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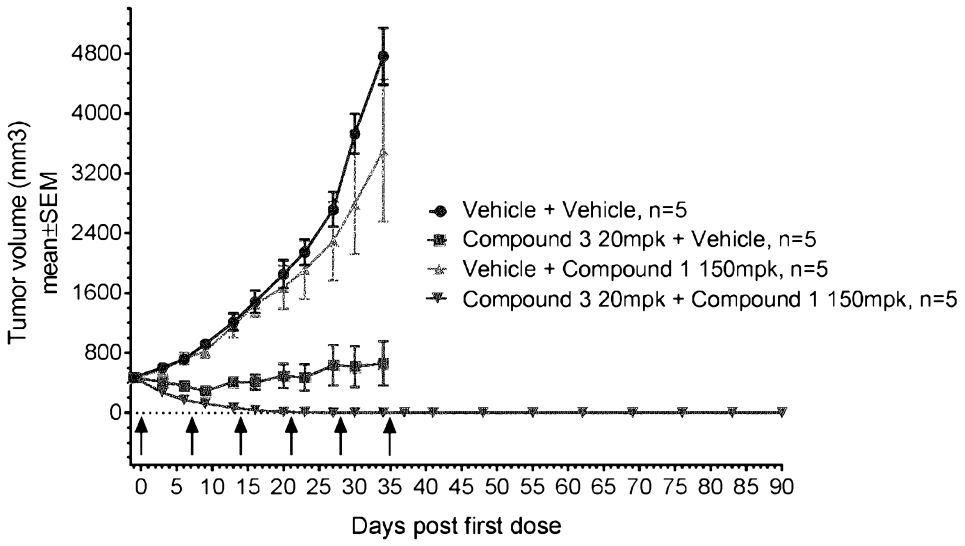
(71) Applicants: **LES LABORATOIRES SERVIER** [—/FR]; 35 rue de Verdun, 92284 SURESNES (FR). **NOVARTIS AG** [CH/CH]; Lichtstrasse 35, 4056 Basel (CH).

(72) Inventors: **WEI, Andrew**; 14 The Avenue, Victoria, Surrey Hills, Victoria 3127 (AU). **MOUJALLED, Donia**; 13 Parfrey avenue, Victoria, Lalor, Victoria 3075 (AU). **POMILIO, Giovanna**; 1 Hyde Street, Victoria, Hadfield, Victoria 3046 (AU). **MARAGNO, Ana Leticia**; 4, Allée Anastasi, 78290 Croissy-sur-Seine (FR). **GENESTE, Olivier**; Bâtiment A, 17, rue Crevel Duval, 92500 RUEIL MALMAISON (FR). **CLAPERON, Audrey**; 30, rue du 22 septembre, 92400 Courbevoie (FR). **MAACKE, Heiko**; c/o Novartis Pharma AG, Postfach, Novartis Institutes for Biomed. Research, 4002 Basel (CH). **HALILOVIC, Ensar**; c/o Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts MA 02139 (US). **PORTER, Dale**; c/o Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts MA 02139 (US). **MORRIS, Erick**; c/o Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts 02139 (US). **WANG, Youzhen**; c/o Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts MA 02139 (US). **SANGHAVI, Sneha**; c/o Novartis Institutes for Biomedical Research, Inc., 250 Massachusetts Avenue, Cambridge, Massachusetts MA 02139 (US). **MISTRY, Prakash**; c/o Novartis Pharma AG, Postfach, Novartis Institutes for Biomed. Research, 4002 Basel (CH).

(54) Title: COMBINATION OF A BCL-2 INHIBITOR AND A MCL-1 INHIBITOR, USES AND PHARMACEUTICAL COMPOSITIONS THEREOF

Figure 5. Anti-tumor effects of Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in lymphoma Karpass422 xenograft model in rats.



(57) Abstract: A combination comprising a BCL-2 inhibitor and a MCL1 inhibitor, and compositions and uses thereof.

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FIELD OF THE INVENTION

The present invention relates to a combination of a BCL-2 inhibitor and a MCL1 inhibitor. The invention also relates to the use of said combination in the treatment of cancer, in particular leukaemia, lymphoma, multiple myeloma, neuroblastoma and lung cancer, and more especially acute myeloid leukaemia, T-cell acute lymphoblastic leukemia, B-cell acute lymphoblastic leukemia, mantle cell lymphoma, diffuse large B-cell lymphoma and small cell lung cancer. Also provided are pharmaceutical formulations suitable for the administration of such combinations.

BACKGROUND OF THE INVENTION

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, MCL1 and BCL-2a1; and (iii) the pro-apoptotic effector proteins BAX and BAK (Czabotar et al, *Nature Reviews Molecular cell biology* 2014 Vol 15:49-63). Overexpression of the anti-apoptotic members of BCL-2 family is observed in many cancers, particularly in hematological malignancies such as mantle cell lymphoma (MCL), follicular lymphoma/diffuse large B-cell lymphoma (FL/D) and multiple myeloma (Adams and Cory *Oncogene* 2007 Vol 26:1324-1337). Pharmacological inhibition of the anti-apoptotic proteins BCL-2, BCL-XL and Bcl-w by the recently developed BH3-mimetics drugs such as ABT-199 and ABT-263 has emerged as a therapeutic strategy to induce apoptosis and cause tumor regression in cancer (Zhang et al, *Drug Resist Updat* 2007 Vol 10(6):207-17). Nevertheless, mechanisms of resistance to these drugs have been observed and investigated (Choudhary GS et al, *Cell Death and Disease* 2015 Vol 6, e1593; doi:10.1038/cddis.2014.525).

Acute myeloid leukaemia (AML) is a rapidly fatal blood cancer arising from clonal transformation of hematopoietic stem cells resulting in paralysis of normal bone marrow

function and deaths due to complications from profound pancytopenia. AML accounts for 25% of all adult leukaemias, with the highest incidence rates occurring in the United States, Australia and Europe (WHO. GLOBOCAN 2012. Estimated cancer incidence, mortality and prevalence worldwide in 2012. International Agency for Research on Cancer). Globally, there are approximately 88,000 new cases diagnosed annually. AML continues to have the lowest survival rate of all leukaemias, with expected 5-year survival of only 24%. Although the standard therapy for AML (cytarabine in combination with anthracyclines) was conceived over 4 decades ago, the introduction of successful targeted therapies for this disease has remained an elusive goal. Furthermore, there remains a need for a chemotherapy-free treatment option for patients with AML. The concept of targeted therapy in AML has been hampered by the realisation that this disease evolves as a multi-clonal hierarchy, with rapid outgrowth of leukaemic sub-clones as a major cause of drug resistance and disease relapse (Ding L et al, *Nature* 2012 481:506-10). Recent clinical investigations have demonstrated the efficacy of BCL-2 inhbibitors in the treatment of AML (Konopleva M et al, *American Society of Hematology* 2014:118). Although these inhibitors are clinically active, it is likely that other BCL-2 family members will need to be targeted in order to enhance the overall efficacy in AML. In addition to BCL-2, MCL1 has also been identified as an important regulator of cell survival in AML (Glaser SP et al, *Genes & development* 2012 26:120-5).

Multiple myeloma (MM) is a rare and incurable disease that is characterized by the accumulation of clonal plasma cells in the bone marrow (BM) and accounts for 10% of all haematological malignancies. In Europe, there are approximately 27,800 new cases each year. Due to the availability of new agents in recent years including bortezomib and lenalidomide, and autologous stem cell transplant (ASCT), the survival rate has improved. However, the response to these new agents is frequently not durable and it became an evidence that new treatments are needed, especially for relapsed/ refractory patients and patients with unfavorable prognostic (unfavorable cytogenetic profil). Recent investigations suggest a promising activity of BCL-2 inhibitors in a sub-group of multiple myeloma patients (Touzeau C, Dousset C, Le Gouill S, et al. *Leukemia*. 2014; 28(1):210-212). MCL1 has also been identified as an important regulator of cell survival in multiple

myeloma (Derenne S, Monia B, Dean NM, et al. *Blood*. 2002;100(1):194-199; Zhang B, Gojo I, Fenton RG. *Blood*. 2002;99(6):1885-1893).

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common type (25-35%) of Non-Hodgkin Lymphoma with 24 000 new patients/year. DLBCL is a heterogeneous disease with over a dozen subtypes, including double-hit/MYC translocation, Activated B-Cell (ABC) and Germinal Center B-cell (GCB). Modern immune chemotherapy (R-CHOP) cures approximately 60% of patients with DLBCL, but for the 40% remaining, there is little therapeutic option and the prognostic is poor. Thus, there is a high medical need to increase cure rates and clinical outcomes in high risk DLBCL such as ABC subtype (35% of DLBCL) that display constitutive activation of the prosurvival NF-κB pathway.

Neuroblastoma (NB) is the most common extra-cranial solid tumor in infants and children, representing 8%-10% of all childhood tumors stratified currently into low-, intermediate-, or high-risk. It accounts for approximately 15% of all cancer-related deaths in the pediatric population. The incidence of NB is 10.2 cases per million children under 15 years of age, and nearly 500 new cases are reported annually. The median age of diagnosis is 22 months. Outcomes in patients with NB have improved steadily over the last 30 years with 5-year survival rates rising from 52% to 74%. However, it is estimated that 50-60% of patients in the high-risk group experience relapse, and as such, they have only seen a modest decrease in mortality. The median time to relapse was 13.2 months, and 73% of those who relapsed were 18 months or older. Taken together, NB overall survival rates remain quite abysmal (~20% at 5 years) despite more aggressive therapies (Colon and Chung, *Adv Pediatr* 2013 58:297-311). The mainstay of treatment consists of chemotherapy, surgical resection, and/or radiotherapy. However, many aggressive NB have developed resistance to chemotherapeutic agents, making the likelihood of relapse quite high (Pinto et al, *J Clin Oncol* 201533:3008-11). Standards of care for NB depending on risk stratification are frequently carboplatin, cisplatin cyclophosphamide, doxorubicin, etoposide, cytokines (GM-CSF and IL2), and vincristine. Relapse after initial response to chemotherapy is the major reason for treatment failure especially in high-risk NB.

Chemoresistance may derive from the activation of prosurvival BCL-2 proteins (*e.g.* BCL-2 and MCL1 proteins). NB express high level of BCL-2 and MCL1 and low level of BCL-

XL. Inhibition of BCL-2 sensitizes cell to death and induces NB tumor regression *in vivo* (Ham et al, *Cancer Cell* 29:159-172). Antagonisms of BCL-2 and MCL1 restore chemotherapy in high-risk NB (Lestini et al, *Cancer Biol Ther* 2009 8:1587-1595; Tanos et al, *BMC Cancer* 2016 16:97). Thus, there is strong rational to combine BCL-2 and MCL1 inhibitors in naïve or resistant patients.

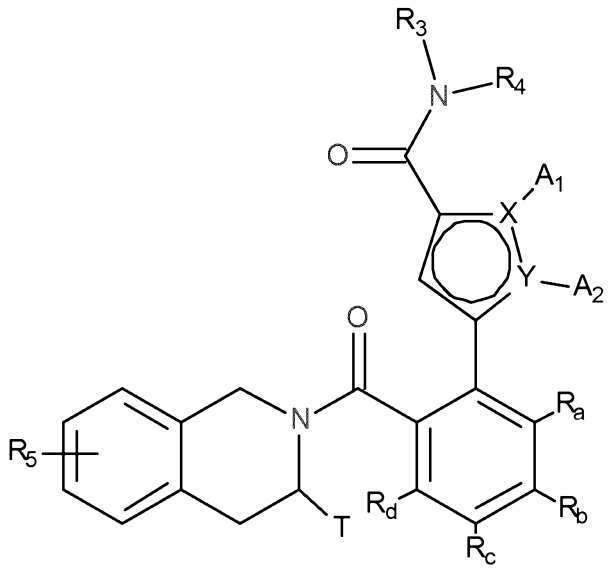
The present invention provides a novel combination of a BCL-2 inhibitor and a MCL1 inhibitor. The results show that with the development of potent small molecules targeting BCL-2 and MCL1, highly synergistic pro-apoptotic activity is revealed in primary human AML samples (Figure 2A and 17) as well as in AML (Figures 9, 13 and 14), multiple myeloma (Example 4), lymphoma (Figures 4 and 12), neuroblastoma (Figure 10), T-ALL, B-ALL cell lines (Figure 11) and in small cell lung cancer cell lines (Figures 15 (a)-(e)). We also show that combined BCL-2 and MCL1 targeting *in vivo* is efficacious at tolerated doses in AML and lymphoma xenograft models in mouse and rats (Figures 2, 5, 6, 7, 8 and 16), and dramatically increases time to relapse in AML (Figures 2B and 2C). Furthermore, in clonogenic assays, we demonstrate that BCL-2+MCL1 targeting is specifically toxic to leukemogenic cells, but not normal hematopoietic stem cells (Figure 3), in contrast to prior MCL1 gene targeting experiments in mice. Prior to the development of these potent and selective inhibitors, the feasibility of targeting both BCL-2 and MCL1, remained uncertain. Previous lineage-specific deletion models indicated potential risk to cardiac (Wang X et al, *Genes & development*. 2013;27(12):1351-1364; Thomas RL et al, *Genes & development*. 2013;27(12):1365-1377), granulocyte/hematopoietic (Opferman J et al, *Science's STKE*. 2005;307(5712):1101; Dzhagalov I et al, *Blood*. 2007;109(4):1620-1626; Steimer DA et al, *Blood*. 2009;113(12):2805-2815), thymocyte (Dunkle A et al, *Cell Death & Differentiation*. 2010;17(6):994-1002), neuronal (Arbour N et al, *Journal of Neuroscience*. 2008;28(24):6068-6078) and liver function (Hikita H et al, *Hepatology*. 2009;50(4):1217-1226 ; Vick B et al, *Hepatology*. 2009;49(2):627-636) resulting from long-term ablation of MCL1. Despite these concerns, weekly, twice weekly and even daily (during 5 consecutive days) intravenous delivery of a new potent short-acting pharmacological inhibitor of MCL1 has recently been shown to be well tolerated and active against a range of cancers *in vivo*, including AML (Kotschy A et al, *Nature*. 2016;538(7626):477-482; WO 2015/097123). The short half-life of MCL1 protein may permit sufficient time for its

regeneration in critical organs, thereby permitting physiological tolerance to MCL1 inhibitors short-term exposure (Yang T et al, *Journal of cellular physiology*. 1996;166(3):523-536). Until now, pulsatile inhibition of BCL-2 and MCL1 mimicking a drug-like effect has not been possible using genetically engineered approaches. The studies using BCL-2 and MCL1 inhibitors according to the present invention provide proof-of-concept demonstration that intermittent exposure to these drugs may be sufficient to trigger apoptosis and clinical response among highly sensitive diseases, such as AML, without concurrent toxicity to major organ systems.

The synergistic effect of targeting both BCL-2 and MCL1 *in vitro* and *in vivo* and the non-toxicity to normal marrow production when targeting both anti-apoptotic proteins have only been demonstrated through combination of potent small molecule inhibitors. These aspects were not anticipated by the results of gene targeting experiments, which would predict that MCL1 deletion is poorly tolerated by hematopoietic stem cells.

SUMMARY OF THE INVENTION

The present invention relates to a combination comprising (a) a BCL-2 inhibitor of formula (I):



(I)

wherein:

- X and Y represent a carbon atom or a nitrogen atom, it being understood that they may not simultaneously represent two carbons atoms or two nitrogen atoms,

- A_1 and A_2, together with the atoms carrying them, form an optionally substituted, aromatic or non-aromatic heterocycle Het composed of 5, 6 or 7 ring members which may contain, in addition to the nitrogen represented by X or by Y, from one to 3 hetero atoms selected independently from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be substituted by a group representing a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a group -C(O)-O-Alk wherein Alk is a linear or branched (C_1-C_6)alkyl group,
  or A_1 and A_2 independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_1-C_6)alkyl group or a cycloalkyl,
- T represents a hydrogen atom, a linear or branched (C_1-C_6)alkyl group optionally substituted by from one to three halogen atoms, a group (C_1-C_4)alkyl-NR_1R_2, or a group (C_1-C_4)alkyl-OR_6,
- R_1 and R_2 independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
  or R_1 and R_2 form with the nitrogen atom carrying them a heterocycloalkyl,
- R_3 represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a cycloalkyl group, a (C_3-C_{10})cycloalkyl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched, a heterocycloalkyl group, an aryl group or a heteroaryl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_4 represents an aryl group, a heteroaryl group, a cycloalkyl group or a linear or branched (C_1-C_6)alkyl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_5 represents a hydrogen or halogen atom, a linear or branched (C_1-C_6)alkyl group, or a linear or branched (C_1-C_6)alkoxy group,
- R_6 represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- R_a, R_b, R_c and R_d, each independently of the others, represent R_7, a halogen atom, a linear or branched (C_1-C_6)alkoxy group, a hydroxy group, a linear or branched (C_1-C_6)polyhaloalkyl group, a trifluoromethoxy group, -NR_7R_7', nitro, R_7-CO-(C_0-C_6)alkyl-, R_7-CO-NH-(C_0-C_6)alkyl-, NR_7R_7'-CO-(C_0-C_6)alkyl-,

NR_7R_7'-CO-(C_0-C_6)alkyl-O-, R_7-SO_2-NH-(C_0-C_6)alkyl-, R_7-NH-CO-NH-(C_0-C_6)alkyl-, R_7-O-CO-NH-(C_0-C_6)alkyl-, a heterocycloalkyl group, or the substituents of one of the pairs (R_a,R_b), (R_b,R_c) or (R_c,R_d) form together with the carbon atoms carrying them a ring composed of from 5 to 7 ring members, which may contain from one to 2 hetero atoms selected from oxygen and sulphur, it also being understood that one or more carbon atoms of the ring defined hereinbefore may be deuterated or substituted by from one to 3 groups selected from halogen and linear or branched (C_1-C_6)alkyl,

- R_7 and R_7' independently of one another represent a hydrogen, a linear or branched (C_1-C_6)alkyl, a linear or branched (C_2-C_6)alkenyl, a linear or branched (C_2-C_6)alkynyl, an aryl or a heteroaryl, or R_7 and R_7' together with nitrogen atom carrying them form a heterocycle composed of from 5 to 7 ring members,

it being understood that when the compound of formula (I) contains a hydroxy group, the latter may be optionally converted into one of the following groups: –OPO(OM)(OM'), -OPO(OM)($O^-M_1^+$), –OPO($O^-M_1^+$)($O^-M_2^+$), –OPO(O^-)(O^-)M_3^{2+}, –OPO(OM)(O[CH_2CH_2O]$_n$$CH_3$), or –OPO($O^-M_1^+$)(O[$CH_2CH_2O$]$_n$$CH_3$), wherein M and M' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a cycloalkyl or a heterocycloalkyl, both composed of from 5 to 6 ring members, while M_1^+ and M_2^+ independently of one another represent a pharmaceutically acceptable monovalent cation, M_3^{2+} represents a pharmaceutically acceptable divalent cation, and n is an integer from 1 to 5,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 4 hetero atoms selected from oxygen, sulphur and nitrogen (including quaternary nitrogens),
- "cycloalkyl" means any mono- or bi-cyclic, non-aromatic, carbocyclic group containing from 3 to 10 ring members,

- "heterocycloalkyl" means any mono- or bi-cyclic, non-aromatic, condensed or spiro group composed of 3 to 10 ring members and containing from 1 to 3 hetero atoms selected from oxygen, sulphur, SO, SO_2 and nitrogen,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the groups alkyl, alkenyl, alkynyl and alkoxy to be substituted by from 1 to 3 groups selected from: linear or branched (C_1-C_6)alkyl optionally substituted by a hydroxyl, a morpholine, 3-3-difluoropiperidine or a 3-3-difluoropyrrolidine; (C_3-C_6)spiro; linear or branched (C_1-C_6)alkoxy optionally substituted by a morpholine; (C_1-C_6)alkyl-S-; hydroxyl; oxo; *N*-oxide; nitro; cyano; -COOR'; -OCOR'; NR'R"; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; (C_1-C_6)alkylsulphonyl; halogen; aryl optionally substituted by one or more halogens; heteroaryl; aryloxy; arylthio; cycloalkyl; heterocycloalkyl optionally substituted by one or more halogen atoms or alkyl groups, wherein R' and R" independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group optionally substituted by a methoxy,

it being possible for the Het group defined in formula (I) to be substituted by from one to three groups selected from linear or branched (C_1-C_6)alkyl, hydroxy, linear or branched (C_1-C_6)alkoxy, $NR_1'R_1"$ and halogen, it being understood that R_1' and $R_1"$ are as defined for the groups R' and R" mentioned hereinbefore,

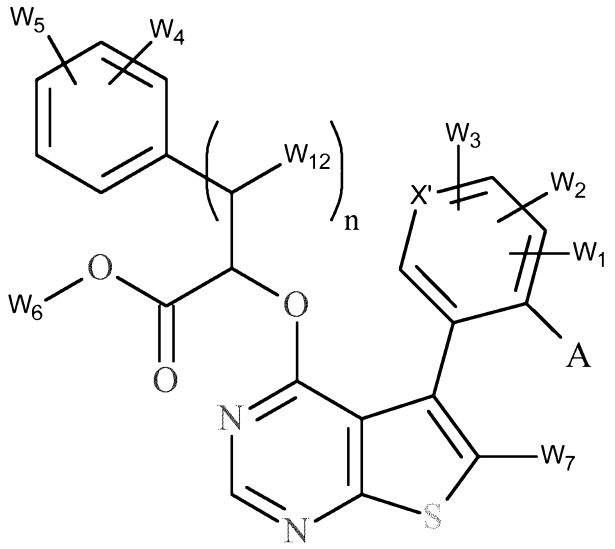
or its enantiomers, diastereoisomers, or addition salts thereof with a pharmaceutically acceptable acid or base,

and (b) a MCL1 inhibitor.

Said compounds of formula (I), their synthesis, their use in the treatment of cancer and pharmaceutical formulations thereof, are described in WO 2013/110890, WO 2015/011397, WO 2015/011399 and WO 2015/011400, the contents of which are incorporated by reference.

In certain embodiments, the MCL1 inhibitor is selected from A-1210477 (*Cell Death and Disease* 2015 6, e1590; doi:10.1038/cddis.2014.561) and the compounds described in WO 2015/097123, WO 2016/207216, WO 2016/207217, WO 2016/207225, WO 2016/207226, or in WO 2016/033486, the contents of which are incorporated by reference.

The present invention also relates to a combination comprising (a) a BCL-2 inhibitor and (b) a MCL1 inhibitor of formula (II):



(II)

wherein:

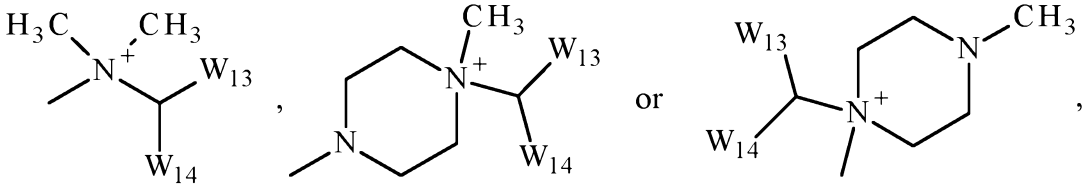
- A represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a linear or branched (C_1-C_6)polyhaloalkyl, a hydroxy group, a cyano, -$NW_{10}W_{10}$', -Cy_6 or an halogen atom,
- W_1, W_2, W_3, W_4 and W_5 independently of one another represent a hydrogen atom, a halogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)polyhaloalkyl, a hydroxy group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a cyano, a nitro group, -alkyl(C_0-C_6)-NW_8W_8', -O-Cy_1, -alkyl(C_0-C_6)-Cy_1, -alkenyl(C_2-C_6)-Cy_1, -alkynyl(C_2-C_6)-Cy_1, -O-alkyl(C_1-C_6)-W_9, -C(O)-OW_8, -O-C(O)-W_8,

-C(O)-NW_8W_8', -NW_8-C(O)-W_8', -NW_8-C(O)-OW_8', -alkyl(C_1-C_6)-NW_8-C(O)-W_8', -SO_2- NW_8W_8', -SO_2-alkyl(C_1-C_6),

or the substituents of one of the pairs (W_1, W_2), (W_2, W_3), (W_1, W_3), (W_4, W_5) when grafted onto two adjacent carbon atoms, form together with the carbon atoms carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that resulting ring may be substituted by a group selected from a linear or branched (C_1-C_6)alkyl group, -$NW_{10}W_{10}$', -alkyl(C_0-C_6)-Cy_1 or an oxo,

- X' represents a carbon or a nitrogen atom,
- W_6 represents a hydrogen, a linear or branched (C_1-C_8)alkyl group, an aryl, an heteroaryl group, an arylalkyl(C_1-C_6) group, an heteroarylalkyl(C_1-C_6) group,
- W_7 represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, -Cy_3, -alkyl(C_1-C_6)-Cy_3, -alkenyl(C_2-C_6)-Cy_3, -alkynyl(C_2-C_6)-Cy_3, -Cy_3-Cy_4, -alkynyl(C_2-C_6)-O-Cy_3, -Cy_3-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_4, an halogen atom, a cyano, -C(O)-W_{11}, -C(O)-$NW_{11}W_{11}$',
- W_8 and W_8' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, or -alkyl(C_0-C_6)-Cy_1,

  or (W_8, W_8') form together with the nitrogen atom carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain in addition to the nitrogen atom from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be substituted by a group representing a hydrogen atom, or a linear or branched (C_1-C_6)alkyl group and it being understood that one or more of the carbon atoms of the possible substituents, may be deuterated,
- W_9 represents -Cy_1, -Cy_1-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-NW_8-alkyl(C_0-C_6)-Cy_2, -Cy_1-Cy_2-O-alkyl(C_0-C_6)-Cy_5, -C(O)-NW_8W_8', -NW_8W_8', -OW_8,-NW_8-C(O)-W_8', -O-alkyl(C_1-C_6)-OW_8, -SO_2-W_8, -C(O)-OW_8, -NH-C(O)-NH-W_8,



it being possible for the ammonium so defined to exist as a zwitterionic form or to have a monovalent anionic counterion,

- W_{10}, W_{10}', W_{11} and W_{11}' independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- W_{12} represents a hydrogen or a hydroxy group,
- W_{13} represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- W_{14} represents a -O-P(O)(O^-)(O^-) group, a -O-P(O)(O^-)(OW_{16}) group, a -O-P(O)(OW_{16})(OW_{16}') group, a -O-SO_2-O^- group, a -O-SO_2-OW_{16} group, -Cy_7, a -O-C(O)-W_{15} group, a -O-C(O)-OW_{15} group or a -O-C(O)-$NW_{15}W_{15}$' group,
- W_{15} and W_{15}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a linear or branched amino(C_1-C_6)alkyl group,
- W_{16} and W_{16}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or an arylalkyl(C_1-C_6) group,
- Cy_1, Cy_2, Cy_3, Cy_4, Cy_5, Cy_6 and Cy_7 independently of one another, represent a cycloalkyl group, a heterocycloalkyl group, an aryl or an heteroaryl group,
- n is an integer equal to 0 or 1,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl, indanyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 3 heteroatoms selected from oxygen, sulphur and nitrogen,
- "cycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members,
- "heterocycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members, and containing from 1 to 3 heteroatoms selected from oxygen, sulphur and nitrogen, which may include fused, bridged or spiro ring systems,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the alkyl, alkenyl, alkynyl, alkoxy, to be substituted by from 1 to 4 groups selected from linear or branched (C_1-C_6)alkyl which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy which may be substituted by a linear or branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, hydroxy, halogen, oxo, -NW'W'', -O-C(O)-W', or -CO-NW'W''; linear or branched (C_2-C_6)alkenyl group; linear or branched (C_2-C_6)alkynyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; linear or branched (C_1-C_6)alkoxy which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_2-C_6)alkynyl, -NW'W'', or hydroxy; (C_1-C_6)alkyl-S- which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; hydroxy; oxo; *N*-oxide; nitro; cyano; -C(O)-OW'; -O-C(O)-W'; -CO-NW'W''; -NW'W''; -(C=NW')-OW''; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; or halogen; it being understood that W' and W'' independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; and it being understood that one or more of the carbon atoms of the preceding possible substituents, may be deuterated,

its enantiomers, diastereoisomers or atropisomers, or addition salts thereof with a pharmaceutically acceptable acid or base.

