Abstract:
The present invention relates to improved methods of administering TLR3 agonists, particularly in combination with antigens and anti-cancer agents. Such methods are useful for example in the treatment of cancers.
IMPROVED TREATMENT OF CANCER USING TLR3 AGONISTS IN COMBINATION WITH ANOTHER THERAPEUTIC AGENT

[001] The present invention relates generally to the fields of genetics and medicine. More specifically, the present invention relates to improved methods of treating cancers using TLR3 agonists in combination with other anti-cancer agents.

INTRODUCTION

[002] Current cancer treatment options, such as surgery, chemotherapy and radiation treatment, are oftentimes either ineffective or present serious side effects. Recently, cancer therapies include treatment with immunotherapeutic agents. The therapeutic compounds include TLR3 agonists (WO 06/054 177, WO 06/014 653, Lacour et al Clinical Research 1984, Michelson et al Proc Soc Exp Biol Med 1985), although therapy yielded mixed results. TLR3 agonists have been reported to have a use in vaccine applications (WO 03/078 595, WO 03/028 656), but had never been reported to have applicability to the treatment of established tumors. It has also been recently proposed by Zitvogel et al (Nature Review 2008) that certain anti-cancer chemotherapeutic agents may have immunostimulatory effects in addition to their e.g. cytotoxic effects. However all current approaches pose significant drawbacks for the patient. Surgery, for example, may not completely remove the neoplastic tissue. Despite the availability of a variety of chemotherapeutic agents, chemotherapy has only a limited efficiency. Even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even those agents that act by mechanisms different from the mechanisms of action of the drugs used in the specific treatment; this phenomenon is termed pleiotropic drug or multidrug resistance.

Thus, because of drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols. There is thus a need for a novel method for treating a subject having a cancer, said method being capable of stopping the spread of the tumor and leading to increases in survival or well-being of the patient.

[003] Therefore, a method for treating a subject comprising administering to said subject a first dose of an antigen optionally together with an adjuvant followed by the administration of a TLR3 agonist.

SUMMARY OF THE INVENTION

[004] The present invention describes therapeutic regimen that lead to a more efficient treatment of cancer, comprising immunizing to subject in need thereof a first dose of an antigen optionally together
with an adjuvant and administering to said subject an effective amount of a TLR3 agonist, in a preferred embodiment, the method further comprises the administration of another anti-cancer agent.

[005] The present invention describes the treatment of a subject comprising administering to a subject in need thereof a first dose of an antigen optionally together with an adjuvant and administering to said subject an effective amount of a TLR3 agonist, preferably an antibody or other TLR3 binding protein, a phosphate containing small molecule, a nucleotide, nucleotide-like, nucleotide analog, or nucleic acid molecule, or most preferably a double-stranded RNA. More specifically, the present invention shows, for the first time, that a therapeutic benefit can be achieved when a subject is treated with a TLR3 agonist after a first administration of a relevant antigen to said subject.

[006] The finding is particularly surprising and important for the management of cancer patients because an increase in survival is rarely if ever achieved in any known active regimen in melanoma and other cancers. The usual finding is an improvement in early endpoints (tumor size, response rate and/or time to progression, otherwise known as progression-free survival) but the improvement almost never translates into a significant increase in overall survival or significant decrease of the spread of the tumor, as exhibited in vivo in mice treated in accordance with the present invention.

[007] Furthermore, the finding that TLR3 agonist therapy administered after the administration of a first dose of an antigen optionally together with an adjuvant can increase survival, will permit therapeutic strategies that are expected to be efficacious and a suitable treatment for tumors, including particularly melanoma tumors. Likewise, the TLR3 agonists may find use in melanoma (and other) cancers that are metastatic, recurring and/or refractory.

[008] Accordingly, in another aspect, the present invention provides a method of treating a subject having a tumor comprising administering to said subject, a first dose of an antigen optionally together with an adjuvant, and an effective amount of TLR-3 agonist optionally together with another anti-cancer agent. In a preferred embodiment, said subject has a solid tumor. In a preferred embodiment, said subject has a melanoma.

[009] Therefore, the present invention concerns the use of a TLR3 agonist for the manufacture of a medicament for treating cancer in a subject, wherein said medicament is administered in combination with another anti-cancer treatment. Preferably, the TLR3 agonist is a double-stranded RNA molecule. Optionally, the cancer is a metastatic cancer. In a particular embodiment, the present invention concerns the use of a double-stranded polyA:U RNA molecule for the manufacture of a medicament
for treating a cancer in a subject, wherein said medicament is administered together with another anti cancer treatment. Preferably the cancer is a solid tumor or a carcinoma, for example a melanoma.

[0010] In a preferred embodiment of the methods and uses according to the present invention, the subject is a human subject.

[0011] It will be appreciated that the methods of treatment mentioned herein can be used as prophylactic treatment; in any of the embodiments herein, a prophylactically effective amount of the TLR3 agonist can be interchanged with a therapeutically effective amount of a TLR3 agonist.

[0012] In a preferred embodiment of the methods and uses according to the present invention, the TLR3 agonist is a double-stranded RNA molecule, preferably a polyA:U molecule. In an other preferred embodiment of the methods and uses according to the present invention, the double-stranded RNA molecule is a polyLC molecule.

[0013] The present invention provides a method for administering a composition to a subject comprising the steps of: administering to said subject an antigen; and administering to said subject, in combination with said antigen, a TLR3 agonist, wherein the TLR3 agonist is administered after said antigen. In an embodiment, the method further comprises administering to said subject an anti-cancer agent in combination with the TLR3 agonist.

[0014] The present invention provides the use of a TLR3 agonist for the preparation of a medicament for treating or preventing a disease, wherein the TLR3 agonist is administered in combination with an antigen, and wherein the TLR3 agonist is administered after said antigen. In addition, the present invention also provides a TLR3 agonist for (the use for) treating or preventing a disease, wherein the TLR3 agonist is administered in combination with an antigen, and wherein the TLR3 agonist is administered after said antigen.

[0015] The present invention also provides the use of an antigen for the preparation of a medicament for treating or preventing a disease, wherein the antigen is administered in combination with a TLR3 agonist, and wherein the TLR3 agonist is administered after said antigen. In addition, the present invention also provides an antigen for (the use for) treating or preventing a disease, wherein the antigen is administered in combination with a TLR3 agonist, and wherein the TLR3 agonist is administered after said antigen.

[0016] In an embodiment, the anti-cancer agent is administered to the subject in combination with the TLR3 agonist.
[0017] The present invention also provides the use of an anti-cancer agent for the preparation of a medicament for treating or preventing a disease, wherein the anti-cancer agent is administered in combination with a TLR3 agonist and an antigen. In addition, the present invention also provides an anti-cancer agent for (the use for) treating or preventing a disease, wherein the anti-cancer agent is administered in combination with a TLR3 agonist and an antigen.

[0018] In any embodiment of the methods or uses provided by the present invention, the TLR3 agonist is administered between 1 and 21 days after the antigen, optionally at least 4 days after the antigen (e.g. between 4 and 21 days). In any embodiment of the methods or uses provided by the present invention, the antigen is administered in an effective amount to induce a CD8+ T cell response against said antigen, and the TLR3 agonist is administered at a time after the antigen sufficient to permit the expansion of CD8+ T cells, optionally wherein the TLR3 agonist is administered between 1 and 21 days after the antigen, optionally at least 4 days after the antigen (e.g. between 4 and 21 days, between 7 and 21 days, 7 and 14 days, etc.). In an embodiment, the antigen is co-administered with an adjuvant. Optionally the method can comprise a step of detecting an expansion of CD8+ T cells directed to the antigen.

[0019] According to an embodiment of the present invention, the anti-cancer agent is administered on the same day as the TLR3 agonist. According to an embodiment of the present invention, the anti-cancer agent is administered before the TLR3 agonist. According to an embodiment of the present invention, the anti-cancer agent is administered after the TLR3 agonist.

[0020] According to an embodiment of the present invention, the TLR3 agonist is administered more than once. In an embodiment two successive administrations of TLR3 agonist are separated by at least 24 hours.

[0021] In another embodiment, the antigen, adjuvant, TLR3 agonist and/or anti-cancer agent are administered in a therapeutically effective amount.

[0022] In a preferred embodiment of the present invention, the TLR3 agonist is a dsRNA, preferably polyA:U or polyLC, in an embodiment, the polyA:U has a chain length of more than 1000 base pairs.

[0023] In an embodiment of the present invention, the subject has a tumor or a disease. The tumor can be a solid tumor, for example, a melanoma, a breast cancer, a prostate cancer, a lung cancer.

[0024] According to the present invention, the antigen is a cancer antigen selected in the list consisting of MAGE, MART-1/Melan-A, gp100, dipeptidyl peptidase IV (DPPIV), adenosine
deaminase-binding protein (ADAbp), cyclophilin β, colorectal associated antigen, an activated oncogene, a fetal antigen, an activation marker, a tyrokinases, carcinoembryonic antigen, prostate specific antigen, prostate-specific membrane antigen, T-cell receptor/CD3-zeta chain, GAGE tumor antigens.

[0025] According to the present invention, the anti-cancer agent is selected from the list consisting of x rays, oxaliplatin, anthracyclins, taxanes, alkylating agents, mitotic inhibitors, imatinib mesylate, antivascular flavonoids, anti-metabolites, pyrimidine analogues and dacarbazine.

[0026] According to the present invention, the adjuvant is selected from the list consisting of aluminum salts, oil-in-water emulsion formulations, saponin adjuvants, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA), cytokines, detoxified mutants of a bacterial ADP- I ribosylating toxin, MPL or 3-0 deacylated MPL (3dMPL), oligonucleotides comprising CpG motifs, a polyoxyethylene ether or a polyoxyethylene ester, a saponin and an immunostimulatory oligonucleotide such as a CpG oligonucleotide, an immunostimulant and a particle of metal salt, dsRNA molecules inducing IFNα, MPL derivatives, imidazoquinolones, muramyl peptides or combinations thereof.

DESCRIPTION OF THE FIGURES

[0027] Figure 1 to 8: Figures 1 to 8 represent the tumor growth for each condition. For each mouse, the tumor growth is represented in thin line and the mean tumor growth is represented in bolded line. Tumor size (in mm³) is represented in ordinates, days are represented in axis.

Figure 1: NaCl treated group

Figure 2: OVA-CpG treated group

Figure 3: OVA CpG polyA:U treated group

Figure 4: OVA CpG oxaliplatin treated group

Figure 5: OVA CpG polyA:U oxaliplatin treated group

Figure 6: OVA CpG docetaxel treated group

Figure 7: OVA CpG polyA:U docetaxel treated group

Figure 8: OVA CpG polyLC docetaxel treated group

[0028] Figure 9 to 11: Figures 9 to 11 represent the compared mean tumor growth for various conditions. Tumor size (in mm³) is represented in ordinates, days are represented in axis.

