy within seven days or cancer therapy (chemotherapy, radiation therapy, and immunotherapy) within 21 days of C. novyi-NT spore treatment were ineligible. Dogs were required to have a performance score of 0 or 1 (Table 7) and to be available for the full duration of the study for enrollment. Concurrent use of anticancer agents and participation in other clinical trials were prohibited. Dogs that were pregnant or likely to become pregnant were not included in the study. Also, dogs that may have been unavailable for the entire study duration, and dogs that were considered unsuitable for study enrollment by the Investigator or Medical Director were not included in the study.

Table 7
Performance status evaluations

Score	Description
0	Normal activity
1	Restricted activity: decreased activity from pre-disease status
2	Compromised: ambulatory only for vital activities, able to consistently defecate and urinate in acceptable areas
3	Disabled: must be force fed and/or unable to confine urination and defecation to acceptable areas
4	Death

[0156] During a screening visit, each dog was assigned a unique study dog identification number consisting of a 5-digit numeric code (which may not have been sequentially in order of the screening dog number). The first 2 digits indicated the study site (01 to 99), the middle digit indicated the study 'R', and the last 2 digits described the study dog number within a study site (01 to 99). For example the 11th dog enrolled at Site 9 was assigned study dog number 09-R11. Study dog numbers were assigned chronologically in the order that dogs were enrolled at a given study site. A dog was considered enrolled in the study when it satisfied the inclusion and exclusion criteria.

[0157] Gross pathology and histopathology was performed in accordance with Food and Drug Administration's CVM Guidance for Industry 185. At necropsy, the following tissues (Table 8) were assessed for gross pathology and for histopathology and described in the necropsy report. Samples of brain, heart, lung, liver, spleen, kidney, muscle, bone, small intestine, large intestine and any tissue with gross abnormality were collected for microbiology.

Table 8List of tissues to be examined by gross pathology and histopathology

Pituitary gland	Brain	Bone and marrow
Thyroid gland	Spinal cord	Marrow smear
Parathyroid gland	Eyes	Spleen
Adrenal gland	Lung	Stomach
Pancreas	Muscle	Duodenum
Ovaries	Mammary gland	Jejunum
Uterus	Liver	Ileum
Testes	Gall bladder	Colon
Prostate	Kidneys	Cecum
Epididymis	Urinary bladder	Thymus
Heart	Lymph nodes	Injection site
Ventricles	Skin	Any abnormal tissues

[0158] All dogs were hospitalized from day 0 (D0) to day 4 (D4), and then optionally (at the Investigator's discretion) for 24 to 48 hours after each subsequent treatment for clinical observation. Fluids were administered to all study dogs during hospitalization following C. novyi NT treatment. On dosing days all dogs were administered intravenous (IV) crystalloids at 4 ml/kg/h for 2 hours. Dogs were closely monitored for six hours after each IT injection of C. novyi-NT spores. At the next visit (4 days later) all dogs were administered subcutaneous (SQ) crystalloids at 20 ml/kg. If a dog was hospitalized and receiving IV crystalloids on the day that SQ crystalloids were to be administered, it was not necessary to give the SQ dose.

[0159] Study visits and events are summarized in Table 9 as an example of a 4-dose treatment regimen. The dosing interval was suggested to be on a weekly basis, if the dog was to be treated with repeated dosing. Treatment delays for repeated dosing occurred during the course of the study due to adverse events or the decision of the investigator.

Table 9
Summary of study evaluations

	Pretreatment Screening^a	Day 0^b	Day 4	Day 7^b	Day 11	Day 14^b	Day 18	Day 21^b	Day 25	Day 60	Day 90
Informed Consent	X										
Medical History & Demographics	X										
Physical Exam	X	X	X	X	X	X	X	X	X	X	X
Weight & Vital Signs	X	X	X	X	X	X	X	X	X	X	X
Performance Score	X										
Inclusion & Exclusion Criteria	X										
Laboratory Values ^c	X	X	X	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Imaging ^d	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Biopsy	X										
Research	X										

	Pretreatment Screening ^a	Day 0 ^b	Day 4	Day 7 ^b	Day 11	Day 14 ^b	Day 18	Day 21 ^b	Day 25	Day 60	Day 90
Bloodwork											
Tumor Measurements & Photographs	X	X		X		X		X		X	X
Assign study dog number	X										
Enrollment	X										
IT <i>C. novyi</i> -NT		X		X		X		X		X	
IV Fluid Therapy ^e		X		X		X		X		X	
SQ Fluid Therapy ^f			X		X		X		X		
Study completion ^g											X

^a Screening evaluations undertaken 1-14 days prior to treatment. ^b Patient monitored 6 hours post-treatment. Evaluation made every 15 minutes for 1st hour post-treatment, every 30 minutes for 2nd hour post treatment and every 60 minutes for 3rd - 6th hour post-treatment. ^c Laboratory values include: complete blood count, serum biochemistry panel, prothrombin time, thromboplastin time and urinalysis. (X) - at discretion of the investigator. ^d Diagnostic imaging including: radiographs, ultrasound examination, or computed tomography. ^e Crystalloid at 4mL/kg/hr for two hours. ^f Crystalloid at 20mL/kg. ^g Following study completion and if systemic antibiotics were required to manage adverse events, it was recommended to administer doxycycline 5-10 mg/kg orally twice a day (PO BID) to dogs for 3 months.

[0160] Sixteen dogs, 9 neutered males, 1 entire (intact) male, and 6 neutered females, were enrolled in the study. (Table 6). Their demographics and tumor characteristics are given in Table 6. Enrolled cases exhibited diverse breeds, weights and ages. Cases were previously diagnosed with naturally occurring cancers representing a variety of histological origins: 13 dogs had a diagnosis of soft tissue sarcoma (81.3%), 1 osteosarcoma (6.3%), 1 melanoma (6.3%) and 1 mast cell tumor (6.3%). Of the 13 soft tissue sarcomas, histologic subtype was available for 11 and included: 4 hemangiopericytomas (30.8%), 3 peripheral nerve sheath tumors (23.1%), 1 synovial cell sarcoma (7.7%), 1 myxosarcoma (7.7%), 1 rhabdosarcoma (7.7%) and 1 fibrosarcoma (7.7%). The mean weight of dogs in the trial was 29.4 kg (range 8.1 – 44.3 kg) and their mean age was 10.9 years (range: 7.2 – 14.3 years). Thirteen dogs had a diagnosis of soft tissue sarcoma, and one each had a diagnosis of osteosarcoma, malignant melanoma, and mast cell tumor. Of the 13 soft tissue sarcomas, six were available for immunohistochemistry (IHC). All six were positive for S100 and negative for smooth muscle actin, suggesting the diagnosis of a sarcoma subtype called peripheral nerve sheath tumors. Seven of the tumors were grade I, five were grade II, and four were grade III. Eight dogs had previous surgical therapy for their cancers.

Preparation and IT injection of C. novyi-NT spores in spontaneous canine tumors

[0161] C. novyi-NT spores for use in the comparative canine study were produced as previously described (Dang, et al., 2001, Bettegowda, et al., 2006). In brief, bacteria were cultured in sporulation medium for at least

two weeks to ensure maximum yield of mature spores. Mature spores were purified through two consecutive, continuous Percoll gradients followed by four washes and re-suspensions in PBS. Sterility testing of the final product was performed by culturing product in Soybean-Casein Digest Medium and Thioglycollate Medium in accordance with FDA 21CFR610.12 guidelines (Nelson Laboratories, Salt Lake City, UT). Germination efficiency assays were performed under anaerobic conditions on Brucella agar with 5% horse blood to ensure the spores meet preset viability criteria. Spores were packaged in sterile 1.8 mL cryovials with O-ring sealed screw caps (Simport, Beloeil, Canada) at a volume of 1000 μ L and a concentration of 1×10^9 spores/mL. *C. novyi*-NT cryovials were stored at 2-8°C. For dosing, a 0.4 mL aliquot of the stock spore solution was packaged into 0.5 mL cryovials. After dosing, the cryovials and unused *C. novyi*-NT spores were discarded according to applicable regulations for disposal of Biosafety Level 2 material. Prior to IT injection, spores were re-suspended with a vortex, mixing at maximum speed for 10 seconds for a total of three times before being withdrawn into a 1mL syringe. The injection site was aseptically prepared. If available, ultrasound or computed tomography (CT) was used to identify a necrotic region of the tumor. If a necrotic region was not identified, the injection was directed to the center of the tumor. The needle was inserted once into the pre-defined region and 100 μ L of spore suspension (1×10^8 *C. novyi*-NT spores) were dispensed with even pressure. The injection needle was removed slowly and the injection site sterilized. All dogs received at least 1 cycle of an IT dose of 1×10^8 spores in 100 μ L saline (biosurgery): 3 dogs received a single treatment cycle, 13 dogs received more than 1 and up to 4 treatment cycles. Dogs

could receive up to 4 cycles of biosurgery with a one-week interval between cycles. Treated dogs were followed for at least 90 days after the first IT injection. Extended follow-up for disease progression and survival were warranted when available. Early withdrawal from the study was allowed for toxicity or progressive disease.