Said compounds of formula (II), their synthesis, their use in the treatment of cancer and pharmaceutical formulations thereof, are described in WO 2015/097123, the content of which is incorporated by reference.

In certain embodiments, the BCL-2 inhibitor is selected from the following compounds: 4-(4-{[2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-*N*-[(3-nitro-4-{[(oxan-4-yl)methyl]amino}phenyl)sulfonyl]-2-[(1*H*-pyrrolo[2,3-*b*]pyridin-5-yl)oxy]benzamide (venetoclax or ABT-199); 4-(4-{[2-(4-chlorophenyl)-5,5-

dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-*N*-(4-{[(2*R*)-4-(morpholin-4-yl)-1-(phenylsulfanyl)butan-2-yl]amino}-3-(trifluoromethanesulfonyl)benzenesulfonyl] benzamide (navitoclax or ABT-263); oblimersen (G3139); obatoclax (GX15-070); HA14-1; (±)-gossypol (BL-193); (-)-gossypol (AT-101); apogossypol; TW-37; antimycin A, ABT-737 (Oltersdorf T et al, *Nature* 2005 June 2;435(7042):677-81) and compounds described in WO 2013/110890, WO 2015/011397, WO 2015/011399 and WO 2015/011400, the contents of which are incorporated by reference.

According to a first aspect of the invention, there is provided a combination comprising:

(a) a BCL-2 inhibitor of formula (I) as described herein, and

(b) a MCL1 inhibitor of formula (II) as described herein.

In another embodiment, the invention provides a combination comprising:

(a) Compound 1: *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide, or a pharmaceutically acceptable salt thereof, and

(b) a MCL1 inhibitor,

for simultaneous, sequential or separate use.

In another embodiment, the invention provides a combination comprising:

(a) Compound 4: 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl}phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide, or a pharmaceutically acceptable salt thereof, and

(b) a MCL1 inhibitor,

for simultaneous, sequential or separate use.

Alternatively, the invention provides a combination comprising:

(a) a BCL-2 inhibitor, and

(b) Compound 2: (*2R*)-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)-1H-pyrazol-5-yl]methoxy}phenyl)propanoic acid,

for simultaneous, sequential or separate use.

In another embodiment, the invention provides a combination comprising:
(a) a BCL-2 inhibitor, and
(b) Compound 3: (*2R*)-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,
for simultaneous, sequential or separate use.

In another embodiment, the invention provides a combination as described herein, for use in the treatment of cancer.

In another embodiment, the invention provides the use of a combination as described herein, in the manufacture of a medicament for the treatment of cancer.

In another embodiment, the invention provides a medicament containing, separately or together,
(a) a BCL-2 inhibitor of formula (I) and
(b) a MCL1 inhibitor,
or
(a) a BCL-2 inhibitor and
(b) a MCL1 inhibitor of formula (II),
for simultaneous, sequential or separate administration, and wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in effective amounts for the treatment of cancer.

In another embodiment, the invention provides a method of treating cancer, comprising administering a jointly therapeutically effective amount of:
(a) a BCL-2 inhibitor of formula (I) and
(b) a MCL1 inhibitor,
or
(a) a BCL-2 inhibitor and
(b) a MCL1 inhibitor of formula (II),

to a subject in need thereof.

In another embodiment, the invention provides a method for sensitizing a patient 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 jointly therapeutically effective amount of:

(a) a BCL-2 inhibitor of formula (I) and

(b) a MCL1 inhibitor,

or

(a) a BCL-2 inhibitor and

(b) a MCL1 inhibitor of formula (II),

to said patient.

In a particular embodiment, the BCL-2 inhibitor is *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide hydrochloride (Compound 1, HCl).

In a particular embodiment, the BCL-2 inhibitor is 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl}phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide hydrochloride (Compound 4, HCl).

In another embodiment, the BCL-2 inhibitor is ABT-199.

In another embodiment, the MCL1 inhibitor is (*2R*)-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).

In another embodiment, the MCL1 inhibitor is (*2R*)-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 3).

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. **Expression of BCL-2 and MCL1 is prevalent in AML.** 7 AML cell lines and 13 primary AML samples with >70% blasts were immunoblotted for indicated proteins, showing that BCL-2 and MCL1 proteins are dominantly expressed in contrast to BCL-XL, which was expressed in a lower proportion of samples.

Figure 2. **Combined BCL-2/MCL1 targeting has synergistic activity in AML *in vitro* and *in vivo*.** (A) 54 primary AML samples were incubated with a 6-log concentration range of Compound 1 (HCl salt), Compound 2 or a 1:1 concentration in RPMI/15% FCS for 48h and the LC_{50} determined (B) Four cohorts of NSG mice were engrafted with luciferase expressing MV4;11 cells. Tumour engraftment was verified on day 10 (baseline) and then Compound 1, HCl 100mg/d orally on weekdays (expressed as the free base) or Compound 2 25mg/kg IV twice weekly administration commenced for 4 weeks. The impact of Compound 2 and the combination with Compound 1 was evidenced by reduced luciferase bulk on days 14 and 28 after starting therapy and increased overall survival (C).

Figure 3. **Toxicity assessment of combined BCL-2/MCL1 targeting on normal CD34+ cells from normal donors or leukaemic blasts.** Sorted normal CD34+ or leukaemic blasts were plated and treated with Compound 1, HCl and Compound 2 at 1:1 ratio at the indicated concentrations. Combined Compound 1 + Compound 2 is toxic to leukaemic but not normal CD34+ progenitors.

Figure 4. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 in combination with Compound 1, HCl in DB cells (A) and Toledo cells (B).** 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 3 and

Compound 1, HCl at the concentrations tested. Synergistic effects occurred across a broad range of single agent concentrations.

Figure 5. **Anti-tumor effects of Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in lymphoma Karpass422 xenograft model in rats.**

Figure 6. **Body weight changes in animals treated with Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in lymphoma Karpass422 xenograft model in rats.**

Figure 7. **Anti-tumor effects of Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in DLBCL Toledo xenograft model in mice.**

Figure 8. **Body weight changes in animals treated with Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in DLBCL Toledo xenograft model in mice.**

Figure 9. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the AML cell line OCI-AML3 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 3 and Compound 1, HCl at the concentrations tested. Synergistic effects occurred across a broad range of single agent concentrations.

Figure 10. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the NB cell line LAN-6 in two independent experiments (N1: upper**

panel; N2: lower panel). 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 3 and Compound 1, HCl at the concentrations tested.

Figure 11. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the B-ALL cell line NALM-6 in two independent experiments (N1: upper panel; N2: lower panel)**

Figure 12. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Com pound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the MCL cell line Z-138.**

Figure 13. **Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with ABT-199 (BCL-2 inhibitor) in AML cell line OCI-AML3 in two independent experiments (N1: upper panel; N2: lower panel).**

Figure 14. **Exemplary cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 4, HCl (BCL-2 inhibitor) in AML cell lines (ML-2 cells in A and OCI-AML-3 in B).**

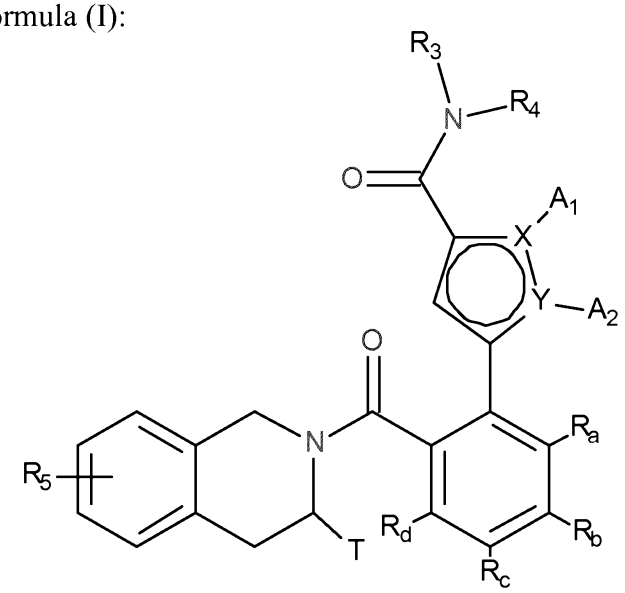
Figures 15 (a)-(e). **Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.**

Figures 16 (a)-(b). **Anti-tumor effects of Compound 1, HCl, ABT-199, Compound 3 and the combination of Compound 1, HCl or ABT-199 + Compound 3 in Patient-derived primary AML model HAMLX5343 in mice.**

Figure 17. **Heat-map comparison of AML sensitivity (LC_{50}) to BH3-mimetic monotherapy, or drug combinations (tested in 1:1 ratio), relative to chemotherapy (idarubicin) after 48h exposure. Cell viability of each primary AML samples after 48h in DMSO is shown.**

DETAILED DESCRIPTION OF THE INVENTION

The invention therefore provides in Embodiment E1, a combination comprising (a) a BCL-2 inhibitor of formula (I):



(I)

wherein:

- X and Y represent a carbon atom or a nitrogen atom, it being understood that they may not simultaneously represent two carbons atoms or two nitrogen atoms,
- A_1 and A_2, together with the atoms carrying them, form an optionally substituted, aromatic or non-aromatic heterocycle Het composed of 5, 6 or 7 ring members which may contain, in addition to the nitrogen represented by X or by Y, from one to 3 hetero atoms selected independently from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be substituted by a group

representing a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a group -C(O)-O-Alk wherein Alk is a linear or branched (C_1-C_6)alkyl group,
or A_1 and A_2 independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_1-C_6)alkyl group or a cycloalkyl,

- T represents a hydrogen atom, a linear or branched (C_1-C_6)alkyl group optionally substituted by from one to three halogen atoms, a group (C_1-C_4)alkyl-NR_1R_2, or a group (C_1-C_4)alkyl-OR_6,
- R_1 and R_2 independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
  or R_1 and R_2 form with the nitrogen atom carrying them a heterocycloalkyl,
- R_3 represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a cycloalkyl group, a (C_3-C_{10})cycloalkyl-(C_1-C_6)alkyl group wherein the alkyl moiety is linear or branched, a heterocycloalkyl group, an aryl group or a heteroaryl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_4 represents an aryl group, a heteroaryl group, a cycloalkyl group or a linear or branched (C_1-C_6)alkyl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_5 represents a hydrogen or halogen atom, a linear or branched (C_1-C_6)alkyl group, or a linear or branched (C_1-C_6)alkoxy group,
- R_6 represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- R_a, R_b, R_c and R_d, each independently of the others, represent R_7, a halogen atom, a linear or branched (C_1-C_6)alkoxy group, a hydroxy group, a linear or branched (C_1-C_6)polyhaloalkyl group, a trifluoromethoxy group, -NR_7R_7', nitro, R_7-CO-(C_0-C_6)alkyl-, R_7-CO-NH-(C_0-C_6)alkyl-, NR_7R_7'-CO-(C_0-C_6)alkyl-, NR_7R_7'-CO-(C_0-C_6)alkyl-O-, R_7-SO_2-NH-(C_0-C_6)alkyl-, R_7-NH-CO-NH-(C_0-C_6)alkyl-, R_7-O-CO-NH-(C_0-C_6)alkyl-, a heterocycloalkyl group, or the substituents of one of the pairs (R_a,R_b), (R_b,R_c) or (R_c,R_d) form together with the carbon atoms carrying them a ring composed of from 5 to 7 ring members, which may contain from one to 2 hetero atoms selected from oxygen and

sulphur, it also being understood that one or more carbon atoms of the ring defined hereinbefore may be deuterated or substituted by from one to 3 groups selected from halogen and linear or branched (C_1-C_6)alkyl,

- R_7 and R_7' independently of one another represent a hydrogen, a linear or branched (C_1-C_6)alkyl, a linear or branched (C_2-C_6)alkenyl, a linear or branched (C_2-C_6)alkynyl, an aryl or a heteroaryl, or R_7 and R_7' together with nitrogen atom carrying them form a heterocycle composed of from 5 to 7 ring members,

it being understood that when the compound of formula (I) contains a hydroxy group, the latter may be optionally converted into one of the following groups: –OPO(OM)(OM'), -OPO(OM)($O^-M_1^+$), –OPO($O^-M_1^+$)($O^-M_2^+$), –OPO(O^-)(O^-)M_3^{2+}, –OPO(OM)(O[CH_2CH_2O]$_n$$CH_3$), or –OPO($O^-M_1^+$)(O[$CH_2CH_2O$]$_n$$CH_3$), wherein M and M' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a cycloalkyl or a heterocycloalkyl, both composed of from 5 to 6 ring members, while M_1^+ and M_2^+ independently of one another represent a pharmaceutically acceptable monovalent cation, M_3^{2+} represents a pharmaceutically acceptable divalent cation, and n is an integer from 1 to 5,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 4 hetero atoms selected from oxygen, sulphur and nitrogen (including quaternary nitrogens),
- "cycloalkyl" means any mono- or bi-cyclic, non-aromatic, carbocyclic group containing from 3 to 10 ring members,
- "heterocycloalkyl" means any mono- or bi-cyclic, non-aromatic, condensed or spiro group composed of 3 to 10 ring members and containing from 1 to 3 hetero atoms selected from oxygen, sulphur, SO, SO_2 and nitrogen,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the groups alkyl, alkenyl, alkynyl and alkoxy to be substituted by from 1 to 3 groups

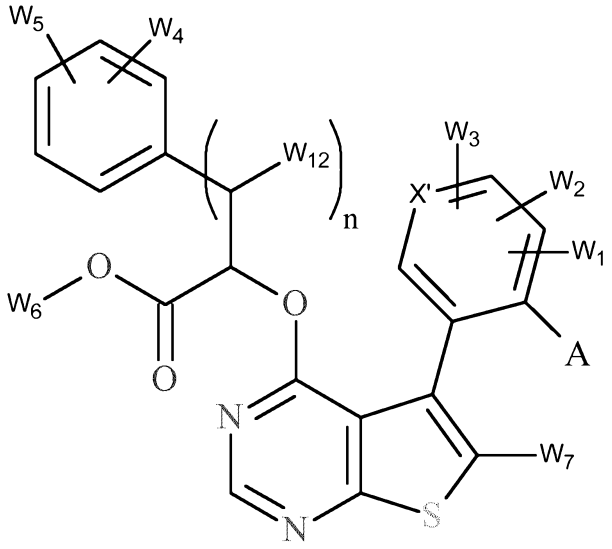
selected from: linear or branched (C_1-C_6)alkyl optionally substituted by a hydroxyl, a morpholine, 3-3-difluoropiperidine or a 3-3-difluoropyrrolidine; (C_3-C_6)spiro; linear or branched (C_1-C_6)alkoxy optionally substituted by a morpholine; (C_1-C_6)alkyl-S-; hydroxyl; oxo; *N*-oxide; nitro; cyano; -COOR'; -OCOR'; NR'R"; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; (C_1-C_6)alkylsulphonyl; halogen; aryl optionally substituted by one or more halogens; heteroaryl; aryloxy; arylthio; cycloalkyl; heterocycloalkyl optionally substituted by one or more halogen atoms or alkyl groups, wherein R' and R" independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group optionally substituted by a methoxy,

it being possible for the Het group defined in formula (I) to be substituted by from one to three groups selected from linear or branched (C_1-C_6)alkyl, hydroxy, linear or branched (C_1-C_6)alkoxy, $NR_1'R_1"$ and halogen, it being understood that R_1' and $R_1"$ are as defined for the groups R' and R" mentioned hereinbefore,

or its enantiomers, diastereoisomers, or addition salts thereof with a pharmaceutically acceptable acid or base,

and (b) a MCL1 inhibitor,
for simultaneous, sequential or separate use.

The invention also provides in embodiment E2 a combination comprising (a) a BCL-2 inhibitor and (b) a MCL1 inhibitor of formula (II):



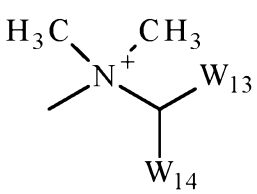
(II)

wherein:

- A represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a linear or branched (C_1-C_6)polyhaloalkyl, a hydroxy group, a cyano, -$NW_{10}W_{10}$', -Cy_6 or an halogen atom,
- W_1, W_2, W_3, W_4 and W_5 independently of one another represent a hydrogen atom, a halogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)polyhaloalkyl, a hydroxy group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a cyano, a nitro group, -alkyl(C_0-C_6)-NW_8W_8', -O-Cy_1, -alkyl(C_0-C_6)-Cy_1, -alkenyl(C_2-C_6)-Cy_1, -alkynyl(C_2-C_6)-Cy_1, -O-alkyl(C_1-C_6)-W_9, -C(O)-OW_8, -O-C(O)-W_8, -C(O)-NW_8W_8', -NW_8-C(O)-W_8', -NW_8-C(O)-OW_8', -alkyl(C_1-C_6)-NW_8-C(O)-W_8', -SO_2- NW_8W_8', -SO_2-alkyl(C_1-C_6),
  or the substituents of one of the pairs (W_1, W_2), (W_2, W_3), (W_1, W_3), (W_4, W_5) when grafted onto two adjacent carbon atoms, form together with the carbon atoms carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that resulting ring may be substituted by a group selected from a linear or branched (C_1-C_6)alkyl group, -$NW_{10}W_{10}$',

-alkyl(C_0-C_6)-Cy_1 or an oxo,

- X' represents a carbon or a nitrogen atom,
- W_6 represents a hydrogen, a linear or branched (C_1-C_8)alkyl group, an aryl, an heteroaryl group, an arylalkyl(C_1-C_6) group, an heteroarylalkyl(C_1-C_6) group,
- W_7 represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, -Cy_3, -alkyl(C_1-C_6)-Cy_3, -alkenyl(C_2-C_6)-Cy_3, -alkynyl(C_2-C_6)-Cy_3, -Cy_3-Cy_4, -alkynyl(C_2-C_6)-O-Cy_3, -Cy_3-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_4, an halogen atom, a cyano, -C(O)-W_{11}, -C(O)-$NW_{11}W_{11}$',
- W_8 and W_8' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, or -alkyl(C_0-C_6)-Cy_1,
  or (W_8, W_8') form together with the nitrogen atom carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain in addition to the nitrogen atom from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be substituted by a group representing a hydrogen atom, or a linear or branched (C_1-C_6)alkyl group and it being understood that one or more of the carbon atoms of the possible substituents, may be deuterated,
- W_9 represents -Cy_1, -Cy_1-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-NW_8-alkyl(C_0-C_6)-Cy_2, -Cy_1-Cy_2-O-alkyl(C_0-C_6)-Cy_5, -C(O)-NW_8W_8', -NW_8W_8', -OW_8,-NW_8-C(O)-W_8', -O-alkyl(C_1-C_6)-OW_8, -SO_2-W_8, -C(O)-OW_8, -NH-C(O)-NH-W_8,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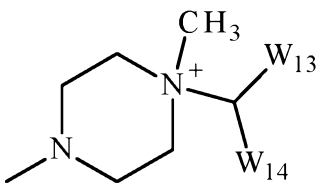
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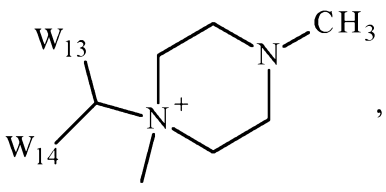

,

it being possible for the ammonium so defined to exist as a zwitterionic form or to have a monovalent anionic counterion,

- W_{10}, W_{10}', W_{11} and W_{11}' independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- W_{12} represents a hydrogen or a hydroxy group,
- W_{13} represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,

- W_{14} represents a -O-P(O)(O^-)(O^-) group, a -O-P(O)(O^-)(OW_{16}) group, a -O-P(O)(OW_{16})(OW_{16}') group, a -O-SO_2-O^- group, a -O-SO_2-OW_{16} group, -Cy_7, a -O-C(O)-W_{15} group, a -O-C(O)-OW_{15} group or a -O-C(O)-$NW_{15}W_{15}$' group,
- W_{15} and W_{15}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a linear or branched amino(C_1-C_6)alkyl group,
- W_{16} and W_{16}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or an arylalkyl(C_1-C_6) group,
- Cy_1, Cy_2, Cy_3, Cy_4, Cy_5, Cy_6 and Cy_7 independently of one another, represent a cycloalkyl group, a heterocycloalkyl group, an aryl or an heteroaryl group,
- n is an integer equal to 0 or 1,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl, indanyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 3 heteroatoms selected from oxygen, sulphur and nitrogen,
- "cycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members,
- "heterocycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members, and containing from 1 to 3 heteroatoms selected from oxygen, sulphur and nitrogen, which may include fused, bridged or spiro ring systems,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the alkyl, alkenyl, alkynyl, alkoxy, to be substituted by from 1 to 4 groups selected from linear or branched (C_1-C_6)alkyl which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy which may be substituted by a linear or branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, hydroxy, halogen, oxo, -NW'W'', -O-C(O)-W', or -CO-NW'W''; linear or branched (C_2-C_6)alkenyl group; linear or branched (C_2-C_6)alkynyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; linear or branched (C_1-C_6)alkoxy which may be substituted by a group representing a linear or

branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_2-C_6)alkynyl, -NW'W'', or hydroxy; (C_1-C_6)alkyl-S- which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; hydroxy; oxo; *N*-oxide; nitro; cyano; -C(O)-OW'; -O-C(O)-W'; -CO-NW'W''; -NW'W''; -(C=NW')-OW''; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; or halogen; it being understood that W' and W'' independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; and it being understood that one or more of the carbon atoms of the preceding possible substituents, may be deuterated,

its enantiomers, diastereoisomers or atropisomers, or addition salts thereof with a pharmaceutically acceptable acid or base,

for simultaneous, sequential or separate use.

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.

E3. A combination according to E1, wherein the MCL1 inhibitor is a compound of formula (II) as defined in E2.

E4. A combination according to any of E1 to E3, wherein the BCL-2 inhibitor is *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl) carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide.

E5. A combination according to any of E1 to E3, wherein the BCL-2 inhibitor is 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl} phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide.

E6. A combination according to E4, wherein *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide is in the form of the hydrochloride salt.

E7. A combination according to E5, wherein 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl} phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide is in the form of the hydrochloride salt.

E8. A combination according to E4 or E6, wherein the dose of *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide during the combination treatment is from 50 mg to 1500 mg.

E9. A combination according to any of E1 to E8, wherein the BCL-2 inhibitor is administered once a week.

E10. A combination according to E6 or E8, wherein *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide is administered during the combination treatment once a day.

E11. A combination according to any of E1 to E3, wherein the BCL-2 inhibitor is ABT-199.

E12. A combination according to any of E1 to E11, wherein the MCL1 inhibitor is (*2R*)-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)-1H-pyrazol-5-yl]methoxy}phenyl)propanoic acid.

E13. A combination according to any of E1 to E11, wherein the MCL1 inhibitor is (*2R*)-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.

E14. A combination according to any of E1 to E13, wherein the BCL-2 inhibitor and the MCL1 inhibitor are administered orally.

E15. A combination according to any of E1 to E13, wherein the BCL-2 inhibitor is administered orally and the MCL1 inhibitor is administered intravenously.

E16. A combination according to any of E1 to E13, wherein the BCL-2 inhibitor and the MCL1 inhibitor are administered intravenously.

E17. A combination according to any of E1 to E16, for use in the treatment of cancer.

E18. The combination for use according to E17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in amounts which are jointly therapeutically effective for the treatment of cancer.

E19. The combination for use according to E17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in amounts which are synergistically effective for the treatment of cancer.

E20. The combination for use according to E17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in synergistically effective amounts which enable a reduction of the dose required for each compound in the treatment of cancer, whilst providing an efficacious cancer treatment, with eventually a reduction in side effects.

E21. The combination for use according to any of E17 to E20, wherein the cancer is leukaemia.

E22. The combination for use according to E21, wherein the cancer is acute myeloid leukaemia, T-ALL or B-ALL.

E23. The combination for use according to any of E17 to E20, wherein the cancer is myelodysplastic syndrome or myeloproliferative disease.

E24. The combination for use according to any of E17 to E20, wherein the cancer is lymphoma.

E25. The combination for use according to any of E24, wherein the lymphoma is a non-Hodgkin lymphoma.

E26. The combination for use according to any of E25, wherein the non-Hodgkin lymphoma is diffuse large B-cell lymphoma or mantle-cell lymphoma.

E27. The combination for use according to any of E17 to E20, wherein the cancer is multiple myeloma.

E28. The combination for use according to any of E17 to E20, wherein the cancer is neuroblastoma.

E29. The combination for use according to any of E17 to E20, wherein the cancer is small cell lung cancer.

E30. A combination according to any of E1 to E16, further comprising one or more excipients.

E31. The use of a combination according to any of E1 to E16, in the manufacture of a medicament for the treatment of cancer.

E32. The use according to E31, wherein the cancer is leukaemia.

E33. The use according to E32, wherein the cancer is acute myeloid leukaemia, T-ALL or B-ALL.

E34. The use according to E31, wherein the cancer is myelodysplastic syndrome or myeloproliferative disease.

E35. The use according to E31, wherein the cancer is lymphoma.

E36. The use according to E35, wherein the lymphoma is a non-Hodgkin lymphoma.

E37. The use according to E36, wherein the non-Hodgkin lymphoma is diffuse large B-cell lymphoma or mantle-cell lymphoma.

E38. The use according to E31, wherein the cancer is multiple myeloma.

E39. The use according to E31, wherein the cancer is neuroblastoma.

E40. The use according to E31, wherein the cancer is small cell lung cancer.

E41. A medicament containing, separately or together,
(a) a BCL-2 inhibitor of formula (I) as defined in E1, and
(b) a MCL1 inhibitor,
for simultaneous, sequential or separate administration, and wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in effective amounts for the treatment of cancer.

E42. A medicament containing, separately or together,
(a) a BCL-2 inhibitor, and
(b) a MCL1 inhibitor of formula (II) as defined in E2,
for simultaneous, sequential or separate administration, and wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in effective amounts for the treatment of cancer.

E43. A method of treating cancer, comprising administering a jointly therapeutically effective amount of (a) a BCL-2 inhibitor of formula (I) as defined in E1, and
(b) a MCL1 inhibitor,
to a subject in need thereof.

E44. A method of treating cancer, comprising administering a jointly therapeutically effective amount of (a) a BCL-2 inhibitor, and
(b) a MCL1 inhibitor of formula (II) as defined in E2,
to a subject in need thereof.

E45. A method for sensitizing a patient 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 jointly therapeutically effective amount of (a) a BCL-2 inhibitor of formula (I) as defined in E1, and (b) a MCL1 inhibitor, to said patient.

E46. A method for sensitizing a patient 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 jointly therapeutically effective amount of (a) a BCL-2 inhibitor, and (b) a MCL1 inhibitor of formula (II) as defined in E2, to said patient.

"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 "co-agent") 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.

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.

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.

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.

"Cancer" means a class of disease in which a group of cells display uncontrolled growth. Cancer types include haematological cancer (lymphoma and leukaemia) and solid tumors including carcinoma, sarcoma, or blastoma. In particular "cancer" refers to leukaemia, lymphoma or multiple myeloma, and more especially to acute myeloid leukaemia.

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.

"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.

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.

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.

In another aspect, 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 BCL-2 inhibitor of formula (I) in combination with a MCL1 inhibitor, as described herein, to the patient. A patient who is sensitized is a patient who is responsive to the treatment involving administration of a BCL-2 inhibitor of formula (I) in combination with a MCL1 inhibitor, as described herein, or who has not developed resistance to such treatment.

“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.

‘AML’ means acute myeloid leukaemia.

‘T-ALL’ and ‘B-ALL’ means T-cell acute lymphoblastic leukemia and B-cell acute lymphoblastic leukemia.

‘free base’ refers to compound when not in salt form.

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 %.

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.

The pharmaceutical compositions according to the invention comprise one or more excipients or carriers selected from diluents, lubricants, binders, disintegration agents, stabilisers, preservatives, absorbents, colourants, sweeteners, flavourings etc.

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.

The compounds of the combination may be administered simultaneously or sequentially. The administration route is preferably the oral route, 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.

Preference is given to the pharmaceutical compositions being tablets.

