(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 3 565 547 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
13.03.2024 Bulletin 2024/11

(21) Application number: **18700861.0**

(22) Date of filing: **05.01.2018**

(51) International Patent Classification (IPC):
A61K 31/337 *(2006.01)* ***A61K 31/519*** *(2006.01)*
A61K 45/06 *(2006.01)* ***A61P 35/00*** *(2006.01)*

(52) Cooperative Patent Classification (CPC):
(C-Sets available)
A61K 31/337; A61K 31/519; A61P 35/00; A61P 43/00 (Cont.)

(86) International application number:
PCT/EP2018/050298

(87) International publication number:
WO 2018/127575 (12.07.2018 Gazette 2018/28)

(54) COMBINATION OF A MCL-1 INHIBITOR AND A TAXANE COMPOUND, USES AND PHARMACEUTICAL COMPOSITIONS THEREOF

KOMBINATION VON EINEM MCL-1 MIT EINEM TAXAN-VERBINDUNG, IHRE VERWENDUNG UND PHARMAZEUTISCHE ZUSAMMENSETZUNGEN DARAUS

COMBINAISON D'UN INHIBITEUR DE MCL-1 AVEC COMPOSE DU TAXANE, UTILISATIONS ET COMPOSITIONS PHARMACEUTIQUES ASSOCIÉES

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
Designated Validation States:
MA MD TN

(30) Priority: **06.01.2017 US 201762443082 P**
24.02.2017 EP 17157779
30.06.2017 US 201762527360 P

(43) Date of publication of application:
13.11.2019 Bulletin 2019/46

(73) Proprietors:
- **Les Laboratoires Servier**
  92284 Suresnes Cedex (FR)
- **Novartis AG**
  4056 Basel (CH)

(72) Inventors:
- **PORTER, Dale**
  Cambridge, Massachusetts MA 02139 (US)
- **HALILOVIC, Ensar**
  Cambridge, Massachusetts MA 02139 (US)
- **CHANRION, Maïa**
  92130 Issy les Moulineaux (FR)
- **MARAGNO, Ana Leticia**
  78290 Croissy-sur-Seine (FR)
- **GENESTE, Olivier**
  92500 Rueil Malmaison (FR)
- **MERINO, Delphine**
  Fitzroy, Victoria 3065 (AU)
- **WHITTLE, James**
  Fitzroy North, Victoria 3068 (AU)
- **VAILLANT, François**
  Geelong West, Victoria 3218 (AU)
- **VISVADER, Jane**
  Kew, Victoria 3101 (AU)
- **LINDEMAN, Geoffrey**
  Kew, Victoria 3101 (AU)
- **LESSENE, Guillaume**
  Bundoora, Victoria 3083 (AU)
- **MARANGONI, Elisabetta**
  75014 Paris (FR)

Processed by Luminess, 75001 PARIS (FR)

(Cont. next page)

(74) Representative: **Bernad, Stéphane**
Les Laboratoires Servier
35, rue de Verdun
92284 Suresnes Cedex (FR)

(56) References cited:
EP-A1- 2 886 545 **WO-A1-2015/097123**
WO-A1-2016/207216 **WO-A1-2016/207217**
WO-A1-2016/207226 **WO-A1-2017/125224**
US-A1- 2015 352 097

- **TING SONG ET AL: "Mechanism of synergy of BH3 mimetics and paclitaxel in chronic myeloid leukemia cells: Mcl-1 inhibition", EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES., vol. 70, 14 January 2015 (2015-01-14), pages 64-71, XP055393531, NL ISSN: 0928-0987, DOI: 10.1016/j.ejps.2015.01.003**
- **DATABASE WPI Week 201654 Thomson Scientific, London, GB; AN 2016-12691Q XP002772476, -& CN 105 311 016 A (CAS DALIAN CHEM & PHYSICAL INST) 10 February 2016 (2016-02-10)**
- **Liu: "MicroRNA-101 inhibits cell progression and increases paclitaxel sensitivity by suppressing MCL-1 expression in human triplenegative breast cancer", Oncotarget, Vol. 6, No. 24,20070-20083, 8 May 2015 (2015-05-08), XP055393625, Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4652988/pdf/oncotarget-06-20070.pdf [retrieved on 2017-07-25]**
- **SHUTARO HABATA ET AL: "BAG3-mediated Mcl-1 stabilization contributes to drug resistance via interaction with USP9X in ovarian cancer", INTERNATIONAL JOURNAL OF ONCOLOGY, 21 April 2016 (2016-04-21), XP055393544, GR ISSN: 1019-6439, DOI: 10.3892/ijo.2016.3494**

(52) Cooperative Patent Classification (CPC): (Cont.)

C-Sets
A61K 31/337, A61K 2300/00;
A61K 31/519, A61K 2300/00

Description

FIELD OF THE INVENTION [CLEAN]

[0001] The present invention relates to a combination of a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid and a taxane compound which is paclitaxel or docetaxel. The invention also relates to the use of said combination in the treatment of breast and lung cancers. Also provided are pharmaceutical formulations suitable for the administration of such combinations.

BACKGROUND OF THE INVENTION

[0002] Cancer is characterized by uncontrolled cell proliferation. Antimitotic agents and antimicrotubule agents have been explored for cancer therapy because of their important effect in the cell division. Inhibition of the mitotic machinery results in a diverse array of outcomes, primarily leading to cell cycle arrest and cell death. Antimicrotubule agents, such as taxanes are currently being used in clinical setting. For example, paclitaxel and docetaxel have a similar spectrum of clinical activity including ovarian, lung, breast, bladder, and prostate cancers. Taxanes are anti-mitotic agents that bind to tubulin and inhibit microtubule depolymerization thereby disrupting the normal equilibrium involved in microtubule assembly and deconstruction and therefore impair microtubule functioning. Microtubules are essential to cell division and cells exposed to taxanes can fail to divide. Cell cycle arrest after treatment with taxanes may eventually result in cell death due to unsuccessful mitosis. Despite the advances in anticancer therapy, there exists a long-felt need for more effective therapies with limited toxicities. Indeed, the toxicities associated with paclitaxel and docetaxel include neutropenia as the major dose limiting toxicity, along with significant peripheral neuropathy. In fact, dose reductions are frequent in heavily pretreated patients to mitigate the severity of these toxicities. On top of that, the development of resistance to taxanes also limits its use in the clinic.

[0003] Apoptosis is a highly regulated cell death pathway that is initiated by various cytotoxic stimuli, including oncogenic stress and chemotherapeutic agents. It has been shown that evasion of apoptosis is a hallmark of cancer and that efficacy of many chemotherapeutic agents is dependent upon the activation of the intrinsic mitochondrial pathway. Three distinct subgroups of the BCL-2 family proteins control the intrinsic apoptosis pathway: (i) the pro-apoptotic BH3 (the Bcl-2 homology 3)-only proteins; (ii) the pro-survival members such as BCL-2 itself, BCL-xL, BCL-W, MCL-1 and BCL-2A1; and (iii) the pro-apoptotic effector proteins BAX and BAK (Czabotar et al., Nature Reviews Molecular Cell Biology 2014, 15, 49-63). Overexpression of the anti-apoptotic members of BCL-2 family is observed in many cancers, (Adams and Cory, Oncogene 2007, 26, 1324-1337) and the pharmacological inhibition of the anti-apoptotic proteins by BH3-mimetics drugs such as ABT-199 (venetoclax), ABT-263 (navitoclax) and S63845 has emerged as a therapeutic strategy to induce apoptosis and cause tumor regression in cancer (Zhang et al., Drug Resist. Updat. 2007, 10, 207-217; Kotschy et al., Nature 2016, 538, 477-482). Nevertheless, mechanisms of resistance to BH3 mimetics have been observed (Choudhary et al., Cell Death and Disease 2015, 6, e1593) and the use of combination therapies could improve efficacy and delay or even abrogate resistance development. For example, a combination with S1, a pan Bcl-2/Bcl-XL/Mcl-1 inhibitor, and paclitaxel produces a synergistic effect on CML-derived cell lines (Song et al., European J. Pharm. Sc. 2015, 70, 64-71).

[0004] Breast cancer is a heterogeneous disease and can be stratified into at least five major subgroups based on gene expression profiling: Luminal A, Luminal B (estrogen positive, ER^+), HER2-amplified, basal-like (predominantly triple negative breast cancer or TNBC) and normal-like (Curtis et al., Nature 2012, 486, 346-352; Perou et al., Nature 2000, 406, 747-752). These subtypes generally predict clinical behavior with respect to response and resistance to therapy, patterns of metastasis, and overall survival. Multiple mechanisms contribute to tumor progression and resistance to cancer therapy, including the evasion of cell death due to deregulation of the balance between anti- and pro-apoptotic members of the BCL2 family (Merino et al., Oncogene 2016, 35, 1877-1887; Beroukim et al., Nature 2010, 463, 899-905; Wertz et al., Nature 2011, 471, 110-114; Goodwin et al., Cell Death Differ. 2015). In the triple-negative breast cancer subgroup, residual disease after neo-adjuvant therapy is associated with higher risk of metastatic recurrence compared to patients achieving a pathological complete response (Liedtke et al., J. Clin. Oncol. 2008, 26(8), 1275-1281). About 70 % of TNBC patients do not achieve complete response after neo-adjuvant chemotherapy (such as paclitaxel or docetaxel) and suffer a dramatically worse outcome, with a higher probability of metastatic relapse and a 3-year overall survival of only 60-70 %.

[0005] Lung cancer is classified into non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), and carcinoid. Approximately 80 % of all lung cancers correspond to NSCLC, which can be further classified into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Lung cancer is the most common cause of cancer-related death in

men and second most common in women after breast cancer. Although lung cancer therapy has evolved significantly with the appearance of targeted therapy, there is still a strong need to develop novel therapies in order to improve treatment efficacy, reduce side effects and avoid appearance of resistance (Rothschild Cancers 2015, 7(2), 930-949).

[0006] The present invention provides a novel combination of a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{ [1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl 1-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid and a taxane compound which is paclitaxel or docetaxel. The results show that with the association of potent small molecules targeting MCL-1 with antimicrotubule agents is highly synergistic in breast and lung cancer cell lines (Figures 1 to 7; Tables 1 and 4). We also show that combined disrupting microtubule function and MCL-1 targeting *in vivo* is efficacious at tolerated doses in breast cancer PDX (Patient Derived Xenograft) models (Figures 8 and 9) and at different doses in female nude rats bearing MDA-MB-231 xenograft, a model of triple negative breast cancer (Figures 10 to 14). The positive combination was also observed on different PDX models of TNBC using different schedules of administration (Figure 15). The synergistic effect of targeting MCL-1 and disrupting microtubule function *in vitro* and *in vivo* at tolerated doses have been demonstrated through combination of potent small molecule inhibitors. Finally, residual tumor analysis after neo-adjuvant treatment is a major and under-explored field to study chemoresistance and, thus, we perform experiments in PDX models resistant to chemotherapy showing that the combination of a MCL-1 inhibitor and a taxane compound is efficient (Example 9).

SUMMARY OF THE INVENTION

[0007] The present invention relates to a combination for use in the treatment of breast cancer or lung cancer comprising:

(a) a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid,

and (b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate use.

[0008] Said Mcl-1 inhibitors, their synthesis, their use in the treatment of cancer and pharmaceutical formulations thereof, are described in WO 2015/097123, WO 2016/207216, WO 2016/207217, WO 2016/207225, WO 2016/207226 and WO 2017/125224.

[0009] In another embodiment, the invention provides a combination for use in the treatment of breast cancer or lung cancer comprising:

(a) Compound 1: (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl) ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid, or a pharmaceutically acceptable salt thereof, and
(b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate use.

[0010] Alternatively, the invention provides a combination for use in the treatment of breast cancer or lung cancer comprising:

(a) Compound 2: (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl) ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid, or a pharmaceutically acceptable salt thereof, and
(b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate use.

[0011] In another embodiment, the invention provides a medicament containing, separately or together,

(a) a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid, and

(b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate administration, and wherein the MCL-1 inhibitor and the taxane compound are provided in effective amounts for the treatment of breast cancer or lung cancer.

[0012] In another embodiment, the MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl] oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid (Compound 1).

[0013] In another embodiment, the MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid (Compound 2).

[0014] In another embodiment, the taxane compound is paclitaxel.

[0015] In another embodiment, the taxane compound is docetaxel.

BRIEF DESCRIPTION OF THE FIGURES

[0016]

Figure 1 illustrates matrices for inhibition, Loewe excess inhibition and growth inhibition for paclitaxel combinations with Compound 1 in representative MDA-MB-453 breast cancer cell line.

Figure 2 illustrates matrices for inhibition, Loewe excess inhibition and growth inhibition for paclitaxel combinations with Compound 1 in representative MDA-MB-468 breast cancer cell line.

Figure 3 illustrates matrices for inhibition, Loewe excess inhibition and growth inhibition for paclitaxel combinations with Compound 1 in representative H522 lung cancer cell line.

Figure 4 illustrates exemplary cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 2 in combination with paclitaxel in the breast cancer cell line MDA-MB-453 in two independent experiments. Values in the effect matrix range from 0 (no inhibition) to 100 (total inhibition). Values in the synergy matrix represent the extent of growth inhibition in excess of the theoretical additivity calculated based on the single agent activities of Compound 2 and paclitaxel at the concentrations tested.

Figure 5 illustrates exemplary cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 2 in combination with paclitaxel in the lung cancer cell line H522 in two independent experiments. Values in the effect matrix range from 0 (no inhibition) to 100 (total inhibition). Values in the synergy matrix represent the extent of growth inhibition in excess of the theoretical additivity calculated based on the single agent activities of Compound 2 and Paclitaxel at the concentrations tested.

Figure 6 illustrates synergistic effect of Compound 2 with docetaxel. SK-BR-3 cells were treated with docetaxel (2 nM), or left untreated in the presence of Compound 2 (30 nM), with or without QVD (10 μM) for 72 hours before viability analysis with propidium iodide (PI) staining. Results are presented as a percent of untreated cells and represent 3-5 independent experiments.