Figure 9: The graph represents the tumor growth in C57bl/6 mice who have been inoculated with B16OVA cancer cells and treated either by NaCl (black full line, full lozenges), OVA-CpG (black dotted line, full squares) or OVA-CpG and polyA:U (black dashed line, open triangles). The graph
shows a dramatic reduction of tumor growth when mice are first treated with OVA-CpG and then treated with polyA:U.

Figure 10: The graph represents the tumor growth in C57bl/6 mice who have been inoculated with B16OVA cancer cells and treated either by NaCl (black full line, full lozenges), OVA-CpG (black dotted line, full squares), OVA-CpG and oxaliplatin (black dashed line, full squares) or OVA-CpG, polyA:U and oxaliplatin (full black line, open triangles). The graph shows a reduction of tumor growth when mice are treated with OVA-CpG and oxaliplatin. This effect is even more dramatic when mice are also treated with polyA:U.

Figure 11: The graph represents the tumor growth in C57bl/6 mice who have been inoculated with B16OVA cancer cells and treated either by NaCl (black full line, full lozenges), OVA-CpG (black dotted line, full squares), OVA-CpG and docetaxel (dashed line, full lozenges), OVA-CpG, polyA:U and docetaxel (full black line, open points) and OVA-CpG, polyLC and docetaxel (full black line, open triangles). The graph shows an important reduction of tumor growth when mice are treated with OVA-CpG, oxaliplatin and a dsRNA (polyA:U or polyLC).

[0029] Figure 12: Treatment sequence scheme: The scheme shows the setting of the experiment. Mice (C57bl/6 mice) were injected with B16OVA cell lines on day 0. Days are represented in axis. Depending on the group of mice considered, mice received OVA-CpG on day 4 (injected in footpad, in underlined, black full arrow) after tumor engraftment (B16OVA injection) and then either polyA:U, polyLC or saline i.p. on days 9, 12, 16 and 19 (in bolded, dotted arrows) and docetaxel, oxaliplatin or saline on day 10 (in italics, double arrow).

[0030] Figure 13: Survival curves for the group of mice treated with OVA-CpG, polyA:U and oxaliplatin (dashed black line) versus NaCl (full black line). Again the claimed setting led to a longer survival time. The difference in survival time is statistically significant (P<0.0001). Days are represented in axis, percentage of survival in ordinates (100% equals no death).

**DETAILED DESCRIPTION OF THE INVENTION**

[0031] Primary tumors are commonly treated by a combination of therapies, in most cases including surgery, local radiotherapy and chemotherapy. Even when the tumor has apparently been defeated, micrometastases of dormant tumor cells frequently lead to tumor relapse and therapeutic failure. Some therapeutic programmes can elicit specific cellular responses that render tumor-cell death immunogenic. Other drugs may have side effects that stimulate the immune system, through transient lymphodepletion, by the subversion of immunosuppressive mechanisms or through direct or indirect stimulatory effects on immune effectors. The challenge is to create a situation where the host immune system is capable of controlling any residual disease.
Toll-like receptors (TLR) have emerged as key players in the detection of pathogens and the induction of anti-microbial immune response. TLR recognize Pathogen Associated Molecular Patterns (PAMP), and upon specific ligand binding, they trigger an anti-microbial innate immunity. Beside its involvement in the innate immune response, TLR engagement by specific ligands also shapes the adaptive immunity. To date, 13 TLRs have been identified (TLRl-IO in humans). Among them, TLR3 has been reported to recognize dsRNA. TLR3 agonist activate dendritic cells expressing TLR3, thereby leading to the secretion of pro-inflammatory mediators such as IL-6 and TNF, various chemokines, as well as activation and recruitment of effectors T cells, dendritic cells and natural killer (NK). Several recent publications have shown that in addition to its immunostimulatory properties, TLR3 can directly induce cell death upon specific ligand binding, e.g. in macrophages. Some epithelial cancer cells also express TLR3. In response to TLR3 ligands stimulation in vitro, they secrete mainly chemokines such as MCP-I, IP-10 or RANTES, as well as inflammatory cytokine IL-6, probably resulting in intratumoral attraction of immune effector cells. Moreover, in vitro, TLR3 ligands mediate apoptosis in cancer cells. Therefore, TLR3 is able to mediate both immunostimulatory effect and direct apoptotic effect on cancer cells.

The studies disclosed herein present in vivo results obtained from C57bl/6 mice bearing B16 melanoma tumors. Mice treated with a purified tumor antigen (OVA), followed by treatment with both a TLR3 agonist (polyAU or poly IC) and optionally an anti-cancer agent, demonstrated dramatically decreased tumor growth over time. Without wishing to be bound by theory, it is believed that the TLR3 agonist includes a mechanism of action which synergizes with the CD8+ T cell response induced by the tumor antigen, for example by enhancing and/or directing the T cells response, and/or by stimulating antigen-specific T cells generated by the tumor antigen, and that the optimal use of the TLR3 agonist in this administration setting will involve permitting at least a minimal amount of time to pass between the tumor antigen administration and the TLR3 agonist administration so as to permit the activation of T cells (e.g. CD8+ T cells) and the initiation of a T cell response to the antigen and thus to the tumor cells. Such activation can generally take place or be observed within approximately 1 week following administration of a tumor antigen. Again without wishing to be bound by theory, it is believed that TLR3 agonists involve a mechanism of action where they induce the production of cytokines by tumor cells, which in turn can render the tumor more immunogenic (e.g. attracting CD8+ T cells). As a result it will generally be advantageous to administer the TLR3 agonist at a time after tumor antigen administration sufficient to permit the tumor antigen to induce an expansion of activated CD8+ T cells. The greatest expansion of activated CD8+ T cells typically observed as early as about 4 days following antigen administration in humans (shorter in mice), but CD8+ T cell expansion may be present earlier to a lesser degree and/or may vary slightly between individuals. A CD8+ T cell response can generally be detected by well known methods, for example detecting the presence of
CD8+ T cells that are specific for a tumor antigen and/or that express cytokines (e.g. using ELISPOT assays), and/or the presence of expanded CD8+ T cells. Accordingly, it will be appreciated that it may be advantageous to further administer, in combination with the antigen, any additional agent as an adjuvant to enhance the T cell response (e.g. ThI, CD8+ T cell response) generated by administration of the antigen. Many such compounds are known, and in the present examples a CpG motif-containing oligonucleotide that is believed to act as an agonist of Toll-like receptor 9 (TLR9) was administered at the same time as the tumor antigen. Finally, TLR3 agonists may also involve a mode of action whereby they induce apoptosis of tumors so as to make the tumor of a size or extent which is more amenable to eradication by an established and ongoing T cell response.

[0034] It was also discovered that use of an additional anti-cancer agent (other than the TLR3 agonist and tumor antigen), when used in combination with the aforementioned tumor antigen and TLR3 agonist therapy, produces strong decrease in tumor growth in C57bl/6 mice bearing B16 melanoma tumors. Thus, a particularly advantageous therapeutic strategy will involve treating a subject having a tumor with a tumor antigen followed by a TLR3 agonist, and further administering an additional anti-cancer agent, e.g. a chemotherapeutic or cytotoxic agent. The administration of an anti-cancer agent can also increase the pre-activation of T lymphocytes, thereby mounting an effective adaptive and innate immune response, which may potentiate the action of the TLR3 agonist by targeting more efficiently the tumor cells. Thus, another particularly advantageous therapeutic strategy will involve treating a subject having a tumor with a tumor antigen and an additional anti-cancer agent, having preferably an immunomodulating effect, followed by a TLR3 agonist. The chemotherapeutic agents used in the present examples appear to provide an increase in efficacy that is greater than would be expected by cumulating the effects observed individually. The agent may, via cytotoxic activity on tumor cells, reduce the tumor load to a level where its growth can be controlled by immunotherapy, and/or may render the tumor additionally immunogenic. The chemotherapeutic agent can generally be administered in any suitable manner, temporal juxtaposition, or dose so long as the treatment does not counteract the immune response (e.g. a CD8+ T cell response) induced by the tumor antigen, optionally as enhanced and the immune effect of the TLR3 agonist. Certain chemotherapeutic agents have been reported to have immunostimulatory effects themselves, and such agents will be preferred for use in combination with the tumor antigen-TLR3 agonist treatment.

[0035] It is believed that the dramatic improvement in tumor size control observed upon treatment with an antigen, TLR3 agonist and additional anti-cancer agent is due to a combination of factors that act synergistically. Vaccination is a known therapeutic approach to stimulate the immune system and sensitize the immune system against a further challenge; the prior antigen administration sensitizes the immune system to the antigen and potentiates an immune response. Optionally, the antigen can be administered with an adjuvant, to enhance the sensitization and lead to a more efficient pre-activation
of the immune system for the coming treatments. The efficacy of the TLR3 agonist administration, which acts on TLR3 present in immune cells and can lead to their activation, may thus be improved. In addition, the conjoint administration of the anti-cancer agent may enhance the effect of the TLR3 agonist by an additional immunomodulatory effect and/or by reducing tumor burden (e.g. via a direct cytotoxic effect) to a level where the host immune system can control the tumor. Anti-cancer agents best suited for the present invention combine two roles: a direct cytotoxic effect on tumor cells and an immunostimulating effect. This immunostimulating effect is related to the signals that are delivered by stressed or dying tumor cells under the influence of anti-cancer agents which can be expected to regulate antigen uptake, as well as antigen processing and presentation by APCs. Other immunostimulating effects can also be: activation of tumor-suppressor proteins, as well as transcription factors, upregulation of MHC class I molecule, thereby enhancing antigen presentation, stimulation the tumor-reactive effector T cells following lymphoablation, action on regulatory T cells. The stimulation of the immune system will enhance the capacity of antigen-presenting cells to engulf dying tumor cells and then to process and present tumor antigens to T cells. In addition, stressed tumor cells may upregulate stimulatory ligands thereby increasing their susceptibility to lysis by endogenous immune effectors. The combination of these two effects may indeed lead to direct reduction or weakening of tumor cells and enhancement of the secondary immune response via indirect immune system stimulation.

[0036] The prior vaccination with an antigen, preferably in the presence of an adjuvant, will generate first a peripheric antigen-specific immune response to tumor antigen, and also pre-sensitize the immune system to a subsequent antigenic stimulation. The subsequent administration of a TLR3 agonist will not only re-activate this immune system in the periphery, in a non specific manner, but also mediate secretion of pro-inflammatory cytokines and chemoattractant mediators by tumor cells, resulting in attraction and intratumoral activation of effector cells. The TLR3 agonist administration will take place in a more favorable context when an antigen is first administered as the antigen administration will generate tumor-specific immune cells which will then be further re-activated and attracted at the tumor site by TLR3 ligands. Moreover, upon TLR3 treatment, target cells might also appear as more antigenic to immune cells, express more TLR3 receptors and thus become more prone to apoptosis.

[0037] The action of the anticancer agent will be to reduce the tumor burden by direct cytotoxicity and optionally stimulate the immune system through various mechanisms depending on the anticancer agent used, for example enhance antigen uptake and presentation, secretion of co-stimulating factors such as chemokines, expression of apoptosis inducing factors on target cells, etc. Altogether, this setting leads to a very favorable context for the reduction of the tumor burden leading possibly to a cure of the patient. One possible side effect of anticancer agent could be to create an inflammatory
site, and to induce up-regulation of TLR3 on cancer cells, thus become more prone to apoptosis mediated by subsequent TLR3 stimulation.

