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(54) **Title:** THERAPY AND METHODS OF INTRODUCING IMMATURE DENDRITIC CELLS AND/OR CYTOTOXIC T LYMPHOCYTE AND ANTI PD-1 / PD-L1 ANTIBODY FOR TREATMENT OF TUMORS

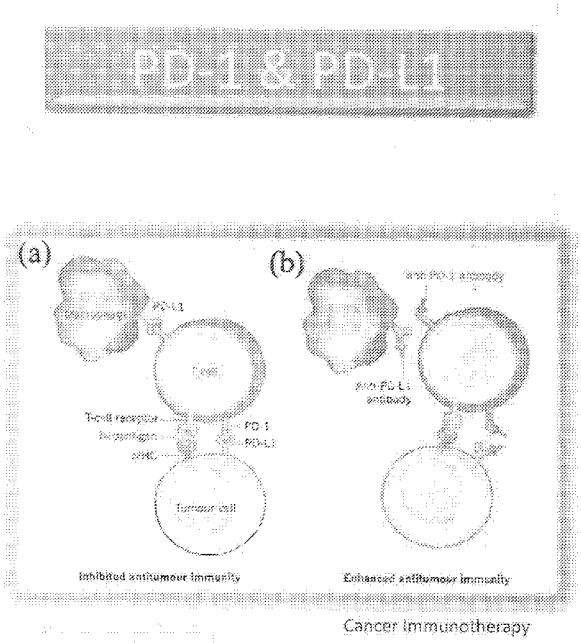

FIG. 1A

(57) **Abstract:** The invention relates to therapy and methods of applying the therapy to a patient. The invention includes the introduction of immature dendritic cells into the patient and the introduction of PD-1 and/or PD-L1 inhibitor into the patient. The immature dendritic cells are introduced intratumorally and/or through vessel and the PD-1 and/or PD-L1 inhibitor is introduced intratumorally and/or through vessel and/or subcutaneously. The immature dendritic cells can be formed by collecting monocyte cells from the patient and culturing the cells in a culture medium. The invention can be effective to regress, reduce or eliminate tumor cells in tumor tissue of the patients, including metastasized tumors. Further, the treatment of the invention is effective in the absence of conventional therapy, such as radiotherapy and chemotherapy.

WO 2020/227279 A1

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THERAPY AND METHODS OF INTRODUCING IMMATURE DENDRITIC CELLS AND/OR CYTOTOXIC T LYMPHOCYTE AND ANTI PD-1 / PD-L1 ANTIBODY FOR TREATMENT OF TUMORS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119(e) to U.S. Patent Application No. 16/404,091, filed on May 6, 2019, entitled "THERAPY AND METHODS OF INTRODUCING IMMATURE DENDRITIC CELLS AND/OR CYTOTOXIC T LYMPHOCYTE AND ANTI-TNF ANTIBODY FOR TREATMENT OF TUMORS", the contents of which are incorporated herein.

Field of the Invention

[0001] The invention relates to tumor cell and tumor tissue therapies, and methods for administering the therapies to treat cancer cells in a patient. The invention includes introducing intratumorally and/or through vessel immature dendritic cells and/or cytotoxic T lymphocytes (CTLs), and introducing intratumorally and/or through vessel and/or subcutaneously PD-1 and/or PD-L1 inhibitors to a cancer patient. The tumor therapies and methods of the invention are effective to treat the cancer patient in the presence or absence of conventional therapies, such as chemotherapy and/or radiotherapy.

Description of the Prior Art

[0002] Cytotoxic T lymphocytes (CTLs) are an important component of cellular immunity. They play a critical role in the control of many infections and cancers. These T cells are responsible for "hunting down" other cells of the body that are infected by viruses or contain cancer, and destroying them. For example, when a virus or cancer is using a cell to reproduce, the cell displays some of the viral proteins or cancer components on its surface. The cytotoxic T cells can recognize these proteins or components and hone-in to destroy the infected or cancer-containing cells before they release the infection or cancer into the bloodstream. Many vaccines are effective, at least

in part, by stimulating this type of T cell activation or response. CTLs can also create chemicals known as cytokines which assist in coordinating how the immune system fights against disease.

[0003] PD-1 is a protein that is present on CTLs. PD-L1 is present on cancer, e.g., tumor, cells. PD-1 and PD-L1 are checkpoint proteins that help keep the body's immune system in check. Since an important part of the immune system is its ability to distinguish between normal cells in the body and those that are "foreign" as well as attack the foreign cells, checkpoints provide a means for activating or inactivating the immune system to start or halt, respectively, an immune response.

[0004] PD-1 normally acts as a type of "off switch" or "brake" that blocks or halts CTLs from attacking other cells including cancer cells. Therefore, the association or interaction of PD-1 on CTLs and PD-L1 on cancer, e.g., tumor, cells decreases the ability of the CTLs to attack and kill the cancer cells. As a result of binding PD-1 and PD-L1, cancer cells can evade immune attack, and allows them to survive and grow.

[0005] PD-1 and/or PD-L1 inhibitors (e.g., anti-PD-1 and/or anti-PD-L1) suppress the physiologic response to PD-1 and/or PD-L1. The use of immune checkpoint inhibitors that block association/interaction, e.g., binding, of PD-1 and PD-L1 increases the ability of T cells to attack and kill cancer cells and, in turn, decreases the ability of cancer cells to evade the immune system. These inhibitors restore immune function through activation of T-cells and cell-mediated immune responses against cancer/tumor cells.

[0006] Inhibition of PD-1 and/or PD-L1 effects can be achieved with monoclonal antibody that target either PD-1 or PD-L1 to block their binding and, in turn, boost the immune response against cancer cells. Drugs that target these checkpoints can be potentially effective for treating cancer cells. Non-limiting examples of known PD-1 inhibitors include Pembrolizumab, Nivolumab and Cemiplimab, which are commercially available under the trade names Keytruda®, Opdivo® and Libtayo®, respectively. These drugs have been used in the treatment of cancer including melanoma of the skin, non-small cell lung cancer, kidney cancer, bladder cancer, head and neck cancers, and Hodgkin lymphoma. Non-limiting examples of known PD-L1 inhibitors include Atezolizumab, Avelumab and Durvalumab, which are commercially available under the

trade names Tecentriq®, Bavencio® and Imfinzi®, respectively. These drugs have been used to treat cancer including bladder cancer, non-small cell lung cancer and Merkel cell skin cancer (Merkel cell carcinoma).

[0007] There is a concern that known cancer treatment drugs allow the immune system to attack normal organs in the body, in addition to the targeted cancer cells. This concern can lead to serious side effects in patients that are taking the drugs. The serious effects can include serious problems in the lungs, intestines, liver, kidneys, hormone-making glands and other organs.

[0008] According to published data, i.e., from the American Cancer Society, it is estimated that 1,665,540 new cancer cases will be diagnosed and 585,720 cancer deaths will occur in 2014, in the United States. Cancer remains the second most common cause of death in the United States and accounts for nearly 1 of every 4 deaths.

[0009] Known cancer treatment procedures can be expensive, time-consuming and invasive. Further, these known procedures may not be capable of regressing, reducing or eliminating cancer in certain patients.

[0010] Thus, there is a need in the art to develop new cancer therapies and methods of administering the cancer therapies to regress, reduce or eliminate tumor cells in tumor tissue of a patient. It is desirable for the therapies and methods to be effective in a reasonable period of time and further, desirable for the therapies and methods to be as minimally invasive to the patient as reasonably possible. Furthermore, it is advantageous for the therapy and methods to be effective in the presence or absence of subjecting the patient to conventional therapy regimens, such as, radiotherapy and/or chemotherapy. It is an object of the invention to provide drugs and cancer treatments that are effective to target PD-1 or PD-L1 while not causing serious side effects to the patient.

SUMMARY OF THE INVENTION

[0011] The invention resolves the above needs by providing effective therapies and methods for regression, reduction or elimination of tumor cells in tumor tissue of a patient, as well as tumor cells in metastasized tumors. In one aspect, the invention provides a method of introducing intratumorally and/or through vessel a therapeutically

effective amount of immature dendritic cells and/or CTLs to the patient, and introducing intratumorally and/or through vessel and/or subcutaneously a therapeutically effective amount of anti PD-1/PD-L1 antibody to the patient.

[0012] In certain embodiments, the method can further include collecting monocyte cells and/or the CTLs from a patient, culturing the monocyte cells and/or the CTLs, forming immature dendritic cells from the monocyte cells, and re-introducing the immature dendritic cells and/or the cultured CTLs to the patient. The re-introducing of the cultured CTLs can be conducted prior to or following or coincident with the introducing of the immature dendritic cells.

[0013] The monocyte cells can be cultured in a medium including IL-4, GM-CFS, and mixtures thereof to form the immature dendritic cells. The CTLs can be cultured in a medium including IL-2, CD3, and mixtures thereof.

[0014] In certain embodiments, the introducing of the anti PD-1/PD-L1 antibody can be coincident with the introducing of the immature dendritic cells and/or CTLs. In other embodiments, the introducing of the anti PD-1/PD-L1 antibody can be immediately following or following a short time thereafter the introducing of the immature dendritic cells and/or CTLs. A short period of time can include seconds or minutes or hours or days. The anti PD-1/PD-L1 antibody may be introduced when there is a sufficient amount of CTLs present in the auto-immune system of the patient.

[0015] The introducing of the immature dendritic cells and/or CTLs, and/or the introducing of the anti PD-1/PD-L1 antibody can be in conjunction with an anti-inflammatory agent.

[0016] The introducing of the immature dendritic cells and/or CTLs can be in conjunction with an adjuvant. The immature dendritic cells and/or CTLs and adjuvant can be combined to form a composition, and the composition can be introduced intratumorally and/or through vessel into the patient. The adjuvant can be selected from the group consisting of lipid-based, protein-based and polysaccharides-based adjuvants, such as lymphocyte cultured medium, Marignase, Agaricus,OK432, BCG, Lentinan (shiitake),Reishi,Sarunokoshikake, TNF Meshimakobu, Froint's complete or incomplete adjuvant, LPS, fatty acids, TW80, phospholipids, cytokines or a virus, and mixtures

thereof. In certain embodiments, the adjuvant can be a leukocyte cultured medium (LCM) adjuvant. The LCM adjuvant can include at least three cytokines selected from the group consisting of eotaxin, FGF, G-CSF, GM-CSF, IFNγ, IP10, IL1β, IL1ra, IL2, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12, IL13, IL15, IL17, MCP1, MIP1α, MIP1β, PDGFbb, RANTES, TNFα and VEGF.

