The problem is existing immunotherapy approaches to treating cancer can lack efficacy when neoantigen expression is low. Accordingly, there is a need for a drug that can selectively target cancer cells to increase their neoantigen expression, so immunotherapy treatment can help eradicate the cancer cells.

SUMMARY

The selective activation of procaspase-3 to caspase-3 in tumors leads to quantitative cleavage of MLH1, resulting in dMMR/MSI, thereby markedly increasing the efficacy of immunotherapies. PAC-1 is used herein to selectively induce immune stimulation in cancer, including MLH1 cleavage that convert MSS tumors to dMMR/MSI tumors, thus making tumors more susceptible to treatment with immunotherapies. Results suggested that immune stimulation with PAC-1 promotes induction of a stress response, thereby altering the tumor microenvironment to increase the extent of immune inflammation. Such results bring the power of immunotherapy -- dramatic and durable responses -- to a greater number of cancer patients.

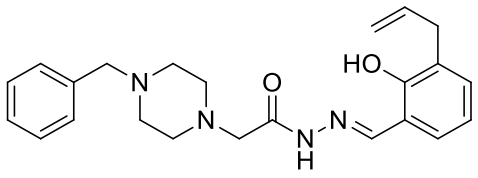
Accordingly, this disclosure provides a composition comprising:

(a) a procaspase-3 activator;

(b) at least one second active agent, wherein the second active agent is a check-point inhibitor, cancer vaccine, metabolic modulator, macrophage inhibitor, or immune-stimulator or modulator; and

(c) optionally a pharmaceutically acceptable diluent, excipient, or carrier.

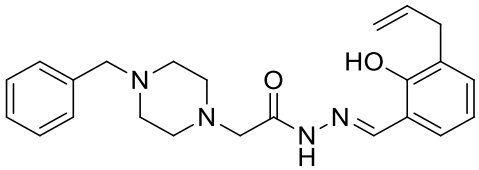
In various ~~embodiments~~examples, the procaspase-3 activator is PAC-1:



(PAC-1).

In one aspect, the present disclosure provide a composition comprising:

(a) a procaspase-3 activator PAC-1,



(PAC-1);

(b) at least one second active agent, wherein the second active agent is a check-point inhibitor, cancer vaccine, metabolic modulator, macrophage inhibitor, or immune-stimulator or modulator; and

(c) optionally a pharmaceutically acceptable diluent, excipient, or carrier

wherein the second active agent has an effect in a cancer cell that induces apoptosis and the procaspase-3 activator PAC-1 enhances the effect of the second active agent by an amount greater than an additive effect, wherein the procaspase-3 activator PAC-1 primes the vulnerability of the cancer cell to the second active agent.

This disclosure also provides a method of treating a cancer comprising administering to a subject in need thereof, concurrently or sequentially, a therapeutically effective amount of a procaspase-3 activator and an effective amount of a second active agent, wherein the second active agent is an immunotherapeutic; wherein the effect of the second active agent is enhanced by the administration of the procaspase-3 activator.

In another aspect, the present disclosure provides an *in vitro* method of inhibiting the growth or proliferation of cancer cells comprising contacting the cancer cells with an effective amount of a composition as described herein, thereby inhibiting growth or proliferation of the cancer cells.

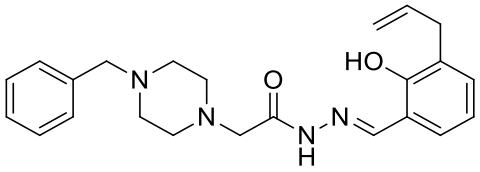
In another aspect, the present disclosure provides an *in vitro* method of inducing apoptosis in a cancer cell comprising contacting the cancer cell with an effective amount of a composition as described herein, wherein the apoptosis is induced by suppressing mismatch-repair (MMR) proteins in the cancer cell.

One certain ~~embodiment~~ example of a method of treating cancer comprises administering to a subject PAC-1 and an anti-PD-1 antibody wherein the PAC-1 is administered daily for 21 or more consecutive days such that a total administered dose per day of the PAC-1 is about 100 mg/kg to

about 125 mg/kg and the anti-PD-1 antibody is administered two times or four times over the 21 or more consecutive days, wherein a dose of the anti-PD-1 antibody is about 10 mg/kg and each of the dose of the anti-PD-1 antibody are administered on separate days.