Pharmaceutical composition of Compound 1 HCl salt film-coated tablet containing 50 mg and 100 mg of drug substance

	Amount (mg)		Function
Tablet	**50 mg strength**	**100 mg strength**	
Compound 1 HCl salt	52,58	105,16	Drug Substance
equivalent in base to	50	100	
Lactose monohydrate	178,51	357,02	Diluent
Maize starch	66,6	133,2	Disintegrant
Povidone	23,31	46,62	Binder
Magnesium stearate	3,33	6,66	Lubricant
Silica, colloidal anhydrous	0,67	1,34	Flow agent
Sodium starch glycolate (Type A)	10	20	Disintegrant
For an uncoated tablet with a mass of	335	670	
Film-Coating			
Glycerol	0,507	1,014	Plasticizing agent
hypromellose	8,419	16,838	Film-coating agent
Macrogol 6000	0,538	1,076	Smoothing agent
Magnesium stearate	0,507	1,014	Lubricant
Titanium dioxide	1,621	3,242	Pigment
Intermediary Vehicule			
Water, purified	qs.	qs.	Solvent
For a film-coated tablet with a mass of	346,6	693,2	

PHARMACOLOGICAL DATA

MATERIAL AND METHOD FOR EXAMPLES 1-3:

Primary AML patient samples: Bone marrow or peripheral blood samples from patients with AML were collected after informed consent in accordance with guidelines approved by The Alfred Hospital Human research ethics committees. Mononuclear cells were isolated by Ficoll-Paque (GE Healthcare, VIC, Aus) density-gradient centrifugation, followed by red cell depletion in ammonium chloride (NH_4Cl) lysis buffer at 37°C for 10 minutes. Cells were then re-suspended in phosphate-buffered saline containing 2% Fetal Bovine serum (Sigma, NSW, Aus). Mononuclear cells were then suspended in RPMI-1640 (GIBCO VIC, Aus) medium containing penicillin and streptomycin (GIBCO) and heat inactivated fetal bovine serum 15% (Sigma).

Cell lines, cell culture and generating luciferase reporter cell lines: Cell lines MV4;11, OCI-AML3, HL-60, HEL, K562, KG-1 and EOL-1 were maintained at 37°C, 5% CO_2 in RPMI-1640 (GIBCO) supplemented with 10% (v/v) fetal bovine serum (Sigma) and penicillin and streptomycin (GIBCO). MV4;11 luciferase cell lines were generated by lentivral transductions.

Antibodies: Primary antibodies used for western blot analysis were MCL1, BCL-2, Bax, Bak, Bim, BCL-XL (generated in-house WEHI) and tubulin (T-9026,Sigma).

Cell Viability: Freshly purified mononuclear cells from AML patient samples were adjusted to a concentration of $2.5x10^5$/ml and 100µL of cells aliquoted per well into 96 well plates (Sigma). Cells were then treated with Compound 1, HCl, Compound 2, ABT-199 (Active Biochem, NJ, USA) or idarubicin (Sigma), over a 6 log concentration range from 1nM to 10µM for 48hr. For combinations assays, drugs were added at a 1:1 ratio from 1nM to 10uM and incubated at 37°C 5% CO_2. Cells were then stained with sytox blue nucleic acid stain (Invitrogen, VIC, Aus) and fluorescence measured by flow cytometric analysis using the LSR-II Fortessa (Becton Dickinson, NSW, Aus). FACSDiva

software was used for data collection, and FlowJo software for analysis. Blast cells were gated using forward and side scatter properties. Viable cells excluding sytox blue were determined at 6 concentrations for each drug and the 50% lethal concentration (LC_{50}, in μM) determined.

LC_{50} determination and synergy: Graphpad Prism was used to calculate the LC_{50} using non-linear regression. Synergy was determined by calculating the Combination Index (CI) based on the Chou Talalay method as described (Chou Cancer Res; 70(2) January 15, 2010).

Colony assays: Colony forming assays were performed on freshly purified and frozen mononuclear fractions from AML patients. Primary cells were cultured in duplicate in 35mm dishes (Griener-bio, Germany) at 1 x 10^4 to 1 x 10^5. Cells were plated in 0.6% agar (Difco NSW, Aus): AIMDM 2x (IMDM powder-Invitrogen), supplemented with $NaHCO_3$, dextran, Pen/Strep, B mercaptoethanol and asparagine):Fetal Bovine Serum (Sigma) at a 2:1:1 ratio. For optimal growth conditions all plates contained GM-CSF (100ng per plate), IL-3(100ng/plate R&D Systems, USA) SCF(100ng/plate R&D Systems) and EPO (4U/plate) (Growth was for 2-3 weeks in the presence and absence of drug at 37°C at 5% CO_2 in a high humidity incubator. After incubation plates were fixed with 2.5% glutaraldehyde in saline and scored using the GelCount from Oxford Optronix (Abingdon, United Kingdom).

Western Blotting: Lysates were prepared in NP40 lysis buffer (10 mM Tris-HCl pH 7.4, 137 mM NaCl, 10% glycerol, 1% NP40), supplemented with protease inhibitor cocktail (Roche, Dee Why, NSW, Australia). Protein samples were boiled in reducing loading dye before separation on 4–12% Bis-Tris polyacrylamide gels (Invitrogen, Mulgrave, VIC, Australia), and transferred to Hybond C nitrocellulose membrane (GE, Rydalmere, NSW, Australia) for incubation with specified antibodies. All membrane-blocking steps and antibody dilutions were performed using 5% (v/v) skim milk in PBS containing 0.1% (v/v) Tween-20 phosphate-buffered saline (PBST) or Tris-buffered-saline, and washing steps performed with PBST or TBST. Western blots were visualized by enhanced chemiluminescence (GE).

In vivo experimentation AML engraftment: Animal studies were performed under the institutional guidelines approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee, MV4;11 cells transduced with the luciferase reporter (pLUC2) were intravenously injected at 1×10^5 cells into irradiated (100Rad) non-obese diabetic/severe combined immunodeficient (NOD/SCID/ IL2rγnull) mice as previously described (Jin et al., *Cell Stem Cell* 2 July 2009, Volume 5, Issue 1, Pages 31–42). Engraftment was measured at day 7 by quantifying the percentage of hCD45+ cells in the PB by flow cytometry and by IVIS imaging of bioluminescent MV4;11 cells. At day 10, mice received daily oral gavage of Compound 1, HCl (200µL 100mg/kg – dosage expressed as the free base) dissolved in PEG400 (Sigma), absolute ethanol (Sigma) and distilled H_20 40:10:60 or Compound 2 (200µL 25mg/kg) twice weekly dissolved in 50% 2-hydroxypropyl)-β-cyclodextrin (Sigma) and 50% 50mM HCl or the drug combination or vehicle, over 4 weeks. Blood counts were determined using a hematology analyzer (BioRad, Gladesville, NSW).

IVIS imaging: Bioluminescent imaging was performed using the Caliper IVIS Lumina III XR imaging system. Mice were anaesthetised with isofleurine and injected intraperitoneally with 100µL of 125 mg/kg luciferin (Perkin Elmer, Springvale, VIC).

MATERIAL AND METHOD FOR EXAMPLE 4:

Cell lines: Human myeloma cell lines (HMCLs) were derived from primary myeloma cells cultured in RPMI 1640 medium supplemented with 5% fetal calf serum from and 3 ng/mL recombinant IL-6 for IL-6 dependent cell lines. HMCLs are representative of phenotypic and genomic heterogeneity and the variability in patient's response to therapy.

MTT assay: Cell viability is measured using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric survival assay. Cells are incubated with compounds in 96-well plates containing a final volume of 100 µl/well time. (*2R*)-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) is used at 9 different concentrations accordingly to single agent sensitivity. *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide hydrochloride (Compound 1, HCl) is used at a fixed dose – 1 µM. At the end of each treatment, cells are incubated with 1 mg/mL MTT (50 µl MTT solution 2.5 mg/ml for each well) at 37°C for 3 hours allowing the MTT to be metabolized. Lysis buffer (100 µl Lysis buffer: DMF (2:3) /SDS (1:3)) is added into each well to dissolve formazan cristals and after 18h of incubation, absorbance in viable cells is measured at 570 nm using a spectrophotometer.

As control, cells are incubated with medium alone and with medium containing 0.1% DMSO. As myeloma cell growth control, myeloma cell absorbance is recorded every day (D0, D1, D2, D3 and D4).

All experiments are repeated 3 times, and each experimental condition is repeated at least in triplicate wells in each experiment.

The inhibition effect is calculated with the following formula:

Inhibition effect (%) = (1-Absorbance value of treated cells/Absorbance value of control cells)*100

EXAMPLE 1: BCL-2 and MCL1 are the dominant pro-survival proteins expressed in AML

7 AML cell lines and 13 primary AML samples with >70% blasts were immunoblotted for proteins indicated in Figure 1.

As illustrated in Figure 1, a proteomic survey of the expression of BCL-2 family members in AML showed that, in addition to BCL-2, most primary AML samples and AML cell lines co-expressed the pro-survival protein MCL1. BCL-XL is less frequently expressed in AML.

EXAMPLE 2: Combined BCL-2 and MCL1 targeting displays synergistic killing in AML

54 AML patient samples were incubated with a 6-log concentration range of Compound 1 (HCl salt), Compound 2 or a 1:1 concentration in RPMI/15% FCS for 48h and the LC_{50} determined (Figure 2A).

Approximately 20% of primary AML samples were highly sensitive to either Compound 1 or Compound 2, with the lethal concentration of drug required to kill 50% of primary AML blasts after 48 hours (LC_{50}) in the low nanomolar range (LC_{50}<10nM) (Figure 2A). In contrast, when Compound 1 and Compound 2 were combined, the proportion of AML samples that were sensitive increased dramatically to 70%, indicating synergistic activity when BCL-2 and MCL1 were simultaneously targeted (Figure 2A). Some results are displayed in Figure 17.

To verify the *in vivo* activity of this approach, luciferase expressing MV4;11 AML cells were engrafted into NSG mice and treated with Compound 1 (HCl salt) or Compound 2 alone, or in combination and tumour burden assessed after 14 and 21 days of therapy (Figure 2B). At the completion of 28 days of therapy, mice were followed for survival (Figure 2C). These experiments showed that the combination of Compound 1 and Compound 2 was highly effective *in vivo*, validating the impressive activity observed using primary AML cells *in vitro*.

The data presented in Figures 2A-2C herein show the synergistic combination activity between Compound 1, HCl and Compound 2 in AML.

EXAMPLE 3: Combined BCL-2 and MCL1 inhibition targets leukaemic, but not normal progenitor function

To assess the toxicity of BCL-2 inhibition combined with MCL1 inhibition on normal human CD34+ cells or ficolled blasts from patients with AML, clonogenic potential was assessed after 2 weeks exposure to combined therapies. Colonies were grown in agar supplemented with 10% FCS, IL3, SCF, GM-CSF and EPO over 14 days and colonies enumerated with an automated Gelcount® analyser. Assays for primary AML samples were performed in duplicate and averaged. Errors for CD34+ represent mean +/- SD of 2 independent normal donor samples. Results were normalised to the number of colonies

counted in DMSO control. Indicated drug concentrations were plated on D1. Notably, Compound 1 + Compound 2 suppressed AML colony forming activity without affecting the function of normal CD34+ colony growth.

Taken altogether, Examples 2 and 3 show that dual pharmacological inhibition of BCL-2 and MCL1 is a novel approach to treating AML without need for additional chemotherapy and with an acceptable therapeutic safety window.

EXAMPLE 4: *In vitro* evaluation of multiple myeloma cell survival in response to a MCL1 inhibitor as a single agent or in combination with a BCL-2 inhibitor

The sensitivity of 27 human multiple myeloma cell lines to Compound 1, Compound 2 or to Compound 2 in the presence of 1μM of Compound 1 was analyzed by using MTT cell viability assay. 50% inhibitory concentrations (IC_{50}, in nM) were determined.

The results are displayed in the following table:

Cell lines	Compound 1, HCl (IC_{50} nM)	Compound 2 (IC_{50} nM)	IC_{50} of Compound 2 in the presence of 1 μM of Compound 1, HCl (nM)
AMO1	8610,3	0,5	0,2
ANBL6	1905,0	79,5	20,8
BCN	22217,0	1111,4	59,3
JIM3	>30000	56,3	25,9
JJN3	2692,0	15,6	2,4
KMM1	23926,3	57,8	8,6
KMS11	10486,7	44,1	3,9
KMS12BM	1393,7	44,1	0,03
L363	7581,3	7,6	3,4
LP1	9770,0	158,2	2,9
MM1S	21407,0	138,5	23,0
NAN1	6659,0	5,7	1,4

NAN3	8241,3	1,5	1,2
NAN6	4074,8	7,1	1,8
NAN8	9096,3	75,4	32,5
NAN9	23157,6	9,7	1,1
NCI-H929	15688,3	2,3	1,0
OPM2	6460,7	9,4	1,2
RPMI8226	3204,0	27,4	3,0
SBN	21273,7	221,1	14,6
U266	>30000	170,1	14,9
XG1	9779,7	5,9	0,2
XG11	7912,0	374,7	8,3
XG2	15297,7	6,4	2,7
XG3	7224,7	6,1	1,3
XG6	8544,3	19,2	0,5
XG7	18121,7	16,3	8,0

Strong synergistic activity was demonstrated when combining Compound 1 and Compound 2 in the majority of the cell lines as compared to the compounds alone.

EXAMPLE 5: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of 17 Diffuse Large B-Cell Lymphoma (DLBCL) cell lines

Material and Method

Cell lines were sourced and maintained in the basic media supplemented with FCS (Fetal Calf Serum) as indicated in Table 1. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). Unless otherwise mentioned, culture media and supplements were from Amimed/Bioconcept (Allschwil, Switzerland).

Cell lines were cultured at 37°C in a humidified atmosphere containing 5% CO_2 and expanded in T-75 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 (Omni Life Science, Bremen, Germany) prior to plating 25 ul/well at

the densities indicated in Table 1 into 384-well plates (Corning). All cell lines were determined to be free of mycoplasma contamination by PCR assay performed at Idexx Radil (Columbia, MO, USA) and misidentification ruled out by assessment of a panel of 48 Small Nucleotide Polymorphisms (SNPs) at Asuragen (Austin, TX, USA) or in-house.

Stock solutions of compounds were prepared at a concentration of 10 mM in DMSO (Sigma) and stored at -20°C. Where necessary to afford a full dose-response curve, the stock solutions were pre-diluted in DMSO to 1'000-fold the desired start concentration (see Table 2). On the day after cell seeding, eight 2.5-fold serial dilutions of each compound were dispensed, either individually or in all possible permutations in a checkerboard fashion, directly into the cell assay plates using a non-contact 300D Digital Dispenser (TECAN, Männedorf, Switzerland) as outlined in Figure 4. The final concentration of DMSO was 0.2% in all wells.

Effects of the single agents as well as their checkerboard combinations on cell viability were assessed after 2 days of incubation at 37 °C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo (Promega, Madison, WI, USA) at 25 μL reagent/well and n=2 replicate plates per condition. Luminescence was quantified on a M1000 multipurpose platereader (TECAN, Männedorf, Switzerland). The number/viability of cells at time of compound addition was likewise assessed and used to assess the degree of the population doubling time of a particular cell line.

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*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666). All calculations were performed using the Combination Analysis Module in-house software. IC_{50} are defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the vehicle (DMSO) control.

The interpretation of the Synergy Score is as follows:
SS ~ 0 → Additive

SS >1 → Weak Synergy

SS >2 → Synergy

Table 1. Identity and assay conditions for the 17 Diffuse Large B-Cell Lymphoma cell lines used in the combination experiments.

Cell line	Medium (source)	%FCS	Doubling time (hours)	Cell number seeded/well
DB	RPMI (ATCC)	10	31.7	500
DOHH-2	RPMI (DSMZ)	10	25.3	500
HT	RPMI (ATCC)	10	34.3	2000
JM1	Iscove's MEM*(ATCC)	10	22.8	500
KARPAS-422	RPMI (DSMZ)	10	26.5	500
NU-DHL-1	RPMI (DSMZ)	20	28.0	500
OCI-LY-19	MEM alpha (DSMZ)	20	25.8	500
Pfeiffer	RPMI (ATCC)	10	46.2	2000
RL	RPMI (ATCC)	10	28.9	500
SU-DHL-10	RPMI (DSMZ)	20	105.7	1000
SU-DHL-4	RPMI (DSMZ)	10	25.2	500
SU-DHL-5	RPMI (DSMZ)	20	25.9	500
SU-DHL-6	RPMI (DSMZ)	20	30.1	500
SU-DHL-8	RPMI (DSMZ)	20	23.6	500
Toledo	RPMI (ATCC)	10	49.6	2000
U-937	RPMI (ATCC)	10	28.7	500
WSU-DLCL2	RPMI (DSMZ)	10	26.1	500

*This medium was further complemented with 50 µM 2-mercaptoethanol. Doubling times were calculated based on the difference in ATP levels at the end compared to the beginning of compound incubation.

Table 2. Single agent IC_{50} values for Compound 3 and Compound 1, HCl, as well as the synergy scores for their combination are indicated. Interactions were deemed synergistic when scores ≥ 2.0 where observed.

Cell Line	Compound 3			Compound 1, HCl			Combination	
	Start conc [uM]	Abs IC_{50} [uM]	Max Inh [%]	Start conc [uM]	Abs IC_{50} [uM]	Max Inh [%]	Synergy Score	Synergy Score Error
DB	1	0.0129	99.2	10	2.76	95.7	17.3	0.18
DOHH-2	0.1	0.00122	98.3	10	0.156	101.9	2.90	0.11
HT	1	0.00638	99.3	10	≥10	37.2	2.35	0.06
JM1	1	0.0588	99.7	10	0.697	99.1	5.83	0.25
KARPAS-422	1	0.00214	98.3	10	2.18	90.9	9.74	0.32
NU-DHL-1	1	0.0579	98.1	10	0.0515	102.1	4.97	0.12
OCI-LY-19	1	0.0604	98.5	10	0.0895	100.1	3.92	0.07
Pfeiffer	1	0.0426	82.7	10	4.50	92.0	3.44	0.19
RL	10	3.03	95.4	10	0.281	99.0	12.7	0.38
SU-DHL-10	1	0.00384	98.3	10	≥10	43.9	2.44	0.26
SU-DHL-4	1	0.0178	99.5	10	0.86	96.9	10.8	0.28
SU-DHL-5	0.1	0.00094	98.2	10	≥10	49.6	1.45	0.14
SU-DHL-6	1	0.00213	99.1	10	0.614	101.0	4.57	0.21
SU-DHL-8	10	0.305	95.6	10	≥10	28.7	9.88	0.20
Toledo	1	≥1	45.8	10	0.137	101.9	11.1	0.51
U-937	1	0.00832	97.1	10	6.83	62.1	5.63	0.20
WSU-DLCL2	1	0.00792	99.0	10	1.02	99.3	8.67	0.13

"Start conc" means start concentration.

"Abs IC_{50}" means absolute IC_{50}.

"Max Inh" means maximum inhibition.

Results

The effect on proliferation of combining the MCL1 inhibitor Compound 3 with the BCL-2 inhibitor Compound 1, HCl was assessed in a panel of 17 Diffuse Large B-Cell Lymphoma (DLBCL) cell lines.

Compound 3 as single agent strongly inhibited the growth of the majority of the 17 DLBCL lines tested (Table 1). Thus, 14 cell lines displayed IC_{50}s below 100 nM, and an additional 1 cell lines displayed IC_{50}s between 100 nM and 1 uM. Only 2 cell lines displayed an IC_{50} above 1 uM.

Compound 1, HCl as single agent also inhibited the growth of the majority of the 17 DLBCL lines tested, although slightly less potent (Table 2). Thus, 2 cell lines displayed IC_{50}s below 100 nM, and 6 cell lines displayed IC_{50}s between 100 nM and 1 uM. Nine cell line displayed an IC_{50} above 1 uM (four of which above 10 uM).

In combination, Compound 3 and Compound 1, HCl treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2 - Lehar *et al*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666) in 16 out of 17 DLBCL cell lines tested (Table 2). In 5 cell lines, the synergy effect was marked, with synergy scores between 5 and 10. In 4 cell lines, the synergy effect was exceptional, achieving synergy scores between 10 and 17.3. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of Compound 3 and Compound 1 that did not display an anti-proliferative effect on their own. For example, in DB cells, Compound 3 and Compound 1 at the second lowest concentration tested elicited a growth inhibition of only 1 and 2 %, respectively, while the respective combination of the two compounds afforded a growth inhibition of 96% (Figure 4A, left panel), thus being 91% above the additivity calculated based on the single agent activities (Figure 4A, right panel). As an additional example, in Toledo cells, in which Compound 3 was less potent and achieved only partial growth inhibition (46%) at the highest concentration tested, the combination with the second lowest concentrations of Compound 1 resulted in synergistic growth inhibition of 98% (Figure 4B, left panel), thus being 52% above the additivity calculated based on the single agent activities (Figure 4B, right panel).

Furthermore, it is noteworthy that the synergistic effects occurred across a broad range of single agent concentrations, which should prove beneficial *in vivo* with respect to flexibility concerning dosing levels and scheduling.

In summary, the combination of Compound 3 and Compound 1 afforded strong to exceptional synergistic growth inhibition in the majority of DLBCL cell lines tested.

EXAMPLE 6: *In vivo* efficacy in Karpas422 xenografts with combination of a MCL1 inhibitor (Compound 3) and a BCL-2 inhibitor (Compound 1)

Material and Method

Tumour Cell Culture and Cell Inoculation

Karpas 422 human B-cell non-Hodgkin's lymphoma (NHL) cell line was established from the pleural effusion of a patient with chemotherapy-resistant NHL. The cells were obtained from the DSMZ cell bank and cultured in RPMI-1640 medium (BioConcept Ltd. Amimed,) supplemented with 10% FCS (BioConcept Ltd. Amimed), 2 mM L-glutamine (BioConcept Ltd. Amimed), 1 mM sodium pyruvate (BioConcept Ltd. Amimed) and 10 mM HEPES (Gibco) at 37°C in an atmosphere of 5% CO_2 in air. Cells were maintained between 0.5 and 1.5 x 106 cells/mL. To establish Karpas 422 xenografts cells were harvested and re-suspended in HBSS (Gibco) and mixed with Matrigel (BD Bioscience) (1:1 v/v) before injecting 200 µL containing 1x107 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 5Gy over 2 minutes using a ɤ-irradiator.

Tumour Growth

Tumour growth was monitored regularly post cell inoculation and animals were randomised into treatment groups (n=5) when tumour volume reached appropriate volume. During the treatment period tumour volume was measured about twice a week using calipers. Tumour size, in mm^3, was calculated from: (L x W2 x π/6). Where W = width and L = length of the tumour.

Treatment

Tumour bearing animals (rats) were enrolled into treatment groups (n=5) when their tumours reached an appropriate size to form a group with a mean tumour volume of about

450 mm^3. The treatment groups were as outlined in Table 3. The vehicle for Compound 1, HCl or Compound 1, HCl was administered by oral (*po*) gavage 1 h before vehicle for Compound 3 or Compound 3 which was administered by 15 minutes *iv* infusion. For *iv* infusion animals were anesthetized with isoflurane/O_2 and the vehicle or Compound 3 administered via a cannula in the tail vein. Animals were weighed at dosing day(s) and dose was body weight adjusted, dosing volume was 10 ml/kg for both compounds.

Body weights

Animals were weighed at least 2 times per week and examined frequently for overt signs of any adverse effects.

Data analysis and statistical evaluation

Tumour 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 ± SEM.

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

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

Tumour regression was calculated according to:

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

wherein Δtumour volumes represent the mean tumour volume on the evaluation day minus the mean tumour volume at the start of the experiment.

Table 3. Treatment groups for combination efficacy in Karpass422 xenograft bearing rats

Groups	Treatment	Dose (expressed as the free base)	Schedule	Number of rats
1	Vehicle for Compound 1, HCl (PEG400/EtOH/Phosal 50 PG (30/10/60)), *po* 1h before vehicle for Compound 3, 15 minutes *iv* infusion 10 ml/kg	10 ml/kg + 10 ml/kg	QW, *po* 1h before + QW, *iv* infusion	5
2	Vehicle for Compound 1, HCl + Compound 3	0 mg/kg + 20 mg/kg	QW, *po* 1h before + QW, *iv* infusion	5
3	Compound 1, HCl + Vehicle for Compound 3	150 mg/kg + 0 mg/kg	QW, *po* 1h before + QW, *iv* infusion	5
4	Compound 1, HCl + Compound 3	150 mg/kg + 20 mg/kg	QW, *po* 1h before + QW, *iv* infusion	5

Treatments were initiated when the average tumour volume was about 450 mm^3. Compound 1, HCl was formulated in PEG400/EtOH/Phosal 50 PG (30/10/60) and Compound 3 was placed in solution.

QW means once-weekly.

Results

Combination treatment with Compound 1 free base at 150 mg/kg *po* 1h before Compound 3 at 20 mg/kg *iv* infusion induces complete regression in all Karpas422 tumours by day 30 from start of treatment (Figure 5). All animals in the treatment group have remained tumour free after treatment was stopped on day 35 up to day 90. A positive combination effect is observed in the combination group compared with single agent activity. On day 34 the tumour response in the single agent Compound 3 and the combination group are

significantly different from the vehicle group ($p<0.05$).The combination treatment is well tolerated based on body weight changes (Figure 6).

EXAMPLE 7: *In vivo* efficacy in DLBCL Toledo xenograft with combination of a MCL1 inhibitor (Compound 3) and a BCL-2 inhibitor (Compound 1, HCl)

Material and Method

Cell implantation

The xenograft model was established by direct subcutaneous (sc) implantation of 3 million Toledo cell suspension with 50% matrigel into the subcutaneous area of SCID/beige mice. All procedures were carried out using aseptic technique. The mice were anesthetized during the entire period of the procedure.

In general, a total of 6 animals per group were enrolled in efficacy study. For single-agent and combination studies, animals were dosed via oral gavage (po) for Compound 1 and intravenously (iv) via tail vein for Compound 3. Compound 1, HCl was formulated as solution in PEG300/EtOH/water (40/10/50), and Compound 3 was placed in solution. When tumors reached approximately 220 mm^3 at day 26 post cell implantation, tumour-bearing mice were randomized into treatment groups.

The design of the study including dose schedule for all treatment groups are summarized in the table below. Animals were weighed at dosing day(s) and dose was body weight adjusted, dosing volume was 10 ml/kg. Tumour dimensions and body weights were collected at the time of randomization and twice weekly thereafter for the study duration. The following data was provided after each day of data collection: incidence of mortality, individual and group average body weights, and individual and group average tumour volume.

Groups	Treatment	Dose (expressed as the free base)	Schedule	Number of mice
1	Vehicle	PEG300/EtOH/water (40/10/50)	QW, *po*	6
2	Compound 1, HCl	100 mg/kg	QW, *po*	6
3	Compound 3	25 mg/kg	QW, *iv*	6
4	Compound 1, HCl + Compound 3	100 mg/kg 25 mg/kg	QW, *po* QW, *iv*	6

For the study in Toledo model, treatments were initiated on day 26 following cell implantation, when the average tumour volume was ~218 to 228 mm^3.

QW means once-weekly.

Body Weight (BW)

The % change in body weight was calculated as $(BW_{current} - BW_{initial})/(BW_{initial}) \times 100$. Data is presented as percent body weight change from the day of treatment initiation.

Tumour Volume and percent mice remaining on the study

Percent treatment/control (T/C) values were calculated using the following formula:

$\% T/C = 100 \times \Delta T/\Delta C$ if $\Delta T > 0$

$\%$ Regression $= 100 \times \Delta T/T_0$ if $\Delta T < 0$

where:

T = mean tumour volume of the drug-treated group on the final day of the study;

ΔT = mean tumour volume of the drug-treated group on the final day of the study – mean tumour volume of the drug-treated group on initial day of dosing;

T_0 = mean tumour volume of the drug-treated group on the day of cohort;

C = mean tumour volume of the control group on the final day of the study; and

ΔC = mean tumour volume of the control group on the final day of the study – mean tumour volume of the control group on initial day of dosing.

Percent mice remaining on the study = 6- number of mice reaching end point/6*100

Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). Delta tumour volume and percent body weight changes were used for statistical analysis. Between group comparisons were carried out using the One way ANOVA followed by a post hoc Tukey test. For all statistical evaluations, the level of significance was set at $p < 0.05$. Significance compared to the vehicle control group is reported unless otherwise stated.