[0017] In another aspect, the invention provides a method of regressing, reducing or eliminating tumor cells in a patient, which includes obtaining monocyte cells from the patient by isolating the monocyte cells from peripheral blood mononuclear cells, differentiating the monocyte cells to produce immature dendritic cells, combining a first sample of the immature dendritic cells with adjuvant and keyhole limpet to form a first mixture of the immature dendritic cells, introducing intratumorally and/or through vessel the first mixture of the immature dendritic cells into the patient, preparing CTLs from the monocyte-depleted peripheral blood mononuclear cells, introducing intratumorally and/or through vessel a first sample of the CTLs into the patient subsequent to introducing the first mixture of immature dendritic cells, combining a second sample of the immature dendritic cells with adjuvant to form a second mixture of the immature dendritic cells, introducing intratumorally and/or through vessel the second mixture of the immature dendritic cells to the patient, and introducing intratumorally and/or through vessel a second sample of the CTLs to the patient subsequent to introducing the second mixture of the immature dendritic cells Furthermore, the method includes introducing intratumorally and/or through vessel and/or subcutaneously a therapeutically effective amount of anti PD-1/PD-L1 antibody into the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] A further understanding of the invention can be gained from the following description of the preferred embodiments when read in conjunction with the accompanying drawings in which:

[0019] FIG. 1A is a schematic showing effectiveness of anti-PD-1 and/or anti-PD-L1 to suppress/inhibit the interaction and binding of PD-1 and/or PD-L1 wherein view (a) shows inhibited antitumor immunity with PD-1 and PD-L1 and view (b) shows enhanced

antitumor immunity with anti-PD-1 antibody and anti-PD-L1 antibody; FIG. 1B shows images of antitumor immunity wherein view (c) shows a dendritic cell and memory T-cell and view (d) shows an autologous cancer cell and CTL, in accordance with certain embodiments of this invention;

[0020] FIG. 2 is a plot showing a cancer treatment protocol and the number of tumors for every 3-months check-ups of a female cancer patient, in accordance with certain embodiments of this invention;

[0021] FIG. 3A is an image showing conditions of cancer tumors of the female patient before administration of treatment protocol HITV; FIG. 3B is a plurality of images showing before and after conditions of the cancer tumors of the female patient administered the cancer treatment protocol as shown in FIG. 2 wherein views (a) and (b) show before and after IMRT, respectively, views (c) and (d) shown before and after RFA, respectively, views (e) and (f) show before and after DC, respectively, and views (f) and (g) show before and after DC+PD-1, respectively, in accordance with certain embodiments of the invention;

[0022] FIG. 4 is a plot showing a cancer treatment protocol and the number of tumors for every 3-months check-ups of a female cancer patient as shown, in accordance with certain embodiments of this invention;

[0023] FIG. 5A is a plurality of images in views (a), (b) and (c) showing the female patient's lymphoma tumors on January 1, 2016, and FIG. 5B shows images in view (c), (d) and (e) on April 22, 2016, indicative of the reduction of the lymphoma of the female patient administered of a cancer treatment protocol as shown in FIG. 4, in accordance with certain embodiments of the invention;

[0024] FIG. 6A is an image showing a carcinoma of a male patient on April 13, 2016, and FIG. 6B is an image showing reduction of the carcinoma of the male patient on June 15, 2016, as a result of administration of a cancer treatment protocol, in accordance with certain embodiments of the invention;

[0025] FIG. 7A is an image showing a carcinoma of a female patient on February 5, 2016, and FIG. 7B is an image showing reduction of the carcinoma of the female patient

on May 13, 2016, as a result of administration of a cancer treatment protocol, in accordance with certain embodiments of the invention; and

[0026] FIG. 8A is a plot comparison for five assays on tumor cell staining (whole cohort) and FIG. 8B is a plot comparison for five assays on tumor cell staining (non-small cell lung cancer, cytology excluded), for the various cancer protocols shown in Table 7, in accordance with certain embodiments of the invention.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0027] The invention includes introducing to a patient immature dendritic cells and/or cytotoxic T lymphocytes (CTLs) and PD-1 and/or PD-L1 inhibitor (anti PD-1/PD-L1 antibody). The immature dendritic cells and/or CTLs are introduced to the patient intratumorally and/or through vessel, and the PD-1 and/or PD-L1 inhibitor is introduced to the patient intratumorally and/or through vessel and/or subcutaneously. The immature dendritic cells may be introduced coincident with introduction of the CTLs. Alternatively, the immature dendritic cells may be introduced prior to (e.g., immediately before or a short time before), or following (e.g., immediately thereafter or a short time thereafter), introduction of the CTLs. Similarly, the CTLs may be introduced coincident with introduction of the immature dendritic cells; alternatively, the CTLs may be introduced prior to (e.g., immediately before or a short time before), or following (e.g., immediately thereafter or a short time thereafter), introduction of the immature dendritic cells. The time between introducing the immature dendritic cells and/or CTLs can vary. In certain embodiments, the time period may range from zero (they are introduced simultaneously) to several seconds or zero to several minutes or zero to several hours or zero to several days or zero to several weeks. Further, the PD-1 and/or PD-L1 inhibitor may be introduced coincident with introduction of the immature dendritic cells and/or the CTLs. Alternatively, the PD-1 and/or PD-L1 inhibitor may be introduced immediately following introduction of the immature dendritic cells and/or the CTLs, or a short time thereafter the introduction of the immature dendritic cells and/or the CTLs, or a longer time thereafter the introduction of the immature dendritic cells and/or the CTLs.

[0028] Certain embodiments of the invention include a three phase cancer treatment method or protocol. The three phases are vaccination phase, CTL induction phase, and maintenance phase. The vaccination phase includes a first introduction of immature dendritic cells followed by radiation therapy to the patient, which is followed by a second introduction of immature dendritic cells to the patient. The CTL induction phase includes the introduction of immature dendritic and CTLs to the patient. The maintenance phase includes the introduction of the PD-1 and/or PD-L1 inhibitor to the patient. The maintenance phase may also include the introduction of dendritic cells with the PD-1 and/or PD-L1 inhibitor. The immature dendritic cells and CTLs may be produced by collecting monocyte cells and/or CTLs from a patient; culturing the monocyte cells and/or culturing/activating the CTLs collected from the patient; forming immature dendritic cells from the monocyte cells; and forming cultured CTLs from the natural CTLs. As aforementioned, the immature dendritic cells and CTLs may be introduced intratumorally and/or through vessel, and the PD-1 and/or PD-L1 inhibitor is introduced to the patient intratumorally and/or through vessel and/or subcutaneously.

[0029] The administration and combination of the three phases are described herein in additional detail.

[0030] The therapies and methods of the invention are effective to induce regression, reduction or elimination of cancerous tumor cells, cancerous tumor tissue, and one or more cancerous tumors including those formed by metastasis. Further, this regression, reduction or elimination can be achieved in the presence or absence of conventional therapy, such as, but not limited to, radiotherapy and chemotherapy. In certain embodiments, therapies and methods of the invention are administered to a patient absent of subjecting the patient to radiotherapy and/or chemotherapy.

[0031] As used herein, “patient(s)” include mammal(s), which include human(s).

[0032] As used herein, the term "therapeutically effective amount" refers to that amount of CTLs, PD-1 and/or PD-L1 inhibitor, immature dendritic cells, anti-inflammatory agent, adjuvant, TNF/IL-10 inhibitor or combinations thereof, required to bring about a desired effect in a human or other mammal. In all instances, at its most basic level, the desired effect is a regression, reduction or elimination of tumor cells in tumor tissue of

the cancer patient when compared to the tumor cells in the tumor tissue of the cancer patient prior to employing the therapies and methods of the invention.

[0033] As used herein, the term "subcutaneous" and related terms employing "subcutaneous" or "subcutaneously" refer to therapy that includes the introduction of PD-1 and/or PD-L1 inhibitor beneath the skin of a patient, e.g., hypodermic.

[0034] As used herein, the term "through vessel" and related terms employing "through vessel" refer to therapy that includes the introduction of immature dendritic cells and/or CTLs and/or PD-1 and/or PD-L1 inhibitor into a channel, such as, a vein or artery, in a patient's body for carrying fluid.

[0035] As used herein, the term "intratumoral therapy" and related terms employing "intratumoral" or "intratumorally" refer to therapy that includes the introduction (e.g., injection) of immature dendritic cells and/or CTLs and/or PD-1 and/or PD-L1 inhibitor directly into the tumor tissue of a patient.

[0036] The PD-1 and/or PD-L1 inhibitor can be in various forms known in the art. The PD-1 and/or PD-L1 inhibitor can be incorporated into a delivery mechanism, such as a carrier or medium, to facilitate introduction into the patient. In certain embodiments, the PD-1 and/or PD-L1 inhibitor can be included or contained in a liquid for ease of introduction, e.g., injection.

[0037] In general, PD-1 and PD-L1 are referred to as checkpoint proteins because they keep the immune system in check. The PD-1 is found on T cells, e.g., CTLs, and PD-L1 is found on cells including cancer cells. As aforementioned, the association or interaction of PD-1 and PD-L1, such as when PD-1 binds to PD-L1, results in PD-1 acting as an "off switch" or "brake" to prevent or block the CTLs from attacking other cells in the body. Since large amounts of PD-L1 can be found on some cancer cells, the binding of PD-1 with PD-L1 contributes to the ability of cancer cells to evade immune system attack.

[0038] It has been found that PD-1 and/or PD-L1 inhibitors, e.g., anti-PD-1 and/or anti-PD-L1, can be effective to suppress/inhibit the interaction and binding of PD-1 and PD-L1, and some monoclonal antibodies are effective PD-1 and/or PD-L1 inhibitors. These monoclonal antibodies that target either PD-1 or PD-L1 can block their binding and therefore, boost the immune response against cancer cells. As shown in FIG. 1A, view

(a), antitumor immunity is inhibited and as shown in FIG. 1A, view (b), antitumor immunity is enhanced. Thus, in accordance with the invention, anti PD-1/PD-L1 antibody is combined with CTLs to inhibit binding and, in turn, preclude evasion of immune attack. In certain embodiments, the combination of anti PD-1/PD-L1 antibody and CTLs is coincident, such as being co-introduced or co-injected in the patient.

[0039] In the therapies and methods of the invention, introduction of anti PD-1/PD-L1 antibody intratumorally and/or through vessel and/or subcutaneously is effective to suppress the binding of PD-1 and PD-L1. Suppression of this binding, the CTLs, e.g., induced by the introduction of the immature dendritic cells and/or CTLs, are not blocked from attacking and/or killing cancer cells in the body, and may be effective to destroy tumor cells both locally and systemically in the patient. The result of this immunological function can vary and may depend on the amount of tumor cells, and CTLs in the patient's body. In certain embodiments, these factors are used to determine a therapeutically effective amount of anti PD-1/PD-L1 antibody to be introduced or administered to the patient in order to achieve a favorable outcome, e.g., to maximize the regression, reduction or elimination of tumor cells (local and metastasized) in the patient. It is contemplated that an appropriate balance of the amount of tumor cells and PD-1 and/or PD-L1 present in the patient's body, the amount of CTLs induced by the introduction intratumorally and/or through vessel of immature dendritic cells and/or CTLs, and the amount of anti PD-1/PD-L1 antibody introduced intratumorally and/or through vessel and/or subcutaneously in the patient's body results in maximizing the effect of the therapy and the regression of tumors.

[0040] It is found that the therapies and methods of the invention which control, *e.g.*, suppress, the binding of the PD-1 and PD-L1 are effective to treat tumors. In certain embodiments, the therapies and methods of the invention are particularly effective to treat multiple metastasized tumors.

[0041] Further, without intending to be bound by any particular theory, it is believed that CTLs, which are naturally induced as part of the autoimmune response above-described, may not be potent against tumors especially when the tumors are at an advanced stage or aggressively growing. The reasons may be that these CTLs are not

induced in an adequate quality and/or in an adequate quantity and/or in a timely manner to defend the patient's body from the tumor invasion. Since CTLs are produced by the patient, in certain embodiments, CTLs can be collected from the patient, cultured, and then introduced, *e.g.*, returned, to the same patient's body. The culture medium can vary and may be selected from those known in the art. Non-limiting examples include, but are not limited to, IL-2, CD3, and mixtures thereof.

[0042] In certain embodiments, the invention includes collecting monocyte cells from the patient. The monocyte cells are cultured to form immature dendritic cells which are then introduced, *e.g.*, returned, to the same patient's body. The culture medium can vary and may be selected from those known in the art. Non-limiting examples include, but are not limited to, IL-4, GM-CFS, and mixtures thereof. In certain embodiments, CTLs are collected from the patient following collection, culture and return of the immature dendritic cells into the patient. Subsequent to their collection, the CTLs are cultured in a suitable medium and then the cultured CTLs are introduced, e.g., returned, to the same patient's body. The immature dendritic cells and/or CTLs are introduced intratumorally and/or through vessel into the patient. In alternative embodiments, only immature dendritic cells are introduced or only CTLs are introduced or a combination of immature dendritic cells and CTLs are introduced into the patient.

