(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2019 (21.03.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/054865 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number:
PCT/NL2018/050601

(22) International Filing Date:
13 September 2018 (13.09.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2019548 14 September 2017 (14.09.2017) NL
2020886 07 May 2018 (07.05.2018) NL

(71) Applicant: **ACADEMISCH ZIEKENHUIS LEIDEN (H.O.D.N. LUMC)** [NL/NL]; Albinusdreef 2, 2333 ZA Leiden (NL).

(72) Inventors: **VAN DER BURG, Sjoerd, Henricus**; C/o LUMC, Medical Oncology, Albinusdreef 2, 2333 ZA Leiden (NL). **ARENS, Ramon**; C/o LUMC, Albinusdreef 2, 2333 ZA Leiden (NL). **VAN DER SLUIS, Tetje, Cornelia**; C/o Lumc, Albinusdreef 2, 2333 ZA Leiden (NL). **MELIEF, Cornelis, J.M.**; C/o Lumc, Albinusdreef 2, 2333 ZA Leiden (NL).

(74) Agent: **HGF BV**; Zuid-Hollandlaan 7, 2596AL The Hague (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(54) Title: T-CELL BASED IMMUNOTHERAPY

(57) Abstract: The present invention provides novel mechanisms for enhancing a T cell response in vivo, ex vivo or in vitro, particularly in the context of vaccination, adoptive cell therapy and T-cell based immunotherapies involving immune checkpoint blockade. Corresponding methods and uses are also described.

WO 2019/054865 A1

T-cell based immunotherapy

The present invention provides novel mechanisms for enhancing a T cell response *in vivo*, *ex vivo* or *in vitro*, particularly in the context of vaccination, adoptive cell therapy and T-cell based immunotherapies involving immune checkpoint blockade. Corresponding methods and uses are also described.

Background

The immune system is actively involved in the eradication of malignant cells or disease-causing agents from the body. Several immunotherapeutic strategies have been designed and tested for use in the clinic. Examples of relevant immunotherapies include vaccination (both prophylactic and therapeutic), adoptive cell therapy (ACT), oncolytic virus therapy and immunotherapies involving immune checkpoint blockade. Such immunotherapies are currently being developed for use in several different treatment programs, including but not limited to the treatment of cancer and chronic infections.

Although ACT and immune check point blockade have shown clear clinical benefit under specific circumstances (1, 2, and 3), neither immunotherapy is currently successful enough to treat the majority of the cancer patients. Improvement of these immunotherapies is therefore required.

Therapeutic vaccines serve to enlarge antigen-specific T-cell numbers. In comparison to the clinical results of immune checkpoint blockade and ACT, those of therapeutic vaccines have been less impressive. Some of the best currently available vaccine platforms can achieve high immune response rates of properly polarized T-cell immune responses and encouraging efficacy was obtained when patients with chronically infected and premalignant lesions were treated. Real clinical success by vaccination is less evident in patients with cancer (reviewed in (4)) but recent preclinical data has shown that this may be improved by combinations of therapeutic vaccines with chemotherapy (5 to 7).

Therapeutic vaccination targeting tumor-specific antigens has recently been shown by the inventors to induce a tumor specific CD8 T cell response *in vivo*, where therapeutic vaccination with overlapping synthetic long peptides (SLP) targeting the oncogenes E6 and E7 was shown to result in the induction of cytokine producing CD8 T cells and in clinical responses in mice and patients with premalignant lesions. Furthermore, a combination of certain chemotherapeutic agents including topotecan were shown to improve survival when

combined with an SLP vaccine (5). Although these results looked promising, the mechanism underlying the improved effect was not clear.

One potential obstacle to the success of T-cell based immunotherapies is the relatively slow build-up in the number of tumor-specific T-cells and the transient nature of the peak response. Stimulation of T-cells via their T-cell receptor (TCR) (signal 1) and multiple costimulatory signals (signal 2) are required for their expansion, survival and differentiation (8). T-cells also receive important signals mediated by certain cytokines (signal 3), such as IL-12 and interferons (9). For CD8 T-cells, autocrine IL-2 production is also crucial for their expansion potential (10, 11). The complexity of T-cell stimulation lies in the spatio-temporal regulation of the signals they receive.

After receiving the above described signals, T-cells can undergo massive proliferation leading to a population expansion than can exceed >10.000 fold (12). The molecular machinery involved in the cell cycle regulation has been extensively studied, and includes key regulatory proteins such as cyclins, cyclin-dependent kinases (CDKs) that initiate cyclic transition between phases (13) and ubiquitination enzymes (14). The cell cycle of lymphocytes seems to correlate with the differentiation of T-cells, and in this respect it is suggested that CDKs can directly regulate factors involved in T-cell differentiation (15). Despite a molecular understanding, no mechanism explains the semi-stochastic nature of cycling cells, which leads to kinetic variation in the G1/S/G2/M phases (16), and as a consequence leads to a variable number of cells that can attack infected or malignant cells at the same time.

Brief summary of the disclosure

The inventors have investigated the kinetics of the T cell cycle during T cell activation to understand why a combination treatment of topotecan and vaccination resulted in improved survival (5). In depth studies *in vivo* and *ex vivo* have now revealed that, surprisingly, the improvement results from cell cycle synchronization of the vaccine-stimulated T-cells in the presence of topotecan, followed by a fast and strong expansion of the activated T-cells after topotecan clearance and a delayed contraction thereafter. The marked effect of topotecan-mediated cell cycle synchronisation of T cells on the disease outcome was unexpected.

Advantageously, the inventors have elucidated the mechanism by which topotecan can be utilised to enhance T cell response. Elucidation of this mechanism has enabled the inventors to identify alternative agents that may also be able to perform this function. Identification of other agents is useful, for example, because topotecan has been found to be relatively toxic

to T cells e.g. when administered to T cells *ex vivo*. Other agents that act as transient cell cycle inhibitors have been tested by the inventors and confirmed to synchronise the cell cycle of T cells. Such other agents include the less toxic CDK1 blocker RO-3306, which arrests cells at the G2-phase; irinotecan, which arrests at the S-phase, and thymidine, which arrests cells in G1/S.

The results presented herein show the utility of a transient cell cycle inhibitor when administered with a vaccine to a subject. The transient cell cycle inhibitor synchronises the T cell cycle such that the subsequent T cell response is swift, coordinated and prolonged.

The aggregated data from many different (pre)clinical studies with vaccines, adoptive cell transfer and immune checkpoint inhibitors indicates that chronic infections and cancer can successfully be treated with immunotherapy, provided that specific T-cells are activated, are present in large numbers when attacking the infected or malignant cells, and can exert their function for a long time.

Advantageously, the inventors have now shown that T cell synchronisation can also be achieved *ex vivo*, and thus would be beneficial in the context of immunotherapies such as adoptive cell therapy.

Advantageously, the swift, coordinated and prolonged T cell response that is induced by cell cycle synchronisation of T cells is also of benefit to treatments involving immune checkpoint blockade.

The results presented herein show that a transient cell cycle inhibitor can be used to synchronise the T cell cycle in a subject such that the subsequent T cell response in the subject is swift, coordinated and prolonged. This finding has particular utility for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

For such patient subgroups, a novel, unexpected and efficacious treatment regimen is provided herein, as these patients have not previously been thought to benefit from transient cell cycle inhibition.

Accordingly, in one aspect, an effective amount of a vaccine for use in therapy is provided, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

In another aspect, an effective amount of a transient cell cycle inhibitor for use in therapy is provided, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

In another aspect, an effective amount of a vaccine for use in the manufacture of a medicament is provided, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

In another aspect, an effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament is provided, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

In another aspect, an effective amount of a vaccine for use in therapy is provided, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

In another aspect, an effective amount of a transient cell cycle inhibitor for use in therapy is provided, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential

administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

In another aspect, an effective amount of a modulator of an immune suppressive mechanism for use in therapy is provided, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

In another aspect, an effective amount of a vaccine for use in the manufacture of a medicament is provided, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

In another aspect, an effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament is provided, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

In another aspect, an effective amount of a modulator of an immune suppressive mechanism for use in the manufacture of a medicament is provided, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

The modulator of an immune suppressive mechanism may be a chemotherapeutic agent.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

is provided, the method comprising:

i) administering to the subject an effective amount of a vaccine; and,

ii) administering to the subject an effective amount of a transient cell cycle inhibitor,

wherein steps i) and ii) are separate, simultaneous or sequential, and in any order, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

is provided the method comprising:

i) administering to the subject an effective amount of a vaccine;

ii) administering to the subject an effective amount of a transient cell cycle inhibitor; and

iii) administering to the subject an effective amount of a modulator of an immune suppressive mechanism,

wherein steps i), ii) and iii) are separate, simultaneous or sequential, and in any order.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

is provided, the method comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

is provided, the method comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

is provided, the method comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor and an effective amount of a modulator of an immune suppressive mechanism.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

is provided, the method comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a modulator of an immune suppressive mechanism.

In another aspect, a method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

is provided, the method comprising administering to the subject an effective amount of a modulator of an immune suppressive mechanism, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a transient cell cycle inhibitor.

The transient cell cycle inhibitor may arrest the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

The transient cell cycle inhibitor may be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole), and a HDAC6 inhibitor (such as tubastatin A).

In another aspect, there is provided an effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with

an effective amount of a transient cell cycle inhibitor. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a vaccine for use in T-cell based immunotherapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use T-cell based immunotherapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided an effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is

further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a modulator of an immune suppressive mechanism for use in therapy, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a vaccine for use in T-cell based immunotherapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use T-cell based immunotherapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a modulator of an immune suppressive mechanism for use T-cell based immunotherapy, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided an effective amount of a modulator of an immune suppressive mechanism for use in the manufacture of a medicament, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

Suitably, the modulator of an immune suppressive mechanism may be a chemotherapeutic agent.

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising:
i) administering to the subject an effective amount of a vaccine; and,
ii) administering to the subject an effective amount of a transient cell cycle inhibitor,
wherein steps i) and ii) are separate, simultaneous or sequential, and in any order. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising:
i) administering to the subject an effective amount of a vaccine;

ii) administering to the subject an effective amount of a transient cell cycle inhibitor; and
iii) administering to the subject an effective amount of a modulator of an immune suppressive mechanism,
wherein steps i), ii) and iii) are separate, simultaneous or sequential, and in any order. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor. Optionally the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine. Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor and an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a modulator of an immune suppressive mechanism. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

In another aspect, there is provided a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising

administering to the subject an effective amount of a modulator of an immune suppressive mechanism, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a transient cell cycle inhibitor. Optionally, the specific combination of cell cycle inhibitor and modulator is not topotecan (inhibitor) and cisplatin (modulator).

Suitably, the transient cell cycle inhibitor may arrest the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

Suitably, the transient cell cycle inhibitor may be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole), and a HDAC6 inhibitor (such as tubastatin A).

Suitably, the subject may be afflicted with:

i) cancer; preferably wherein the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas, brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

ii) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

In another aspect, there is provided an *ex vivo* method of preparing a T cell population suitable for adoptive cell therapy, the method comprising:

i) activating the T cell population; and

ii) contacting the T cell population with a transient cell cycle inhibitor;

wherein steps i) and ii) may be in any order.

Suitably, the method further may comprise removing contact between the activated T cell population and the transient cell cycle inhibitor, thereby expanding the T cell population.

Suitably, contact between the T cell population and the transient cell cycle inhibitor may be removed by washing.

Suitably, the T cell population may be activated by providing the T cell population with antigen-loaded antigen presenting cells that stimulate cognate T cell receptors on the T cell population. Optionally, the antigen-loaded antigen presenting cells are dendritic cells.

Suitably, the T cell population may be activated by providing the T cell population with T cell-activating antibodies. Optionally, the T cell population is additionally provided with at least one cytokine.

Suitably, the cell cycle inhibitor may arrest the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

Suitably, the cell cycle inhibitor may be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A).

In another aspect, there is provided a T cell preparation suitable for adoptive cell therapy, obtained from the method of the invention.

Suitably, the T cell preparation may be formulated in a pharmaceutically acceptable carrier.

In another aspect, there is provided an effective amount of T cell preparation according to the invention for use in adoptive cell therapy in a human subject in need thereof.

In another aspect, there is provided an effective amount of T cell preparation according to the invention for use in the manufacture of a medicament for adoptive cell therapy.

In another aspect, there is provided a method of adoptive T cell therapy in a subject in need thereof, the method comprising administering to the subject an effective amount of a T cell preparation according the invention.
Suitably, the adoptive T cell therapy may comprise administration of an effective amount of at least one immune checkpoint inhibitor to the subject.

Suitably, the adoptive T cell therapy may comprise administration of an effective amount of a vaccine comprising an antigen that stimulates cognate T cell receptors on the T cell population.

Suitably, the subject may be afflicted with:

a) cancer, preferably where the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas; brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

b) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

Suitably, the subject may be immunodeficient.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in T-cell based immunotherapy, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

In another aspect, there is provided an effective amount of an immune checkpoint inhibitor for use in T-cell based immunotherapy, wherein the immune checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

In another aspect, there is provided an effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

In another aspect, there is provided an effective amount of an immune checkpoint inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy, wherein the immune checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

In another aspect, there is provided a method of T-cell based immunotherapy in a subject in need thereof, comprising

i) administering to the subject an effective amount of an immune checkpoint inhibitor; and,

ii) administering to the subject an effective amount of a transient cell cycle inhibitor,

wherein steps i) and ii) are separate, simultaneous or sequential, and in any order.

In another aspect, there is provided a method of T-cell based immunotherapy in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of an immune checkpoint inhibitor.

In another aspect, there is provided a method of T-cell based immunotherapy in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint inhibitor, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor.

Suitably, the subject may be afflicted with:

i) cancer; preferably wherein the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas; brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

ii) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

Suitably, the cell cycle inhibitor may arrest the cell cycle at one of following stages; G1/S, G2, G2/M, and S-phase.

Suitably, the cell cycle inhibitor may be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A).

Suitably, the immune checkpoint inhibitor may block CTLA-4, PD-1, PD-L1, TIM3, TIGIT, VISTA or LAG-3.

Suitably, the immune checkpoint inhibitor may be an antibody, an siRNA or a small molecule.

Various aspects of the invention are described in further detail below.

Brief description of the drawings

Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

Figure 1 shows the temporal proliferation blockade of vaccine-stimulated CD8 T cells results in a massive expansion of tumor-specific CD8 T cells and superior tumor control. A-B) Wild-type C57BL/6 mice were injected s.c. with $1x10^5$ TC-1 tumor cells. Eight days later, when tumors were palpable, mice were treated systemically with chemotherapeutics with or without HPV16 $E7_{43-77}$ peptide in Montanide in the opposing flank (Cisplatin (4 mg/kg, intraperitoneal injection (i.p.)) was provided on day 8 and 15, topotecan (2 mg/kg, i.p) was provided on days 8, 9, 10, 11 and 15, 16, 17, 18)). Chemotherapy was repeated 1 week after initial treatment and vaccination was repeated 2 weeks after initial treatment. A) Schematic diagram of the therapy regimen is shown in the top left corner. Average tumor growth per group (until the first animal had to be killed for ethical reasons) is shown. B) Mice were treated according to the setup as shown in A; however, one group (diamonds) received CD8 depleting antibodies from day 7 and then every six days. C) To ensure similar tumor sizes on day 17, animals were treated with vaccine on day 8 and 6 days later with chemotherapy (cisplatin on day 14, topotecan on day 14-16). Tumors were dissected on day 17 and analyzed by flow cytometry. Shown is the percentage of CD8 T cells and vaccine-specific CD8 T cells (as determined by H2-Db $E7_{49-57}$ (RAHYNIVTF) tetramer staining) within the tumor (live gate). Indicated is the fold decrease between "peptide" and "peptide + cisplatin + topotecan" group. Shown is the mean + SEM as determined by one-way ANOVA followed by Tukey post hoc analysis (*, $P < 0.05$ and **, $P < 0.01$, ***, $P < 0.001$). This clearly shows that topotecan suppresses the expansion of tumor-antigen-specific T cells while they are stimulated. D) Shown is the Ki-67 expression on H2-Db $E7_{49-57}$ Tetramer+ T cells on day 17 and day 21 and this revealed that while under normal circumstances (peptide only) the antigen-specific T cells start to contract, the topotecan treated T cells start to expand. E) To specifically chase the development of specific T cells during and after chemotherapy wild-type C57BL/6 mice were injected s.c. with $1x10^5$ TC-1 tumor cells. Animals were treated with vaccine on day 9 and 6 days later with chemotherapy (cisplatin (4 mg/kg, intraperitoneal injection (i.p.)) on day 15, topotecan (2 mg/kg, i.p) on day 15-17). Presented is a quantification of the percentage vaccine-specific cells in the blood, as determined by H2-Db $E7_{49-57}$ tetramer staining. Small bar graph in shows the quantification on day 28 after tumor challenge. Shown is the mean + SEM. Clearly, only when topotecan is given, the specific T cells start to expand at a later phase and are present at higher levels at the time that in the other conditions the specific T cells have already contracted.

Figure 2 shows that topotecan impacts expansion and contraction of SLP vaccine-induced T cells. A) 0.5 × 10^6 Thy1.1+ congenically labelled OT-I T cells were injected i.v. in Thy1.2+ recipient mice on day -1. The next day (day 0), mice were vaccinated with the SLP containing the (SIINFEKL) epitope. Cisplatin (4mg/kg (i.p.)) was provided on day 0, topotecan (2 mg/kg (i.p.) on day 0-2. The percentage of OT-I T cells in blood was measured on day 3, 6, 9 and 13. Samples from one treatment group were pooled and the geometric mean of Ki-67 expression on each time point was plotted. B) Percentage of SLECs of OT-I cells on day 6 is plotted in the left section, each symbol represents an individual animal (analyzed by Mann Whitney T test). Representative dot plots are shown on the right. C) To show that the effects seen on T cells was a direct effect of topotecan on T cells and not an effect bestowed upon by tumor cells in response to topotecan treatment, wild-type C57BL/6 mice were vaccinated. Six days later, animals were treated with systemic chemotherapy (topotecan on day 6-8). Indicated are significant differences between “peptide” and “peptide + topotecan” treated animals. N = 4-8 mice per group, data is representative for 2 individual experiments. Shown is the mean + SEM (*, $P < 0.05$ and **, $P < 0.01$, ***, $P < 0.001$). Clearly, the mice treated with topotecan showed a much stronger peptide-induced T-cell expansion, than those not treated with topotecan. Also, the antigen-specific T cells contracted slower. D) Model for the effect of topotecan on the antigen specific T cells. A typical CD8 T cell response to vaccination is shown in blue. T cells clonally expand upon presentation of antigen by antigen presenting cells followed by a contraction phase. Some remaining memory CD8 T cells can persist for years. A CD8 T cell response to vaccination in the presence of topotecan is shown in red. Vaccine-specific CD8 T cells are paused in their proliferation by topotecan. Upon topotecan washout the T cells rigorously proliferate, resulting in a strong expansion of the number of vaccine specific T cells. The contraction phase is prolonged resulting in more T cells long after vaccination.

Figure 3 shows that T-cell proliferation is unprecedented high after temporal cell cycle inhibition. Proliferation plots after treatment with Topotecan. Purified CD8 T cells were CFSE labelled, stimulated with aCD3/aCD28 antibodies, and 1 hour later treated with topotecan or irinotecan. At day 3, the topotecan (A) or irinotecan (B) was washed out or not, and the next day the proliferation was determined by flow cytometry. C) Cell numbers of T-cell cultures at day 4. T cells were stimulated with aCD3/aCD28 antibodies, and 1 hour later treated with RO-3306 (10 μM) or with thymidine (4 mM). At day 3, the cell cycle inhibitors were washed out or not. Cell numbers were assessed at day 4. This clearly shows that when the cell cycle inhibitor is washed out, the T cells start to proliferate vigorously and expand in a much shorter time period to similar levels as those T cells that were not cell cycle inhibited.

Figure 4 shows flow cytometry data comparing *ex vivo* T cell treatment with the two cell cycle inhibitors topotecan or irinotecan. A: Here the timing of cell cycle inhibition is studied. The plots indicate the proliferation of T cells after treatment with different Topotecan concentrations at different time points. B: $CD8^+$ T cell viability represented as % $7AAD^-$ cells after treatment with 1 µg/ml Topotecan either with and without IL-2 (10 Units/ml) in different treatment schemes, showing that topotecan is toxic, even at a lower concentration when used in vitro, and is viability is not rescued when IL-2 is provided. However, Irinotecan, which has the same capacity to stimulate unprecedented high proliferation after temporal cell cycle inhibition, is less toxic in vitro, as shown in C: $CD8^+$ T cell viability represented as % $7AAD^-$ cells after treatment with different Irinotecan concentrations. D: CD8+ T cell proliferation represented as % divided cells after treatment with different Irinotecan concentrations.