Figure 7 illustrates synergistic effect of Compound 2 with docetaxel. SK-BR-3 cells were treated with increasing concentrations of Compound 2 and docetaxel for 72 hours, then subjected to viability assays using Cell Titer Glo followed by BLISS score analysis. BLISS synergy values are > 0.0 on vertical axis.

Figure 8 illustrates efficacy of Compound 2 with docetaxel in improving of animal survival in TNBC PDX models. Kaplan-Meier survival curves of mice bearing 110T (n = 10-12 mice per arm), 838T (n = 12 mice per arm), or PDX OD-BRE-0589 (8 mice per arm) treated with vehicle alone (black line), docetaxel (10 mg/kg i.p. on day 0 and 21) plus vehicle for Compound 2 (dark grey line), Compound 2 (25 mg/kg i.v. once weekly for 6 weeks) plus vehicle for docetaxel (light grey line), or combined docetaxel and Compound 2 (dotted line).

Right panels: individual tumor volume curves. The PDX 838T model was terminated at 120 days due to age related illness, independent of therapy. Log rank (Mantel-Cox) p value is showing for combination therapy versus docetaxel alone.

Figure 9 illustrates the maintaining of normal body weight during therapy. NOD SCID IL2 gamma receptor knockout mice were treated with docetaxel (15 mg/kg, once i.p.) and Compound 2 at 25 or 50 mg/kg (3 mice per group, i.v. injections, once per week for 3 weeks). Their body weight was monitored three times per week for 3 weeks. Compound 2 therapy as single agent or combined with docetaxel was well tolerated.

Figure 10 illustrates efficacy of paclitaxel and Compound 1 (named as Compound A in the Figure) alone and in combination in female nude rats bearing MDA-MB-231 xenografts, a model of TNBC. Tumors were established in female nude rats by subcutaneous inoculation of human TNBC MDA-MB-231 cells (1 x 10^7 cells/200 μL HBSS/Matrigel 1:1 v/v). Animals with appropriate size tumors were randomized into groups (n = 7-8) with a mean tumor volume about 400 mm^3. Tumor volumes were estimated using the two largest diameters according to (L x W^2 x π/6) and body weights were measured 2-3 times per week. Data are presented as means ± SEM or as individual tumor volumes. * $p < 0.05$, compared with vehicle group (one way ANOVA with post hoc Dunnett's test).

Figure 11 illustrates tolerability of paclitaxel and Compound 1 (named as Compound A in the Figure) alone and in combination in female nude rats bearing MDA-MB-231 xenografts, a model of TNBC. Tumors were established in female nude rats by subcutaneous inoculation of human TNBC MDA-MB-231 cells (1 x 10^7 cells/200 μL HBSS/Matrigel 1:1 v/v). Animals with appropriate size tumors were randomized into groups (n = 7-8) with a mean tumor volume about 400 mm^3. Tumor volumes were estimated using the two largest diameters according to (L x W^2 x π/6) and body weights were measured 2-3 times per week.

Figure 12 illustrates efficacy of paclitaxel and Compound 1 (named as Compound A in the Figure) alone and in combination at different doses schedules in female nude rats bearing MDA-MB-231 xenografts, a model of TNBC. Tumors were established in female nude rats by subcutaneous inoculation of human TNBC MDA-MB-231 cells (1 x 10^7 cells/200 μL HBSS/Matrigel 1:1 v/v). Animals with appropriate size tumors were randomized into groups (n = 7-8) with a mean tumor volume about 400 mm^3. Tumor volumes were estimated using the two largest diameters according to (L x W^2 x π/6) and body weights were measured 2-3 times per week. Data are presented as means ± SEM or as individual tumor volumes. * $p < 0.05$, compared with vehicle group (one way ANOVA with post hoc Dunnett's test).

Figure 13 illustrates tolerability of paclitaxel and Compound 1 (named as Compound A in the Figure) alone and in combination at different doses schedules in female nude rats bearing MDA-MB-231 xenografts, a model of TNBC. Tumors were established in female nude rats by subcutaneous inoculation of human TNBC MDA-MB-231 cells (1 x 10^7 cells/200 μL HBSS/Matrigel 1:1 v/v). Animals with appropriate size tumors were randomized into groups (n = 7-8) with a mean tumor volume about 400 mm^3. Tumor volumes were estimated using the two largest diameters according to (L x W^2 x π/6) and body weights were measured 2-3 times per week.

Figure 14 illustrates synergistic effect of Compound 1 with docetaxel.

Figure 15 illustrates antitumor activity of docetaxel and Compound 1 administrated IV alone and in combination in female SCID mice bearing patient derived TNBC model. Docetaxel was administrated first followed either 30 minutes or 72 hours later with Compound 1.

DETAILED DESCRIPTION OF THE INVENTION

[0017] The invention therefore provides in Embodiment E1, a combination for use in the treatment of breast cancer or lung cancer comprising:

(a) a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid,

and (b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate use.

[0018] Further enumerated embodiments (E) of the invention are described herein. It will be recognized that features specified in each embodiment may be combined with other specified features to provide further embodiments of the present invention.

[0019] E2. A combination according to E1, wherein the MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl] methoxy }phenyl)propanoic acid.

[0020] E3. A combination according to E1, wherein the MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy} phenyl)propanoic acid.

[0021] E4. A combination according to E1, wherein the taxane compound is paclitaxel.

[0022] E5. A combination according to E1, wherein the taxane compound is docetaxel.

[0023] E6. A combination according to E1, wherein MCL-1 inhibitor is (2*R*)-2-{ [(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy} phenyl)propanoic acid and the taxane compound is paclitaxel.

[0024] E7. A combination according to E1, wherein MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl] methoxy}phenyl)propanoic acid and the taxane compound is paclitaxel.

[0025] E8. A combination according to E1, wherein MCL-1 inhibitor is (2*R*)-2- {[(5S_a)-5-{ 3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy} phenyl)propanoic acid and the taxane compound is docetaxel.

[0026] E9. A combination according to E1, wherein MCL-1 inhibitor is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl) thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl] methoxy}phenyl)propanoic acid and the taxane compound is docetaxel.

[0027] E10. A combination according to E3, wherein the dose of (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*] pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl) propanoic acid during the combination treatment is from 25 mg to 1500 mg.

[0028] E11. A combination according to E3 or E10, wherein (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*] pyrimidin-4-yl]oxy}-3-(2-{[2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl) propanoic acid is administered during the combination treatment once a week.

[0029] E12. A combination according to any of E1 to E11, wherein the MCL-1 inhibitor is administered orally and the taxane compound is administered intravenously.

[0030] E13. A combination according to any of E1 to E11, wherein the MCL-1 inhibitor and the taxane compound are administered intravenously.

[0031] E14. The combination according to E1, wherein the breast cancer is triple negative breast cancer, particularly chemoresistant triple negative breast cancer, more particularly, triple negative breast cancer resistant to taxane therapy.

[0032] E15. The combination according to E1, wherein the lung cancer is non-small cell lung cancer or small cell lung cancer.

[0033] E16. A medicament containing, separately or together,

(a) a MCL-1 inhibitor as defined in E1, and
(b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate administration, and wherein the MCL-1 inhibitor and the taxane compound are provided in effective amounts for the treatment of breast cancer or lung cancer.

[0034] 'Combination' refers to either a fixed dose combination in one unit dosage form (e.g., capsule, tablet, or sachet), non-fixed dose combination, or a kit of parts for the combined administration where a compound of the present invention and one or more combination partners (e.g. another drug as explained below, also referred to as 'therapeutic agent' or 'coagent') may be administered independently at the same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, e.g. synergistic effect.

[0035] The terms 'co-administration' or 'combined administration' or the like as utilized herein are meant to encompass administration of the selected combination partner to a single subject in need thereof (*e.g.* a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

[0036] The term 'fixed dose combination' means that the active ingredients, e.g. a compound of formula (I) and one or more combination partners, are both administered to a patient simultaneously in the form of a single entity or dosage.

[0037] The term 'non-fixed dose combination' means that the active ingredients, e.g. a compound of the present invention and one or more combination partners, are both administered to a patient as separate entities either simultaneously or sequentially, with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient. The latter also applies to cocktail therapy, e.g. the administration of three or more active ingredients.

[0038] 'Cancer' means a class of disease in which a group of cells display uncontrolled growth. Cancer types include

solid tumors including carcinoma, sarcoma, or blastoma. In particular 'cancer' refers to breast and lung cancer.

[0039] The term 'jointly therapeutically effective' means that the therapeutic agents may be given separately (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals that they prefer, in the warm-blooded animal, especially human, to be treated, still show a (preferably synergistic) interaction (joint therapeutic effect). Whether this is the case can, *inter alia,* be determined by following the blood levels, showing that both compounds are present in the blood of the human to be treated at least during certain time intervals.

[0040] 'Synergistically effective' or 'synergy' means that the therapeutic effect observed following administration of two or more agents is greater than the sum of the therapeutic effects observed following the administration of each single agent.

[0041] As used herein, the term 'treat', 'treating' or 'treatment' of any disease or disorder refers in one embodiment, to ameliorating the disease or disorder (*i.e.,* slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment 'treat', 'treating' or 'treatment' refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, 'treat', 'treating' or 'treatment' refers to modulating the disease or disorder, either physically, (*e.g.,* stabilization of a discernible symptom), physiologically, (*e.g.,* stabilization of a physical parameter), or both.

[0042] As used herein, a subject is 'in need of a treatment if such subject would benefit biologically, medically or in quality of life from such treatment.

[0043] In another aspect that is not claimed, provided is a method for sensitizing a human who is (i) refractory to at least one chemotherapy treatment, or (ii) in relapse after treatment with chemotherapy, or both (i) and (ii), wherein the method comprises administering a MCL-1 inhibitor in combination with a taxane compound, as described herein, to the patient. A patient who is sensitized is a patient who is responsive to the treatment involving administration of a MCL-1 inhibitor in combination with a taxane compound, as described herein, or who has not developed resistance to such treatment.

[0044] 'Medicament' means a pharmaceutical composition, or a combination of several pharmaceutical compositions, which contains one or more active ingredients in the presence of one or more excipients.

[0045] In the pharmaceutical compositions according to the invention, the proportion of active ingredients by weight (weight of active ingredients over the total weight of the composition) is from 5 to 50 %.

[0046] Among the pharmaceutical compositions according to the invention there will be more especially used those which are suitable for administration by the oral, parenteral and especially intravenous, per- or trans-cutaneous, nasal, rectal, perlingual, ocular or respiratory route, more specifically tablets, dragées, sublingual tablets, hard gelatin capsules, glossettes, capsules, lozenges, injectable preparations, aerosols, eye or nose drops, suppositories, creams, ointments, dermal gels etc.

[0047] The pharmaceutical compositions according to the invention comprise one or more pharmaceutically acceptable excipients or carriers selected from diluents, lubricants, binders, disintegration agents, stabilizers, preservatives, absorbents, colorants, sweeteners, flavorings etc.

[0048] By way of non-limiting example there may be mentioned:

- *as diluents:* lactose, dextrose, sucrose, mannitol, sorbitol, cellulose, glycerol,
- *as lubricants:* silica, talc, stearic acid and its magnesium and calcium salts, polyethylene glycol,
- *as binders:* magnesium aluminium silicate, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose and polyvinylpyrrolidone,
- *as disintegrants:* agar, alginic acid and its sodium salt, effervescent mixtures.

[0049] The compounds of the combination may be administered simultaneously or sequentially. The administration route is preferably the intravenous infusion or injection, and the corresponding pharmaceutical compositions may allow the instantaneous or delayed release of the active ingredients. The compounds of the combination may moreover be administered in the form of two separate pharmaceutical compositions, each containing one of the active ingredients, or in the form of a single pharmaceutical composition, in which the active ingredients are in admixture.

[0050] The useful dosage regimen varies according to the sex, age and weight of the patient, the administration route, the nature of the cancer and of any associated treatments and ranges from 25 mg to 1500 mg of MCL-1 inhibitor per week, more preferably from 50 mg to 1400 mg per week. The dose of the taxane compound will be the same as that used when it is administered on its own.

PHARMACOLOGICAL DATA

EXAMPLE 1: *In vitro* effect on proliferation of combining MCL-1 inhibitor (Compound 1) with paclitaxel in breast and lung cancer cell lines

[0051] The effect on proliferation of combining MCL-1 inhibitor (Compound 1) with paclitaxel was assessed in a panel of 19 breast cancer cell lines (BT-20, BT-474, BT-549, Cal-148, HCC1143, HCC1395, HCC1500, HCC1937, HCC1954, HCC38, HCC70, Hs 578T, MCF7, MDA-MB-157, MDA-MB-231, MDA-MB-436, MDA-MB-453, MDA-MB-468 and SK-BR-3) and 3 lung cancer cell line (H522, H23 and A549).

Material and method

[0052] Compounds were dissolved in 100 % DMSO (Sigma, Catalog #D2438-50ML) at a stock concentration of 10 mM and stored at -20 °C until use. Compounds were arrayed in 2 ml deep 96-well plates (Greiner bio-one, catalog number 780271) serially diluted 3-fold. Compound 1 was used over a concentration range of 0.0 - 10.0 μM. Paclitaxel was used over a concentration range of 0.0 - 1.0 μM in breast cancer cells and 0.0 - 2.0 μM range in lung cancer cells.

[0053] All cell lines were purchased from the American Type Culture Collection and cultured according to vendor recommendations. All lines were supplemented with 10 % FBS (GIBCO, Catalog number 10099-141). All cell lines were determined to be free of mycoplasma contamination by a PCR detection assay performed at Idexx Radii (Columbia, MO, USA) and authenticated by SNP analysis. Cells were thawed from frozen stocks, expanded through ≥ 1 passage and grown at 37 °C in 5 % CO_2. Cells were expanded to T-75 flasks and assessed for viability using a Beckman-Coulter ViCell counter prior to plating. To split and expand cell lines, cells were dislodged from flasks using 0.25 % Trypsin-EDTA (Corning Costar, Catalog #25-053-CL).