[0038] The anticancer administration can take place before or after the TLR3 agonist administration. When administered before, the immunostimulatory effect of the anticancer agent will play a more central role as it will potentiate the tumor-specific immune response, further activated and attracted at tumor site by TLR3 agonist. When administered after, the direct cytotoxicity of the anticancer agent will mostly enable to reduce the tumor burden, enabling the tumor-specific immune response to control the growth of remaining tumor cells. Anticancer agent can also be administered many times before and/or after the TLR3 agonist.

[0039] Similarly, the TLR3 agonist administration can take place before or after the anticancer agent administration. Then TLR3 agonist can also be administered many times before and/or after the anticancer agent.

15 DEFINITIONS

[0040] As used in the specification, "a" or "an" may mean one or more. As used in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more.

[0041] Where "comprising" is used, this can preferably be replaced by "consisting essentially of, more preferably by "consisting of.

[0042] The terms "cancer" and "tumor" as used herein are defined as a new growth of cells or tissue comprising uncontrolled and progressive multiplication. In a specific embodiment, upon a natural course the cancer is fatal. In specific embodiments, a cancer is invasive, metastatic, and/or anaplastic (loss of differentiation and of orientation to one another and to their axial framework).

[0043] The term "invasive" as used herein refers to cells which have the ability to infiltrate surrounding tissue. In a specific embodiment, the infiltration results in destruction of the surrounding tissue. In another specific embodiment, the cells are cancer cells. In a preferred embodiment, the cells are breast cancer cells, and the cancer spreads out of a duct into surrounding breast epithelium. In a specific embodiment, "metastatic" breast cancer is within the scope of "invasive."

[0044] The term "metastatic" as used herein is defined as the transfer of cancer cells from one organ or part to another not directly connected with it. In a specific embodiment, breast cancer cells spread to another organ or body part, such as lymph nodes.
[0045] "Weekly" stands for "about once a week" (meaning that more than one treatment is made with an interval of about one week between treatments), the about here preferably meaning +/-1 day (that is, translating into "every 6 to 8 days"); most preferably, "weekly" stands for "once every 7 days".  

5 [0046] As used herein, the terms "co-administration" or "co-administering" refer to two agents that are administered in temporal juxtaposition. The co-administration may be effected by the two agents being mixed into a single formulation, or by the two agents being administered separately but simultaneously, or separately and within a short time of each other. By way of illustration, in an embodiment, the antigen is co-administered simultaneously with an adjuvant.  

10 [0047] As used herein, the term "in combination" refers to the use of more than one therapies (e.g., more than one prophylactic agent and/or therapeutic agent). The use of the term "in combination" does not restrict the order in which therapies (e.g., prophylactic or therapeutic agents) are administered to a subject with a disease. A first therapy can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject with disease. For the avoidance of doubt, two agents used "in combination" may be, but are not necessarily, co-administered. By way of illustration, the agents may be administered in either order, i.e. a TLR3 agonist may be administered first, or a chemotherapeutic agent may be administered first. In another embodiment of the instant invention, the two agents are co-administered in a single formulation, or are co-administered simultaneously.  

15 [0048] As used herein, the terms "prevent", "preventing" and prevention refer the inhibition of the development or onset of a disease (e.g. a cancer) or the prevention, recurrence, onset, or development of one or more symptoms of disease (e.g. a cancer), in a subject resulting from the administration of therapy (e.g., a prophylactic or therapeutic agent) or a combination of therapies (e.g., a combination of prophylactic and/or therapeutic agents).  

20 [0049] As used herein, the term "prophylactically effective amount" refers to that amount of the prophylactic agent sufficient to result in the prevention of the recurrence or onset of a disease (e.g. a cancer) or one or more symptoms thereof.  

25 [0050] As used herein, the term "small molecules" and analogous terms include, but are not limited
to, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than 1,000 grams per mole. In a preferred embodiment, "small molecules" encompass organic or inorganic compounds having a molecular weight less than 750 grams per mole. In yet another specific embodiment, "small molecules" encompass organic or inorganic compounds having a molecular weight less than 500 grams per mole. Salts, esters, and other pharmaceutically acceptable forms of such compounds are also encompassed.

[0051] As used herein, the terms "subject(s)" and "patient(s)", used interchangeably, refer to an animal, preferably a mammal including, but not limited to, a non-primate (e.g., a cow, pig, horse, cat, dog, rat, and mouse) and a non-human primate (e.g., a monkey such as a cynomolgous monkey), and more preferably a human. In a specific embodiment, the subject is a human with cancer.

[0052] As used herein, the term "synergistic" refers to a combination of therapies (e.g., prophylactic or therapeutic agents) which is more effective than the additive effects of any two or more single agents. For example, a synergistic effect of a combination of therapies (e.g., prophylactic or therapeutic agents) permits the use of lower dosages of one or more of the agents and/or less frequent administration of said therapies to a subject with cancer.

[0053] The ability to utilize lower dosages of therapies (e.g., prophylactic or therapeutic agents) and/or to administer said therapies less frequently reduces the toxicity associated with the administration of said therapies to a subject without reducing the efficacy of said therapies in the prevention or treatment of cancer. In addition, a synergistic effect can result in improved efficacy of therapies in the prevention or treatment of cancer. Finally, synergistic effect of a combination of therapies may avoid or reduce adverse or unwanted side effects associated with the use of any single therapy.

[0054] As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to any agent(s) which can be used in the treatment, management, or amelioration of disease (e.g., cancer) or one or more symptoms thereof. In certain embodiments, the term "therapeutic agent" refers to a TLR3 agonist. In certain other embodiments, the term "therapeutic agent" does not refer to a TLR3 agonist. In yet other embodiments, the term "therapeutic agents" refers to a TLR3 agonist and a cancer therapy other than a TLR3 agonist. Preferably, a therapeutic agent is an agent which is known to be useful for, or has been or is currently being used for the treatment, management, or amelioration of disease (e.g., cancer) or one or more symptoms thereof. Therapeutic agents may be characterized as different agents based upon one or more effects the agents have in vivo and/or in vitro.

[0055] As used herein, the term "therapeutically effective amount" refers to that amount of a therapy
(e. g., a therapeutic agent) which is sufficient to reduce or ameliorate the severity, duration and/or progression of a disease or one or more symptoms thereof. For example, when referring to cancer, a therapeutically effective amount may refer to that amount which is sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue, ameliorate cancer or one or more symptoms thereof, or prevent the advancement of cancer, cause regression of cancer, or enhance or improve the therapeutic effect (s) of another therapy (e. g., a therapeutic agent). A therapeutically effective amount, when referring to cancer, may refer to the amount of a therapy (e. g., a therapeutic agent) sufficient to delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of a therapy (e. g., a therapeutic agent) that provides a therapeutic benefit in the treatment or management of cancer. Further, a therapeutically effective amount with respect to a therapeutic agent of the invention means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of cancer. Used in connection with an amount of a TLR3 agonist, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergizes with another therapy (e. g., a therapeutic agent).

[0056] As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity, and/or duration of disease (e.g. cancer), or one or more symptoms thereof that results from the administration of one or more therapies (e.g., one or more prophylactic and/or therapeutic agents).

[0057] As used herein, the terms "prevent", "preventing", and "prevention" refer to the prevention of the recurrence, onset, or development of disease (e.g. cancer) or one or more symptoms thereof in a subject, said prevention resulting from a therapy (e.g., the administration of a prophylactic or therapeutic agent), or a combination therapy (e.g., the administration of a combination of prophylactic or therapeutic agents).

[0058] As used herein, the terms "therapies" and "therapy" can refer to any protocol (s), method(s) and/or agent(s) that can be used in the prevention, treatment, management or amelioration of cancer or one or more symptoms thereof. In certain embodiments, the terms "therapy" and "therapies" refer to cancer chemotherapy, radiation therapy, hormonal therapy, biological therapy, and/or other therapies useful for the prevention, management, or treatment of cancer known to an oncologist skilled in the art.

[0059] As used herein, the terms "manage", "managing" , and "management" refer to the beneficial effects that a subject derives from a therapy (e. g., a prophylactic or therapeutic agent), which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more therapies
(e.g., prophylactic or therapeutic agents) to "manage" a disease so as to prevent the progression or worsening of the disease.

[0060] The term "TLR3 agonist" refers to an affinity agent (i.e., a molecule that binds a target molecule) capable of activating a TLR3 polypeptide to induce a full or partial receptor-mediated response. For example, an agonist of TLR3 induces TLR3-mediated signaling, either directly or indirectly. A TLR3 agonist, as used herein, may but is not required to bind a TLR3 polypeptide, and may or may not interact directly with the TLR3 polypeptide. A "nucleotide agonist" or "nucleic acid agonist" refers to the situation where the affinity agent comprises or consists of nucleotides and/or nucleic acid(s). An "antibody agonist" refers to the situation where the affinity agent is an antibody.

[0061] The terms "polynucleotide" and "nucleic acid", used interchangeably herein, refer to polymeric forms of nucleotides of any length, either ribonucleotides or deoxynucleotides. Thus, these terms include, but are not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. These terms further include, but are not limited to, mRNA or cDNA that comprise intronic sequences (see, e.g., Niwa et al. (1999) Cell 99(7):691-702). The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidites and thus can be an oligodeoxynucleoside phosphoramidate or a mixed phosphoramidate-phosphodiester oligomer. Peyrottes et al. (1996) Nucl. Acids Res. 24:1841-1848; Chaturvedi et al. (1996) Nucl. Acids Res. 24:23181 0 2323. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars, and linking groups such as fluororibose and thioate, and nucleotide branches. The sequence of nucleotides may be interrupted by nonnucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides, or a solid support.

[0062] The terms "antibody", designates a polyclonal antibody, a monoclonal antibody, as well as fragments or derivatives thereof having substantially the same antigen specificity. Fragments include Fab, Fab′2, CDR regions, etc. Derivatives include single-chain antibodies, humanized antibodies, poly-functional antibodies, etc.
[0063] As used herein, the term "host cell" includes a particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell.

[0064] As used herein, "T" cells refers to a sub-population of lymphocytes that mature in the thymus, and which display, among other molecules T cell receptors on their surface. T cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including the TCR, CD4 or CD8, the ability of certain T cells to kill tumor cells, the ability of certain T cells to activate other cells of the immune system, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response. Any of these characteristics and activities can be used to identify T cells, using methods well known in the art. CD8+ T cells, also known as cytotoxic T cells, Tc, CTLs, T-Killer cells or killer T cells, are generally known to be capable of inducing the death of infected or tumor cells or are otherwise damaged or dysfunctional, and most cytotoxic T cells express T-cell receptors (TcRs) that can recognize a specific antigenic peptide bound to Class I MHC molecules.