[0043] The anti PD-1/PD-L1 antibody can be introduced intratumorally and/or thorough vessel and/or subcutaneously into the patient in a therapeutically effective amount prior to, coincident with, or following introducing the cultured CTLs and/or immature dendritic cells. The amount or dose of anti PD-1/PD-L1 antibody administered can vary and may depend on the type and progression of the cancer, as well as the mechanism for administration/introduction. In certain embodiments, about 10 mg (e.g., 10 mg/ml) or about 20 mg (e.g., 20 mg/ml) of the anti PD-1/PD-L1 is injected intratumorally or an amount within the range of about 10 mg (e.g., 10 mg/ml) to about 20 mg (e.g., 20 mg/ml) is injected intratumorally. For intravenous administration, e.g., DIV/IV infusions, about 50 mg or about 100 mg of the anti PD-1/PD-L1 is introduced or an amount within the range of about 50 mg to about 100 mg is introduced. The anti PD-1/PD-L1 may be a

single administration or dose, e.g., injection or infusion, or may include multiple administrations or doses within a treatment protocol.

[0044] In accordance with the invention, an adequate quantity and quality of CTLs are provided in the patient's body and, in particular, at the tumor site(s), to regress, reduce or eliminate tumor cells. Further, the anti PD-1/PD-L1 antibody is introduced to inhibit binding of PD-1 and PD-L1, and in turn inhibit "turning off" or blocking the CTLs from attacking cancer cells in the body. Further, the invention provides for collecting the natural CTLs from the patient, culturing or activating these collected cells, and re-introducing them, e.g., and anti PD-1/PD-L1 antibody, into the same patient in a quantity and quality which is sufficient (e.g., a therapeutically effective amount) to regress, reduce or eliminate tumor cells.

[0045] In certain embodiments, the CTLs include CD8+NK T cell population.

[0046] In certain embodiments, the invention is a human-initiated therapeutic vaccine with immature dendritic cells and/or CTLs in combination with anti-PD-1 and/or anti-PD-L1 antibody.

[0047] In certain embodiments, the immature dendritic cells and/or CTLs are introduced in conjunction with an adjuvant. The adjuvant can be introduced intratumorally and/or through vessel into the patient prior to, coincident with or following intratumoral and/or through vessel introduction of the immature dendritic cells and/or CTLs. In certain embodiments, the immature dendritic cells and/or CTLs and the adjuvant can be combined to form a composition that can be introduced intratumorally and/or through vessel to the patient. Further, the immature dendritic cells and/or CTLs, and optionally adjuvant, are introduced intratumorally and/or through vessel prior to, coincident with or following introducing intratumorally and/or through vessel and/or subcutaneously the anti PD-1/PD-L1 antibody to the patient.

[0048] Suitable adjuvants for use in the invention can include, without limitation, lipid-based, protein-based and polysaccharides-based adjuvants, such as lymphocyte cultured medium, Marignase, Agaricus,OK432, BCG, Lentinan (shiitake), Reishi, Sarunokoshikake, TNF Meshimakobu, Froint's complete or incomplete adjuvant, LPS, fatty acids, TW80, phospholipids, cytokines or a virus. In certain embodiments, the

adjuvant can be a leukocyte cultured medium (LCM) adjuvant. The LCM adjuvant can include at least three cytokines selected from the group consisting of eotaxin, FGF, G-CSF, GM-CSF, IFNγ, IP10, IL1β, IL1ra, IL2, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12, IL13, IL15, IL17, MCP1, MIP1α, MIP1β, PDGFbb, RANTES, TNFα and VEGF.

[0049] In certain embodiments, the immature dendritic cells and/or CTLs can be introduced to the patient in conjunction with an anti-inflammatory agent. Suitable anti-inflammatory agents can include those that are known in the art. The anti-inflammatory agent can be introduced prior to, coincident with or following introduction of the immature dendritic cells and/or CTLs. It is typical for introduction of the immature dendritic cells, CTLs and anti-inflammatory agent to be simultaneous or substantially simultaneous, or for the elapsed time between introducing the immature dendritic cells and/or CTL and the anti-inflammatory agent to be relatively short in duration.

[0050] The invention can optionally include a precursor treatment. That is, prior to introducing the dendritic cells and/or CTLs, the patient may be administered a treatment selected from chemotherapy, radiotherapy, antibody therapy, and combinations thereof. These regimens are well known in the art. Further, optionally, it is contemplated that the use of these regimens may be employed at various other times throughout the method of the invention. However, it is further understood, that these regimens are not necessary. The therapy and methods of the invention are effective to regress, reduce or eliminate tumor tissue in a patient in the absence of chemotherapy, radiotherapy, antibody therapy, and combinations thereof.

[0051] The method of administering the therapy according to certain embodiments of the invention includes the following steps.

[0052] Step 1: Collecting monocyte cells and/or CTLs from a patient. Various conventional techniques known in the art can be employed for their collection.

[0053] Step 2: Culturing the monocyte cells and/or culturing/activating the CTLs collected from the patient. The monocyte cells form immature dendritic cells. Various conventional techniques known in the art can be employed to culture the monocyte cells and/or to culture/activate the CTLs, and various culture mediums known in the art can be used, such as those disclosed herein.

[0054] Step 3: Introducing intratumorally and/or through vessel, such as but not limited to injecting, a therapeutically effective amount of the immature dendritic cells and/or CTLs into the patient. The immature dendritic cells and/or CTLs can be mixed or combined with an adjuvant and the mixture or combination can be introduced intratumorally and/or through vessel into the patient. The adjuvant can be selected from those known in the art and disclosed herein. Further, optionally, an anti-inflammatory agent may be present with the immature dendritic cells and/or CTLs and adjuvant as disclosed herein.

[0055] Step 4: Introducing intratumorally and/or through vessel and/or subcutaneously, such as, but not limited to, injecting, a therapeutically effective amount of anti PD-1/PD-L1 antibody to the patient. In certain embodiments, from 10 mg to 20 mg of the anti PD-1/PD-L1 antibody is introduced intratumorally and/or through vessel and/or subcutaneously to the patient. This amount may constitute a single dose. Treatment protocols in accordance with the invention, can include the introduction of multiple doses of anti PD-1/PD-L1 antibody wherein each dose is from 10 mg to 20 mg. Further, in certain embodiments, a combination of dendritic cells and anti PD-1/PD-L1 antibody may be introduced.

[0056] The time allowed to lapse between and within the above-identified steps can vary. For example, the time allowed to lapse between Steps 3 and 4 can vary. In certain embodiments, Step 3 can be performed coincident with Step 4 and in other alternate embodiments, Step 4 can be performed following Step 3, such as a couple of seconds, hours, days, or weeks after Step 3.

[0057] The time allowed to lapse within Step 3, i.e., between the introduction of the immature dendritic cells and CTLs, can also vary. In certain embodiments, their introduction is simultaneous or substantially simultaneous. In other embodiments, there is a pre-determined or desired time period which is allowed to lapse between the introduction of each. It is typical for this period of time to be relatively short in duration.

[0058] Further, as previously indicated, the CTLs can be collected from the patient coincident with collection of the monocyte cells or at a time thereafter. In certain embodiments, the monocyte cells are collected and cultured to form immature dendritic

cells and the dendritic cells are introduced into the patient prior to collection of CTLs from the patient.

[0059] Furthermore, as previously indicated, conventional therapy, such as, radiation or chemotherapy, may be conducted at any time during Steps 1 through 4.

[0060] In certain embodiments, the invention can include the treatment steps of: introducing intratumorally and/or through vessel immature dendritic cells in a therapeutically effective amount into the tumor tissue of the patient; collecting from the patient CTLs induced by the introduction of the immature dendritic cells; re-introducing intratumorally and/or through vessel the collected CTLs into the tumor tissue of the same patient; and introducing intratumorally and/or through vessel and/or subcutaneously anti PD-1/PD-L1 antibody to the patient. The introduction of immature dendritic cells may be a prerequisite to the collection and/or introduction of the CTLs cells. In certain embodiments, the introduction of the immature dendritic cells is a prerequisite to introduction of the anti PD-1/PD-L1 antibody. Further, the introduction of the immature dendritic cells may be combined, e.g., simultaneous, with introduction of the anti PD-1/PD-L1 antibody.

[0061] In certain embodiments, the inducement of CTLs by the immature dendritic cells is sufficient (e.g., a therapeutically effective amount) such that the natural CTLs are not removed from the patient and not re-introduced into the same patient. Thus, the anti PD-1/PD-L1 antibody can be introduced with the immature dendritic cells (e.g., absent of re-introducing CTLs).

[0062] Without intending to be bound by any particular theory, it is believed that immature dendritic cells which are formed by culturing monocyte cells collected from a patient and CTLs which are produced by a patient and collected from the patient, provide for an enhanced desired effect when injected into the same patient as compared to immature dendritic cells and CTLs produced and obtained by other means. It appears that immature dendritic cells which are formed from the patient's own monocyte cells which have been collected and CTLs which have been collected, cultured and re-introduced intratumorally and/or through vessel, provide improved coupling or interaction with other cells in the body of the patient.

[0063] In certain embodiments, the invention provides regression, reduction or elimination of tumor cells in tumor tissue which can be visually detected by MRI and/or CT and/or Echo scan.

[0064] Further, in certain embodiments of the invention, a combination of immunotherapy and local radiation is administered to a cancer patient. Without intending to be bound by any particular theory, it is believed that this combination of treatments provides for a therapeutic protocol to stimulate a systemic adaptive immune response against malignant cells. In accordance with this protocol, monocyte cells are obtained from the patient for differentiation into immature dendritic cells. The monocyte cells are isolated from peripheral blood mononuclear cells (PBMCs). The monocyte cell-depleted, T-cell enriched fraction of the PBMCs is then used to prepare activated T-cells, e.g., CTLs. The immature dendritic cells are combined with LCM, a multi-cytokine-based adjuvant, and keyhole limpet hemocycanin (KLH). This mixture is injected into the patient. Subsequently, such as but not limited to, on the following day, activated T-cells, e.g., CTLs, are infused. After a period of time, such as, but not limited to, about seven days, local radiation is administered to the patient. After a period of time, such as, but not limited to, about another seven days, a second sample of immature dendritic cells and activated T-cells, e.g., CTLs, is administered to the patient. For example, immature dendritic cells suspended in LCM are injected and then, e.g., on the following day, activated T-cells, e.g., CTLs, are infused. It has been found that this protocol results in an increase in $CD8^+$ $CD56^+$ cells. Without intending to be bound by any particular theory, it is believed that these cells are capable of killing cancer cells.

[0065] Moreover, in certain embodiments, the cancer treatment in accordance with the invention includes the introduction of a therapeutically effective amount of TNF inhibitor (anti TNF), such as TNF antibody, and/or IL-10 inhibitor (anti IL-10), such as IL-10 antibody to the patient. The TNF/IL-10 inhibitor may be administered with the dendritic cells and/or CTLs. For instance, the TNF/IL-10 inhibitor is introduced coincident with one or both of dendritic cells and CTLs; alternatively, the TNF/IL-10 inhibitor is introduced prior to or following introduction of one or both of dendritic cells and CTLs. The TNF/IL-10 inhibitor may be administered intratumorally and/or through vessel

and/or subcutaneously to the patient. It is contemplated that the intratumoral injection of immature dendritic cells results in inducement of CTLs as part of an immunoresponse. This immunoresponse may promote a TNF response, which may in turn induce inflammation. Thus, the TNF/IL-10 inhibitor introduced with CTLs, e.g., co-introduced or co-injected, may inhibit or reduce such inflammation.

[0066] Accordingly, TNF antibody (anti TNF) is effective to suppress the systemic inflammation caused by TNF and by suppressing the inflammation, the CTLs are not limited in their immunological function. The TNF antibody may be introduced when there is a sufficient amount of CTLs presenting in the autoimmune system of the patient such that the immune response of CTLs is supported by suppressing the activity of the TNF. The TNF antibody can be administered in various forms, such as, incorporated in a delivery mechanism, e.g., liquid carrier/medium, to facilitate introduction/injection to the patient.