[0054] Cell proliferation was measured in 72 hours CellTiter-Glo™ (CTG) assays and all results shown are the result of at least triplicate measurements. The cells were dispensed into tissue culture treated 96-well plates (Costar, catalog number 3904) with a final volume of 80 μL of medium and at density of 3000 cells per well. 16 to 24 hours after plating, 20 μL of each compound dilution series were transferred to plates containing the cells, resulting in compound concentration ranges stated above and a final DMSO concentration of 0.16 %. Additionally a day zero plate was assayed at this time using the CellTiter-Glo® Luminescent Cell Viability Assay, as described below. After 72 hours of compound treatment the effects of compounds on cell proliferation was determined using the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega, Catalog #G7573). This is a homogeneous method to determine the number of viable cells in culture based on quantitation of the ATP present, which signals the presence of metabolically active cells. The method is described in detail in the Technical Bulletin, TB288 Promega. Briefly, 100 μl of CTG Reagent was added to plates and plates were incubated for 20-30 minutes on an orbital shaker. Plates were then read on the Perkin Elmer Victor™ X4 plate reader.

[0055] The percent growth inhibition, excess inhibition and growth inhibition were calculated using Combo Module software using the Loewe synergy model (as described in Lehar et al., Nature Biotechnology 2009, 27(7), 659-66), which measures the effect on growth above what would be expected if two drugs behaved in a dose additive manner. Positive numbers represent areas of increasing synergy. The percentage of growth inhibition relative to DMSO is displayed in the panel labelled *"Inhibition".* The amount of inhibition in excess of the expected amount is in the panel labelled *"Loewe Excess Inhibition".* The amount of inhibition normalized to day zero is displayed in the panel labelled *"Growth Inhibition".* Concentrations of Compound 1 are shown along the bottom row from left to right and increasing concentrations of paclitaxel along the left most column from bottom to top. All remaining points in the grids display results from a combination of the two inhibitors that correspond to the single agent concentrations denoted on the two axes. Absolute IC_{50} was determined by finding the compound concentration where the calculated curve crosses the 50 % activity mark. Absolute IC_{50} and synergy score were calculated in Combo module software as described in Lehar et al. 2009.

Synergy Score

[0056]

SS ~ 0 → Dose Additive
SS >2 → Synergy
SS >1 → Weak Synergy

Table 1. Single agent absolute IC_{50} values for each compound and synergy score measurements for the combination of Compound 1 and paclitaxel are indicated. Interactions were deemed synergistic when scores ≥ 2.0 were observed.

Cell Line	Linage	Compound 1	Paclitaxel	Combination
		Abs IC_{50} (nM)	Abs IC_{50} (nM)	Synergy Score (SS)
BT-20	**Breast**	653	5.97	7.93
BT-474	**Breast**	1262.5	1.7	4.7
BT-549	**Breast**	>10000	8.82	9.01
Cal-148	**Breast**	1330	4.25	13.8
HCC1143	**Breast**	>10000	9.43	2.58
HCC1395	**Breast**	9915	8.03	1.52
HCC1500	**Breast**	257.5	>1000	6.38
HCC1937	**Breast**	137	11.6	9.4
HCC1954	**Breast**	158.5	6.5	3.69
HCC38	**Breast**	3385	4.33	5.19
HCC70	**Breast**	704	8.39	11.9
Hs 578T	**Breast**	519.5	0.363	8.49
MCF7	**Breast**	1292.5	10.4	3.82
MDA-MB-157	**Breast**	>10000	>1000	0.389
MDA-MB-231	**Breast**	>10000	17.5	4.86
MDA-MB-436	**Breast**	>10000	14.7	1.41
MDA-MB-453	**Breast**	>10000	4.2	23.9
MDA-MB-468	**Breast**	9917.5	3.07	11.3
SK-BR-3	**Breast**	301	2.66	4.52
NCI-H522	**Lung**	713	0.48	14.9
NCI-H23	**Lung**	160	0.22	6.97
A549	**Lung**	>10000	0.163	4.82

Results

[0057] Compound 1 as single agent inhibit the growth of 7/19 breast cancer cell lines and 2/3 lung cancer cell lines, with IC_{50} below 1000 nM (Table 1).

[0058] Paclitaxel as single agent inhibited the growth of the 17/19 breast cancer cell lines and 3/3 lung cancer cell lines, with IC_{50} below 1000 nM.

[0059] In combination, Compound 1 and paclitaxel treatment caused synergistic growth inhibition (i.e. Synergy Scores above 2 (Lehar et al, 2009)) in 16/19 breast cancer cell lines and 3/3 lung cancer cell lines (Table 1). In 11 cell lines, the synergy effect was marked, with synergy scores above 6. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and the synergistic effects occurred across a broad range of single agent concentrations (Figures 1, 2 and 3), which should prove beneficial *in vivo* with respect to flexibility concerning dosing levels and scheduling.

EXAMPLE 2: *In vitro* effect on proliferation of combining MCL-1 inhibitor (Compound 2) with paclitaxel in breast and lung cancer cell lines

[0060] The effect on proliferation of combining MCL-1 inhibitor (Compound 2) with paclitaxel was assessed in a panel of 2 breast cancer cell lines (MDA-MB-453 and MDA-MB-468) and one lung cancer cell line (H522).

Material and method

[0061] Cell lines were sourced and maintained in the basic media supplemented with fetal bovine serum as indicated in Table 2. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM).

[0062] Cell lines were cultured at 37 °C in a humidified atmosphere containing 5 % CO_2 and expanded in T-150 flasks. In all cases cells were thawed from frozen stocks, expanded through ≥ 1 passage using appropriate dilutions, counted and assessed for viability using a CASY cell counter prior to plating 150 μl/well at the densities indicated in Table 2 into 96-well plates. All cell lines were determined to be free of mycoplasma contamination in-house.

[0063] Stock solutions of compounds were prepared at a concentration of 5 mM in DMSO and stored at -20 °C.

[0064] In order to analyze the activity of the compounds in single agent or in combination, cells were seeded and treated with seven or eight 3.16-fold serial dilutions of each compound dispensed, either individually or in all possible permutations in a checkerboard fashion, directly into the cell assay plates as indicated in Figures 5 and 6. Effects of the single agents as well as their checkerboard combinations on cell viability were assessed after 3 days of incubation at 37 °C/5 % CO_2 by quantification of cellular ATP levels using CellTiterGlo at 75 μL reagent/well. At least two independent experiments, each one performed in duplicates, were performed. Luminescence was quantified on a multipurpose plate reader.

[0065] Single agent IC_{50}s were calculated using standard four-parametric curve fitting. Potential synergistic interactions between compound combinations were assessed using the Excess Inhibition 2D matrix according to the Loewe additivity model and are reported as Synergy Score (Lehar et al. 2009). All calculations were performed using Clalice™ Bioinformatics Software available in Horizon website.

[0066] The doubling time indicated in Table 2 is the mean of the doubling time obtained in the different passages (in T-150 flasks) performed from the thawing of the cells to their seeding in the 96-weel plates.

Synergy Score

[0067]

SS ~ 0 → Additive
SS >1 → Weak Synergy
SS >2 → Synergy

Table 2. Identity and assay conditions for the cell lines used in the combination experiments.

Cell line	Linage	Medium	%FBS	Source	Doubling time (hours)	Cell number seeded/well
MDA-MB-453	**Breast**	L-15	10	ATCC	41.4	15000
MDA-MB-468	**Breast**	L-15	20	ATCC	34.3	30000
H522	**Lung**	RPMI	10	ATCC	67.2	30000

Table 3. Single agent IC_{50} values for Compound 2 and paclitaxel are indicated. Compounds were incubated with the cells during 3 days.

Cell Line	Compound 2		Paclitaxel	
	Start cone [μM]	IC_{50} [μM]	Start cone [μM]	IC_{50} [μM]
MDA-MB-453	2.00	>2	1.0	> 1
MDA-MB-468	2.00	>2	1.0	0.0009
H522	2.00	0.140	0.01	0.0002

Table 4. Synergy scores for Compound 2 and paclitaxel combination are indicated. Interactions were deemed synergistic when scores ≥ 2.0 where observed. Start concentrations of compounds, mean of max inhibition and the standard deviation (sd) of the synergy scores are indicated. Compounds were incubated with the cells during 3 days.

Cell Line	Compound 2		Paclitaxel		Combination	
	Start cone [μM]	Mean of Max Inh [%]	Start cone [μM]	Mean of Max Inh [%]	Mean of Synergy Score (SS)	Synergy Score Error (sd)
MDA-MB-453	2.0	21.0	1.00	37.0	16.9	0.5
MDA-MB-468	2.0	24.0	1.00	75.0	6.5	0.2
H522	2.0	92.0	0.01	61.0	3.7	0.6

Results

[0068] Compound 2 as single agent inhibited the growth of 1/3 cell lines tested, with IC_{50} of 140 nM for H522 cell line (Table 3).

[0069] Paclitaxel as single agent inhibited the growth of the 2/3 lines tested, with IC_{50} below 1 nM. In combination, Compound 2 and paclitaxel treatment caused synergistic growth inhibition (i.e. Synergy Scores above 2 (Lehar et al. 2009)) in the three cell lines tested (Table 4). In 2 cell lines, the synergy effect was marked, with synergy scores of 6.5 and 16.9. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and the synergistic effects occurred across a broad range of single agent concentrations (Figures 4 and 5), which should prove beneficial *in vivo* with respect to flexibility concerning dosing levels and scheduling.

EXAMPLE 3: Synergy between MCL-1 inhibitor and docetaxel *in vitro*

[0070] We investigated whether MCL-1 inhibitor (Compound 2) elicited synergistic activity with agents currently used in the treatment of TNBC. Compound 2 was combined with docetaxel in SK-BR-3 cells.

Material and method

[0071] *Cell Lines:* The breast cancer cell line SK-BR-3 was maintained in RPMI-1640 plus GlutaMAX-1 (Gibco) supplemented with 10 % fetal calf serum (FCS) and 10 μg/ml insulin. For viability assays, cells were plated at 2 x 10^5 cells/ml in 96 well plates, in RPMI-1640 medium (Gibco) supplemented with 10 % FCS and 10 μg/ml insulin, and treated with increasing concentrations of Compound 2.

[0072] *Cell Viability:* Cell viability was assessed using the Cell Titer Glo Luminescent Assay (Promega) as per the manufacturer's instructions. The broad-spectrum caspase inhibitor QVD-OPh hydrate (Sigma-Aldrich) was used at 10 μM. Propidium iodide exclusion (5 μg/ml) was analyzed by flow cytometry. For *in vitro* cell assays to address synergy between different drugs, combination effects were determined using the Bliss independence method (Prichard et al., Antimicrobial Agents and Chemotherapy 1991, 35, 1060-5).

Results

[0073] Docetaxel and MCL-1 inhibitors showed marked synergy at very low concentrations of both components. Particularly, docetaxel and Compound 2 showed marked synergy at very low concentrations of docetaxel (2 nM) and Compound 2 (31 nM) (Figures 6 and 7). Inhibition of caspases with the pan-caspase inhibitor QVD-OPH efficiently blocked cell death, confirming that cell death was triggered via apoptosis (Figure 6).

EXAMPLE 4: MCL-1 inhibition sensitizes PDX tumors to taxane treatment *in vivo*

[0074] Since *in vitro* assays revealed that breast cancer cell lines were sensitive to Compound 2 in combination therapy, we next determined their therapeutic effect *in vivo* in three PDX models, representing three TNBCs (110T, 838T and PDX OD-BRE-0589).

Material and method

[0075] Human breast cancer tissues were obtained from consenting patients through the Royal Melbourne Hospital Tissue Bank, the Victorian Cancer Biobank and Georges-Francois Leclerc Center with relevant institutional review board approval. Human Ethics approval was obtained from the Walter and Eliza Hall Institute (WEHI) Human Research Ethics Committee and from the Georges-Francois Leclerc Center Human Research Ethics Committee. NOD SCID IL2 gamma receptor knockout mice or SCID mice were bred and maintained according to institutional guidelines. All animal experiments were approved by the WEHI and Servier Research Institute (IdRS) Animal Ethics Committee.

[0076] Compound 2 (25 mg/kg) or its vehicle was injected i.v. weekly for six weeks. Compound 2 was dissolved in 20 % (2-hydroxypropyl)-β-cyclodextrin and 25 mM hydrochloric acid. Docetaxel (10 mg/kg i.p.) or its vehicle was prepared as previously described (Oakes et al., Proceedings of the National Academy of Sciences of the USA 2012, 109, 2766-71) and injected i.p. weekly one day prior to Compound 2. Mice were monitored for tumor development three times weekly and tumor size measured using electronic vernier calipers. Tumor volume was estimated by measuring the minimum and maximum tumor diameters using the formula: $(\text{minimum diameter})^2(\text{maximum diameter})/2$. Once tumors arose, mice were randomized into treatment arms. Treatment was initiated when the tumor volume reached 80-120 mm^3. Randomization and tumor measurements were managed using the Study Director software (v 3.0, studylog). Mice were sacrificed at the first measurement for which tumor volume exceeded 600 mm^3 or animal health deterioration that are not due to disease progression or drug toxicity (mice censored).

Results

[0077] Compound 2 alone was insufficient in inhibiting tumor growth. However, we observed a superior activity in combination with docetaxel as compared to docetaxel administered as a single agent, resulting in significantly improved animal survival in the three PDX models (Figure 8).

[0078] These results indicate that MCL-1 inhibitors combined with taxane compounds are likely to significantly enhance tumor response and clinical outcome.

EXAMPLE 5: MCL-1 inhibitor combined with docetaxel is well tolerated *in vivo*

[0079] NOD SCID IL2 gamma receptor knockout mice were treated with docetaxel (15 mg/kg, once i.p.) and Compound 2 at 25 or 50 mg/kg (3 mice per group, i.v. injections, once per week for 3 weeks). Their body weight was monitored three times per week for 3 weeks. Compound 2 combined with docetaxel was well-tolerated and did not induce significant body weight loss (Figure 9).