Figure 5 shows that the cell cycle inhibitor synergizes with PDL-1 blockade to prevent tumor progression. MC-38 tumor cells (3×10^5) were injected s.c. in the flank of wild-type C57BL/6 mice (day 0) and 8, 11 and 14 days later groups of mice (group 3 and 4) were treated with antibodies blocking PDL-1 (clone MIH5, 200 ug in PBS) provided i.p. At day 11, 12 and 13 post tumor challenge, groups of mice (group 1 and 4) were treated with topotecan (2 mg/kg in PBS) provided i.p. One group (group 2) did not receive treatment. Tumor progression was monitored twice a week. A: Schematic of the experimental setup. B: Percentage of tumor-bearing mice. C: Percentage of tumor-free mice.

Detailed description

The inventors have surprisingly found that transient cell cycle inhibitors can be used to synchronise the cell cycle of T cells, followed by a fast and strong expansion of the activated T-cells after removal or clearance of the cell cycle inhibitor resulting in a massive, swift and prolonged T cell response.

The invention has utility in any aspect that can benefit from an improved or enhanced T cell response. Examples of such aspects include vaccination, adoptive cell therapy and immunotherapies involving immune checkpoint blockade.

Vaccines, cell cycle inhibitors and modulators for use in therapy

As used herein, "vaccine" refers to a biological preparation that provides active acquired immunity to a particular disease or malignancy in a subject. In this context, a vaccine contains an agent that induces an agent-specific T cell response. The agent is typically an

antigen that can be presented in the context of an antigen-presenting cell to a cognate T cell receptor on a T cell, thereby inducing an antigen-specific T cell response.

The vaccine may be prophylactic (e.g. to prevent or ameliorate the effects of a future infection or malignancy) or therapeutic (e.g. to treat the effects of a current infection or malignancy).

The vaccine may comprise a disease-associated antigen (e.g. a tumor-presented antigen) that is recognised by a cognate T cell receptor on a T cell.

The vaccine may comprise an antigen that is in a form that is suitable for directly loading onto MHC, or it may be in a form that requires further processing (e.g. trimming) prior to MHC presentation.

In a particular example, a vaccine comprises an antigen that stimulates a cognate T cell receptor on a T cell, wherein the T cell is for use in adoptive T cell therapy.

By way of example, but not by way of limitation, appropriate vaccines may comprise a protein (e.g. including a fusion protein) expressing one or more disease associated antigen(s), a recombinant virus expressing one or more disease associated antigen(s), a nucleic acid (e.g. DNA or RNA) that encodes one or more disease associated antigen(s), a dendritic cell that is loaded with one or more disease antigen(s), a dendritic cell (e.g. recombinant dendritic cell) engineered to express one or more disease antigen(s), a cell lysate of cells that express one or more disease antigen(s), a cell (e.g. a recombinant cell) that is engineered to express one or more disease antigen(s) etc.

In a particular example, the vaccine is a synthetic long peptide. The use of synthetic long peptides as vaccines is well known in the art.

The vaccine may include one or more cancer or tumor antigens, for example, when the therapy is a neoadjuvant therapy (see below). In some examples, the cancer antigen is, but is not limited to, acute lymphoblastic leukemia (etv6, amll, cyclophilin b), B cell lymphoma (Ig-idiotype), glioma (E-cadherin, a-catenin, P-catenin, y-catenin, pl20ctn), bladder cancer (p21ras), biliary cancer (p21ras), breast cancer (MUC family, HER2/neu, c-erbB-2), cervical carcinoma (p53, p21ras), colon carcinoma (p21ras, HER2/neu, c-erbB-2, MUC family), colorectal cancer (Colorectal associated antigen (CRC)-C017-1A/GA733, APC), choriocarcinoma (CEA), epithelial cell cancer (cyclophilin b), gastric cancer (HER2/neu, c-

erbB-2, ga733 glycoprotein), hepatocellular cancer (a-fetoprotein), Hodgkins lymphoma (lmp-1, EBNA-1), lung cancer (CEA, MAGE-3, NY-ESO-1), lymphoid cell-derived leukemia (cyclophilin b), melanoma (p15 protein, gp75, oncofetal antigen, GM2 and GD2 gangliosides, Melan-A/MART-1, cdc27, MAGE-3, p21ras, gplOO"), myeloma (MUC family, p21ras), non-small cell lung carcinoma (HER2/neu, c-erbB-2), nasopharyngeal cancer (lmp-1, EBNA-1), ovarian cancer (MUC family, HER2/neu, c-erbB-2), prostate cancer (Prostate Specific Antigen (PSA) and its antigenic epitopes PSA-1, PSA-2, and PSA-3, PSMA, HER2/neu, c-erbB-2, ga733 glycoprotein), renal cancer (HER2/neu, c-erbB-2), squamous cell cancers of the cervix, vulva, anus, vagina, esophagus, larynx, oropharynx, penis, skin (viral products such as human papilloma virus proteins, merkel cell virus proteins), testicular cancer (NY-ESO-1), and T cell leukemia (HTLV-1 epitopes).

As used herein, a "transient cell cycle inhibitor" refers to an agent that reversibly blocks the cell cycle of T cells (it may also block the cell cycle of other cells as well). The terms "transient", "temporal" and "reversible" can be used interchangeably in this context. Furthermore, the term "transient cell cycle inhibitor" is interchangeable with, and abbreviated to, "cell cycle inhibitor" herein. A transient cell cycle inhibitor may also be called a cell cycle synchronizing agent or an agent that induces cell cycle arrest (i.e. an inducer of cell cycle arrest). "Cell cycle arrest" or any grammatical equivalent thereof, refers to a state wherein a cell does not exit one cell cycle and enter another, e.g. does not exit G2 to enter mitosis (M phase) such that the cell is considered to be 'arrested' at the G2 phase, or e.g. does not exit G1 to enter S phase such that the cell is considered to be arrested at the G1 phase etc.

The cell cycle is a ubiquitous and essential process that occurs during the growth and proliferation of cells. Based on morphological changes, the cell cycle can be broadly subdivided into inter-phase and mitotic (M)-phase stages. The M-phase includes several sub-phases, including prophase, metaphase, anaphase, and telophase. Similarly, inter-phase encompasses the G1, S, and G2-phases, where G1 and G2-phase represent "pauses" in the cell cycle that occur between DNA synthesis and mitosis. G1-phase is the first pause in which cells prepare for DNA synthesis. In S-phase, cells synthesize DNA and thus have aneuploidic DNA content between 2N and 4N. Conversely, G2-phase is the second pause of the cell cycle in which the cell prepares for mitosis (M-phase). Based on the cell cycle stages, checkpoints for DNA damage are classified into three stages: G1/S (G1), intra-S phase, and G2/M checkpoints.

In the context of immunotherapy, artificial regulation of the cell cycle is very important because cells in different stages of the cell cycle react differently even when maintained

under the same environmental conditions. Since cells are unsynchronized in their natural state, non-toxic specific agents may be used to induce cell synchronization. Such agents are known as transient cell cycle inhibitors herein (or by the alternative names of a cell cycle synchronizing agent or an inducer of cell cycle arrest as described above). The term "cell synchronization" refers to a process by which cells at different stages of the cell cycle are brought to the same phase.

The transient cell cycle inhibitor may arrest the cell cycle at any one of following stages: interphase, G0 phase, G0/G1 phase, early G1 phase, G1 phase, late G1 phase, G1/S phase, S phase, G2/M phase, or M phase.

The transient cell cycle inhibitor may be any agent that reversibly blocks or arrests the cell cycle of a T cell. Several cell cycle inhibitors are known in the art. Different known methods can be used to identify a cell cycle inhibitor. For example, fluorescence-activated cell sorting (FACS) assay, imaging using molecular probes, and Western blot analysis using cell cycle-dependent proteins can be used to determine whether or not an agent induces cell cycle synchronisation and thus induces cell cycle arrest. The method may comprise contacting a population of cells that have been treated with the agent with a dye, that allows for the differentiation of cells at different stages. In a particular example, the ability of an agent to block cells in a particular phase of growth can be determined by cell cycle analysis methods known in the art, i.e., uniform suspensions of nuclei are stained with propidium iodide (PI) using the detergent-trypsin method of Vindelov et al., Cytometry, 3: 323-327 (1983) to determine relative cellular DNA content by flow cytometric analysis. Events are gated using doublet discrimination to exclude doublets, and the data are modeled using ModFit LT Cell Cycle Analysis(TM) software (Verity Software House).

The transient cell cycle inhibitor induces the cells to synchronise their cell cycle by blocking the transition from one cell cycle phase to another. In other words, the cell cycle inhibitor induces the percentage of cells in one specific stage of the cell cycle to increase. It is not meant that all cells in the population will be found in the same stage of the cell cycle after treatment with the transient cell cycle inhibitor, just that it increases the proportion of cells that are in a particular phase of the cell cycle. In one example, contact with the cell cycle inhibitor will induce at least about 50% of the contacted T cell population to be in the desired cell cycle phase (i.e. at least 50% of the contacted cells will be synchronised to the desired cell cycle stage selected from interphase, G0 phase, G0/G1 phase, early G1 phase, G1 phase, late G1 phase, G1/S phase, S phase, G2/M phase, or M phase) after 1 day (i.e. 24 hours) of contact with the cell cycle inhibitor. In another example, contact with the cell cycle

inhibitor will induce at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at least about 95% of the contacted T cell population to be in the desired cell cycle phase after 1 day (i.e. 24 hours) of contact with the cell cycle inhibitor.

Preferably, a transient cell cycle inhibitor is one that, upon contact with a T cell population *ex vivo*, induces at least about 50% of the contacted T cell population to be in the desired cell cycle phase after 24 hours of contact, without substantially reducing the viability of the T cell population e.g. such that at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% of the cells remain viable after 24 hours of contact and preferably at least 50% of these viable cells are in the desired cell cycle phase. Suitable methodology for testing these parameters is set out in detail in the examples section below (under "Optimisation of T cell treatment with topotecan or irinotecan *ex vivo").* Suitable methods to determining cell viability are also well known (e.g. propidium iodide staining). In addition, upon removal of contact with the transient cell cycle inhibitor (e.g. by washing in accordance with the methods set out in detail in the examples section below (under "Optimisation of T cell treatment with topotecan or irinotecan ex vivo")) the cell cycle arrest is reversed such that at least 70%, at least 75%, at least 80%, at least 90%, or at least 95% of the viable cells return to the cell cycle. Methods for determining which proportion of the cells are no longer arrested are well known in the art, and have been discussed in detail above.

Preferably, the transient cell cycle inhibitor is provided at an effective dose. The actual dose used will depend on a number of parameters (e.g. if it is for in vivo or ex vivo use). However, a range of suitable ex vivo doses may be determined using the parameters set out in the paragraph above (i.e. a dose that induces the specified level of cell cycle arrest whilst retaining the specified level of cell viability). Appropriate doses for use in vivo are known in the art.

In one example, a cell cycle inhibitor may be an inhibitor of a metabolic pathway involved in the cell cycle, including for example one of thymidine, aphidicolin, colchicine, nocodazole or 5-fluorodeoxyuridine. As a particular example, thymidine could be used to arrest the cells in G1/S phase (e.g. using 4 mM thymidine for 16-24 hours). Alternatively, aphidicolin or colchicine can be used to arrest a cell at G2/M phase. In one example, the inhibitor of the metabolic pathway is not gemcitabine.

In an alternative example, the cell cycle inhibitor may be an inhibitor of a transcription factor, enzyme (e.g., a kinase or phosphorylase), or cellular pathway. For example, the cell cycle

inhibitor may be a cyclin dependent kinase inhibitor, a cyclin dependent kinase 4 inhibitor, a cyclin dependent kinase 6 inhibitor, a DNA polymerase inhibitor, a FDVIG CoA reductase inhibitor, an inhibitor of nucleotide biosynthesis, or an inhibitor of microtubule polymerization.

A cell cycle inhibitor may arrest the cell cycle by modulating a CEK interacting protein (cip) pathway, kinase inhibitory protein (kip) pathway, inhibitor of kinase 4 (INK4a) pathway, or alternative reading frame (ARF) pathway. For example, the cip/kip family members p21, p27, and p57 arrest the cell cycle at G1 phase.

A cell cycle inhibitor may arrest the cell cycle by modulating a cyclin D pathway, e.g. inhibiting cyclin dependent kinase 1 (CDK1), cyclin dependent kinase 2 (CDK2), cyclin dependent kinase 4 (CDK4) or cyclin dependent kinase 6 (CDK6). Arresting the cell cycle may comprise contacting the cell with a cyclin dependent kinase inhibitor. Non-limiting examples of CDK1 inhibitors include AZD5438, SCH 727965, Seliciclib. Non-limiting examples of CDK2 inhibitors include flavopiridol, AZD5438, SNS-032, Bryostatin-1, Seliciclib, and SCH 727965. The cyclin dependent kinase inhibitor may also be, for example, a cyclin dependent kinase 4 inhibitor or a cyclin dependent kinase 6 inhibitor. Palbociclib, flavopiridol, AZD5438, and PD0332991 are non-limiting examples of selective inhibitors of both CDK4 and CDK6. In some examples, the cyclin dependent kinase inhibitor is RO-3306, dinaciclib, palbociclib, ribociclib, vomciclib, or abemaciclib (see also PCT Patent Application Publication No. WO 2014/109858; hereby incorporated by reference).

A cell cycle inhibitor may arrest the cell cycle by inhibiting microtubule polymerization in the cell. Inhibiting microtubule polymerization may comprise contacting the cell with an inhibitor of microtubule polymerization, such as nocodazole, or paclitaxel.

A cell cycle inhibitor may arrest the cell cycle by inhibiting HMG CoA reductase in the cell. Inhibiting HMG CoA reductase may comprise contacting the cell with an HMG CoA reductase inhibitor, such as lovastatin.

A cell cycle inhibitor may arrest the cell cycle by inhibiting topoisomerase I. Examples of topoisomerase I inhibitors include topotecan, irinotecan, camptothecin and lamellarin D.

A cell cycle inhibitor may arrest the cell cycle by inhibiting HDAC6. An example of a HDAC6 inhibitor is tubastatin A.

A cell cycle inhibitor may arrest the cell cycle by inhibiting DNA polymerase in the cell. Inhibiting DNA polymerase may comprise contacting the cell with a DNA polymerase inhibitor, such as aphidicolin.

A cell cycle inhibitor may be one of abemaciclib, aminopterin, aphidicolin, blebbistatin, butyrate, cathinone, colcemid, colchicine, compactin, cytochalasin D, cytosine arabinoside, fluorodeoxyuridine, hydroxyurea, lovastatin, methotrexate, mevinolin, MG132, mimosine, nocodazole, noscapine, palbociclib, pantopon, razoxane, reveromycin A, RO-3306, roscovitine, ribociclib, vincristine, or voruciclib.

The cell cycle inhibitor may be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A).

In each aspect described herein, there is a specific example wherein the cell cycle inhibitor is not topotecan.

In each aspect described herein, there is a specific example wherein the cell cycle inhibitor is not gemcitabine.

In each aspect described herein, there is a specific example wherein the cell cycle inhibitor is not paclitaxel.

The transient cell cycle inhibitor is a reversible inhibitor of the T cell cycle, e.g., the T cell can restart its cell cycle after the cell cycle inhibitor is removed. In preferred examples, the agent is non-toxic, e.g., a concentration of the agent that is non-toxic to the cell is sufficient to arrest the cell cycle of the cell.

The cell may be in contact (in vivo, ex vivo or in vitro) with the cell cycle inhibitor for at least one day. In one example, the cell may be in contact with the cell cycle inhibitor for at least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours. The contact may be for a period of up to 5 days. The period of time may depend on different factors, e.g., because different cells and different cell culture conditions result in cell cycles of varying duration.

The cell cycle inhibitor may subsequently be removed from the T cell population. The removal may be direct (e.g. by washing of e.g. a T cell population *ex vivo*). Alternatively, the removal may be *in vivo*, e.g. by the cell cycle inhibitor losing its activity over time, or being degraded/cleared from the body. In either case, removal of the cell cycle inhibitor results in "adaptation" or "adaptation to cell cycle arrest" which is a lifting or abrogation of the cell cycle arrest, such that formerly arrested cells re-enter the cell cycle. Such a process is also referred to as escaping from cell cycle arrest. This is discussed in more detail below.

The vaccine and/or transient cell cycle inhibitor described herein may be prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

When the invention comprises a modulator of an immune suppressive mechanism, the transient cell cycle inhibitor may be any transient cell cycle inhibitor, including topotecan (or gemcitabine, or paclitaxel). In other words, topotecan (or gemcitabine, or paclitaxel) may be the transient cell cycle inhibitor of choice when the cell cycle inhibitor is for administration with (or prepared for administration with) a vaccine and a modulator of an immune suppressive mechanism. In a preferred example of this aspect, the cell cycle inhibitor may therefore be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A). In a very specific example of this aspect however, the cell cycle inhibitor and the modulator are not the specific combination of topotecan (cell cycle inhibitor) and cisplatin (modulator).

As used herein, “a modulator of an immune suppressive mechanism” (also referred to as “modulator” herein) refers an agent that modulates (e.g. reduces/inhibits/arrests/reverses) an immune suppressive mechanism in the subject being treated. Modulators of immune suppressive mechanisms are well known and include, but are not limited to chemotherapy, CSF1 signalling blockers, immune checkpoint inhibitors, aspirin; trabectedin; compounds that inhibit Arginase 1, iNOS or IDO, compounds that inhibit the hypoxia pathway or influence the pH of cells e.g. proton pump inhibitors or bicarbonates etc.

Several examples of appropriate modulators are known (see e.g. Huber et al, Seminars in cancer Biology 43(2017): 74-89 which is incorporated herein in its entirety). Examples of appropriate modulators referred to by Huber et al include inhibitors for cytokines (IL-6, IL-10 and transforming growth factor, TGF B1) chemokines (C-C motif chemokine ligand 2 (CCL-

2), C-X-C motif chemokine ligand 1 (CXCL1) and CXCL5)) growth factors (vascular endothelial growth factor, VEGF and granulocyte-macrophage colony-stimulating factor, GM-CSF) and soluble factors (prostaglandin, PGE2) that are produced by tumor cells and other TME components (TAMs, MDSCs and Tregs) and affect the differentiation, maturation, and functionality of DCs into a skewing towards regulatory DCs, which, in turn, produce immunosuppressive factors. Specifically appropriate modulators described above include inhibitors of IL-6, IL-10 and transforming growth factor, TGF B1; vascular endothelial growth factor, VEGF; and prostaglandin, PGE2.

As described by Huber et al, lactic acid produced by tumor cells or by DCs cultured at high density, or added to culture medium during DC differentiation, reduced both basal CD1 expression and toll-like receptor (TLR)-induced expression of CD1a, CD83, and HLA-DR. These DCs displayed a tolerogenic phenotype, characterized by reduced IL-12 and increased IL-10 secretion in response to TLR stimulation, and impaired migratory response to lymph node-derived chemokine CCL-19. Moreover, lactic acid inhibited the proliferation of allogeneic T cells during MLR and the proliferation of antigen-specific CTLs stimulated with autologous peptide-pulsed DCs. The restoration of physiological pH or the inhibition of LDH with oxamic acid (in vitro) or diclofenac (in vivo) reversed this suppressive effect. Similarly, LDHA inhibition by diclofenac, which is associated with a reduction of lactate production by glioma cells, promotes a regained ability of tumor-infiltrating DCs to produce IL-12 upon TLR stimulation. Accordingly, appropriate modulators include an inhibitor of lactic acid, or an inhibitor of IL-10. Similarly, modulation may also be achieved by restoration of restoration of physiological pH or the inhibition of LDH with oxamic acid or the inhibition of LDH or LDHA with diclofenac.

An appropriate modulator may also inhibit Tregs e.g. pharmacological inhibition of the mevalonate pathway through the administration of statins. As described by Huber at al, these cholesterol-lowering drugs appear to impair Treg suppressive activity. Interestingly, treatment with etomoxir, an inhibitor of carnitine palmitoyl-transferase 1 (CPT1), a FAO mitochondrial enzyme, abrogates Treg development. Accordingly, in a specific example, a modulator may include statins or etomoxir.

The terms ""a modulator of an immune suppressive mechanism" and "modulator" are used interchangeably herein, unless the context indicates otherwise.

As mentioned above, an example of an appropriate modulator is a chemotherapeutic agent. As used herein, the phrase "chemotherapeutic agent" refers to (but is not limited to)

compounds that are used in chemotherapy for the treatment of proliferative disorders such as cancer. For the avoidance of doubt, reference to a chemotherapeutic agent herein refers to an additional agent to the cell cycle inhibitor. Accordingly, if the cell cycle inhibitor is also known to function as a chemotherapeutic agent, and the claims or description refers to a cell cycle inhibitor and a chemotherapeutic agent, an additional chemotherapeutic agent is intended, in addition to the cell cycle inhibitor (wherein the additional chemotherapeutic agent may or may not be a second transient cell cycle inhibitor).

Several chemotherapeutic agents are known, some of which are clinically approved or awaiting approval as cancer therapies. Suitable examples include nucleoside analogues, topoisomerase inhibitors, platinum complexes, and combinations thereof. A specific example of a nucleoside analogue is gemcitabine, although many others are well known. Another specific example of a nucleoside analogue is fluorouracil (also known as 5-FU). A specific example of a topoisomerase inhibitor is irinotecan, although many others are well known. A specific example of a platinum complex is oxaliplatin, although many others are well known.