Results

Treatment	T/C% at day 42
Vehicle	100
Compound 1, HCl	37
Compound 3	102
Compound 1, HCl + Compound 3	3

In Toledo model, Compound 1 free base at 100 mg/kg produced statistically significant anti-tumour effects with 37% T/C. Compound 3 at 25 mg/kg resulted in no anti-tumour effects with 102% T/C (Figure 7). Combination of Compound 1 + Compound 3 led to tumour stasis with 3% T/C, which is statistically significant compared to Vehicle, Compound 1 and Compound 3 treated tumors ($p<0.05$, by one-way ANOVA test).

Therefore, combined inhibition of BCL-2 and MCL1 in DLBCL may provide a therapeutic benefit in the clinic. In addition, the mean body weight change for Toledo is shown in Figure 8. Treatment of mice with Compound 1, HCl and Compound 3 exhibit body weight gain (1.081% and 2.3%, respectively). The combination group showed slight body weight loss (-3.2%). No other signs of adverse events were observed in this study. All 6 animals survived throughout the study.

Taken altogether, Examples 2, 6 and 7 show that the combination of a MCL1 inhibitor and a BCL-2 inhibitor is efficacious at tolerated doses in mice and rats bearing xenografts of acute myeloid leukemia and lymphoma human derived cell lines, suggesting that a suitable therapeutic window is achievable with this combination in these diseases.

EXAMPLE 8: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of 13 Acute Myeloid Leukemia (AML) cell lines.

Material and Method

Cell lines were sourced and maintained in the basic media supplemented with FBS (Fetal Bovine Serum) as indicated in Table 1. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM).

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 ul/well at the densities indicated in Table 1 into 96-well plates. All cell lines were determined to be free of mycoplasma contamination in-house.

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

In order to analyse the activity of the compounds as single agents, cells were seeded and treated with nine 2-fold serial dilutions of each compound dispensed individually directly into the cell assay plates. Effects of the compounds 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. All the experiments were performed in triplicates. Luminescence was quantified on a multipurpose plate reader. Single agent IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the vehicle (DMSO) control.

In order to analyse the activity of the compounds 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 Figure 9. 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. Two independent experiments, each one performed in duplicates, were performed. Luminescence was quantified on a multipurpose plate reader.

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*, *Nat Biotechnol*. **2009** July ; 27(7): 659–666). All calculations were performed using Clalice™ Bioinformatics Software.

The doubling time indicated in Table 3 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.

The interpretation of the Synergy Score is as follows:

SS ~ 0 → Additive

SS >1 → Weak Synergy

SS >2 → Synergy

Table 3. Identity and assay conditions for the 13 Acute Myeloid Leukemia (AML) cell lines used in the combination experiments.

Cell line	Medium (source)	%FBS	Doubling time (hours)	Cell number seeded/well
MV4;11	RPMI (ATCC)	10	31.0	56520
MOLM-13	RPMI (DSMZ)	10	32.4	56520
PL-21	RPMI (DSMZ)	10	32.4	56520
ML-2	RPMI (DSMZ)	10	31.6	56520
Nomo-1	RPMI (DSMZ)	10	43.5	56520
THP-1	RPMI (ATCC)	10	49.6	56520
HL-60	IMDM (ATCC)	20	34.8	56520
Kasumi-1	RPMI (ATCC)	20	59.4	56520
OCI-AML3	MEM alpha (DSMZ)	20	25.7	56520
EOL-1	RPMI (DSMZ)	10	37.6	113040
GDM-1	RPMI (ATCC)	10	31.6	56520
KG1	IMDM (ATCC)	20	45.7	56520
KG1a	IMDM (ATCC)	20	36.5	56520

Table 4a. Single agent IC_{50} values for Compound 3, Compound 1, HCl and ABT-199 in 13 AML cell lines are indicated. Compounds were incubated with the cells during 3 days.

Cell Line	Compound 3		Compound 1, HCl		ABT-199	
	Start conc [uM]	IC_{50} [uM]	Start conc [uM]	IC_{50} [uM]	Start conc [uM]	IC_{50} [uM]
MV4;11	0.01	0.001	0.1	0.03	n.d.	n.d.
MOLM-13	0.01	0.002	0.1	0.04	n.d.	n.d.
PL-21	0.10	0.065	30.0	2.78	15.0	3.300
ML-2	0.10	0.005	2.0	0.04	n.d.	n.d.
Nomo-1	0.05	0.013	30.0	7.45	15.0	5.000
THP-1	0.10	0.017	30.0	0.75	2.0	0.900
HL-60	0.10	0.025	30.0	1.42	15.0	2.100
Kasumi-1	2.00	0.033	30.0	0.77	n.d.	n.d.
OCI-AML3	2.00	0.146	30.0	8.09	15.0	8.500
EOL-1	0.10	0.001	2.0	0.04	0.2	0.004
GDM-1	0.10	0.008	2.0	0.06	n.d.	n.d.
KG1	30.00	0.390	30.0	4.70	15.0	3.400
KG1a	30.00	2.000	30.0	1.75	15.0	0.900

Table 4b. Single agent IC_{50} values for Compound 4, HCl in 5 AML cell lines are indicated. Compound was incubated with the cells during 3 days.

Cell Line	Compound 4, HCl	
	Start conc [uM]	IC_{50} [uM]
MV4;11	0.5	0.01
MOLM-13	0.5	0.012
ML-2	0.5	0.01
OCI-AML3	15	5.41
GDM-1	0.5	0.002

Table 5a. Synergy scores for Compound 3 and Compound 1 combination in 13 AML cell lines 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 3		Compound 1, HCl		Combination (a)	
	Start conc [uM]	Mean of Max Inh [%]	Start conc [uM]	Mean of Max Inh [%]	Mean of SynergyScore	Synergy Score Error (sd)
MV4;11	0.1	100.0	0.3	99.5	4.3	0.7
MOLM-13	0.1	99.5	0.3	90.0	8.2	1.3
PL-21	0.3	91.5	5.0	75.0	17.9	2.7
ML-2	0.1	99.5	0.3	88.0	10.9	1.8
Nomo-1	0.3	97.0	5.0	31.0	11.8	1.0
THP-1	0.3	99.0	5.0	55.5	13.2	0.1
HL-60	0.3	97.5	5.0	61.5	12.9	1.7
Kasumi-1	0.3	84.5	2.0	52.5	10.5	0.5
OCI-AML3	2.0	100.0	5.0	53.5	19.8	0.2
EOL-1	0.1	100.0	1.0	100.0	5.8	0.8
GDM-1	0.1	99.0	0.3	87.0	11.1	1.4
KG1	2.0	80.5	5.0	53.0	14.6	1.7
KG1a	2.0	28.5	5.0	73.0	13.0	0.9

Table 5b. Synergy scores for Compound 3 and ABT-199 combination in 8 AML cell lines 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 3		ABT-199		Combination (b)	
	Start conc [uM]	Mean of Max Inh [%]	Start conc [uM]	Mean of Max Inh [%]	Mean of SynergyScore	Synergy Score Error (sd)
PL-21	0.3	89.0	2.0	41.5	19.7	2.7
Nomo-1	0.3	95.5	2.0	45.5	10.7	1.6
THP-1	0.3	97.0	0.3	47.0	12.4	0.7
HL-60	0.3	97.5	2.0	56.0	12.9	1.6
OCI-AML3	2.0	100.0	2.0	58.5	16.4	0.5
EOL-1	0.1	100.0	0.1	97.5	4.0	0.3
KG1	2.0	89.0	2.0	54.5	12.2	0.8
KG1a	2.0	57.5	2.0	73.0	17.6	0.1

Table 5c. Synergy scores for Compound 3 and Compound 4, HCl combination in 5 AML cell lines 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 3		Compound 4, HCl		Combination (c)	
	Start conc [uM]	Mean of Max Inh [%]	Start conc [uM]	Mean of Max Inh [%]	Mean of SynergyScore	Synergy Score Error (sd)
MV4;11	0.01	100.0	0.03	70	3.37	0.75
MOLM-13	0.1	100	0.1	99	3.84	0.02
ML-2	0.1	100	0.1	99	7.09	0.96
OCI-AML3	2.0	100.0	5.0	53.5	16.53	1.62
GDM-1	0.1	100	0.1	99	7.03	0.52

Results

Combination (a). The effect on proliferation of combining the MCL1 inhibitor Compound 3 with the BCL-2 inhibitor Compound 1 was assessed in a panel of 13 Acute Myeloid Leukemia (AML) cell lines.

Compound 3 as single agent strongly inhibited the growth of the majority of the 13 AML lines tested (Table 4a). Thus, 10 cell lines displayed IC_{50}s below 100 nM, and an additional 2 cell lines displayed IC_{50}s between 100 nM and 1 uM. Only 1 cell lines displayed an IC_{50} above 1 uM.

Compound 1, HCl as single agent also inhibited the growth of the several AML lines tested, although slightly less potent (Table 4a). Thus, 5 cell lines displayed IC_{50}s below 100 nM, and 2 cell lines displayed IC_{50}s between 100 nM and 1 uM. Six cell lines displayed an IC_{50} above 1 uM.

In combination, Compound 3 and Compound 1, HCl treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2) in the entire 13 cell lines tested (Table 5a). In 2 cell lines, the synergy effect was marked, with synergy scores between 5 and 10. In 10 cell lines, the synergy effect was exceptional, achieving synergy scores between 10 and 19.8.

Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of Compound 3 and Compound 1 that did not have an anti-proliferative effect on their own. For example, in OCI-AML3 cells, Compound 3 and Compound 1 at the third lowest concentration tested elicited a growth inhibition of 5 and 1%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 84% (Figure 9A, top left panel), thus being 79% above the additivity calculated based on the single agent activities (Figure 9A, top right panel).

Furthermore, it is noteworthy that the synergistic effects occurred across a broad range of single agent concentrations, which should prove beneficial in vivo with respect to flexibility concerning dosing levels and scheduling.

In summary, the combination of Compound 3 and Compound 1 afforded synergistic growth inhibition in all the 13 AML cell lines tested. Importantly, exceptional synergistic growth inhibition was observed in the majority AML cell lines tested (10/13).

Combination (b). The effect on proliferation of combining the MCL1 inhibitor Compound 3 with the BCL-2 inhibitor ABT-199 was assessed in a panel of 8 Acute Myeloid Leukemia (AML) cell lines.

Compound 3 as single agent strongly inhibited the growth of the majority of the 8 AML lines tested (Table 4a). Thus, 5 cell lines displayed IC_{50}s below 100 nM, and an additional 2 cell lines displayed IC_{50}s between 100 nM and 1 uM. Only 1 cell lines displayed an IC_{50} above 1 uM.

ABT-199 as single agent also inhibited the growth of AML lines, although with less potency (Table 4a). Thus, only one cell line displayed IC_{50}s below 100 nM, and 2 cell lines displayed IC_{50}s between 100 nM and 1 uM. Five cell lines displayed IC_{50} above 1 uM.

In combination, MCL1 inhibitor and ABT-199 treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2) in the entire panel of 8 cell lines tested (Table 5b). In the majority of the cell lines, the synergy effect was exceptional, achieving synergy scores between 10 and 17.6. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of MCL1 inhibitor and ABT-199 that did not have an anti-proliferative effect on their own. For example, in OCI-AML3 cells, MCL1 and ABT-199 at the third lowest concentration tested

elicited a growth inhibition of 26% and 18%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 91% (Figure 13, top left panel).

Furthermore, it is noteworthy that the synergistic effects occurred across a broad range of single agent concentrations, which should prove beneficial in vivo with respect to flexibility concerning dosing levels and scheduling.

In summary, the combination of Compound 3 and ABT-199 afforded synergistic growth inhibition in all the 8 AML cell lines tested. Importantly, exceptional synergistic growth inhibition was observed in the majority AML cell lines tested (7/8).

Combination (c). The effect on proliferation of combining the MCL1 inhibitor Compound 3 with the BCL-2 inhibitor Compound 4 was assessed in a panel of 5 Acute Myeloid Leukemia (AML) cell lines.

Compound 3 as single agent strongly inhibited the growth of the 5 AML lines tested (Table 4b). Thus, all cell lines displayed IC_{50}s below 200 nM. Compound 4, HCl as single agent also inhibited the growth of the 4 out of 5 cell lines tested with IC_{50} below or equal to 40 nM, one cell line being resistant to Compound 4 with an IC_{50} of 10μM. In combination, Compound 3 and Compound 4, HCl treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2) in the entire 5 cell lines tested (Table 5c). In 2 cell lines, the synergy effect was marked, with synergy scores between 5 and 10. In 1 cell line, the synergy effect was exceptional, achieving synergy score of 16.5. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of Compound 4, HCl and Compound 3 that have no or low anti-proliferative effect on their own. For example, in OCI-AML3 cells, Compound 4, HCl and Compound 3 at the third lowest concentration tested elicited a growth inhibition of 1 and 40%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 98% (Figure 1A, left panel; representative of two independent experiments), thus being 53% above the additivity calculated based on the single agent activities (Figure 14A, right panel). In ML-2, Compound 4, HCl and Compound 3 at the fifth lowest concentration tested elicited a growth inhibition of 18 and 26%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 100% (Figure 14B, left panel; representative of two independent experiments), thus being

51% above the additivity calculated based on the single agent activities (Figure 15, right panel)

In summary, the combination of Compound 4 and Compound 3 afforded synergistic growth inhibition in all the 5 AML cell lines tested.

EXAMPLE 9: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of of 12 neuroblastoma (NB) cell lines

Materials and methods

Cell lines were sourced and maintained in the basic media supplemented with FBS as indicated in Table 1. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 µg/ml) and L-glutamine (2 mM). 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 ul/well at the densities indicated in Table 6 into 96-well plates. All cell lines were determined to be free of mycoplasma contamination in-house.

Stock solutions of compounds were prepared at a concentration of 5 mM in DMSO and stored at -20°C. In order to analyse the activity of the compounds as single agents, cells were seeded and treated with nine 3.16-fold serial dilutions of each compound dispensed individually directly into the cell assay plates. Effects of the compounds on cell viability were assessed after 2 or 3 days of incubation (as indicated in Table 6) at 37 °C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo at 150 µL reagent/well. Two independent experiments, each one performed in duplicates were performed. All the experiments were performed in triplicates. Luminescence was quantified on a multipurpose platereader. Single agent IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the vehicle (DMSO) control.

Identical experiments were performed to assess potential synergistic interactions between compound combinations. Synergy Score were assessed using the Excess Inhibition 2D matrix according to the Loewe additivity model (Lehar *et al*, *Nat Biotechnol.* **2009** July ;

27(7): 659–666). All calculations were performed using Chalice TM Bioinformatics Software.

The doubling time indicated in Table 6 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.

The interpretation of the Synergy Score is as follows:

SS ~ 0 → Additive

SS >1 → Weak Synergy

SS >2 → Synergy

Table 6. Identity and assay conditions for the 12 neuroblastoma (NB) cell lines used in the combination experiments.

Cell line	Medium (source)	%FBS	Doubling time (hours)	Cell number seeded/well	Days of incubation with cpds
SK-N-AS	DMEM (ATCC)	10	33	9375	3
SK-N-BE	EMEM/Ham F12 (ATCC)	10	50	37500	3
SK-N-DZ	DMEM (ATCC)	10	42	37500	3
LAN-6	DMEM (DSMZ)	20	100	9375	3
NBL-S	Iscove's MDM (DSMZ)	10	46	18750	3
SIMA	RPMI (DSMZ)	10	60	18750	3
KELLY	RPMI (ECACC)	10	34	3750	2
IMR-32	EMEM (ATCC)	10	55	28125	2
SH-SY-5Y	1/2 EMEM no glutamin + 1/2 Ham F12 + 2mM Glutamin + NEAA (ECACC)	15	35	3750	2
SK-N-SH	EMEM (ATCC)	10	65	3750	2
NB-1	RPMI (JCRB)	10	35	15000	2
SK-N-FI	DMEM (ATCC)	10	60	7500	2

Table 7. Single agent IC_{50} values for Compound 3 and Compound 1, HCl, are indicated. Compounds were incubated with the cells during 2 or 3 days.

Cell Line	Compound 3	Compound 1, HCl
	IC_{50} [uM]	IC_{50} [uM]
SK-N-AS	0.26	> 1
SK-N-BE	>2	>2
SK-N-DZ	>2	>2
LAN-6	>2	>2
NBL-S	>2	>2
SIMA	>2	>2
NB1	0.123	>3
SK-N-SH	>3	>3
SH-SY5Y	>3	>3
Kelly	0.031	>3
SK-N-FI	>3	>3

Table 8. Synergy scores for combination with Compound 3 and Compound 1, HCl are indicated. Interactions were deemed synergistic when scores ≥ 2.0 where observed. Compounds were incubated with the cells during 2 or 3 days.

Cell Line	Compound 3		Compound 1, HCl		Combination	
	Start conc [uM]	Mean of Max Inh [%]	Start conc [uM]	Max Inh [%]	Synergy Score	Synergy Score Error
SK-N-AS	2	84	1	9	2.78	0.46
SK-N-BE	2	27	2	27	10.72	0.78
SK-N-DZ	2	2.5	2	10	0.34	0.06
LAN-6	2	17.5	2	26	10.51	0.39
NBL-S	2	13	2	10	17.81	3.7
SIMA	2	0	2	48.5	2.41	0.75
NB1	3	99	3	11	10.72	4.33
SK-N-SH	3	40	3	15	4.07	0.23
SH-SY5Y	3	24	3	10	10.21	0.54
Kelly	3	99	3	27	9.62	0.48
SK-N-FI	3	33	3	6	4.35	0.91

Results

The effect on proliferation of combining the MCL1 inhibitor Compound 3 with the BCL-2 inhibitor Compound 1 was assessed in a panel of 12 neuroblastoma cell lines. Three out of the 12 cell lines tested are sensitive to Compound 3 as single agent (Table 7). One cell lines displayed IC_{50}s below 100 nM, and an additional 2 cell lines displayed IC_{50}s between 100 nM and 1 uM.

All cell lines are resistant to Compound 1, HCl as single agent with all cell lines tested displaying an IC_{50} above 1μM. In combination, Compound 3 and Compound 1 treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2 - Lehar *et al*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666) in 11 out of 12 NB cell lines tested (Table 8). In 5 cell lines, the synergy effect was exceptional, achieving synergy scores between 10 and 17.81. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of Compound 3 and Compound 1, HCl that did not have an anti-proliferative effect on their own. For example, in LAN-6 cells, Compound 3 and Compound 1, HCl at 630 nM elicited a growth inhibition of only 12% and 0%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 95% (Figure 10, upper left panel), thus being 76% above the additivity calculated based on the single agent activities (Figure 10, upper right panel). In summary, the combination of Compound 3 and Compound 1 afforded strong to exceptional synergistic growth inhibition in the majority of neuroblastoma cell lines tested.

EXAMPLE 10: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of 8 B-cell acute lymphoblastic leukaemia (B-ALL) and 10 T-cell acute lymphoblastic leukaemia (T-ALL) cell lines.

Materials and methods

Cell lines were sourced and maintained in the basic media supplemented with FBS as indicated in Table 1. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM). 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 ul/well at

the densities indicated in Table 9 into 96-well plates. All cell lines were determined to be free of mycoplasma contamination in-house.

Stock solutions of compounds were prepared at a concentration of 5 mM in DMSO and stored at -20°C. In order to analyse the activity of the compounds as single agents, cells were seeded and treated with nine 2-fold serial dilutions of each compound dispensed individually directly into the cell assay plates. Effects of the compounds 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. All the conditions were tested in triplicates. Luminescence was quantified on a multipurpose plate reader. Single agent IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the vehicle (DMSO) control.

In order to analyse the activity of the compounds 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 Figure 1. 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. For B-ALL cell lines, two independent experiments, each one performed in duplicates, were performed. For T-ALL cell lines, one experiment performed in triplicate was performed. Luminescence was quantified on a multipurpose plate reader.

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*, *Nat Biotechnol*. **2009** July ; 27(7): 659–666). All calculations were performed using Chalice TM Bioinformatics Software available in Horizon website.

The doubling time indicated in Table 9 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.

The interpretation of the Synergy Score is as follows:

SS ~ 0 → Additive

SS >1 → Weak Synergy

SS >2 → Synergy

Table 9. Identity and assay conditions for the 8 B-ALL and 10 T-ALL cell lines used in the combination experiments.

Cell line	Cancer type	Medium (source)	%FBS	Doubling time (hours)	Cell number seeded/well
TOM-1	**B-ALL**	RPMI (DSMZ)	20	70.0	112500
SUP-B15	**B-ALL**	McCoy (DSMZ)	20	35.0	112500
NALM-21	**B-ALL**	RPMI (DSMZ)	10	50.0	112500
NALM-6	**B-ALL**	RPMI (DSMZ)	10	27.0	56250
TANOUE	**B-ALL**	RPMI (DSMZ)	10	26.0	30000
Kasumi-2	**B-ALL**	RPMI (DSMZ)	10	52.0	112500
RS4;11	**B-ALL**	RPMI (ATCC)	10	42.0	90000
BALL-1	**B-ALL**	RPMI (DSMZ)	10	38.0	112500
BE-13	**T-ALL**	RPMI 1640 (DSMZ)	10	37.0	13875
MOLT-4	**T-ALL**	RPMI 1640 (ATCC)	10	24.0	28125
TALL-104	**T-ALL**	IMDM (ATCC)	20	68.0	13875
HPB-ALL	**T-ALL**	RPMI 1640 (DSMZ)	20	42.0	56250
DND-41	**T-ALL**	RPMI 1640 (DSMZ)	10	38.0	56250
CML-T1	**T-ALL**	RPMI 1640 (DSMZ)	10	32.0	112500
J45.01	**T-ALL**	RPMI 1640 (ATCC)	10	25.0	56250
CCRF-CEM	**T-ALL**	RPMI 1640 (ATCC)	10	24.0	56250
J.RT3 T3.5	**T-ALL**	RPMI 1640 (ATCC)	10	24.0	56250
Loucy	**T-ALL**	RPMI 1640 (ATCC)	10	61.0	112500

Table 10. Single agent IC_{50} values for Compound 3 and Compound 1, HCl in the 8 B-ALL and 10 T-ALL cell lines are indicated. Compounds were incubated with the cells during 3 days.

Cell Line	Cancer type	Treatment duration(h)	Compound 3		Compound 1, HCl	
			Start conc [uM]	IC_{50} [uM]	Start conc [uM]	IC_{50} [uM]
TOM-1	**B-ALL**	**72**	0.10	0.024	0.15	0.019
SUP-B15	**B-ALL**	**72**	2.00	0.078	0.90	0.025

NALM-21	**B-ALL**	**72**	0.10	0.012	0.50	0.095
NALM-6	**B-ALL**	**72**	2.00	0.120	30.00	3.630
TANOUE	**B-ALL**	**72**	30.00	6.540	30.00	17.000
Kasumi-2	**B-ALL**	**72**	2.00	0.030	2.00	0.209
RS4;11	**B-ALL**	**72**	0.90	0.079	9.00	0.020
BALL-1	**B-ALL**	**72**	0.25	0.063	0.10	0.019
BE-13	**T-ALL**	**72**	0.15	0.015	30.00	6.700
MOLT-4	**T-ALL**	**72**	2.00	0.026	30.00	3.290
TALL-104	**T-ALL**	**72**	2.00	0.044	30.00	15.900
HPB-ALL	**T-ALL**	**72**	2.00	0.660	30.00	4.500
DND-41	**T-ALL**	**72**	30.00	7.000	30.00	9.000
CML-T1	**T-ALL**	**72**	30.00	6.000	30.00	15.000
J45.01	**T-ALL**	**48**	0.60	0.029	30.00	9.000
CCRF-CEM	**T-ALL**	**48**	0.90	0.047	30.00	7.500
J.RT3 T3.5	**T-ALL**	**48**	1.88	0.063	30.00	10.000
Loucy	**T-ALL**	**48**	0.90	0.064	3.75	0.231

Table 11. Synergy scores for Compound 3 and Compound 1, HCl combination in 8 B-ALL and 10 T-ALL cell lines 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	Cancer type	Treatment duration (h)	Compound 3		Compound 1, HCl		Combination	
			Start conc [uM]	Mean of Max Inh [%]	Start conc [uM]	Mean of Max Inh [%]	Mean of Synergy Score	Synergy Score Error (sd)
TOM-1	**B-ALL**	**72**	0.3	98.5	0.1	90.5	4.1	0.4
SUP-B15	**B-ALL**	**72**	2.0	99.0	0.3	97.0	5.6	0.4
NALM-21	**B-ALL**	**72**	0.3	99.0	0.3	84.5	9.6	0.0
NALM-6	**B-ALL**	**72**	2.0	71.5	5.0	56.5	15.9	1.4
TANOUE	**B-ALL**	**72**	5.0	78.5	5.0	13.0	1.0	0.6
Kasumi-2	**B-ALL**	**72**	0.3	99.0	2.0	82.0	10.1	1.9
RS4;11	**B-ALL**	**72**	0.3	87.5	0.3	98.0	7.0	1.3
BALL-1	**B-ALL**	**72**	0.3	96.0	0.3	100.0	6.3	0.3
BE-13	**T-ALL**	**72**	1.0	95.0	5.0	0.0	8.8	0.4
MOLT-4	**T-ALL**	**72**	1.0	99.0	5.0	63.0	4.4	0.1
TALL-104	**T-ALL**	**72**	1.0	99.0	2.0	29.0	15.1	0.5

HPB-ALL	**T-ALL**	**72**	1.0	49.0	5.0	15.0	5.6	0.3
DND-41	**T-ALL**	**72**	2.0	17.0	5.0	24.0	10.3	0.3
CML-T1	**T-ALL**	**72**	2.0	22.3	5.0	17.0	3.3	0.2
J45.01	**T-ALL**	**48**	2.0	100.0	2.0	23.0	2.9	0.1
CCRF-CEM	**T-ALL**	**48**	2.0	92.0	2.0	55.0	4.1	1.0
J.RT3 T3.5	**T-ALL**	**48**	2.0	99.0	2.0	32.0	3.8	0.1
Loucy	**T-ALL**	**48**	2.0	100.0	2.0	77.0	11.3	0.6

Results

The effect on proliferation of combining the MCL1 inhibitor with the BCL-2 inhibitor was assessed in a panel of 8 B-ALL and 10 T-ALL cell lines.

MCL1 inhibitor as single agent strongly inhibited the growth of the majority of the ALL cell lines tested (Table 10). Thus, 13 ALL cell lines displayed IC_{50}s below 100 nM, and an additional 2 ALL cell lines displayed IC_{50}s between 100 nM and 1 uM. Only 3 ALL cell lines displayed IC_{50} above 1 uM.

BCL-2 inhibitor as single agent also inhibited the growth of several ALL cell lines tested, although it was less potent (Table 10). Thus, 5 cell lines displayed IC_{50}s below 100 nM, and 2 cell lines displayed IC_{50}s between 100 nM and 1 uM. Eleven ALL cell lines displayed an IC_{50} above 1 uM.

In combination, MCL1 inhibitor and BCL-2 inhibitor treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2 - Lehar *et al*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666) in the entire 17/18 ALL cell lines tested (Table 11). In 6 cell lines, the synergy effect was marked, with synergy scores between 5 and 10. In 5 cell lines, the synergy effect was exceptional, achieving synergy scores between 10 and 15.9. Importantly, the synergy was not dependent on single agent anti-proliferative effects, and in fact was particularly strong at concentrations of MCL1 inhibitor and BCL-2 inhibitor that did not have an anti-proliferative effect on their own. For example, in NALM-6 cells, MCL1 inhibitor and BCL-2 inhibitor at the fourth lowest concentration tested elicited a growth inhibition of 6 and 8%, respectively, while the respective combination of the two compounds afforded a growth inhibition of 61% (Figure 11, top left panel).

Furthermore, it is noteworthy that the synergistic effects occurred across a broad range of single agent concentrations, which should prove beneficial in vivo with respect to flexibility concerning dosing levels and scheduling.

In summary, the combination of MCL1 inhibitor and BCL-2 inhibitor afforded synergistic growth inhibition in the majority (17/18) of ALL cell lines tested. Importantly, exceptional synergistic growth inhibition was observed in 5/18 ALL cell lines tested.

EXAMPLE 11: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of 5 Mantle Cell Lymphoma (MCL) cell lines.

Materials and methods

Cell lines were sourced and maintained in the basic media supplemented with FBS as indicated in Table 12. In addition, all media contained penicillin (100 IU/ml), streptomycin (100 μg/ml) and L-glutamine (2 mM).

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 ul/well at the densities indicated in Table 12 into 96-well plates. All cell lines were determined to be free of mycoplasma contamination in-house.