EXAMPLE 6: Efficacy of Compound 1 in combination with paclitaxel in MDA-MB-231 breast xenograft model in nude rats

Methods

[0080] This study evaluated the antitumor activity and tolerability of Compound 1 in combination with paclitaxel in the triple negative breast cancer (TNBC) model, MDA-MB-231, in female NTac:NIH-Whn nude rats (Taconic).

[0081] Compound 1 (free base) and paclitaxel (Sandoz) was used in these studies. Paclitaxel was diluted as per manufacturer's instruction with sterile 5 % (w/v) glucose solution to 1.5 mg/ml to administer 7.5 mg/kg in 5 ml/kg dose volume [final ethanol and Cremophor EL concentration was 10 and 15 %, respectively in 5 % (w/v) glucose solution]. Compound 1 was formulated in a liposomal formulation (Novartis) at 5 mg/ml to administer 50 mg/kg dose in 10 ml/kg dose volume.

[0082] MDA-MB-231, a triple negative breast cancer cell line, was obtained from ATCC cell bank. The cells were cultured at 37 °C in an atmosphere of 5 % CO_2 in air in DMEM high glucose medium (BioConcept Ltd. Amimed) supplemented with 10 % FCS (BioConcept Ltd. Amimed, # 2-01F36-I) and 4 mM L-glutamine (BioConcept Ltd. Amimed, #5-10K00-H). To establish MDA-MB-231 xenografts cells were harvested and re-suspended in HBSS (Gibco, #14175) and mixed with Matrigel (BD Bioscience, #354234) (1:1 v/v) before injecting 200 μL containing 1 x 10^7 cells subcutaneously in the right flanks of animals which were anesthetized with isoflurane. Twenty four hours prior to cell inoculation all animals were irradiated with 5 Gy over 2 minutes using a γ-irradiator.

[0083] Tumor growth was monitored regularly post cell inoculation and animals were randomised into treatment groups (n = 7-8) with a mean tumor volume of about 400 mm^3. Groups were treated with:

1) the vehicle used for formulating paclitaxel, iv plus liposomal vehicle iv; or
2) 7.5 mg/kg iv bolus paclitaxel plus liposomal vehicle iv; or

3) the vehicle used for formulating paclitaxel iv plus Compound 1 at 50 mg/kg iv; or
4) 7.5 mg/kg iv paclitaxel plus 50 mg/k iv Compound 1.

[0084] The vehicle for paclitaxel or paclitaxel was administered once a week (QW) as a slow bolus dose via a caudal vein 0.5 h or 16 h before the vehicle for Compound 1 or Compound 1 itself in liposomal formulation and these were administered by iv infusion over 15 minutes in a caudal vein. For bolus administrations and 15 minute infusions animals were anesthetized for about 5 and 25 minutes, respectively with isoflurane/O_2.

[0085] Tumor volumes were measured using calipers 2-3 times per week. Tumor size, in mm^3, was calculated from: $(L \times W^2 \times \pi/6)$, where W = width and L = length of the tumor. Animals were also weighed 2-3 times per week and examined frequently for overt signs of any adverse effects.

[0086] Tumor and body weight change data were analyzed statistically using GraphPad Prism 7.00 (GraphPad Software). If the variances in the data were normally distributed, the data were analyzed using one-way ANOVA with post hoc Dunnett's test for comparison of treatment versus control group. The post hoc Tukey's test was used for intragroup comparison. Otherwise, the Kruskal-Wallis ranked test post hoc Dunn's was used. When applicable, results are presented as mean $\pm$ SEM.

[0087] As a measure of efficacy the %T/C value is calculated at the end of the experiment according to:

$$(\Delta\text{tumor volume}^{\text{treated}}/\Delta\text{tumor volume}^{\text{control}})*100$$

[0088] Tumor regression was calculated according to:

$$-(\Delta\text{tumor volume}^{\text{treated}}/\text{tumor volume}^{\text{treated at start}})*100$$

where Δtumor volumes represent the mean tumor volume on the evaluation day minus the mean tumor volume at the start of the experiment.

Results: efficacy and tolerability

[0089] Compound 1 50 mg/kg dosed 0.5 h or 16 h after vehicle for paclitaxel [ethanol : Cremophor EL : 5 % (w/v) glucose (10 : 15 : 75 %)] is well tolerated.

[0090] Compound 1 (50 mg/kg QW) in liposomal formulation exhibited no efficacy in the MDA-MB-231 xenograft model after QWx7 iv infusion administration (Figures 10 and 12). Paclitaxel 7.5 mg/kg caused tumor growth delay (T/C % = 34 %) and was significantly ($p < 0.05$) different from the vehicle treated group (Figures 10 and 12).

[0091] Combination of 7.5 mg/kg iv paclitaxel plus 50 mg/kg iv Compound 1 administered 0.5 h or 16 h apart caused 82 and 59 % regression, respectively on day 28 after start of treatment in the surviving animals (3/8 in both groups) (Figure 10). On day 46 from start of treatment, tumor regression was 92 and 81 %, in the surviving animals 2/8 and 3/8, respectively. The tumor volume in animals from both of these combination groups was significantly different ($p < 0.05$) from that in animals treated with paclitaxel or Compound 1 alone on day 28 and 46 (Figure 10).

[0092] Combination of 7.5 mg/kg iv paclitaxel plus 25 mg/kg iv Compound 1 administered 16 h apart caused tumor stasis (15 % regression on day 28 and the T/C % values was 2 % on day 49 after start of treatment) in the surviving (7/7) animals (Figure 12). This dose schedule was well tolerated based on body weight changes and clinical signs.

[0093] Combination of 3.75 mg/kg iv paclitaxel plus 50 mg/kg iv Compound 1 administered 16 h apart caused tumor stasis up to day 35 (3 % regression on day 28 and the T/C % values was 20 % on day 49 after start of treatment) in the surviving (7/7) animals (Figure 12). This dose schedule was well tolerated based on body weight changes and clinical signs (Figures 11 and 13). These data indicate that combination of paclitaxel and Compound 1 has a marked positive effect on antitumor activity compared to either agent alone.

EXAMPLE 7: MCL-1 inhibition sensitizes PDX tumors to taxane treatment *in vivo*

[0094] We determined the *in vivo* therapeutic effect of Compound 1 in combination therapy, in TNBC 110T PDX model.

Material and method

[0095] Human breast cancer tissues were obtained from consenting patients through the Royal Melbourne Hospital Tissue Bank, the Victorian Cancer Biobank and Georges-Francois Leclerc Center with relevant institutional review board approval. Human Ethics approval was obtained from the Walter and Eliza Hall Institute (WEHI) Human Research Ethics

Committee and from the Georges-Francois Leclerc Center Human Research Ethics Committee. NOD SCID IL2 gamma receptor knockout mice or SCID mice were bred and maintained according to institutional guidelines. All animal experiments were approved by the WEHI and Servier Research Institute (IdRS) Animal Ethics Committee.

[0096] A cohort of 40 female NSG mice was seeded with thawed single cell suspensions of early passage human breast tumors (TNBC PDX110). Briefly, 100,000 cells were resuspended in 10 μl of transplantation buffer (50 % fetal calf serum, 10 % of a 0.04 % trypan blue solution and 40 % PBS) and growth-factor-reduced Matrigel [BD] at a ratio of 3:1, and injected into the cleared mammary fat pads of 3- or 4-week-old NOD-SCID-IL2R$\gamma_c^{-/-}$ female mice. Mice were monitored for tumor development three times weekly and tumor size measured using electronic vernier calipers. Tumor volume was estimated by measuring the minimum and maximum tumor diameters using the formula: (minimum diameter)^2(maximum diameter)/2. Once tumors reached a volume of 60-110 mm^3, mice were randomized into treatment arms and treatment commenced. Docetaxel or its vehicle was prepared by dissolving stock solution (20 mg/ml) with PBS and injected i.p. every 21 days for two treatment cycles. The duration of therapy is indicated by the bar. Compound 1 was dissolved in 20 % (2-Hydroxypropyl)-β-cyclodextrin and 25 mM hydrochloric acid. Compound 1 (15 mg/kg) or its vehicle was injected i.v. twice weekly for six weeks. Mice were sacrificed at the first measurement where tumor volume exceeded 600 mm^3, or if their health deteriorated for reasons other than disease progression or drug toxicity (censored event). n = 9-10 mice per treatment group.

Results

[0097] Compound 1 alone was insufficient in inhibiting tumor growth. However, we observed a superior activity in combination with docetaxel as compared to docetaxel administered as a single agent, resulting in significantly improved animal survival in the PDX model (Figure 14).

[0098] These results indicate that MCL-1 inhibitors combined with taxane compounds are likely to significantly enhance tumor response and clinical outcome.

EXEMPLE 8: Antitumor activity of docetaxel and Compound 1 in female SCID mice bearing patient derived TNBC model

[0099] This study evaluated the antitumor activity Compound 1 in combination with docetaxel in a TNBC PDX model OD-BRE-00589, in female SCID mice.

Methods

[0100] Docetaxel was formulated in 5 % ethanol, 5 % PS80 and 90 % Glucose at 0.67 mg/ml to administer 10 mg/kg. Compound 1 was formulated in a liposomal formulation (Novartis) at 7.5 mg/ml to administer 70 mg/kg.

[0101] OD-BRE-00589 is a triple negative breast cancer PDX, obtained from the IMODI consortium. Consenting patients was obtained from the Georges-Francois Leclerc Center Human Research Ethics Committee. It was grafted on SCID mice as fragments of 27 mm^3 volume.

[0102] Tumor growth was monitored regularly post fragment grafting and animals were randomized 11 days after grafting into treatment groups (n = 8) with a mean tumor volume of about 200 mm^3. Control group was not treated and the other groups were treated with:

1) 70 mg/kg of Compound 1 iv, or
2) 10 mg/kg of docetaxel iv, or
3) 10 mg/kg of docetaxel iv followed by 30 min later with 70 mg/kg of Compound 1, or
4) 10 mg/kg of docetaxel iv followed by 72h later with 70 mg/kg of Compound 1.

[0103] The administrations were performed once in the caudal vein.

[0104] Tumor volumes were measured using calipers 2-3 times per week. Tumor volume was calculated using the formula: length x width^2/2. Animals were also weighed 2-3 times per week and examined frequently for overt signs of any adverse effects.

Results

[0105] Compound 1 alone was insufficient in inhibiting tumor growth. However, we observed a superior activity in combination with docetaxel as compared to docetaxel administered as a single agent, resulting in significantly improved antitumor activity in the PDX model (Figure 15).

[0106] These results indicate that MCL-1 inhibitors combined with taxane compounds are likely to significantly enhance

tumor response and clinical outcome.

EXAMPLE 9: Efficacy studies of Compound 1 combined to paclitaxel in PDX models resistant to docetaxel

Methods

[0107] PDX models whose resistance to docetaxel has been confirmed *in vivo* were tested. Each treatment group included 5 female Swiss Nude mice, aged from 6 to 8 weeks old. The treatment started when xenografts reached a mean tumor volume of ~ 120-150 mm^3. Groups of mice were then randomly affected to the different treatments. The number of grafted animals depended on the homogeneity of the tumor growth.

[0108] Formulated in a liposomal formulation, Compound 1 was administered intravenously at 70 mg/kg once a week. The formulation had to be prepared extemporaneously. Paclitaxel diluted in 0.9 % NaCl was given ip at 25 mg/kg. Paclitaxel was given QW 16 hours before the administration of Compound 1. The administration schedule of the different treatments is defined as follow:

Group	Dose (mg/kg)	Schedule
Control (vehicle)	-	-
Compound 1	70	IV, Q7D, 4w: D1-D8-D15-D22
Paclitaxel	25	IP, Q7D, 4w: D1-D8-D15-D22
Compound 1 + paclitaxel	Compound 1: 70 Paclitaxel: 25	Q7D, 4w Paclitaxel, IP: D1-D8-D15-D22 Compound 1, IV: D1-D8-D15-D22

[0109] Tumor sizes were measured twice a week and weights of individual mice were measured once a week. Treatments were done until the median tumor volume of the most responsive group started to regrow. Mice were followed after the stop of the treatment to compare the time to relapse between the groups. Using Statview software, tumor volume and/or relative tumor volume (RTV, ratio of the volume at the time t divided by the initial volume at day 1 and multiplied by 100), optimal growth inhibition (ratio of RTV (x 100) in the treated group divided by the RTV in the controls), growth delay (the time in days necessary to multiply by 4 an initial tumor volume of 200 mm^3 in treated group and control group) and body weight change were compared:

[0110] If relapse was observed after paclitaxel treatment, tumors were rechallenged with the Compound 1 + paclitaxel combination. This was done either by including 10 mice in the paclitaxel treated group and then treated tumor relapsing mice with paclitaxel alone (5 animals) or in combination (5 animals), or by using not randomized mice from the efficacy study. If initial response to paclitaxel was observed, additional animals left after randomization were included for this study.

Results

[0111] Compound 1 alone was insufficient in inhibiting tumor growth. However, we observed a superior activity in combination with paclitaxel as compared to paclitaxel administered as a single agent, resulting in significantly improved antitumor activity in the PDX model resistant to a taxane compound.

[0112] These results indicate that MCL-1 inhibitors combined with taxane compounds are likely to significantly enhance tumor response and clinical outcome.

Claims

1. A combination for use in the treatment of breast cancer or lung cancer comprising:

(a) a MCL-1 inhibitor which is (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-d]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2,2-trifluoroethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propanoic acid or (2*R*)-2-{[(5S_a)-5-{3-chloro-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorophenyl)thieno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{ [2-(2-methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propanoic acid,
and (b) a taxane compound which is paclitaxel or docetaxel,

for simultaneous, sequential or separate use.

2. A combination for use according to claim 1, wherein the dose of MCL-1 inhibitor during the combination treatment is from 25 mg to 1500 mg.

3. A combination for use according to claim 1, wherein the MCL-1 inhibitor is administered during the combination treatment once a week.