Examples of chemotherapeutic agents include alkylating agents such as thiotepa and CYTOXAN(TM) cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin; bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN(TM) doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin

and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amisacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKO polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., TAXOL(R) paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.) and TAXOTERE(R) doxetaxel (Rhône-Poulenc Rorer, Antony, France); chlorambucil; GEMZAR(TM) gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE(TM)0 vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumours such as anti-oestrogens and selective oestrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX(TM) tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON(TM) toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGAS(TM) megestrol acetate, AROMASIN(TM) exemestane, formestane, fadrozole, RIVISOR(TM) vorozole, FEMARA(TM) letrozole, and

ARIMIDEX(TM) anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in abherant cell proliferation, such as, for example, PKC-alpha, Raf, and H-Ras; ribozymes such as a VEGF expression inhibitor (e.g., ANGIOZYME(R) ribozyme) and a HER2 expression inhibitor; vaccines such as gene therapy vaccines, for example, ALLOVECTIN(TM) vaccine, LEUVECTIN(TM) vaccine, and VAXID(TM) vaccine; PROLEUKIN(TM) rIL-2; LURTOTECAN(TM) topoisomerase I inhibitor; ABARELIX(TM) rGnRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above. The selection of suitable chemotherapeutic agents may depend on the specific proliferative disorder being treated.

The vaccine, transient cell cycle inhibitor and modulator are for administration in an “effective amount”. An “effective amount” (or “therapeutically effective amount”) is an amount that alone, or together with further doses, produces the desired (therapeutic) response. The (therapeutically) effective amount to be used will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the subject. A suitable dosage for a given subject can be determined by an attending physician, taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Accordingly, in one example, a suitable dose (of e.g. a vaccine, or a transient cell cycle inhibitor, or a modulator of an immune suppression mechanism, or an immune check point inhibitor as described herein) is selected based on the body weight of the subject. The dosages and schedules may be varied according to the particular disease state and the overall condition of the patient. For example, it may be necessary or desirable to reduce the doses of the components of the combination treatment in order to reduce toxicity. Suitable doses may also be determined for subgroups of subjects, e.g. based on their heredity and/or pharmocogenetic profile(s).

Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods.

The vaccines (or transient cell cycle inhibitors, or modulators of an immune suppression mechanism, or immune check point inhibitors) described herein are therefore administered to a subject in an effective amount to produce the desired response. An example of the desired response to a vaccine is the induction of a T-cell response. An example of the desired response to the cell cycle inhibitor is the vigorous expansion of T cells

following release. An example of the desired response to modulator is the release of immune suppression. An example of the desired response to the check point inhibitor is alleviation of the checkpoint block. Specific examples of the therapeutic response in the context of e.g. a cancer patient include a reduction in the tumor to lower risk levels, a reduction in the severity of tumor, and increase in survival rate. Methods for measuring these responses are well known.

Preferably, the combination(s) described herein will provide a benefit to the treatment of a disease in a subject in need thereof. For example, the combination may have an additive or synergistic effect on the treatment of a disease in a subject in need thereof. A combination treatment is defined as affording an "additive effect" "synergistic effect" or a "synergistic treatment" if the effect is therapeutically superior, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, to that achievable on dosing one or other of the components of the combination treatment at its conventional dose. For example, the effect of the combination treatment is additive if the effect is therapeutically superior to the effect achievable with vaccine alone or transient cell cycle inhibitor alone. For example, the effect of the combination treatment may be synergistic if the effect of the combination treatment supersedes the effect of the individual treatments added together. Further, the effect of the combination is beneficial (e.g. additive or synergistic) if a beneficial effect is obtained in a group of subjects that does not respond (or responds poorly) to vaccine or transient cell cycle inhibitor alone. In addition, the effect of the combination treatment is defined as affording a benefit (e.g additive or synergistic effect) if one of the components is dosed at its conventional dose and the other component is dosed at a reduced dose and the therapeutic effect, as measured by, for example, the extent of the response, the response rate, the time to disease progression or the survival period, is equivalent to or better than that achievable on dosing conventional amounts of either one of the components of the combination treatment. In particular, a benefit is deemed to be present if the conventional dose of vaccine or transient cell cycle inhibitor may be reduced without detriment to one or more of the extent of the response, the response rate, the time to disease progression and survival data, in particular without detriment to the duration of the response, but with fewer and/or less troublesome side-effects than those that occur when conventional doses of each component are used.

Two or more of the vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) may be provided in a form which is suitable for sequential (consecutive), separate and/or simultaneous (concurrent)

administration to the subject, in any order. For example, a vaccine may be provided in a form that is suitable for sequential, separate and/or simultaneous administration with a transient cell cycle inhibitor (or vice versa). Preferably, a vaccine may be administered to the subject at the same time or before the transient cell cycle inhibitor is administered. Alternatively, a vaccine may be administered to the subject at the same time or after the transient cell cycle inhibitor is administered. In cases where they are administered simultaneously, the vaccine and transient cell cycle inhibitor may be administered as separate compositions that are administered at the same time, or may be administered as a combined composition that includes both. The same applies for the optional addition of a modulator of an immune suppressive mechanism (i.e. it may be provided in a form which is suitable for sequential (consecutive), separate and/or simultaneous (concurrent) administration with the vaccine and/or the cell cycle inhibitor, in any order, as described in detail above).

Equally, a transient cell cycle inhibitor may be provided in a form that is suitable for sequential, separate and/or simultaneous administration with an immune checkpoint inhibitor (or vice versa). Preferably, a transient cell cycle inhibitor may be administered to the subject at the same time or after the immune checkpoint inhibitor is administered. Alternatively, a transient cell cycle inhibitor may be administered to the subject at the same time or before the immune checkpoint inhibitor is administered. In cases where they are administered simultaneously, the immune checkpoint inhibitor and transient cell cycle inhibitor may be administered as separate compositions that are administered at the same time, or may be administered as a combined composition that includes both.

The transient cell cycle inhibitor and the vaccine (and optionally the modulator of an immune suppressive mechanism) may be administered in a manner that allows the T cell population in the subject to be activated at the same time as, or in advance of, contact with the cell cycle inhibitor (e.g. contact with the cell cycle inhibitor may occur within 12 hours, or within 24 hours of T cell activation commencing). Alternatively, the transient cell cycle inhibitor and the vaccine (and optionally the modulator of an immune suppressive mechanism) may be administered in a manner that allows the T cell population in the subject to be in contact with the cell cycle inhibitor prior to T cell activation. A person of ordinary skill in the art is able to identify an appropriate administration regimen. By way of example, the transient cell cycle inhibitor and vaccine (and optionally the modulator of an immune suppressive mechanism) may be administered simultaneously. The dosage form and regimen used may be such that, preferably, the inhibitory effects of the transient cell cycle inhibitor are maintained for at least the 1 day, at least the 2 days, or at least 3 days of vaccination.

The vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) described herein can be administered to the subject by any conventional route, including oral administration (for example in tablet form), injection or by gradual infusion over time. The administration may, for example, be topical, oral, parenteral, intravenous, intraperitoneal, intramuscular, intravascular, intracavity, intranasal, intracerebral, intratracheal, intralesional, intraperitoneal, rectal, subcutaneous, transdermal, epidural, percutaneous, or by infusion. Well established routes of administration are known for several cell cycle inhibitors of interest such as topotecan, irinotecan, thymidine, RO-3306, dinaciclib, palbociclib, nocodazole, paclitaxel and tubastatin A. By way of a further example, irinotecan can be administered by injection (e.g. as an intravenous infusion or by continuous infusion).

The vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) described herein may therefore be in a form suitable for the appropriate mode of administration. For example, suitable forms for oral administration include a tablet or capsule; suitable forms for nasal administration or administration by inhalation include a powder or solution; suitable forms for parenteral injection (including intravenous, subcutaneous, intramuscular, intravascular or infusion) include a sterile solution, suspension or emulsion; suitable forms for topical administration include a patch, an ointment or cream; and suitable forms for rectal administration include a suppository. Alternatively, the route of administration may be by injection (e.g. i.v).

The vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) are advantageously presented in unit dosage form. Dosage forms (also called unit doses) are pharmaceutical drug products in the form in which they are marketed for use, with a specific mixture of active ingredients and inactive components (excipients), in a particular configuration (such as a capsule shell, for example), and apportioned into a particular dose. Depending on the route of administration, dosage forms include liquid, solid, and semisolid dosage forms. Common dosage forms include pills, tablet, capsule, drinks or syrups.

Where the administration of the separate formulations of two components (e.g. two or more of vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate)) is sequential or separate, the delay in administering the second formulation should not be such as to lose the beneficial effect of the combination therapy.

The vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) may be part of a composition (e.g. a pharmaceutical composition) that comprises the compound (i.e. the vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate)) and one or more other components. A composition may be a pharmaceutical composition that comprises the vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) and a pharmaceutically acceptable excipient, diluent and/or carrier. Pharmaceutical compositions may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents or compounds.

As used herein, "pharmaceutically acceptable" refers to a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected compound without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

Excipients are natural or synthetic substances formulated alongside an active ingredient (e.g. the vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate)), included for the purpose of bulking-up the formulation or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating drug absorption or solubility. Excipients can also be useful in the manufacturing process, to aid in the handling of the active substance concerned such as by facilitating powder flowability or non-stick properties, in addition to aiding *in vitro* stability such as prevention of denaturation over the expected shelf life. Pharmaceutically acceptable excipients are well known in the art. A suitable excipient is therefore easily identifiable by one of ordinary skill in the art. By way of example, suitable pharmaceutically acceptable excipients include water, saline, aqueous dextrose, glycerol, ethanol, and the like.

Diluents are diluting agents. Pharmaceutically acceptable diluents are well known in the art. A suitable diluent is therefore easily identifiable by one of ordinary skill in the art.

Carriers are non-toxic to recipients at the dosages and concentrations employed and are compatible with other ingredients of the formulation. The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to

facilitate the application. Pharmaceutically acceptable carriers are well known in the art. A suitable carrier is therefore easily identifiable by one of ordinary skill in the art.

The vaccine, cell cycle inhibitor, modulator of an immune suppression mechanism, or immune check point inhibitor (as appropriate) may be administered using any suitable method and dosage form, as described in detail above.

The vaccine, cell cycle inhibitor, modulator and/or immune check point inhibitor (as appropriate) are for use in therapy. As used herein, "therapy" refers to the prevention or treatment of a disease or disorder. Therapy may be prophylactic or therapeutic (these terms are defined above in the context of vaccines and apply equally here). Therapy includes immunotherapy i.e. the prevention or treatment of a disease or disorder in a subject using substances that stimulate the immune response of the subject (e.g. increase an immune response in a subject). By way of example, the vaccine, cell cycle inhibitor and/or modulator may be for use in T-cell based immunotherapy. As used herein "T-cell based immunotherapy" refers to the prevention or treatment of a disease or disorder in a subject using substances that stimulate a T-cell mediated immune response in the subject (e.g. enhance a T cell response in the subject). Methods that can be used to identify T cell based immunotherapies, including methods that determine whether a T cell response (i.e. T cell activation) is induced/enhanced are well known. By way of illustration, methods for measuring T cell response are described in the examples section below.

The vaccine, cell cycle inhibitor and/or modulator may be for use in the manufacture of a medicament. As used herein "a medicament" refers to a substance used for medical treatment (i.e. a medicine). The medicament may be, e.g. a T cell product that is for use in adoptive cell transfer.

The vaccines, cell cycle inhibitors, and optionally modulators described above can be used in a method for enhancing a T cell response in a subject in need thereof or a method for increasing an immune response in a subject in need thereof.

Accordingly, a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof is also provided, comprising:
i) administering to the subject an effective amount of a vaccine; and,
ii) administering to the subject an effective amount of a cell cycle inhibitor,
wherein steps i) and ii) are separate, simultaneous or sequential, and in any order.

Optionally, the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Preferably, step i) is carried out at the same time, or before, step ii). Alternatively, step ii) may be carried out at the same time, or before, step i).

Optionally, the method may further comprise the step of iii) administering to the subject an effective amount of a modulator of an immune suppressive mechanism,
wherein steps i), ii) and iii) are separate, simultaneous or sequential, and in any order. In this aspect (i.e. when a modulator is also administered) the transient cell cycle inhibitor may be selected from any transient cell cycle inhibitor i.e. may also be topotecan. In a very specific example of this aspect however, the cell cycle inhibitor and the modulator are not the specific combination of topotecan (cell cycle inhibitor) and cisplatin (modulator).

As stated previously, preferably, step i) is carried out at the same time, or before, step ii). Alternatively, step ii) may be carried out at the same time, or before, step i).

Several of the terms have been described in detail above and apply equally here.

As used herein, "increasing" or "enhancing" an immune response refers to an increase in the immune response (e.g. a cell mediated immune response such as a T cell mediated immune response) of the subject during or after treatment compared to their immune response prior to treatment. An induced or enhanced immune response (e.g. a T cell response) therefore encompasses any measurable increase in the immune response. Several known methods for measuring the immune response (including measuring T cell activation) are well known in the art.

As used herein the term "subject" refers to an individual, e.g., a human, having or at risk of having (i.e. susceptible to developing) a disorder or disease (e.g. a disorder or disease that may be prevented, ameliorated or treated using a T-cell based immunotherapy e.g. by enhancing a T cell response or increasing a T cell response in the subject). Subjects that can also be other primate subjects, such as monkeys and apes for veterinary medicine purposes. The subjects can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. Other suitable subjects may include livestock (e.g. cattle, sheep, pigs etc) and domesticated animals (e.g. cats, dogs, horses etc).

The subject may be a patient i.e. a subject in need of treatment. The subject may have already received treatment for the disorder or symptom. Alternatively, the subject has not been treated prior to treatment in accordance with the present invention, or may be treated in accordance with the invention whilst they are (already) undergoing treatment with a second specified treatment (e.g. according to the invention an effective amount of vaccine may be administered to the subject, wherein the subject is (already) undergoing treatment with an effective amount of a transient cell cycle inhibitor (or vice versa)) etc. This applies to all of the permutations set out herein.

As used herein, a subject that is “undergoing treatment” with a specified agent (e.g. a transient cell cycle inhibitor, a vaccine, a modulator of an immune suppression mechanism, or an immune check point inhibitor (as appropriate)) means that the subject has already commenced treatment with the specified agent (the subject may be in any phase of the treatment e.g. induction phase, maintenance phase, recovery phase etc).

As used herein, the terms “treat”, “treating” and "treatment" are taken to include an intervention performed with the intention of preventing the development or altering the pathology of a disorder or symptom. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted disorder or symptom. Accordingly, the term “treating” encompasses treating and/or preventing the development of a disorder or symptom.

Subjects may be afflicted with cancer, including but not limited to colon, lung, liver, breast, prostate, ovarian, skin (including melanoma), bone, cervix, vulva, head and neck region, anus, oropharynx, larynx, pancreas and brain cancer, etc. In some cases, the tumor presented antigens are known, such as melanoma, breast cancer, squamous cell carcinoma, cervix cancer, vulva cancer, cancer of the head and neck region, anal cancer, oropharyngeal cancer, colon cancer, leukaemia, myeloma, prostate cancer, etc. (in these examples memory T cells can be isolated or engineered by introducing the T cell receptor genes). In other cases, the tumor presented antigens can be targeted with genetically modified T cells expressing an engineered immunoreceptor. Examples include but are not limited to B cell lymphoma, breast cancer, prostate cancer, and leukaemia.
In some embodiments, the subject has a bladder, brain, breast, bladder, bone, cervical, colon, esophageal, kidney, liver, lung, ovarian, pancreatic, proximal or distal bile duct, prostate, skin, stomach, thyroid, or uterine cancer. In some embodiments, the subject has a metastatic cancer. In some embodiments, the subject has a cancer that is acute lymphoblastic leukemia, acute lymphoblastic leukemia, acute myeloid leukemia, acute

promyelocytic leukemia, adenocarcinoma, adenoma, adrenal cancer, adrenocortical carcinoma, AIDS-related cancer, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain cancer, carcinoma, cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumor, visual pathway or hypothalamic glioma, breast cancer, bronchial adenoma/carcinoid, Burkitt lymphoma, carcinoid tumor, carcinoma, central nervous system lymphoma, cervical cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorder, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma. epidermoid carcinoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumor, extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancer/intraocular melanoma, eye cancer/retinoblastoma, gallbladder cancer, gallstone tumor, gastric/stomach cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, giant cell tumor, glioblastoma multiforme, glioma, hairy-cell tumor, head and neck cancer, heart cancer, hepatocellular/liver cancer, Hodgkin lymphoma, hyperplasia, hyperplastic corneal nerve tumor, in situ carcinoma, hypopharyngeal cancer, intestinal ganglioneuroma, islet cell tumor, Kaposi's sarcoma, kidney/renal cell cancer, laryngeal cancer, leiomyoma tumor, lip and oral cavity cancer, liposarcoma, liver cancer, non-small cell lung cancer, small cell lung cancer, lymphomas, macroglobulinemia, malignant carcinoid, malignant fibrous histiocytoma of bone, malignant hypercalcemia, malignant melanomas, marfanoid habitus tumor, medullary carcinoma, melanoma, merkel cell carcinoma, mesothelioma, metastatic skin carcinoma, metastatic squamous neck cancer, mouth cancer, mucosal neuromas, multiple myeloma, mycosis fungoides, myelodysplastic syndrome, myeloma, myeloproliferative disorder, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neck cancer, neural tissue cancer, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, ovarian epithelial tumor, ovarian germ cell tumor, pancreatic cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma, pituitary adenoma, pleuropulmonary blastoma, polycythemia vera, primary brain tumor, prostate cancer, rectal cancer, renal cell tumor, reticulum cell sarcoma, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, seminoma, Sezary syndrome, skin cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck carcinoma, stomach cancer, supratentorial primitive neuroectodermal tumor, testicular cancer, throat cancer, thymoma, thyroid cancer, topical skin lesion, trophoblastic tumor, urethral cancer, uterine/endometrial cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenström's macroglobulinemia or Wilm's tumor.

In some embodiments, the subject has a solid tumor. In some embodiments, the subject has a sarcoma, carcinoma, a neurofibromatoma or a lymphoma. In some embodiments, the subject has a colon cancer. In some embodiments, the subject has a lung cancer. In some embodiments, the subject has an ovarian cancer. In some embodiments, the subject has a pancreatic cancer. In some embodiments, the subject has a prostate cancer. In some embodiments, the subject has a proximal or distal bile duct carcinoma. In some embodiments, the subject has a breast cancer. In some embodiments, the subject has a HER2-positive breast cancer. In some embodiments, the subject has a HER2-negative breast cancer.

In some embodiments, the cancer is a hematologic cancer. In some embodiments, cancer is a leukemia, a lymphoma, or a myeloma. In some embodiments, cancer is a non-Hodgkin lymphoma. In some embodiments, cancer is a Hodgkin lymphoma. In some embodiments, cancer is a B-cell malignancy. In some embodiments, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), germinal center diffuse large B-cell lymphoma (GCB DLBCL), primary mediastinal B-cell lymphoma (PMBL), Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma (MCL), B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some embodiments, cancer is a T-cell malignancy. In some embodiments, the T-cell malignancy is peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL), blastic NK-cell lymphoma, enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphomas, or treatment-related T-cell lymphomas. In some embodiments, the subject has multiple myeloma. In some embodiments, the regression of a cancer ceases.

In some embodiments, the subject has a relapsed or refractory cancer. In some embodiments, the relapsed or refractory cancer is a bladder cancer. In some embodiments, the relapsed or refractory cancer is a colon cancer. In some embodiments, the relapsed or refractory cancer is a lung cancer. In some embodiments, the relapsed or refractory cancer

is an ovarian cancer. In some embodiments, the relapsed or refractory cancer is a pancreatic cancer. In some embodiments, the relapsed or refractory cancer is a prostate cancer. In some embodiments, the relapsed or refractory cancer is a proximal or distal bile duct carcinoma. In some embodiments, the relapsed or refractory cancer is a breast cancer.

In some embodiments, the subject has a relapsed or refractory hematologic cancer. In some embodiments, the relapsed or refractory hematologic cancer is a leukemia, a lymphoma, or a myeloma. In some embodiments, the relapsed or refractory hematologic cancer is a non-Hodgkin lymphoma. In some embodiments, the relapsed or refractory hematologic cancer is a Hodgkin lymphoma. In some embodiments, the relapsed or refractory hematologic cancer is a B-cell malignancy. In some embodiments, the B-cell malignancy is chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL), germinal center diffuse large B-cell lymphoma (GCB DLBCL), primary mediastinal B-cell lymphoma (PMBL), Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma (MCL), B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, Waldenström macroglobulinemia, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some embodiments, the relapsed or refractory hematologic cancer is a T-cell malignancy. In some embodiments, the T-cell malignancy is peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL), blastic NK-cell lymphoma, enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphomas, or treatment-related T-cell lymphomas. In some embodiments, the subject has a relapsed or refractory multiple myeloma. In some embodiments, the regression of a relapsed or refractory cancer ceases.