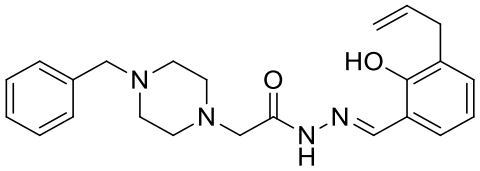
The disclosure also provides for the use of the compositions described herein for use in medical therapy. The medical therapy can be treating cancer, for example, breast cancer, triple negative breast cancer, ovarian cancer, lung cancer, endometrial cancer, pancreatic cancer, prostate cancer, lymphoma, melanoma, leukemia, multiple myeloma, glioblastoma, liver cancer, non-small cell lung cancer, osteosarcoma, meningioma, renal cancer, metastatic renal cell carcinoma, thyroid cancer, or colon cancer. Embodiments of the disclosure also provide for the use of a composition as described herein for the manufacture of a medicament to treat a disease in a mammal, for example, cancer in a human. The medicament can include a pharmaceutically acceptable diluent, excipient, or carrier.

In another aspect, the present disclosure provides a use of a procaspase-3 activator PAC-1



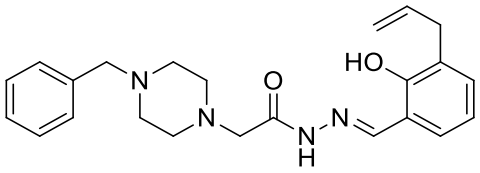
(PAC-1), and a second active agent in the manufacture of a medicament for combination therapy of cancer, wherein the procaspase-3 activator PAC-1 and the second active agent are to be administered concurrently or sequentially, wherein the second active agent is an immunotherapeutic, and wherein the effect of the immunotherapeutic is enhanced by the administration of the procaspase-3 activator PAC-1.

In another aspect, the present disclosure provides a use of a procaspase-3 activator PAC-1



(PAC-1), and an anti-PD-1 antibody in the manufacture of a medicament for combination therapy of cancer, wherein the procaspase-3 activator PAC-1 is to be administered daily for 21 or more consecutive days such that a total dose to be administered per day of the procaspase-3 activator PAC-1 is about 100 mg/kg to about 125 mg/kg and the anti-PD-1 antibody is to be administered two times or four times over the 21 or more consecutive days, wherein a dose of the anti-PD-1 antibody is about 10 mg/kg and each of the dose of the anti-PD-1 antibody is to be administered on separate days.

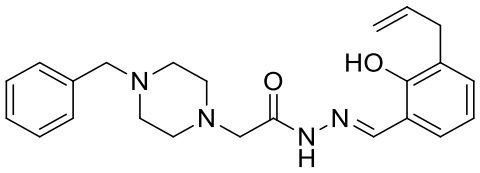
In another aspect, the present disclosure provides a procaspase-3 activator PAC-1



(PAC-1),

and a second active agent for use in combination therapy of cancer, wherein the procaspase-3 activator PAC-1, and the second active agent are to be administered concurrently or sequentially, wherein the second active agent is an immunotherapeutic, and wherein the effect of the immunotherapeutic is enhanced by the administration of the procaspase-3 activator PAC-1.

In another aspect, the present disclosure provides a procaspase-3 activator PAC-1



(PAC-1),

and an anti-PD-1 antibody for use in combination therapy of cancer, wherein the procaspase-3 activator PAC-1 is to be administered daily for 21 or more consecutive days such that a total dose to be administered per day of the procaspase-3 activator PAC-1 is about 100 mg/kg to about 125 mg/kg and the anti-PD-1 antibody is to be administered two times or four times over the 21 or more consecutive days, wherein a dose of the anti-PD-1 antibody is about 10 mg/kg and each of the dose of the anti-PD-1 antibody is to be administered on separate days.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the specification and are included to further demonstrate certain embodiments or various aspects of the disclosure. In some instances, embodiments of the disclosure can be best understood by referring to the accompanying drawings in combination with the detailed description presented herein. The description and accompanying drawings may highlight a certain specific example, or a certain aspect of the disclosure. However, one skilled in the art will understand that portions of the example or aspect may be used in combination with other examples or aspects of the disclosure.