4. A combination for use according to claim 1, wherein the MCL-1 inhibitor and the taxane compound are administered intravenously.

5. A combination for use according to claim 1, wherein the MCL-1 inhibitor is administered orally and the taxane compound is administered intravenously.

6. A medicament containing, separately or together,

(a) a MCL-1 inhibitor as defined in claim 1, and
(b) a taxane compound as defined in claim 1,

for simultaneous, sequential or separate administration, and wherein the MCL-1 inhibitor and the taxane compound are provided in effective amounts for the treatment of breast cancer or lung cancer.

Patentansprüche

1. Kombination zur Verwendung bei der Behandlung von Brustkrebs oder Lungenkrebs, umfassend:

(a) einen MCL-1-Inhibitor, der (2*R*)-2-{[(5Sa)-5-{3-Chlor-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(5-fluorofuran-2-yl)thieno[2,3-d]pyrimidin-4-yl]oxy}-3-(2-{[1-(2,2, 2-trifluorethyl)-1*H*-pyrazol-5-yl]methoxy}phenyl)propansäure oder (2*R*)-2-{[(5S_a)-5-{3-chlor-2-methyl-4-[2-(4-methylpiperazin-1-yl)ethoxy]phenyl}-6-(4-fluorphenyl)thieno[2,3-d]Pyrimidin-4-yl]oxy}-3-(2-{[2-(2-Methoxyphenyl)pyrimidin-4-yl]methoxy}phenyl)propansäure,
und (b) eine Taxanverbindung, die Paclitaxel oder Docetaxel ist,

zur gleichzeitigen, aufeinanderfolgenden oder getrennten Verwendung.

2. Kombination nach Anspruch 1, wobei die Dosis des MCL-1-Inhibitors während der Kombinationsbehandlung 25 mg bis 1500 mg beträgt.

3. Kombination nach Anspruch 1, wobei der MCL-1-Inhibitor während der Kombinationsbehandlung einmal pro Woche verabreicht wird.

4. Kombination nach Anspruch 1, wobei der MCL-1-Inhibitor und die Taxanverbindung intravenös verabreicht werden.

5. Kombination nach Anspruch 1, bei der der MCL-1-Inhibitor oral und die Taxanverbindung intravenös verabreicht wird.

6. Arzneimittel, enthaltend, getrennt oder zusammen

(a) einen MCL-1-Inhibitor wie in Anspruch 1 definiert, und
(b) eine Taxanverbindung, wie in Anspruch 1 definiert,

zur gleichzeitigen, aufeinanderfolgenden oder getrennten Verabreichung, und wobei der MCL-1-Inhibitor und die Taxanverbindung in wirksamen Mengen für die Behandlung von Krebs bereitgestellt werden.

Revendications

1. Combinaison destinée à être utilisée dans le traitement du cancer du sein ou du cancer du poumon comprenant :

(a) un inhibiteur de MCL-1 qui est l'acide (2*R*)-2-{[(5S_a)-5-{3-chloro-2-méthyl-4-[2-(4-méthylpipérazin-1-yl)éthoxy]phényl}-6-(5-fluorofuran-2-yl)thiéno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{ [1-(2,2,2-trifluoroéthyl)-1*H*-pyrazol-5-yl]méthoxy}phényl)propanoïque ou l'acide (2*R*)-2-{[(5S_a)-5-{3-chloro-2-méthyl-4-[2-(4-méthylpipérazin-1-yl)éthoxy]phényl}-6-(4-fluorophényl)thiéno[2,3-*d*]pyrimidin-4-yl]oxy}-3-(2-{[2-(2-méthoxyphényl)pyrimidin-4-yl]méthoxy}phényl)propanoïque,
et (b) un composé de taxane qui est le paclitaxel ou le docétaxel,

pour une utilisation simultanée, séquentielle ou séparée.

2. Combinaison destinée à être utilisée selon la revendication 1, dans laquelle la dose d'inhibiteur de MCL-1 au cours du traitement combiné est de 25 mg à 1500 mg.

3. Combinaison destinée à être utilisée selon la revendication 1, dans laquelle l'inhibiteur de MCL-1 est administré au cours du traitement combiné une fois par semaine.

4. Combinaison destinée à être utilisée selon la revendication 1, dans laquelle l'inhibiteur de MCL-1 et le composé de taxane sont administrés par voie intraveineuse.

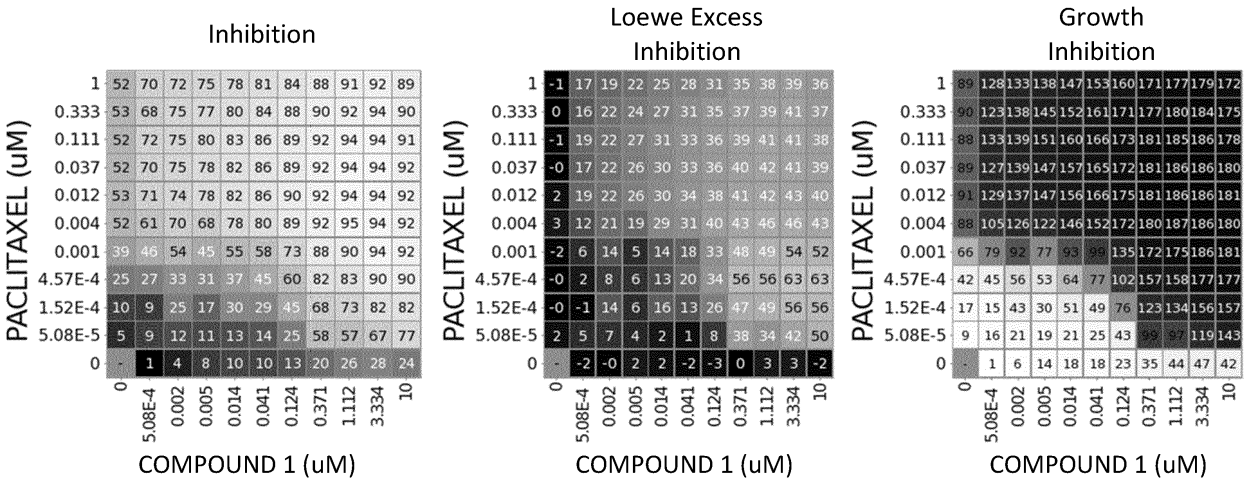
5. Combinaison destinée à être utilisée selon la revendication 1, dans laquelle l'inhibiteur de MCL-1 est administré par voie orale et le composé de taxane est administré par voie intraveineuse.

6. Médicament contenant, séparément ou ensemble,

(a) un inhibiteur de MCL-1 tel que défini dans la revendication 1, et
(b) un composé de taxane tel que défini dans la revendication 1,

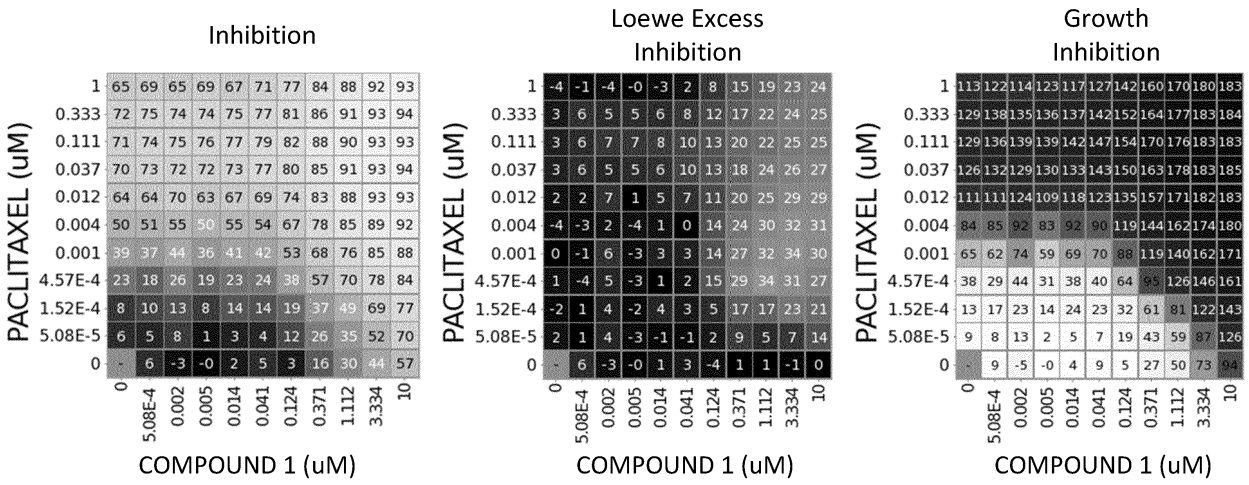
pour une administration simultanée, séquentielle ou séparée, et dans lequel l'inhibiteur de MCL-1 et le composé de taxane sont fournis en quantités efficaces pour le traitement du cancer du sein ou du cancer du poumon.

Figure 1



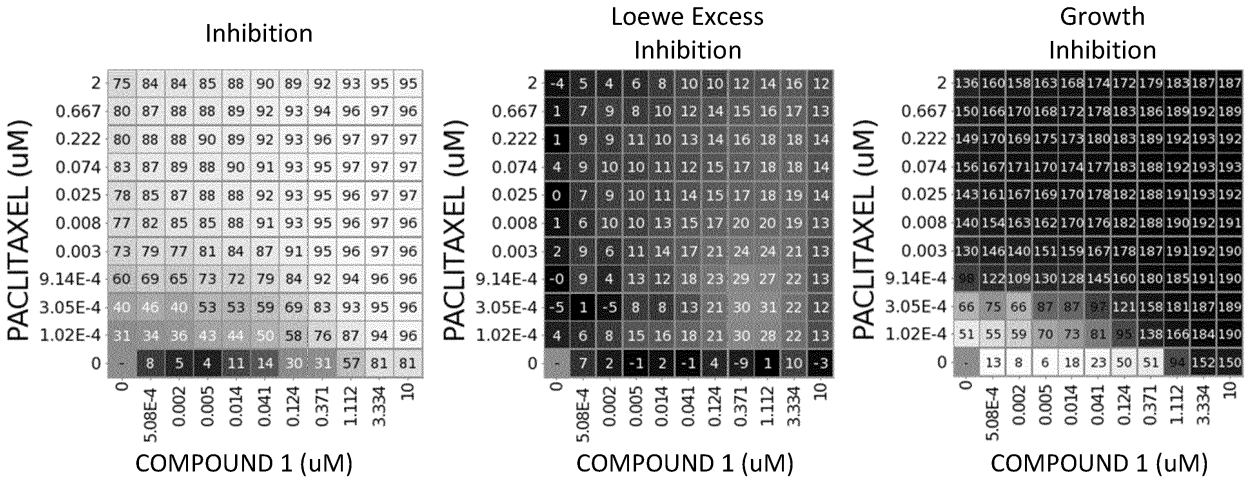
Synergy Score: 23.9

Figure 2



Synergy Score: 11.3

Figure 3



Synergy Score: 14.9

Figure 4

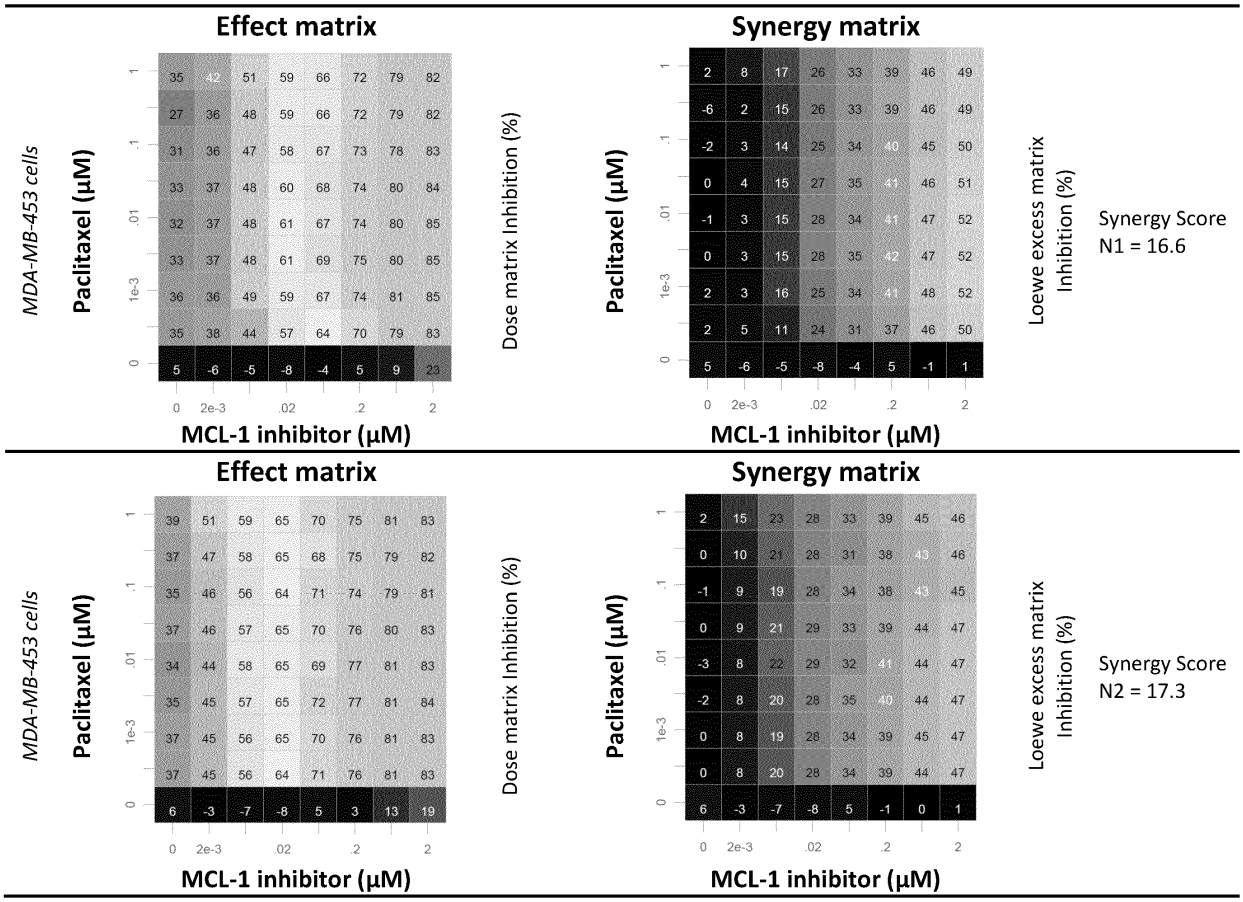

Effect matrix
Synergy matrix
MDA-MB-453 cells
Paclitaxel (μM)
MCL-1 inhibitor (μM)
Dose matrix Inhibition (%)
Loewe excess matrix Inhibition (%)
Synergy Score N1 = 16.6
Synergy Score N2 = 17.3