[0067] The invention includes one or more of the following features:

[0068] Anti PD-1/PD-L1 antibody induces tolerance break between cancer cells and CTLs;

[0069] A combination of dendritic cells and Nivolumab has been shown to control growth of new tumors through IV/IA infusions (12) or intra-tumoral injections (43); and

[0070] A higher regulation rate has been shown by intra-tumoral injection (60.5%) for CR/PR than IV/IA infusion (16.7%) for PR.

EXAMPLES

[0071] As shown by the clinical data in Table 1, multiple cancer patients having a variety of cancer diagnoses (e.g., liver, esophageal, breast, lung, colon, ovarian, rectal, cervical, uterine, lymphoma, pancreatic, and bone) were administered a treatment protocol at the various sites, e.g., organs and tissues, in the body corresponding to the particular diagnosis, in accordance with certain embodiments of the invention. The patients experienced partial response or regression (PR) or complete response or

regression (CR) of cancer tumors, or the results showed progressive disease (PD) or stable disease (SD). Table 1 shows that the treatment protocol for most of the patients included administration of dendritic cells and PD-1 inhibitor, which was an anti-PD-1 antibody known generically as Nivolumab and commercially available under the tradename Opdivo®.

[0072] Table 2 shows the efficacy of Nivolumab for Stage III lung cancer, Stage IV pancreatic cancer, malignant lymphoma and colon cancer, as well as for various recurrent cancers. In addition, Table 2 demonstrates the favorable results of the treatment protocol including intratumoral administration of Nivolumab. The 43 intratumoral injections resulted in the following outcome: 14 complete responses or regression (CR), 12 partial responses or regression (PR), 1 stable disease (SD) and 16 progressive diseases (PD). Thus, 26 out of the 43 injections, i.e., 60.5%, provided CR or PR. As shown in Table 2, intratumoral injection resulted in a higher regulation rate for CR/PR than the IV/IA infusions. There were 2 partial responses or regression (PR) based on 12 infusions, i.e., 16.7%.

[0073] Table 3 shows a treatment protocol in accordance with certain embodiments of the invention, which was administered to a female patient (Patient ID: 1548EM) diagnosed with endometrial cancer and metastasis. Chemotherapy was administered to the patient, followed by apheresis to collect monocyte cells and CTLs from the patient. The monocyte cells and CTLs were cultured. Immature dendritic cells produced from the monocyte cells were introduced to the patient, followed by introduction of the cultured CTLs and subsequently, the introduction of dendritic cells and a PD-1 inhibitor. As shown in Table 3, on January 16, 2018, dendritic cells and Nivolumab (anti-PD-1 antibody) were administered to the patient. As shown in FIG. 2 and FIGS. 3A and 3B (views (a) through (g)), administration of this protocol according to certain embodiments of the invention resulted in a reduction and subsequent elimination of a number of tumors. In FIGS. 3A and 3B (views (a) through (g)), the arrows within the images point to the location of the tumors.

[0074] Table 4 shows a treatment protocol in accordance with certain embodiments of the invention, which was administered to a female patient (Patient ID: 0677FS)

diagnosed with malignant lymphoma. Chemotherapy was administered to the patient, and apheresis was performed on the patient to collect monocyte cells and CTLs. The monocyte cells and CTLs were cultured. The immature dendritic cells produced from the monocyte cells were introduced to the patient, followed by introduction of the cultured CTLs and subsequently, the introduction of a PD-1 inhibitor. As shown in Table 4, on February 9, 2016, Nivolumab (anti-PD-1 antibody) was administered to the patient. As shown in FIG. 4 and FIGS. 5A (views (a) through (c)) and 5B (views (d) through (f)), administration of this protocol according to certain embodiments of the invention resulted in a reduction and subsequent elimination of a number of tumors. In FIG. 5A (views (a) through (c)), the arrows within the images point to the location of the tumors.

[0075] Table 5 shows a treatment protocol in accordance with certain embodiments of the invention, which was administered to a male patient (Patient ID: 1119HY) diagnosed with recurrence of renal cancer with developing multiple lung metastasis. Apheresis was performed on the patient to collect monocyte cells from the patient. The monocyte cells were cultured. The immature dendritic cells produced from the monocyte cells and PD-1 inhibitor were administered on April 13, 2016, July 12, 2016, October, 19, 2016, August 22, 2018, and October 22, 2018, as shown in Table 5. On January 21, 2019 and February 18, 2019, dendritic cells and PD-L1 inhibitor (Durvalumab) were administered to the patient. As shown in FIGS. 6A and 6B, administration of this protocol according to certain embodiments of the invention resulted in a significant reduction of clear cell carcinoma. In FIGS. 6A and 6B, the arrows within the images point to the location of the tumors.

[0076] Table 6 shows a treatment protocol in accordance with certain embodiments of the invention, which was administered to a female patient (Patient ID: 1089KD) diagnosed with recurrence of cervical uterine carcinoma. Chemotherapy was administered to the patient, followed by apheresis to collect monocyte cells and CTLs from the patient. The monocyte cells and CTLs were cultured. The immature dendritic cells produced from the monocyte cells were introduced to the patient, followed by introduction of the cultured CTLs and subsequently, the introduction of dendritic cells and a PD-1 inhibitor. Table 6 shows dendritic cells and PD-1 inhibitor were administered

on February 18, 2016. As shown in FIGS. 7A and 7B, administration of this protocol according to certain embodiments of the invention resulted in significant regression of the clear cell carcinoma. In FIGS. 7A and 7B, the arrows within the images point to the location of the tumors.

[0077] Table 7 shows immune checkpoint inhibitors and corresponding PD-L1 assays. The drugs identified are commercially available under the corresponding trade name and from the associated manufacturer/producer. Nivolumab (Opdivo) and Pembrolizumab (Keytruda) target PD-1, whereas Atezolizumab (Tecentriq), Durvalumab (Imfinzi) and Avelumab (Bavencio) target PD-L1. FIGS. 8A and 8B show two plots to compare these five assays on tumor cell staining.

[0078] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications that are within the spirit and scope of the invention, as defined by the appended claims.

TABLE 1

Nivolumab (antiPD-1) Clinical Data

Patient ID	Diagnosis	Treated Sites	DC	anti-PD-1	Ad. Date	Method	Ch-T	Ev. Time	Result
#1177SC	Rec. of Malignant Thymoma Op.	Pleura		100mg	2016.01.21	DIV	No	3M	PR
#1404NS	Rec. of esophageal Ca. Op	Multiple LN Meta.		100mg	2016.01.20	DIV	Yes	1M	PD
		Multiple LN Meta.		50mg	2016.04.20	DIV	No	2M	PD
		LN x4	4U	10mg x4	2016-06-06	IT	No	5M	PD
		LN x4	4U	10mg x4	2016.07.05	IT	No		PD
#918TW	Rec. of Adenocystic NPC Op.	Lung & Pleura		100mg	2006.01.21	DIV	Yes	4H	PD
	Lung & Pleural Meta.								
#1316TH	Rec. of Gastric Ca. Op.	Liver, Bil-adrenal	2U	100mg	2016.01.20	DIV	No	2M	PD
		LN Meta. Ascitis							
#1212RK	Rec. of right breast Ca. Op.	Left Pleura		100mg	2016.01.26	DIV	Yes	1M	PR
			1U	100mg	2016.03.22	DIV	Yes	1M	SD
			1U	50mg	2016.04.19	DIV	Yes	1M	SD
		Botallo LN, Pleura	2U	10mg x2	2016.05.19	IT	Yes	1M	PR
		Th5, Pleura	2U	10mg x2	2016.06.15	IT	Yes	1.5M	PR
		Pleura x2	2U	10mg x2	2016.08.22	IT	No	1M	PD
#1340KD	Pancreatic Ca.		3U	100mg	2016.01.15	DIV	Yes	1M	PD
	Liver, LN metastasis								
#677FS	Malignant Lymphoma (St. IV)	Lt. SCLN x2	2U	10mg x2	2016.02.09	IT	No	2M	CR
		Lt. Gluteal LN	1U	10mg	2016.02.09	IT	No	2M	CR
#1122MW	Rt. Lung Ca.	Rt. S6 Primary	1U	10mg	2916.02.24	IT	No	0.5M	PR
		Rt. S6 Primary	1U	10mg	2016.03.30	IT	No	3M	CR
		Rt. Pleural Effusion	1U	10mg	2016.06.21	IP	No	2M	CR
#1326AA	Rec. of Breast Ca. Op.	Rt. SCLN	1U	10mg	2016.01.20	IT	No	2M	SD
#1000MN	Rec. of Malignant Thymoma	Lt. Upper Med. LN	1U	10mg	2016.03.08	IT	No	1M	PR
		Paratracheal LN	1U	10mg	2016.03.08	IT	No	2M	CR
		Lt. SCLN x3	3U	10mg x3	2016.04.05	IT	No	1M	PR

TABLE 1 (CONT'D)

Nivolumab (antiPD-1) Clinical Data

Patient ID	Diagnosis	Treated Sites	DC	Anti-PD-1	Ad. Date	Method	Ch-T	Ev. Time	Result
#1210AO	Colon Ca.	Ovarium	2U	20mg	2016.02.24	IT	Yes	3M	PR
	Lung, Liver & Ovarial Meta.			50mg	2016.04.20	DIV	Yes	1.5M	PD
		Botallo LN	1U	10mg	2016.06.27	IT	Yes	2M	PD
		Rt. Lung S3	1U	10mg	2016.06.27	IT	Yes	2M	PD
		Liver S3 & S8	2U	10mg x2	2016.07.25	IT	Yes	1M	PD
#910KK	Rec. of Rectal Ca. Op.	Rt. Ilian LN	1U	100mg	2016.01.21	DIV	Yes	6M	PD
	Peritoneal Dissemination	Rt. Iliac LN	1U	40mg	2016.05.12	1A	Yes	1.5M	PD
	Sacrum & Dauglas Meta.	Rt. Iliac LN x2	2U	10mg x2	2016.08.30	IT	Yes		PD
#1089AD	Rec. of Cervical Uterine Ca. Op.	Sacral Soft Tissue	1U	10mg	2016.02.18	IT	No	3M	CR
	Lt. Pelvic LN & Sacral bone Met.								
#433YI	Rec. of Rt. Breast Ca. Op.	Th11	1U	10mg	2016.03.29	IT	No	2M	CR
	Multi. Bone Meta.	Parasternal LN	1U	10mg	2016.03.29	IT	No	2M	CR
#1119HY	Rec. of Lt. Renal Ca.	Lt. Lung S3	2U	20mg	2016.04.13	IT	No	2M	PR
	Mult. Lung Met.								
#884SM	Rec. of Lt. Breast Ca. Op.	PALN	1U	10mg	2016.04.26	IT	No	2M	PR~CR
	Mult. LN Meta>	PALN	1U	10mg	2016.06.02	IT	No	1M	CR
#677FS	Malignant Lymphoma (St. IV)	Lt. Glutal LN	1U	10mg	2016.02.09	IT	No	3M	CR
		Lt. SCLN	1U	10mg	2016.02.09	IT	No	3M	CR
		Lt. ICLN	1U	10mg	2016.02.09	IT	No	3M	CR

TABLE 2

Diagnosis and Efficacy of Nivolumab for 17 Cases

Stage III		**Stage IV**		**Recurrent**	
Lung Ca	1	Pancreatic Ca	1	Malig. Thymoma	2
		Mali. Lymphoma	2	Esophageal Ca	1
		Colon Ca	1	NPC	1
				Gastric Ca	1
				Breast Ca	4
				Rectal Ca	1
				Uterus Ca	1
				Renal Ca	1