[0162] Study evaluations were undertaken as described in Table 9. Pre-screening evaluations were conducted 1 to 14 days before the first cycle of biosurgery. Dogs were monitored periodically on both an inpatient and outpatient basis during the study. Laboratory samples were taken as defined in Table 9 and included a complete blood count, serum biochemistry, prothrombin time, partial thromboplastin time, and urinalysis. Imaging was performed at screening and included regional CT, thoracic radiography, and abdominal ultrasonography. Additional imaging may be conducted during the study at the investigator's discretion.

[0163] Adverse events were evaluated, where possible, using the Veterinary Co-operative Oncology Group – Common Terminology Criteria for Adverse Events (VCOG-CTCAE) v1.0 (Veterinary Co-operative Oncology Group, 2004), with terminology from the Veterinary Dictionary for Drug Related Affairs (VeDDRA) rev.4 (European Medicines Agency, 2012). Terminologies for adverse events related to C. novyi-NT germination (target lesion reactions) are defined in Table 10. Clinical observations without appropriate VeDDRA or target lesion reaction terminology were classified separately as uncoded signs (Table 11). Relationship to C. novyi-NT therapy was determined by the reporting investigator.

Table 10

Coded terms to describe tumor adverse events associated with *C. novyi*-NT activity

System Organ Class (SOC) Term	High Level Term (HLT)	Preferred Term (PT)	Low Level Term (LLT)
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor abscess
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor closed wound
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor malodorous
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor necrosis
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor open wound
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor tissue loss
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor tissue sloughing
Target lesion reaction	Tumor inflammation	Tumor abscess	Tumor ulceration
Target lesion reaction	Tumor inflammation	Tumor consistency change	Tumor consistency change
Target lesion reaction	Tumor inflammation	Tumor consistency change	Tumor firmer
Target lesion reaction	Tumor inflammation	Tumor consistency change	Tumor softer
Target lesion reaction	Tumor inflammation	Tumor discharge	Tumor bleeding
Target lesion reaction	Tumor inflammation	Tumor discharge	Tumor bloody discharge
Target lesion reaction	Tumor inflammation	Tumor discharge	Tumor discharge
Target lesion reaction	Tumor inflammation	Tumor discharge	Tumor purulent discharge
Target lesion	Tumor	Tumor discharge	Tumor serous

System Organ Class (SOC) Term	High Level Term (HLT)	Preferred Term (PT)	Low Level Term (LLT)
reaction	inflammation		discharge
Target lesion reaction	Tumor inflammation	Tumor inflammation	Increased tumor heat
Target lesion reaction	Tumor inflammation	Tumor inflammation	Increased tumor warmth
Target lesion reaction	Tumor inflammation	Tumor inflammation	Tumor edematous
Target lesion reaction	Tumor inflammation	Tumor inflammation	Tumor inflammation
Target lesion reaction	Tumor inflammation	Tumor inflammation	Tumor inflammatory reaction
Target lesion reaction	Tumor inflammation	Tumor inflammation	Tumor pruritis
Target lesion reaction	Tumor inflammation	Tumor inflammation	Tumor swollen
Target lesion reaction	Tumor inflammation	Tumor pain	Tumor pain
Target lesion reaction	Tumor inflammation	Tumor skin disorder	Tumor bruising
Target lesion reaction	Tumor inflammation	Tumor skin disorder	Tumor discoloration
Target lesion reaction	Tumor inflammation	Tumor skin disorder	Tumor erythema
Target lesion reaction	Tumor inflammation	Tumor skin disorder	Tumor petichiation
Target lesion reaction	Tumor inflammation	Other tumor disorder	Other tumor disorder
Target lesion reaction	Tumor inflammation	Tumor pain	Tumor discomfort

Table 11

Signs not attributable in VeDDRA to underlying clinical entity or *C. novyi-NT* related target lesion reaction

Adverse Event (Preferred Term)	G-I	G-II	G-III	G-IV	# of dogs (with at least 1 occurrence of AE)	Total
Uncoded sign	15	2		1 ^a	5	18

^a Grade IV decrease in blood eosinophils reported by investigator.

[0164] Longest diameter tumor measurements of the target (injected) lesion were made on day 0, day 7, day 14, day 21, day 60 and day 90 post-treatment (Table 9). Non-target and new lesions were recorded but not measured. The best overall target response was evaluated on or after the day 21 study visit: complete response (CR) was defined as the complete disappearance of the target lesion; partial response (PR) was defined as at least a 30% decrease in the longest diameter of the target lesion; and progressive target disease (PD) was defined as at least a 20% increase in the longest diameter of the target lesion or the appearance of new nontarget lesions. Stable disease (SD) was defined as insufficient decrease or increase in the longest diameter of the target lesion to qualify as CR, PR, or PD. In the case of *C. novyi-NT* related abscesses, medical, or surgical debridement of necrotic tissue was at the discretion of the investigator.

[0165] Evaluation of surgical samples and necropsies were conducted by board certified veterinary pathologists. Tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Slides stained with H&E and or gram stained slides were prepared for evaluation according to standard procedure guidelines. For immunohistochemistry (IHC), formalin-

fixed, paraffin-embedded tumor tissue was sectioned at 5 μ m, deparaffinized in xylene, and rehydrated through graded alcohols. Antigen retrieval was done using unmasking solution (Vector Laboratories, Burlingame, CA). Primary antibodies S100 (DAKO, Carpinteria, CA) and anti-smooth muscle actin (DAKO, Carpinteria, CA) were used at 1:100. Secondary antibodies (Vector Laboratories, Burlingame, CA) labeled with DAB were used at 1:500. Sections were incubated with ABC reagent (Vector Laboratories, Burlingame, CA) and counterstained with hematoxylin. Tumor grades were assigned to each based on published criteria (Dennis, et al., 2011, Patnaik, et al., 1984, Smedley, et al., 2011, Sabattini, et al., 2014).

Example 7

Intratumoral (IT) administration of *C. novyi-NT* – Study 1 Results

[0166] All dogs received at least one cycle of biosurgery, with 53 cycles given of a maximum of 64 planned. The majority of dogs, 10 of 16, received the intended four cycles. Cycles of biosurgery were typically one week apart. No placebo control or masking was used.

[0167] For dogs showing early tumor responses, toxicity, or progressive disease after the first cycle, subsequent cycles were stopped. The most common adverse events were consistent with local infection at the *C. novyi-NT* spore injection site, including: fever (17 incidents), tumor inflammation (12 incidents), tumor abscess (10 incidents), anorexia (nine incidents), and lethargy (six incidents) (Table 12). Clinical signs of an inflammatory response at the injected target lesion site was observed in 14 of 16 dogs (87.5%), including: tumor inflammation (12/14), tumor abscess (7/14), tumor pain (5/14), and tumor discharge (4/14) (Table 13).

Table 12

Summary of adverse events observed throughout study

Adverse Event (Preferred Term)	G-I	G-II	G-III	G-IV	# of dogs (with at least 1 occurrence of AE)	Total
Hyperthermia	14	3			10	17
Tumor inflammation	7	4	1		12	12
Tumor abscess	6	3	1		8	10
Anorexia	7	2			8	9
Lethargy	3	2	1		6	6
Lameness	5		1		6	6
Oedema	5	1			5	6
Hypertension	6				4	6
Neutrophilia	6				6	6
Tumor discharge	6				4	6
Anaemia	4		1		5	5
Diarrhoea		3	1		2	4
Tumor pain	3	1			4	4
Leucocytosis	4				3	4
Lymphadenitis	4				4	4
Tumor consistency change	3				3	3
Leucopenia		1		1	1	2
Thrombocytopenia	1			1	2	2
Localized pain		1	1		2	2
Lymphopenia	1		1		2	2
Change in blood protein	1	1			2	2
Emesis	1	1			2	2
Fluid in abdomen	1	1			1	2
General pain	1	1			2	2
Electrolyte disorder	2				2	2
Impaired consciousness	2				2	2
Tumor skin disorder	2				2	2

Adverse Event (Preferred Term)	G-I	G-II	G-III	G-IV	# of dogs (with at least 1 occurrence of AE)	Total
Neutropenia				1	1	1
Malaise			1		1	1
Muscle weakness			1		1	1
Recumbency			1		1	1
Steatitis			1		1	1
Digestive tract haemorrhage		1			1	1
Skin and tissue infection		1			1	1
Arrhythmia	1				1	1
Bone and joint disorder	1				1	1
Cardiac enlargement	1				1	1
Digestive tract disorder	1				1	1
Eosinophilia	1				1	1
Erythema	1				1	1
Hepatomegaly	1				1	1
Hepatopathy	1				1	1
Injection site pruritus	1				1	1
Lymphocytosis	1				1	1
Murmur	1				1	1
Nausea	1				1	1
Palpable mass	1				1	1
Pulmonary disorder	1				1	1
Skin haemorrhage	1				1	1
Urine abnormalities	1				1	1
Total						153

Table 13

Summary of clinical evidence of germination and response from *C. novyi*-NT therapy

Case ID	Clinical evidence of germination ^a	Clinical response ^b
01-R02	Tumor inflammation, skin disorder and discharge	PD
04-R01	Tumor inflammation and pain	CR
04-R02	Tumor inflammation and abscess	PR
04-R03	Tumor inflammation, consistency change, discharge and tumor pain	CR
04-R04	Tumor inflammation and pain	NE
04-R05	Tumor inflammation, consistency change, skin disorder and pain	PR
04-R06	Tumor inflammation, abscess and discharge	CR
04-R08	Tumor abscess and discharge	NE
10-R01	-	PD
10-R02	Tumor inflammation, abscess and pain	SD
11-R01	Tumor inflammation and abscess	PR
11-R02	Tumor inflammation	SD
11-R04	Tumor abscess and consistency change	SD
16-R02	Tumor inflammation	PD
16-R03	Tumor inflammation and abscess	SD
26-R01	-	SD

^aClinical evidence of *C. novyi*-NT germination on or after day 0 of the study and includes target lesion reactions (Figure 5). ^bBest response of the target lesion, as defined by the study protocol, after day 21 of the study: CR - complete response; PR - partial response; SD - stable disease; PD - progressive disease; NE - not evaluable for response after on or after day 21 of the study.