Figure 5

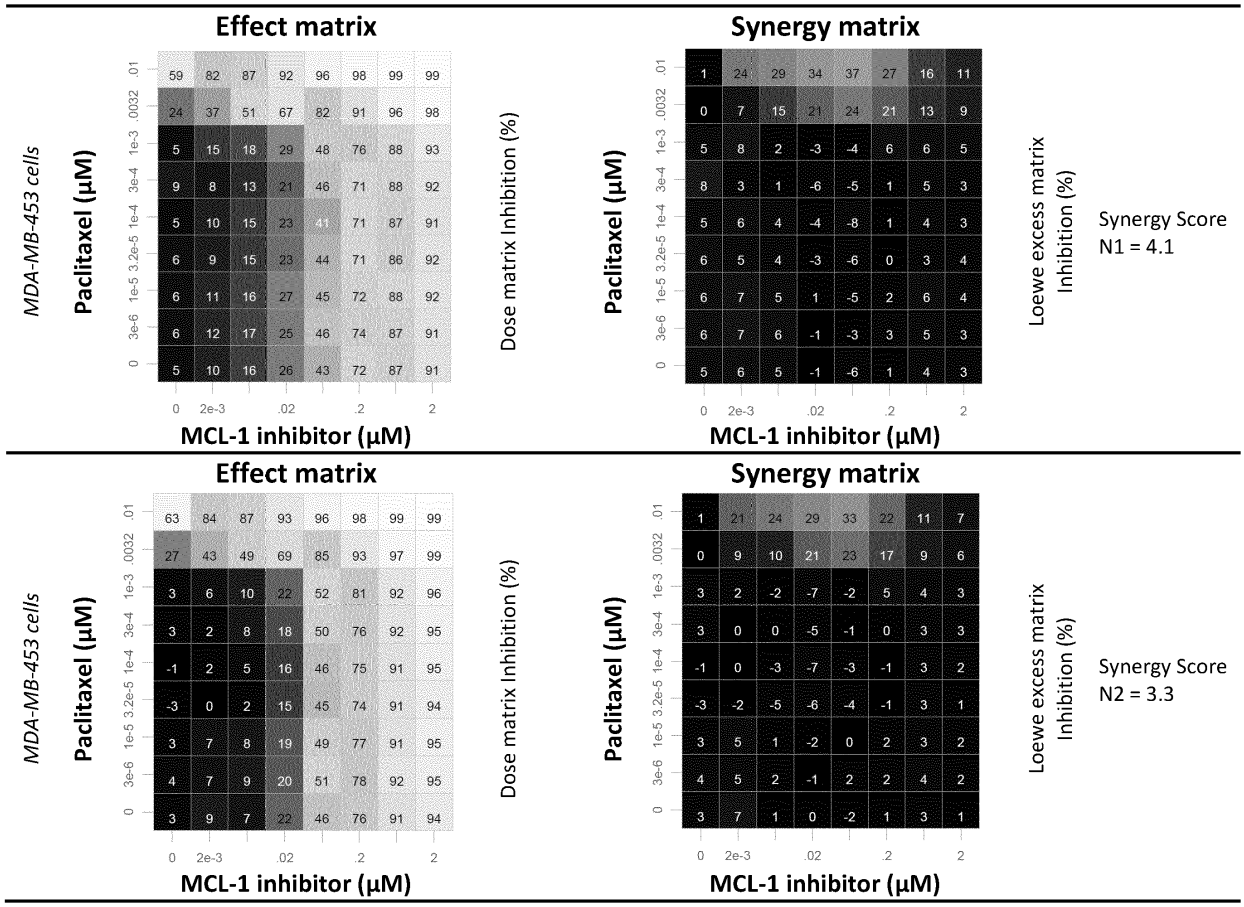

Effect matrix
Synergy matrix
MDA-MB-453 cells
Paclitaxel (µM)
MCL-1 inhibitor (µM)
Dose matrix Inhibition (%)
Loewe excess matrix Inhibition (%)
Synergy Score N1 = 4.1
Synergy Score N2 = 3.3

Figure 6

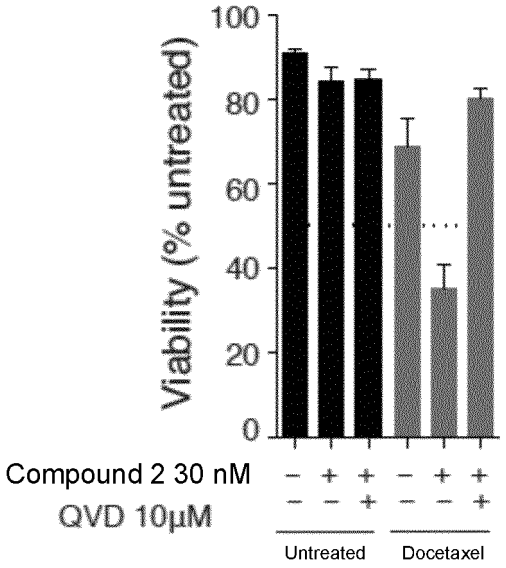

Viability (% untreated)
100
80
60
40
20
0
Compound 2 30 nM
QVD 10µM
Untreated
Docetaxel

Figure 7

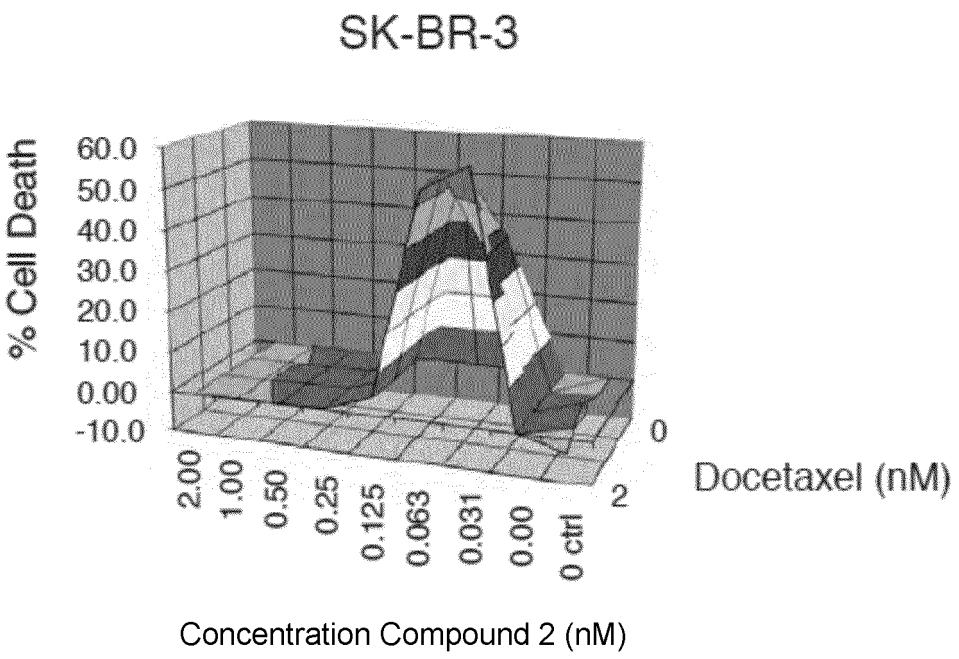

SK-BR-3
% Cell Death
60.0
50.0
40.0
30.0
20.0
10.0
0.00
-10.0
2.00
1.00
0.50
0.25
0.125
0.063
0.031
0.00
0 ctrl
0
2
Docetaxel (nM)
Concentration Compound 2 (nM)

Figure 8

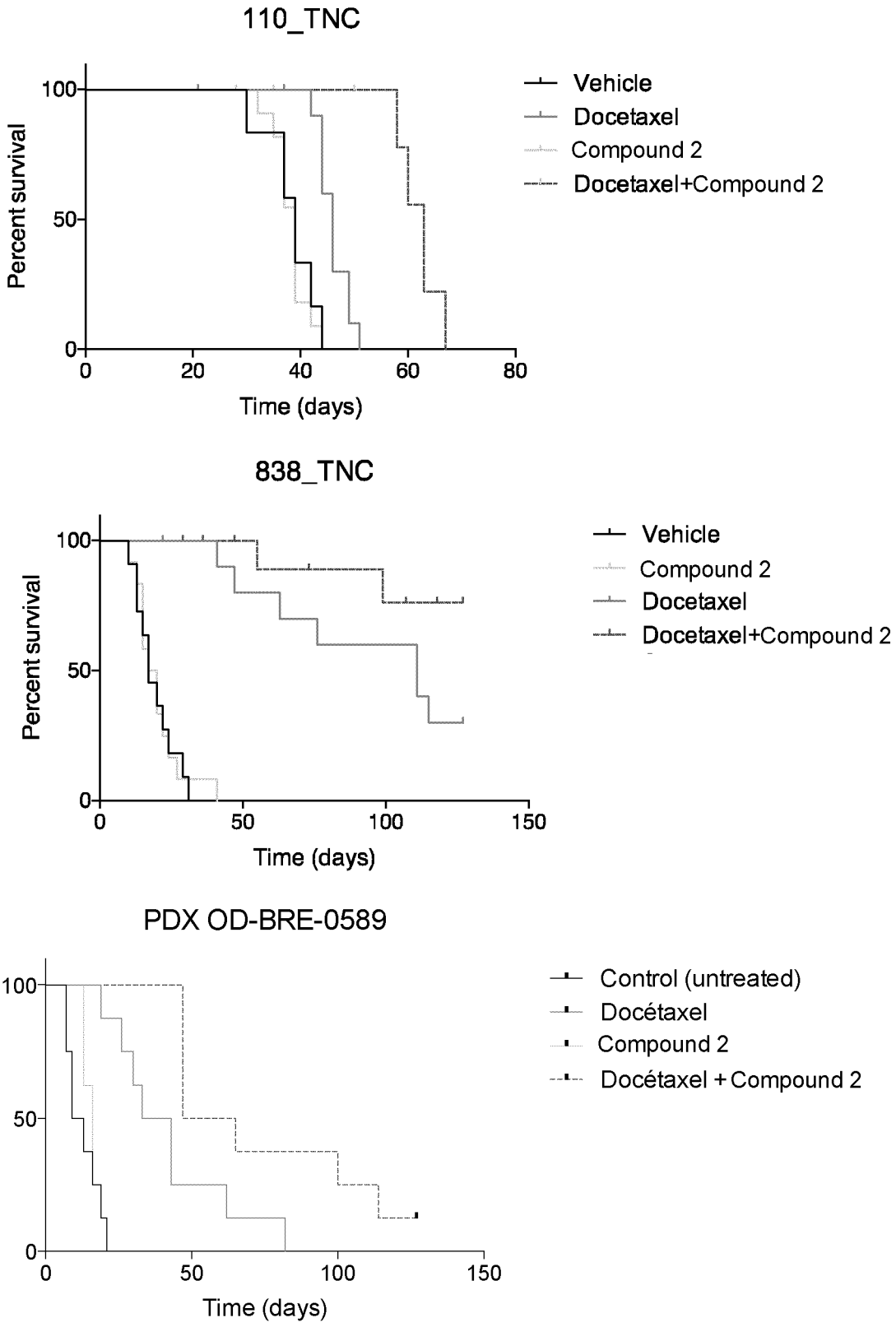

110_TNC
Percent survival
100
50
0
0
20
40
60
80
Time (days)
Vehicle
Docetaxel
Compound 2
Docetaxel+Compound 2
838_TNC
Percent survival
100
50
0
0
50
100
150
Time (days)
Vehicle
Compound 2
Docetaxel
Docetaxel+Compound 2
PDX OD-BRE-0589
Percent survival
100
50
0
0
50
100
150
Time (days)
Control (untreated)
Docétaxel
Compound 2
Docétaxel + Compound 2

Figure 9

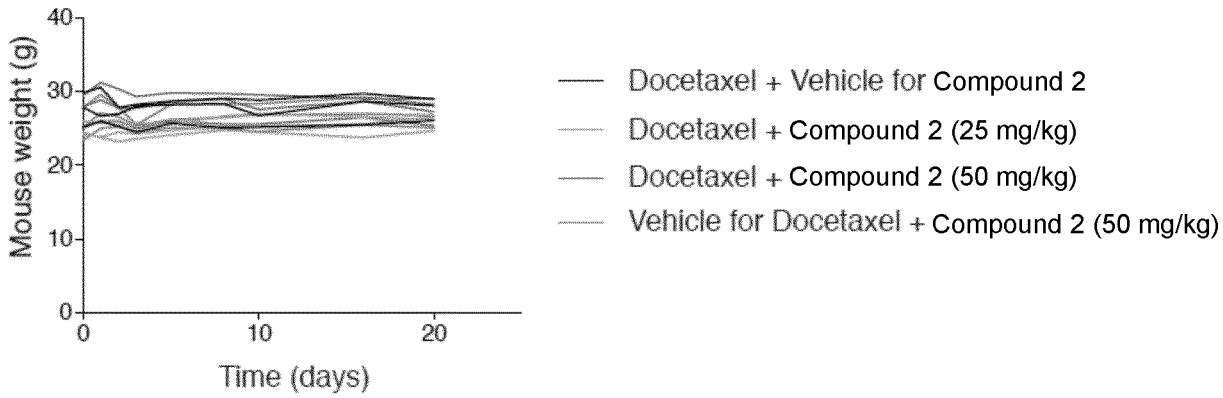

Mouse weight (g)
40
30
20
10
0
0
10
20
Time (days)
Docetaxel + Vehicle for Compound 2
Docetaxel + Compound 2 (25 mg/kg)
Docetaxel + Compound 2 (50 mg/kg)
Vehicle for Docetaxel + Compound 2 (50 mg/kg)