DIV or 1A (12 Infusions)			
CR	PR	SD	PD
0	2	2	8
16.7%			

IT: Intra-tumoral (43 Injections)			
CR	PR	SD	PD
14	12	1	16
60.5%			

TABLE 3

Patient ID: 1548EM
Gender: Female
DOB: 1960/02/06

Clinical Diagnosis: Rec. of endometrial cancer,Virchow & multiple LN metastasis
Pathology: adenocarcinoma
Stage: Rec.
Present History:
2015.11 Endometrial cancer diagnosed,PALN metastasis
2015.12.14 Radical hysterectomy+ peivic & paraaortic adenectomy 2016.01.14 TC chemo x6 course
2016.04.28 Virchow LN metastasis
2016.05.18 AP chemo xl termination by side effect
2016.07.01 Apheresis(1)
2016.07.04 PET-CT(lt cervical LN,lt SCLN x2,lt ICLN x2,caval LN,pararectal LN)
2016.07.21 DC(08/09,11/10,11/30,12/27,2017/02/21,04/19,07/19,08/29, 09/27,10/26,11/21,12/21,2018/01/16,02/15,05/10,08/02) 2016.07.25 IMRT 48.5Gy/10Fr/12D(lt cervical LN,lt SCLN x2,lt ICLN x2,caval LN,pararectal LN) 2016.09.20 PET-CT Evaluation(1)

Treated sites: lt cervical LN SD,lt SCLN x2 PR,lt ICLN x2 PR,caval LN CR,pararectal LN PR
New Lesion: rt iliac LN
2016.10.04 CT(lt cervical LN,lt SCLN,rt iliac LN)

TABLE 3 (CONT'D)

Patient ID: 1548EM
Gender: Female
DOB: 1960/02/06

2016.11.10 Apheresis (2), rt iliac LN PD
2016.12.05 IMRT 60.2Gy/14Fr/18D (rt iliac LN)
2017.02.08 PET-CT Evaluation (2)
Treated sites: lt cervical LN CR, lt SCLN x2 CR, lt ICLN x2 CR, caval LN CR, pararectal LN CR, rt iliac LN PR
New Lesion: liver S6
2017.03.21 CTL (liver S6) PD
2017.06.05 RFA (liver S6)
2017.07.18 Apheresis (3)
2017.09.19 PET-CT Evaluation (3)
Treated sites: rt iliac LN CR, liver S6 CR
New lesion: retroduo LN
2017.10.04 Xeloda 1200mg/day
2018.01.09 PET-CT Evaluation (4)
Treated sites: retroduo LN PD
New lesion: none
2018.01.16 Apheresis (4)
DC + Nivolumab (PD-1) 10mg (retroduo LN)
2018.04.09 PET-CT Evaluation (5)
Treated sites: retroduo LN CR
New lesion: none
2018.07.10 PET-CT Evaluation (6): normal study

TABLE 4

Patient ID: 0677FS

Gender: Female
DOB: 1950/03/22

Clinical Diagnosis: Malignant lymphoma
Pathology: Follicular lymphoma
Present History:

2011.10.12 PET-CT (lt SCLN x4, PALN x9, caval LN x8, rt renal pelvic LN x2, peritoneum x10, rt iliac LN x6, lt iliac LN x10, lt iliopsoas M, lt inguinal LN)
2011.10.18 Lt. inguinal LN biopsy: follicular lymphoma
2011.10.21 Apheresis (1)
2011.10.26 R-CHOP 5 course started, finished 2012.01.27, postponed 6th course due to side effect
2011.11.10 DC (11/29, 12/20, 2012/01/19, 07/03, 2013/02/26, 03/14, 05/16, 06/10, 07/11, 08/12, 11/21, 2014/05/28, 07/02, 08/04, 09/02, 10/07, 11/04, 12/02, 2015/01/06, 02/05, 03/05, 03/31, 05/14, 08/04, 10/22, 12/17, 2016/03/08)
2012.01.12 (1) PET-CT mostly CR, remaining lesion : lt psoas muscle
2012.02.09 CTL apheresis
2012.03.01 (2) PET-CT remaining lesion : lt psoas muscle
2012.03.19 CTL (12/12, 2013/01/07, 05/17, 06/11, 07/12, 08/13, 09/10, 10/08, 2014/01/14, 05/28, 12/02, 12/17, 2015/02/05)
2012.06.08 (3) PET-CT PD : lt psoas muscle, new lesion : PALN, lt iliac LN
2012.06.12 Apheresis (2)
2012.06.20 IMRT 32.14Gy/5Fr/7D (lt psoas Mx2, PALNx2, lt iliac LNx3, rt iliac LN, lt retro-peritoneum)

TABLE 4 (CONT'D)

Patient ID: 0677FS

2012.07.24 CTL apheresis
2012.08.11 (4) PET-CT Evaluation, CR all treated sites, new lesion: none
2012.11.10 (5) PET-CT Evaluation, PR lt psoas M, new lesion: rt retro-diaphragm Lig (Th11), lt iliac LNx2
2013.02.09 (6) PET-CT Evaluation, CR lt psoas M, lt iliac LN (1), PD rt retro-diaphragm Lig. (Th11), lt iliac LN (2), new lesion: rt rib 10, Th10 (right), Th11 (right), L1
2013.02.28 Apheresis (3)
2013.03.04 IMRT
2013.04.23 (7) PET-CT Evaluation
Treated sites: rt rib10, Th10, Th11 x2, caval LN, lt iliac LN all CR
New lesion: none
2013.07.24 (8) PET-CT Evaluation
Treated sites: CR
New lesion: lt SCLN x3
2013.10.24 (9) PET-CT Evaluation
Treated sites: lt SCLN x3 all CR
New lesion: lt gluteal LN
2014.01.30 (10) PET-CT Evaluation
Treated sites: lt gluteal LN PR
New lesion: none
2014.04.22 (11) PET-CT Evaluation
Treated sites: lt gluteal LN CR
New lesion: new lt gluteal LN
2014.07.22 (12) PET-CT Evaluation
Treated lesion: new lt gluteal LN CR
New lesion: none

TABLE 4 (CONT'D)

Patient ID: 0677FS

2014.09.02 Apheresis (4)
2014.10.25 (13) PET-CT Evaluation
Treated stes: It gluteal LN PD
New lesion: It acc LN
2015.01.24 (14) PET-CT Evaluation
Treated sites: It acc LN CR, It gluteal LN SD
New lesion: It acc LN (2)
2015.04.17 (15) PET-CT Evaluation
Treated sites: It acc LN (2), It gluteal LN PR
New lesion: none
2015.07.10 (16) PET-CT Evaluation
Treated sites: It acc LN (2) CR, It gluteal LN PD
New lesion: It acc LN (3), It cervical LN, It ICLN
2015.07.15 Apheresis (5)
2015.10.09 (17) PET-CT Evaluation
Treated sites: It cervical LN, It acc LN (3) CR, It ICLN, It gluteal LN PR
New lesion: none
2016.01.08 (18) PET-CT Evaluation
Treated sits: It cervical LN PR, It gluteal LN SD, It SCLN, It ICLN PD
New lesion: It SCLN (2)
2016.02.09 PD-1 (It SCLN, It SCLN (2), It ICLN, It gluteal LN)
2016.04.22 (19) PET-CT Evaluation
Treated sites: It SCLN, It SCLN (2), It ICLN, It gluteal LN CR
New lesion: none

TABLE 5

Patient ID: 1119HY
Gender: Male
DOB: 1948/03/22
Clinical Diagnosis: Recurrence of Lt. renal cancer post-operative
Pathology: Clear cell carcinoma

Present History:

2011.11 Left renal cell carcinoma, Lt. nephrectomy
2013.03 Multiple lung metastasis
2013.05 Sunitinib started
2014.02 CT evaluation: PD
2014.03 Changed to Axitinib
2014.05.29 Apheresis
2014.06.12 DC (07/09, 08/05, 08/21, 09/04, 09/18, 10/02, 10/16, 11/19, 12/10, 12/24, 2015/01/15, 02/04, 02/26, 03/26, 04/23, 05/21, 06/18, 07/16, 08/10, 09/03, 10/13, 11/11, 12/10, 2016/02/10, 04/13, 05/19, 06/15, 07/12, 08/17, 09/14, 10/19, 11/17, 12/15, 2017/01/25, 02/24, 03/23, 04/24, 05/15, 06/22, 07/19, 08/23, 09/26, 2018/02/15, 04/17, 06/14, 08/22, 09/20, 10/22, 2019/01/21, 02/18, 03/11, 04/08)
2014.09.19 CT evaluation
Rt. lung S3-1 PR, S3-2 PR, S5 SD, S6-1 PR, S6-2 SD, S8 PR
Lt. lung S1+2 SD, S3-1 SD, S3-2 SD, S3-3 SD
2015.06.10 CT evaluation
Rt. lung S3-1 SD, S3-2 CR, S5 SD, S6-1 SD, S6-2 SD, S8 PR
Lt. lung S1+2 SD, S3-1 PR, S3-2 PR, S3-3 SD
2015.09.18 CT evaluation
Rt. lung S3 SD, S5 PD, S6-1 PD, S6-2 PD, S8 SD
Lt. lung S1+2 PD, S3-1 PD, S3-2 PD, S3-3 SD
2015.10.13 DC intra-tumoral (Rt. S5, S6-1. S6-2)

TABLE 5 (CONT'D)

Patient ID: 1119HY

2015.11.11 DC intra-tumoral (Lt. S1;2, S3-1, S3-3)
2016.01.13 CT evaluation
Rt. lung S3 SD, S5 SD, S6-1 PR, S6-2 PR
Lt. lung S1+2 SD, S3-1 PR, S3-2 SD, S3-3 SD
2016.04.08 CT evaluation
Rt. lung S3 SD, S5 PR, S6-1 PR, S6-2 SD
Lt. lung S1+2 PR, S3-1 PD, S3-2 SD, S3-3 SD, S3-4 PD
2016.04.13 DC + PD-1 20mg, intra-tumoral (Lt. S3-1)...(1)
2016.06.15 CT evaluation
Rt. lung S3 SD, S5 PR, S6-1 PR, S6-2 PR
Lt. lung S1+2 SD, S3-1 PR, S3-2 PR, S3-3 SD, S3-4 PR
2016.07.12 DC + PD-1 10mg (Rt. S3)
2016.10.01 PET-CT evaluation
Treated sites: Rt. S3 SD, Lt. S3-1 PD
New lesion: None
2016.10.19 DC + PD-1 10mg (Lt. S3-1)...(2)
2017.01.14 PET-CT evaluation
Treated sites: Rt. S3 PR, Lt. S3-1 PR
2017.01.28 Brain MRI: Normal study
2017.02.02 Apheresis
2017.10.14 PET-CT evaluation, acute pancreatitis
2017.12.07 ERCP, pancreatitis
2018.05.18 Apheresis
2018.08.18 PET-CT evaluation
Treated sites: Rt. S3 SD, S8 PD, Lt. S1+2, S3 x2 SD
New lesion: Lt. hilar LN

TABLE 5 (CONT'D)

Patient ID: 1119HY

2018.08.22 DC + PD-1 20mg (Lt. hilar LN)
2018.10.22 DC + PD-1 10mg (Rt. S8)
2018.12.15 PET-CT evaluation
Treated sites: Rt. S8 SD, Lt. hilar LN PR
New lesion: None
2019.01.21 DC + Durvalumab (PD-L1) 25mg (Rt. S8)
2019.02.18 DC + Durvalumab (PD-L1) 50mg (Lt. hilar LN)
2019.03.11 CT evaluation
Rt. lung S3 PR, S5 PR, S6 PR, S8 PR
Lt. lung S1+2 SD, S3-1 CR, hilar LN PR

TABLE 6

Patient ID: 1089KD
Gender: Female
DOB: 1958/02/14
Clinical Diagnosis: Recurrence of cervical uterine carcinoma post-operative
Pathology: Clear cell carcinoma

Present History:

2009.06	Cervical uterine carcinoma, abdominal radical hysterectomy, Stage IIB (pT2bpN0, Clear cell carcinoma, vascular invasion ++) CDDP+CPT11 6 course
2011.07.26	Lt. iliac LN metastasis operation, Path: Clear cell carcinoma
2012.09	Lt. Int. & Ext. iliac LN metastasis operation + radiation 50.4Gy/28Fr
2014.02.22	PET-CT (lt iliac LN)
2014.03.24	Apheresis
2014.04.03	DC (04/23, 06/26, 07/23, 08/27, 10/09, 11/13, 12/04, 2015/01/15, 03/19, 04/02, 06/18, 07/16, 11/12, 2016/01/14, 02/18, 03/17, 04/14)
2014.04.11	IMRT25Gy/5F/7D (lt iliac LN)
2014.06.06	(1) PET-CT Evaluation Treated sites: lt Pelvic LN① PR, lt Pelvic LN②③ CR New lesion: none
2014.06.26	CTL (lt Iliac LN①) (07/23)
2014.12.11	(2) PET-CT Evaluation Treated sites: CR, New lesion: S1
2014.12.19	CTL (S1)
2015.02.19	(3) PET-CT Evaluation Treated sites: S1 PD, New lesion: lt Iliac LN x2
2015.03.23	IMRT40Gy/5F/5D (lt S1-2, lt iliac LN)
2015.04.17	CECT: lt iliac vein embolism
2015.04.30	PS3 (05/07, 05/14, 06/11, 06/18, 07/29, 09/16)

TABLE 6 (CONT'D)

Patient ID: 1089KD

2015.05.12 (4) PET-CT Evaluation
Treated sites: S1 CR, lt iliac LN PR~CR
New lesion: lt sacrum, caval LN
2015.08.27 (5) PET-CT Evalusation
Treated sites: S1 SD, caval LN PR, lt iliac LN SD, lt sacrum SD
New lesion: none
Pelvis MRI: lt sacral fracture
2015.09.03 Macrophage (lt iliac LN)
2015.10.22 Macrophage (S1)
2015.11.27 (6) PET-CT Evaluation
Treated sites: S1 CR, caval LN CR, lt iliac LN CR, lt sacrum PR
New lesion: none
2016.02.05 (7) PET-CT Evaluation
Treated sites: lt sacrum CR
New lesion: soft tissue close to lt sacrum
2016.02.18 DC + PD-1 10mg (soft tissue close to lt sacrum)
2016.03.31 Pelvis MRI: improvement of lt iliac vein embolism
2016.05.13 (8) PET-CT Evaluation
Treated sites: soft tissue close to lt sacrum CR
New lesion: none

TABLE 7

Immune checkpoint inhibitors and matching

PD-L1 assay

Drug	Company	Target	PDL-1 IHC assay	PDL-1 antibody Epitopes	Auto stainer	Detection system
Nivolumab (Opdivo)	BMS	PD-1	28-8	Extracellular	Dako Link 48	Envision Flex
Pembrolizumab (Keytruda)	Merck	PD-1	22C3	Extracellular		
Atezolizumab (Tecentriq)	Roche/ Genetech	PD-L1	SP142	Cytoplasmic	Ventana Benchmark	Optiview + Amplification
Durvalumab (Imfinzi)	Astra Zeneca	PD-L1	SP263	Cytoplasmic		Optiview
Avelumab (Bavencio)	Pfizer	PD-L1	73-10	Cytoplasmic	Dako Link 48	Envision Flex

We claim:

1. A method of regressing, reducing or eliminating tumor cells in tumor tissue of a patient comprising:

introducing intratumorally and/or through vessel a therapeutically effective amount of immature dendritic cells and/or CTLs into the patient; and

introducing intratumorally and/or through vessel and/or subcutaneously a therapeutically effective amount of PD-1 and/or PD-L1 inhibitor to the patient.

2. The method of claim 1, further including:

collecting monocyte cells and/or CTLs from the patient;

culturing the monocyte cells and/or the CTLs; and

forming immature dendritic cells from the monocyte cells.

3. The method of claim 1, wherein the introducing of the immature dendritic cells and/or CTLs, is coincident with the introducing of the PD-1 and/or PD-L1 inhibitor .

4. The method of claim 1, wherein the patient is a human.

5. The method of claim 1, wherein the introducing of the immature dendritic cells and/or CTLs is in conjunction with an adjuvant.

6. The method of claim 5, wherein the immature dendritic cells and/or CTLs and adjuvant are combined to form a composition and the composition is introduced intratumorally and/or through vessel into the patient.

7. The method of claim 5, wherein the adjuvant is selected from the group consisting of lipid-based, protein-based and polysaccharides-based adjuvants, and mixtures thereof.

8. The method of claim 7, wherein the adjuvant is selected from the group consisting of lymphocyte cultured medium, Marignase, Agaricus,OK432, BCG, Lentinan (shiitake),Reishi,Sarunokoshikake, TNF Meshimakobu, Froint's complete or incomplete adjuvant, LPS, fatty acids, TW80, phospholipids, cytokines or a virus, and mixtures thereof.

9. The method of claim 7, wherein the adjuvant comprises a leukocyte cultured medium (LCM).

10. The method of claim 9, wherein the LCM comprises at least three cytokines selected from the group consisting of eotaxin, FGF, G-CSF, GM-CSF, IFNγ, IP10, IL1β, IL1ra, IL2, IL4, IL5, IL6, IL7, IL8, IL9, IL10, IL12, IL13, IL15, IL17, MCP1, MIP1α, MIP1β, PDGFbb, RANTES, TNFα and VEGF.

11. The method of claim 1, wherein the introducing of the PD-1 and/or PD-L1 inhibitor is immediately following or a short time after the introducing of the immature dendritic cells and/or CTLs.

12. The method of claim 2, wherein culturing the monocyte cells is carried out in a culture medium selected from the group consisting of IL-4, GM-CFS, and mixtures thereof.

13. The method of claim 2, wherein culturing the CTLs is carried out in a culture medium selected from the group consisting of IL-2, CD3, and mixtures thereof.

14. The method of claim 1, wherein the tumor cells are present in metastasized tumor tissue.

15. The method of claim 11 wherein a short time after can be in a range from a couple of seconds to a couple of minutes to a couple of hours to a couple of days.

16. The method of claim 1, wherein the method is carried out in the absence of conventional therapy.

17. The method of claim 16, wherein conventional therapy is selected from chemotherapy, radiotherapy and combinations thereof.

18. The method of claim 1, further comprising introducing anti-IL-10, anti-IL-6 or mixtures thereof.

19. A method of regressing, reducing or eliminating tumor cells in a patient comprising:

obtaining monocyte cells from the patient by isolating the monocyte cells from peripheral blood mononuclear cells;

differentiating the monocyte cells to produce immature dendritic cells;

combining a first sample of the immature dendritic cells with adjuvant and Keyhole limpet to form a first mixture of the immature dendritic cells;

injecting the first mixture of the immature dendritic cells into the patient;

preparing CTLs from the monocyte-depleted peripheral blood mononuclear cells;

introducing a first sample of the CTLs into the patient subsequent to introducing the first mixture of immature dendritic cells;

combining a second sample of the immature dendritic cells with adjuvant to form a second mixture of the immature dendritic cells;

introducing the second mixture of the immature dendritic cells to the patient;

introducing a second sample of the CTLs to the patient subsequent to introducing the second mixture of the immature dendritic cells; and

introducing PD-1 and/or PD-L1 inhibitor to the patient.

PD-1 & PD-L1
(a)
PD-L1
T cell
T-cell receptor
PD-1
PD-L1
MHC
Tumour cell
Inhibited antitumour immunity
(b)
Anti-PD-L1 antibody
Enhanced antitumour immunity
Cancer immunotherapy
(c)
(d)

FIG. 1A

FIG. 1B

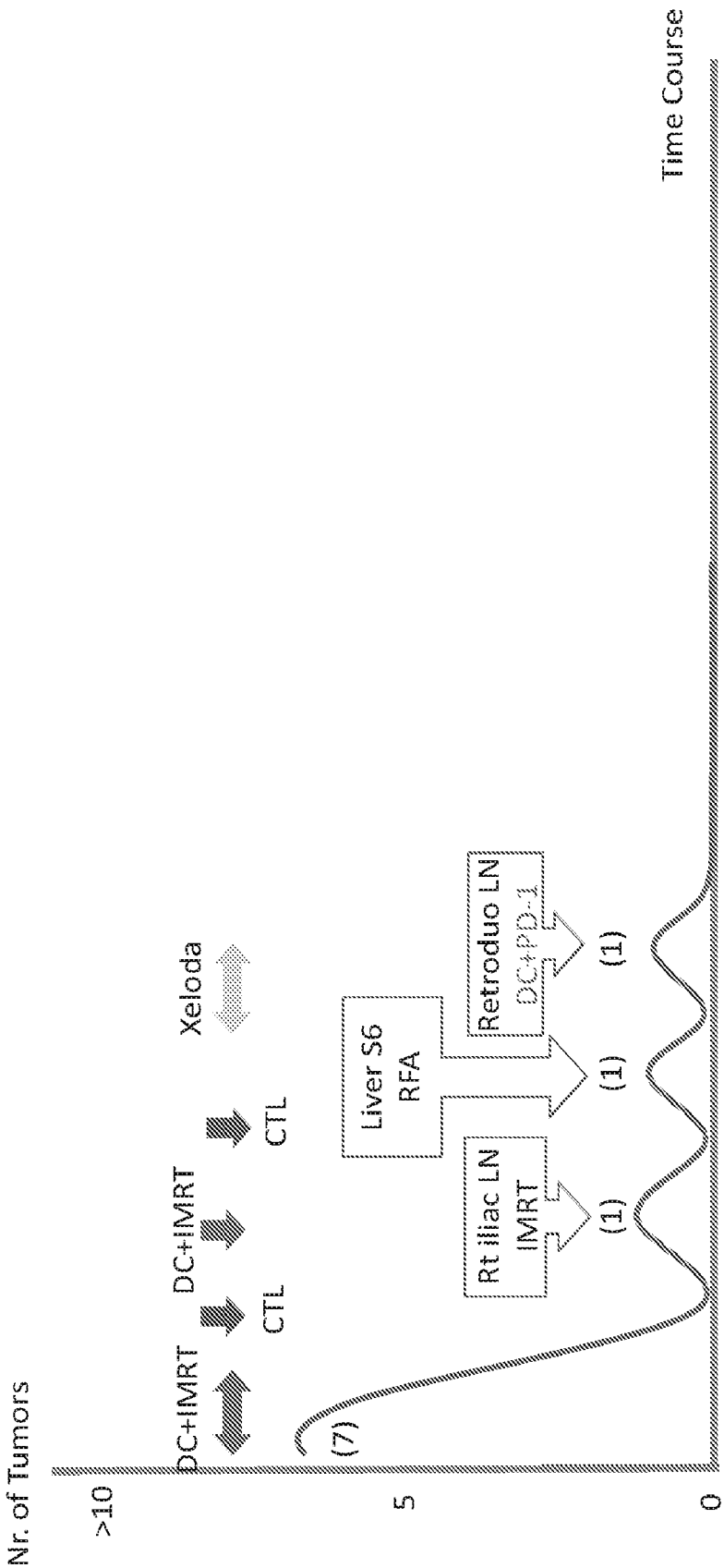

Protocol HITV IIe-2
Rec. of Endometrial Cancer Op, Virchow & multiple
LN Metastasis (E/M, 57Y Female, ###1548)
Nr. of Tumors
>10
5
0
Before
1st Year
2nd Year
3rd Year
Time Course
DC+IMRT
CTL
DC+IMRT
CTL
Xeloda
(7)
Liver S6
RFA
Rt iliac LN
IMRT
Retroduo LN
DC+PD-1
(1)
(1)
(1)
(1)
Every 3 months check ups