Stock solutions of compounds were prepared at a concentration of 5 mM in DMSO and stored at -20°C. In order to analyse the activity of the compounds as single agents 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. Effects of the single agents as well as their checkerboard combinations on cell viability were assessed after 2 days of incubation at 37 °C/5% CO_2 by quantification of cellular ATP levels using CellTiterGlo at 150 μL reagent/well. All the conditions were tested in triplicates. Luminescence was quantified on a multipurpose plate reader.

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*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666). All calculations were performed using Chalice™ Bioinformatics Software available in Horizon website.

Single agent IC_{50}s were calculated using standard four-parametric curve fitting. IC_{50} is defined as the compound concentration at which the CTG signal is reduced to 50% of that measured for the vehicle (DMSO) control.

The doubling time indicated in Table 12 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

SS ~ 0 → Additive

SS >1 → Weak Synergy

SS >2 → Synergy

Table 12. Identity and assay conditions for the 5 Mantle Cell Lymphoma cell lines used in the combination experiments.

Cell line	Medium	%FBS	Source	Doubling time (hours)	Cell number seeded/well
Z-138	RPMI	10	ATCC	22.5	37500
Jeko	RPMI	20	ATCC	26.0	27000
Mino	RPMI	15	ATCC	31.1	56250
JVM-2	RPMI	10	ATCC	76.0	56250
REC-1	RPMI	10	ATCC	36.0	56250

Table 13. Single agent IC_{50} values for Compound 3 and Compound 1, HCl in the 5 Mantle Cell Lymphoma cell lines are indicated. Compounds were incubated with the cells during 2 days.

Cell Line	Compound 3		Compound 1, HCl	
	Start conc [uM]	IC_{50} [uM]	Start conc [uM]	IC_{50} [uM]
Z-138	2	0.448	5	> 5
Jeko	2	0.023	5	> 5
Mino	2	0.008	2	0.091
JVM-2	2	>2	5	> 5
REC-1	2	0.077	2	0.703

Table 14. Synergy scores for Compound 3 and Compound 1, HCl combination in 5 Mantle Cell Lymphoma cell lines are indicated. Interactions were deemed synergistic when scores ≥ 2.0 where observed. Start concentrations of compounds, max inhibition and the synergy scores are indicated. Compounds were incubated with the cells during 2 days.

Cell Line	Compound 3		Compound 1, HCl		Combination
	Start conc [uM]	Max Inh [%]	Start conc [uM]	Max Inh [%]	Synergy Score
Z-138	2.0	96.0	5.0	25.0	11.1
Jeko	2.0	100.0	5.0	35.0	9.7
Mino	2.0	100.0	2.0	91.0	5.7
JVM-2	2.0	19.0	5.0	38.0	3.4
REC-1	2.0	99.0	2.0	78.0	5.1

Results

The effect on proliferation of combining the MCL1 inhibitor with the BCL-2 inhibitor was assessed in a panel of 5 Mantle Cell Lymphoma cell lines.

As single agents, MCL1 inhibitors displayed superior activity as compared with BCL-2 inhibitor. Thus, 3 cell lines displayed IC_{50}s below 100 nM for MCL1 inhibitor while only one cell line displayed IC_{50}s below 100 nM for BCL-2 inhibitor (Table 13).

In combination, MCL1 inhibitor and BCL-2 inhibitor treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2 - Lehar *et al*, *Nat Biotechnol.* **2009** July ; 27(7): 659–666) in all cell lines tested (Table 14), as examplified in Figure 12. Importantly, in 4/5 cell lines, the synergy effect was marked, with synergy scores above 5.

EXAMPLE 12: *In vitro* effect on proliferation of combining a MCL1 inhibitor with a BCL-2 inhibitor in a panel of 5 Small Cell Lung Cancer (SCLC) cell lines.

All cell lines were obtained from ATCC. Culture media containing RPMI1640 (Invitrogen) supplemented with 10% FBS (HyClone) was used for COR-L95, NCI-H146, NCI-H211, SHP-77, SW1271, NCI-H1339, NCI-H1963, and NCI-H889. Culture media containing Waymouth's MB 752/1 (Invitrogen) with 10% FBS was used for DMS-273. Culture media containing DMEM/F12 (Invitrogen) containing 5% FBS, and supplemented with 0.005 mg/ml insulin, 0.01 mg/ml transferrin, and 30 nM sodium selenite solution (Invitrogen), 10

nM hydrocortisone (Sigma), 10 nM beta-estradiol (Sigma), and 2 mM L-glutamine (HyClone) was used for NCI-H1105.

Cell lines were cultured in 37°C and 5% CO2 incubator and expanded in T-75 flasks. In all cases cells were thawed from frozen stocks, expanded through ≥1 passage using 1:3 dilutions, counted and assessed for viability using a ViCell counter (Beckman-Coulter), prior to plating in 384-well. To split and expand cell lines, cells were dislodged from flasks using 0.25% Trypsin-EDTA (GIBCO). All cell lines were determined to be free of mycoplasma contamination as determined by a PCR detection methodology performed at Idexx Radil (Columbia, MO, USA) and correctly identified by detection of a panel of SNPs.

Cell proliferation was measured in 72hr CellTiter-Glo™ (CTG) assays (Promega G7571) and all results shown are the result of at least triplicate measurements. For CellTiter-Glo™ assays, cells were dispensed into tissue culture treated 384-well plates (Corning 3707) with a final volume of 35 μL of medium and at density of 5000 cells per well. 24 hrs after plating, 5 μL of each compound dilution series were transferred to plates containing the cells, resulting in compound concentration ranges from 0-10 uM and a final DMSO (Sigma D8418) concentration of 0.16%. Plates were incubated for 72 hrs and the effects of compounds on cell proliferation was determined using the CellTiter-Glo™ Luminescent Cell Viability Assay (Promega G7571) and a Envision plate reader (Perkin Elmer).

The CellTiter-Glo® Luminescent Cell Viability Assay 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, cells were plated in Opaque-walled multiwell plates in culture medium as described above. Control wells containing medium without cells were also prepared to obtain a value for background luminescence. 15 uL of CellTiter-Glo® Reagent was then added and contents mixed for 10 minutes on an orbital shaker to induce cell lysis. Next, luminescence was recorded using the plate reader.

The percent growth inhibition and excess inhibition were analysed using the Chalice software (CombinatoRx, Cambridge MA). The percentage of growth inhibition relative to

DMSO is displayed in the panel labelled inhibition, and the amount of inhibition in excess of the expected amount in the panel labelled ADD Excess Inhibition (Figures 15 (a)-(e)). Concentrations of Compound 1, HCl are shown along the bottom row from left to right and increasing concentrations of Compound 3 along the leftmost 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. Data analysis of cell proliferation was performed using Chalice Analyser as described in Lehar *et al*, *Nat Biotechnol*. **2009** July ; 27(7): 659–666. Excess inhibition was calculated using the Loewe synergy model which measures the effect on growth relative to what would be expected if two drugs behave in a dose additive manner. Positive numbers represent areas of increasing synergy.

Synergy Score

SS ~ 0 → Dose Additive

SS >2 → Synergy

SS >1 → Weak Synergy

Results

In combination, Compound 1 and Compound 3 treatment caused synergistic growth inhibition (*i.e.* Synergy Scores above 2) in 8/10 small cell lung cancer cell lines. Importantly, in 6 cell lines, the synergy effect was marked, with synergy scores above 6.

EXAMPLE 13: *In vivo* efficacy in Patient-derived primary AML model HAMLX5343 with combination of a MCL1 inhibitor (Compound 3) and a BCL-2 inhibitor (Compound 1, HCl or ABT-199)

Materials and Methods

Materials

Animals

NOD *scid* gamma (NSG) female mice weighing 17-27 grams (Jackson Laboratories) were allowed to acclimate with access to food and water ad libitum for 3 days prior to manipulation.

Primary tumor models

Patient-derived primary AML model HAMLX5343 carrying *KRAS* mutation and wild type *FLT3* were obtained from Dana Farber Cancer Institute.

Test compounds, formulations

Compound 1, HCl was formulated in 5% Ethanol, 20% Dexolve-7 as a solution for intravenous administration or formulated in PEG300/EtOH/water (40/10/50) for oral administration. ABT-199 was formulated in PEG300/EtOH/water (40/10/50) for oral administration. All of them are stable for at least one week at 4°C. Compound 3 was formulated in Liposomal formulation as a solution for intravenous formulation, which is stable for three weeks at 4°C. Vehicle and compound dosing solutions were prepared as needed. All animals were dosed at 10 mL/kg with Compound 1 (expressed as the free base) or ABT-199, or 5 mL/kg with Compound 3.

Methods

Study design

Eight treatment groups were used in study 7844HAMLX5343-XEF as summarized in Table 15. All treatments were initiated when the average tumor burden (%CD-45 positive cells) was between 8% and 15%.

In this study, Compound 1 was administered by oral gavage (po) or intravenous administration at 50 mg/kg once a week, ABT-199 was administered at 25mg/kg by oral gavage (po) once a week, either as a single agent or in combination with Compound 3 at 12.5mg/kg once a week, respectively, for 18 days.

Both Compound 1 (expressed as the free base) and ABT-199 were administered at 10 mL/kg. Compound 3 was administered at 5 mL/kg. The dose was body weight adjusted. Bodyweights were recorded twice/week and tumor burden was recorded once/week.

Table 15. Doses* and dose schedules for 7844HAMLX5343-XEF

Treatment groups	Number of animals	Dosing regimen
Vehicle (10 mL/kg)	4	QW
Compound 1 (50mg/kg po)	4	QW
Compound 1 (50mg/kg iv)	4	QW

Treatment groups	Number of animals	Dosing regimen
ABT-199 (25mg/kg po)	4	QW
Compound 3 (12.5mg/kg iv)	4	QW
Compound 1 + Compound 3 (po/iv)	4	QW + QW
Compound 1 + Compound 3 (iv/iv)	4	QW + QW
ABT-199 + Compound 3 (po/iv)	4	QW + QW

* *Doses are expressed as the free base*

Primary AML model

For this experiment, 32 mice were implanted with primary AML line HAMLX5343. Mice were injected intravenously with 2.0 million leukemia cells. When the tumor burden was between 8%-15%, animals were randomized into eight groups of four mice each for vehicle, Compound 1 (po), Compound 1 (iv), ABT-199, Compound 3, or combination treatment. After 18 days of treatment, the study was terminated when the tumor burden reached 99%. Tumor burden was measured by FACS analysis.

Animal monitoring

Animal well-being and behavior, including grooming and ambulation were monitored twice daily. General health of mice was monitored and mortality recorded daily. Any moribund animals were sacrificed.

Tumor measurement

Mice were bled via tail snip once per week. Blood was split into an IgG control well and a CD33/CD45 well of a 96-well plate. Blood was lysed with 200µl RBC lysis buffer twice at RT, then washed once with FACS buffer (5% FBS in PBS). Samples were then incubated for 10-30 minutes at 4C in 100µl blocking buffer (5% mouse Fc Block + 5% human Fc Block + 90% FACS buffer). 20µl IgG control mix (2.5µl Mouse igG1 K isotype control-PE + 2.5µl Mouse igG1 K isotype control-APC + 15µl FACS buffer) were added to the IgG control wells and 20ul CD33/CD45 mix (2.5µl Mouse anti-human CD33-PE + 2.5µl Mouse anti-human CD45-APC + 15µl FACS buffer). Samples were incubated for 30-60 minutes at 4C then washed twice prior to analysis. Samples were run on Canto with

FACSDiva software. Analysis was performed with FloJo software. The percent of CD45-positive, live, single cells was reported as the tumor burden.

Data analysis

Percent treatment/control (T/C) values were calculated using the following formula:

$\%T/C = 100 \times \Delta T/\Delta C$ if $\Delta T > 0$

$\%Regression = 100 \times \Delta T/T_{initial}$ if $\Delta T < 0$

where:

T = mean tumor burden of the drug-treated group on the final day of the study;

ΔT = mean tumor burden of the drug-treated group on the final day of the study – mean tumor burden of the drug-treated group on initial day of dosing;

$T_{initial}$ = mean tumor burden of the drug-treated group on initial day of dosing;

C = mean tumor burden of the control group on the final day of the study; and

ΔC = mean tumor burden of the control group on the final day of the study – mean tumor burden of the control group on initial day of dosing.

All data were expressed as Mean ± SEM. Delta tumor burden and body weight were used for statistical analysis. Between-groups comparisons for final measurements were performed using ANOVA with Tukey's test. Statistical analysis was carried out using GraphPad Prism.

Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Delta tumor volume and body weight were used for statistical analysis. Between-group comparisons were carried out using the Kruskal-Wallis ANOVA followed by a post hoc Dunn's test or Tukey's test. For all statistical evaluations, the level of significance was set at $p < 0.05$. Significance compared to the vehicle control group is reported unless otherwise stated. The standard protocols used in pharmacology studies are not pre-powered to demonstrate statistically significant superiority of a combination over the respective single agent treatment. The statistical power is often limited by potent single agent response and/or

model variability. The p-values for combination vs single agent treatments are, however, provided.

Results

Synergistic anti-tumor effect of combined MCL1 and BCL-2 inhibition

In the 7844HAMLX5343-XEF study, Compound 1, ABT-199 or Compound 3 alone did not show anti-tumor activity in the HAMLX5343 model carrying the *KRAS* mutation, when administered at 50mg/kg (oral or *iv*), 25mg/kg (oral) or 12.5mg/kg (*iv*) once a week, respectively (%T/C of 98, 92, 98 or 99%, respectively, $p>0.05$).

When orally administered, Compound 1 at 50mg/kg or ABT-199 at 25mg/kg in combination with Compound 3 (12.5mg/kg *iv*) once a week resulted in tumor stasis (%T/C of 3% or 6%, respectively, $p<0.05$) in this model.

On the other hand, the combination of intravenously administered Compound 1 with Compound 3 induced near complete tumor regression (%Regression of 100%), which is significantly different from either single agent ($p<0.05$) or Compound 1/Compound 3 po/*iv* combination. The mean tumor burden for each treatment group is plotted against time for the 18 day treatment period, as shown in Figure 1. The change in tumor burden, %T/C or %Regression is presented in Table 16 and in Figures 16 (a)-(b).

Table 16. Summary of anti-tumor effect in 7844HAMLX5343-XEF study

Treatment	**T/C %**	**Regression %**
Vehicle	100	
Compound 1 50mpk *po*	98	
Compound 1 50mpk *iv*	92	
ABT-199 25mpk *po*	98	
Compound 3 12.5mpk *iv*	99	
Compound 1 + Compound 3 *(po/iv)*	3*	
Compound 1 + Compound 3 *(iv/iv)*		100**

ABT-199 + Compound 3 ***(po/iv)***	6*	

* $p < 0.05$ versus Vehicle and single agents (ANOVA, Tukey's test)

** $p < 0.05$ versus *po/iv* combination (ANOVA, Tukey's test)

Conclusion

AML is an aggressive and heterogeneous hematologic malignancy, caused by the transformation of hematopoietic progenitor cells due to acquisition of genetic alterations (Patel *et al, New England Journal of Medicine* 2012 366:1079-1089). The 5-year survival rate of AML has been low due to lack of effective therapies. Evasion of apoptosis is a hallmark of cancer (Hanahan *et al Cell* 2000 100:57-70). One of the primary means by which cancer cells evade apoptosis is by up-regulating the pro-survival BCL-2 family proteins such as BCL-2, BCL-xL and MCL1.

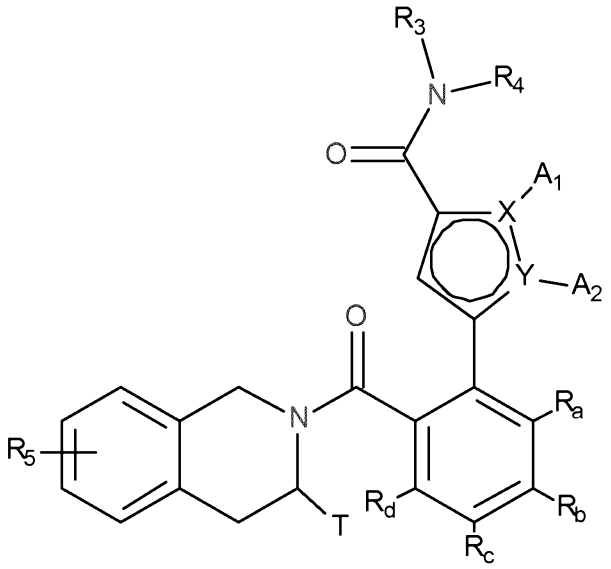
MCL1 gene is of the most commonly amplified gene in cancer patients (Beroukhim *et al*, Nature 2010 463:899-905). Moreover, both BCL-2 and MCL1 are highly expressed in AML. Therefore, the combination of Compound 1 (BCL-2i) and Compound 3 (MCL1) may provide synergy by enhancing pro-apoptotic signals as a general mechanism against AML.

We show here that BCL-2 inhibitor Compound 1 or ABT-199 in combination with Compound 3 (MCL1 inhibitor) has a dramatic synergistic effect in treating AML in an AML xenograft model with KRAS mutation (wt FLT3). The *iv/iv* Compound 1/Compound 3 combination is superior to the *po/iv* combination treatment at the same dose level. The results indicate that the combination of and MCL1 inhibitors would be an effective therapy for AML.

CLAIMS

1. A combination comprising:

(a) a BCL-2 inhibitor of formula (I):



(I)

wherein:

- X and Y represent a carbon atom or a nitrogen atom, it being understood that they may not simultaneously represent two carbons atoms or two nitrogen atoms,
- A_1 and A_2, together with the atoms carrying them, form an optionally substituted, aromatic or non-aromatic heterocycle Het composed of 5, 6 or 7 ring members which may contain, in addition to the nitrogen represented by X or by Y, from one to 3 hetero atoms selected independently from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be substituted by a group representing a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a group -C(O)-O-Alk wherein Alk is a linear or branched (C_1-C_6)alkyl group,
  or A_1 and A_2 independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_1-C_6)alkyl group or a cycloalkyl,
- T represents a hydrogen atom, a linear or branched (C_1-C_6)alkyl group optionally substituted by from one to three halogen atoms, a group (C_1-C_4)alkyl-NR_1R_2, or a group (C_1-C_4)alkyl-OR_6,

- R_1 and R_2 independently of one another represent a hydrogen atom or a linear or branched $(C_1\text{-}C_6)$alkyl group,
  or R_1 and R_2 form with the nitrogen atom carrying them a heterocycloalkyl,
- R_3 represents a linear or branched $(C_1\text{-}C_6)$alkyl group, a linear or branched $(C_2\text{-}C_6)$alkenyl group, a linear or branched $(C_2\text{-}C_6)$alkynyl group, a cycloalkyl group, a $(C_3\text{-}C_{10})$cycloalkyl-$(C_1\text{-}C_6)$alkyl group wherein the alkyl moiety is linear or branched, a heterocycloalkyl group, an aryl group or a heteroaryl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_4 represents an aryl group, a heteroaryl group, a cycloalkyl group or a linear or branched $(C_1\text{-}C_6)$alkyl group, it being understood that one or more of the carbon atoms of the preceding groups, or of their possible substituents, may be deuterated,
- R_5 represents a hydrogen or halogen atom, a linear or branched $(C_1\text{-}C_6)$alkyl group, or a linear or branched $(C_1\text{-}C_6)$alkoxy group,
- R_6 represents a hydrogen atom or a linear or branched $(C_1\text{-}C_6)$alkyl group,
- R_a, R_b, R_c and R_d, each independently of the others, represent R_7, a halogen atom, a linear or branched $(C_1\text{-}C_6)$alkoxy group, a hydroxy group, a linear or branched $(C_1\text{-}C_6)$polyhaloalkyl group, a trifluoromethoxy group, $-NR_7R_7'$, nitro, $R_7\text{-}CO\text{-}(C_0\text{-}C_6)$alkyl-, $R_7\text{-}CO\text{-}NH\text{-}(C_0\text{-}C_6)$alkyl-, $NR_7R_7'\text{-}CO\text{-}(C_0\text{-}C_6)$alkyl-, $NR_7R_7'\text{-}CO\text{-}(C_0\text{-}C_6)$alkyl-O-, $R_7\text{-}SO_2\text{-}NH\text{-}(C_0\text{-}C_6)$alkyl-, $R_7\text{-}NH\text{-}CO\text{-}NH\text{-}(C_0\text{-}C_6)$alkyl-, $R_7\text{-}O\text{-}CO\text{-}NH\text{-}(C_0\text{-}C_6)$alkyl-, a heterocycloalkyl group, or the substituents of one of the pairs (R_a,R_b), (R_b,R_c) or (R_c,R_d) form together with the carbon atoms carrying them a ring composed of from 5 to 7 ring members, which may contain from one to 2 hetero atoms selected from oxygen and sulphur, it also being understood that one or more carbon atoms of the ring defined hereinbefore may be deuterated or substituted by from one to 3 groups selected from halogen and linear or branched $(C_1\text{-}C_6)$alkyl,
- R_7 and R_7' independently of one another represent a hydrogen, a linear or branched $(C_1\text{-}C_6)$alkyl, a linear or branched $(C_2\text{-}C_6)$alkenyl, a linear or branched $(C_2\text{-}C_6)$alkynyl, an aryl or a heteroaryl, or R_7 and R_7' together with nitrogen atom carrying them form a heterocycle composed of from 5 to 7 ring members,

it being understood that when the compound of formula (I) contains a hydroxy group, the latter may be optionally converted into one of the following groups: –OPO(OM)(OM'), -OPO(OM)($O^-M_1^+$), –OPO($O^-M_1^+$)($O^-M_2^+$), –OPO(O^-)(O^-)M_3^{2+}, –OPO(OM)(O[CH_2CH_2O]$_n$$CH_3$), or –OPO($O^-M_1^+$)(O[$CH_2CH_2O$]$_n$$CH_3$), wherein M and M' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a cycloalkyl or a heterocycloalkyl, both composed of from 5 to 6 ring members, while M_1^+ and M_2^+ independently of one another represent a pharmaceutically acceptable monovalent cation, M_3^{2+} represents a pharmaceutically acceptable divalent cation, and n is an integer from 1 to 5,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 4 hetero atoms selected from oxygen, sulphur and nitrogen (including quaternary nitrogens),
- "cycloalkyl" means any mono- or bi-cyclic, non-aromatic, carbocyclic group containing from 3 to 10 ring members,
- "heterocycloalkyl" means any mono- or bi-cyclic, non-aromatic, condensed or spiro group composed of 3 to 10 ring members and containing from 1 to 3 hetero atoms selected from oxygen, sulphur, SO, SO_2 and nitrogen,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the groups alkyl, alkenyl, alkynyl and alkoxy to be substituted by from 1 to 3 groups selected from: linear or branched (C_1-C_6)alkyl optionally substituted by a hydroxyl, a morpholine, 3-3-difluoropiperidine or a 3-3-difluoropyrrolidine; (C_3-C_6)spiro; linear or branched (C_1-C_6)alkoxy optionally substituted by a morpholine; (C_1-C_6)alkyl-S-; hydroxyl; oxo; *N*-oxide; nitro; cyano; -COOR'; -OCOR'; NR'R"; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; (C_1-C_6)alkylsulphonyl; halogen; aryl optionally substituted by one or more halogens; heteroaryl; aryloxy; arylthio; cycloalkyl; heterocycloalkyl optionally substituted by one or more halogen atoms or alkyl groups,

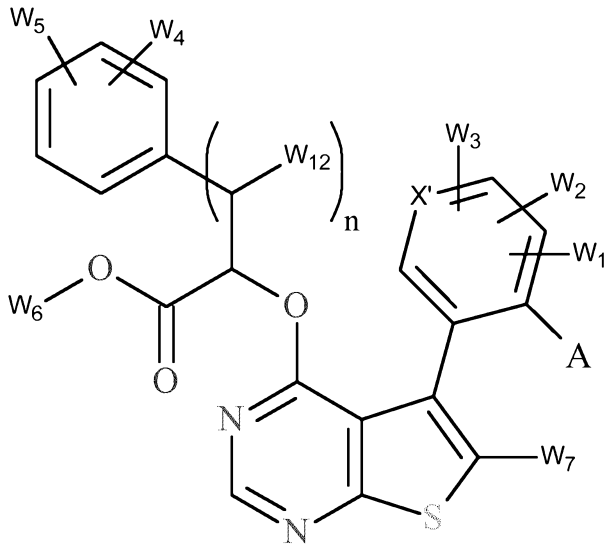
wherein R' and R" independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group optionally substituted by a methoxy,

it being possible for the Het group defined in formula (I) to be substituted by from one to three groups selected from linear or branched (C_1-C_6)alkyl, hydroxy, linear or branched (C_1-C_6)alkoxy, $NR_1'R_1"$ and halogen, it being understood that R_1' and $R_1"$ are as defined for the groups R' and R" mentioned hereinbefore,

or its enantiomers, diastereoisomers, or addition salts thereof with a pharmaceutically acceptable acid or base,

and (b) a MCL1 inhibitor,
for simultaneous, sequential or separate use.