[0065] Within the context of this invention, "active" or "activated" T cells designate biologically active T cells, more particularly T cells having the capacity of cytolysis or of stimulating an immune response by, e.g., secreting cytokines. For instance, an "active" CD8+ T cell is able to stimulate the killing of tumor cells. Active cells can be detected in any of a number of well known methods, including functional assays and expression-based assays such as the expression of cytokines such as TNF-alpha. The activation of cytotoxic T cells is generally dependent on several simultaneous interactions between molecules expressed on the surface of the T cell and molecules on the surface of the antigen presenting cell (APC). For instance, the two signal model for Tc cell activation involves an interaction between the T cell receptor on the T cell and peptide-bound MHC class I molecules on an APC, and an interaction between the CD28 molecule on the T cell and CD80 or CD86 molecules on an APC. Once activated, the Tc cell undergoes clonal expansion with the help of a cytokine called Interleukin-2 (IL-2) that is a growth and differentiation factor for T cells. This increases the number of cells specific for the target antigen that can then travel throughout the body in search of antigen-positive somatic cells.

[0066] "TLR3", "TLR3 polypeptide" and "TLR3 receptor", used interchangeably, are used herein to refer to Toll Like Receptor 3, a member of the Toll-like receptor (TLRs) family. As mentioned, it will be appreciated that any TLR3 polypeptide fragment or homologue can be used in accordance with the present methods. In one aspect, the TLR3 polypeptide may comprise an amino acid sequence of at least about 25, 30, 35, 40, 45, 50, 60, 70, 80, 100, 200, 300, 400, 500, 600, 700, 800, 900 or 904 amino acid residues in length, of which at least about 50-80%, preferably at least about 60-70%. More preferably at least about 65%, 75%, 80%, 85% or 90%. 95%, 98%, 99% or 99.5% of the amino acid
residues are identical or similar amino acids to the sequence of the full-length native human TLR3. Identity or similarity may be determined using any desired algorithm; the percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity number of identical overlapping positions/total number of positions x 100%). In one embodiment, the two sequences are the same length. The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, wordlength=12 to obtain nucleotide sequences used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0067] Toll Like Receptor 3 is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs are highly conserved from Drosophila to humans and share structural and functional similarities. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression. This receptor is most abundantly expressed in placenta and pancreas, and is restricted to the dendritic subpopulation of the leukocytes. It recognizes dsRNA associated with viral infection, and induces the activation of NF-kappaB and the production of type I interferons. It may thus play a role in host defense against viruses. TLR3 mRNA sequence is described in NCBI accession number NM_003265, the sequence of which is described in WO 98/50547 (the disclosure of which is incorporated herein by reference).

[0068] A TLR3 polypeptide designates any protein or polypeptide encoded by a TLR3 gene as disclosed above. The term "polypeptide" refers to any molecule comprising a stretch of amino acids. This term includes molecules of various lengths, such as peptides and proteins. The polypeptide may be modified, such as by glycosylations and/or acetylations and/or chemical reaction or coupling, and may contain one or several non-natural or synthetic amino acids. A specific example of a TLR3 polypeptide comprises all or part of NP_003256 sequence.

TLR3 agonists
[0069] The TLR3 agonists according to the present invention can be selected from any suitable agent. For example, TLR3 agonists can be selected from a range of nucleic acid agonists; other agonists, whether nucleic acid based, proteinaceous or small molecules, can be tested using known assays.

[0070] Generally, any proteinaceous, nucleic acid or small molecule candidate TLR3 agonist can be identified using known assays. For example, assays for detecting TLR3 agonism of test compounds are described, for example, in PCT publication nos. WO 03/31573, WO 04/053057, WO 04/053452, and WO 04/094671, the disclosures of each of which are incorporated herein by reference.

[0071] Regardless of the particular assay employed, a compound can be identified as an agonist of TLR3 if performing the assay with the compound results in at least a threshold increase of some biological activity mediated by TLR3. Conversely, a compound may be identified as not acting as an agonist of TLR3 if, when used to perform an assay designed to detect biological activity mediated by TLR3, the compound fails to elicit a threshold increase in the biological activity. Unless otherwise indicated, an increase in biological activity refers to an increase in the same biological activity over that observed in an appropriate control. An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR3 agonism of a compound in a particular assay. The precise threshold increase of TLR3-mediated biological activity for determining whether a particular compound is or is not an agonist of TLR3 in a given assay may vary according to factors known in the art including but not limited to the biological activity observed as the endpoint of the assay, the method used to measure or detect the endpoint of the assay, the signal-to-noise ratio of the assay, the precision of the assay. For example, regardless of the particular assay employed, a compound can generally be identified as an agonist of TLR3 if performing the assay with a compound results in at least a threshold increase of some biological activity mediated by TLR3.

[0072] An assay may or may not be performed in conjunction with the appropriate control. With experience, one skilled in the art may develop sufficient familiarity with a particular assay (e.g., the range of values observed in an appropriate control under specific assay conditions) that performing a control may not always be necessary to determine the TLR3 agonism of a compound in a particular assay, and whether the same assay is being used to determine the agonism of a compound for multiple TLRs.

[0073] The precise threshold increase of TLR3-mediated biological activity for determining whether a particular compound is or is not an agonist of TLR3 in a given assay may vary according to factors
known in the art including but not limited to the biological activity observed as the endpoint of the assay, the method used to measure or detect the endpoint of the assay, the signal-to-noise ratio of the assay, the precision of the assay, and whether the same assay is being used to determine the agonism of a compound for multiple TLRs. Accordingly it is not practical to set forth generally the threshold increase of TLR3-mediated biological activity required to identify a compound as being an agonist or a non-agonist of TLR3 for all possible assays. Those of ordinary skill in the art, however, can readily determine the appropriate threshold with due consideration of such factors.

[0074] Assays employing HEK293 cells transfected with an expressible TLR3 structural gene may use a threshold of, for example, at least a three-fold increase in a TLR3-mediated biological activity (e.g., NF-KB activation) when the compound is provided at a concentration of, for example, from about 1 μM to about 10 μM for identifying a compound as an agonist of the TLR3 transfected into the cell. However, different thresholds and/or different concentration ranges may be suitable in certain circumstances. Also, different thresholds may be appropriate for different assays.

[0075] In certain embodiments, the TLR3 agonist can be a natural agonist of a TLR3 or a synthetic TLR3 agonist compound.

[0076] In preferred embodiments of the invention, a TLR3 agonist is used to treat a patient. TLR3 agonists are well known in the art and suitable TLR3 agonists are available. Further TLR3 agonists, or derivatives or analogs of known TLR3 agonists can be readily identified, made and/or assessed.

[0077] The most commonly used TLR3 agonist are nucleic acid based agonists. Thus in preferred aspects, a TLR3 agonist for use according to the present invention are nucleotide or nucleic acid based. Nucleotide or nucleic-acid based compounds can be assessed for their ability to act as an TLR3 agonist using readily available methods. The nucleic acid based TLR3 agonist can be single-stranded or double-stranded or a mixture thereof. The nucleic acid based TLR3 agonist can comprise deoxyribonucleotides, or ribonucleotides or a mixture thereof. The nucleotides can be natural or synthetic, and may be derivatives or analogs of natural nucleotides, such as for example in Kandimalla et al. (2003) Nucl. Acid. Res. 31(9): 2393-2400). In an embodiment, the TLR3 agonist has no or low homology (e.g., less than 10, 20, 30, 40 %) with the subject genome, in particular with human genome.

[0078] The particular TLR3 agonist on which the present invention is based was a double stranded RNA compound (dsRNA) referred to as polyadenylic-polyuridylic acid, i.e., poly(A):poly(U), pApU, polyA or polyA:U each of these terms being equivalent. PolyAU is generally an at least partially double stranded molecule made of polyadenylic acid(s) and polyuridylic acid(s), each optionally substituted with other monomers so long as the biological function (e.g. TLR3 agonism) is preserved.
Double-stranded RNA which represents either genomic or life cycle intermediate material of many viruses activates cells through binding to the dsRNA-dependent protein kinase (PKR), a kinase that initiates a complex molecular anti-viral program (Gil, J., Alcamí, J., and Esteban, M. (1999). Mol Cell Biol 19:4653-4663). dsRNA was however only recently suggested to act through TLR3 (Alexopoulou et al, R.A. (2001) Nature 413:732-738). It was reported that dsRNA triggers the production of type 1 IFN, and dsRNA has been reported to have promise for certain clinical applications such as anti-viral therapies. A dsRNA compound referred to as Ampligen, for example, has been studied for its ability induce type 1 IFN production and as a consequence to treat viral infection.

[0079] Within the context of the present invention, the term "double-stranded RNA" molecule designates any therapeutically or prophylactically effective (synthetic) double-stranded RNA compound. Such compounds are typically active per se, i.e., they do not encode a polypeptide or do not require translation to be active. dsRNA TLR3 agonists can have any suitable length. Preferably, a dsRNA molecule TLR3 agonist has a length of at least about 10 base pairs (bp), 20bp, 30bp, 50bp, 80bp, 100bp, 200bp, 400bp, 600bp, 800bp or 1000bp. In one aspect the dsRNA molecule is a short dsRNA having a chain length of less than 30bp, 50bp, 80bp, 100bp or 200bp. In another embodiment, the dsRNA molecule is a longer dsRNA, but having a chain length of less than 400bp, 600bp, 800bp or 1000bp. In another embodiment, the dsRNA molecule is a long dsRNA having a chain length of greater than 1000bp.

[0080] In one aspect, a dsRNA composition comprises a heterogenous mixture of dsRNA molecules, wherein a plurality of molecules have differing lengths. Preferably the dsRNA molecules have on average a length of at least about 10bp, 20bp, 30bp, 50bp, 80bp, 100bp, 200bp, 400bp, 600bp, 800bp or 1000bp. In another embodiment, a dsRNA composition comprises a plurality dsRNA molecules where at least 20%, 50%, 80%, 90% or 98% of dsRNA molecules have a length of at least about 10bp, 20bp, 30bp, 50bp, 80bp, 100bp, 200bp, 400bp, 600bp, 800bp or 1000bp. In a preferred embodiment dsRNA composition has a substantially homogenous mixture of dsRNA molecules, where substantially all the molecules do not differ in chain length by more than 30bp, 50bp, 80bp, 100bp or 200bp. Average chain length of nucleic acid TLR3 agonists can be determined easily, for example, by gel permeation chromatography.

[0081] Previous studies of double-stranded RNA (dsRNA) assessing their ability to be effective interferon inducers suggested that dsRNA agents must possess the secondary structure of a double stranded helix. Other dsRNA agents which have also been shown to be suitable as TLR3 agonist include double-stranded polynucleotides which are not complementary or not perfectly complementary; these have been known as, so-called "mismatched" or "loop-out" structures and exist in naturally occurring RNAs such as transfer tRNAs, ribosomal RNAs and the viral RNA secondary
structures. One commonly cited dsRNA compound, Ampligen, comprises a structure where a few parts of cytidine in the poly L-poly C (also named poly(I):poly(C), pIpC, polyLC or polyLC) structure are replaced with uridine (i.e. mismatched RNA); this compound has been reported to have physiological activity similar to that of the parent polyLC. However it will be appreciated that TLR3 agonists of any type and configuration can be used in accordance with this invention.