FIG. 2

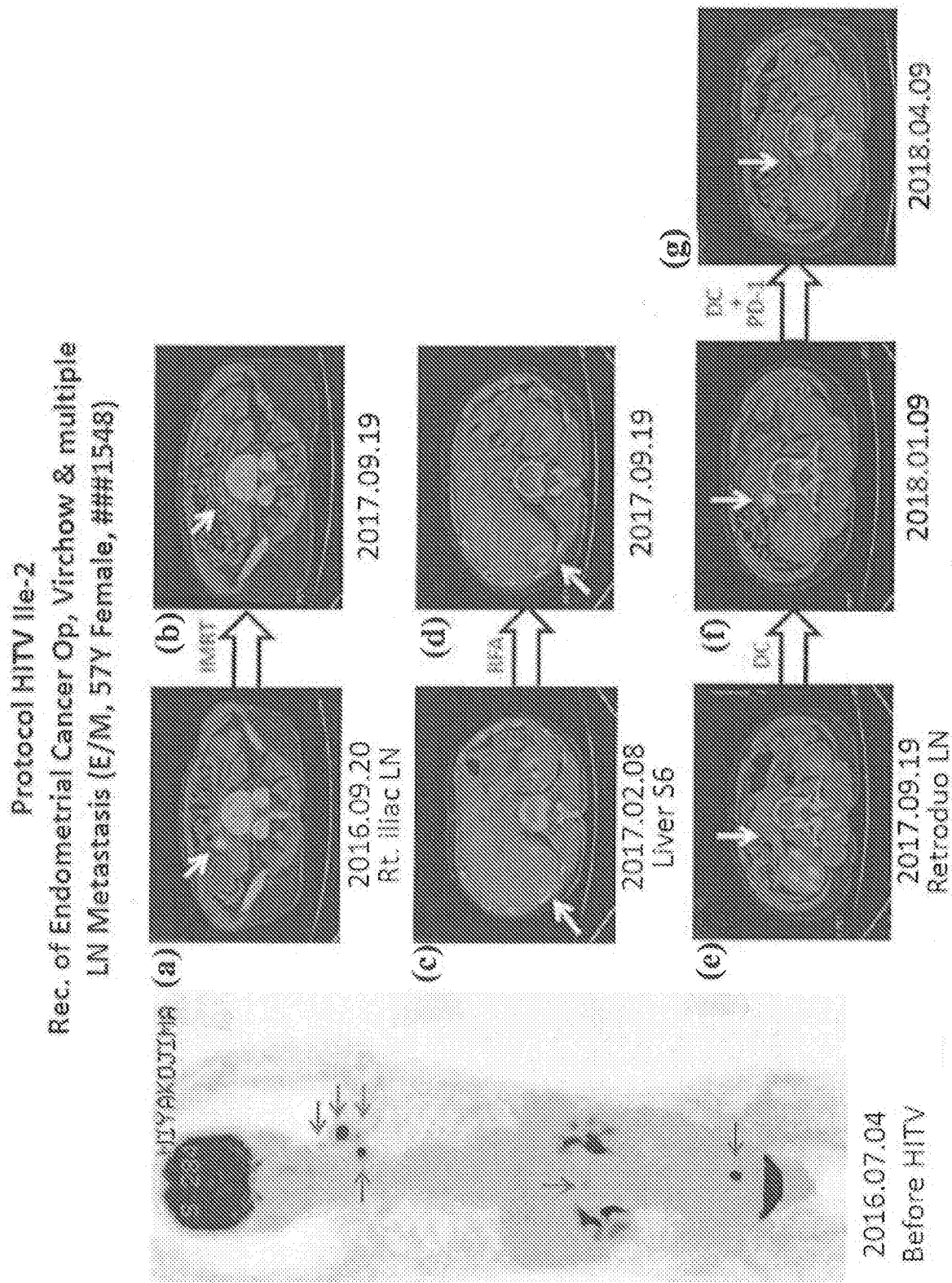

Protocol HITV Ile-2
Rec. of Endometrial Cancer Op, Virchow & multiple LN Metastasis (E/M, 57Y Female, ###1548)
(a)
2016.09.20
Rt. iliac LN
(b)
IV/IAT
2017.09.19
(c)
2017.02.08
Liver S6
(d)
IA
2017.09.19
(e)
2017.09.19
Retroduo LN
(f)
IVC
2018.01.09
(g)
IVC
+
PD-1
2018.04.09
2016.07.04
Before HITV

FIG. 3A

FIG. 3B

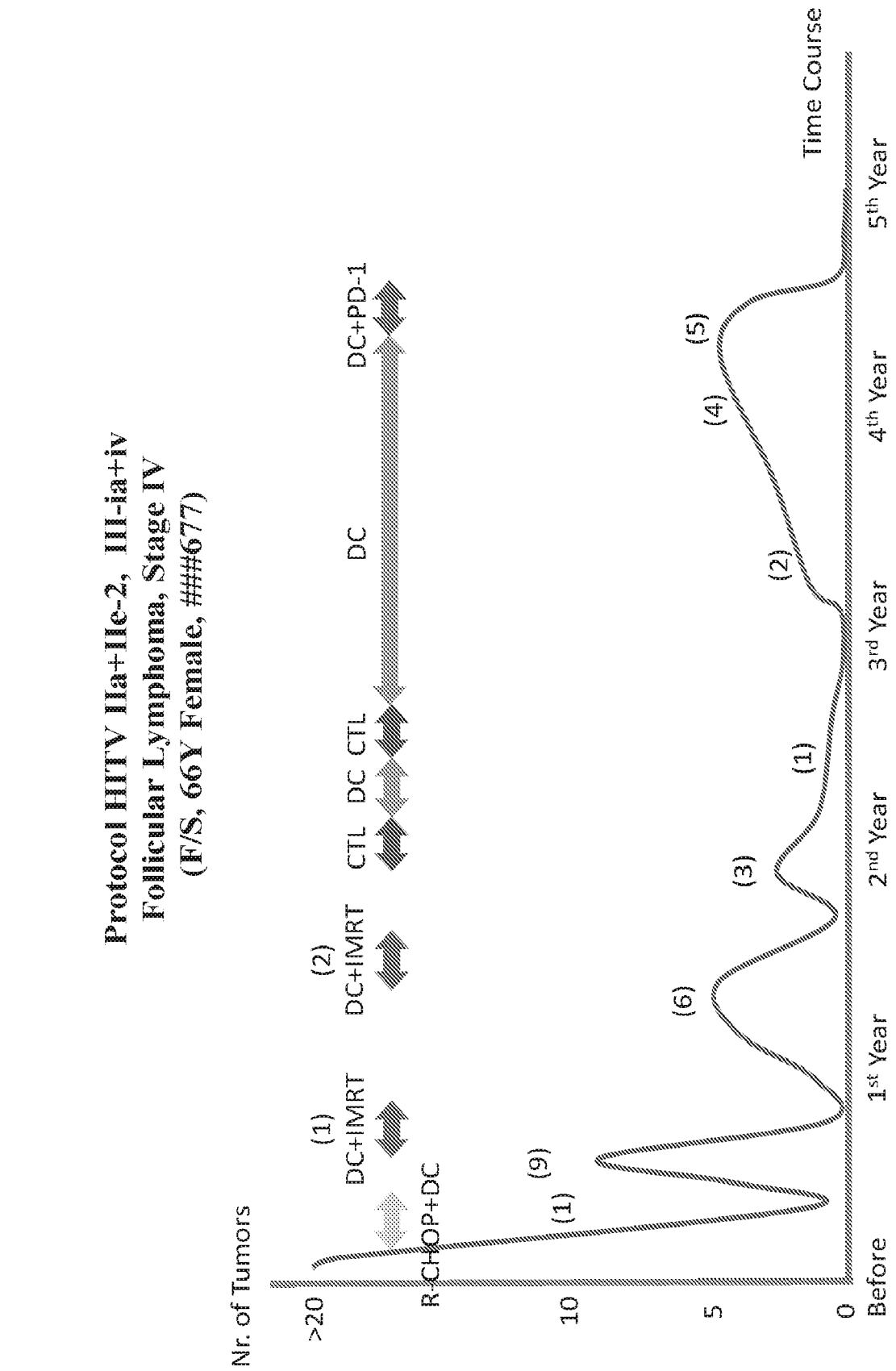
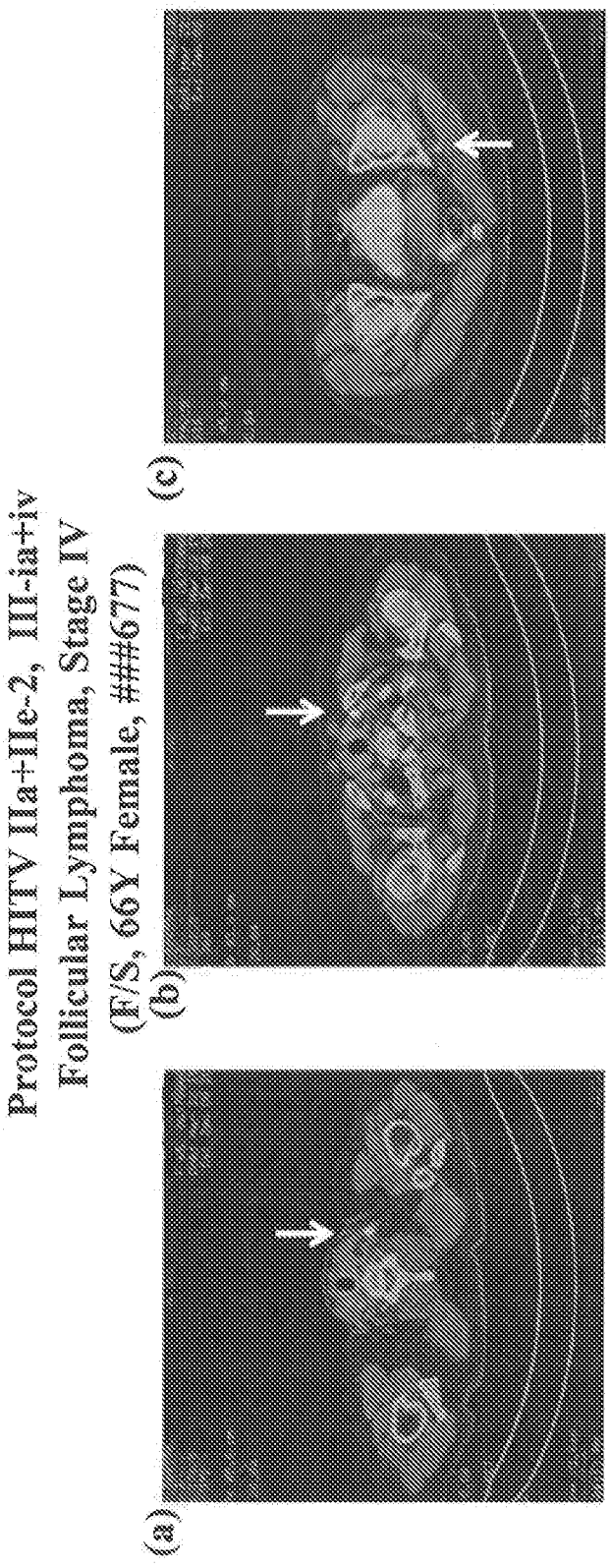
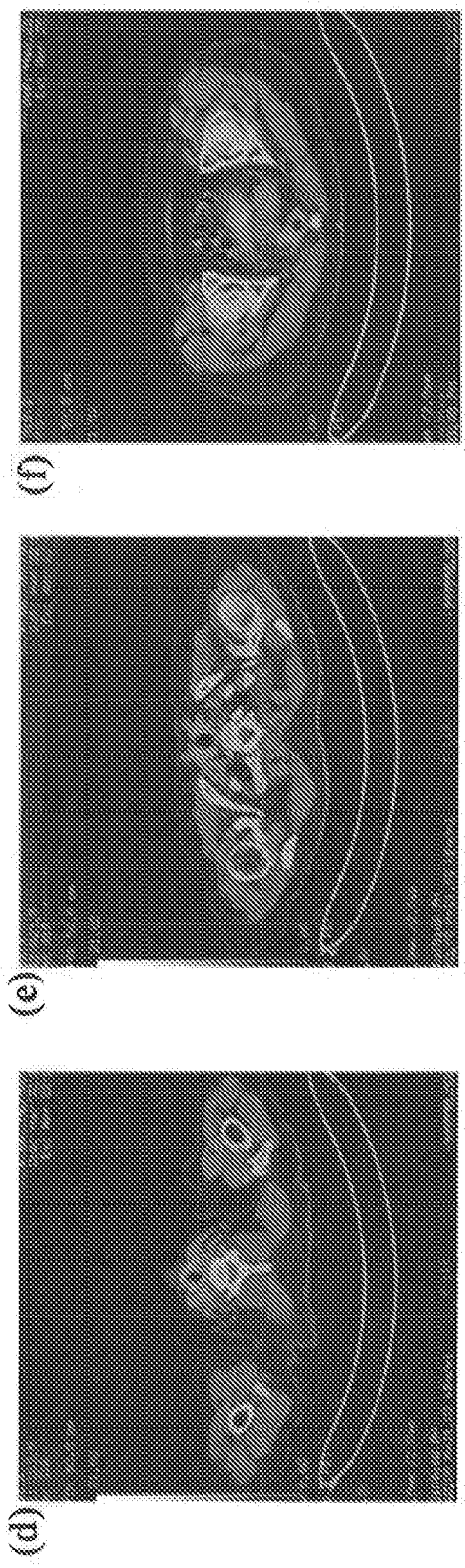