2. A combination comprising:
(a) a BCL-2 inhibitor and
(b) a MCL1 inhibitor of formula (II):



(II)

wherein:

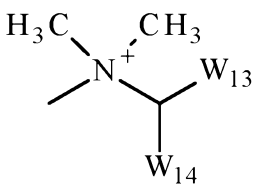
- A represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a linear or branched

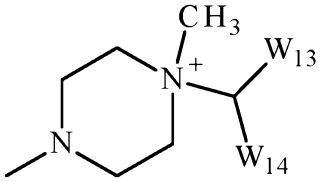
(C_1-C_6)polyhaloalkyl, a hydroxy group, a cyano, -$NW_{10}W_{10}$', -Cy_6 or an halogen atom,

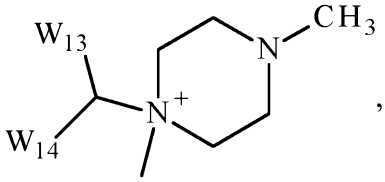
- W_1, W_2, W_3, W_4 and W_5 independently of one another represent a hydrogen atom, a halogen atom, a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, a linear or branched (C_1-C_6)polyhaloalkyl, a hydroxy group, a linear or branched (C_1-C_6)alkoxy group, -S-(C_1-C_6)alkyl group, a cyano, a nitro group, -alkyl(C_0-C_6)-NW_8W_8', -O-Cy_1, -alkyl(C_0-C_6)-Cy_1, -alkenyl(C_2-C_6)-Cy_1, -alkynyl(C_2-C_6)-Cy_1, -O-alkyl(C_1-C_6)-W_9, -C(O)-OW_8, -O-C(O)-W_8, -C(O)-NW_8W_8', -NW_8-C(O)-W_8', -NW_8-C(O)-OW_8', -alkyl(C_1-C_6)-NW_8-C(O)-W_8', -SO_2- NW_8W_8', -SO_2-alkyl(C_1-C_6),
  or the substituents of one of the pairs (W_1, W_2), (W_2, W_3), (W_1, W_3), (W_4, W_5) when grafted onto two adjacent carbon atoms, form together with the carbon atoms carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that resulting ring may be substituted by a group selected from a linear or branched (C_1-C_6)alkyl group, -$NW_{10}W_{10}$', -alkyl(C_0-C_6)-Cy_1 or an oxo,
- X' represents a carbon or a nitrogen atom,
- W_6 represents a hydrogen, a linear or branched (C_1-C_8)alkyl group, an aryl, an heteroaryl group, an arylalkyl(C_1-C_6) group, an heteroarylalkyl(C_1-C_6) group,
- W_7 represents a linear or branched (C_1-C_6)alkyl group, a linear or branched (C_2-C_6)alkenyl group, a linear or branched (C_2-C_6)alkynyl group, -Cy_3, -alkyl(C_1-C_6)-Cy_3, -alkenyl(C_2-C_6)-Cy_3, -alkynyl(C_2-C_6)-Cy_3, -Cy_3-Cy_4, -alkynyl(C_2-C_6)-O-Cy_3, -Cy_3-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_4, an halogen atom, a cyano, -C(O)-W_{11}, -C(O)-$NW_{11}W_{11}$',
- W_8 and W_8' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group, or -alkyl(C_0-C_6)-Cy_1,
  or (W_8, W_8') form together with the nitrogen atom carrying them an aromatic or non-aromatic ring composed of from 5 to 7 ring members, which may contain in addition to the nitrogen atom from one to 3 heteroatoms selected from oxygen, sulphur and nitrogen, it being understood that the nitrogen in question may be

substituted by a group representing a hydrogen atom, or a linear or branched (C_1-C_6)alkyl group and it being understood that one or more of the carbon atoms of the possible substituents, may be deuterated,

- W_9 represents -Cy_1, -Cy_1-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-O-alkyl(C_0-C_6)-Cy_2, -Cy_1-alkyl(C_0-C_6)-NW_8-alkyl(C_0-C_6)-Cy_2, -Cy_1-Cy_2-O-alkyl(C_0-C_6)-Cy_5, -C(O)-NW_8W_8', -NW_8W_8', -OW_8,-NW_8-C(O)-W_8', -O-alkyl(C_1-C_6)-OW_8, -SO_2-W_8, -C(O)-OW_8, -NH-C(O)-NH-W_8,



,


or


,

it being possible for the ammonium so defined to exist as a zwitterionic form or to have a monovalent anionic counterion,

- W_{10}, W_{10}', W_{11} and W_{11}' independently of one another represent a hydrogen atom or an optionally substituted linear or branched (C_1-C_6)alkyl group,
- W_{12} represents a hydrogen or a hydroxy group,
- W_{13} represents a hydrogen atom or a linear or branched (C_1-C_6)alkyl group,
- W_{14} represents a -O-P(O)(O^-)(O^-) group, a -O-P(O)(O^-)(OW_{16}) group, a -O-P(O)(OW_{16})(OW_{16}') group, a -O-SO_2-O^- group, a -O-SO_2-OW_{16} group, -Cy_7, a -O-C(O)-W_{15} group, a -O-C(O)-OW_{15} group or a -O-C(O)-$NW_{15}W_{15}$' group,
- W_{15} and W_{15}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or a linear or branched amino(C_1-C_6)alkyl group,
- W_{16} and W_{16}' independently of one another represent a hydrogen atom, a linear or branched (C_1-C_6)alkyl group or an arylalkyl(C_1-C_6) group,
- Cy_1, Cy_2, Cy_3, Cy_4, Cy_5, Cy_6 and Cy_7 independently of one another, represent a cycloalkyl group, a heterocycloalkyl group, an aryl or an heteroaryl group,
- n is an integer equal to 0 or 1,

it being understood that:

- "aryl" means a phenyl, naphthyl, biphenyl, indanyl or indenyl group,
- "heteroaryl" means any mono- or bi-cyclic group composed of from 5 to 10 ring members, having at least one aromatic moiety and containing from 1 to 3

heteroatoms selected from oxygen, sulphur and nitrogen,

- "cycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members,
- "heterocycloalkyl" means any mono- or bi-cyclic non-aromatic carbocyclic group containing from 3 to 10 ring members, and containing from 1 to 3 heteroatoms selected from oxygen, sulphur and nitrogen, which may include fused, bridged or spiro ring systems,

it being possible for the aryl, heteroaryl, cycloalkyl and heterocycloalkyl groups so defined and the alkyl, alkenyl, alkynyl, alkoxy, to be substituted by from 1 to 4 groups selected from linear or branched (C_1-C_6)alkyl which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy which may be substituted by a linear or branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, hydroxy, halogen, oxo, -NW'W'', -O-C(O)-W', or -CO-NW'W''; linear or branched (C_2-C_6)alkenyl group; linear or branched (C_2-C_6)alkynyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; linear or branched (C_1-C_6)alkoxy which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy, a linear or branched (C_1-C_6)polyhaloalkyl, a linear or branched (C_2-C_6)alkynyl, -NW'W'', or hydroxy; (C_1-C_6)alkyl-S- which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; hydroxy; oxo; *N*-oxide; nitro; cyano; -C(O)-OW'; -O-C(O)-W'; -CO-NW'W''; -NW'W''; -(C=NW')-OW''; linear or branched (C_1-C_6)polyhaloalkyl; trifluoromethoxy; or halogen; it being understood that W' and W'' independently of one another represent a hydrogen atom or a linear or branched (C_1-C_6)alkyl group which may be substituted by a group representing a linear or branched (C_1-C_6)alkoxy; and it being understood that one or more of the carbon atoms of the preceding possible substituents, may be deuterated,

their enantiomers, diastereoisomers and atropisomers, and addition salts thereof with a pharmaceutically acceptable acid or base,

for simultaneous, sequential or separate use.

3. A combination according to claim 1, wherein the MCL1 inhibitor is a compound of formula (II) as defined in claim 2.

4. A combination according to any of claims 1 to 3, wherein the BCL-2 inhibitor is *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl) carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide.

5. A combination according to any of claims 1 to 3, wherein the BCL-2 inhibitor is 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl} phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide.

6. A combination according to claim 4, wherein *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide is in the form of the hydrochloride salt.

7. A combination according to claim 5, wherein 5-(5-chloro-2-{[(3*S*)-3-(morpholin-4-ylmethyl)-3,4-dihydroisoquinolin-2(1*H*)-yl]carbonyl} phenyl)-*N*-(5-cyano-1,2-dimethyl-1*H*-pyrrol-3-yl)-*N*-(4-hydroxyphenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide is in the form of the hydrochloride salt.

8. A combination according to claim 4 or 6, wherein the dose of *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide during the combination treatment is from 50 mg to 1500 mg.

9. A combination according to any of claims 1 to 8, wherein the BCL-2 inhibitor is administered once a week.

10. A combination according to claim 6 or 8, wherein *N*-(4-hydroxyphenyl)-3-{6-[((3*S*)-3-(4-morpholinylmethyl)-3,4-dihydro-2(1*H*)-isoquinolinyl)carbonyl]-1,3-benzodioxol-5-yl}-*N*-phenyl-5,6,7,8-tetrahydro-1-indolizine carboxamide is administered during the combination treatment once a day.

11. A combination according to any of claims 1 to 3, wherein the BCL-2 inhibitor is ABT-199.

12. A combination according to any of claims 1 to 11, wherein the MCL1 inhibitor is (*2R*)-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.

13. A combination according to any of claims 1 to 11, wherein the MCL1 inhibitor is (*2R*)-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.

14. A combination according to any of claims 1 to 13, wherein the BCL-2 inhibitor and the MCL1 inhibitor are administered orally.

15. A combination according to any of claims 1 to 13, wherein the BCL-2 inhibitor is administered orally and the MCL1 inhibitor is administered intravenously.

16. A combination according to any of claims 1 to 13, wherein the BCL-2 inhibitor and the MCL1 inhibitor are administered intravenously.

17. A combination according to any of claims 1 to 16, for use in the treatment of cancer.

18. The combination for use according to claim 17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in amounts which are jointly therapeutically effective for the treatment of cancer.

19. The combination for use according to claim 17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in amounts which are synergistically effective for the treatment of cancer.

20. The combination for use according to claim 17, wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in synergistically effective amounts which enable a reduction of the dose required for each compound in the treatment of cancer, whilst providing an efficacious cancer treatment, with eventually a reduction in side effects.

21. The combination for use according to any of claims 17 to 20, wherein the cancer is leukaemia.

22. The combination for use according to claim 21, wherein the leukaemia is acute myeloid leukaemia, T-ALL or B-ALL.

23. The combination for use according to any of claims 17 to 20, wherein the cancer is myelodysplastic syndrome or myeloproliferative disease.

24. The combination for use according to any of claims 17 to 20, wherein the cancer is lymphoma.

25. The combination for use according to claim 24, wherein the lymphoma is a non-Hodgkin lymphoma.

26. The combination for use according to claim 25, wherein the non-Hodgkin lymphoma is diffuse large B-cell lymphoma or mantle-cell lymphoma.

27. The combination for use according to any of claims 17 to 20, wherein the cancer is multiple myeloma.

28. The combination for use according to any of claims 17 to 20, wherein the cancer is neuroblastoma.

29. The combination for use according to any of claims 17 to 20, wherein the cancer is small cell lung cancer.

30. A combination according to any of claims 1 to 16, further comprising one or more excipients.

31. The use of a combination according to any of claims 1 to 16, in the manufacture of a medicament for the treatment of cancer.

32. The use according to claim 31, wherein the cancer is leukaemia.

33. The use according to claim 32, wherein the leukaemia is acute myeloid leukaemia, T-ALL or B-ALL.

34. The use according to claim 31, wherein the cancer is myelodysplastic syndrome or myeloproliferative disease.

35. The use according to claim 31, wherein the cancer is lymphoma.

36. The use according to claim 35, wherein the lymphoma is a non-Hodgkin lymphoma.

37. The use according to claim 36, wherein the non-Hodgkin lymphoma is diffuse large B-cell lymphoma or mantle-cell lymphoma.

38. The use according to claim 31, wherein the cancer is multiple myeloma.

39. The use according to claim 31, wherein the cancer is neuroblastoma.

40. The use according to claim 31, wherein the cancer is small cell lung cancer.

41. A medicament containing, separately or together,
(a) a BCL-2 inhibitor of formula (I) as defined in claim 1, and
(b) a MCL1 inhibitor,
for simultaneous, sequential or separate administration, and wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in effective amounts for the treatment of cancer.

42. A medicament containing, separately or together,
(a) a BCL-2 inhibitor, and
(b) a MCL1 inhibitor of formula (II) as defined in claim 2,
for simultaneous, sequential or separate administration, and wherein the BCL-2 inhibitor and the MCL1 inhibitor are provided in effective amounts for the treatment of cancer.

43. A method of treating cancer, comprising administering a jointly therapeutically effective amount of (a) a BCL-2 inhibitor of formula (I) as defined in claim 1, and
(b) a MCL1 inhibitor,
to a subject in need thereof.

44. A method of treating cancer, comprising administering a jointly therapeutically effective amount of (a) a BCL-2 inhibitor, and
(b) a MCL1 inhibitor of formula (II) as defined in claim 2,
to a subject in need thereof.

45. A method for sensitizing a patient 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 jointly therapeutically effective amount of (a) a BCL-2 inhibitor of formula (I) as defined in claim 1, and (b) a MCL1 inhibitor, to said patient.

46. A method for sensitizing a patient 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 jointly therapeutically effective amount of (a) a BCL-2 inhibitor, and (b) a MCL1 inhibitor of formula (II) as defined in claim 2, to said patient.

Figure 1. Expression of BCL-2 and MCL1 is prevalent in AML. Immunoblot analysis showing the expression of BCL-2 family members in primary AML samples and AML cell lines. BCL-2 and MCL1 proteins are dominantly expressed in contrast to BCL-XL, which was expressed in a lower proportion of primary AML samples and AML cell lines.

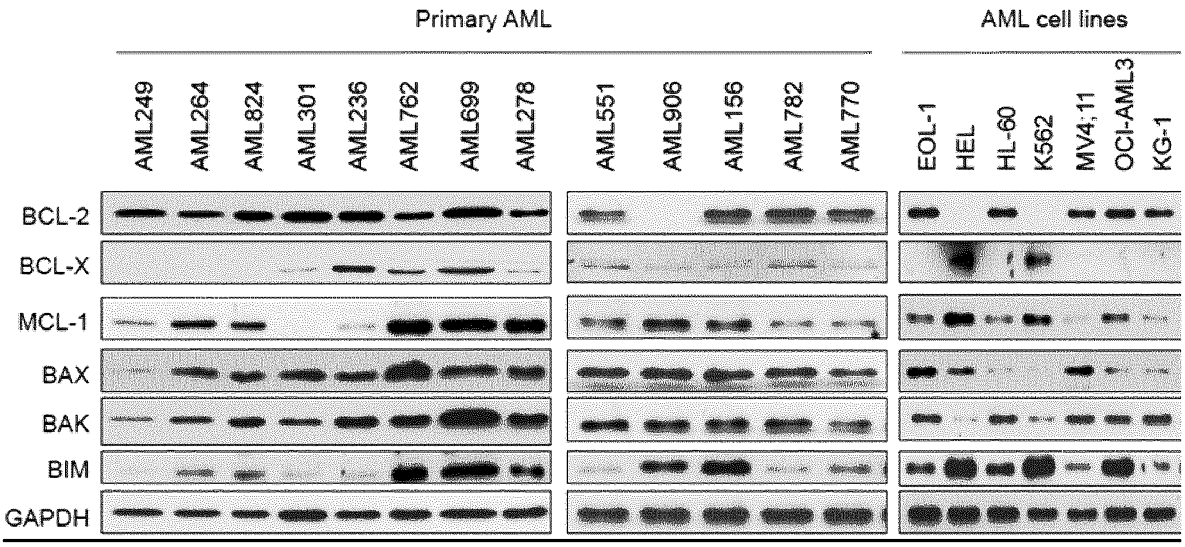
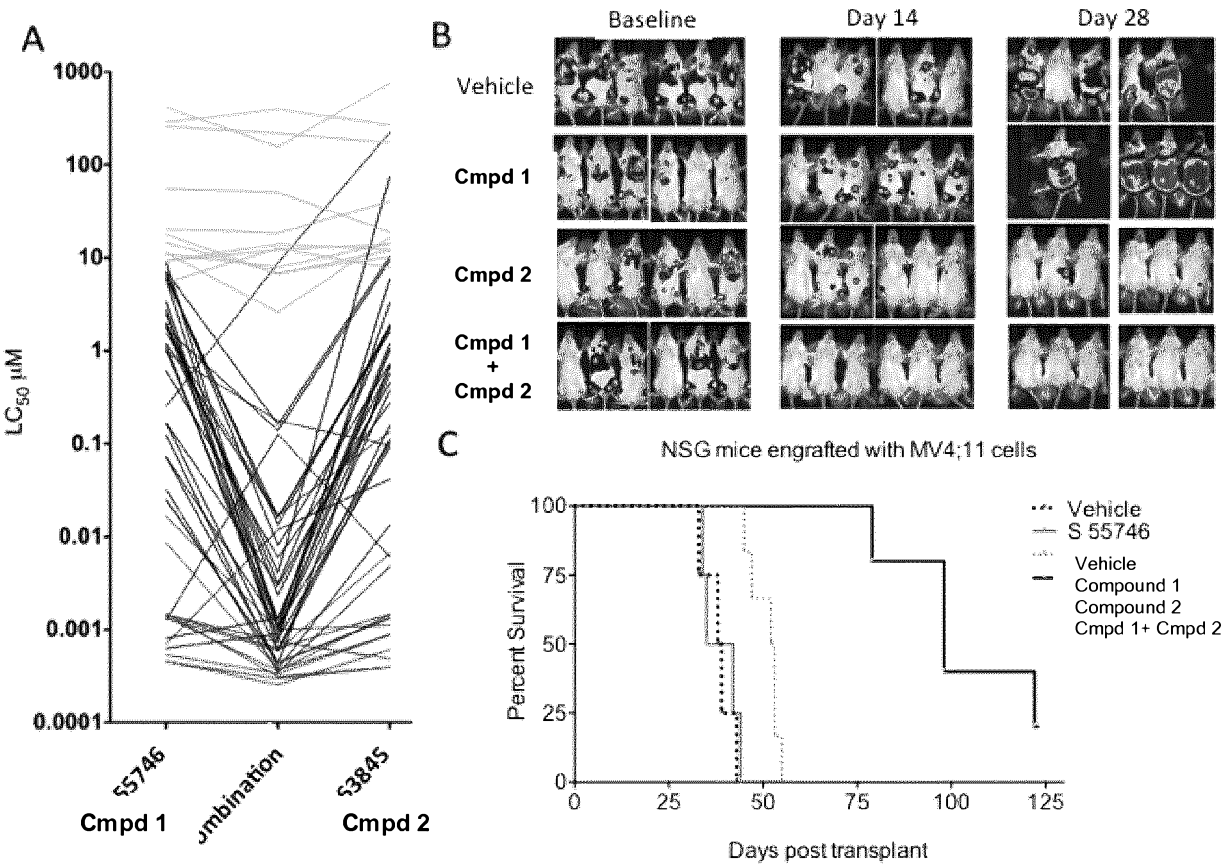
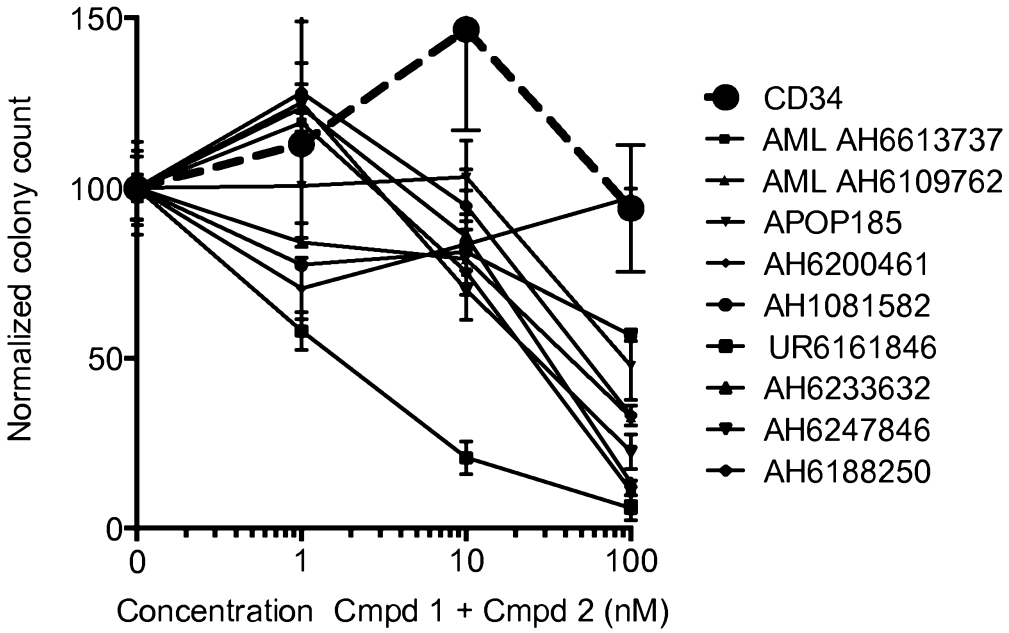


Figure 2. Combined BCL-2/MCL1 targeting has synergistic activity in AML. (A) A series of primary AML samples were incubated with Compound 1 / Cmpd 1 (HCl salt) and Compound 2 / Cmpd 2 alone, or in combination and the LC50 killing effect determined. This showed substantial synergy of this combination in a large proportion of primary AML samples. In (B), cohorts of 6 NSG mice were engrafted with luc-MV4;11 AML cells and after engraftment confirmed, mice were treated after day 10 with Compound 1, HCl (100mg/kg po weekdays – dosage expressed as the free base) or Compound 2 (25mg/kg IV twice weekly) alone, or in combination for 4 consecutive weeks. A vehicle only arm served as control. Bioimaging of mice on day 14 and 28 after engraftment showed marked suppression of AML in Compound 2 and Compound 1 + Compound 2 treated mice. In (C), however, the survival of Compound 1 + Compound 2 treated mice was significantly extended, compared to other treatment arms, confirming the clinical benefit of dual BCL-2/MCL1 targeted



therapy *in vivo*.

Figure 3. **Toxicity assessment of BCL-2 targeting (with Compound 1 / Cmpd 1 ; HCl salt) in combination with MCL1 targeting (with Compound 2 / Cmpd 2), on clonogenic function of normal CD34+ cells from healthy donors or leukaemic blasts from patients with AML. Drugs at indicated concentrations were incubated with cells and colonies counted after 14-21 days using a Gelcount® analyzer. Colonies were normalized to DMSO control. Compound 1 in combination with Compound 2 suppressed AML colony forming activity without affecting the function of normal CD34+ colony growth.**



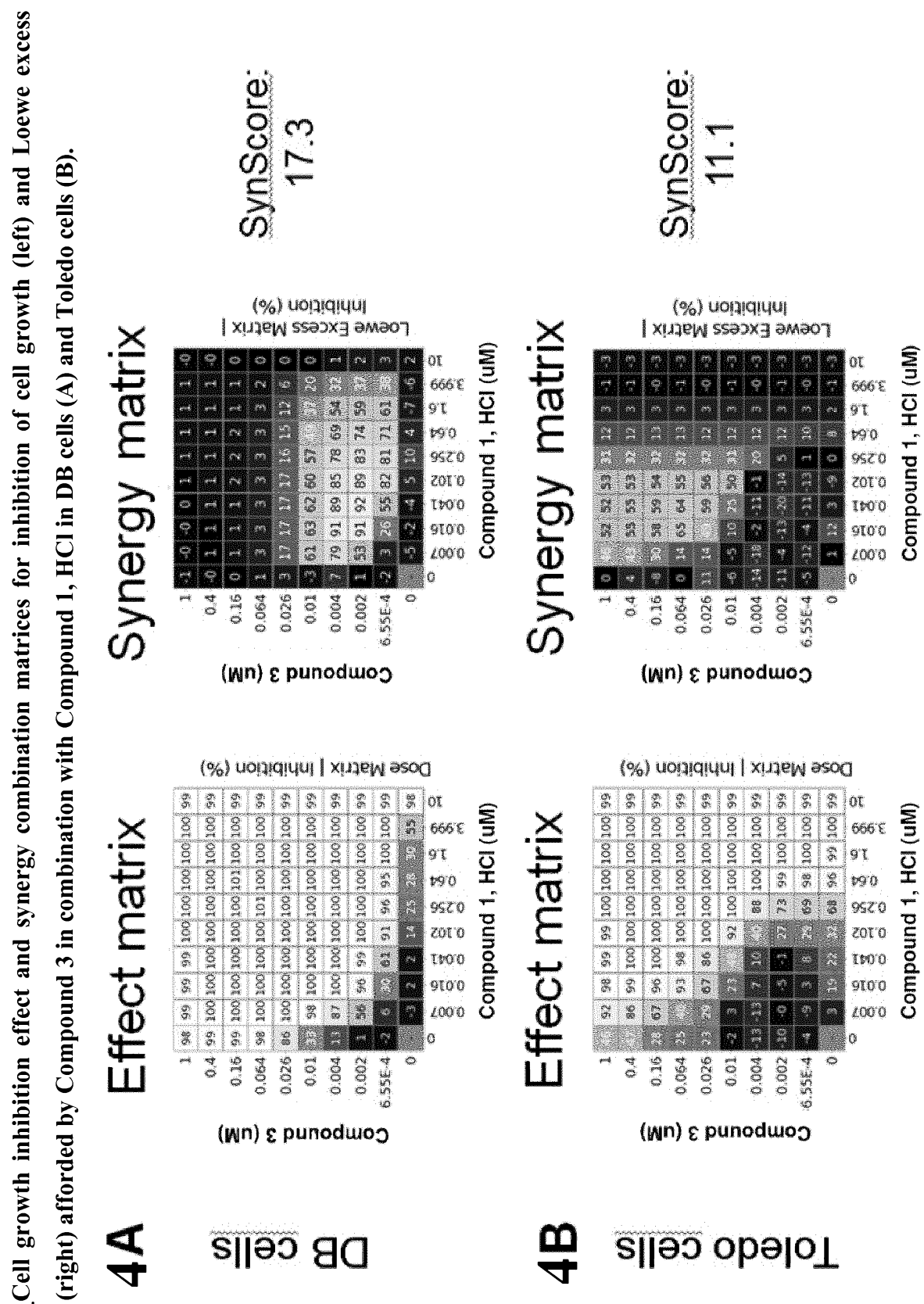


Figure 4. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 in combination with Compound 1, HCl in DB cells (A) and Toledo cells (B).

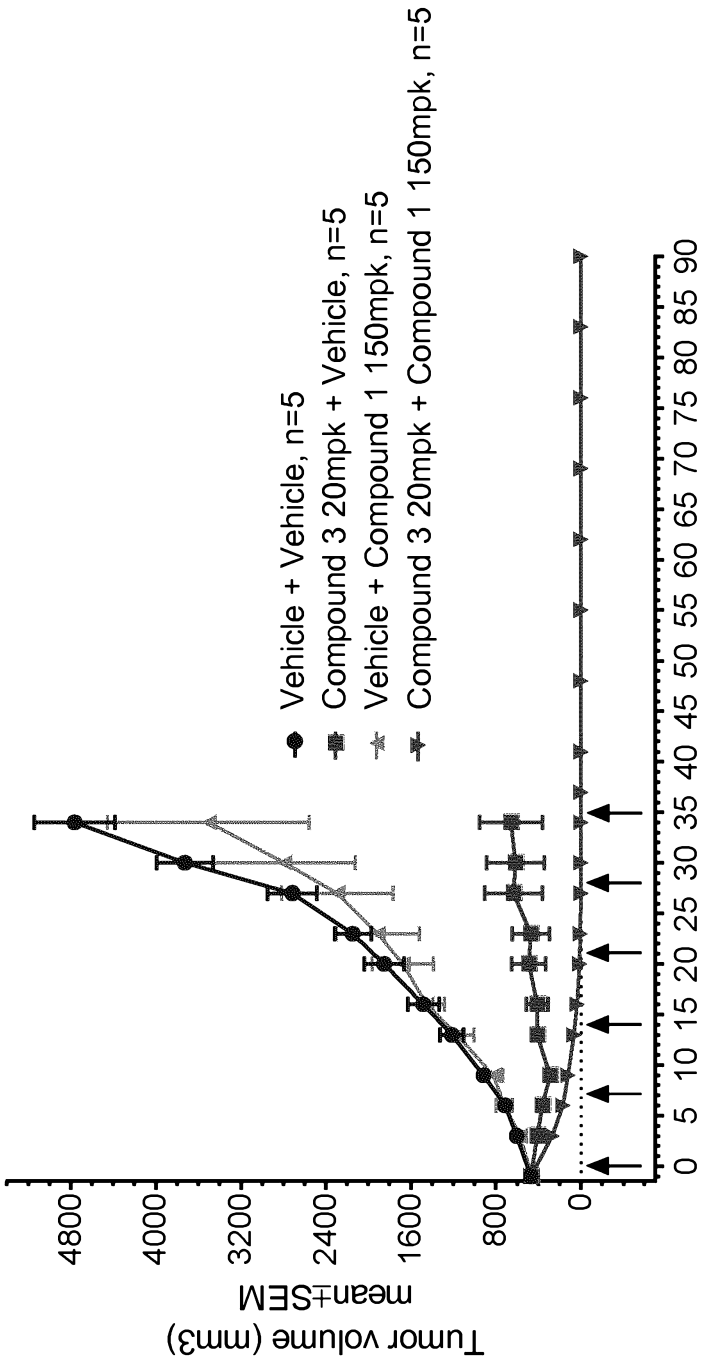


Figure 5. Anti-tumor effects of Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in lymphoma Karpass422 xenograft model in rats.

Figure 6. Body weight changes in animals treated with Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in lymphoma Karpass422 xenograft model in rats.