Early-onset adverse events

[0168] Early-onset adverse events refer to the events occurring within the first 7 days following the first treatment cycle (13 dogs) or a single treatment cycle (3 dogs). A variety of adverse (AE) event findings were noted

across multiple cases. The early-onset adverse events that occurred within 7 days either after the 1st treatment cycle (13 dogs that have received multiple cycles) or after the single treatment cycle (3 dogs that have received only one cycle) are summarized in Table 14.

Table 14

Summary of early onset^a adverse events of any grade during the first treatment cycle

Adverse Event	Type	Number of dogs ^b (N=16)	Incidence (%)
Tumor inflammation	Target Lesion reaction	9	56.3%
Anorexia	General signs or symptoms	4	25.0%
Edema	General signs or symptoms	4	25.0%
Fever	General signs or symptoms	4	25.0%
WBC increased	Blood and lymphatic system	2	12.5%
Hypertension	Circulatory disorders	2	12.5%
Lethargy	General signs or symptoms	2	12.5%
Pain	General signs or symptoms	2	12.5%
Tumor abscess	Target Lesion reaction	2	12.5%
Hb decreased	Blood and lymphatic system	1	6.3%
MCV decreased	Blood and lymphatic system	1	6.3%
Neutrophils increased	Blood and lymphatic system	1	6.3%
RBC decreased	Blood and lymphatic system	1	6.3%
WBC decreased	Blood and lymphatic system	1	6.3%
Blood in feces	Digestive tract disorders	1	6.3%
Diarrhea	Digestive tract disorders	1	6.3%
Nausea	Digestive tract disorders	1	6.3%
Regurgitation	Digestive tract disorders	1	6.3%
Vomiting	Digestive tract disorders	1	6.3%
Injection site pruritus	Injection site reactions	1	6.3%
Tumor bleeding	Target Lesion reaction	1	6.3%
Tumor erythema	Target Lesion reaction	1	6.3%

^a Up to and less than 7 days after first treatment. ^b Number of dogs with at least one adverse event of any grade

[0169] Common early onset adverse event findings included: target tumor lesion reactions, alterations in general signs and symptoms, and blood and lymphatic system abnormalities. The majority of early onset adverse

events were mild to moderate (Grade I-II), with tumor inflammation, anorexia, tumor edema, and fever being the most commonly observed events. Grade III tumor abscess and Grade III tumor inflammation were noted in two cases (10-R02 and 16-R03). Early onset adverse event findings appear consistent with the anticipated tumor inflammatory reactions resulting from the mechanism of action of the *C. novyi-NT* therapeutic.

Late-onset adverse events

[0170] A subset of 3 dogs received only a single treatment cycle (as of December 2, 2012). Late-onset adverse events refer to the events occurring after 7 days following the single treatment cycle and are summarized in Table 15 for the 3 dogs (04-R04, 10-R02, and 11-R01). The majority of late-onset adverse events were mild to moderate (Grade I-II) and 11 of the 12 later onset findings were noted in a single subject 04-R04. This dog presented with chondroblastic osteosarcoma of the right forelimb with a LD measurement of 94.5 mm at baseline (CT measurement not available). Amputation was pursued 20 days after *C. novyi-NT* spore injection due to progressive disease. The other two subjects have well tolerated the single treatment cycle. Their late-onset AE was exclusively limited to a mild fever (Grade I).

Table 15

Summary of later onset^a adverse events of any grade after first treatment cycle

Adverse Event	Type	Number of dogs ^b (N=3)	Incidence (%)	Days to Finding ^c
Fever	General signs or symptoms	1	33.3%	9
Pain	General signs or symptoms	1	33.3%	20
Surgical site disorder	Systemic disorders NOS	1	33.3%	24
Neutrophils increased	Blood and lymphatic system	1	33.3%	34
RBC decreased	Blood and lymphatic system	1	33.3%	34
Eosinophils increased	Blood and lymphatic system	1	33.3%	61
WBC increased	Blood and lymphatic system	1	33.3%	61
Tumor new mass	Neoplasia	1	33.3%	82
Lymphadenopathy	Lymph node disorders	1	33.3%	82
Thrombocytes decreased	Blood and lymphatic system	1	33.3%	93

^a After 7 days following a single treatment only. ^b Number of dogs with at least one adverse event of any grade. ^c From day of first treatment.

[0171] In summary, the safety profile observed following one treatment cycle of *C. novyi-NT* IT administration of 1×10^8 spores suggested suitable tolerability. The early- onset and late-onset adverse events were consistent with the anticipated tumor inflammatory reactions resulting from the mechanism of action of *C. novyi-NT*. The adverse events have been monitored and managed effectively as disclosed herein.

[0172] The adverse events noted when dogs were given multiple treatment cycles of *C. novyi-NT* by IT administration are summarized in Table 9 for adverse events (AEs) of any Grades and in Table 10 for AEs of Grade III and above.

[0173] The variety and incidence of adverse event findings following multiple cycles of treatment was broadly similar to that observed following a single treatment cycle. Likewise, the onset of events appeared to be largely consistent with what was observed following a single treatment cycle: of 169

findings across all cases, only 30 were noted more than seven days following a prior dose. Similarly, tumor inflammation, anorexia, and fever were the most commonly observed events. Adverse events that occurred in more than one case included: target lesion reactions, alterations in general signs and symptoms, blood and lymphatic system abnormalities, lameness, hypertension, lymphadenopathy, diarrhea, and new masses. The majority (about 95%) of findings were mild to moderate in intensity (Grade I to II).

Severe adverse events

[0174] Severe adverse events (Grade III and greater) were noted in 5 cases (Table 16). Subject 04-R05 experienced a Grade III increase in neutrophil count. Subject 10-R01 experienced Grade III anemia, lethargy, muscle weakness, myositis, pain and recumbency. Extensive metastatic disease, while not observed at baseline, was diagnosed following necropsy of case 10-R01 at Day 60; progressive disease may have influenced adverse event findings in this case. Subject 10-R02 experienced a Grade III tumor abscess. Subject 11-R01 experienced a Grade IV decreased thrombocyte count 93 days after first treatment cycle which resolved without intervention. Symptoms resolved 21 days after the Day 93 visit without any medical treatment. Notably, this subject also exhibited Grade I and Grade III symptoms of thrombocytopenia at screening and baseline, respectively. Subject 16-R03 experienced Grade III diarrhea, lameness and tumor inflammation that resolved within one week.

Table 16

Summary of adverse events greater than or equal to Grade III for all treatment cycles

Adverse Event	Type	Number of dogs ^a (N=16)	Incidence (%)
Lameness	Musculoskeletal disorders	3	18.8%
Pain	General signs or symptoms	2	12.5%
Anemia	Blood and lymphatic system	1	6.3%
Neutrophils decreased	Blood and lymphatic system	1	6.3%
Thombocytes decreased	Blood and lymphatic system	1	6.3%
Diarrhea	Digestive tract disorders	1	6.3%
Lethargy	General signs or symptoms	1	6.3%
Steatitis	General signs or symptoms	1	6.3%
Myositis	Musculoskeletal disorders	1	6.3%
Tumor abscess	Target Lesion reaction	1	6.3%
Tumor inflammation	Target Lesion reaction	1	6.3%

^a Number of dogs with at least one adverse event of any grade.

[0175] Two dogs had documented new masses during the study. A rectal mass was identified in subject 04-R04 on Day 82 and a lytic vertebral lesion of T1 in subject 10-R01 on Day 9. These findings may represent a metastasis or a second distinct pathology. In both cases, the relationship to *C. novyi-NT* therapy was unclear.

Response from *C. novyi-NT* therapy

[0176] In summary, *C. novyi-NT* IT treatment in companion dogs at a dose of 1×10^8 spores per cycle of therapy for up to 4 cycles is well tolerated. Most adverse events possibly or probably related to drug that were greater than Grade III resolved within one week. Expected adverse events have been largely associated with local inflammatory changes following intratumoral therapy and generally resolved within one week. The adverse events and serious adverse events have been monitored and managed effectively as disclosed herein.