[0082] Generally, the polynucleotides need to be resistant to nucleases in order to remain as macromolecules for a sufficient length of time; polynucleotides are less sensitive to nuclease attack when they are in a helical complex. However, certain analogs such as Ampligen™ appear to retain their TLR3 agonist activity.

[0083] In a particular embodiment, each strand of these dsRNAs can have a length comprised between about 5 and 50 bases, more preferably between 5 and 40, 35, 30, 25 or 20 bases. Each strand is preferably perfectly complementary to the other. Preferred examples of such dsRNAs are homopolyRNAs, i.e., dsRNAs in which each strand consists essentially of a repeat of the same base; or comprise a homopolyRNA region.

[0084] The base may be any naturally occurring base (e.g., polyA, polyU, polyC, polyG) or non-naturally occurring (e.g., chemically synthesized or modified) base (e.g., polyI). Polynucleotides typified by polyinosinic-polycytidylic acid, i.e., poly(I):poly(C), pIpC or polyLC and polyadenylic-polyuridylic acid, i.e., poly(A):poly(U), pApU or polyA:U, are well-known compounds in the art and have been known to induce interferon production by immune cells. Thus in preferred embodiments, the TLR3 agonist for use according to the invention is a double stranded nucleic acid selected from the group consisting of: polyinosinic acid and polycytidylic acid, polyadenylic acid and polyuridylic acid, polyinosinic acid analogue and polycytidylic acid, polyinosinic acid and polycytidylic acid analogue, polyinosinic acid analogue and polycytidylic acid analogue, polyadenylic acid analogue and polyuridylic acid, polyadenylic acid and polyuridylic acid analogue, and polyadenylic acid analogue and polyuridylic acid analogue.

[0085] It will be appreciated that nucleic acid-based agonists of TLR3 can be designed using any suitable method. Preferably, the basic requirement of stability and resistance to nuclease attack and the preferences for chain length are taken into account, and that structural changes can be tested and assessed with reference to the a rA_n:rU_n or rI_n:rC_n complex for example. Measures can be taken to increase stability and resistance to nucleases, or to increase or optionally decrease interferon-inducing action.
[0086] Other examples of dsRNA include nucleic acids described in U.S. Patent Nos. 5,298,614 and 6,780,429. U.S. Patent no. 5,298,614 reports that when chain length of the double stranded nucleic acid derivatives is limited to certain ranges, the resulting substances exhibit desired physiological activity with markedly less toxicity, providing polynucleotides having a length of about 50 to 10,000 as calculated by base pair numbers. Also described are derivative wherein the purine or pyrimidine ring in the nucleic acid polymer is substituted with at least one SH group, or said derivative contains a disulphide bond, or both (preferred ratio of number of sulphur atoms to cytidyl acid present in the poly C are 1:6 to 39). U.S. Patent No. 6,780,429 describes a particular type of dsRNA compounds that are "chain-shortened" having lengths of about 100 to 1,000 as calculated by base pair numbers, or preferably from 200 to 800, and more preferably from 300 to 600. The latter compounds are reported to contain low numbers of 2'-5' phosphodiester bonds by a method designed to avoid phosphate groups causing intramolecular rearrangement from 3' position to 2' position through a mechanism called pseudo rotation simultaneously that can occur during hydrolysis of polynucleotides, resulting in a portion of 3'-5' phosphodiester bonds in the chain-shortened polynucleotide molecule being replaced by 2'-5' phosphodiester bonds. The disclosures of each of these references are incorporated herein by reference.

[0087] Other nucleic acid agonists that can be suitable for use as TLR3 agonists are provided in: Field et al: Proc. Nat. Acad. Sci. U.S. 58, 1004, (1967); Field et al: Proc. Nat. Acad. Sci. U.S. 58, 2102, (1967); Field et al: Proc. Nat. Acad. Sci. U.S. 61, 340, (1968); Tytell et al: Proc. Nat. Acad. Sci. U.S. 58, 1719, (1967); Field et al: J. Gen. Physiol. 56, 905 (1970); De Clercq et al: Methods in Enzymology, 78, 291 (1981). A number of synthetic nucleic acid derivatives have been described, including homopolymer-homopolymer complexes (Double Strand Nucleic Acid Polymer such as those in which polyLC or polyA:U are a parent structure, where these homopolymer-homopolymer complexes contain: (1) base modifications, exemplified by polyinosinic acid-poly(5-bromocytidyl acid), polyinosinic acid-poly(2-thiocytidyl acid), poly(7-deazainosinic acid)-polyctydylacid, poly(7-deazainosinic acid)-poly(5-bromocytidyl acid), and polyinosinic acid-poly(5-thiouridyl acid); (2) Sugar Modifications, exemplified by poly(2'-azidoinosinic acid)-polyctydylacid; and (3) phosphoric Acid Modifications, exemplified by polyinosinic acid-poly(cytidyl-5'-thiophosphoric acid). Other synthetic nucleic acid derivatives that have been described include interchanged copolymers, exemplified by poly(adenylc acid-uridylic acid) or polyA:U; and homopolymer-copolymer complexes, exemplified by polyinosinic acid-poly(cytidyl acid-uridylic acid) or polyLC and polyinosinic acid-poly(citydylc acid-4-thiouridyl acid). Other synthetic nucleic acid derivatives that have been described include complexes of synthetic nucleic acid with polycation, exemplified by polyinosinic acid-polycytidyl acid-poly-L-lysinecarboxy-methylcellulose complex (called "Poly ICLC"). Yet another example of synthetic nucleic acid derivative is polyinosinic acid-poly(l-vinylcytosine).
One example of a TLR3 agonist is Ampligen™ (Hemispherx, Inc., of Rockville, Md, U.S.A.), a dsRNA formed by complexes of polyriboinosinic and polyribocytidylic/uridylic acid, such as \( r_I^n \cdot r(C_x U \text{ or } G)_n \) where \( x \) has a value from 4 to 29, e.g., \( r_I^n \cdot r(C_{21} U)_n \). Many mismatched dsRNA polymers which behave similarly to Ampligen™ have been studied; mismatched dsRNA based on polyLC have included complexes of a polyinosinate and a polycytidylate containing a proportion of uracil bases or guanidine bases, e.g., from 1 in 5 to 1 in 30 such bases. The key therapeutic advantage of mismatched dsRNAs over other forms of natural and/or synthetic dsRNAs a reported reduction in toxicity over compounds such as those described in Lampson et al in U.S. Patent No. 3,666,646.

Specific examples of double-stranded RNA according to the present invention further include Polyadenur™ (Ipsen) and Ampligen™ (Hemispherx). Polyadenur™ is a polyA:U RNA molecule, i.e., contains a polyA strand and a polyU strand. Polyadenur™ has been developed for the potential treatment of hepatitis B virus (HBV) infection. Ampligen™ is of a poly(I):poly(C) compound (or a variant thereof comprising a poly(I):poly(C12U) RNA molecule). Ampligen is disclosed for instance in EP 281 380 or EP 113 162. Ampligen™ has been proposed for the treatment of cancer, viral infections and immune disorders. It was developed primarily for the potential treatment of myalgic encephalomyelitis (ME, or chronic fatigue syndrome/chronic fatigue immune dysfunction syndrome, CFS/CFIDS).

Further dsRNAs have been disclosed in the literature or may be developed, which can be used within the present invention. More generally, any synthetic double-stranded homopolyRNA may be used in the context of this invention.

TLR3 agonist can also be any organic or inorganic substance, such as a lipid, peptide, polypeptide, small molecule, etc., in isolated or in mixture with other substances. The TLR3 agonist candidate may be selected from a combinatorial library of products, for instance. In a preferred embodiment, the TLR3 agonist is an antibody directed against TLR3 receptor and which is capable of activating a TLR3 receptor to induce a full or partial receptor-mediated response. The TLR3 agonist can also be an antibody fragment or derivative of an antibody directed against TLR3 receptor and which is capable of activating a TLR3 receptor to induce a full or partial receptor-mediated response.
Adjuvants

[0092] According to the present invention, the treatment comprises the administration to the subject of an adjuvant and a cancer antigen. There are two classes of adjuvants: vaccine delivery systems (e.g. emulsions, microparticles, immune-stimulating complexes ISCOMs, liposomes) and immunostimulatory adjuvants (e.g. lipopolysaccharide, monophosphoryl lipid A (MPL), CpG DNA, or muramylpeptides). In the context of the invention, adjuvants that can orient the antibody response towards a Th1 profile or that can enhance a T cell or CD8+ T cell response, are particularly preferred. An adjuvant is Th1 oriented when its administration favors the secretion of IgG2a and IgG2b antibodies. On the contrary, an adjuvant favoring the secretion of IgG1 and IgG3 is considered as a Th2 oriented adjuvant.

[0093] Oligonucleotides containing unmethylated CpG motifs have been shown to induce activation of B cells, NK cells and antigen-presenting cells (APCs), such as monocytes and macrophages. See e.g. US Patent 6,207,646. Ths, adjuvants derived from the CpG family of molecules, CpG dinucleotides and synthetic oligonucleotides which comprise CpG motifs (see e.g. Krieg et al; Nature (1995) 374:546 and Davis et al; J Immunol (1998) 160:870-876) such as any of the various immunostimulatory CpG oligonucleotides disclosed in US Patent 6,207,646 may be used in the subject methods and compositions. Such CpG oligonucleotides generally comprise at least 8 up to about 100 basepairs, preferably 8 to 40 basepairs, more preferably 15-25 basepairs, and any number of basepairs between these values. For example, oligonucleotides comprising the consensus CpG motif, represented by the Formula 5'-XiCGX_2-3', where Xi and X2 are nucleotides and C is unmethylated, will find use as immunostimulatory CpG molecules. Generally, Xi is A, G or T, and X2 is C or T. Other useful CpG molecules include those captured by the formula 5'-X^CGXsX^-3', where Xi and X2 are a sequence such as GpT, GtG, GpA, ApA, ApT, ApG, CpT, CpA, CpG, TpA, TpT pr TpG and X3 and X4 areTpT, CpT, ApT, ApG, CpG, TpC, ApC, CpC, TpA, ApA, GpT, CpA or TpG, wherein "p" signifies a phosphate bond. Preferably, the oligonucleotides do not include a GCG sequence at or near the 5' and/or 3' terminus. Additionally, the PcG is preferably flanked on its 5'-end with two purines (preferably GpA dinucleotide) or with a purine and a pyrimidine (preferably GpT) and flanked on its 3'-end with two pyrimidines, preferably TpT or TpC dinucleotide. Thus, preferred molecules will comprise the sequence GACGTT, GACGTC, GTCGTT or GTCGCT, and these sequences will be flanked by several additional nucleotides. The nucleotides outside of this central core area appear to be extremely amendable to change.