Figure 1. A) Microsatellite Instability (MSI) and B) MLH1-silencing are strongly correlated with increased numbers of somatic mutations, shown here for colon cancer in data from Vogelstein and co. (Proc Natl Acad Sci U S A **2015**, *112*, 118). MSS, Microsatellite Stable.

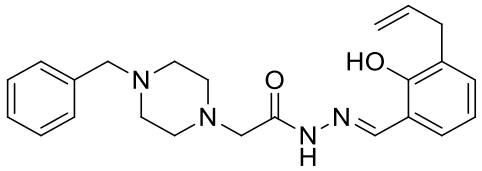
Figure 2. The synergistic effect of PAC-1 plus immunotherapy. PAC-1-induced caspase-3 cleaves certain proteins that sensitize cancer to various immunotherapy approaches.

Figure 3. PAC-1 treatment leads to MLH1 cleavage in the absence of Apoptotic Death Markers. Cell lines were incubated with indicated concentrations of PAC-1 for 72 hours, followed by western blot analysis for MLH1 protein level, as well as PARP-1, cleaved PARP-1 (c-PARP-1 is an apoptosis maker), and beta-actin (loading control). The type of cell line is denoted with the normal cell line, HFF-1 specifically highlighted, demonstrating cancer cell specific MLH1 cleavage.

What is claimed is:

1. A composition comprising:

(a) a procaspase-3 activator PAC-1,



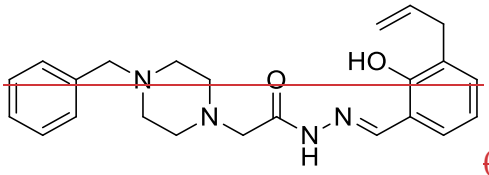
(PAC-1);

(b) at least one second active agent, wherein the second active agent is a check-point inhibitor, cancer vaccine, metabolic modulator, macrophage inhibitor, or immune-stimulator or modulator; and

(c) optionally a pharmaceutically acceptable diluent, excipient, or carrier

wherein the second active agent has an effect in a cancer cell that induces apoptosis and the procaspase-3 activator PAC-1 enhances the effect of the second active agent by an amount greater than an additive effect, wherein the procaspase-3 activator PAC-1 primes the vulnerability of the cancer cell to the second active agent.

~~2. The composition of claim 1 wherein the procaspase-3 activator is PAC-1:~~



~~(PAC-1).~~

~~3. The composition of claim 1 wherein the second active agent has an effect in a cancer cell that induces apoptosis and PAC-1 enhances the effect of the second active agent by an amount greater than an additive effect, wherein PAC-1 primes the vulnerability of the cancer cell to the second active agent.~~

~~4~~2. The composition of claim 1 wherein the second active agent modulates indoleamine-pyrrole 2,3-dioxygenase (IDO), adenosine A_{2A} receptor (A2AR), transforming growth factor beta (TGF-β), C-X-C chemokine receptor type 4 (CXCR-4), C-C chemokine receptor type 4 (CCR4), tumor necrosis factor receptor (CD27), interleukin-2 receptor subunit beta (CD122), death receptor 5 (DR5), inhibitors of apoptosis proteins (IAP), glutaminase, colony stimulating factor 1 receptor (CSF1R), toll-like receptors (TLRs), dendritic cells (DC), or a combination thereof.

~~5~~3. The composition of claim 1 wherein the second active agent is ADXS11-001, ADXS31-142, AMP-224, AMP-514, atezolimumab, atezolizumab, avelumab, bevacizumab, cemiplimab, BLZ945, BMS-936559, BMS986016, BMS986156, BMS986205, CB839, CIMAvax, CMP001, CP870893, CPI-444, CRS207, CV301, DC vaccine, DNX2401, DS-8273a, durvalumab, epacadostat, FAZ053, FPA008, GDC0919, GSK3174998, GVAX, GWN323, IMCgp100, IMP321, imprime PGG, indoximid, ipilimumab, JTX-2011, LAG525, LCL161, LK-301, LY2157299, LY2510924, LY3022855, MBG453, MEDI0562, MEDI0680, MEDI6469, MEDI9447, MGN1703, mogamulizumab, MOXR0916, neoantigen vaccine, NEO-PV-01, NIS793, nivolumab, NKTR-214, PBF509, PDR001, pembrolizumab, peptide vaccine, pexidartinib (PLX3397), PF-04518600, PF-3512676, REGN2810, REGN3767, RO7009789, SD101, talimogene laherparepvec, TPIV200/huFR-1, tremelimumab, TroVax, TSR022, ulocuplumab, urelumab, utomilumab, varlilumab, viagenpumatucel-L (HS-110), or a combination thereof.