[0177] Given that *C. novyi-NT* IT administration was accompanied by broad evidence of biological activity, a preliminary assessment of primary tumor response using RECIST 1.1 was made and is summarized in Table 17 below.

Table 17

Summary of clinical evidence of germination and response from *C. novyi-NT* therapy

Case ID	Clinical evidence of germination^a	Clinical Response^b
01-R02	Tumor inflammation, skin disorder and disorder	PD
04-R01	Tumor inflammation and pain	CR
04-R02	Tumor inflammation and abscess	PR
04-R03	Tumor inflammation, consistency change, discharge and tumor pain	CR
04-R04	Tumor inflammation and pain	NE
04-R05	Tumor inflammation, consistency change, skin disorder and pain	PR
04-R06	Tumor inflammation, abscess and discharge	CR
04-R08	Tumor abscess and discharge	NE
10-R01	-	PD
10-R02	Tumor inflammation, abscess and pain	SD
11-R01	Tumor inflammation and abscess	PR
11-R02	Tumor inflammation	SD
11-R04	Tumor abscess and consistency change	SD

Case ID	Clinical evidence of germination ^a	Clinical Response ^b
16-R02	Tumor inflammation	PD
16-R03	Tumor inflammation and abscess	SD
26-R01	-	SD

[0178] Dogs were evaluated for best response on or after day 21 of the study. Three had a complete response (CR) to therapy, three had partial responses (PR), five had stable disease (SD), three had progressive disease (PD), and two dogs (04-R04 and 04-R08) were not evaluable for response because the injected tumor was surgically resected before day 21. The objective response rate for biosurgery was 37.5% (6 of 16 dogs; 95 percent confidence interval: 15.2 – 64.6%). Tumor abscesses and responses occurred after one to four cycles of biosurgery. Dog 11-R01 experienced a PR after a single cycle, 04-R03 had a CR after three cycles, dogs 04-R02 and 04-R05 had PRs after four cycles, while 04-R01 and 04-R06 had CRs after four cycles. Figures 7A-F and Figures 8A-F show representative changes in dogs with partial (11-R01) and complete responses (04-R03), respectively. Resolution of abscesses occurred with debridement and wound healing was complete after 2 to 4 weeks. However, overt abscess formation was not always observed before an objective response. Dogs 04-R01 and 04-R06 received 4 cycles of biosurgery, with tumor inflammation, but not abscessation, observed up to the day 21 study visit. Even so, complete responses were noted on the day 42 (unscheduled visit) and day 60 study visits in these two dogs, respectively.

[0179] Individual subjects are discussed in more detail below:

[0180] Andy (11-R01, Figures 7A-F), a 10 year-old, neutered male, Maltese, presented with a grade II soft tissue sarcoma on the left pinna. His treatment history included surgery prior to enrollment. He received a single dose of *C. novyi-NT* spores on June 18, 2012. Andy experienced Grade I tumor swelling on Day 1 (June 19, 2012). Abscess formation led to ulceration of the tumor and discharge of purulent, necrotic material. The resulting wound healed without complication. During the extended follow-up period, a Grade IV thrombocytopenia was observed on Day 93 (September 19, 2012) that resolved at a routine follow-up visit a few weeks later. A thickened cutaneous area of approximately 8 mm remained after wound healing (see Figure 9 for a time course of tumor measurements over the course of the study). This may have represented scar tissue or residual tumor.

[0181] Molly (11-R02), a 12 year-old, neutered female, Labrador Retriever, presented with a grade II soft tissue sarcoma on the left stifle. She had no treatment history prior to enrollment. She received 3 cycles of IT *C. novyi-NT* spores, followed by 1 IV dose of 1×10^8 *C. novyi-NT* spores, 7 days after the 3rd IT dose. Her 1st, 2nd and 3rd IT doses on July 11, 2012, July 18, 2012, and July 25, 2012, respectively. The single IV dose of *C. novyi-NT* spores was given on August 1, 2012 due to lack of biological activity seen with the prior IT doses. The only adverse event noted was Grade I hypertension after the 3rd IT dose. Hypertension was transient and self-limiting, resolving within 1 hour. Molly's tumor was surgically removed on Day 30 (August 10, 2012) for histologic analysis. The mass was confirmed to be a soft tissue sarcoma with areas of necrosis and inflammation. Bacteria were not present on gram stains, supporting lack of biological activity in this case.

[0182] Ricky (10-R01), a 13 year-old, male neutered, Golden retriever, presented with oral melanoma. His treatment history included surgery prior to enrollment. He received 2 cycles of IT *C. novyi-NT* spores. *C. novyi-NT* IT treatments were administered on August 2, 2012 and August 9, 2012. On Day 9 (August 11, 2012), Ricky developed sudden onset of cervical pain and rear leg neurological deficits 2 days after the 2nd treatment cycle. Grade III anemia was also noted. An MRI was performed and revealed probable cervical steatitis and cervical spinal cord compression. Corticosteroids and gastrointestinal protectants were administered and Ricky recovered after 3 days. No changes in the oral melanoma were noted and no additional *C. novyi-NT* treatments were administered. On Day 21 (August 23, 2012), an MRI was performed and showed improvement in the previously described steatitis; however, metastatic pulmonary nodules were noted on CT of the thorax. Excision of the oral melanoma was performed. A human tyrosinase melanoma vaccine was started on August 30, 2012. On Day 42 (September 13, 2012), Ricky presented with recurrent cervical pain and forelimb pain (2 weeks after discontinuation of corticosteroids) and 2 weeks after receiving the melanoma vaccine. Medical management with pain medication did not result in improvement after 4 days so corticosteroids were restarted. On Day 46, Grade III anemia and elevated BUN were noted. A presumptive gastrointestinal bleed was treated with gastrointestinal protectants. On Day 60, Ricky collapsed and developed hematemesis. Humane euthanasia was performed. A necropsy revealed disseminated metastatic melanoma including submandibular lymph node, mediastinal lymph node, mesenteric lymph node, kidney, and perispinal fat in the region of the cervical spine. No

evidence of gastric or intestinal ulceration was found. The presumed cause for the two episodes of spinal pain is metastatic melanoma. The relationship to *C. novyi-NT* is uncertain.

[0183] Finnegan (04-R02), an 11 year-old, entire male, Golden Retriever, presented with a soft tissue sarcoma (hemangiopericytoma) on the right lateral metacarpus. His treatment history included surgery prior to enrollment. He received 4 cycles of IT *C. novyi-NT* spores. Adverse events were mild and well tolerated. Complete ablation of the tumor occurred after 4 cycles of treatment, leaving a margin of normal tissue about the site of the tumor. Finnegan received his 1st, 2nd, 3rd and 4th treatment cycles on August 3, 2012, August 10, 2012, August 17, 2012 and August 24, 2012, respectively. Administration of *C. novyi-NT* was associated with only Grade I adverse events reported after the 1st, 2nd and 3rd cycles. Grade I and II adverse events were noted 48 hours after the 4th dose. Tumor infection was noted and consisted of fever, leukocytosis, neutrophilia and tumor-associated pain and abscess formation. Infection progressed to abscess formation and ablation of the entire tumor with minimal debridement occurring 96 hours after the 4th dose. Tumor measurements at this visit were recorded in the morning prior to complete ablation of gross tumor later that day. Amputation of the limb was pursued instead of open-wound management on Day 25 (August 28, 2012) and antibiotics were given. Finnegan recovered uneventfully from surgery and remains grossly tumor free 94 days (November 05, 2012) after his first treatment.

[0184] Drake (04-R01, Figure 10A), a 7 year-old, neutered male, Golden Retriever, presented with a soft tissue sarcoma (fibrosarcoma) in the

right mid maxillary region. He had no treatment history prior to enrollment. He received 4 cycles of IT *C. novyi-NT* spores. Adverse events were mild and well tolerated. Complete ablation of the tumor occurred after 4 cycles, leaving a margin of normal tissue about the site of the tumor. Drake received his 1st, 2nd, 3rd, and 4th treatments on August 13, 2012, August 20, 2012, August 27, 2012, and September 4, 2012, respectively. The intervals between 1st, 2nd, and 3rd doses were 7 days; while the interval between 3rd and 4th doses was 8 days in observance of a national holiday. Administration of *C. novyi-NT* was associated with mild adverse events, including Grade I lethargy and inappetence and Grade II vomiting and hematochezia reported 24-48 hours after the 1st cycle. These AEs were treated successfully with an anti-emetic and antibiotic. AEs were noted within 24 hours of the 4th dose, including Grade I tumor pain and swelling. Further evidence of tumor infection and abscess formation was not observed. Ablation of the tumor was evident on day 60 (October 12, 2012) and the tumor was not measurable (see Figure 10B for a time course of tumor measurements over the course of the study). The region was firm and remained slightly swollen and a CT scan was performed. Drake remains free of tumor on day 86 (November 7, 2012) after 1st dose.