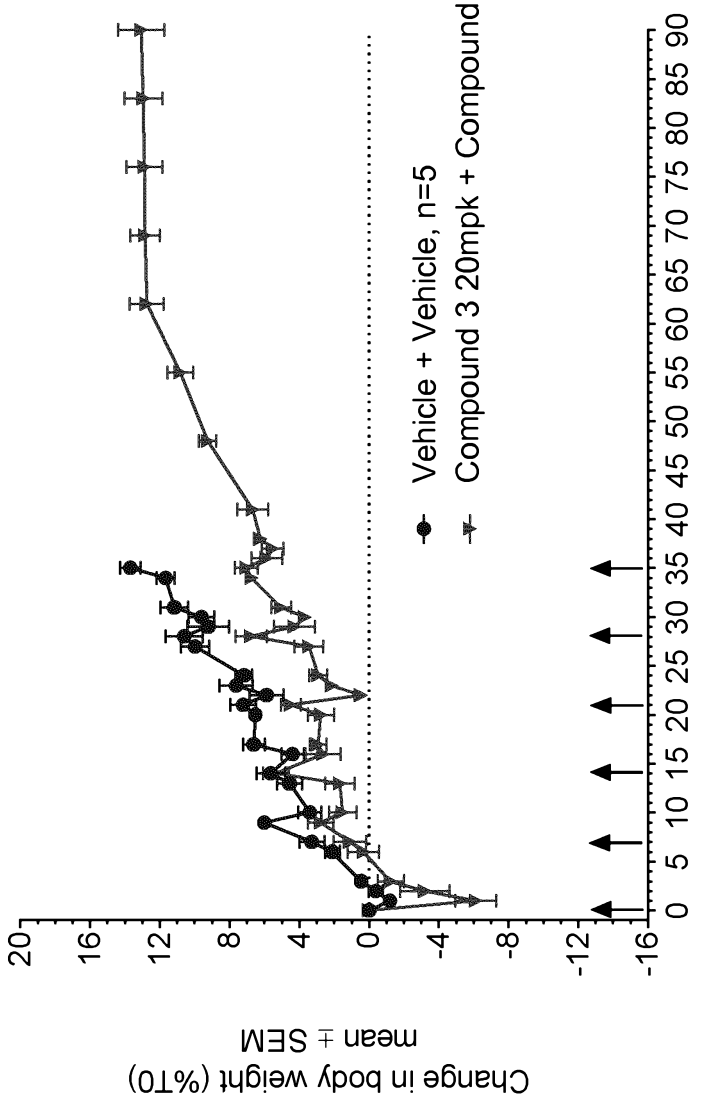
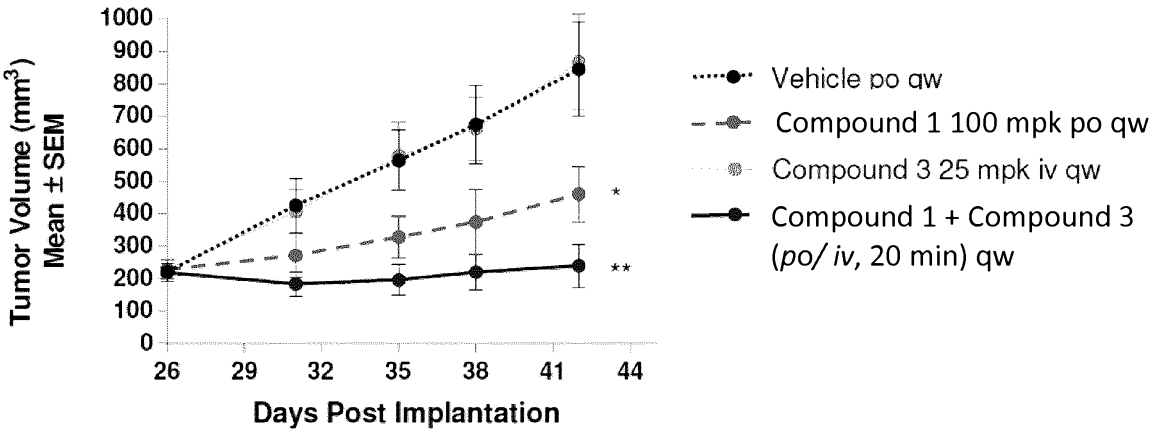


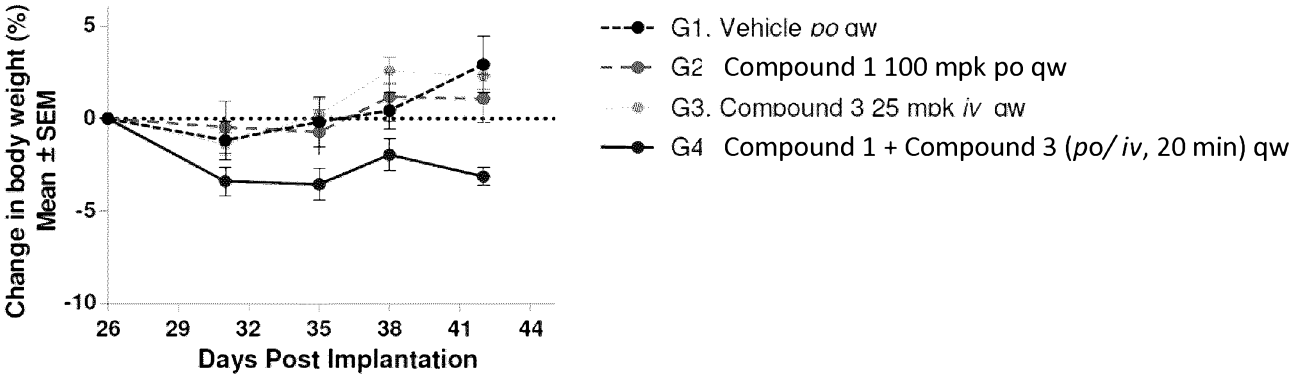
Figure 7. Anti-tumor effects of Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in DLBCL Toledo xenograft model in mice.



* p<0.05 vs Vehicle

** p<0.05 vs Vehicle, Compound 1, HCl and Compound 3

Figure 8. Body weight changes in animals treated with Compound 1, HCl, Compound 3 and the combination of Compound 1, HCl + Compound 3 in DLBCL Toledo xenograft model in mice.



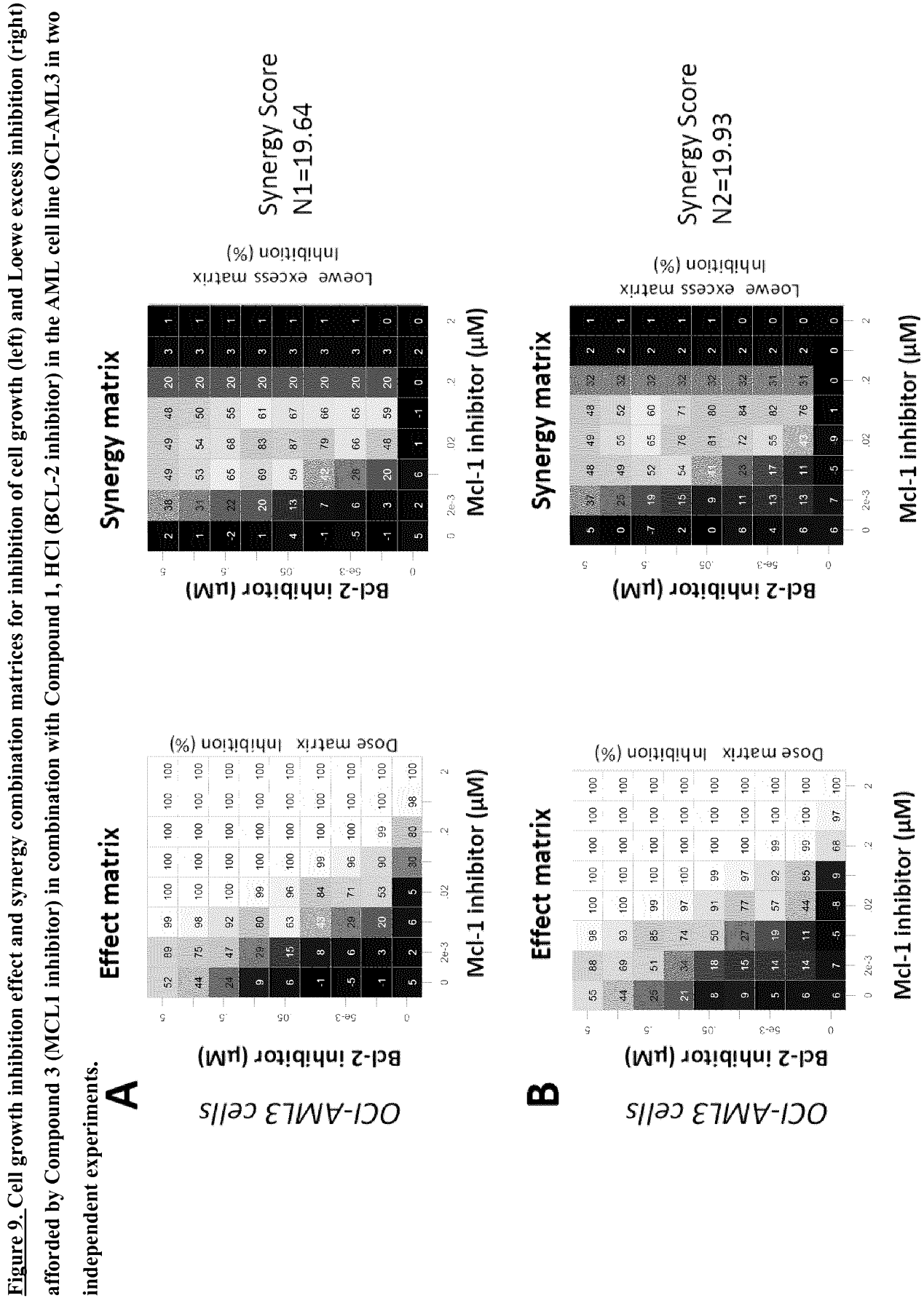


Figure 9. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the AML cell line OCI-AML3 in two independent experiments.

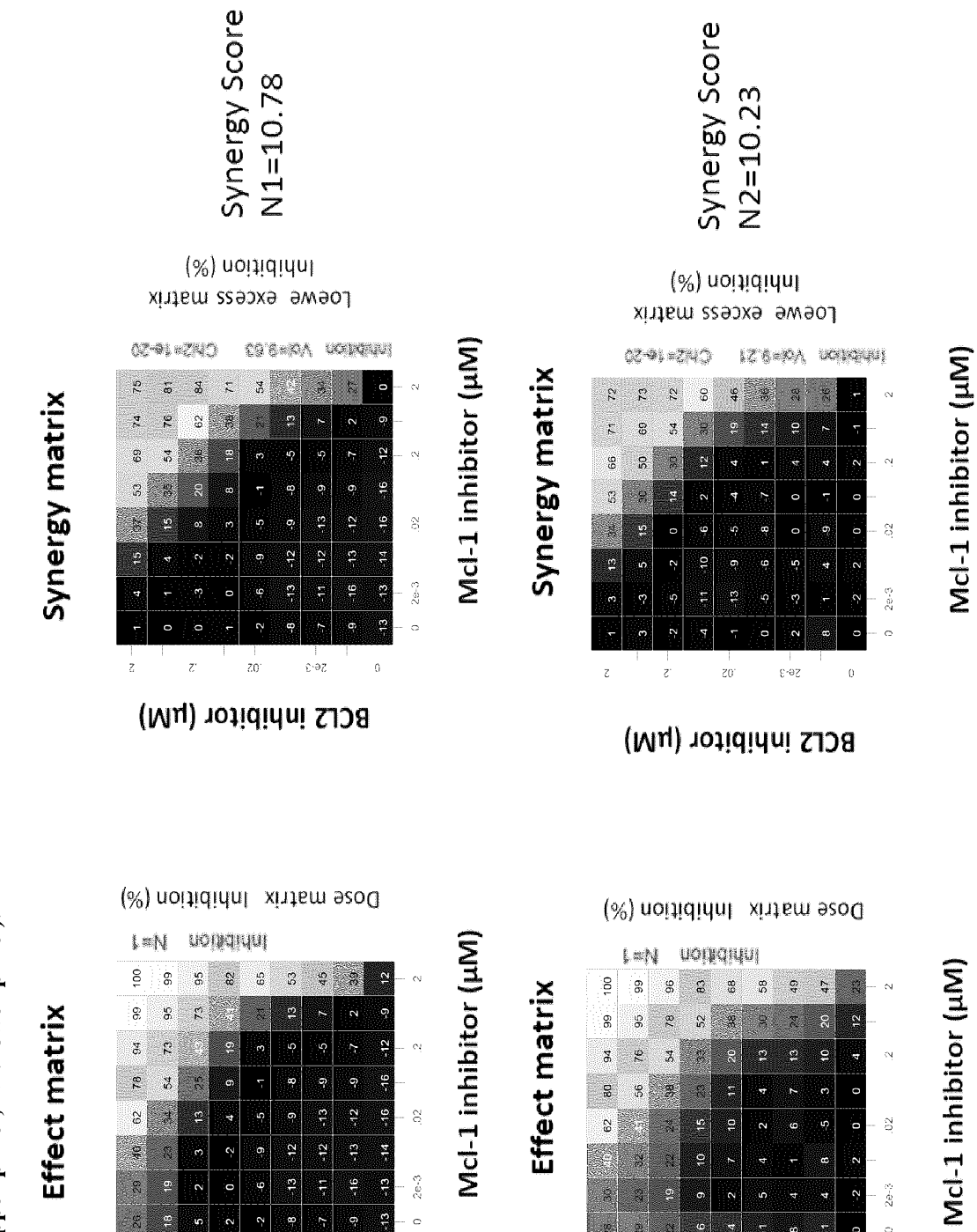


Figure 10. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the NB cell line LAN-6 in two independent experiments (N1: upper panel; N2: lower panel).

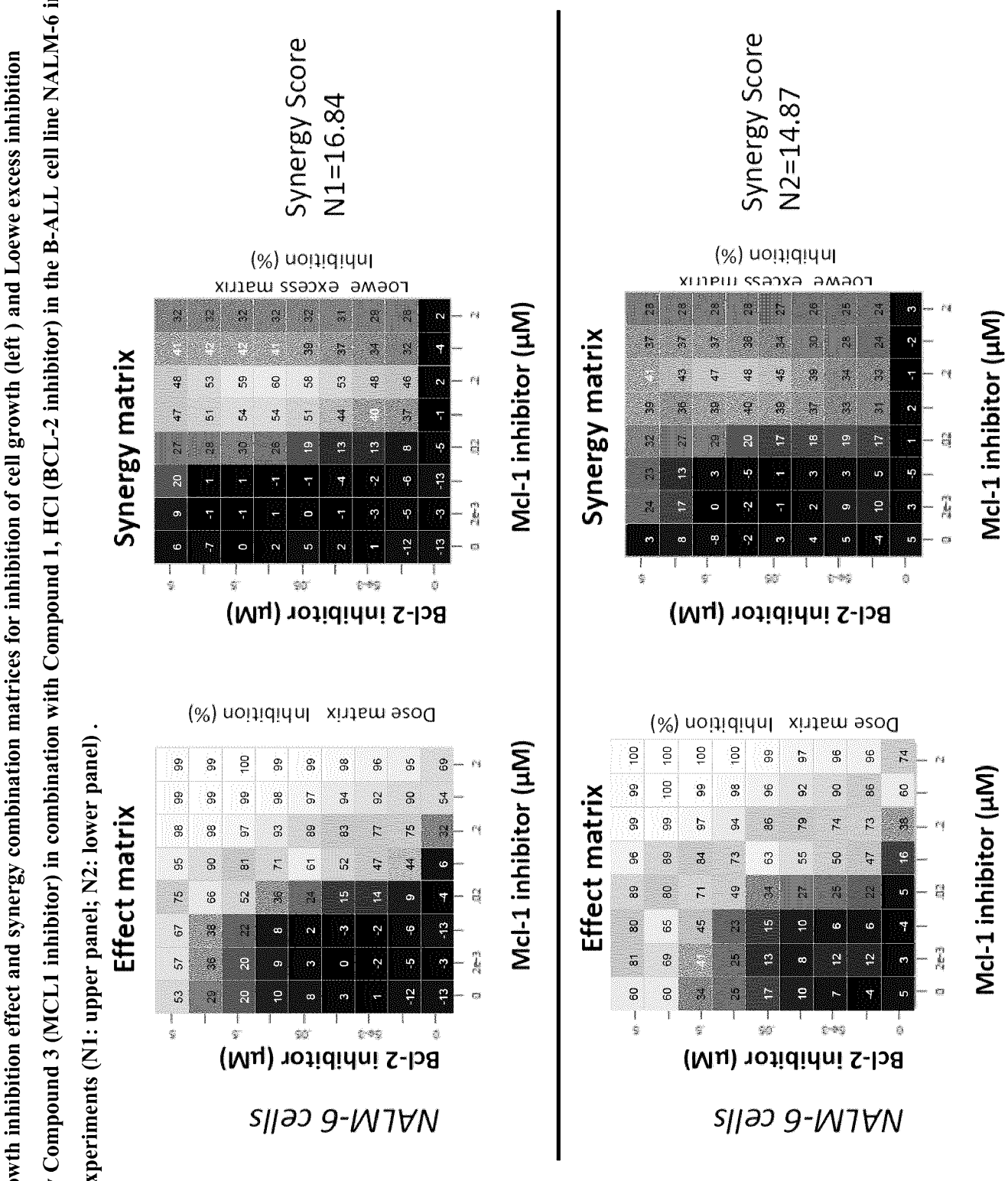


Figure 11. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the B-ALL cell line NALM-6 in two independent experiments (N1: upper panel; N2: lower panel) .

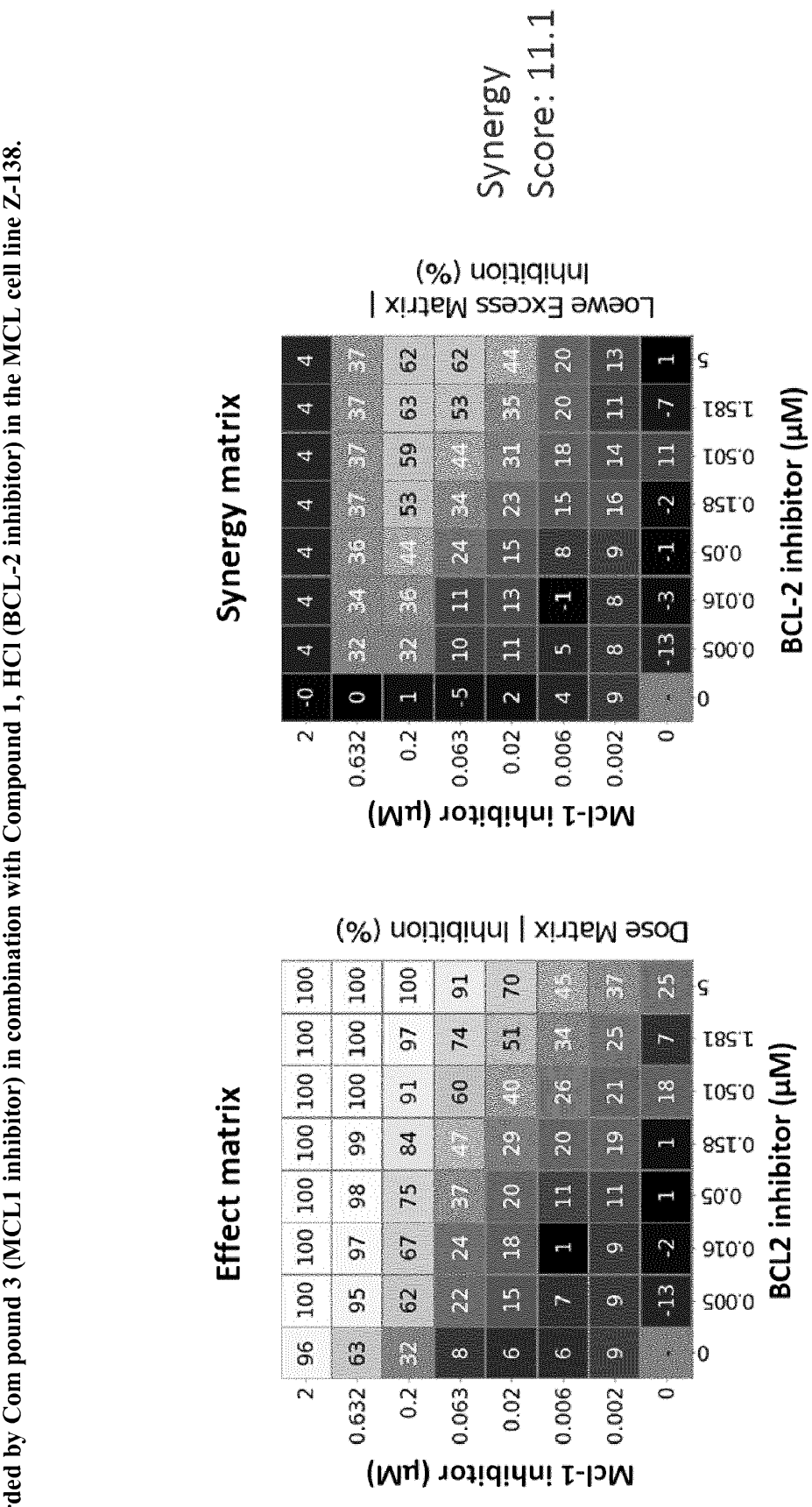


Figure 12. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Com pound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in the MCL cell line Z-138.

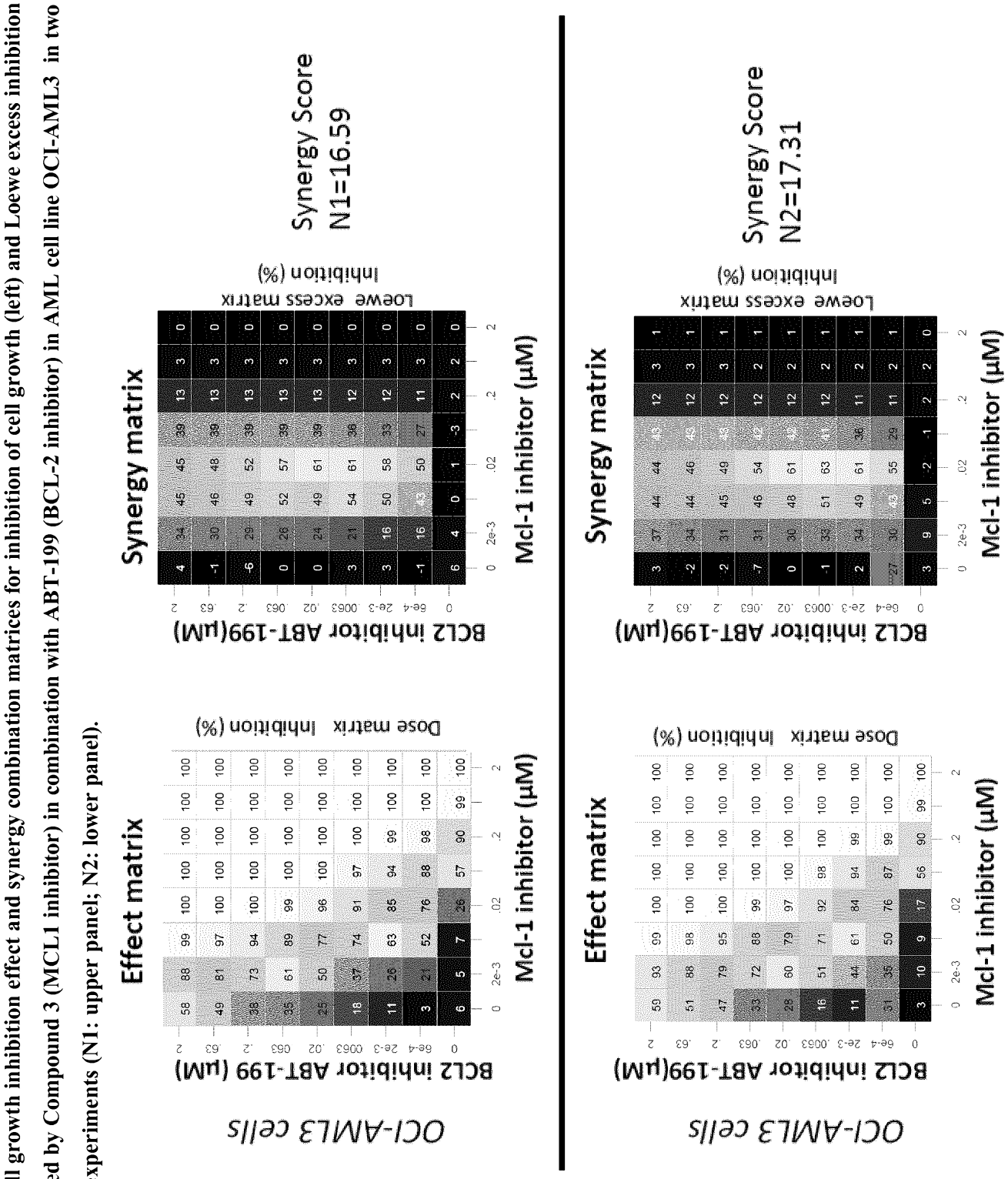


Figure 13. Cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with ABT-199 (BCL-2 inhibitor) in AML cell line OCI-AML3 in two independent experiments (N1: upper panel; N2: lower panel).

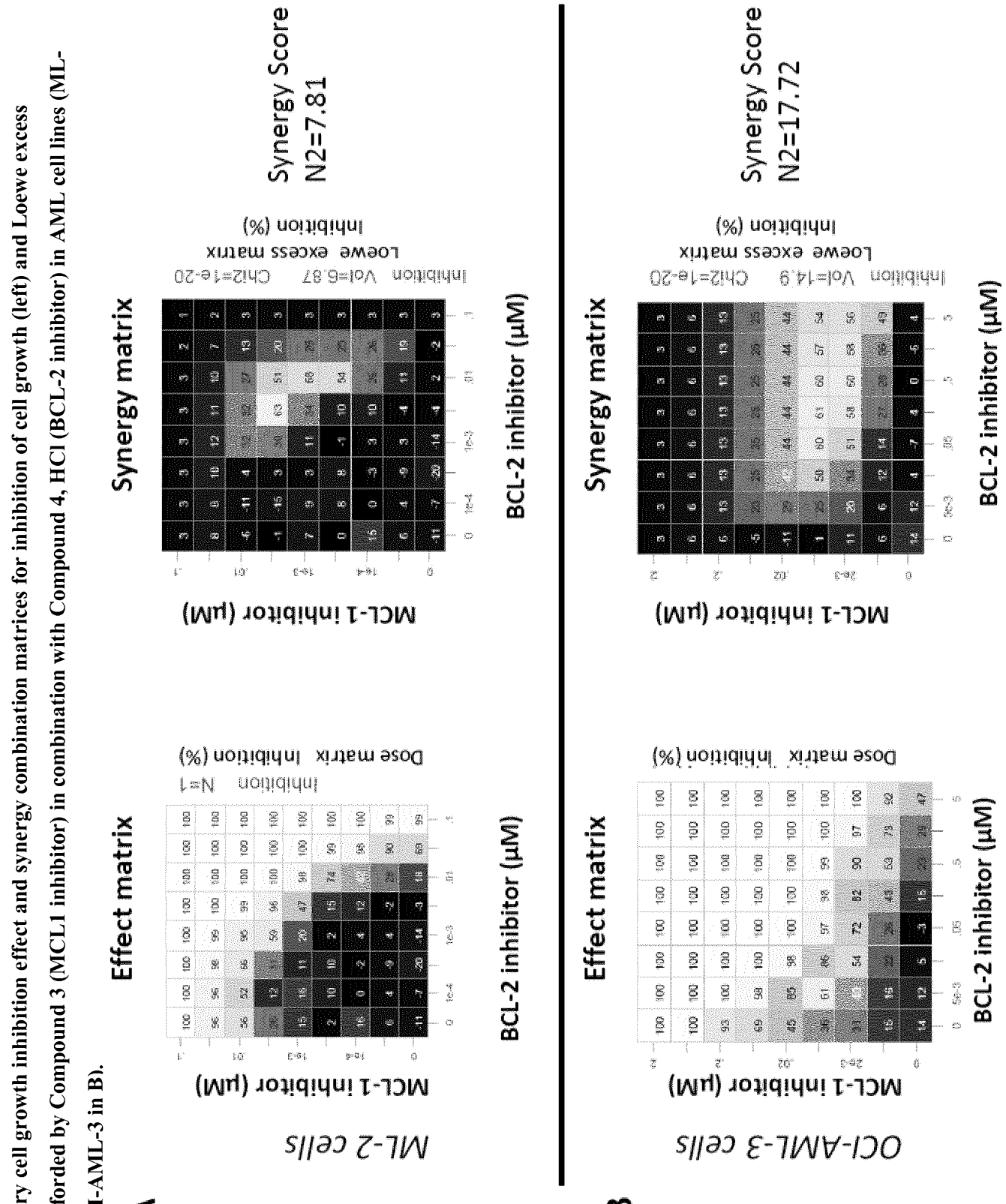


Figure 14. Exemplary cell growth inhibition effect and synergy combination matrices for inhibition of cell growth (left) and Loewe excess inhibition (right) afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 4, HCl (BCL-2 inhibitor) in AML cell lines (ML-2 cells in A and OCI-AML-3 in B).

Figure 15 (a): Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.