Figure 10

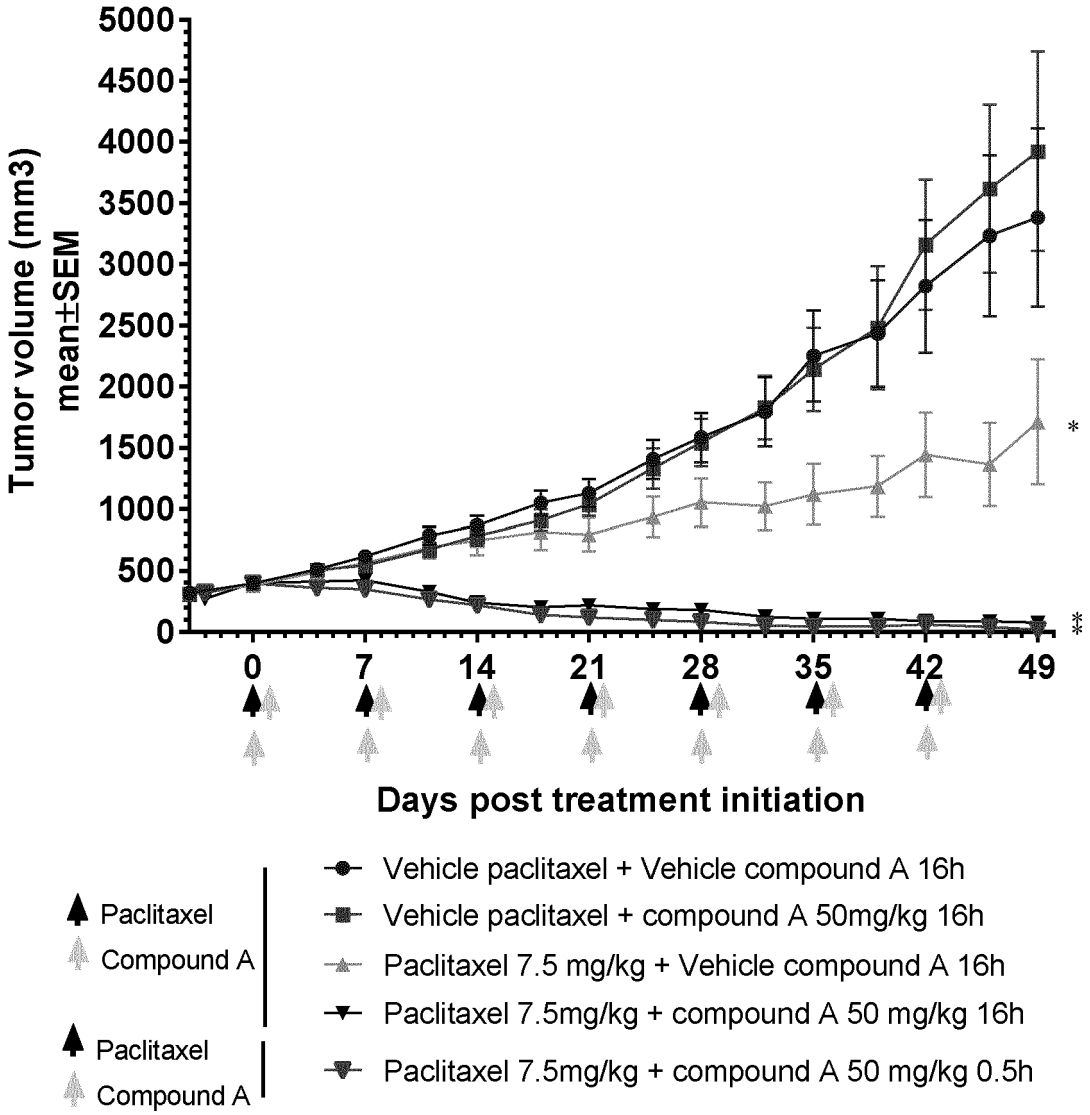

Tumor volume (mm3)
mean±SEM
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
*
**
Days post treatment initiation
Paclitaxel
Compound A
Paclitaxel
Compound A
Vehicle paclitaxel + Vehicle compound A 16h
Vehicle paclitaxel + compound A 50mg/kg 16h
Paclitaxel 7.5 mg/kg + Vehicle compound A 16h
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 16h
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 0.5h

Figure 11

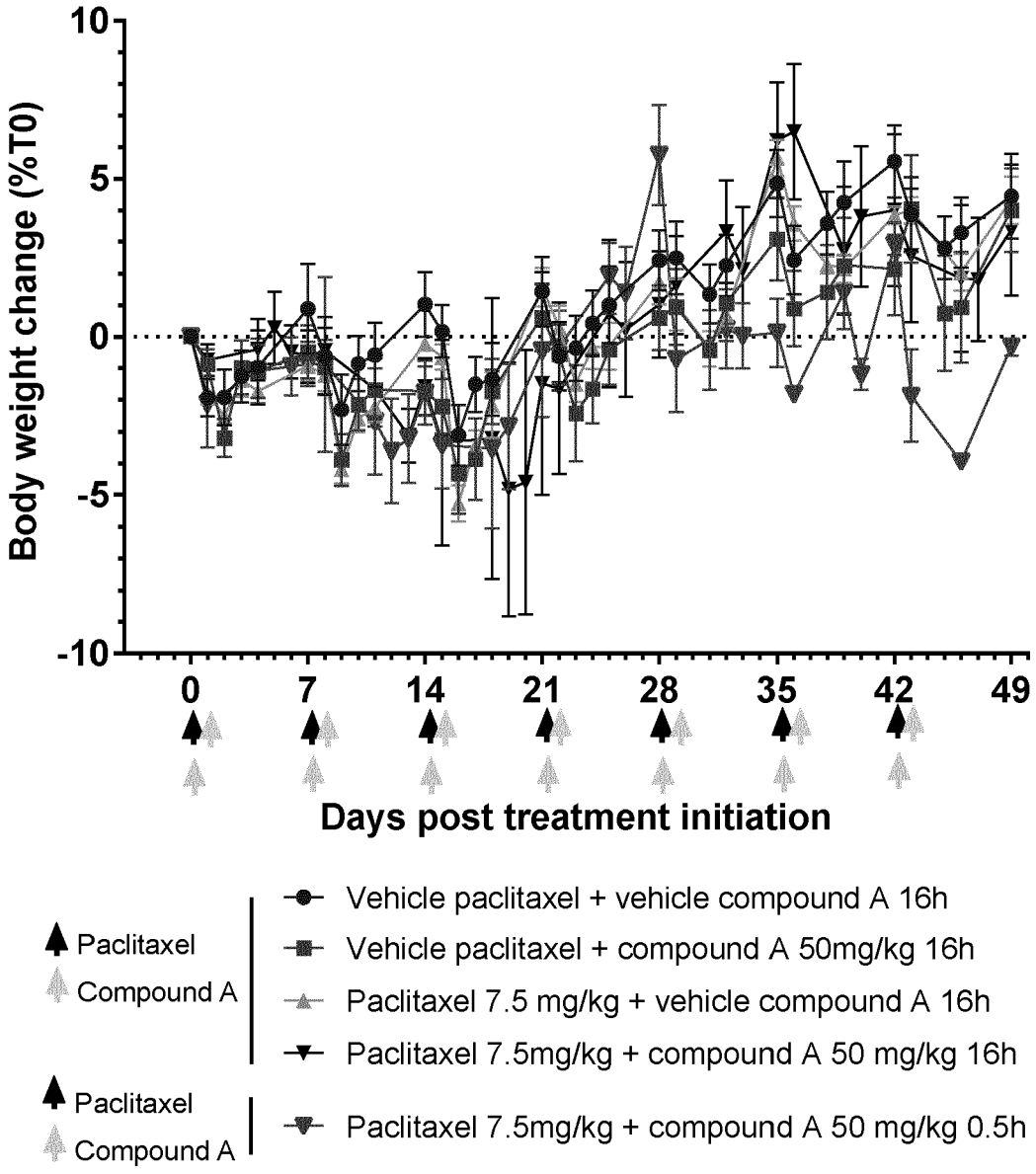

10
5
0
-5
-10
Body weight change (%T0)
0
7
14
21
28
35
42
49
Days post treatment initiation
Paclitaxel
Compound A
Vehicle paclitaxel + vehicle compound A 16h
Vehicle paclitaxel + compound A 50mg/kg 16h
Paclitaxel 7.5 mg/kg + vehicle compound A 16h
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 16h
Paclitaxel
Compound A
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 0.5h

Figure 12

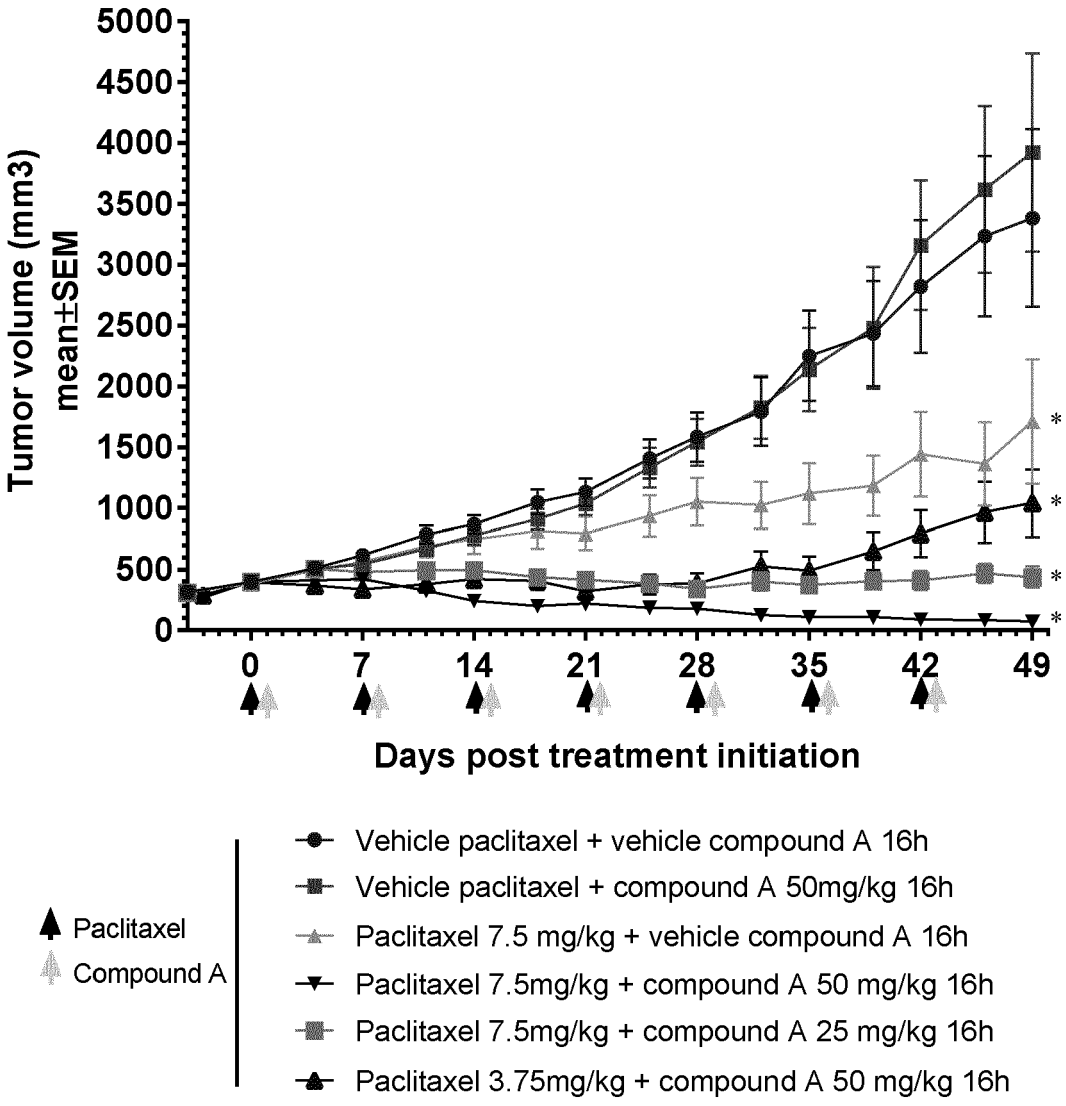

Tumor volume (mm3)
mean±SEM
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
Days post treatment initiation
*
*
*
*
Paclitaxel
Compound A
Vehicle paclitaxel + vehicle compound A 16h
Vehicle paclitaxel + compound A 50mg/kg 16h
Paclitaxel 7.5 mg/kg + vehicle compound A 16h
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 16h
Paclitaxel 7.5mg/kg + compound A 25 mg/kg 16h
Paclitaxel 3.75mg/kg + compound A 50 mg/kg 16h