[0185] Baxter (04-R03, Figures 8A-F), a 9 year-old, neutered male, Boxer, presented with a grade II soft tissue sarcoma on the left medial antebrachium. He had no treatment history prior to enrollment. He received three cycles of IT *C. novyi-NT* spores. Adverse events were mild and well tolerated. Complete ablation of the tumor occurred after three injections, leaving a margin of normal tissue about the site of the tumor. Baxter received

his 1st, 2nd and 3rd doses of *C. novyi-NT* spores on August 17, 2012, August 24, 2012, and August 31, 2012, respectively. Administration of *C. novyi-NT* was well tolerated, with no study agent related toxicity reported after the 1st or 2nd dose. Study-related adverse events were noted 24 hours after the 3rd dose. These adverse events were associated with tumor infection and consisted of fever, anorexia, lethargy and tumor-associated pain, swelling and bleeding. Adverse events were mild (Grade II or lower) and were managed with supportive care and analgesics. *C. novyi-NT* related tumor infection progressed to involve the entire tumor and abscess formation. Surgical debridement of the tumor on September 2, 2012 resulted in rapid resolution of AEs. Wound healing was without complication and complete by October 16, 2012. Baxter remains grossly tumor free at 94 days (November 19, 2012) after his first treatment (see Figure 11 for a time course of tumor measurements over the course of the study).

[0186] Harley (26-R01), a 7 year-old, neutered male, Labrador Retriever, presented with a grade II soft tissue sarcoma (hemangiopericytoma) on the right paw. He had no treatment history prior to enrollment. He received 4 cycles of IT *C. novyi-NT* spores. The 1st, 2nd, 3rd and 4th doses were given on August 20, 2012, August 27, 2012, September 4, 2012 and September 10, 2012. The interval between doses was 6-8 days. A baseline elevation of temperature was noted at the time of the 1st and 2nd doses. IT treatment of *C. novyi-NT* spores was well tolerated with no adverse events reported. There was no response to therapy.

[0187] Ursula (04-R-04), an 11 year old, female spayed, Saint Bernard mix, presented with chondroblastic osteosarcoma of the right forelimb. Her

treatment history included surgery prior to enrollment. She received a single IT dose of *C. novyi-NT* spores. No metastatic disease was present at enrollment. Following the first treatment on August 31, 2012, tumor abscess formation and peritumoral inflammation was evident within the first 24 hours and medically managed with pain medication, warm compresses and intravenous crystalloids. After no improvement, the tumor/abscess was lanced on Day 2 (September 2, 2012). Moderate serosanguineous fluid was present. An anaerobic culture isolated *C. novyi*. Antibiotics were administered starting on Day 4 (September 4, 2012). The incision was managed as an open wound until Day 20 (September 20, 2012) when amputation was pursued for progressive disease. Histopathology revealed severe necrosis and hemorrhage along with persisting chondroblastic osteosarcoma. Following amputation, an incision site infection was noted. Cultures did not reveal *C. novyi*. No adjuvant therapy was pursued following amputation. On Day 81 (November 21, 2012), Ursula presented for rectal prolapse and was found to have rectal polyps. Thoracic radiographs performed at the time of this evaluation revealed pulmonary metastasis.

[0188] Gabriel (16-R02), a 9 year-old, neutered male, Labrador Retriever, presented with a grade I soft tissue sarcoma on the left lateral thigh. His treatment history included surgery prior to enrollment. He received 4 cycles of IT *C. novyi-NT* spores. IT administration of *C. novyi-NT* was generally well tolerated with a 1 week delay between the 1st and 2nd doses due to Grade II diarrhea that responded to medical management. Gabriel received his 1st, 2nd, 3rd and 4th doses on September 12, 2012, September 26, 2012, October 3, 2012 and October 10, 2012 respectively. Toxicity was

mild and consisted mainly of diarrhea and constitutive symptoms. Grade II diarrhea was noted after each dose and responded well to medical management. After the 1st dose, a 1- week dose delay was implemented resulting in a 14 day interval between the 1st and 2nd doses. Dose delays were not implemented for further doses for Grade II diarrhea. Additionally, Grade II tumor swelling was observed on Day 4 (September 16, 2012). Tumor size remained stable from D0 (September 12, 2012) to D63 (November 14, 2012), the most recent study visit.

[0189] Buddy (04-R05), a 13 year-old, neutered male, Shetland sheepdog, presented with soft tissue sarcoma (rhabdomyosarcoma) on the right antebrachium. His treatment history included surgery, chemotherapy, and a previous *C. novyi-NT* clinical trial prior to enrollment. No metastatic disease was noted at the time of study entry. He received 4 cycles of IT *C. novyi-NT* spores. Clinically significant adverse events contemporaneous with *C. novyi-NT* were isolated to a Grade III neutropenia and fever following the 3rd cycle of therapy. This event resolved within 48 hours of medical management with intravenous antibiotics and fluid therapy. Buddy received his 1st, 2nd, 3rd and 4th treatment cycles on September 20, 2012, September 27, 2012, October 5, 2012, and October 12, 2012. Mild tumor inflammation (erythema, warmth, swelling) was noted associated with 2 of the 4 cycles. A transient decrease in tumor size was noted at Day 4 (September 24, 2012). A new non-target lesion was noted near the primary tumor site on Day 21 (October 12, 2012). The primary target tumor was stable at Day 61.

[0190] Amber (16-R03), a 10 year-old, neutered female, Shepherd, presented with a grade I soft tissue sarcoma on the left paw, palmar and

dorsal surfaces. Her treatment history included surgery prior to enrollment. She received 4 cycles of IT *C. novyi-NT* spores. The 1st, 2nd, 3rd and 4th doses were given on September 26, 2012, October 3, 2012, October 15, 2012, and October 24, 2012. The interval between doses was 7-12 days. Amber experienced Grade II tumor swelling and pain after her 1st and 2nd doses. Grade I inappetence was noted on Day 2 (September 28, 2012). On Day 8 (October 4, 2012, 1 day after 2nd dose), a Grade I fever, Grade II tumor warmth and Grade III lameness was noted. Her tumor was lanced and analgesics were given. A Grade III diarrhea was noted on Day 11 (October 7, 2012) and managed medically. Due to the tumor associated adverse events and diarrhea, the 3rd dose was delayed until Day 19 (October 15, 2012). Grade II tumor swelling was again observed on Day 19, after the 3rd dose of *C. novyi-NT* and this was managed with analgesics. No adverse events were noted after the 4th dose.

[0191] Six (11-R04), a 9 year-old, neutered male, Husky, presented with a grade I soft tissue sarcoma on the right paw. She had no treatment history prior to enrollment. She received 4 cycles of IT *C. novyi-NT* spores. Six received the 1st, 2nd, 3rd and 4th doses on October 1, 2012, October 8, 2012, October 15, 2012, and October 22, 2012, respectively. Administration of *C. novyi-NT* spores was well tolerated with only mild adverse events observed. After the 1st dose, Grade I hypertension and fever were noted. Fever and hypertension were self-limiting and resolved within 1 and 2 hours of dosing respectively. On Day 4 (October 5, 2012), the tumor was subjectively softer and a small area of ulceration (Grade I) was observed at the site of a previous biopsy. Ulceration continued to Day 31 (November 1, 2012), the

most current study visit. This ulceration may be associated with either the study agent or a complication of the biopsy required for study enrollment.

[0192] Belle (04-R06), an 11 year-old, female spayed, Labrador retriever, presented with a mast cell tumor (originally aspirated as a soft tissue sarcoma) on the right rear digit 3 with metastasis to the popliteal lymph node. She had no treatment history prior to enrollment. She received 4 cycles of IT *C. novyi-NT* spores. Adverse events were mild and limited to Grade I fever and Grade I tumor inflammation. Belle received the 1st, 2nd, 3rd and 4th treatment cycles on October 19, 2012, October 26, 2012, November 2, 2012, and November 9, 2012. Grade I fever contemporaneous with *C. novyi-NT* treatment and tumor inflammation. Fever and inflammation were self-resolving without the need for medical management other than protocol required subcutaneous fluids administered on scheduled study visits. Ulceration of the tumor was noted on Day 21 (November 9, 2012). Photographs of the tumor sent to the investigator by the dog owner showed resolution of the ulceration and marked regression in the mass. An unscheduled visit was performed on Day 46 (December 4, 2012) to capture tumor response assessment. Complete regression of the tumor was noted.

[0193] Frida (11-R01), a 7 year-old, female spayed, German shepherd mix, presented with a soft tissue sarcoma (hemangiopericytoma) on the right rear paw with possible lymph node metastasis (based on CT). Her treatment history included surgery prior to enrollment. She traveled with her owner from Mexico to participate in this clinical trial. She received 3 cycles of IT *C. novyi-NT* spores. Adverse events were limited to a waxing and waning fever for 48 hours, which resolved with intravenous fluids and NSAIDs. Frida received the

1st, 2nd, and 3rd cycles of therapy on November 6, 2012, November 14, 2012, and November 21, 2012. The only significant adverse events included Grade I fever requiring hospitalization and fluids starting on Day 4 (November 10, 2012) and progressing to Grade II fever on Day 5 (November 11, 2012). The fever resolved after 48 hours. A Grade I fever was also noted after the 3rd cycle of therapy on Day 18 (November 24, 2012). Tumor progression prompted amputation on Day 21 (November 27, 12).