In some embodiments, the subject exhibits one or more symptoms of a hematologic cancer. In some embodiments, the subject exhibits one or more symptoms of a B-cell malignancy. In some embodiments, the subject exhibits one or more symptoms of a T-cell malignancy. In some embodiments, the subject exhibits one or more symptoms of a leukemia, a lymphoma, or a myeloma. In some embodiments, the subject exhibits one or more symptoms such as, but not limited to, abnormal B-cell function, abnormal B-cell size or shape, abnormal B-cell count, fatigue, fever, night sweats, frequent infection, enlarged lymph nodes, paleness,

anemia, easy bleeding or bruising, loss of appetite, weight loss, bone or joint pain, headaches, etc.

In some embodiments, the subject has a high risk of developing cancer or a high risk of cancer recurrence. In some embodiments, a high risk of cancer (developing cancer or recurrence of cancer) is determined based on the expression or presence of a biomarker. In some embodiments, the biomarker includes PMSB1 P11A G/C heterozygote, CD68, suppressor of cytokine signaling 1 (SOCS1), LIM domain only 2 (LMO2), CD137, or a combination thereof.

In some embodiments, a high risk cancer includes bladder, colon, lung, ovarian, pancreatic, prostate, proximal or distal bile duct and breast cancer. In some embodiments, a high risk of bladder, colon, lung, ovarian, pancreatic, prostate and proximal or distal bile duct cancer (developing the cancer or cancer recurrence) is determined based on the expression or presence of a biomarker. In some embodiments, biomarkers for bladder cancer include BTA Stat, BTA Track, NMP 22, Bladder Chek, immunocyt, UroVysion, cytokeratins 8, 18 and 19, telomerase TRAP, hTert and hTR, BLCA-4, survivn, hyaluronic acid/hyaluronidase, DD23 monoclonal antibody, fibronectin and HCG. In some embodiments, biomarkers for colon cancer include CEA, CA 19-9, CYFRA 21-1, ferritin, osteopontin, p53, seprase and EGFR. In some embodiments, biomarkers for lung cancer include ERCC-1, NSE, ProGRP, SCC, beta-tubulin, RRM1, EGFR, VEGF, CYFRA-21-1, CEA, CRP, LDH, CA125, CgA, NCAM and TPA. In some embodiments, biomarkers for ovarian cancer include CA125, Her-2/neu, Akt-2, inhibin, HLA-G, TATI, CASA, TPA, CEA, LPA, PAI-1, IL-6, kallikreins 5, 6, 7, 8, 9,10, 11, 13, 14, 15, hCGpcf, prostasin, osteopontin, HE4, mitogen-activated protein kinase, IGFBP-2, RSF-1 and NAC-1. In some embodiments, biomarkers for pancreatic cancer include CA19-9, CEA, TIMP-1, CA50, CA242, MUC1, MUC5AC, Claudin 18 and annexin A8. In some embodiments, biomarkers for prostate cancer include PSA, human kallikrein 2, IGF-1, IGFBP-3, PCA3, AMACR, GSTPi, CDKN1B, Ki-67, PTEN, and PSCA. In some embodiments, biomarkers for proximal or distal bile duct carcinoma include CA125, CA19-9, CEA, CgA, MUC1, MUC5AC, PML, p53, DPC4, Ki67, matrix metalloproteinases, alpha-fetoprotein, N-cadherin, VEGF-C, claudins, thrombospondin-1, cytokeratins and CYFRA 21-1. In some embodiments, biomarkers for breast cancer include HER-1, -2, -3, -4; EGFR and HER-2/neu.

A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof is also provided, comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor (optionally wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine); or vice versa (i.e. a method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine (optionally wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine)). In either aspect, the subject may additionally be undergoing treatment with an effective amount of a modulator of an immune suppressive mechanism (as defined in detail above). In these aspects, when the subject is also undergoing treatment with a modulator, the transient cell cycle inhibitor may be selected from any transient cell cycle inhibitor i.e. it may also be topotecan, paclitaxel or gemcitabine. The cell cycle inhibitor may therefore be selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A). In a very specific example of this aspect however, the cell cycle inhibitor and the modulator are not the specific combination of topotecan (cell cycle inhibitor) and cisplatin (modulator).

The invention also finds particular utility in methods for the treatment and/or prevention of pathogenic infections, and methods of adjuvant or neoadjuvant therapy for cancer. These aspects are described in more detail below. The details of the vaccines, transient cell cycle inhibitors and/or modulators described above apply equally to these methods (and this the specific details given above in respect of the vaccine, transient cell cycle inhibitor and/or modulator combinations are also useful in treating and/or preventing pathogenic infections (as described below) and are also useful as adjuvant or neoadjuvant therapies for cancer (as is also detailed below).

Prevention and/or treatment of infections

As stated above, the inventors have surprisingly found that transient cell cycle inhibitors can be used to synchronise the cell cycle of T cells ex vivo or in vivo, followed by a fast and strong expansion of the activated T-cells after removal or clearance of the cell cycle inhibitor resulting in a massive, swift and prolonged T cell response. The invention has utility in any aspect that can benefit from an improved or enhanced T cell response. Examples of such

aspects include the treatment and/or prevention of infections by intracellular pathogens, clearance of which from the body requires a T cell mediated response.

The vaccine, transient cell cycle inhibitor (and optionally modulator) combinations described herein are therefore useful for treating and/or preventing an intracellular pathogenic infection or disease in a subject.

It is well known that T cell mediated responses can be a highly effective protection mechanism against intracellular agents such as viruses, protozoans, fungi and intracellular bacteria. The vaccines, cell cycle inhibitors, and optionally modulators described above can therefore be used to prevent and/or treat any suitable intracellular pathogenic infection in a subject. The vaccines, cell cycle inhibitors, and optionally modulators may therefore be used prophylactically and/or therapeutically.

As used herein, a "pathogen" is a microorganism that can cause disease. An "intracellular pathogen" is a pathogen that can be found within host cells (i.e. can be located within or occur within host cells). Several examples of intracellular pathogens are well known, and include viruses, protozoans, fungi and intracellular bacteria.

Viruses are obligate intracellular pathogens. Examples of viral and retroviral infections that may be treated and/or prevented by the vaccine, cell cycle inhibitor, and optionally modulator combinations described herein include infections by human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), Human Papilloma virus (HPV), Human T cell lymphocytotropic virus (HTLV-1), measles virus, adenovirus, cytomegalovirus (CMV) and hepatitis C virus (HCV) (summarised in Huber *et al.*, review article frontiers in immunology; April 2014; vol 5; article 171). Another example includes BK polyomavirus infections in transplant patients. Other suitable examples of viruses that cause infection (and thus can be treated and/or prevented as described herein) are readily identifiable by a person of skill in the art, and are not limited to Retroviridae (including human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III: and other isolates, such as HIV-LP; Picornaviridae (e.g., polio viruses, hepatitis A virus: enteroviruses, human coxsackie viruses, rhinoviruses, echovircases); Calciviridae (e.g., strains that cause gastroenteritis); Togaviridae (e.g., equine encephalitis viruses, rubella viruses); Flaviridae (e.g., dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g., coronaviruses); Rhabdoviridae (e.g., vesicular stomatitis viruses, rabies viruses): Filoviridae (e.g., ebola viruses); Pararmyxoviridae (e.g., parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g., influenza viruses): Bunyaviridae (e.g.,

Hantaan viruses, bunya viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g., reoviruses, orbiviurses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvoviridae (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes viruses'); Poxyiridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g., African swine fever virus); and unclassified viruses (e.g., the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1=internally transmitted; class 2=parenterally transmitted (i.e., Hepatitis C); Norwalk and related viruses, and astroviruses).

Intracellular protozoans that cause infection include Apicomplexans (e.g. Plasmodium spp., Toxoplasma gondii and Cryptosporidium parvum) and Trypanosomatids (e.g. Leishmania spp. and Trypanosoma spp such as Trypanosoma cruzi). Other suitable examples of intracellular protozoans that cause infection (and thus can be treated and/or prevented as described herein) are readily identifiable by a person of skill in the art.

Intracellular fungal infections include, for example, infections by Histoplasma or Cryptococcus neoformans. Other suitable examples of intracellular fungi that cause infection (and thus can be treated and/or prevented as described herein) are readily identifiable by a person of skill in the art.

Intracellular bacteria include bacteria that have the ability to survive within macrophages, such as mycobacterium spp. (e.g. M. tuberculosis and M. leprae). Other intracellular bacteria include Chlamydia spp. Rickettsia spp. Listeria monocytogenes, Coxiella spp., Salmonella typhimurium, Listeria spp., Legionella pneumophila, and Yersinia pestis. Other suitable examples of intracellular bacteria that cause infection (and thus can be treated and/or prevented as described herein) are readily identifiable by a person of skill in the art.

As used herein, “intracellular pathogenic infection” refers to an invasion of a subject’s (host’s) body tissues by a pathogen that resides within host cells (i.e. is an intracellular pathogen). In other words, the infection is an infection that is associated with or caused by an intracellular pathogen. The infection may be acute (short lived) or persistent (chronic). Persistent (or chronic) infections are typically caused by an inefficient host immune response, resulting in long-lasting symptoms. Examples of persistent infections include latent infections (e.g. with HSV) or slow infections (e.g. with HIV).

The subject may be infected, or be at risk of becoming infected, by an intracellular pathogen as described herein.

Infection of a subject by an intracellular pathogen described herein may cause a disease or disorder. For example, infection by HIV may result in AIDS. The vaccines, cell cycle inhibitors, and optionally modulators described herein may also be used to treat and/or prevent a disease or disorder caused by an intracellular pathogenic infection. The vaccines, cell cycle inhibitor, and optionally modulators may be used prophylactically and/or therapeutically to prevent and/or treat such disease or disorders.

The subject can be afflicted with, or be at risk of developing, a disease or disorder caused by an intracellular pathogenic infection as described herein.

Advantageously, the vaccines, cell cycle inhibitors, and optionally modulators described above can be used to improve vaccine efficacy (compared to administration of the vaccine only).

Adjuvant therapy

For most patients newly diagnosed with operable cancer, the standard treatment is definitive surgery followed by chemotherapy. Such treatment aims at removing as much primary and metastatic disease as possible in order to prevent recurrence and improve survival. Indeed, most of these patients have no macroscopic evidence of residual tumor after surgery. However, many of them would later develop recurrence and may eventually die of their diseases. This occurs because a small number of viable tumor cells became metastasized prior to the surgery, escaped the surgery and went undetected after the surgery due to the limitation of current detection techniques.

Adjuvant therapies can be administered to a subject that has achieved a satisfactory response (e.g. complete response) after standard cancer treatment in respect a previously diagnosed cancer. The adjuvant therapy is used to prevent or slow down recurrence of the disease.

The vaccines, cell cycle inhibitors, and optionally modulators described herein may be used as an adjuvant therapy. They may be administered to a subject, characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, in an effective amount to prevent, reduce the risk of, or delay relapsed or

refractory disease, wherein the first anticancer therapy does not comprise administration of the adjuvant therapy.

The vaccines, cell cycle inhibitors, and optionally modulator combinations described herein (referred to as the "adjuvant therapy" throughout) may also be administered to a subject, characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy (e.g. in an effective amount to extend disease free survival (DFS) or overall survival (OS) in the subject) wherein the first anticancer therapy does not comprise administration of the adjuvant therapy.

As a specific non-limiting example, if the adjuvant therapy is a combination of antigenic epitope PSA-1 ("vaccine") and irinotecan ("transient cell cycle inhibitor"), then the first anticancer therapy cannot comprise the combination of PSA-1 and irinotecan.

In some embodiments, the first anticancer therapy comprises administration of a chemotherapeutic agent, a biologic agent, radiation therapy, bone marrow transplant, surgery, photosensitizing agents, toxins, or a combination thereof. In some embodiments, the chemotherapeutic agent or biologic agent is selected from among an antibody, a B cell receptor pathway inhibitor, a T cell receptor inhibitor, a PI3K inhibitor, an IAP inhibitor, an mTOR inhibitor, a radioimmunotherapeutic, a DNA damaging agent, a histone deacetylase inhibitor, a protein kinase inhibitor, a hedgehog inhibitor, an Hsp90 inhibitor, a telomerase inhibitor, a Jak1/2 inhibitor, a protease inhibitor, an IRAK inhibitor, a PKC inhibitor, a PARP inhibitor, a CYP3A4 inhibitor, an AKT inhibitor, an Erk inhibitor, a proteosome inhibitor, an alkylating agent, an antimetabolite, a plant alkaloid, a terpenoid, a cytotoxin, a topoisomerase inhibitor, or a combination thereof. Specific examples of such first anticancer therapies are well known in the art.

In other embodiments, the first anticancer therapy may comprise surgery, for removal of a tumor. The adjuvant therapy may then be administered to prevent, reduce the risk of, or delay relapsed or refractory disease. In some embodiments, the surgery for removal of a tumor is a definitive surgery. In some embodiments, the subject has no detectable tumors following surgery. In some embodiments, the subject has no detectable circulating tumor cells in a fluid sample (e.g. blood, spinal fluid or urine) following surgery. In some embodiments, the surgery for removal of a tumor is a partial removal of the tumor. In some embodiments, the subject has not been administered chemotherapy for treatment of the cancer. In some embodiments, the subject has been administered a chemotherapeutic agent or biologic agent for treatment of the cancer.

The adjuvant therapy may therefore be administered to a subject having cancer, following treatment of a tumor with a first anticancer therapy to decrease the size of a tumor or eliminate the tumor, wherein the first anticancer therapy does not comprise administration of the adjuvant therapy. The adjuvant therapy is for administration in an effective amount to prevent or delay progression of the tumor, promote further regression of the tumor, or eliminate the tumor.

In some embodiments, the adjuvant therapy described herein extends disease free survival (DFS) or overall survival (OS) in the subject. Disease free survival (DFS) or overall survival (OS) may be assessed one or more years (i.e. 1, 2, 3, 4, 5, ,6 ,7 ,8, 9, 10 or longer) following initiation of administration of the adjuvant therapy described herein. In some embodiments, the adjuvant therapy is initiated following a decrease in size of a tumor (e.g. a primary or metastatic tumor) following administration of the first anticancer therapy. In some embodiments, treatment with the first anticancer therapy is discontinued prior to initiation of treatment with the adjuvant therapy. In some embodiments, treatment with the first anticancer therapy is continued prior to initiation of treatment with the adjuvant therapy.

In some embodiments, the subject has no detectable cancer following treatment of the cancer with the first anticancer therapy and prior to initiation of adjuvant therapy administration. In some embodiments, the subject has no detectable primary or metastatic tumors, or circulating tumor cells in a fluid sample (e.g. blood, spinal fluid or urine) following treatment with the first anticancer therapy, prior to initiation of adjuvant therapy administration.

In some embodiments, the risk of relapsed or refractory disease is reduced by about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater compared to no treatment with the adjuvant therapy. In some embodiments, the subject is disease free for about 6 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years or longer following last administration of the adjuvant therapy. In some embodiments, the subject has a high risk of cancer recurrence prior to administration of the adjuvant therapy.

In some embodiments, the tumor is a sarcoma, carcinoma, lymphoma, or a melanoma. In some embodiments, the lymphoma is an enlarged lymph node or an extranodal lymphoma. In some embodiments, the subject has a brain, breast, bladder, bone, colon, kidney, liver, lung, ovarian, pancreatic, prostate, skin or proximal or distal bile duct carcinoma. In some embodiments, the subject has a hematologic cancer. In some embodiments, the cancer is a

leukemia, a lymphoma, or a myeloma. In some embodiments, the subject has a non-Hodgkin's lymphoma. In some embodiments, the non-Hodgkin's lymphoma is chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma, marginal zone lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, or extranodal marginal zone B cell lymphoma. In some embodiments, the non-Hodgkin's lymphoma is a relapsed or refractory non-Hodgkin's lymphoma. In some embodiments, the subject has a T-cell malignancy. In some embodiments, the T-cell malignancy is peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large cell lymphoma, angioimmunoblastic lymphoma, cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma (ATLL), blastic NK-cell lymphoma, enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphomas, or treatment-related T-cell lymphomas. In some embodiments, the T-cell malignancy is a relapsed or refractory T-cell malignancy. In some embodiments, the risk of a secondary tumor is decreased compared to no treatment with the adjuvant therapy. In some embodiments, DFS or OS is evaluated about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 years or longer after initiation of the adjuvant therapy treatment.

Typically, the cancer for which the subject underwent treatment with the first anticancer therapy is a cancer with a high recurrence risk. For example, the cancer may be a cancer that is predicted to recur in the subject within the first two years after the previous treatment regimen. Examples of cancers with a high recurrence risk include ovarian cancer and pancreatic cancer. Other examples of cancers with a high recurrence risk are described elsewhere herein.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

The cancer may be refractory. By “refractory” in the context of a cancer is intended the particular cancer is resistant to, or non-responsive to, therapy with a particular therapeutic agent. A cancer is refractory to therapy with a particular therapeutic agent either from the onset of treatment with the particular therapeutic agent (i.e., non-responsive to initial exposure to the therapeutic agent), or as a result of developing resistance to the therapeutic agent, either over the course of a first treatment period with the therapeutic agent or during a subsequent treatment period with the therapeutic agent.

As used herein, "survival" refers to the patient remaining alive, and includes disease free survival (DFS) and overall survival (OS). Survival is estimated by the Kaplan-Meier method, and any differences in survival are computed using the stratified log-rank test.

As used herein, "disease free survival (DFS)" refers to the patient remaining alive, without return of the cancer, for a defined period of time such as about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 10 years, or more from initiation of treatment or from initial diagnosis. In one embodiment, DFS is analyzed according to the intent-to-treat principle, i.e., patients are evaluated on the basis of their assigned therapy. The events used in the analysis of DFS include local, regional and distant recurrence of cancer, occurrence of secondary cancer, and death from any cause in patients without a prior event (e.g., cancer recurrence or second primary cancer).

As used herein, "overall survival" refers to the patient remaining alive for a defined period of time, such as about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 10 years, or more from initiation of treatment or from initial diagnosis.

As used herein, "extending survival" or "increasing the likelihood of survival" refers to increasing DFS and/or OS or increasing the probability of remaining alive and/or disease-free at a given point in time in a treated patient relative to an untreated patient (i.e. relative to a patient not treated with an adjuvant therapy described herein), or relative to a control treatment protocol, such as treatment only with the chemotherapeutic agent or biologic agent, such as those use in the standard of care for a particular cancer). Survival is monitored for at least about two months, four months, six months, nine months, or at least about 1 year, or at least about 2 years, or at least about 3 years, or at least about 4 years, or at least about 5 years, or at least about 10 years, etc., following the initiation of treatment or following the initial diagnosis.

As used herein, "adjuvant therapy" refers to a therapy administered to a subject following a successful standard of care cancer therapy (a "first anticancer therapy"), so as to reduce the risk of disease recurrence of the cancer, either local or metastatic. The subject has a history of cancer, but is characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy.

As used herein, "standard of care" therapy refers to a therapy routinely used to treat a particular disease or disorder. As used herein, "standard of care" chemotherapy refers to the chemotherapeutic agents routinely used to treat a particular cancer.

As used herein, “definitive surgery” is used as that term is used within the medical community, and typically refers to surgery where the outcome is potentially curative. Definitive surgery includes, for example, procedures, surgical or otherwise, that result in removal or resection of the tumor, including those that result in the removal or resection of all grossly visible tumor. Definitive surgery includes, for example, complete or curative resection or complete gross resection of the tumor. Definitive surgery includes procedures that occurs in one or more stages, and includes, for example, multi-stage surgical procedures where one or more surgical or other procedures are performed prior to resection of the tumor. Definitive surgery includes procedures to remove or resect the tumor including involved organs, parts of organs and tissues, as well as surrounding organs, such as lymph nodes, parts of organs, or tissues.

The cancer may be benign or malignant. The term cancer also encompasses dormant tumors or micrometastases. The term cancer includes also solid tumors and hematologic cancers. By "early stage cancer" or "early stage tumor" is meant cancer that is not invasive or metastatic or is classified as a Stage 0, 1, or II cancer.

The term "pre-cancerous" refers to a condition or a growth that typically precedes or develops into a cancer. A "pre-cancerous" growth will have cells that an characterized by abnormal cell cycle regulation, proliferation, or differentiation, which can be determined by markers of cell cycle regulation, cellular proliferation, or differentiation.

By "dysplasia" is meant any abnormal growth or development of tissue, organ or cells. Preferably, the dysplasia is high grade or precancerous. By "metastasis" is meant the spread of cancer from its primary site to other places in the body. Cancer cells can break away from a primary tumor, penetrate into lymphatic and blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasize) in normal tissues elsewhere in the body. Metastasis can be local or distant. Metastasis is a sequential process, contingent on tumor cells breaking off from the primary tumor, traveling through the bloodstream, and stopping at a distant site. At the new site, the cells establish a blood supply and can grow to form a life- threatening mass. Both stimulatory and inhibitory molecular pathways within the tumor cell regulate this behavior, and interactions between the tumor cell and host cells in the distant site are also significant. By "micrometastasis" is meant a small number of cells that have spread from the primary tumor to other parts of the body. Micrometastasis may or may not be detected in a screening or diagnostic test.