~~6~~4. The composition of claim 1 wherein the at least one second active agent is at least one check-point inhibitor that regulates an immune response via programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), tumor necrosis factor receptor superfamily-member 4 (TNFRSF4 or OX40), tumor necrosis factor receptor superfamily-member 9 (TNFRSF9 or 4-1BB), glucocorticoid-induced TNFR-related protein (GITR), inducible T-cell costimulator (ICOS), or a combination thereof.

~~7~~5. The composition of claim ~~6~~4 wherein the checkpoint inhibitor is anti-PD-1, anti-CTLA-4, or a combination thereof; wherein the anti-PD-1 is nivolumab or pembrolizumab, the anti-CTLA-4 is ipilimumab or tremelimumab, or a combination thereof.

~~8~~6. The composition of claim 1 wherein the concentration of the procaspase-3 activator PAC-1 is about 0.1 μM to about 50 μM and the concentration of the second active agent is about 1 nM to about 100 μM.

~~9~~7. The composition of claim 1 comprising a pharmaceutically acceptable diluent, excipient, or carrier, wherein a) the carrier comprises water, a buffer, a sugar, a cellulose, a cyclodextrin, dimethyl sulfoxide, polyethylene glycol, tocopherol, a liposome, a micelle, or a combination thereof, or b) the excipient comprises, a binder, a lubricant, a sorbent, a vehicle, a disintegrant, a preservative, or a combination thereof.

~~10~~8. The composition of claim 1 wherein the composition selectively targets cancer cells, wherein the cancer cells are cells of bladder cancer, breast cancer, colon cancer, endometrial cancer, glioblastoma, leukemia, liver cancer, lung cancer, lymphoma, melanoma, meningioma, multiple myeloma, ovarian cancer, osteosarcoma, pancreatic cancer, prostate cancer, renal cancer, or thyroid cancer;

wherein the breast cancer is optionally triple negative breast cancer, lung cancer is optionally non-small cell lung cancer, and renal cancer is optionally metastatic renal cell carcinoma.

~~11~~9. An *in vitro* method of inhibiting the growth or proliferation of cancer cells comprising contacting cancer cells with an effective amount of a composition of any one of claims 1-~~10~~8, thereby inhibiting the growth or proliferation of the cancer cells.

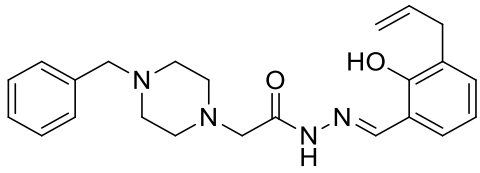
~~12~~10. The *in vitro* method of claim ~~11~~ 9 wherein the growth or proliferation of the cancer cells is inhibited by suppressing mismatch-repair (MMR) proteins, or by caspase-3 activation mediated degradation of MutL homolog 1 (MLH1) proteins;

wherein DNA microsatellite instability (MSI) is induced.

~~13~~11. An *in vitro* method of inducing apoptosis in a cancer cell comprising contacting the cancer cell with an effective amount of a composition of any one of claims 1-~~10~~8, wherein apoptosis is induced by suppressing mismatch-repair (MMR) proteins in the cancer cell.

~~14~~12. The *in vitro* method of claim ~~13~~ 11 wherein the MMR proteins are MutL homolog 1 (MLH1) proteins, wherein degradation of MLH1 proteins, mediated by caspase-3 activation via the procaspase-3 activator PAC-1, induces apoptosis in the cancer cell.