[0094] Moreover, the CpG oligonucleotides for use herein may be double- or single-stranded. Double-stranded molecules are more stable in viva while single-stranded molecules display enhanced immune activity. Additionally, the phosphate backbone may be modified, such as phosphorodithioate-
modified, in order to enhance the immunostimulatory activity of the CpG molecule. As described in U.S. Patent No. 6,207,646, CpG molecules with phosphorothioate backbones preferentially activate B-cells, while those having phosphodiester backbones preferentially activate; monocytic (macrophages, dendritic cells and monocytes) and NK cells.

[0095] CpG molecules can readily be tested for their ability to stimulate an immune response using standard techniques, well known in the art. For example, the ability of the molecule to stimulate a humoral and/or cellular immune response is readily determined using the immunoassays described above. Moreover, the antigen and adjuvant compositions can be administered with and without the CpG molecule to determine whether an immune response is enhanced. If used, the CpG oligonucleotide can be administered either prior to, concurrent with, or subsequent to, delivery of the antigen and/or the adjuvant composition. If administered prior to immunization with the antigen and/or the adjuvant composition, the CpG oligonucleotide can be administered as early as 5-10 days prior to immunization, preferably 3-5 days prior to immunization and most preferably 1-3 or 2 days prior to immunization. If administered separately, the CpG oligonucleotide can be delivered either to the same site of delivery as the antigen and adjuvant composition(s) or to a different delivery site. If simultaneous delivery is desired, the CpG oligonucleotide can be included with the antigen and/or adjuvant composition(s). Generally about 0.5 µg to 1000 µg of the CpG adjuvants will be used, more generally 0.5 µg to about 500 µg, preferably 1 to about 100 µg, preferably about 5 to about 50 µg, preferably 5 to about 30, or any amount within these ranges, of the CpG oligonucleotide per dose, will find use with the present methods.

[0096] As explained above, once the adjuvant composition is formulated, it can be administered to the vertebrate subject, either prior to, concurrent with, or subsequent to, delivery of the antigen. If administered prior to immunization with the antigen, the adjuvant formulations can be administered as early as 5-10 days prior to immunization, preferably 3-5 days prior to immunization and most preferably 1-3 or 2 days prior to immunization with the antigens of interest. If administered separately, the adjuvant formulation can be delivered either to the same site of delivery as the antigen compositions or to a different delivery site. Additionally, if the antigen is to be administered separately, it will generally be delivered in a vaccine composition that includes one or more "pharmacologically acceptable excipients or vehicles" such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances and the like, may be present in such vehicles. Moreover, the vaccine compositions can include carriers, additional adjuvants, additional immunostimulatory agents, and so forth, as described below. Delivery is also as described below.
[0097] If simultaneous delivery is desired, the antigen can be included with the adjuvant composition. Generally, the antigens and adjuvant can be combined by simple mixing, stirring, or shaking. Other techniques, such as passing a mixture of the two components rapidly through a small opening (such as a hypodermic needle) can also be used to provide the vaccine compositions.

[0098] Other adjuvants can also be suited for the present invention, adjuvants can be such as the following adjuvants mentioned but not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837; Chapter 10 in Vaccine design: the subunit and adjuvant approach, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% TWEEN 80™, and 0.5% SPAN 85™ (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y micro fluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalene, 0.4% TWEEN 80™, 5% 1pluronic-blocked polymer L121, and thr-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% TWEEN 80™, and one or more bacterial cell wall components from the group consisting of monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); (3) saponin adjuvants, such as QS21, Quil A or STIMULON™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMs may be devoid of additional detergent, see, e.g., International Publication No. WO 00/07621; (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (International Publication No. WO 99/44636), etc.), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (6) detoxified mutants of a bacterial ADP- I ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an E. coli heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild type amino acid at position 63) LT-R72 (where arginine is substituted for the wild type amino acid at position 72), CT-S109 (where serine is substituted for the wild 20 type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); (7) MPL or 3-0 deacylated MPL (3dMPL) (see, e.g., GB 2220221), EP-A-0689454, optionally in the substantial absence of alum when used with pneumococcal saccharides (see, e.g., 25 International Publication No. W00/56358); (8) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (see, e.g., EP-A-0835318, EP-A 0735898, EP-A-0761231; (9) oligonucleotides comprising CpG motifs (see, e.g., Roman et al. (1997) Nat. Med. 3:849-854; Weiner

Antigen

[0099] In the context of the present invention, the term "antigen" refers to any molecule capable of being recognized by a T-cell antigen receptor or B-cell antigen receptor. The term broadly includes any type of molecule which is recognized by a host immune system as being foreign. Generally, many vaccine compositions will have such properties and will be encompassed by the term "antigen". Antigens generally include but are not limited to cells, cell extracts, proteins, polypeptides, peptides, polysaccharides, polysaccharide conjugates, peptide and non-peptide mimics of polysaccharides and other molecules, small molecules, lipids, glycolipids, polysaccharides, carbohydrates, viruses and viral extracts, and multicellular organisms such as parasites, and allergens. With respect to antigens that are proteins, polypeptides, or peptides, such antigens can include nucleic acid molecules encoding such
antigens. Antigens more specifically include, but are not limited to, cancer antigens, which include cancer cells and molecules, expressed in or on cancer cells; viral antigens, which include whole and attenuated virus and molecules expressed in or on viruses; and allergens. The antigen can be either a viral antigen or a cancer antigen, in a specific embodiment, a cancer antigen is preferred.

[00100] The terms "cancer antigen" and "tumor antigen" are used interchangeably and refer to antigens that are expressed by cancer cells and can thereby be exploited in order to target cancer cells. Cancer antigens are antigens which can potentially stimulate apparently tumor-specific immune responses. Some of these antigens are encoded, although not necessarily expressed, by normal cells. These antigens can be characterized as those which are normally silent (i.e., not expressed) in normal cells, those that are expressed only at certain stages of differentiation and those that are temporally expressed such as embryonic and fetal antigens. Other cancer antigens are encoded by mutant cellular genes, such as oncogenes (e.g., activated ras oncogene), suppressor genes (e.g., mutant p53), fusion proteins resulting from internal deletions or chromosomal translocations. Still other cancer antigens can be encoded by viral genes such as those carried on RNA and DNA tumor viruses.

[00101] A cancer antigen as used herein is a compound, such as a peptide, protein, or glycoprotein, which is associated with a tumor or cancer cell surface and which is capable of provoking an immune response when expressed on the surface of an antigen-presenting cell in the context of a major histocompatibility complex (MHC) molecule. Cancer antigens can be prepared from cancer cells either by preparing crude extracts of cancer cells, for example, as described in Cohen P A et al. (1994) Cancer Res 54:1055-8, by partially purifying the antigens, by recombinant technology, or by de novo synthesis of known antigens. Cancer antigens include but are not limited to antigens that are recombinantly expressed, an immunogenic portion of, or a whole tumor or cancer or cell thereof. Such antigens can be isolated or prepared recombinantly or by any other means known in the art.

[00102] Examples of tumor antigens include MAGE, MART-1/Melan-A (melanoma antigen recognized by T cells), gp100, dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin β, colorectal associated antigen (CRC)-COI7-1A/GA733, an activated oncogene, a fetal antigen, an activation marker, any of the various tyrosinases; mutant ras; mutant p53; p97 melanoma antigen, folate receptor, cyclin D1, insulin-like growth factor binding protein, surviving, telomerase, CA-125, carcinoembryonic antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etvó, amll, prostate specific antigen (PSA) and its immunogenic epitopes PSA-I, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T-cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (e.g., MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A1, MAGE-A2, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-
C3, MAGE-C4, MAGE-C5), as set forth in Boon T, Scientific American (March 1993):82-89, GAGE-family of tumor antigens (e.g., GAGE-I, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-I, NAG, GnT-V, MUM-I, CDK4, tyrosinase, p53, MUC family, VEGF, VEGF receptors, A-Raf, B-Raf, C-Raf, Raf-1, HSP70, HSP90, PDGF, TGF-alpha, EGF, EGF receptor, a member of the human EGF-like receptor family such as HER-2/neu, HER-3, HER-4 or a heterodimeric receptor comprised of at least one HER subunit, gastrin releasing peptide receptor antigen, Muc-1, CA125, α/β3 integrins, αSB1 integrins, allB3-integrins, CTLA-4, CD20, CD22, CD30, CD33, CD52, CD56, CD80, PDGF beta receptor, Src, VE-cadherin, IL-8, hCG, IL-6, IL-6 receptor, IL-15, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, pl20ctn, gpI00.sup.Pmell7, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, pl5, gp75, GM2 and GD2 gangliosides, viral products such as human papillomavirus proteins, Smad family of tumor antigens, imp-1, PIA, EBV-encoded nuclear antigen (EBNA)-I, brain glycogen phosphorylase, SSX-I, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-I and CT-7, and c-erbB-2, or any additional protein target set forth in http://oncologyknowledgebase.com/oksite/TargetedTherapeutics/TTOExhibit2.pdf and http://oncologyknowledgebase.com/oksite/TargetedTherapeutics/TTOExhibit3.pdf, the disclosures of which are herein incorporated by reference. This list is not meant to be limiting. Other tumor antigens can also be.

[00103] As mentioned, an antigen may be in the form of a nucleic acid sequence included in a delivery vector, such as poxviruses or GM-CSF engineered allogeneic tumor cells, preferably that gives rise to expression of a protein or peptide antigen in vivo upon administration. Examples include ALVAC (Sanofi Pasteur), a canary pox virus containing a gene for an antigen (e.g. carcinoembryonic antigen, GP100, etc.). When patients with metastatic melanoma were treated with the ALVAC gpI00M, boosted with gpI00M peptides, immune responses against gpI00M-specific antigens were detected. ALVAC based vaccines have been reported to consistently induce CD8+ T cell responses.

Anti-cancer agents

[00104] According to the present invention, the treatment comprises an additional step of administration of an anti-cancer agent. Such anti-cancer agent include, but are not limited to:

- spindle poisons such as mebendazole, colchicine;
- mitotic inhibitors (taxanes (paclitaxel (Taxol), docetaxel (Taxotere); vinca alkaloids (i.e. vincristine, vinblastine, vinorelbine, vindesine)),
- cytotoxic/antitumor antibiotics: such as anthracyclines (i.e. doxorubicin, daunorubicin, adriamycine, idarubicin, epirubicin and mitoxantrone, valrubicin), streptomyces (i.e. actinomycin, bleomycin, mitomycin, plicamycin)
anti-metabolites (such as pyrimidine analogues (i.e. fluoropyrimidines analogs, 5-fluorouracil (5-FU), flouxuridine (FUDR), Cytosine arabinoside (Cytarabine), Gemcitabine (Gemzar ®), capecitabine; purine analogues (i.e. azathioprine, mercaptopurine, thioguanine, fludarabine, pentostatin, cladribine, capecitabine, clofarabine); folic acid analogues (i.e. methotrexate, folic acid, pemetrexed, aminopterin, raltitrexed, trimethoprim, pyrimethamine),

topoisomerase inhibitors (i.e. camptothecins: irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate, teniposide);
alkylating agents (i.e. busulfan, cisplatin, oxaliplatin, BBR3464, carboplatin, chlorambucil, cyclophosphamide, aldophosphamide, ifosfamide, fotemustine, lomustine, streptozocin, dacarbazaine, procarbazine, temozolomide, thioTEPA, Uramustine, mechloretamine (nitrogen mustard), phosphoramid mustard, melphalan (Akeran®), temozolomide);
DNA methyltransferase inhibitors: 2'-deoxy-5-azacytidine (DAC), 5-azacytidine, 5-aza-2'-deoxycytidine, 1-β-D-arabinofuranosyl-5-azacytosine, dihydro-5-azacytidine;
vascular disrupting agents, such as flavone acetic acid derivatives, 5,6-dimethylxanthenone-4-acetic acid (DMXAA) and flavone acetic acid (FAA);
also other chemotherapeutic drugs such as aprepitant, bortezomib (Velcade ®, Millenium Pharmaceuticals), imatinib mesylate (Gleevec ®), carmustine (BCNU), lomustine (CCNU), tamoxifen, gefitinib, erlotinib, carboxyamidotriazole, efaproxiral, tirapazamine, xcytrin, thymalfasin, vinflunine.