[0194] Mhija (01-R02), a 7 year-old, neutered male, Border Collie, presented with soft tissue sarcoma (peripheral nerve sheath tumor) on the left thoracic flank. She had no treatment history prior to enrollment. She has received 3 cycles of IT *C. novyi-NT* spores. Adverse events were mild and well tolerated. Tumor inflammation, heat and serosanguineous to mucopurulent discharge are probably related to *C. novyi-NT* activity. A 4th cycle of *C. novyi-NT* spores is planned. Mhija received the 1st, 2nd and 3rd doses on November 12, 2012, November 20, 2012, and November 27, 2012, respectively. The interval between 1st and 2nd doses was 8 days; while the interval between 2nd and 3rd doses was 7 days. Administration of *C. novyi-NT* was associated with mild, Grade I – II toxicity. Grade I nausea and regurgitation was noted after the 1st dose, with Grade I inappetence and lethargy noted after the 3rd dose. Toxicities resolved shortly with medical management. Most toxicities were localized to the tumor site, Grade I or II in severity (heat, inflammation, pruritis, serosanguineous to mucopurulent discharge and erythema) and occurring within 2 days of an administration of *C. novyi-NT*. Additionally, Grade I – II ventral edema was observed 2 days after the 1st and 3rd doses.

[0195] Tank (10-R02), a 10 year-old, male neutered, mixbreed, presented with soft tissue sarcoma (hemangiopericytoma) on the right flank. His treatment history included surgery prior to enrollment. He received 1 cycle of IT *C. novyi-NT* spores on November 12, 2012. Grade I fever, decreased appetite, Grade II edema surrounding the tumor, and Grade III tumor abscess were noted on Day 4 (November 16, 2012) following treatment. Medical management including pain medication, IV fluids, and broad-spectrum antibiotics were used to manage the abscess. Tumor inflammation and surrounding edema resolved on Day 11 (November 23, 2012). Tank received a 2nd treatment cycle on December 3, 2012. The interval between cycles was 21 days. The 2nd dose was delayed due to the antibiotics washout period.

[0196] Time courses of tumor measurements from eight of the dogs are shown in Figure 12A. Figure 12B shows three time courses that were shortened due to amputation or data cut-off.

[0197] In summary, *C. novyi-NT* administered by IT injection at a dose of 1×10^8 spores per cycle with up to 4 cycles of treatment exhibits meaningful biological and anti-tumor activities and appears to be well-tolerated in companion dogs with naturally occurring solid tumors. Tumor responses are rapid, with significant tumor necrosis and notable disease regression occurring within days of *C. novyi-NT* administration. Most adverse events are limited to Grade 1 and Grade 2, and are consistent with the mechanism-based tumor inflammatory reactions expected from the *C. novyi-NT* therapeutic. Several cases are currently under long-term follow-up for assessment of progression and survival.

Example 8**Intratumoral (IT) administration of *C. novyi-NT* – Study 2 Methods**

[0198] A study characterizing dose and volume of *C. novyi-NT* administration by IT injection for the treatment of dogs with solid tumors (excluding osteosarcoma or mast cell tumor) is being performed.

[0199] Dogs with solid tumors (except osteosarcoma or mast cell tumor) of any weight, breed, sex, or age were screened for enrollment. Inclusion criteria was similar to that presented in Example 6, with the exception that each dog had a cytologic or histologic diagnosis of any cancer excluding osteosarcoma or mast cell tumor, and that each dog had at least 1 measurable tumor lesion with a longest diameter ≥ 1 cm.

[0200] During the initial screening visit each dog was assigned a unique study dog identification number consisting of a 5-digit numeric code (which may not be sequentially in order of the screening dog number). The first 2 digits indicated the study site (01 to 99), the middle digit indicated the study '5', and the last 2 digits described the study dog number within a study site (01 to 99). For example the 11th dog enrolled at Site 9 was assigned study dog number 09-511. Study dog numbers were assigned chronologically in the order that dogs were enrolled at a given study site. A dog was considered enrolled in the study when it satisfied the inclusion and exclusion criteria.

[0201] Gross pathology, histopathology, and necropsy were performed as described in Example 6.

[0202] *C. novyi-NT* spores were prepared as set forth above prior to shipment at a concentration of 1×10^8 spores/mL and suspended in sterile saline in 2 mL cryovials. Each cycle of *C. novyi* treatment was composed of up to 5 injections of 1 mL spore suspension (1×10^8 spores) for each injection into a single target lesion. The spore suspension containing 1×10^8 spores was packed in individual cryovials for each 1 mL injection, and the vial, syringe, and needle were discarded after each injection.

[0203] The scheme for injection is shown in Figure 13. Five 1 mL injection sites (as represented by squares) were distributed within the tumor: center, and four (4) evenly allocated injection sites within the tumor. The site for each 1 mL injection further consisted of 5 redirection sites (as represented by circles in Figure 13). Each redirection site received 200 μ L of spore suspension. The needle was first directed within the center of the injection site, and then evenly redirected to the four corners of the injection site without withdrawing the needle. Upon the completion of the first 1 mL injection, the needle was withdrawn and the syringe was discarded. The depth of each injection should be adequately distributed such that the best distribution is achieved. The recommended size of syringe was 1 mL for each injection, the recommended needle was between 22-gauge and 25-gauge. Adequate length of needle should be selected based on the depth of the tumor lesion.

[0204] All dogs were hospitalized from D0 to D2, and then at the Investigator's discretion for 24 to 48 hours after each subsequent treatment for clinical observation. Fluids were administered to all study dogs during hospitalization following *C. novyi-NT* treatment. On dosing days all dogs were

administered IV crystalloids at 4 ml/hg/h for 2 hours post-treatment with *C. novyi-NT*.

[0205] Study visits and events are summarized in Table 18, as an example of an 8-cycle treatment regimen. The dosing interval was suggested to be weekly if the intent was to treat the dog with multiple cycles of therapy.

Table 18

Summary of study visits and events

	Screen	Cycle 1*	Cycle 2 - 8†	D70 ± 7 days	D90 ± 7 days
	D-14 to D0	D0	D__		
Informed consent	X				
Demographics	X				
Weight and vitals	X	X	X	X	X
Physical examination	X	X	X	X	X
Lab samples	X	X	(X)	(X)	X
Research blood samples	X	X	X	X	X
Research tumor sample	X				
Diagnostic imaging	X				X***
Performance score	X				
Inclusion/exclusion	X				
Enrollment	X				
Tumor measurement	X	X	X	X	X
<i>C. novyi-NT</i> *		x*	x†		
Crystalloids**		x**	x**		
Study completion					X††

*Owners will leave their dog in clinic from the D0 until D2, and IV crystalloids will be administered to all dogs in hospital. For subsequent cycles, Investigators will fill in the D according to the number of days on study, relative to D0.

**Dogs will be administered IV crystalloids.

*** Thoracic radiographs only.

† Dogs may not receive 8 cycles. For this study, the decision to continue subsequent cycle of dosing will be made on a case by case basis via consultation among the Medical Director, Investigator and Sponsor.

†† Following study completion and if systemic antibiotics were required to manage adverse events, it is recommended to administer doxycycline 5-10 mg/kg PO BID to dogs for 3 months.

Example 9

Intratumoral (IT) administration of *C. novyi-NT* – Study 2 Interim Results

[0206] As of December 2, 2012, two companion dogs have been treated in the study. Both animals received a dose level of 5×10^8 spores administered at 5 unique IT injection sites per treatment cycle.

[0207] The first dog, Buddy (04-503), a 9 year-old, male neutered, Belgian malinois, presented with soft tissue sarcoma on the left carpus with a LD measurement of 69 mm at baseline (4.4 x 3.3 x 0.7 cm by CT). His treatment history included surgery prior to enrollment. He received 2 cycles of IT *C. novyi-NT* spores. Adverse events were mild and limited to Grade I fever and Grade I tumor inflammation. Buddy received the 1st and 2nd treatment cycles on November 21, 2012 and November 28, 2012. Grade I fever and tumor redness, swelling and increased pain were noted within 6 hours of the first injection. The fever resolved within 6 hours following treatment with the NSAID carprofen. Mild tumor ulceration was noted on Day 2 (November 23, 2012) following treatment. At Day 7 (November 28, 2012), a slight decrease in the size of the mass was noted (-12.0%). Each cycle of treatment was well tolerated with no adverse events greater than Grade I.