Figure 13

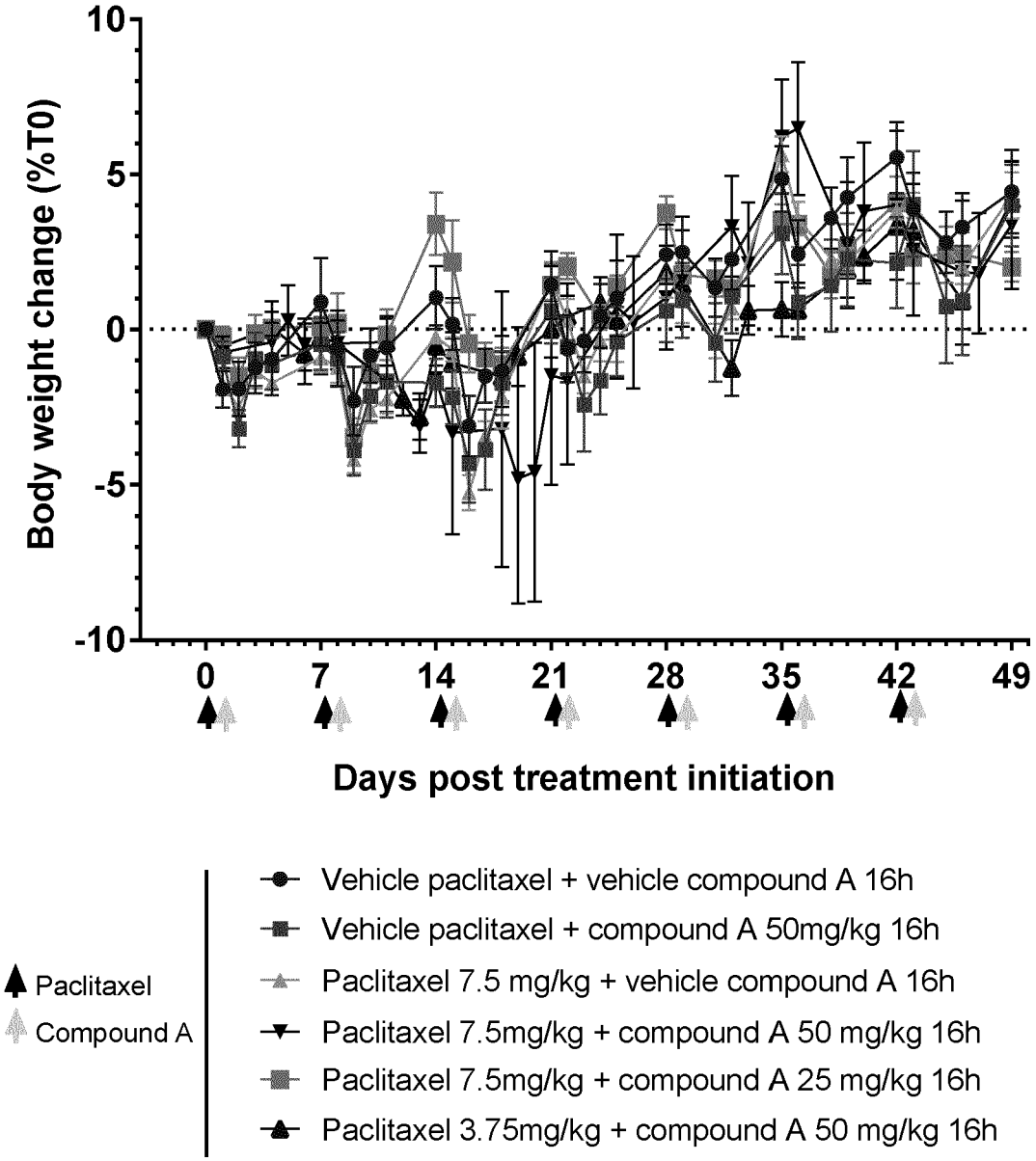

10
5
0
-5
-10
Body weight change (%T0)
0
7
14
21
28
35
42
49
Days post treatment initiation
Paclitaxel
Compound A
Vehicle paclitaxel + vehicle compound A 16h
Vehicle paclitaxel + compound A 50mg/kg 16h
Paclitaxel 7.5 mg/kg + vehicle compound A 16h
Paclitaxel 7.5mg/kg + compound A 50 mg/kg 16h
Paclitaxel 7.5mg/kg + compound A 25 mg/kg 16h
Paclitaxel 3.75mg/kg + compound A 50 mg/kg 16h

Figure 14

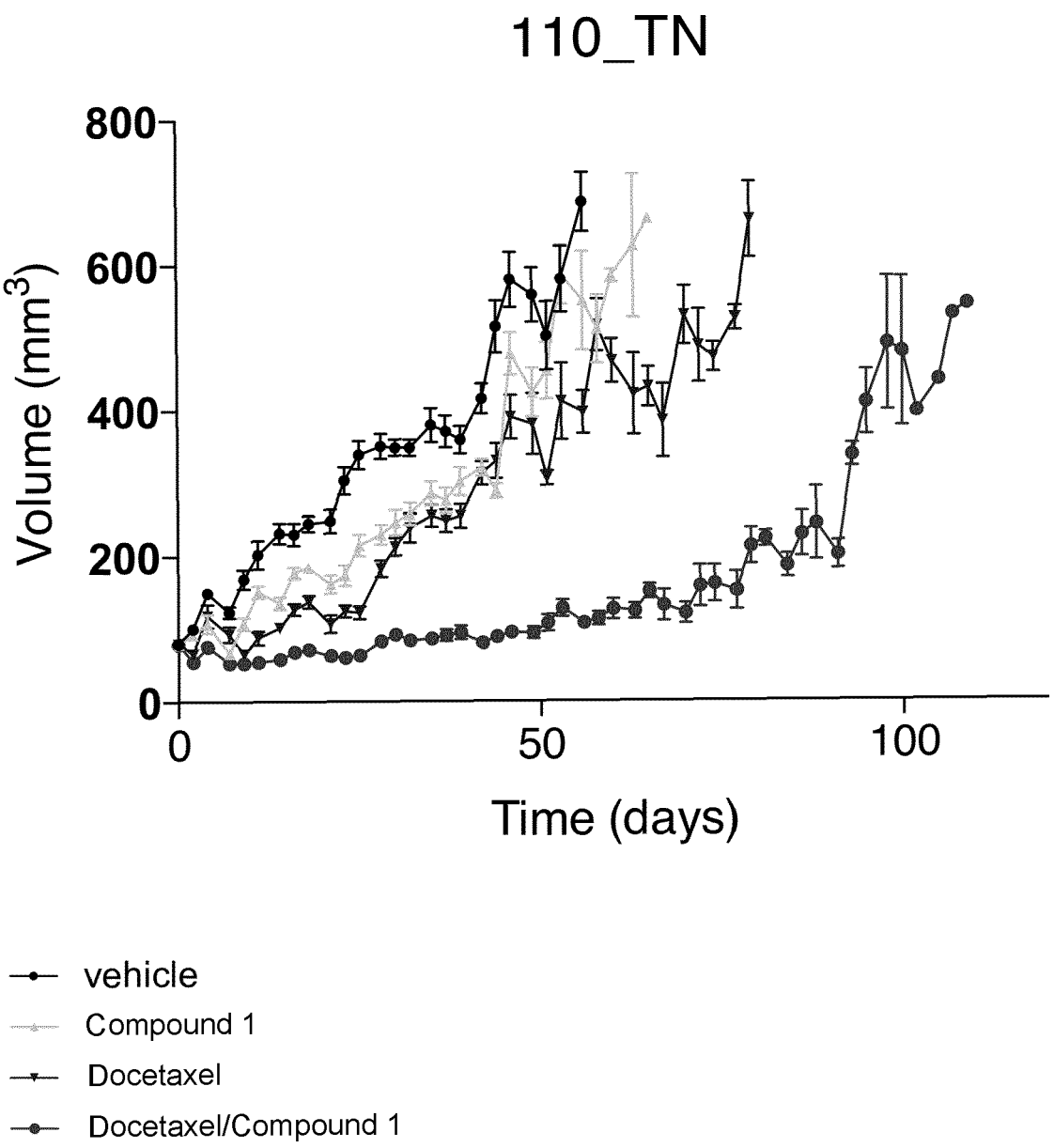
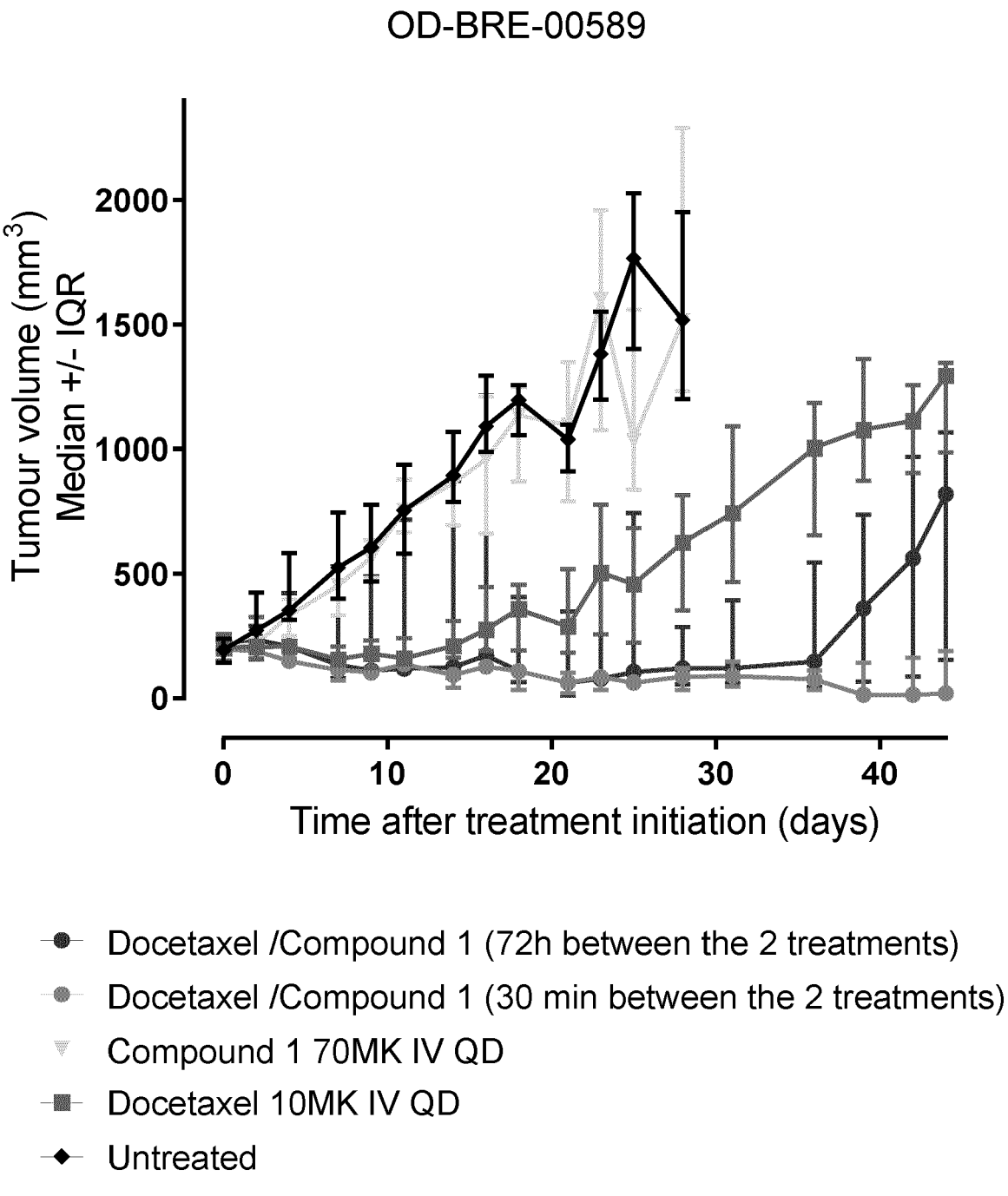

110_TN
800
600
400
200
0
Volume (mm3)
0
50
100
Time (days)
vehicle
Compound 1
Docetaxel
Docetaxel/Compound 1

Figure 15


OD-BRE-00589
Tumour volume (mm3)
Median +/- IQR
2000
1500
1000
500
0
0
10
20
30
40
Time after treatment initiation (days)
Docetaxel /Compound 1 (72h between the 2 treatments)
Docetaxel /Compound 1 (30 min between the 2 treatments)
Compound 1 70MK IV QD
Docetaxel 10MK IV QD
Untreated

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- WO 2015097123 A **[0008]**
- WO 2016207216 A **[0008]**
- WO 2016207217 A **[0008]**
- WO 2016207225 A **[0008]**
- WO 2016207226 A **[0008]**
- WO 2017125224 A **[0008]**

Non-patent literature cited in the description

- **CZABOTAR et al.** *Nature Reviews Molecular Cell Biology,* 2014, vol. 15, 49-63 **[0003]**
- **ADAMS ; CORY.** *Oncogene,* 2007, vol. 26, 1324-1337 **[0003]**
- **ZHANG et al.** *Drug Resist. Updat.,* 2007, vol. 10, 207-217 **[0003]**
- **KOTSCHY et al.** *Nature,* 2016, vol. 538, 477-482 **[0003]**
- **CHOUDHARY et al.** *Cell Death and Disease,* 2015, vol. 6, e1593 **[0003]**
- **SONG et al.** *European J. Pharm. Sc.,* 2015, vol. 70, 64-71 **[0003]**
- **CURTIS et al.** *Nature,* 2012, vol. 486, 346-352 **[0004]**
- **PEROU et al.** *Nature,* 2000, vol. 406, 747-752 **[0004]**
- **MERINO et al.** *Oncogene,* 2016, vol. 35, 1877-1887 **[0004]**
- **BEROUKIM et al.** *Nature,* 2010, vol. 463, 899-905 **[0004]**
- **WERTZ et al.** *Nature,* 2011, vol. 471, 110-114 **[0004]**
- **GOODWIN et al.** *Cell Death Differ.,* 2015 **[0004]**
- **LIEDTKE et al.** *J. Clin. Oncol.,* 2008, vol. 26 (8), 1275-1281 **[0004]**
- *Rothschild Cancers,* 2015, vol. 7 (2), 930-949 **[0005]**
- **LEHAR et al.** *Nature Biotechnology,* 2009, vol. 27 (7), 659-66 **[0055]**
- **PRICHARD et al.** *Antimicrobial Agents and Chemotherapy,* 1991, vol. 35, 1060-5 **[0072]**
- **OAKES et al.** *Proceedings of the National Academy of Sciences of the USA,* 2012, vol. 109, 2766-71 **[0076]**