Administration regimens having daily or multiple doses per week of chemotherapeutic agents will generally be used for orally available agents, including for example HDAC inhibitors, and certain anti-angiogenic therapies such as receptor tyrosine kinase inhibitors and raf or ras kinase inhibitors. Additionally, chemotherapeutic agents such as taxanes, platinum drugs, anti-metabolites, alkylating agents, for example docetaxel, carboplatin, 5-FU, cisplatin, vinorelbin, and gemcitabine having significant toxicity at high (e.g. MTD) doses when administered in a weekly regimen can be administered as lower doses on a more frequent basis. Radiotherapy such as x ray therapy can also be adapted for the present invention.

In an embodiment, the preferred anti-cancer agents are immunomodulating drugs, as detailed in Zitvogel et al 2008 Nat Rev Immunol, such as, but not limited to: alkylating agents (i.e. oxaliplatin), mitotic inhibitors (i.e. docetaxel), imatinib mesylate, antivascular flavonoids, anti-metabolites in particular, pyrimidine analogues (i.e. 5-FU, gemcitabine). In an embodiment, the anti-cancer agent is dacarbazaine.

Generally, antigen(s) and optionally a vaccine adjuvant are administered as a pharmaceutical composition comprising a pharmaceutically acceptable carrier. Anti-cancer agents and
TLR3 agonists are each, in separate compositions or optionally combined in a single composition, also administered generally as a pharmaceutical composition comprising a pharmaceutically acceptable carrier. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. A pharmaceutical carrier can be any compatible, non-toxic substance suitable for delivering the compositions of the invention to a patient.

Methods of administration

[00108] The present invention encompasses treatment protocols that provide better prophylactic or therapeutic profiles than current single agent therapies or combination therapies for cancer. In particular, the invention encompasses the use of a TLR3 agonist, after a first administration with an antigen and an adjuvant, for the prevention, management, treatment or amelioration of cancer or one or more symptoms thereof optionally together with an anti-cancer agent.

[00109] Examples of cancers that can be prevented, managed, treated or ameliorated in accordance with the methods invention include, but are not limited to, solid tumors, and particularly cancers such as melanoma, cancer of the head, neck, eye, mouth, throat, esophagus, chest, bone, lung, colon, rectum, stomach, prostate, breast, ovaries, kidney, liver, pancreas, and brain.

[00110] The invention provides methods for preventing, managing, treating or ameliorating cancer that has the potential to metastasize or has metastasized to an organ or tissue (e.g., bone) or one or more symptoms thereof, said methods comprising administering to a subject in need thereof a first dose of an antigen or a tumor antigen together with an adjuvant, an effective amount of a TLR3 agonist and optionally an effective amount of another anti-cancer agent. Preferably, the TLR3 agonist is a dsRNA compound.

[00111] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight.

[00112] Preferably, the method encompassed by the present invention reduces the size of a tumor or the spread of a tumor in a subject by at least 5%, preferably at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least
60%. at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% relative to a control such as PBS.

_Treatment sequence_

[00113] The active ingredients of the invention can be administered through different schedules. Treatment sequences can be carried out in a number of ways. Generally, a treatment comprises at least one administration of an antigen and the administration of a TLR3 agonist.

[00114] The antigen administration is preferably performed before the TLR3 agonist administration. Preferably, the antigen is administered at least 2, 3, 4 and more days before the TLR3 agonist administration. Although 4 days are sufficient to mount an immune response in mice, the time needed to obtain the same effect in human is longer e.g. 14 days. In an embodiment, the antigen is administered at least 4 days, or between 1 and 21 days, preferably between 7 and 15 days, before the TLR3 agonist. In an other embodiment, the antigen is co-administered together with an adjuvant, e.g. the adjuvant is administered either simultaneously or within a time range of 1, 2, 6 or 12 hours.

[00115] The TLR3 agonist can be administered a single time or over a prolonged period of time. In an embodiment, the TLR3 agonist is administered many times, e.g. 2, 3, 4, 5 or more times. Each administration being separated by 1, 2, 3, 4, 5 or more days.

[00116] In a particular embodiment, an anti-cancer agent is administered in combination with the first TLR3 agonist administration. In a specific embodiment, the anti-cancer agent is administered 6, 12 hours, 1, 2, 3, 4 or more days after the first TLR3 agonist administration. In an other embodiment, the anti-cancer agent is administered 6, 12 hours, 1, 2, 3, 4 or more days prior to the first TLR3 agonist administration. The anti-cancer agent can be administered a single time. In an other embodiment, the anti-cancer agent is administered many times, e.g. 2, 3, 4, 5 or more times.

[00117] In a preferred embodiment, the antigen is administered 1, 2, 3, 4 or more days prior to the first TLR3 agonist administration, the anti-cancer agent is administered 1, 2, 3, 4 or more days after the first TLR3 agonist administration and the TLR3 agonist is administered for at least 2, 3, 4 or more times each administration being separated by 1, 2, 3, 4 or more days.

[00118] The sequence of administration can be repeated several times, each repetition constitutes a cycle of treatment. In an embodiment, after each treatment, there will be a period of wash out lasting 1, 2, 3, 4 or more weeks.
[00119] A specific treatment sequence illustrating the invention, but not intending to limit it is presented in figure 12. The antigen administration occurs on day 4, the TLR3 agonist administration occurs on days 9, 12, 16 and 19, the anti-cancer agent administration occurs on day 10.

[00120] Other treatment sequences are also encompassed by the present invention. For example, the antigen administration occurs prior to the TLR3 agonist treatment. The TLR3 agonist treatment is administered once and the anti cancer agent is administered many times, i.e. 5 times daily. After each cycle of treatment, a wash out of 3 weeks is implemented. The cycle of treatment can be repeated many times, i.e. 1, 2, 3, 4, 5, 6 or more times.

Dosages

[00121] The active compounds of the invention can be administered in various ways. As discussed, specific dosage ranges suitable for the administration of the TLR3 agonist, derived from in vivo experimentation in murine tumor models. Preferably, the TLR3 agonist is administered in a human subject at a dose comprised between 0.01 and 100 mg/kg, preferably between 0.1 and 50 mg/kg, more preferably between 0.1 and 10 mg/kg, or about 1, 2, 3, 4 or 5 mg/kg. In an other embodiment, the TLR3 dose is a low dose i.e. 0.1 to 2 mg/kg. The present disclosure shows that a better effect is obtained when the TLR3 agonist is administered in more than one administration per week. It will be appreciated that appropriate dosages may be deduced from experiments conducted in vitro or in animals, with respect to different TLR3 agonist having different levels of TLR3 agonist activity.

[00122] In one aspect the present invention relates especially to the treatment of a disease, especially a tumor, characterized in that a TLR3 agonist is administered more than once per week, to a human in a dose that is calculated according to the formula (A):

\[
\text{(single dose (mg/kg)=(0.1 to 50))} \times \text{d} \times \text{w} \quad \text{(A)}
\]

where \(d\) is the number of days of treatment, optionally consecutive or non-consecutive, within one week, that is \(d\) is about 1 to about 7, preferably between about 2 to 5, and where \(w\) is the number of weeks of treatment, preferably where \(w\) is 2, 3, 4, 5, 6 or greater. More preferably, the treatment dose is calculated according to the formula B,

\[
\text{(single dose (mg/kg)=(0.5 to 10))} \times \text{d} \times \text{w} \quad \text{(B)}
\]

or according to the formula C,

\[
\text{(single dose (mg/kg)=(less than 1, or about 1, 2, 3, 4 or 5))} \times \text{d} \times \text{w} \quad \text{(C)}
\]

where, in each of formulae A to C, \(d\) is about 1 to about 7, preferably between about 2 to 5, and where \(w\) is the number of weeks of treatment, preferably where \(w\) is 2, 3, 4, 5, 6 or greater.

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[00123] The anti-cancer agent is administered in an effective dose i.e. the dose will be comprised between 0.1 and 100 mg/kg. The dose will be adjusted depending on the disease and the anti-cancer agent administered.

[00124] The adjuvant is administered in an effective dose, i.e. at a dose comprised between 10 µg and 1 g. The antigen is administered at a dose sufficient to induce an activation of the immune system, i.e. at a dose comprised between 1 µg and 1 g. The dose will be adjusted according to current clinical practices according to the immunogenicity of the antigen and the strength of the adjuvant that can optionally be administered to the patient.

[00125] In an embodiment, the compounds can be administered in a low dose, that is to say a dose that is lower than what is usually recommended for a single compound treatment to obtain a therapeutic effect. This low dose is still therapeutically effective due to the synergistic effect of the treatment setting according to the present invention.

Types of cancers

[00126] In various embodiments, the present invention provides methods for determining treatment regimens for cancer subjects. The methods of the invention can be used to determine treatment regimens of any cancer, or tumor, for example, but not limited to, malignancies and related disorders include but are not limited to the following.

[00127] Leukemias such as but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias such as myeloblasts, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as but not limited to Hodgkin's disease, non-Hodgkin's disease; multiple myelomas such as but not limited to smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenstrom's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as but not limited to bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors such as but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma,
pineoblastoma, primary brain lymphoma; breast cancer including but not limited to adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer such as but not limited to pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer such as but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers such as but limited to Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers such as but not limited to ocular melanoma such as iris melanoma, choroidal melanoma, and ciliary body melanoma, and retinoblastoma; vaginal cancers such as squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer such as squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, and sarcoma; cervical cancers such as but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers such as but not limited to endometrial carcinoma and uterine sarcoma; ovarian cancers such as but not limited to, ovarian epithelial carcinoma, borderline tumor, genn cell tumor, and stromal, tumor; esophageal cancers such as but not limited to, squamous cancer, adenocarcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers such as but not limited to, adenocarcinoma, fmingating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers such as but not limited to hepatocellular carcinoma and hepatoblastoma, gallbladder cancers such as adenocarcinoma; cholangiocarcinomas such as but not limited to papillary, nodular, and diffuse; lung cancers such as non-small cell lung cancer, squamous cell carcinoma (epiddennoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers such as but not limited to germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, non seminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers such as but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers such as but not limited to squamous cell carcinoma; basal cancers; salivary gland cancers such as but not limited to adenocarcinoma, mucoepidermoid carcinoma, and adenoid cystic carcinoma; pharynx cancers such as but not limited to squamous cell cancer, and ven-acous; skin cancers such as but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers such as but not limited to renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/or uterine); Wilins' tumor; bladder cancers such as but not limited to transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma,
sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary
adenocarcinomas (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B.
Lippincott Co., Philadelphia and Murphy et al., 1997, Informed Decisions: The Complete Book of
States of America).