[0208] The second dog, Guinness (04-502), a 9 year-old, male neutered, Wheaton terrier, presented with squamous cell carcinoma on the left shoulder with a LD measurement of 122 mm at baseline (9.1 x 9.3 x 14.5 cm by CT), a low-grade hemangiosarcoma on the rear leg, and evidence of

pulmonary metastasis (based on CT). His treatment history included surgery prior to enrollment. Preexisting mitral valve disease was evident based on echocardiography performed prior to enrollment. He received a single dose of IT *C. novyi-NT* spores on November 28, 2012. Grade III fever was noted within 6 hours of treatment and medically managed with IV fluids. On Day 1 (November 29, 2012), abscess of the mass, purulent discharge, and neutrophilia were appreciated. IV fluids were continued and pain medications (including NSAIDs) were started. On Day 2 (November 30, 2012), progressive tumor swelling and evidence of sepsis (fever, neutropenia, hypoglycemia, hypoalbuminemia) prompted lancing of the tumor and irrigation. Broad-spectrum antibiotics, hetastarch and human albumin were administered. On Day 3 (December 1, 2012), progressive decline in status was noted resulting in respiratory distress. Euthanasia solution was administered. A necropsy was performed. Gross clinically significant findings included vegetative endocarditis, suppurative lung nodules, and whole-body subcutaneous hemorrhage and edema. Postmortem aerobic cultures from various tissues and organs (lung, liver, heart, kidney, spleen, GI, stomach) revealed polymicrobial bacterial growth (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Streptococcus* species); anaerobic cultures from all organs and tissues were negative for *C. novyi-NT* growth except in the tumor tissue and urinary bladder. Histopathology of affected tissues are pending. Septic toxemia shock is considered the most likely cause of death and relationship to *C. novyi-NT* therapy is unknown at this time.

Example 10
Intratumoral (IT) administration of *C. novyi-NT* in Humans – Methods

Phase I human clinical trial of IT injected *C. novyi-NT* spores

[0209] An open-label, non-randomized, multi-center phase I safety study of a single IT injection of *C. novyi-NT* spores is currently ongoing in patients with treatment-refractory solid tumors. The clinical study protocol was reviewed and approved by the Institutional Review Board (IRB) of each participating institution, and all regulatory steps were performed under the guidance of the Food and Drug Administration (FDA) (number NCT01924689). All patients were required to sign a written Informed Consent Form (ICF) before inclusion in the study.

[0210] The primary objectives of this phase I study were to determine the safety profile, dose limiting toxicities (DLT), and maximum tolerated dose (MTD) of IT injected *C. novyi-NT*. In addition, the anti-tumor activity of the therapeutic was explored.

Preparation and IT injection of *C. novyi-NT* spores in Phase I study

[0211] The clinical supply of *C. novyi-NT* spores was packaged in a single-use 2 mL sterile and pyrogen-free, Type I borosilicate glass vial with a rubber stopper and aluminum seal with a tamper resistant cap at a concentration of 8.52×10^8 spores/mL suspended in sterile phosphate buffered saline (PBS) with a 1.0 mL fill volume. The vials were stored between 2-8 °C in controlled temperature environment under constant temperature monitoring. The GMP product was manufactured and formulated by Omnia Biologics, Inc. (Rockville, MD).

[0212] After a patient was enrolled in the trial, one vial was shipped to the study site. Further preparation of C. novyi-NT was required and occurred on the same day of the IT injection. Dilution of the concentrated spore suspension was performed in a designated biological safety cabinet using sterile saline (0.9%) infusion bags of appropriate size to achieve the required dose based on the assigned cohort. The injection volume (3 mL) was then withdrawn from the saline bag and injected under radiographic guidance. C. novyi-NT spores were injected with an 18-gauge multi-prong needle (Quadra-Fuse®, Rex-Medical, Conshohocken, PA).

Design and conduct of human clinical trial

[0213] The study was conducted with a standard 3+3 dose-escalation design. Patients must have been diagnosed as having an advanced solid tumor malignancy with a target tumor that was measureable, palpable or clearly identifiable under ultrasound or radiographic guidance and amenable to percutaneous injection of C. novyi-NT spores. The targeted lesion must have a longest diameter ≥ 1 cm and be measurable as defined by RECIST 1.1 criteria. The main eligibility criteria included history of a treatment refractory malignancy; age of at least 18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 ; able to stay within 45 minutes driving time of an emergency room and having a caregiver for 28 days after IT injection. The main exclusion criteria were pregnancy; primary brain malignancy or brain metastases; clinically significant ascites or clinical evidence or history of portosystemic hypertension or cirrhosis; Glasgow Coma Score (GCS) < 15 ; serum creatinine level > 1.5 x the upper limit of normal (ULN), chronic renal failure requiring hemodialysis or peritoneal dialysis;

oxygen saturation (SpO₂) < 95 % (room air); mean arterial blood pressure (BP) < 70 mmHg; platelet count ≤ 100,000 /mm³; hemoglobin < 9.0 g/dL; absolute neutrophil count (ANC) < 1,000 /mm³; clinically significant pleural effusion, pericardial effusion, circumferential pericardial effusion, or any effusion greater than 1.0 cm at any location around the heart; need to ongoing treatment with an immunosuppressive agent; history of solid organ transplantation; systemic or localized infection.

[0214] Eligible patients were admitted and enrolled into a dose cohort. Under the protocol, patients remain hospitalized after spore administration and observed for 8 days, and patients return to the clinical site for routinely scheduled follow-up visits for 12 months, during which time assessments of safety and efficacy were performed.

[0215] Clinical response and progression were evaluated using the RECIST version 1.1. Objective responses were measured by serial CT or MRI scans of the injected tumor, as well as distant metastases (up to 5 target lesions). Safety monitoring for infectious complications or other treatment-emergent adverse events were continuously conducted for 12 months.

Example 11 **Intratumoral (IT) administration of *C. novyi-NT* in Humans – Results**

***C. novyi-NT* causes rapid local tumor destruction in the first human patient**

[0216] The promising outcomes and favorable risk/benefit profiles of biosurgery in the comparative canine trial, in conjunction with the results observed in rats, provided a rationale for attempting biosurgery in humans. Accordingly, a Phase I investigational study in human patients with solid tumors that were either refractory to standard therapy or without an available

standard therapy was initiated (NCT01924689). The first patient enrolled in this trial is reported herein: a 53-year-old female diagnosed with a retroperitoneal leiomyosarcoma in August 2006. The patient underwent several surgical resections and received multiple chemotherapy and radiotherapy treatments, including a right radical nephrectomy and radiation therapy in March 2007, chemotherapy with gemcitabine, taxol, adriamycin, and ifosfamide, resection of liver metastasis in November 2008, multiple wedge resections of right-sided pulmonary metastases in December 2009, trabectedin treatment from March 2010 to April 2011, multiple wedge resection of left-sided pulmonary metastases in December 2010, pazopanib treatment in April 2011, left lower lobectomy in October 2011, HAI abraxane, gemcitabine, and avastin from February 2012 to January 2013, everolimus and pazopanib from February 2013 to July 2013, and bland arterial hepatic embolization in August 2013 and September 2013. However, the patient progressed, with metastatic disease present in her liver, lungs, peritoneum, and soft tissue in the right shoulder and adjacent right humerus.

[0217] Biosurgery was performed with the planned starting dose of 1×10^4 *C. novyi*-NT spores injected into her metastatic right shoulder tumor with an 18-gauge multi-prong needle (day 0, November 19, 2013).

CT-guided intratumoral injection using a three-pronged needle

[0218] The subject was placed under moderate sedation with fentanyl and versed for 35 minutes. An 18-gauge Quadra-Fuse device (Rex Medical) (Figure 16A) was employed for injection under CT guidance by inserting the 3-pronged needle (27g) in the target injection area (Figures 16B and 16C). Three tines (each having 2 through holes, for 4 fluid exits) (Figure 16D) were

deployed at 4, 3, and 2 cm at which location (Figure 16E), a 1 ml aliquot of *C. novyi*-NT spore solution was injected during the staged retraction process. The device was removed after the deployed tines were fully retracted into the needle cannula and manual compression was utilized to achieve hemostasis.

[0219] On day 1, the patient experienced mild right shoulder pain extending to the scapula, which responded to tramadol and acetaminophen. On day 2, her pain required IV patient controlled analgesia with hydromorphone, her leukocyte count increased to 18,300 per μL , and she developed fever with a maximum temperature of 39.2°C . On day 3, the pain in the patient's right shoulder and scapula was difficult to control. Her maximum temperature was 37.8°C . The CT scan of the right upper extremity demonstrated extensive tumor destruction with gas in the soft tissue and bony component of the tumor (Figure 14A). Necrosis of her humerus was discussed. A CT-guided aspirate of her tumor revealed *C. novyi*-NT growth under anaerobic culture conditions. The patient was then started on antibiotics and defervesced shortly after. On day 4, a MRI of the right upper extremity demonstrated markedly diminished enhancement confined to the tumor mass compared to baseline (Figures 14B and 14C). Biopsies from the tumor showed many gram-positive bacteria and an absence of viable tumor cells. At the time of the biopsies, a percutaneous drain was placed within the tumor abscess to drain fluid and debris. The patient remained afebrile and her leukocyte count gradually normalized. She continued on antibiotics and was kept in the hospital for IV analgesia until day 20 when she was transitioned to oral analgesics. She was discharged on orally administered metronidazole and doxycycline per protocol. On day 29, a follow-up MRI

demonstrated an ongoing reduction in tumor enhancement (Figure 14D). On day 55 the patient presented with localized pain as a result of a patient-effort induced pathologic fracture of the right proximal humerus. Subsequent partial resection of the humerus, debridement, and internal fixation with an intramedullary nail and cement spacer resulted in significant improvement in pain and an increase in range of motion. Intraoperative cultures revealed *C. novyi*-NT growth under anaerobic culture conditions. Histopathology demonstrated extensive tumor necrosis with small foci of residual tumor cells. (Figures 15A-D). The patient continues to be monitored and has a performance status of 1 on the Eastern Cooperative Oncology Group scale (ECOG) with no clinical signs of infection.