By "non-metastatic" is meant a cancer that is benign or that remains at the primary site and has not penetrated into the lymphatic or blood vessel system or to tissues other than the primary site. Generally, a non-metastatic cancer is any cancer that is a Stage 0, 1, or II cancer, and occasionally a Stage III cancer.

Reference to a tumor or cancer as a "Stage 0," "Stage I," "Stage II," "Stage III," or "Stage IV" indicates classification of the tumor or cancer using the Overall Stage Grouping or Roman Numeral Staging methods known in the art. Although the actual stage of the cancer is dependent on the type of cancer, in general, a Stage 0 cancer is an in situ lesion, a Stage I cancer is small localized tumor, a Stage II and III cancer is a local advanced tumor which exhibits involvement of the local lymph nodes, and a Stage IV cancer represents metastatic cancer. The specific stages for each type of tumor is known to the skilled clinician.

"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

By "primary tumor" or "primary cancer" is meant the original cancer and not a metastatic lesion located in another tissue, organ, or location in the subject's body. By "benign tumor" or "benign cancer" is meant a tumor that remains localized at the site of origin and does not have the capacity to infiltrate, invade, or metastasize to a distant site.

“Cancer recurrence”, “cancer relapse”, “relapsed or refractory disease” are used interchangeably herein to refer to a return of cancer following treatment, and includes return of cancer in the primary organ, as well as distant recurrence, where the cancer returns outside of the primary organ.

As used herein, a subject at “high risk of cancer recurrence or relapse” is one who has a greater chance of experiencing recurrence of cancer. A subject's risk level can be determined by a skilled physician.

Neoadjuvant therapy

The treatment of early stage or benign tumours is desirable for preventing progression to a malignant or metastatic state, thereby reducing the morbidity and mortality associated with cancer. Neoadjuvant therapy (an adjunctive therapy given before the main definitive cancer treatment) has therefore emerged as an important part of cancer therapy.

The combinations of vaccines, cell cycle inhibitors, and optionally modulators described herein may be useful as a neoadjuvant therapy, for example as a prophylactic means for preventing and/or slowing down cancer progression in a high-risk subject. Advantageously, such neoadjuvant therapies may be used to prime a T cell response in a subject to prevent and/or slow down cancer progression in the subject.

As used herein, "neoadjuvant therapy" refers to a treatment regimen that is given before cancer has been detected or diagnosed in the subject. In other words, the neoadjuvant therapy is administered to the subject pre-emptively, prior to diagnosis of a primary/definitive cancer. The neoadjuvant therapy described herein may therefore also be considered as a cancer vaccine.

Neoadjuvant therapies are therefore provided for treating a benign, pre-cancerous, or non-metastatic cancer in a subject. The vaccine, cell cycle inhibitor and optional modulator are for administration in an effective amount, such that administration of the neoadjuvant therapy prevents the benign, precancerous, or non-metastatic cancer from becoming an invasive or metastatic cancer. For example, the benign, pre-cancerous or non-metastatic cancer can be a stage 0, stage I, or stage II cancer, and in certain embodiments, the administration of the neoadjuvant therapy described herein prevents the benign, pre-cancerous or non-metastatic cancer from progressing to the next stage(s), e.g., a stage I, a stage II, a stage III or stage IV cancer. In certain embodiments, the neoadjuvant therapy described herein is administered for a time and in an amount sufficient to treat the benign, pre-cancerous, or non-metastatic tumor in the subject or to prevent the benign, pre-cancerous, or non-metastatic tumor from becoming an invasive or metastatic cancer. In certain embodiments, administering the neoadjuvant therapy described herein reduces tumor size, tumor burden, or the tumor number of the benign, pre-cancerous, or non-metastatic tumor.

The neoadjuvant therapy described herein can be used to treat, e.g., a stage 0 (e.g., a carcinoma in situ), stage I, or stage II cancer. The neoadjuvant therapy described herein can be used to treat any type of cancer, e.g., benign or malignant. In certain embodiments of the invention, the cancer is an epithelial cell solid tumor, including, but not limited to, gastrointestinal cancer, colon cancer, breast cancer, prostate cancer, renal cancer, lung cancer (e.g., non-small cell lung cancer), melanoma, ovarian cancer, pancreatic cancer, head and neck cancer, liver cancer and soft tissue cancers (e.g., B cell lymphomas such as NHL and multiple myeloma and leukemias such as chronic lymphocytic leukemia). In another embodiment, the benign, pre-cancerous, or non-metastatic tumor is a polyp, adenoma, fibroma, lipoma, gastrinoma, insulinoma, chondroma, osteoma, hemangioma,

lymphangioma, meningioma, leiomyoma, rhabdomyoma, squamous cell papilloma, acoustic neuromas, neurofibroma, bile duct cystanoma, leiomyomas, mesotheliomas, teratomas, myxomas, trachomas, granulomas, hamartoma, transitional cell papilloma, pleomorphic adenoma of the salivary gland, desmoid tumor, dermoid cystpapilloma, cystadenoma, focal nodular hyperplasia, or a nodular regenerative hyperplasia. In another embodiment, the method is desirably used to treat an adenoma. Non-limiting examples of adenomas include liver cell adenoma, renal adenoma, metanephric adenoma, bronchial adenoma, alveolar adenoma, adrenal adenoma, pituitary adenoma, parathyroid adenoma, pancreatic adenoma, salivary gland adenoma, hepatocellular adenoma, gastrointestinal adenoma, tubular adenoma, and bile duct adenoma. Other suitable cancers are described elsewhere herein.

The neoadjuvant therapy described herein can also be used (when administered in an effective amount) to prevent occurrence or recurrence of a benign, pre-cancerous, or non-metastatic cancer in the subject.

The neoadjuvant therapy (i.e. the combinations of vaccines, cell cycle inhibitors, and optionally modulators described herein) is also useful for treating a subject with a family history of cancer, polyps, or an inherited cancer syndrome.

The subject may be a subject that has been determined to have a moderate or high risk of developing cancer. For example, the subject may be in a known high-risk category for cancer (e.g. genetically disposed to developing cancer) and may be asymptomatic. In one example, the subject has a family history of cancer, polyps, or an inherited cancer syndrome (e.g., multiple endocrine neoplasia type 1 (MEN1)). In certain, the subject is at risk of developing a benign, precancerous, or non-metastatic gastrointestinal tumor, a desmoid tumor, or an adenoma (e.g., a gastrointestinal adenoma, a pituitary adenoma, or a pancreatic adenoma).

In some examples, the subject may be a human over the age of 50, that has an inherited cancer syndrome, or has a family history of colon cancer or polyps. Non-limiting examples of inherited gastrointestinal cancer syndromes include familial adenomatous polyposis (FAP), Gardner's syndrome, pancreatic cancer, and hereditary non-polyposis colorectal cancer (HNPCC). For example, the neoadjuvant therapy described herein may be used in an amount and for a time to reduce the number of adenomatous colorectal polyps in a subject having FAP. In certain embodiments, the subject may or may not have previously undergone a colonoscopy.

In another embodiment, the neoadjuvant therapy described herein may be for administration for a time and in an amount sufficient to prevent occurrence of a clinically detectable tumor, or metastasis thereof, or to increase the duration of survival of the subject. The neoadjuvant therapy described herein may also be used to extend disease free survival (DFS) or overall survival (OS) in the subject. These terms are described in detail elsewhere herein.

Generally, alleviation or treatment of a benign, precancerous, non-malignant or early stage cancer using a neoadjuvant therapy described herein involves the lessening of one or more symptoms or medical problems associated with the cancer. The therapeutically effective amount of the neoadjuvant therapy can accomplish one or a combination of the following to reduce (e.g., by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or more) the number of cancer cells in the tumor; to reduce the size of the tumor; to reduce the tumor burden; to inhibit (i.e., to decrease to some extent and/or stop) cancer cell infiltration into peripheral organs; to reduce vessel density in the tumor; to inhibit tumor metastasis; to reduce or inhibit tumor growth or tumor cell proliferation; to reduce or prevent the growth of a dormant tumor; to reduce or prevent the growth or proliferation of a micrometastases; to reduce or prevent the re-growth of a tumor after treatment or removal (e.g., in adjuvant therapy); to increase or extend the DFS or OS of a subject susceptible to or diagnosed with a benign, precancerous, or non-metastatic tumor or a malignant tumor; to reduce the size of a tumor to allow for surgery (e.g., in neoadjuvant therapy); and/or to relieve to some extent one or more of the symptoms associated with the cancer.

The neoadjuvant therapy vaccine component may comprise one or more cancer or tumor antigens. Specific examples of such antigens are given elsewhere herein.

Adoptive cell therapy

The invention may also have utility in preparing a T cell population *ex vivo* e.g. for adoptive T cell therapy. Advantageously, synchronisation of a T cell population in the context of e.g. adoptive T cell therapy enables a rapid, co-ordinated and prolonged T cell response once the T cell population is transferred into the subject.

All of the appropriate terminology described herein in the context of any other aspect (e.g. appropriate transient cell cycle inhibitors, vaccines, modulators, types of subject, effective amount, formulated for simultaneous, separate or sequential administration etc) apply equally to this aspect.

An *ex vivo* method of preparing a T cell population suitable for adoptive T cell therapy is also provided, the method comprising:

a) activating the T cell population; and

b) contacting the T cell population with a transient cell cycle inhibitor; wherein steps a) and b) may be in any order.

In one example, the T cell population is activated and then the activated cell population is contacted with the transient cell cycle inhibitor (e.g. contact with the cell cycle inhibitor occurs after T cell stimulation but before expansion of the T cell population). By way of example, the T cell population may be activated and then contacted with the transient cell cycle inhibitor within 12 hours or within 24 hours of the T cell activation step commencing.

In a different example, the T cell population is contacted with a transient cell cycle inhibitor first, followed by activation of the T cell population (i.e. activation of the T cell population occurs after contact with the cell cycle inhibitor has commenced).

The T cell population may be from any mammalian, preferably primate, species, including monkeys, dogs, and humans. The T cells may be allogenic (from the same species but different donor) as the recipient subject; alternatively, the T cells are autologous (the donor and the recipient are the same); in some examples, the T cells are syngeneic (the donor and the recipients are different but are identical twins).

The T cell population may comprise any appropriate type of T cell, including but not limited to cytotoxic T cells (CTL), CD4+ T cells, CAR T cells and TCRtg T cell. Cytotoxic T lymphocyte (CTL) refers to a T lymphocyte that expresses CD8 on the surface thereof (i.e., a CD8+ T cell). A "T cell population" refers to a plurality of T cells. The T cell population may comprise mixture of cell types. The number of cells needed will depend upon the ultimate use for which the T cell population is intended as will the type of cells included therein. For example, if cells that are specific for a particular antigen are desired, then the population may contain greater than 70%, generally greater than 80%, 85% and 90-95% of such cells.

The T cell population can be collected in accordance with known techniques and enriched for the desired T cells by known techniques such as affinity binding to antibodies such as flow cytometry and/or affinity binding. After enrichment steps, *in vitro* expansion of the desired T cells can be carried out in accordance with known techniques (including but not limited to those described in US Patent No. 6,040,177), or variations thereof that will be apparent to those skilled in the art. For example, the desired T cell population or

subpopulation may be expanded by adding an initial T lymphocyte population to a culture medium *in vitro*, and then adding to the culture medium feeder cells, such as non-dividing peripheral blood mononuclear cells (PBMC), (e.g., such that the resulting population of cells contains at least about 5, 10, 20, or 40 or more PBMC feeder cells for each T lymphocyte in the initial population to be expanded); and incubating the culture (e.g. for a time sufficient to expand the numbers of T cells). The order of additional of the T cells and feeder cells to the culture media can be reversed if desired. The culture can typically be incubated under conditions of temperature and the like that are suitable for the growth of T lymphocytes. For the growth of human T lymphocytes, for example, the temperature will generally be at least about 25 degrees Celsius, preferably at least about 30 degrees, more preferably about 37 degrees.

The T cell population may be a cytotoxic T lymphocyte (CTL) population, where the T cells are specific for an antigen present on a human tumor or a pathogen.

The *ex vivo* method comprises the step of contacting a T cell population with a transient cell cycle inhibitor. The term "transient cell cycle inhibitor" is defined elsewhere in detail herein. As used herein, the term, "contacting" refers to bringing the T cell population into the proximity of the cell cycle inhibitor (e.g. in direct contact by for example adding the cell cycle inhibitor to the buffer or media in which the cells are maintained) such that the cell cycle inhibitor arrests the cell cycle of the T cell population. As indicated elsewhere herein, this is not intended to mean that all of the T cells in the population are arrested such that all the T cells are in the same phase of the cell cycle. Rather, the cell cycle inhibitor functions to increase the proportion of T cells within the desired cell cycle phase (e.g. where the cell cycle inhibitor is thymidine, the proportion of T cells arrested at G1/S is increased etc). The contacting step is therefore performed for a time period and under conditions sufficient to enable the cell cycle inhibitor to induce cell cycle arrest. The contacting step may therefore be performed until e.g. at least about 50% of the contacted T cell population are in the desired cell cycle phase (i.e. at least 50% of the contacted cells are synchronised to the desired cell cycle stage selected from interphase, G0 phase, G0/G1 phase, early G1 phase, G1 phase, late G1 phase, G1/S phase, S phase, G2/M phase, or M phase), or at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at least about 95% of the contacted T cell population to be in the desired cell cycle phase.

The T cell population may be contacted with the transient cell cycle inhibitor for at least one day. In one example, the T cell population may be in contact with the cell cycle inhibitor for at

least 24 hours, at least 36 hours, at least 48 hours, at least 72 hours. The contact may be for a period of up to 5 days. The examples described below give specific details of how the contacting step may be performed. In addition, it is well within the skills of a person of ordinary skill in the art to identify suitable alternative conditions for the contacting step defined herein.

The *ex vivo* method further comprises the step of activating the T cell population. This step may be performed at the same time, before or after the contacting step described above has begun. Preferably, the activating step commences at the same time or before contact with the cell cycle inhibitor. In the example, contact with the cell cycle inhibitor may occur within 12 hour or within 24 hours of T cell activation commencing. Alternatively, the activation step may be at the same time or after the cells have begun contact with the cell cycle inhibitor (e.g. the T cell population cell cycle may be sufficiently arrested at the desired cell cycle phase prior to the activating step).

As used herein, the term "activating" refers to stimulating the T cell. T cell activation (and methods for measuring it) are well known in the art, especially in the context of ex vivo methods such as the preparation of T cells ex vivo for use in adoptive T cell transfer.

The T cell population may be activated by providing the T cell population with antigen-loaded antigen presenting cells that stimulate cognate T cell receptors on the T cell population. By way of example, the antigen-loaded antigen presenting cells may be dendritic cells, however other appropriate antigen presenting cells may also be used. By way of an alternative example, the T cell population may be activated using MHC multimers loaded with the specific antigen, together with a costimulatory antibody. Appropriate methods for activating the T cell population are well known.

The T cell population may be activated by providing the T cell population with T cell-activating antibodies. Appropriate antibodies for activating/stimulating the T cell population include anti-CD3/anti-CD28 antibodies. Other appropriate antibodies may also be used. In one example, when the T cell population is activated by providing the T cell population with T cell-activating antibodies, the T cell population is additionally provided with at least one cytokine. The at least one cytokine may be e.g. IL-2 (where a preferred combination of activating agents is the combination of anti-CD3/anti-CD28 antibodies and IL-2).

The method may further comprise removing contact between the activated T cell population and the transient cell cycle inhibitor, thereby expanding the T cell population. Removing

contact between the activated T cell population and the transient cell cycle inhibitor may occur *ex vivo*, or *in vivo* (e.g. after adoptive T cell transfer of the activated T cell population into a subject has occurred). By way of example, *ex vivo* removal of the contact between the activated T cell population and the transient cell cycle inhibitor may be achieved by washing the cells (e.g. in a suitable buffer or media, such as the buffer or media in which the cells are maintained *ex vivo*, e.g. the equivalent buffer or media into which the transient cell cycle inhibitor was previously added). As noted above, removal of the contact between the activated T cell population and the transient cell cycle inhibitor may also occur *in vivo* (e.g. if contact with the transient cell cycle inhibitor is not removed prior to adoptive T cell transfer, and the body subsequently dilutes, clears or degrades any residual transient cell cycle inhibitor, and thereby removes the contact).

Once the contact between the activated T cell population and the transient cell cycle inhibitor is sufficiently removed, the T cell population expands (i.e. the cell cycle arrest is reversed and the cell cycle is resumed).

The T cell preparations generated herein are suitable for adoptive cell therapy.

The T cell preparation may be formulated in a pharmaceutically acceptable carrier. The T cell preparation can be used adoptive T cell therapy in a human subject in need thereof in an effective amount, or used in the manufacture of a medicament for adoptive T cell therapy. The appropriate terms used are described in detail elsewhere and apply equally here.

A T cell population that is prepared as described above can be utilized in methods and compositions for adoptive immunotherapy in accordance with known techniques, or variations thereof that will be apparent to those skilled in the art based on the instant disclosure. See, e.g., US Patent Application Publication No. 2003/0170238; see also US Patent No. 4,690,915.

The adoptive cell therapy may comprise administration of an effective amount of at least one immune checkpoint inhibitor to the subject. Appropriate immune checkpoint inhibitors are described below in detail and apply equally here.

Alternatively, or in addition, the adoptive cell therapy may comprise administration of an effective amount of a vaccine comprising an antigen that stimulates a cognate T cell receptor on the T cells in the T cell population. The appropriate terms used are described in detail elsewhere and apply equally here.

Check point inhibition

The invention may also have utility in immunotherapy that comprises administration of immune checkpoint inhibitors. Advantageously, the swift, coordinated and prolonged T cell response that is induced by cell cycle synchronisation of T cells in accordance with the invention is also of benefit for treatments involving immune checkpoint blockade.

All of the appropriate terminology described herein in the context of any other aspect (e.g. appropriate transient cell cycle inhibitors, vaccines, modulators, types of subject, effective amount, formulated for simultaneous, separate or sequential administration etc) apply equally to this aspect.

An effective amount of a transient cell cycle inhibitor for use in T-cell based immunotherapy is also provided, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

Equally, an effective amount of an immune checkpoint inhibitor for use in T-cell based immunotherapy is also provided, wherein the immune checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

The immune system plays an important role in controlling and eradicating disorders and malignancies such as cancer. However, in the context of malignancy, multiple mechanisms of immune suppression also exist that may prevent effective antitumor immunity. Agents that are directed to inhibit several negative immunologic regulators (checkpoints) may be used as "immune check point inhibitors" to dampen down these mechanisms of immune suppression. A general review of immune checkpoint inhibitors and their role in immune checkpoint blockade (in the context of cancer therapy) is provided by Postow *et al.*, Journal of Clinical Oncology, 2015 Vol 33, No 17, 1974-1982, which is incorporated herein in its entirety.

In the broadest context, therefore, an immune checkpoint inhibitor may be considered as a specific class of a "modulator of an immune suppressive mechanism" (as defined more broadly elsewhere herein). The term "immune checkpoint inhibitor" refers to e.g. an agent that blocks CTLA-4, PD-1, PD-L1, TIM3, TIGIT, VISTA or LAG-3 activity. By way of example, the immune checkpoint inhibitor may be an antibody, an siRNA or a small molecule. By way of a specific example, and as described in more detail in Postow *et al.*, the immune checkpoint inhibitor may be an antibody that blocks CTLA-1 or PD-1/PD-L1. Such antibodies

are showing real promise in the clinic in the treatment of patients with a variety of malignancies.

As a specific example, the immune checkpoint inhibitor may be an inhibitor of PD-1 and/or PD-L1 activity. In other words, the immune checkpoint inhibitor may result in PD-1 or PD-L1 blockade. The inhibitor of PD-1 and/or PD-L1 activity may be e.g. an antibody that blocks PD-L1 binding to PD1 (or vice versa).

Administration of the cell cycle inhibitor and the immune checkpoint inhibitor may be in any order. Preferably, the cell cycle inhibitor is administered at the same time or after the immune checkpoint inhibitor. Alternatively, the cell cycle inhibitor is administered at the same time or before the immune checkpoint inhibitor.

The transient cell cycle inhibitor and immune check point inhibitor may be administered in a manner that allows the appropriate T cell population in the subject to be activated at the same time, or before, contact with the cell cycle inhibitor. In this context, the T cell population is activated e.g. at the point at which the immune checkpoint is inhibited such that negative immune regulation is reduced/dampened down/inhibited/reversed. In this example, contact with the cell cycle inhibitor may occur within 12 hours or within 24 hours of when T cell activation starts. Alternatively, the transient cell cycle inhibitor and immune check point inhibitor may be administered in a manner that allows the appropriate T cell population in the subject to be in contact with the cell cycle inhibitor prior to T cell activation (e.g. such that a sufficient level of cell cycle arrest is achieved prior to T cell activation). A person of ordinary skill in the art is able to identify an appropriate administration regimen. By way of example, the transient cell cycle inhibitor and immune check point inhibitor may be administered simultaneously. The dosage form and regimen used may be such that, preferably, the inhibitory effects of the transient cell cycle inhibitor and the immune checkpoint inhibitor are maintained for at least the first day, at least the first 2 days, at least the first 3 days of the T cell based immunotherapy.