[00128] Accordingly, the methods of the invention are also useful in the treatment of a variety of
cancers or other abnormal proliferative diseases, including (but not limited to) the following:
carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach,
cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid
lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell
lymphoma, T-cell lymphoma, Burkitt lymphoma; hematopoietic tumors of myeloid lineage, including
acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal
origin, including fibrosarcoma and rhabdomyosarcoma; other tumors, including melanoma,
seminoma, teratocarcinoma, neuroblastoma, glioblastoma and glioma; tumors of the central and
peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and sarcomas; tumors of
mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other
tumors, including melanoma, xeroderma pigmentosum, keratoactanthoma, seminoma, thyroid
follicular cancer, teratocarcinoma and pediatric malignancy. In specific embodiments, malignancy or
dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are
treated in the ovary, bladder, breast, colon, lung, skin, pancreas or uterus. In other specific
embodiments, sarcoma, melanoma, or leukemia is treated.

[00129] In preferred embodiments, the methods of the invention are used for solid tumors. Example
of tumors include melanoma, breast, colon, ovarian, lung, brain and prostate cancers. In a preferred
embodiment, the methods of the invention are directed at treating melanoma.

Treatment of Melanoma

[00130] In specific embodiments, patients with melanoma are administered a prophylactically or
therapeutically effective amount of a TLR3 agonist in combination with the administration of a
prophylactically or therapeutically effective amount of one or more other therapies useful for
melanoma cancer treatment or management including but not limited to: dacarbazine (DTIC),
nitrosoureas such as carmustine (BCNU) and lomustine (CCNU), agents with modest single agent
activity including vinca alkaloids, platinum compounds, and taxanes, the Dartmouth regimen
(cisplatin, BCNU, and DTIC), interferon alpha (IFN-A), and interleukin-2 (IL-2).

[00131] Further aspects and advantages of this invention are disclosed in the following experimental
section, which should be regarded as illustrative and not limiting the scope of this application.

EXAMPLES

Example 1: Effect of the treatment according to the invention on C57bl/6 mice inoculated with B16OVA cells: combination with docetaxel or oxaliplatin.

[00132] 30 C57bl/6 mice were purchased from Charles River Laboratories, France, and were inoculated with B16OVA cancer cells on day 0. B16OVA tumor cells are derived from the B16 melanoma cell line and express the ovalbumin antigen using known methods, for example: B16OVA melanoma cells (H2kb) were derived from B16 cells by transduction with a cDNA encoding the ovalbumin gene. Clones have been selected with G418 and screened for expression of Ova Ag. Mice have been pooled in 8 different groups (Group 1 to Group 8):

[00133] Group 1: On day 4, 9, 10, 12, 16 and 19, mice have been injected intraperitoneally (i.p.) saline. This group is the absolute control.

[00134] Group 2: On day 4, mice have received OVAg + Cpg 5 µg in food pad. On days 9, 10, 12, 16 and 19, mice have been injected intraperitoneally (i.p.) saline.

[00135] Group 3: On days 4 and 10, mice have received intraperitoneally (i.p.) saline. On days 9, 12, 16 and 19, 10 mice have been injected intraperitoneally (i.p.) 100µg of polyA:U.

[00136] Group 4: On day 4, mice have received OVAg + Cpg 5 µg in food pad. On days 9, 12, 16 and 19, mice have been injected intraperitoneally (i.p.) saline. On day 10, mice have been injected intraperitoneally (i.p.) 60 mg/kg of docetaxel.

[00137] Group 5: On day 4, mice have received OVAg + Cpg 5 µg in food pad. On days 9, 12, 16 and 19, 10 mice have been injected intraperitoneally (i.p.) 100µg of polyA:U. On day 10, mice have been injected intraperitoneally (i.p.) saline.

[00138] Group 6: On day 4, mice have received OVAg + Cpg 5 µg in food pad. On days 9, 12, 16 and 19, 10 mice have been injected intraperitoneally (i.p.) 100µg of polyA:U. On day 10, mice have been injected intraperitoneally (i.p.) saline.
[00140] Group 8: On day 4, mice have received OVA 1mg + CpG 5 µg in food pad. On days 9, 12, 16 and 19, 10 mice have been injected intraperitoneally (i.p.) 100µg of polyA:U. On day 10, mice have been injected intraperitoneally (i.p.) 5 mg/kg of oxaliplatin.

5 Materials:

[00141] OVA was purchased from Sigma Chemical Company (St. Louis, MO). CpG was purchased from Invivogen (San Diego, CA). Poly A:U was provided by Innate Pharma. Poly I:C was purchased from Sigma Chemical Co. (St Louis, MO). Docetaxel was purchased from Sanofi Synthelabo, France. Oxaliplatin was purchased from Sanofi Synthelabo, France.

[00142] The tumor growth has been assessed using a caliper.

[00143] In groups where mice have been treated with the antigen and adjuvant, and the TLR3 agonist, a significant delay in tumor growth is observed (figure 8).

[00144] In groups where mice have been treated with the antigen and adjuvant, the TLR3 agonist and the anti-cancer agent, a significant delay in tumor growth is observed as can be observed in figures 1 to 11. Moreover, in the mice group treated with OVA-CpG, polyA:U and oxaliplatin the survival curves have been plotted (figure 13) and there is a significant improvement of the survival compared to saline group (mice live approximately twice longer).

[00145] All publications and patent applications cited in this specification are herein incorporated by reference in their entireties as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[00146] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.
WHAT IS CLAIMED:

1. A method for administering a composition to a subject comprising the steps of:
   a) administering to said subject an antigen; and
   b) administering to said subject a TLR3 agonist,
   wherein the TLR3 agonist is administered after said antigen.

2. The method of claim 1, further comprising administering to said subject an anti-cancer agent in combination with the TLR3 agonist.

3. Use of a TLR3 agonist for the preparation of a medicament for treating or preventing a disease, wherein the TLR3 agonist is administered in combination with an antigen, and wherein the TLR3 agonist is administered after said antigen.

4. Use of an antigen for the preparation of a medicament for treating or preventing a disease, wherein the antigen is administered in combination with a TLR3 agonist, and wherein the TLR3 agonist is administered after said antigen.

5. The use according to claim 3 or 4, wherein an anti-cancer agent is administered to the subject in combination with the TLR3 agonist.

6. Use of an anti-cancer agent for the preparation of a medicament for treating or preventing a disease, wherein the anti-cancer agent is administered in combination with a TLR3 agonist and an antigen.

7. The method or use of any one of the above claims, wherein the TLR3 agonist is administered between 1 and 21 days after the antigen.

8. The method or use of any one of the above claims, wherein the TLR3 agonist is administered at least 4 days after the antigen.

9. The method or use of any one of the above claims, wherein the antigen is administered in an effective amount to induce a CD8+ T cell response against said antigen, and the TLR3 agonist is administered at a time after the antigen sufficient to permit the expansion of CD8+ T cells.
10. The method or use of any one of the above claims, wherein the antigen is co-administered with an adjuvant.

11. The method or use of claims 5 to 10, wherein the anti-cancer agent is administered on the same day as the TLR3 agonist.

12. The method or use of claim 11, wherein the anti-cancer agent is administered before the TLR3 agonist.

13. The method or use of claim 11, wherein the anti-cancer agent is administered after the TLR3 agonist.

14. The method or use of any one of the above claims, wherein the TLR3 agonist is administered more than once.

15. The method or use of claim 14, wherein two successive administrations of TLR3 agonist are separated by at least 24 hours.

16. The method or use of any one of the above claims, wherein said antigen, adjuvant, TLR3 agonist and/or anti-cancer agent are administered in a therapeutically effective amount.

17. The method or use of any one of the above claims, wherein said TLR3 agonist is a dsRNA.

18. The method or use of claim 17, wherein said dsRNA is polyA:U or polyLC.

19. The method or use of claim 17, wherein said polyA:U has a length of more than 1000 base pairs.

20. The method or use of any one of the above claims, wherein said subject has a tumor or said disease is a tumor.

21. The method or use of claim 20, wherein said tumor is a solid tumor.

22. The method or use of claim 21, wherein said solid tumor cancer is a melanoma, a breast cancer, a prostate cancer, a lung cancer.
23. The method or use of any one of the above claims, wherein said antigen is a cancer antigen selected in the list consisting of MAGE, MART-1/Melan-A, gp100, dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin β, colorectal associated antigen, an activated oncogene, a fetal antigen, an activation marker, a tyrokinases, carcinoembryonic antigen, prostate specific antigen, prostate-specific membrane antigen, T-cell receptor/CD3-zeta chain, GAGE tumor antigens.

24. The method or use of claims 2 and 5 to 23, wherein said anti-cancer agent is selected from the list consisting of x rays, oxaliplatine, anthracyclins, taxanes, alkylating agents, mitotic inhibitors, imatinib mesylate, antivascular flavonoids, anti-metabolites, pyrimidine analogues and dacarbazine.

25. The method or use of claims 10 to 24, wherein said adjuvant is selected from the list consisting of aluminum salts, oil-in-water emulsion formulations, saponin adjuvants, Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA), cytokines, detoxified mutants of a bacterial ADP-ribosylating toxin, MPL or 3-O-deacylated MPL (3dMPL), oligonucleotides comprising CpG motifs, a polyoxyethylene ether or a polyoxyethylene ester, a saponin and an immunostimulatory oligonucleotide such as a CpG oligonucleotide, an immunostimulant and a particle of metal salt, dsRNA molecules inducing IFNα, MPL derivatives, imidazoquinolones, muramyl peptides or combinations thereof.
Figure 3
OVA - CpG - polyA:U

Figure 4
OVA - CpG - oxaliplatin
Figure 5
OVA - CpG - polyA:U - oxaliplatin

Figure 6
OVA - CpG - docetaxel
Figure 7
OVA - CpG - polyA:U - docetaxel

Figure 8
OVA - CpG - polyI:C - docetaxel
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

**INV.** A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

**B. RELSD SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and where practical search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>Paragraphs [0009], [0015], [0066], [0166], [0167], [0172]. claims 1,18</td>
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| Date of the actual completion of the international search | 7 August 2009 |
| Date of mailing of the international search report | 24/08/2009 |

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