Protocol HITV IIa+IIe-2, III-ia+iv
Follicular Lymphoma, Stage IV
(F/S, 66Y Female, ###677)
Nr. of Tumors
>20
10
5
0
Before
1st Year
2nd Year
3rd Year
4th Year
5th Year
Time Course
Every 3 months check ups
R-CHOP+DC
(1) DC+IMRT
(2) DC+IMRT
CTL DC CTL
DC
DC+PD-1
(9)
(1)
(6)
(3)
(1)
(2)
(4)
(5)

FIG. 4



2016.01.08
FIG. 5A



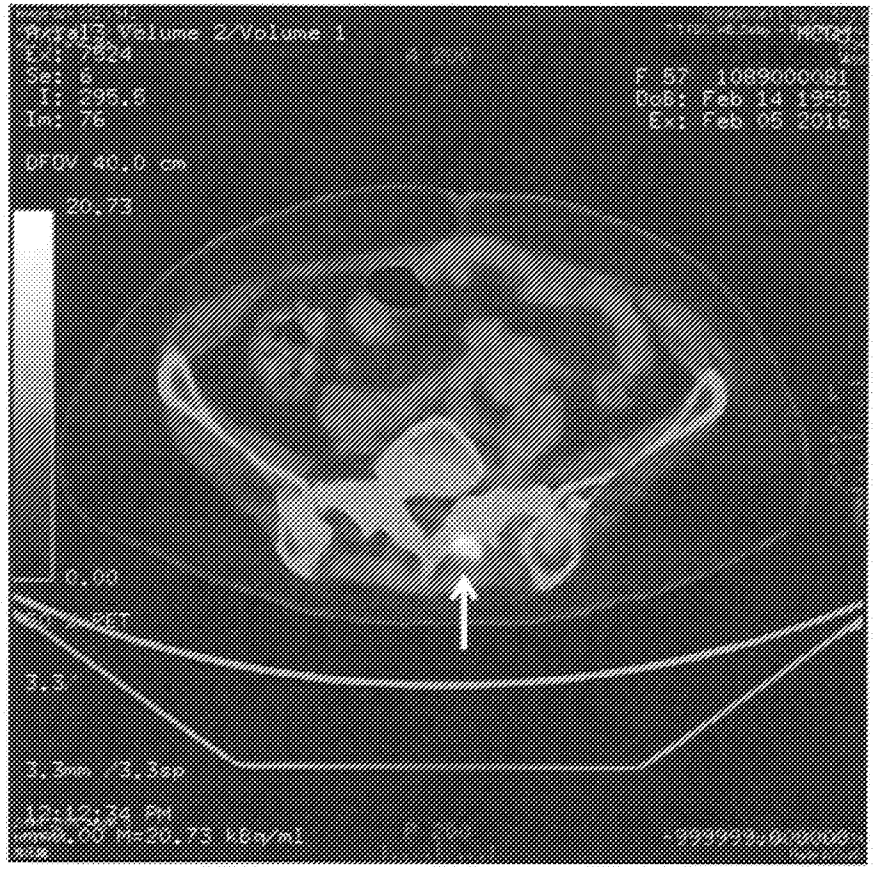
2016.04.22
FIG. 5B

Protocol HITV III-iv, IIa
Clear cell carcinoma, Stage: Rec.
(H/Y, 68Y Male, ###1119)

2016.04.13
FIG. 6A

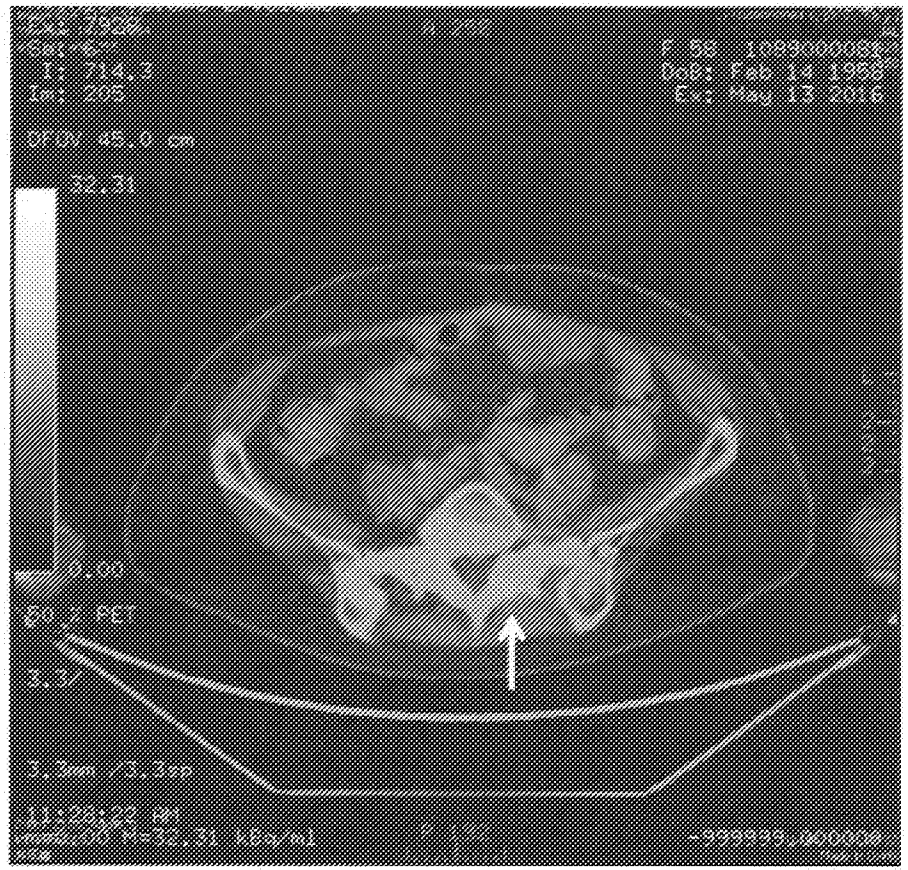
2016.06.15
FIG. 6B

Protocol HITV Ia, IIe-2, III-ia+iv
Cervical Uterine carcinoma
Clear cell carcinoma, Stage: Rec.
(K/D 58Y Female, ###1089)



2016.02.05

FIG. 7A



2016.05.13

FIG. 7B

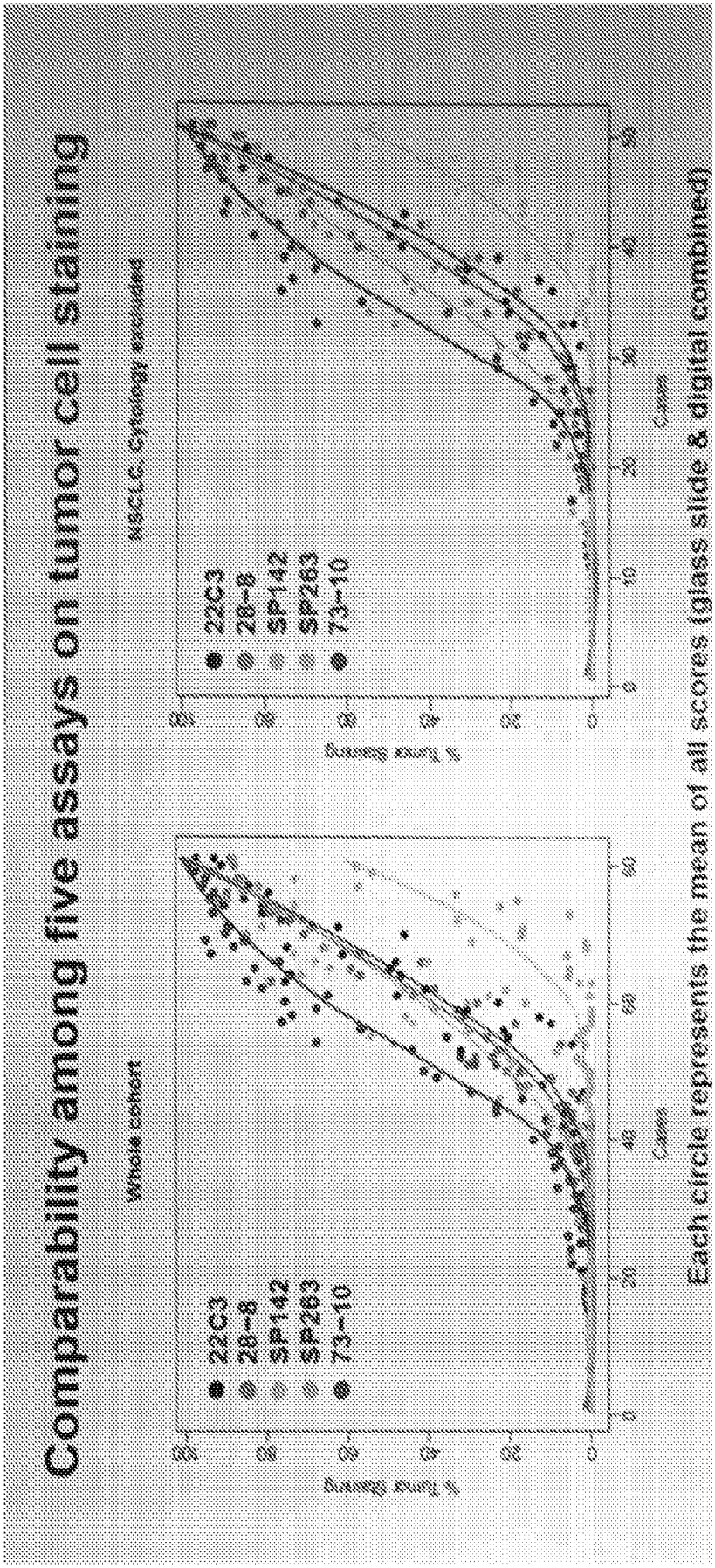


FIG. 8A

FIG. 8B

Hirsch F, WCLC 2017

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/031448

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/395; A61P 35/00; C07K 16/28; C12N 5/00 (2020.01)
CPC - A61K 2039/505; C07K 16/2803; C07K 16/2827; C12N 5/0639; C12N 2506/115 (2020.05)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2018/078145 A1 (BERGEN TEKNOLOGIOVERFØRING AS) 03 May 2018 (03.05.2018) entire document	1-4, 11, 12, 14, 15 ---------- 5-10, 13, 16-19
Y	US 2018/0161367 A1 (HASUMI) 14 June 2018 (14.06.2018) entire document	5-10, 13, 16-19
A	WO 2019/057745 A1 (IMMUNICUM AB) 28 March 2019 (28.03.2019) entire document	1-19
A	US 2018/0078624 A1 (SYZ CELL THERAPY CO.) 22 March 2018 (22.03.2018) entire document	1-19
A	WO 2017/203362 A1 (THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH) 30 November 2017 (30.11.2017) entire document	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2020	06 AUG 2020

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