An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy is also provided, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

Equally, an effective amount of an immune checkpoint inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy is also provided, wherein the immune

checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

The medicament may comprise a T cell product for adoptive cell transfer or infusion into a subject in need thereof.

A method of T-cell based immunotherapy in a subject in need thereof is also provided, the method comprising:
i) administering to the subject an effective amount of an immune checkpoint inhibitor; and,
ii) administering to the subject an effective amount of a transient cell cycle inhibitor,
wherein steps i) and ii) are separate, simultaneous or sequential, and in any order.
Administration of the cell cycle inhibitor and the immune checkpoint inhibitor may be in any order.

A method of T-cell based immunotherapy in a subject in need thereof is also provided, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of an immune checkpoint inhibitor.

Equally, a method of T-cell based immunotherapy in a subject in need thereof is provided, comprising administering to the subject an effective amount of an immune checkpoint inhibitor, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor.

Aspects discussed herein with respect to immune checkpoint inhibitors may also equally apply to aspects discussed herein with respect to treating or preventing pathogenic infections, adjuvant therapies and neoadjuvant therapies (and the respective sub-populations of patients recited in the respective sections above apply equally to this aspect).

Kits

Also provided is a kit comprising:
i) an effective amount of a vaccine; and,
ii) an effective amount of a transient cell cycle inhibitor,
wherein i) and ii) are formulated for simultaneous, separate or sequential administration to a subject.

All of the appropriate terminology described herein in the context of any other aspect (e.g. appropriate transient cell cycle inhibitors, vaccines, modulators, types of subject, effective amount, formulated for simultaneous, separate or sequential administration etc) apply equally to this aspect.

The kit may further comprise iii) an effective amount of a modulator of an immune suppressive mechanism, preferably a chemotherapeutic agent,
wherein i), ii) and iii) are formulated for simultaneous, separate or sequential administration in any order to a subject.

Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For example, Singleton and Sainsbury, Dictionary of Microbiology and Molecular Biology, 2d Ed., John Wiley and Sons, NY (1 94); and Hale and Marham, The Harper Collins Dictionary of Biology, Harper Perennial, NY (1991) provide those of skill in the art with a general dictionary of many of the terms used in the invention. Although any methods and materials similar or equivalent to those described herein find use in the practice of the present invention, the preferred methods and materials are described herein. Accordingly, the terms defined immediately below are more fully described by reference to the Specification as a whole. Also, as used herein, the singular terms "a", "an," and "the" include the plural reference unless the context clearly indicates otherwise. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. It is to be understood that this invention is not limited to the particular methodology, protocols, and reagents described, as these may vary, depending upon the context they are used by those of skill in the art.

Throughout the description and claims of this specification, the words “comprise” and “contain” and variations of them mean “including but not limited to”, and they are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

The patent, scientific and technical literature referred to herein establish knowledge that was available to those skilled in the art at the time of filing. The entire disclosures of the issued patents, published and pending patent applications, and other publications that are cited herein are hereby incorporated by reference to the same extent as if each was specifically and individually indicated to be incorporated by reference. In the case of any inconsistencies, the present disclosure will prevail.

Aspects of the invention are demonstrated by the following non-limiting examples.

EXAMPLES

METHODS

Mice

Six to eight week old female C57BL/6JRccHsd mice were purchased Harlan Laboratories (Horst, Netherlands) and housed in the central animal facility of the Leiden University Medical Center (LUMC; Leiden, the Netherlands). The ovalbumin-specific T cell receptor (TCR) transgenic OT-I mice, on a C57BL/6 background, were bred in house. All mice were housed in individually ventilated cage (IVC) systems under specific pathogen-free conditions. Experiments were approved by the Animal Experiments Committee of the LUMC, in line with the guidelines of the European Committee.

Tumor cell lines and culture conditions

Tumor cell line TC-1 (a kind gift from T.C. Wu, John Hopkins University, Baltimore, MD) was generated by retroviral transduction of lung fibroblasts of C57BL/6 origin with the HPV16 E6 and E7 and c-H-*ras* oncogenes (8). The TC-1 tumor cell line and the colon adenocarcinoma cell line MC-38 were both cultured in Iscove's Modified Dulbecco's Medium (IMDM; BioWhittaker) supplemented with 8% fetal calf serum (FCS), 50 µmol/L 2-mercaptoethanol, and 100 IU/mL penicillin/streptomycin and 2 mmol/L glutamine. TC-1 culture medium was also supplemented 400 µg/ml Geneticin (G418; Life Technologies), nonessential amino acids (Life Technologies), and 1 mM sodium pyruvate (Life Technologies).

Tumor (-treatment) experiments

On day 0, C57BL/6JRccHsd mice were subcutaneously inoculated with 1 × 10^5 TC-1 tumor cells or 1 × 10^5 MC-38 tumor cells in 200-µL PBS and 0.2% BSA in the right flank. When a palpable tumor was present (day 8), mice were split into groups with comparable tumor size and treated with 150 µg synthetic long peptides (SLP) and /or chemotherapy. Cisplatin (4 mg/kg, intraperitoneal injection (i.p.)) was provided on day 8 and 15, topotecan (2 mg/kg, i.p) was provided on days 8, 9, 10, 11 and 15, 16, 17, 18) as previously described (5). TC-1 tumor bearing mice were vaccinated subcutaneously (s.c) in the left flank with synthetic long HPV16 $E7_{43-77}$ peptide (GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR) dissolved in PBS and 1:1 emulsified in Montanide (ISA-51, Seppic). MC-38 tumor bearing animals were treated with synthetic long *Reps1* peptide (ELFRAAQLANDVVLQIMELCA) dissolved in PBS with 30 µg CpG (ODN1826, InvivoGen). Analysis of the systemic T cell response was performed by flow cytometry, using HPV16 $E7_{49-57}$ peptide (RAHYNIVTF) loaded H-$2D^b$ tetramers (HPV16 E7 vaccination experiments) or AQLANDVVL loaded H-$2D^b$ tetramers (*Reps1*/MC-38 vaccination experiments) in combination with 7-aminoactinomycin D (for dead exclusion; Invitrogen), CD3 (clone 500A2) and CD8α (clone 53-6.7).

For *in vivo* $CD8^+$ T-cell depletion, mice were injected i.p. 100 µg of the monoclonal antibody 2.43. All mice used had a >99% depletion as measured by flow cytometry.

Adoptive transfer experiments

For adoptive transfer of OT-I ceels, 0.5 × 10^6 OT-I (Thy1.1 or Ly5.1) T cells were negatively enriched via a $CD8^+$ enrichment kit (BD). The OT-I cells were carboxyfluorescein diacetate succinimidyl ester (CFSE) labelled and the transgenic T cells were then intravenously (i.v.) injected in Ly5.2/Thy1.2-recipient mice. The next day, mice were vaccinated, with the SLP, containing the SIINFEKL epitope to stimulate OT-I cells ($OVA_{241-270}$) together with 20µg CpG emulsified in montanide ISA-51. Cisplatin (4 mg/kg, i.p.) was provided on day 0, topotecan (2 mg/kg, i.p.) on day 0, 1, 2. The kinetics of the OT-I cells were measured in blood by flow cytometry, using CD3 (clone 500A2), CD8α (clone 53-6.7), KLRG1 (clone 2F1), Vβ5.1/5.2 (clone MR9-4), CD90.1 (clone Ox-7) and CD127 (clone A7R34, biotin) in combination with streptavidin (Brilliant Violet 605). Samples were analyzed with a BD LSR II flow cytometer, and results were analyzed using FlowJo software (Tree Star). Slope (*m*) was calculated using the following formula *m = (mean % OT-1 cells of $CD8^+$T cells on peak of response – mean % OT-1 cells of $CD8^+$T cells on next time point measured) / (day of peak of response – day next time point measured)*

Analysis of tumor-infiltrating immune populations

For analysis of intratumoral immune subsets TC-1 tumor bearing mice were vaccinated on day 8, treated with cisplatin on day 14 and treated with topotecan on day 14, 15, 16. On day

17 and 21 mice were sacrificed and transcardially perfused with PBS-EDTA. Isolated tumors were disrupted in small pieces and incubated for 15 minutes at 37°C in IMDM—containing Liberase (Roche) after which the tumors were minced through a 70-µm cell strainer (BD Biosciences) to obtain single cell suspensions. After a short incubation with Fc-block and naïve mouse serum, cells were resuspended in staining buffer (PBS + 2% FSC + 0.05% sodium azide) and incubated with HPV16 $E7_{49–57}$ peptide (RAHYNIVTF) loaded H-$2D^b$ tetramers, 7-aminoactinomycin D (for dead exclusion; Invitrogen), CD19 (clone 1D3, to exclude samples containing > 5% B cells and therefor many PBMCs), CD8α (clone 53–6.7), CD3 (clone 145-2C11), CD45.2 (clone 104), class II (clone M5/114.15.2), for 30 minutes at 4°C. Fluorescent conjugated antibodies were purchased from BD Biosciences and eBioscience. For intracellular Ki-67 staining, surface-stained cells were fixed and permeabilized with the FoxP3 staining buffer, and subsequently incubated with FITC-labeled Ki-67–specific antibodies or isotype control (eBioscience).

Statistical analysis

Statistical analysis Survival for differentially treated mice was compared using the Kaplan–Meier method and the log-rank (Mantel–Cox) test. Additional statistical methods are stated in the legends. All $P < 0.05$ were considered significant.

RESULTS

Triple treatment with vaccine, topotecan and cisplatin in vivo

Temporal cell-cycle blockade of vaccine-stimulated CD8 T-cells *in vivo* results in massive expansion of tumor-specific CD8 T-cells that gradually contract and display superior tumor control.

As part of the inventors' ongoing studies on chemotherapy regimens and immunotherapy combinations they treated tumor-bearing mice with cisplatin, topotecan and vaccination. Mice were subcutaneously inoculated with the HPV16-E6/E7 expressing TC-1 tumor cell line and treatment was started when a palpable tumor was present (Figure 1A). The inventors confirmed that the chemotherapy doublet had, similar to observations in patients (4, 5), a superior clinical control as it delayed tumor growth and improved survival (Figure 1A). SLP vaccination induced a strong but transient regression of tumors. The combination, however, of SLP vaccination and topotecan (25% survival) or cisplatin (65% survival) improved vaccine related survival (Figure 1A) but triple treatment with cisplatin, topotecan and SLP vaccination resulted in durable tumor rejection in nearly all mice (95% survival).

Given the importance of the CD8 T-cells (Figure 1B), the inventors analyzed the intratumoral T-cells. Since tumor size may affect immune infiltration (8, 9) tumors were analyzed at the

peak of the vaccine-induced response in mice with similar tumor sizes (Figure 1C). A strikingly low number of CD8 T-cells was found in the tumors of mice treated with vaccine + topotecan and of mice treated with vaccine + cisplatin + topotecan, while in the tumors of vaccine + cisplatin treated mice sizable amounts of CD8 T-cells were observed (Figure 1C), indicating that topotecan but not cisplatin affected the levels of intratumoral T-cells. Specifically, the tumor-specific (HPV16-E7) CD8 T-cells were affected by topotecan (Figure 1C).

Further analysis revealed that the vaccine-induced CD8 T-cells of the animals not receiving chemotherapy displayed a strong expression of the proliferation marker Ki67 9 days after vaccination (day 17) but 4 days later (day 21) this was low again, suggesting a stop in proliferation and contraction of the T-cell response (Figure 1D). When topotecan was given, the vaccine-induced T-cells displayed a much lower Ki67 expression 9 days after vaccination but 4 days later after the topotecan treatment was stopped high Ki67 levels were observed, suggesting vigorous expansion (Figure 1D) after temporal topotecan treatment. This is consistent with the kinetics of the HPV16/E7-specific CD8 T-cells in TC-1 tumor-bearing animals (Figure 1E), showing a remarkable delayed yet extensive expansion.

Next, the inventors analyzed the response kinetics using T-cells with the same TCR (i.e. OT-I T-cells recognizing the model antigen ovalbumin). Also OT-I cells peaked later in vaccinated mice and displayed a delayed peak in peak in Ki67 expression (Figure 2A). Together these data indicate that topotecan affects antigen-stimulated T-cells, and that after this treatment these T-cells are released from their proliferative block and expand massively. Moreover, analysis of the kinetics of the T-cell response revealed that the contraction of the OT-I response in animals receiving topotecan is prolonged (Figure 2A). As a result more T-cells are present for a longer period of time. Consistent this observation was that the number of short-lived effector cells, which are prone for apoptotic cell death (10), is higher in vaccinated mice without chemotherapy (Figure 2B). To substantiate, the impact of temporal topotecan treatment on the T-cell response kinetics, the inventors analysed the E7-specific CD8 T-cells after vaccination in non-tumor bearing mice (Figure 2C). Strikingly, after a delay of the expansion of the antigen-specific T-cells, the number of vaccine-specific T-cells peaked at higher levels and the contraction phase was prolonged (Figure 2C). The non-vaccine specific CD8 T-cells were not affected by chemotherapy (data not shown). Thus, topotecan treatment induces an atypical kinetic response of T-cells; after treatment the proliferative block is lifted and the T-cells expand more vigorously and display a delayed contraction phase.

Cell cycle synchronization of T-cells *ex vivo* using different transient cell cycle inhibitors (topotecan, irinotecan, RO-3306 or thymidine)

Topotecan is known to prevent T-cells to move through the S-phase of the cell cycle. To recapitulate the effects observed above *in vivo* the inventors set up an *ex vivo* system and showed that the presence of topotecan prevented cell cycle progression. Irinotecan, an analogous topoisomerase I inhibitor, showed a similar prevention of cell cycle progression. Essentially and reminiscent to the *in vivo* data with topotecan, first cell cycle arresting the T-cells and then washing away either topotecan or irinotecan resulted in massive expansion of the T-cells, and even higher numbers of T-cells were observed within 48 hours after the release (Figure 3A-B). This effect of massive expansion after synchronization was not observed with the tumor cells, suggesting this is a unique property of T-cells.

Since it was not clear if only a topotecan-mediated arrest at the G2-phase of the TCR-activated T-cells was enough to obtain massive synchronized proliferation, the inventors tested the cell cycle blockers RO-3306 (CDK1 inhibitor that arrests cells at G2/M) and thymidine. Cell cycle arrest of the cells indeed blocked CD8 T-cell proliferation, and importantly, releasing the cell cycle block resulted in an unparalleled expansion (Figure 3C), thereby recapitulating the *ex vivo*/*in vivo* results with observed with topotecan. Within 36 hours even more T-cells were present than in non-synchronized cultures (data not shown). These results imply that CD8 T-cells that are arrested in their cell cycle and synchronized in e.g. the G2-phase, become remarkably potent during the arrest, and once released are able to expand with an unprecedented speediness. These *ex vivo* results show that this novel method of cell cycle synchronization also applies to adoptive T cell therapy (ACT). Thus, cell cycle synchronization may be utilized as a feature for antigen-specific CD8 T-cell populations in order to improve immunotherapy (e.g. cancer immunotherapy based on vaccination, ACT and/or immune checkpoint blockade).

Optimisation of T cell treatment with topotecan or irinotecan *ex vivo*

Experiments were performed to investigate the effects of different treatment durations of chemotherapy on $CD8^+$ T cell proliferation. In each experiment $CD8^+$ T cells were enriched from spleens of naïve mice resulting in >90% $CD3^+CD8^+$ cells. $CD8^+$ T cells were then labelled with CFSE, and activated with CD3/CD28 beads. After $CD8^+$ T cell activation, the cells were treated with increasing Topotecan and Irinotecan concentrations. At a set time-point, additional chemotherapy was given or chemotherapy treatment was abrogated by washing away the chemotherapeutic agent. The effects of these different treatments on cell viability, cell proliferation and cell cycle were analyzed kinetically.

Methods: On day 0, spleens were collected from naïve C57BL/6 mice mouse and single-cell suspensions were prepared by mincing the spleens through cell strainers (BD Biosciences). $CD8^+$ T cells were isolated using the Mouse CD8 T Lymphocyte Enrichment set (BD Biosciences) according to the manufacturer's protocol. The $CD3^+CD8^+$ lymphocyte purity was analyzed by cell surface staining. Cells were re-suspended in staining buffer (PBS + 2% FCS + 0.05% sodium azide) and incubated with CD3 (clone 500A2), CD4 (clone RM4-5), and CD8a (clone 53-6.7) for 30 minutes at 4°C and analyzed with flow cytometry. To determine the effects on cell proliferation the $CD8^+$ T cells were incubated with 2.5 µM CFSE in PBS/0.1% BSA (Sigma-Aldrich) for 10 minutes at 37°C. Next, the $CD8^+$ T cells were stimulated with CD3/CD28 Dynabeads (Life Technologies) according to the manufacturer's instructions. After 1 hour of activation or the next day (day 1), different concentrations of Topotecan (Accord) and Irinotecan (Fresenius) were added, depending on the experiment and condition. On day 3, chemotherapy was removed from the samples or new chemotherapy was added to the samples that would be analyzed on day 4. On both day 3 and day 4, dead cells were removed with the Dead Cell Removal Kit (Miltenyi Biotec). In addition to the manufacturer's instructions, 50 µl of Dead Cell Removal Microbeads was used, and cells were applied on the MACS column an extra time, or the incubation with microbeads and the application on the MACS column was repeated. The efficacy of the Dead Cell Removal kit was tested with cell surface staining of the 'live' effluent. Cells were re-suspended in staining buffer (PBS + 2% FCS + 0.05% sodium) and incubated with CD3e-APC (clone 145-2c11), CD4-FITC (clone RM4-4), CD8-A700 (clone 53-6.7) and 7-aminoactinomycin D (for dead exclusion; Invitrogen) for 30 minutes at 4°C and analyzed with flow cytometry. After the dead cells were removed, the 'live' effluent was incubated with 10 µM EdU for 2 hours. The cell surface of these cells was stained with CD3-V500 (clone 500A2) and CD8a-PerCP (clone 53-6.7). After fixation, the cells were stained intracellular with FxCycle™ Violet (Life Technologies) according to the instructions of the Click-iT™ Plus EdU Flow Cytometry Assay kit (Invitrogen) and analyzed by flow cytometry.

First, the best time point for the start of cell cycle arrest was determined. $CD8^+$ T cells were activated on day 0 and treated with increasing Topotecan concentrations, a topoisomerase I inhibitor, on day 0 or day 1. On day 3, Topotecan was washed away and medium or medium with Topotecan was added. Cell viability and cell proliferation were analyzed by flow cytometry on both day 3 and day 4. The Topotecan concentrations 1 µg/ml and 3 µg/ml given on day 0, and removal of this agent on day 3 resulted in increased $CD8^+$ T cell proliferation compared with the $CD8^+$ T cells that had continued exposure to Topotecan on day 3 (Fig. 4A). Since this effect was in accordance with the hypothesis based on *in vivo* preliminary findings, day 0 was chosen to start the cell cycle arrest treatment. Next, the

effect of Topotecan treatment in combination with IL-2 on $CD8^+$ T cell viability was tested. IL-2 was given at a concentration of 10 Units/ml at day 0. IL-2 had no effect on cell viability (Fig. 4B) and was therefore not used in further experiments. To study whether the effect of Topotecan on $CD8^+$ T cell proliferation is specific for topoisomerase inhibitors, Irinotecan, another topoisomerase I inhibitor, was tested. Activated $CD8^+$ T cells were treated with different Irinotecan concentrations ranging from 50 µg/ml to 0.08 µg/ml on day 0 for 3 or 4 days. At day 3 or 4, the effects on cell viability and cell proliferation were analyzed with flow cytometry. Irinotecan at a concentration of 10 µg/ml was chosen as the most optimal for further experiments, based on sufficient $CD8^+$ T cell survival (higher than (Fig. 4C). Note, that the survival of T cells is higher with Irinotecan as compared to Topotecan.

A combination of a cell cycle inhibitor and immune checkpoint blockade provides a synergistic effect (Figure 5). In wild-type mice treated with both a cell cycle inhibitor (Topotecan) and immune checkpoint blockade (PDL-1 blockade) a synergistic effect was observed (chi-square test p=0.0290). Tumor-free mice in the double treated group was 50%, while in the PDL-1 treated group 25% of the mice were tumor-free and 0% in the untreated and topotecan only treated group.