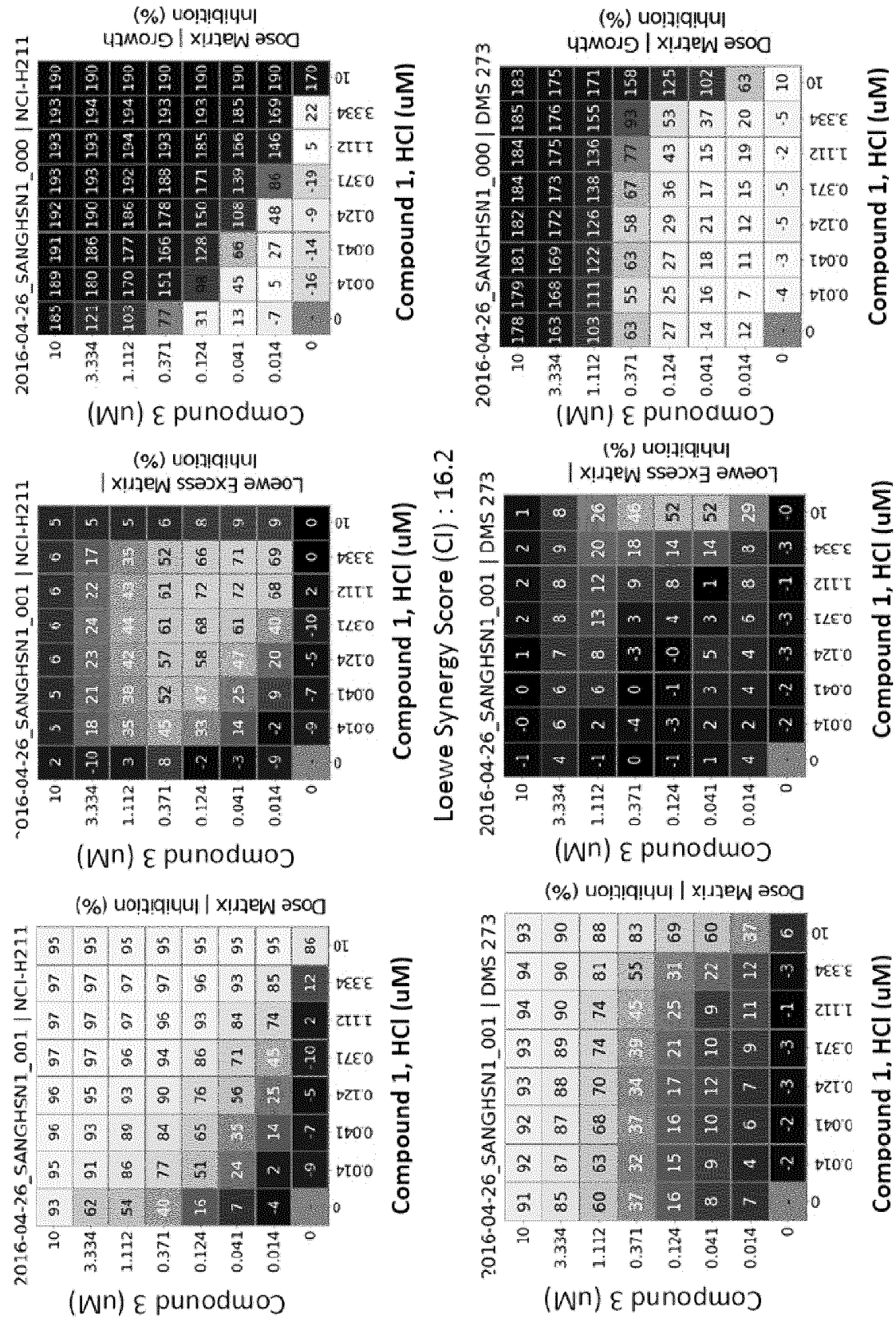


Figure 15 (b): Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.

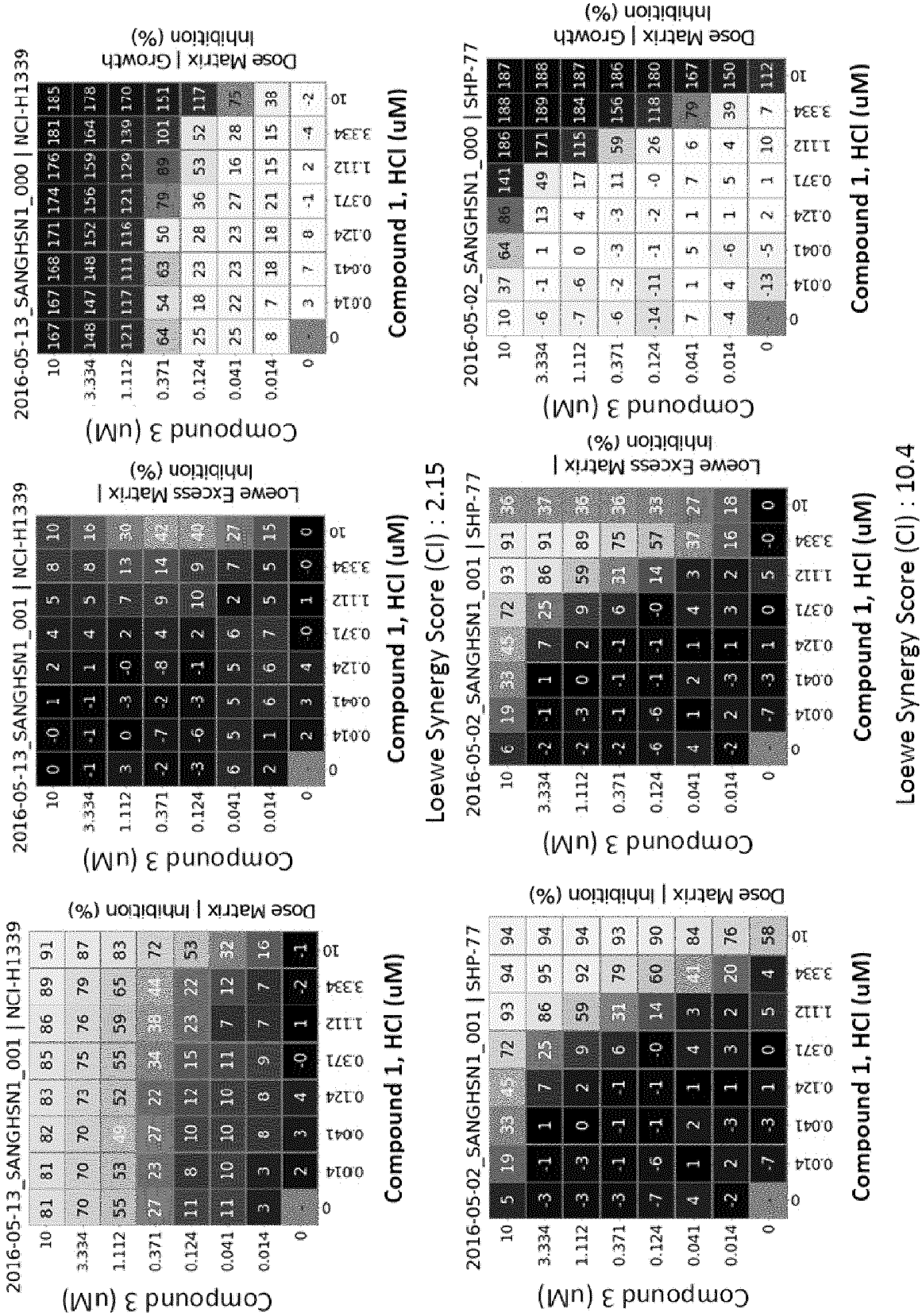


Figure 15 (c): Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.

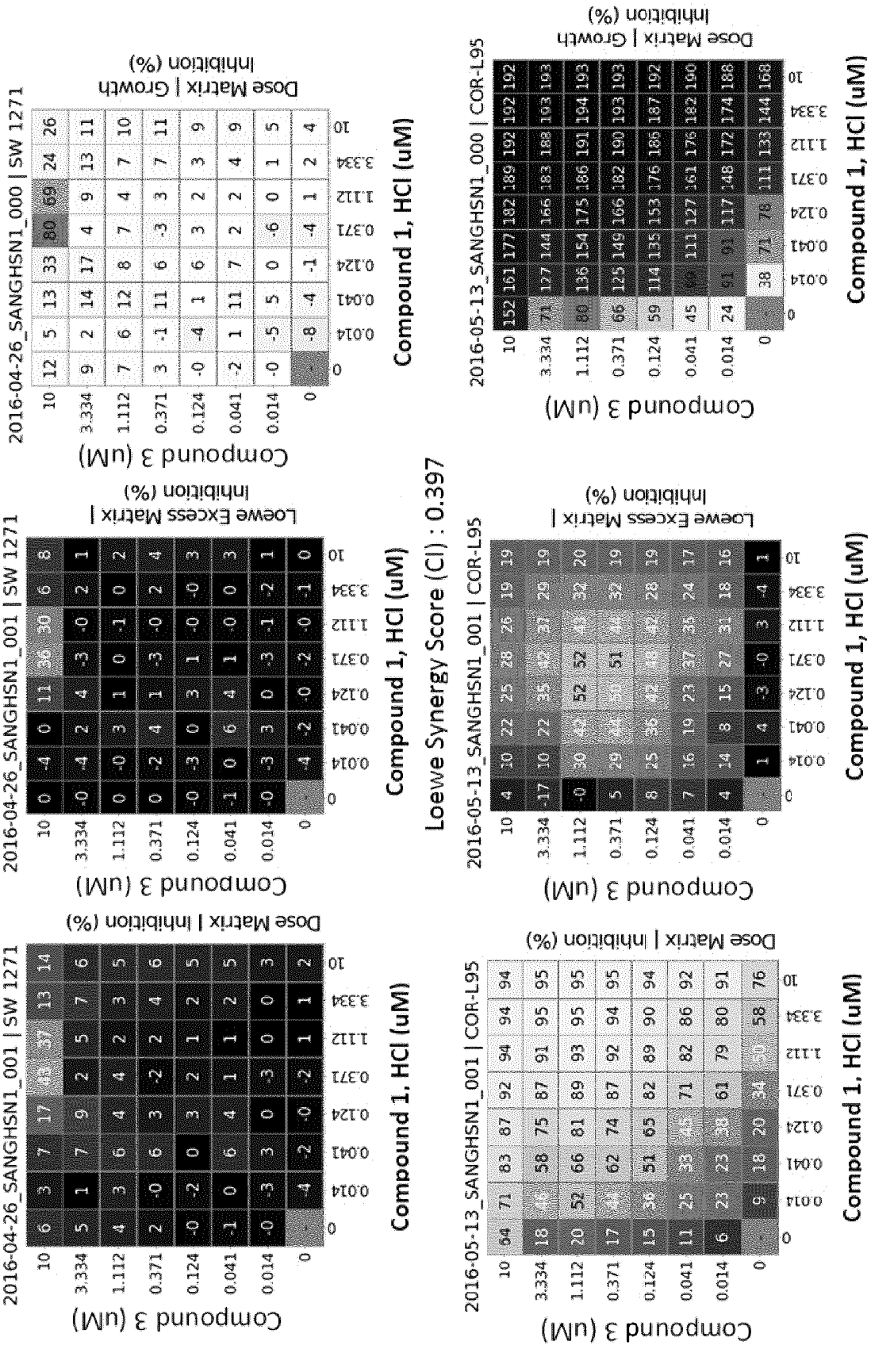


Figure 15 (d): Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.

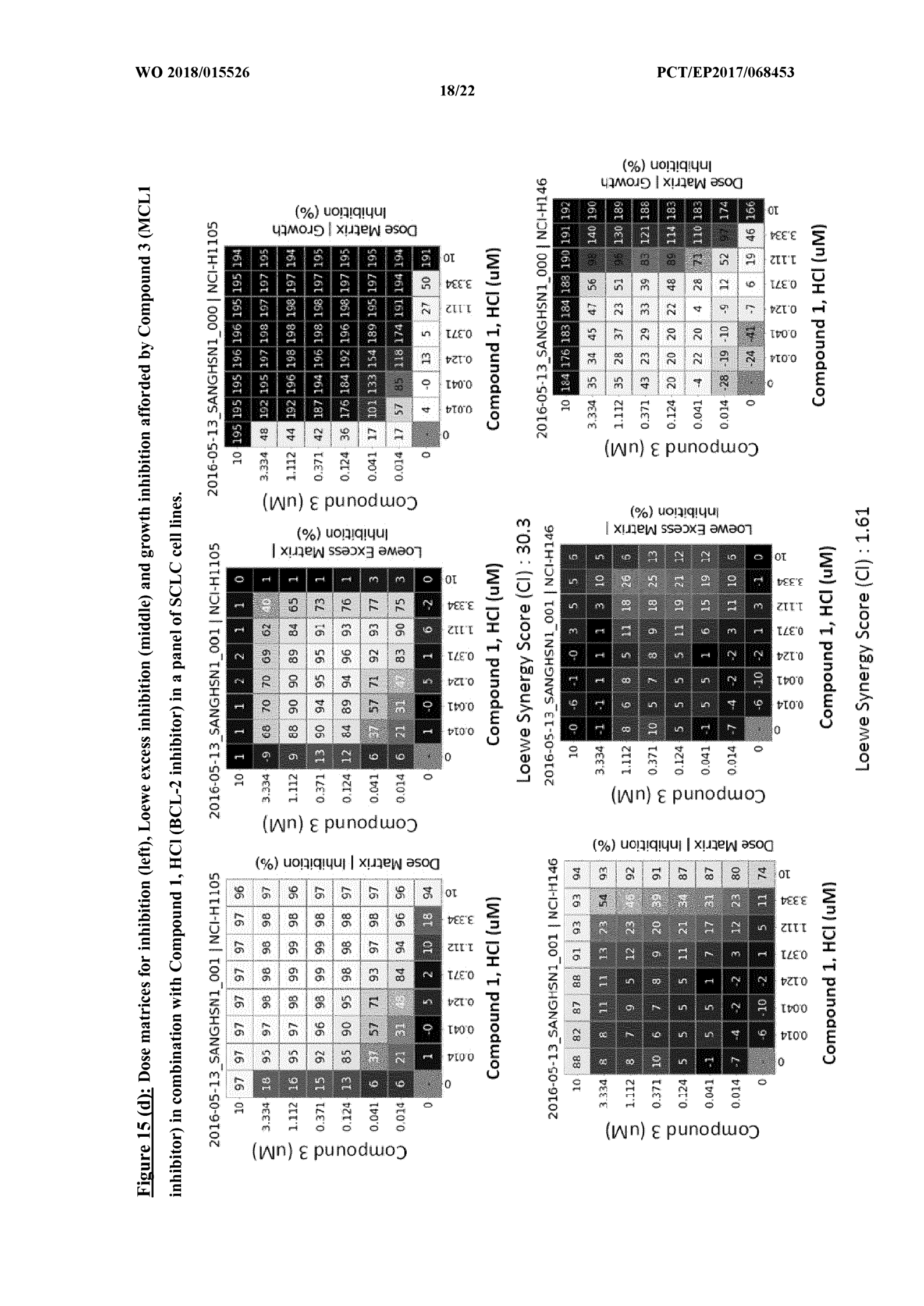


Figure 15 (e): Dose matrices for inhibition (left), Loewe excess inhibition (middle) and growth inhibition afforded by Compound 3 (MCL1 inhibitor) in combination with Compound 1, HCl (BCL-2 inhibitor) in a panel of SCLC cell lines.

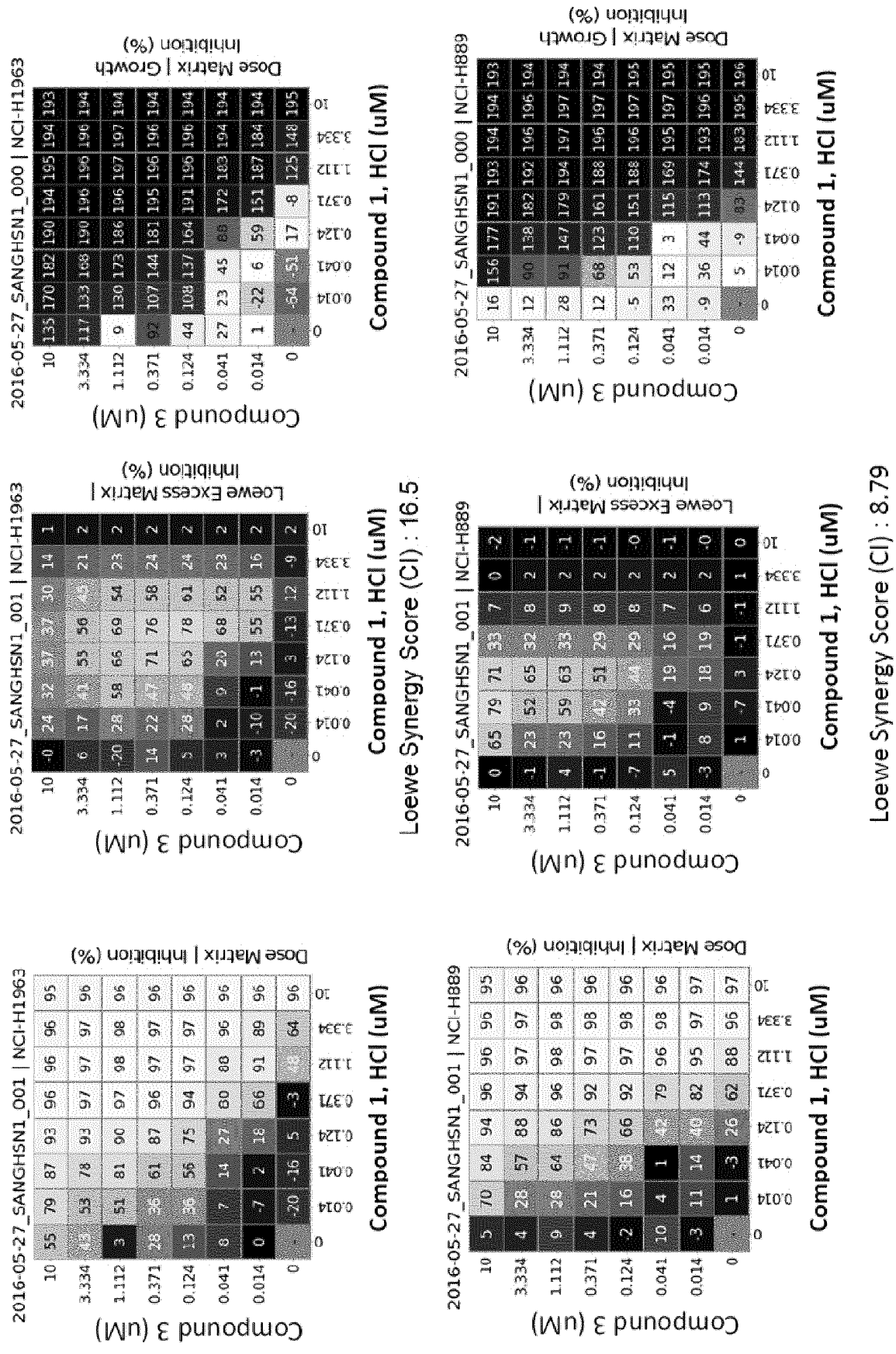
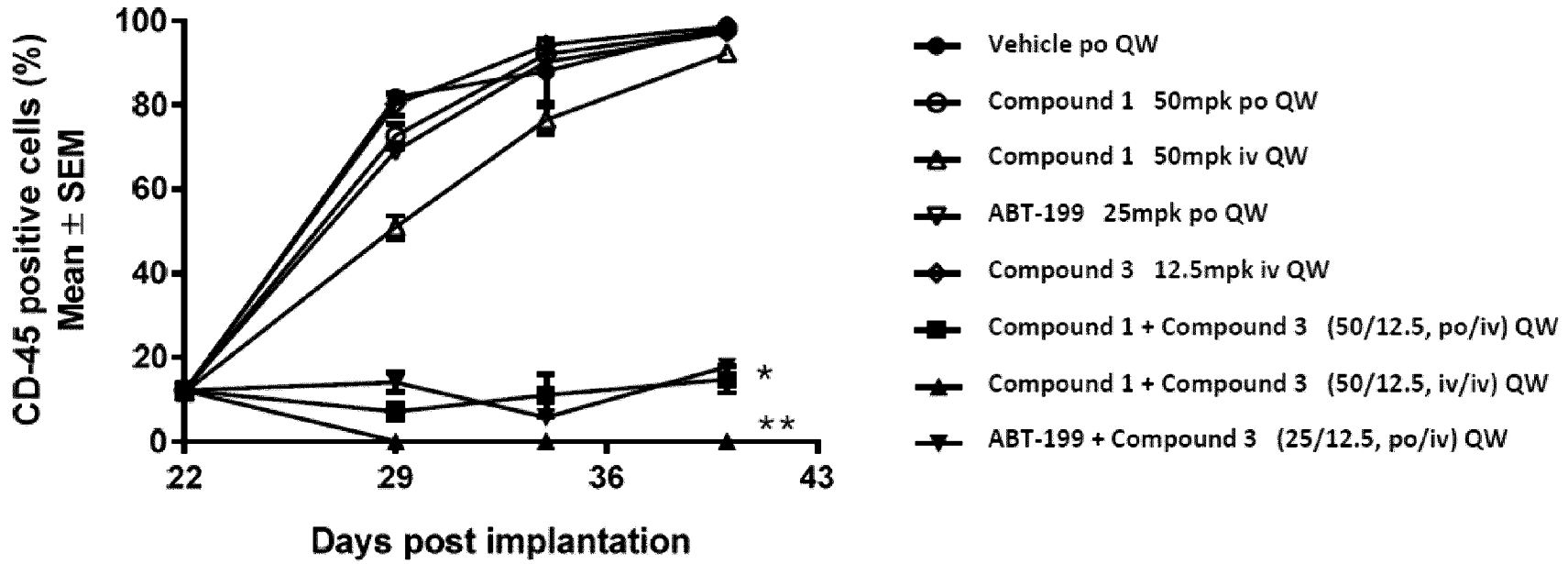


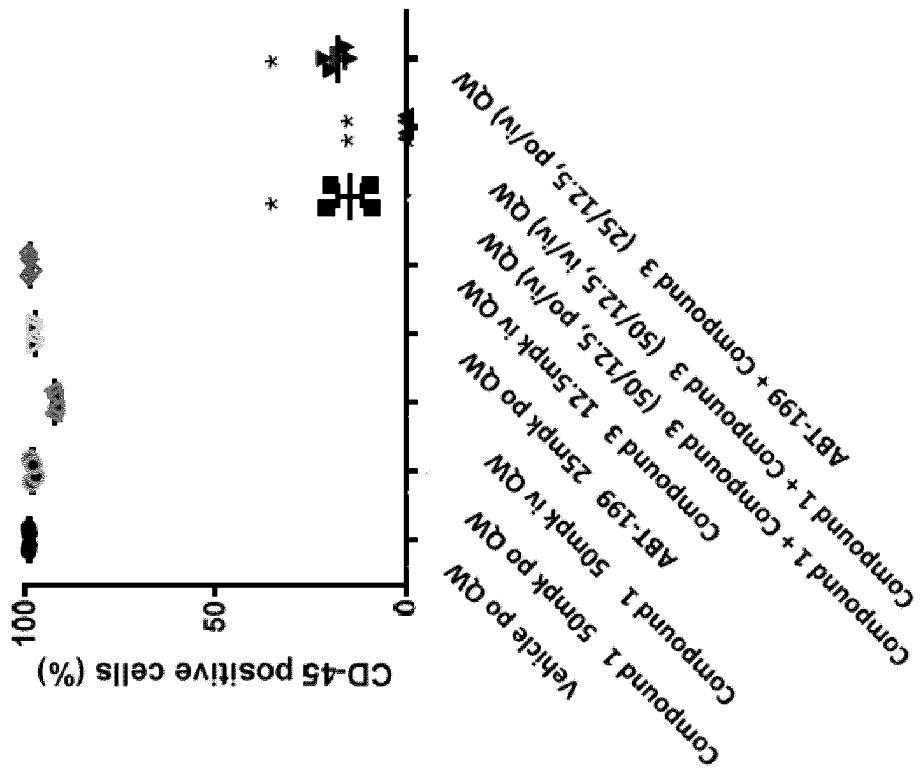
Figure 16 (a). Anti-tumor effects of Compound 1, HCl, ABT-199, Compound 3 and the combination of Compound 1, HCl or ABT-199 + Compound 3 in Patient-derived primary AML model HAMLX5343 in mice.



* $p < 0.05$ versus Vehicle and single agents (ANOVA, Tukey's test)

** $p < 0.05$ versus Vehicle, single agents and *po/iv* combination (ANOVA, Tukey's test)

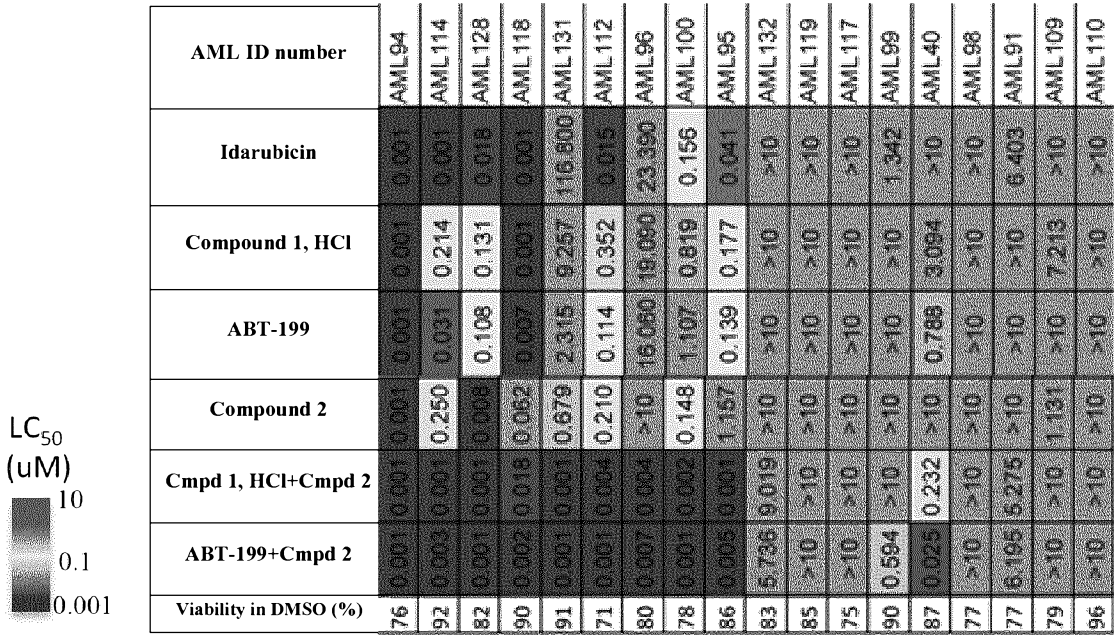
Figure 16 (b). **Anti-tumor effects of Compound 1, HCl, ABT-199, Compound 3 and the combination of Compound 1, HCl or ABT-199 + Compound 3 in Patient-derived primary AML model HAMLX5343 in mice.**



* $p < 0.05$ versus Vehicle and single agents (ANOVA, Tukey's test)

** $p < 0.05$ versus Vehicle, single agents and *po/iv* combination (ANOVA, Tukey's)

Figure 17. Heat-map comparison of AML sensitivity (LC_{50}) to BH3-mimetic monotherapy, or drug combinations (tested in 1:1 ratio), relative to chemotherapy (idarubicin) after 48h exposure. Cell viability of each primary AML samples after 48h in DMSO is shown.



AML ID number	AML94	AML114	AML128	AML118	AML131	AML112	AML96	AML100	AML95	AML132	AML119	AML117	AML99	AML40	AML98	AML91	AML109	AML110
Idarubicin	0.001	0.001	0.018	0.001	116.800	0.015	23.390	0.156	0.041	>10	>10	>10	1.342	>10	>10	6.403	>10	>10
Compound 1, HCl	0.001	0.214	0.131	0.001	9.257	0.352	10.090	0.819	0.177	>10	>10	>10	>10	3.094	>10	>10	7.213	>10
ABT-199	0.001	0.031	0.108	0.007	2.316	0.114	16.060	1.107	0.139	>10	>10	>10	>10	0.788	>10	>10	>10	>10
Compound 2	0.001	0.250	0.008	0.062	0.679	0.210	>10	0.148	1.157	>10	>10	>10	>10	>10	>10	>10	1.131	>10
Cmpd 1, HCl+Cmpd 2	0.001	0.001	0.003	0.018	0.001	0.004	0.004	0.002	0.001	9.019	>10	>10	>10	0.232	>10	5.276	>10	>10
ABT-199+Cmpd 2	0.001	0.003	0.001	0.002	0.001	0.001	0.007	0.001	0.005	5.736	>10	>10	0.594	0.025	>10	6.195	>10	>10
Viability in DMSO (%)	76	92	82	90	91	71	80	78	86	83	85	75	90	87	77	77	79	96