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[0220] All documents cited in this application are hereby incorporated by reference as if recited in full herein.

[0221] Although illustrative embodiments of the present invention have been described herein, it should be understood that the invention is not limited

to those described, and that various other changes or modifications may be made by one skilled in the art without departing from the scope or spirit of the invention.

WHAT IS CLAIMED IS:

1. A method for treating or ameliorating an effect of a solid tumor present in a human comprising administering intratumorally to the human a unit dose of *C. novyi* colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.
2. The method according to claim 1, wherein the solid tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, and melanoma.
3. The method according to claim 1, wherein the solid tumor is leiomyosarcoma.
4. The method according to claim 3, wherein the solid tumor is retroperitoneal leiomyosarcoma.
5. The method according to claim 1, wherein the unit dose comprises from about 1×10^6 - 1×10^7 *C. novyi* CFUs.
6. The method according to claim 1, wherein the unit dose comprises about 1×10^4 *C. novyi* CFUs.
7. The method according to claim 1, wherein the *C. novyi* CFUs are selected from the group consisting of vegetative and spore forms.
8. The method according to claim 1, wherein the *C. novyi* is *C. novyi* NT.
9. The method according to claim 8, wherein the unit dose comprises about 1×10^4 - 1×10^7 *C. novyi* NT spores.
10. The method according to claim 8, wherein the unit dose comprises from about 1×10^6 - 1×10^7 *C. novyi* NT spores.

11. The method according to claim 8, wherein the unit dose comprises about 1×10^4 C. novyi NT spores.
12. The method according to claim 1, wherein the administering step comprises injecting the unit dose at a single location into the tumor.
13. The method according to claim 1, wherein the administering step comprises injecting the unit dose at multiple unique locations into the tumor.
14. The method according to claim 1, wherein the administering step comprises injecting the unit dose at 1-5 unique locations into the tumor.
15. The method according to claim 1, wherein the administering step comprises injecting the unit dose at 5 or more unique locations into the tumor.
16. The method according to claim 1 further comprising administering a plurality of treatment cycles to the human, each treatment cycle comprising injecting one unit dose of the C. novyi CFUs into the solid tumor.
17. The method according to claim 16, wherein 1-10 treatment cycles are administered.
18. The method according to claim 16, wherein 2-4 treatment cycles are administered.
19. The method according to claim 16, wherein the interval between each treatment cycle is about 5-100 days.
20. The method according to claim 16, wherein the interval between each treatment cycle is about 7 days.
21. The method according to claim 9 further comprising administering IV fluids to the human before, during, and/or after each administration of the C. novyi NT spores.

22. The method according to claim 9 further comprising administering a plurality of treatment cycles to the human, each treatment cycle comprising injecting one unit dose of the *C. novyi* NT spores into the solid tumor.

23. The method according to claim 22, wherein 2-4 treatment cycles are administered.

24. The method according to claim 1 further comprising administering IV fluids to the human before, during, and/or after each administration of the *C. novyi*.

25. The method according to claim 1 further comprising providing the human with a first course of antibiotics for a period of time and at a dosage that is effective to treat or alleviate an adverse side effect caused by the *C. novyi*.

26. The method according to claim 25, wherein the antibiotics are administered for two weeks post *C. novyi* administration.

27. The method according to claim 25, wherein the antibiotics are selected from the group consisting of amoxicillin, clavulanate, metronidazole, and combinations thereof.

28. The method according to claim 25 further comprising providing the human with a second course of antibiotics for a period of time and at a dosage that is effective to treat or alleviate an adverse side effect caused by the *C. novyi*.

29. The method according to claim 28, wherein the second course of antibiotics is initiated after completion of the first course of antibiotics and is carried out for 1-6 months.

30. The method according to claim 28, wherein the second course of antibiotics is initiated after completion of the first course of antibiotics and is carried out for 3 months.

31. The method according to claim 28, wherein the antibiotic used in the second course is doxycycline.

32. The method according to claim 1, further comprising administering to the human a therapy selected from the group consisting of chemotherapy, radiation therapy, immunotherapy, and combinations thereof.

33. The method according to claim 32, wherein the immunotherapy comprises administering to the human an immune checkpoint inhibitor.

34. The method according to claim 1, wherein the solid tumor is resistant to a therapy selected from the group consisting of chemotherapy, radiation therapy, immunotherapy, and combinations thereof.

35. The method according to claim 32, wherein the chemotherapy comprises administering to the human an agent selected from the group consisting of an anti-metabolite, a microtubule inhibitor, a DNA damaging agent, an antibiotic, an anti-angiogenesis agent, a vascular disrupting agent, a molecularly targeted agent, and combinations thereof.

36. The method according to claim 32, wherein the chemotherapy comprises administering to the human an agent selected from the group consisting of gemcitabine, taxol, adriamycin, ifosfamide, trabectedin, pazopanib, abraxane, avastin, everolimus, and combinations thereof.

37. The method according to claim 1, wherein the solid tumor is refractory to standard therapy or the solid tumor is without an available standard therapy.

38. The method according to claim 1, which induces a potent localized inflammatory response and an adaptive immune response in the human.

39. A method for microscopically precise excision of tumor cells in a human comprising administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

40. A method for treating or ameliorating an effect of a solid tumor that has metastasized to one or more sites in a human comprising administering intratumorally to the human a unit dose of *C. novyi* NT colony forming units (CFUs) comprising at least about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

41. The method according to claim 40, wherein at least one site is distal to the original solid tumor.

42. A method for debulking a solid tumor present in a human comprising administering intratumorally to the human a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution.

43. The method according to claim 42, wherein the solid tumor is selected from the group consisting of soft tissue sarcoma, hepatocellular carcinoma, breast cancer, pancreatic cancer, and melanoma.

44. A method for debulking a solid tumor present in a human comprising administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores per cycle, each unit dose of *C. novyi* NT being suspended in a pharmaceutically acceptable carrier or solution.

45. A method for treating or ameliorating an effect of a solid tumor present in a human comprising administering intratumorally to the human one to four cycles of a unit dose of *C. novyi* NT spores comprising about 1×10^4 spores

per cycle, each unit dose of *C. novyi* NT spores being suspended in a pharmaceutically acceptable carrier or solution.

46. A method for ablating a solid tumor present in a human comprising administering intratumorally to the human a unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs suspended in a pharmaceutically acceptable carrier or solution, wherein the tumor is ablated leaving a margin of normal tissue.

47. The method according to claim 46, wherein the tumor is a sarcoma.

48. A unit dose of *C. novyi* CFUs comprising about 1×10^3 - 1×10^7 CFUs in a pharmaceutically acceptable carrier or solution, which is effective for treating or ameliorating an effect of a solid tumor present in a human.

49. The unit dose according to claim 48, wherein the *C. novyi* CFUs are selected from the group consisting of vegetative and spore forms.

50. The unit dose according to claim 48, wherein the *C. novyi* is *C. novyi* NT.

51. The unit dose according to claim 50, wherein the unit dose comprises about 1×10^4 - 1×10^7 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

52. The unit dose according to claim 50, wherein the unit dose comprises from about 1×10^6 - 1×10^7 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

53. The unit dose according to claim 50, wherein the unit dose comprises about 1×10^4 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

54. A kit for treating or ameliorating an effect of a solid tumor present in a human comprising a unit dose of *C. novyi* CFUs comprising about 1×10^3 - $1 \times$

10^7 CFUs in a pharmaceutically acceptable carrier or solution and instructions for use of the kit.

55. The kit according to claim 54 further comprising one or more antibiotics, which are effective to treat or alleviate an adverse side effect caused by the *C. novyi* CFUs.

56. The kit according to claim 54, wherein the *C. novyi* CFUs are selected from the group consisting of vegetative and spore forms.

57. The kit according to claim 54, wherein the *C. novyi* is *C. novyi* NT.

58. The kit according to claim 57, wherein the unit dose comprises about 1×10^4 - 1×10^7 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

59. The kit dose according to claim 57, wherein the unit dose comprises from about 1×10^6 - 1×10^7 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

60. The unit dose according to claim 57, wherein the unit dose comprises about 1×10^4 *C. novyi* NT spores in a pharmaceutically acceptable carrier or solution.

61. The kit according to claim 54 further comprising 1-4 unit doses of the *C. novyi* for carrying out 1-4 treatment cycles.

62. The kit according to claim 58 further comprising 1-4 unit doses of the *C. novyi* NT spores for carrying out 1-4 treatment cycles.