REFERENCES

1. Pardoll, D. M. (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252-264

2. Restifo, N. P., Dudley, M. E., and Rosenberg, S. A. (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. *Nat. Rev. Immunol* **12**, 269-281

3. Topalian, S. L., Drake, C. G., and Pardoll, D. M. (2015) Immune checkpoint blockade: a common denominator approach to cancer therapy. *Cancer Cell* **27**, 450-461

4. van der Burg, S. H., Arens, R., Ossendorp, F., van Hall, T., and Melief, C. J. (2016) Vaccines for established cancer: overcoming the challenges posed by immune evasion. *Nature reviews. Cancer*

5. van der Sluis, T. C., van Duikeren, S., Huppelschoten, S., Jordanova, E. S., Beyranvand, N. E., Sloots, A., Boon, L., Smit, V. T., Welters, M. J., Ossendorp, F., van de Water, B., Arens, R., van der Burg, S. H., and Melief, C. J. (2015) Vaccine-induced tumor necrosis factor-producing T cells synergize with cisplatin to promote tumor cell death. *Clin. Cancer Res* **21**, 781-794

6. Welters, M. J., van der Sluis, T. C., van Meir, H., Loof, N. M., van Ham, V. J., van Duikeren, S., Santegoets, S. J., Arens, R., de Kam, M. L., Cohen, A. F., van Poelgeest, M. I., Kenter, G. G., Kroep, J. R., Burggraaf, J., Melief, C. J., and van der Burg, S. H. (2016)

Vaccination during myeloid cell depletion by cancer chemotherapy fosters robust T cell responses. *Sci Transl Med* **8**, 334ra352

7. Beyranvand Nejad, E., van der Sluis, T. C., van Duikeren, S., Yagita, H., Janssen, G. M., van Veelen, P. A., Melief, C. J., van der Burg, S. H., and Arens, R. (2016) Tumor Eradication by Cisplatin Is Sustained by CD80/86-Mediated Costimulation of CD8+ T Cells. *Cancer Res* **76**, 6017-6029

8. Arens, R., and Schoenberger, S. P. (2010) Plasticity in programming of effector and memory CD8 T-cell formation. *Immunol. Rev* **235**, 190-205

9. Curtsinger, J. M., and Mescher, M. F. (2010) Inflammatory cytokines as a third signal for T cell activation. *Curr. Opin. Immunol* **22**, 333-340

10. Feau, S., Arens, R., Togher, S., and Schoenberger, S. P. (2011) Autocrine IL-2 is required for secondary population expansion of CD8(+) memory T cells. *Nat. Immunol* **12**, 908-913

11. Redeker, A., Welten, S. P., Baert, M. R., Vloemans, S. A., Tiemessen, M. M., Staal, F. J., and Arens, R. (2015) The Quantity of Autocrine IL-2 Governs the Expansion Potential of CD8+ T Cells. *J. Immunol* **195**, 4792-4801

12. Butz, E. A., and Bevan, M. J. (1998) Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. *Immunity* **8**, 167-175

13. Nurse, P. (2000) A long twentieth century of the cell cycle and beyond. *Cell* **100**, 71-78

14. King, R. W., Deshaies, R. J., Peters, J. M., and Kirschner, M. W. (1996) How proteolysis drives the cell cycle. *Science* **274**, 1652-1659

15. Wells, A. D., and Morawski, P. A. (2014) New roles for cyclin-dependent kinases in T cell biology: linking cell division and differentiation. *Nat Rev Immunol* **14**, 261-270

16. Dowling, M. R., Kan, A., Heinzel, S., Zhou, J. H., Marchingo, J. M., Wellard, C. J., Markham, J. F., and Hodgkin, P. D. (2014) Stretched cell cycle model for proliferating lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 6377-6382

The invention includes the subject matter reproduced below as numbered paragraphs.

Paragraph 1. An effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 2. An effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 3. An effective amount of a vaccine for use in T-cell based immunotherapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 4. An effective amount of a transient cell cycle inhibitor for use T-cell based immunotherapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 5. An effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 6. An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 7. An effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 8. An effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 9. An effective amount of a modulator of an immune suppressive mechanism for use in therapy, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine.

Paragraph 10. An effective amount of a vaccine for use in T-cell based immunotherapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 11. An effective amount of a transient cell cycle inhibitor for use T-cell based immunotherapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 12. An effective amount of a modulator of an immune suppressive mechanism for use T-cell based immunotherapy, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

Paragraph 13. An effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 14. An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 15. An effective amount of a modulator of an immune suppressive mechanism for use in the manufacture of a medicament, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

Paragraph 16. The effective amount of a vaccine for use according to any of paragraphs 7, 10 or 13, or the effective amount of a transient cell cycle inhibitor for use according to any of paragraphs 8, 11 or 14, or the effective amount of a modulator of an immune suppressive mechanism for use according to any of paragraphs 9, 12 or 15, wherein the modulator of an immune suppressive mechanism is a chemotherapeutic agent.

Paragraph 17. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising:
i) administering to the subject an effective amount of a vaccine; and,
ii) administering to the subject an effective amount of a transient cell cycle inhibitor,
wherein steps i) and ii) are separate, simultaneous or sequential, and in any order, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 18. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising:
i) administering to the subject an effective amount of a vaccine;
ii) administering to the subject an effective amount of a transient cell cycle inhibitor; and
iii) administering to the subject an effective amount of a modulator of an immune suppressive mechanism,
wherein steps i), ii) and iii) are separate, simultaneous or sequential, and in any order.

Paragraph 19. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 20. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is

undergoing treatment with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

Paragraph 21. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor and an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 22. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a modulator of an immune suppressive mechanism.

Paragraph 23. A method of enhancing a T cell response in a subject in need thereof or increasing an immune response in a subject in need thereof, comprising administering to the subject an effective amount of a modulator of an immune suppressive mechanism, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a transient cell cycle inhibitor.

Paragraph 24. The effective amount for use, or the method, according to any one of paragraphs 1 to 23, wherein the transient cell cycle inhibitor arrests the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

Paragraph 25. The effective amount for use, or the method, according to any one of the preceding paragraphs, wherein the transient cell cycle inhibitor is selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole), and a HDAC6 inhibitor (such as tubastatin A).

Paragraph 26. The effective amount for use, or the method according to any one of paragraphs 1 to 25, wherein the subject is afflicted with:

a) cancer; preferably wherein the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas, brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

b) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

Paragraph 27. An *ex vivo* method of preparing a T cell population suitable for adoptive cell therapy, the method comprising:

i) activating the T cell population; and

ii) contacting the T cell population with a transient cell cycle inhibitor;

wherein steps i) and ii) may be in any order.

Paragraph 28. The method of paragraph 27, wherein the method further comprises removing contact between the activated T cell population and the transient cell cycle inhibitor, thereby expanding the T cell population.

Paragraph 29. The method of paragraph 28, wherein contact between the T cell population and the transient cell cycle inhibitor is removed by washing.

Paragraph 30. The method of any one of paragraphs 27 to 29, wherein the T cell population is activated by providing the T cell population with antigen-loaded antigen presenting cells that stimulate cognate T cell receptors on the T cell population.

Paragraph 31. The method of paragraph 30, wherein the antigen-loaded antigen presenting cells are dendritic cells.

Paragraph 32. The method of any one of paragraphs 27 to 29, wherein the T cell population is activated by providing the T cell population with T cell-activating antibodies.

Paragraph 33. The method of paragraph 32, wherein the T cell population is additionally provided with at least one cytokine.

Paragraph 34. The method of any of paragraphs 27 to 33, wherein the cell cycle inhibitor arrests the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

Paragraph 35. The method of any of paragraphs 27 to 34, wherein the cell cycle inhibitor is selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A).

Paragraph 36. A T cell preparation suitable for adoptive cell therapy, obtained from the method of any of paragraphs 27 to 35.

Paragraph 37. A method of any of paragraphs 27 to 35, or a T cell preparation of paragraph 36, wherein the T cell preparation is formulated in a pharmaceutically acceptable carrier.

Paragraph 38. An effective amount of T cell preparation according to paragraph 36 or paragraph 37 for use in adoptive cell therapy in a human subject in need thereof.

Paragraph 39. An effective amount of T cell preparation according to paragraph 36 or paragraph 37 for use in the manufacture of a medicament for adoptive cell therapy.

Paragraph 40. A method of adoptive T cell therapy in a subject in need thereof, the method comprising administering to the subject an effective amount of a T cell preparation according paragraph 36 or paragraph 37.

Paragraph 41. The T cell preparation for use according to paragraph 38 or paragraph 39, or the method according to paragraph 40, wherein the adoptive T cell therapy comprises administration of an effective amount of at least one immune checkpoint inhibitor to the subject.

Paragraph 42. The T cell preparation for use according to paragraph 38, 39 or 41, or the method according to paragraph 40 or 41, wherein the adoptive T cell therapy comprises administration of an effective amount of a vaccine comprising an antigen that stimulates cognate T cell receptors on the T cell population.

Paragraph 43. The T cell preparation for use, or the method, according to any of paragraphs 38 to 42, wherein the subject is afflicted with:

a) cancer, preferably where the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas; brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

b) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

Paragraph 44. The T cell preparation for use, or the method, according to any of paragraphs 38 to 43, wherein the subject is immunodeficient.

Paragraph 45. An effective amount of a transient cell cycle inhibitor for use in T-cell based immunotherapy, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

Paragraph 46. An effective amount of an immune checkpoint inhibitor for use in T-cell based immunotherapy, wherein the immune checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

Paragraph 47. An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

Paragraph 48. An effective amount of an immune checkpoint inhibitor for use in the manufacture of a medicament for T-cell based immunotherapy, wherein the immune checkpoint inhibitor is for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor.

Paragraph 49. A method of T-cell based immunotherapy in a subject in need thereof, comprising
i) administering to the subject an effective amount of an immune checkpoint inhibitor; and,
ii) administering to the subject an effective amount of a transient cell cycle inhibitor,
wherein steps i) and ii) are separate, simultaneous or sequential, and in any order.

Paragraph 50. A method of T-cell based immunotherapy in a subject in need thereof, comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of an immune checkpoint inhibitor.

Paragraph 51. A method of T-cell based immunotherapy in a subject in need thereof, comprising administering to the subject an effective amount of an immune checkpoint inhibitor, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor.

Paragraph 52. The effective amount for use, or the method, according to any one of paragraphs 45 to 51, wherein the subject is afflicted with:

i) cancer; preferably wherein the cancer is colon cancer, lung cancer, liver cancer, breast cancer, prostate cancer, ovarian cancer, skin cancer, bone cancer, cancer of the cervix, vulva, head and neck region, anus, oropharynx, larynx, or pancreas; brain cancer, squamous cell carcinoma, melanoma, leukemia, or myeloma; or

ii) an infectious disease; preferably a viral infection, a bacterial infection, or a protozoal infection.

Paragraph 53. The effective amount for use, or the method, according to any one of paragraphs 45 to 52, wherein the cell cycle inhibitor arrests the cell cycle at one of following stages; G1/S, G2, G2/M, and S-phase.

Paragraph 54. The effective amount for use, or the method, according to any one of paragraphs 45 to 53, wherein the cell cycle inhibitor is selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as topotecan, irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole, paclitaxel), and a HDAC6 inhibitor (such as tubastatin A).

Paragraph 55. The effective amount for use, or the method, according to any one of paragraphs 45 to 54, wherein the immune checkpoint inhibitor blocks CTLA-4, PD-1, PD-L1, TIM3, TIGIT, VISTA or LAG-3.

Paragraph 56. The effective amount for use, or the method, according to any one of paragraphs 45 to 55, wherein the immune checkpoint inhibitor is an antibody, an siRNA or a small molecule.

Claims

1. An effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

2. An effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

3. An effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

4. An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor.

5. An effective amount of a vaccine for use in therapy, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

6. An effective amount of a transient cell cycle inhibitor for use in therapy, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

7. An effective amount of a modulator of an immune suppressive mechanism for use in therapy, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the therapy is selected from:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

8. An effective amount of a vaccine for use in the manufacture of a medicament, wherein the vaccine is prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the vaccine is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

9. An effective amount of a transient cell cycle inhibitor for use in the manufacture of a medicament, wherein the cell cycle inhibitor is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a modulator of an immune suppressive mechanism, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

10. An effective amount of a modulator of an immune suppressive mechanism for use in the manufacture of a medicament, wherein the modulator is prepared for simultaneous, separate or sequential administration with an effective amount of a vaccine, wherein the inhibitor is further prepared for simultaneous, separate or sequential administration with an effective amount of a transient cell cycle inhibitor, wherein the medicament is for:

a) treating and/or preventing an intracellular pathogenic infection or disease in a subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise the vaccine and the transient cell cycle inhibitor and the modulator of an immune suppressive mechanism.

11. The effective amount of a vaccine for use according to claim 5 or 8, or the effective amount of a transient cell cycle inhibitor for use according to claim 6 or 9, or the effective amount of a modulator of an immune suppressive mechanism for use according to claim 7 or 10, wherein the modulator of an immune suppressive mechanism is a chemotherapeutic agent.

12. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

the method comprising:

i) administering to the subject an effective amount of a vaccine; and,

ii) administering to the subject an effective amount of a transient cell cycle inhibitor,

wherein steps i) and ii) are separate, simultaneous or sequential, and in any order, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

13. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

the method comprising:

i) administering to the subject an effective amount of a vaccine;

ii) administering to the subject an effective amount of a transient cell cycle inhibitor; and

iii) administering to the subject an effective amount of a modulator of an immune suppressive mechanism,

wherein steps i), ii) and iii) are separate, simultaneous or sequential, and in any order.

14. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

the method comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

15. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor,

the method comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine, wherein the transient cell cycle inhibitor is not topotecan, paclitaxel or gemcitabine.

16. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

the method comprising administering to the subject an effective amount of a vaccine, wherein the subject is undergoing treatment with an effective amount of a transient cell cycle inhibitor and an effective amount of a modulator of an immune suppressive mechanism.

17. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first

anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

the method comprising administering to the subject an effective amount of a transient cell cycle inhibitor, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a modulator of an immune suppressive mechanism.

18. A method of:

a) treating and/or preventing an intracellular pathogenic infection or disease in the subject;

b) treating a benign tumor, pre-cancerous lesion, or non-metastatic cancer in a subject, or treating a subject with an inherited cancer syndrome; or

c) treating a subject characterised as disease free or having minimal residual disease (MRD) following treatment of a cancer with a first anticancer therapy, wherein the first anticancer therapy does not comprise a vaccine and a transient cell cycle inhibitor and a modulator of an immune suppressive mechanism,

the method comprising administering to the subject an effective amount of a modulator of an immune suppressive mechanism, wherein the subject is undergoing treatment with an effective amount of a vaccine and an effective amount of a transient cell cycle inhibitor.

19. The effective amount for use, or the method, according to any of the preceding claims, wherein the transient cell cycle inhibitor arrests the cell cycle at one of following stages: G1/S, G2, G2/M, or S-phase.

20. The effective amount for use, or the method, according to any one of the preceding claims, wherein the transient cell cycle inhibitor is selected from the group consisting of: a CDK inhibitor (such as RO-3306, dinaciclib, palbociclib), a topoisomerase I inhibitor (such as irinotecan), a G1/S phase inhibitor (such as thymidine), an inhibitor of microtubule dynamics (such as nocodazole), and a HDAC6 inhibitor (such as tubastatin A).

21. An effective amount of a transient cell cycle inhibitor for use in T-cell based immunotherapy, wherein the cell cycle inhibitor is for simultaneous, separate or sequential administration with an effective amount of an immune checkpoint inhibitor.

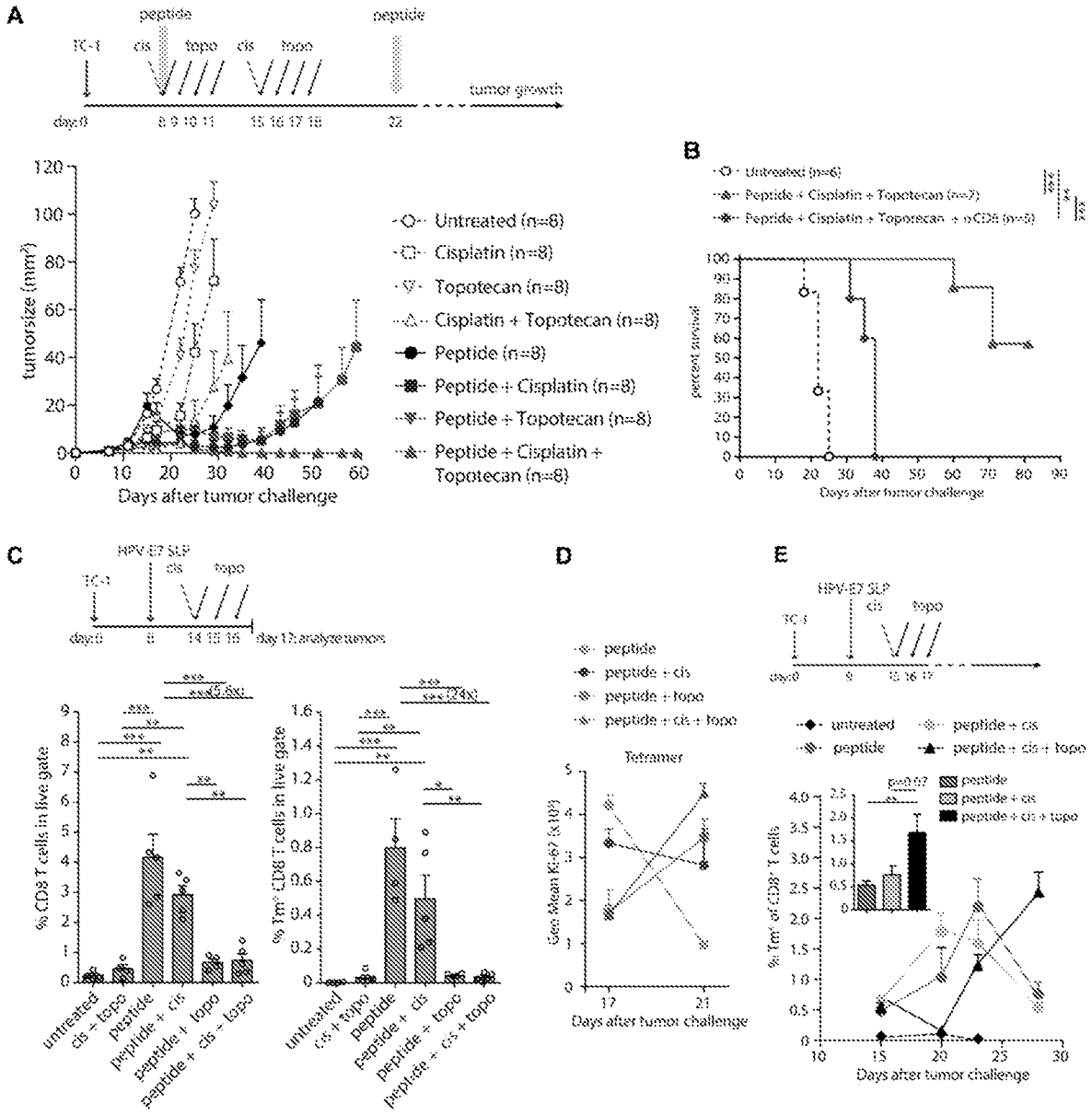

A
peptide
peptide
TC-1
cis
topo
cis
topo
tumor growth
day:0
8 9 10 11
15 16 17 18
22
tumorsize (mm²)
Days after tumor challenge
Untreated (n=8)
Cisplatin (n=8)
Topotecan (n=8)
Cisplatin + Topotecan (n=8)
Peptide (n=8)
Peptide + Cisplatin (n=8)
Peptide + Topotecan (n=8)
Peptide + Cisplatin + Topotecan (n=8)
B
Untreated (n=6)
Peptide + Cisplatin + Topotecan (n=7)
Peptide + Cisplatin + Topotecan + αCD8 (n=8)
percent survival
Days after tumor challenge
C
HPV-E7 SLP
TC-1
cis
topo
day:0
8
14 15 16
day 17: analyze tumors
% CD8 T cells in live gate
% Tm² CD8 T cells in live gate
untreated
cis + topo
peptide
peptide + cis
peptide + topo
peptide + cis + topo
D
peptide
peptide + cis
peptide + topo
peptide + cis + topo
Tetramer
Geo Mean Ki-67 (x10³)
Days after tumor challenge
E
HPV-E7 SLP
TC-1
cis
topo
day:0
9
15 16 17
untreated
peptide
peptide + cis
peptide + cis + topo
peptide
peptide + cis
peptide + cis + topo
% Tm² of CD8⁺ T cells
Days after tumor challenge