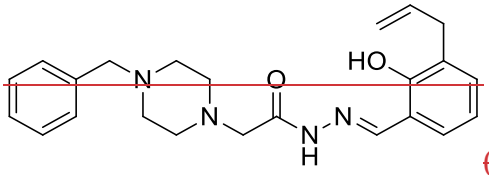
~~1513~~. ~~A method of treating a cancer comprising administering to a subject in need thereof, concurrently or sequentially, a therapeutically effective amount~~ Use of a procaspase-3 activator PAC-1



(PAC-1),

and ~~an effective amount of~~ a second active agent in the manufacture of a medicament for combination therapy of cancer, wherein the procaspase-3 activator PAC-1 and the second active agent are to be administered concurrently or sequentially, wherein the second active agent is an immunotherapeutic, and wherein the effect of the immunotherapeutic is enhanced by the administration of the procaspase-3 activator PAC-1.

~~16. The method of claim 15 wherein the procaspase-3 activator is PAC-1:~~



~~(PAC-1).~~

~~1714~~. The ~~method~~ use of claim ~~16~~ 13 wherein the concentration of the procaspase-3 activator PAC-1 is about 0.1 μM to about 50 μM and the concentration of the second active agent is about 1 nM to about 100 μM.

~~1815~~. The ~~method~~ use of claim ~~16~~ 13 wherein the concentration of the procaspase-3 activator PAC-1 is about 1 μM to about 10 μM and the concentration of the second active agent is about 1 nM to about 1 μM; or

wherein the total ~~administered~~ dose per day of the procaspase-3 activator PAC-1 to be administered is about 10 mg/kg to about 125 mg/kg and the daily total ~~administered~~ dose per day of the second active agent to be administered is about 1 mg/kg to about 100 mg/kg.

~~1916~~. The ~~method~~ use of claim ~~15~~ 13 wherein the second active agent comprises a check-point inhibitor, cancer vaccine, metabolic modulator, macrophage inhibitor, immune-stimulator, or modulator; or a combination thereof.

~~2017~~. The ~~method~~ use of claim ~~15~~ 13 wherein the second active agent is atezolimumab, avelumab, bevacizumab, BMS986016, BMS986156, CP870893, durvalumab, FAZ053, GSK3174998, GWN323, IMP321, ipilimumab, JTX-2011, LAG525, MBG453, MEDI0562,

MEDI0680, MEDI6469, MOXR0916, nivolumab, PDR001, pembrolizumab, PF-04518600, REGN2810, REGN3767, RO7009789, tremelimumab, TSR022, urelumab, utomilumab, or a combination thereof.

~~21~~18. The ~~method~~ use of claim ~~15~~ 13 wherein the immunotherapeutic is a check-point inhibitor, and the check-point inhibitor regulates an immune response via programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), tumor necrosis factor receptor superfamily-member 4 (TNFRSF4 or OX40), tumor necrosis factor receptor superfamily-member 9 (TNFRSF9 or 4-1BB), glucocorticoid-induced TNFR-related protein (GITR), inducible T-cell 36ostimulatory (ICOS), or a combination thereof.

~~22~~19. The ~~method~~ use of claim ~~15~~ 13 wherein the procaspase-3 activator PAC- 1 suppresses mismatch-repair (MMR) proteins;

wherein the MMR proteins comprise MutL homolog 1 (MLH1) proteins, and wherein degradation of MMR proteins, mediated by caspase-3 activation via the procaspase-3 activator, induces a deficiency in MMR proteins, DNA microsatellite instability (MSI), neoantigen expression, or a combination thereof, thereby enhancing the effect of the immunotherapeutic, and wherein the procaspase-3 activator increases tumor-infiltrating lymphocytes in the cancer.

~~23~~20. The ~~method~~ use of claim ~~15~~ 13 wherein the cancer is bladder cancer, breast cancer, colon cancer, endometrial cancer, glioblastoma, leukemia, liver cancer, lung cancer, lymphoma, melanoma, meningioma, multiple myeloma, ovarian cancer, osteosarcoma, pancreatic cancer, prostate cancer, renal cancer, or thyroid cancer;

wherein the breast cancer is optionally triple negative breast cancer, lung cancer is optionally non-small cell lung cancer, and renal cancer is optionally metastatic renal cell carcinoma.