FIG. 1

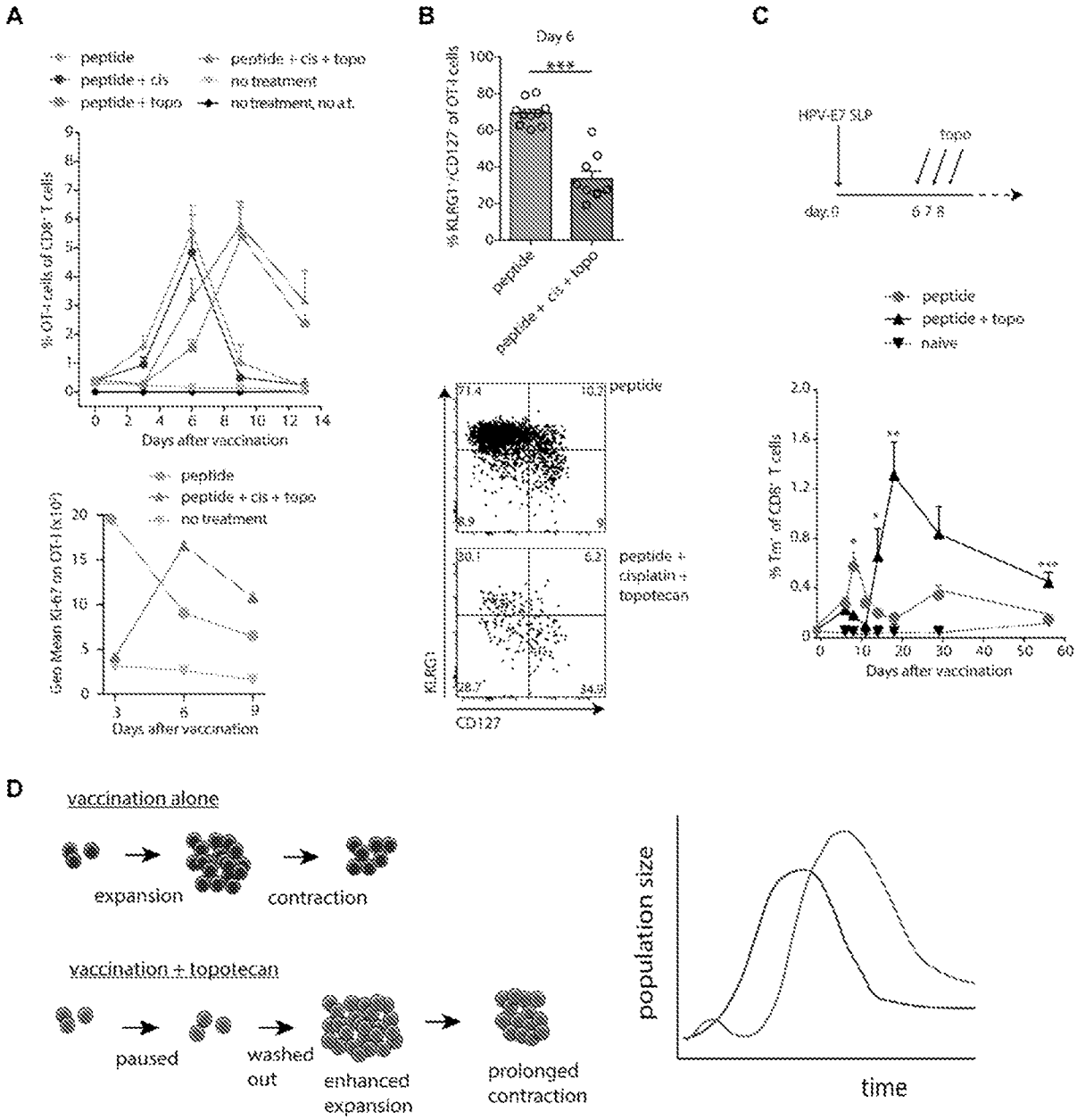

A
peptide
peptide + cis
peptide + topo
peptide + cis + topo
no treatment
no treatment, no a.t.
% OT-1 cells of CD8+ T cells
Days after vaccination
Geo Mean Ki-67 on OT-1 (x10³)
B
Day 6
***
% KLRG1+/CD127- of OT-1 cells
peptide
peptide + cis + topo
peptide
peptide + cisplatin + topotecan
KLRG1
CD127
C
HPV-E7 SLP
topo
day: 0
6 7 8
peptide
peptide + topo
naive
% Tm1 of CD8+ T cells
Days after vaccination
D
vaccination alone
expansion
contraction
vaccination + topotecan
paused
washed out
enhanced expansion
prolonged contraction
population size
time

FIG. 2

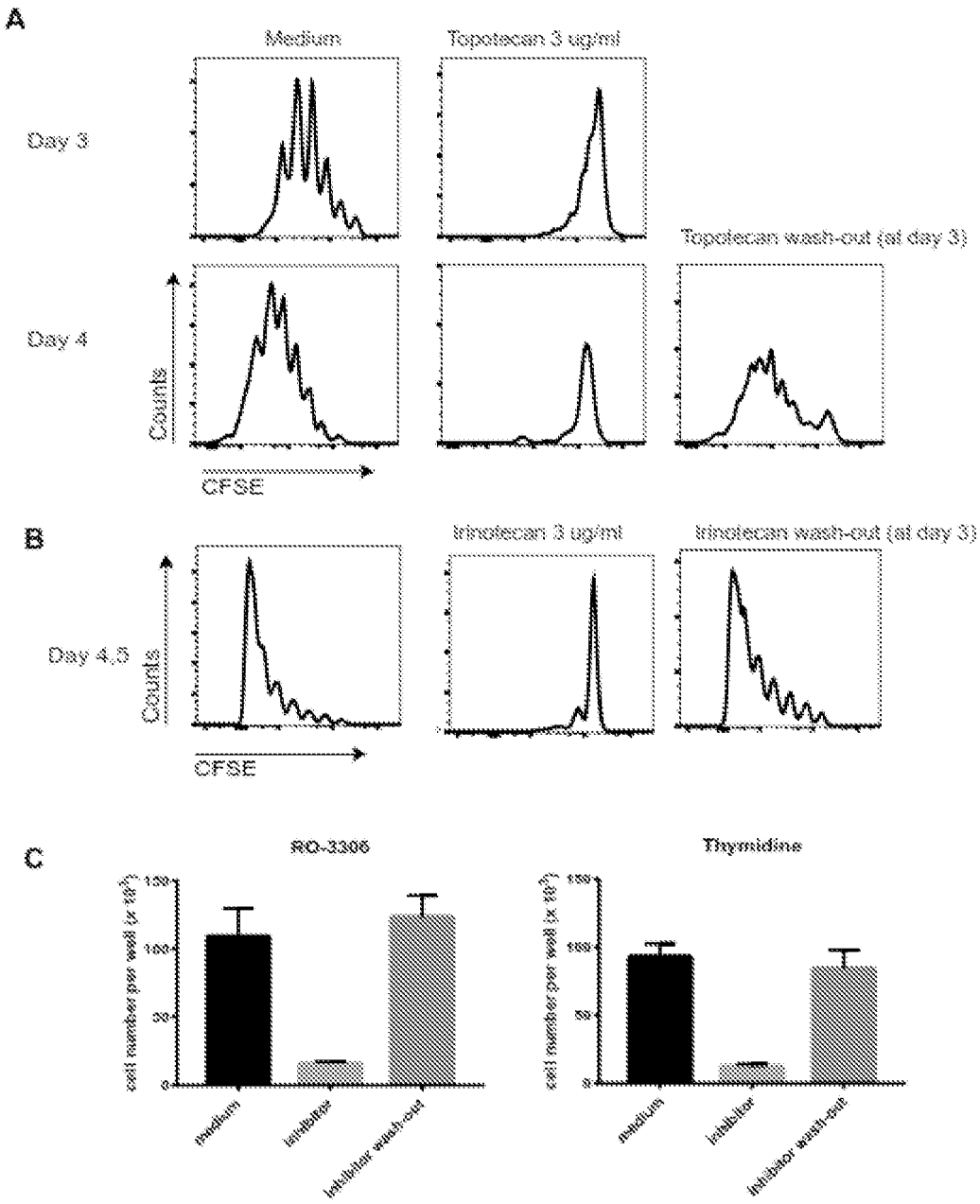

A
Medium
Topotecan 3 ug/ml
Topotecan wash-out (at day 3)
Day 3
Day 4
Counts
CFSE
B
Irinotecan 3 ug/ml
Irinotecan wash-out (at day 3)
Day 4,5
Counts
CFSE
C
RO-3306
Thymidine
cell number per well (x 10⁴)
medium
inhibitor
inhibitor wash-out

FIG. 3

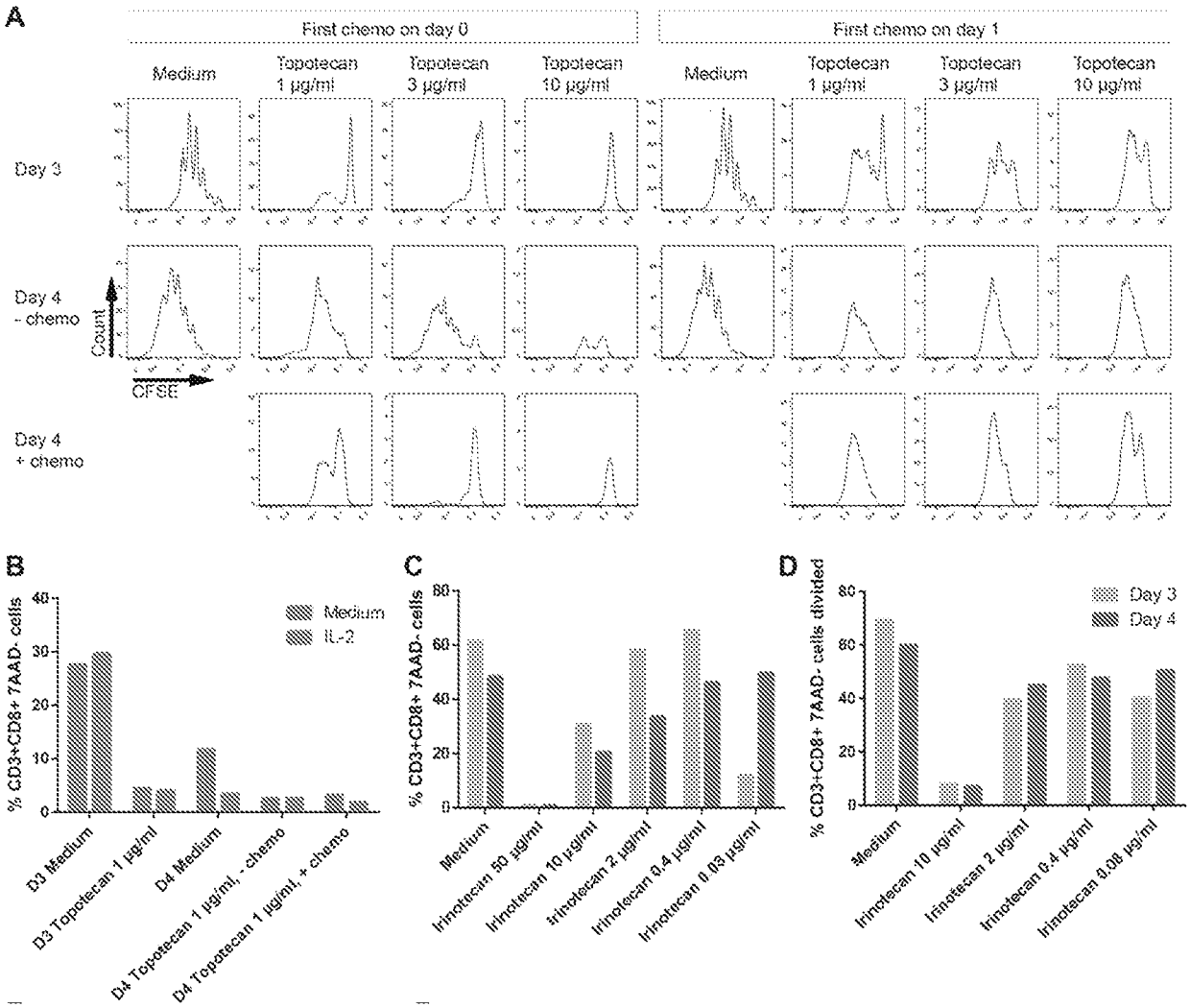
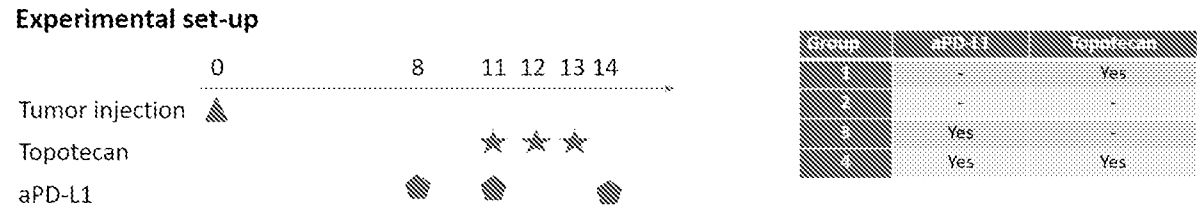
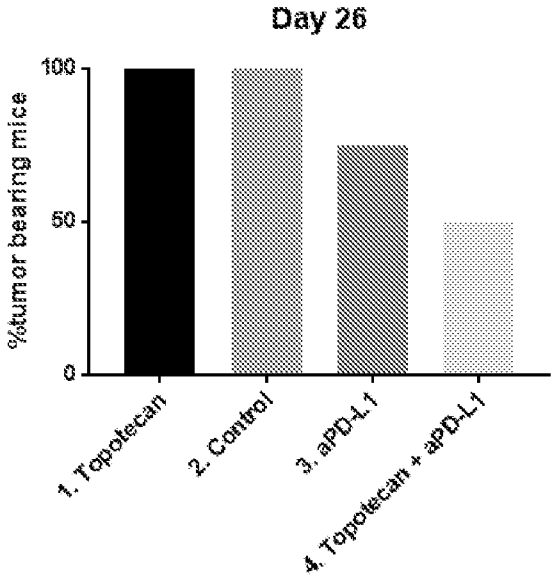

A
First chemo on day 0
First chemo on day 1
Medium
Topotecan 1 µg/ml
Topotecan 3 µg/ml
Topotecan 10 µg/ml
Day 3
Day 4 - chemo
Day 4 + chemo
Count
CFSE
B
% CD3+CD8+ 7AAD- cells
Medium
IL-2
D3 Medium
D3 Topotecan 1 µg/ml
D4 Medium
D4 Topotecan 1 µg/ml, - chemo
D4 Topotecan 1 µg/ml, + chemo
C
% CD3+CD8+ 7AAD- cells
Medium
Irinotecan 50 µg/ml
Irinotecan 10 µg/ml
Irinotecan 2 µg/ml
Irinotecan 0.4 µg/ml
Irinotecan 0.08 µg/ml
D
% CD3+CD8+ 7AAD- cells divided
Day 3
Day 4
Medium
Irinotecan 10 µg/ml
Irinotecan 2 µg/ml
Irinotecan 0.4 µg/ml
Irinotecan 0.08 µg/ml

FIG. 4

A


Experimental set-up
0
8
11 12 13 14
Tumor injection
Topotecan
aPD-L1
Group
aPD-L1
Topotecan
1
-
Yes
2
-
-
3
Yes
-
4
Yes
Yes

B


Day 26
%tumor bearing mice
100
50
0
1. Topotecan
2. Control
3. aPD-L1
4. Topotecan + aPD-L1

C

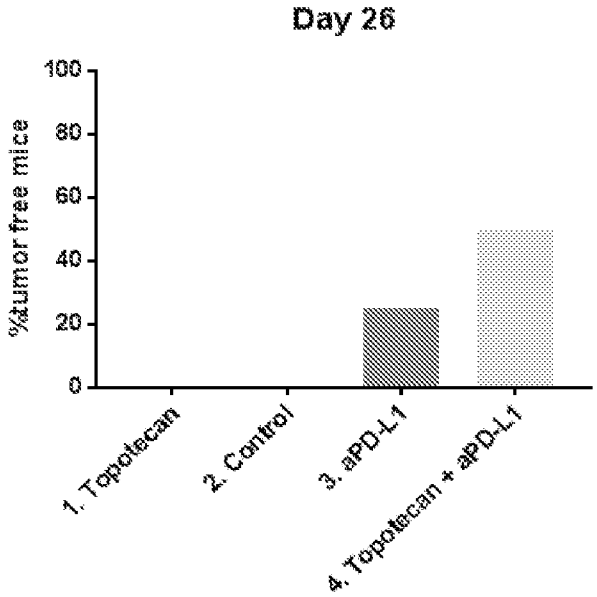

Day 26
%tumor free mice
100
80
60
40
20
0
1. Topotecan
2. Control
3. aPD-L1
4. Topotecan + aPD-L1

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2018/050601

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61K39/00
ADD.

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)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H. M. LEDERMAN ET AL: "Immunologic Effects of Hydroxyurea in Sickle Cell Anemia", PEDIATRICS, vol. 134, no. 4, 1 September 2014 (2014-09-01), pages 686-695, XP055536996, ISSN: 0031-4005, DOI: 10.1542/peds.2014-0571 abstract; figure 1 ----- -/--	1-20

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"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
20 December 2018	15/01/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	van Heusden, Miranda

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E. BEYRANVAND NEJAD ET AL: "Tumor Eradication by Cisplatin Is Sustained by CD80/86-Mediated Costimulation of CD8+ T Cells", CANCER RESEARCH, vol. 76, no. 20, 28 August 2016 (2016-08-28), pages 6017-6029, XP055537015, US ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-16-0881	1-4,11, 12,14, 15,19,20
Y	page 6024, right-hand column, paragraphs 2,3	5-10,13, 16-18

X	KIM H S ET AL: "Dendritic cell vaccine in addition to FOLFIRI regimen improve antitumor effects through the inhibition of immunosuppressive cells in murine colorectal cancer model", VACCINE, ELSEVIER, AMSTERDAM, NL, vol. 28, no. 49, 16 November 2010 (2010-11-16), pages 7787-7796, XP027491390, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2010.09.046 [retrieved on 2010-09-28] abstract page 7788, right-hand column, paragraph 3 page 7787, left-hand column, paragraph 1	1-20

X	TORU MASUZAWA ET AL: "Phase I/II study of S-1 plus cisplatin combined with peptide vaccines for human vascular endothelial growth factor receptor 1 and 2 in patients with advanced gastric cancer", INTERNATIONAL JOURNAL OF ONCOLOGY, vol. 41, no. 4, 25 July 2012 (2012-07-25), pages 1297-1304, XP055408095, GR ISSN: 1019-6439, DOI: 10.3892/ijo.2012.1573	1-4,11, 12,14, 15,19,20
Y	abstract	5-10,13, 16-18

X	WO 2013/134467 A1 (H LEE MOFFITT CANCER CT & RES [US]; UNIV ILLINOIS [US]) 12 September 2013 (2013-09-12)	1-4,11, 12,14, 15,19,20
Y	page 26, line 35 - page 27, line 24; figures 17,27,28 page 23, line 9 - page 24, line 4	5-10,13, 16-18

	-/--	

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MAARTEN SWART ET AL: "Combination Approaches with Immune-Checkpoint Blockade in Cancer Therapy", FRONTIERS IN ONCOLOGY, vol. 6, 1 November 2016 (2016-11-01), XP055437816, DOI: 10.3389/fonc.2016.00233 the whole document	5-10,13, 16-18
Y	WHAY-KUANG CHIA ET AL: "Adoptive T-cell Transfer and Chemotherapy in the First-line Treatment of Metastatic and/or Locally Recurrent Nasopharyngeal Carcinoma", MOLECULAR THERAPY, vol. 22, no. 1, 17 October 2013 (2013-10-17), pages 132-139, XP055329241, US ISSN: 1525-0016, DOI: 10.1038/mt.2013.242 abstract	21
Y	R. HOUOT ET AL: "T-cell-based Immunotherapy: Adoptive Cell Transfer and Checkpoint Inhibition", CANCER IMMUNOLOGY RESEARCH, vol. 3, no. 10, 1 October 2015 (2015-10-01), pages 1115-1122, XP055437818, US ISSN: 2326-6066, DOI: 10.1158/2326-6066.CIR-15-0190 page 1119, right-hand column, paragraph 2 - page 1120, right-hand column; figure 2	21
Y	ARINA AINHOA ET AL: "Enhancing T cell therapy by overcoming the immunosuppressive tumor microenvironment", SEMINARS IN IMMUNOLOGY, vol. 28, no. 1, 10 February 2016 (2016-02-10), pages 54-63, XP029535361, ISSN: 1044-5323, DOI: 10.1016/J.SMIM.2016.01.002 abstract page 55, left-hand column section 3; page 56, right-hand column - page 57, right-hand column; figure 1	21
A,P	WO 2018/106729 A1 (G1 THERAPEUTICS INC [US]) 14 June 2018 (2018-06-14) page 34, line 16 - page 35, line 18	1-21

-/--

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	ELENI PANAGIOTI ET AL: "Features of Effective T Cell-Inducing Vaccines against Chronic Viral Infections", FRONTIERS IN IMMUNOLOGY, vol. 9, 16 February 2018 (2018-02-16), XP055537046, DOI: 10.3389/fimmu.2018.00276 the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2018/050601

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013134467 A1	12-09-2013	AU 2013230881 A1	30-10-2014
		CA 2866707 A1	12-09-2013
		EP 2822926 A1	14-01-2015
		JP 6233812 B2	22-11-2017
		JP 2015510886 A	13-04-2015
		WO 2013134467 A1	12-09-2013
WO 2018106729 A1	14-06-2018	NONE	