~~24~~21. The ~~method~~ use of any one of claims ~~15-23~~ 13-20 wherein:

~~T~~the procaspase-3 activator ~~compound~~ PAC-1 and the second active agent are ~~concurrently~~ to be administered concurrently to ~~the~~ a subject; or

the procaspase-3 activator ~~compound~~ PAC-1 and the second active agent are ~~sequentially~~ to be administered sequentially to ~~the~~ a subject, wherein the procaspase-3

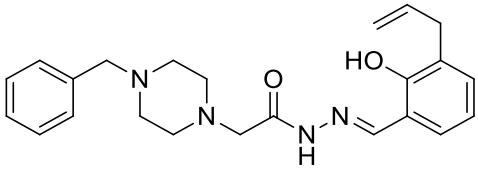
activator ~~compound~~ PAC-1 is to be administered to the subject before the second active agent or the procaspase-3 activator ~~compound~~ PAC-1 is to be administered to the subject after the second active agent.

~~25~~22. The ~~method~~ use of any one of claims ~~15-23~~ 13-20 wherein the procaspase-3 activator ~~compound~~ PAC-1 and the second active agent are to be administered to the subject once daily (*q.d.*), twice a day (*b.i.d.*), three times a day (*t.i.d.*), or four times a day (*q.i.d.*), wherein the total ~~administered~~ dose per day of the procaspase-3 activator PAC-1 to be administered is about 1 mg/kg to about 150 mg/kg; or

each ~~administered~~ dose of the procaspase-3 activator PAC-1 to be administered is about 70 mg, about 175 mg, about 250 mg, about 375 mg, about 450 mg, about 500 mg, about 625 mg, about 750 mg, or about 1000 mg; or

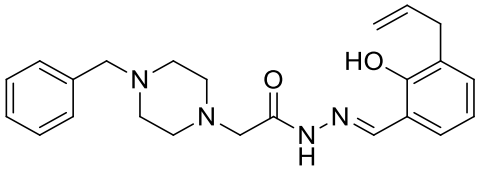
each ~~administered~~ dose of the procaspase-3 activator PAC-1 to be administered is about 50 mg/m^2 to about 250 mg/m^2.

~~26~~23. ~~A method of treating a cancer comprising administering to a subject in need thereof,~~ Use of a procaspase-3 activator PAC-1



(PAC-1), and an anti-PD-1 antibody in the manufacture of a medicament for combination therapy of cancer, wherein the procaspase-3 activator PAC-1 is to be administered daily for 21 or more consecutive days such that a total ~~administered~~ dose to be administered per day of the procaspase-3 activator PAC-1 is about 100 mg/kg to about 125 mg/kg and the anti-PD-1 antibody is to be administered two times or four times over the 21 or more consecutive days, wherein a dose of the anti-PD-1 antibody is about 10 mg/kg and each of the dose of the anti-PD-1 antibody is to be administered on separate days.

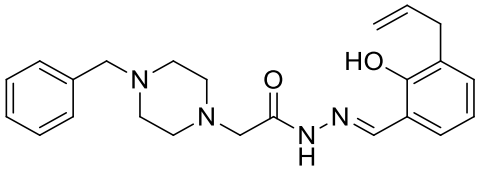
24. The procaspase-3 activator PAC-1



(PAC-1), and a second active agent for use in combination therapy of cancer, wherein the procaspase-3 activator PAC-1, and the second active agent are to be administered concurrently or

sequentially, wherein the second active agent is an immunotherapeutic, and wherein the effect of the immunotherapeutic is enhanced by the administration of the procaspase-3 activator PAC-1.

25. A procaspase-3 activator PAC-1



(PAC-1), and an anti-PD-1 antibody for use in combination therapy of cancer, wherein the procaspase-3 activator PAC-1 is to be administered daily for 21 or more consecutive days such that a total dose to be administered per day of the procaspase-3 activator PAC-1 is about 100 mg/kg to about 125 mg/kg and the anti-PD-1 antibody is to be administered two times or four times over the 21 or more consecutive days, wherein a dose of the anti-PD-1 antibody is about 10 mg/kg and each of the dose of the anti-PD-1 antibody is to be administered on separate days.