

Combinations and uses thereof

Field of the Invention

The present disclosure is related to a pharmaceutical combination of an anti-CD19 antibody and a phosphoinositide 3-kinase inhibitor for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia.

Background

B cells are lymphocytes that play a large role in the humoral immune response. They are produced in the bone marrow of most mammals, and represent 5-15% of the circulating lymphoid pool. The principal function of B cells is to make antibodies against various antigens, and are an essential component of the adaptive immune system.

Because of their critical role in regulating the immune system, dysregulation of B cells is associated with a variety of disorders, such as lymphomas, and leukemias. These include non-Hodgkin's lymphoma ("NHL"), chronic lymphocytic leukemia ("CLL") and acute lymphoblastic leukemia ("ALL").

NHL is a heterogeneous malignancy originating from lymphocytes. In the United States (U.S.), the incidence is estimated at 65,000/year with mortality of approximately 20,000 (American Cancer Society, 2006; and SEER Cancer Statistics Review). The disease can occur in all ages, the usual onset begins in adults over 40 years, with the incidence increasing with age. NHL is characterized by a clonal proliferation of lymphocytes that accumulate in the lymph nodes, blood, bone marrow and spleen, although any major organ may be involved. The current classification system used by pathologists and clinicians is the World Health Organization (WHO) Classification of Tumours, which organizes NHL into precursor and mature B-cell or T-cell neoplasms. The PDQ is currently dividing NHL as indolent or aggressive for entry into clinical trials. The indolent NHL group is comprised primarily of follicular subtypes, small lymphocytic lymphoma, MALT (mucosa-associated lymphoid tissue), and marginal zone; indolent encompasses approximately 50% of newly diagnosed B-cell NHL patients. Aggressive NHL includes patients with histologic diagnoses of primarily diffuse large B cell (DLBL, DLBCL, or DLCL) (40% of all newly diagnosed patients have diffuse large cell), Burkitt's, and mantle cell. The clinical course of NHL is highly variable. A major determinant of clinical course is the histologic subtype. Most indolent types of NHL are considered to be incurable disease. Patients respond initially to either chemotherapy or antibody therapy and most will relapse. Studies to date have not demonstrated an improvement in survival with early intervention. In asymptomatic patients, it is acceptable to "watch and wait" until the patient becomes symptomatic or the disease pace appears to be accelerating. Over time, the disease may transform to a more aggressive histology. The median survival is 8 to 10 years, and indolent patients often

receive 3 or more treatments during the treatment phase of their disease. Initial treatment of the symptomatic indolent NHL patient historically has been combination chemotherapy. The most commonly used agents include: cyclophosphamide, vincristine and prednisone (CVP); or cyclophosphamide, adriamycin, vincristine, prednisone (CHOP). Approximately 70% to 80% of patients will respond to their initial chemotherapy, duration of remissions last on the order of 2-3 years. Ultimately the majority of patients relapse. The discovery and clinical use of the anti-CD20 antibody, rituximab, has provided significant improvements in response and survival rate. The current standard of care for most patients is rituximab + CHOP (R-CHOP) or rituximab + CVP (R-CVP). Interferon is approved for initial treatment of NHL in combination with alkylating agents, but has limited use in the U.S. Rituximab therapy has been shown to be efficacious in several types of NHL, and is currently approved as a first line treatment for both indolent (follicular lymphoma) and aggressive NHL (diffuse large B cell lymphoma). However, there are significant limitations of anti-CD20 monoclonal antibody (mAb), including primary resistance (50% response in relapsed indolent patients), acquired resistance (50% response rate upon re-treatment), rare complete response (2% complete response rate in relapsed population), and a continued pattern of relapse. Finally, many B cells do not express CD20, and thus many B-cell disorders are not treatable using anti-CD20 antibody therapy.

In addition to NHL there are several types of leukemias that result from dysregulation of B cells. CLL is a type of adult leukemia caused by an abnormal accumulation of B lymphocytes. In CLL, the malignant lymphocytes may look normal and mature, but they are not able to cope effectively with infection. CLL is the most common form of leukemia in adults. Men are twice as likely to develop CLL as women. However, the key risk factor is age. Over 75% of new cases are diagnosed in patients over age 50. More than 10,000 cases are diagnosed every year and the mortality is almost 5,000 a year (American Cancer Society, 2006; and SEER Cancer Statistics Review). CLL is an incurable disease but progresses slowly in most cases. Many people with CLL lead normal and active lives for many years. Because of its slow onset, early-stage CLL is generally not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time. Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease. There are dozens of agents used for CLL therapy. Combination chemotherapy regimens such as FCR (fludarabine, cyclophosphamide and rituximab), and BR (Idelalisib and rituximab) are effective in both newly-diagnosed and relapsed CLL. Allogeneic bone marrow (stem cell) transplantation is rarely used as a first-line treatment for CLL due to its risk.

Another type of leukemia is ALL is also known as acute lymphocytic leukemia. ALL is characterised by the overproduction and continuous multiplication of malignant and immature white blood cells (also known as lymphoblasts) in the bone marrow. 'Acute' refers to the undifferentiated,

immature state of the circulating lymphocytes ("blasts"), and that the disease progresses rapidly with life expectancy of weeks to months if left untreated. ALL is most common in childhood with a peak incidence of 4-5 years of age. Children of age 12- 16 die more easily from it than others. Currently, at least 80% of childhood ALL are considered curable. Under 4,000 cases are diagnosed every year and the mortality is almost 1,500 a year (American Cancer Society, 2006; and SEER Cancer Statistics Review).

The human CD 19 molecule is a structurally distinct cell surface receptor expressed on the surface of human B cells, including, but not limited to, pre-B cells, B cells in early development (i.e., immature B cells), mature B cells through terminal differentiation into plasma cells, and malignant B cells. CD 19 is expressed by most pre-B acute lymphoblastic leukemias (ALL), non-Hodgkin's lymphomas, B cell chronic lymphocytic leukemias (CLL), pro-lymphocytic leukemias, hairy cell leukemias, common acute lymphocytic leukemias, and some Null-acute lymphoblastic leukemias (Nadler et al, J. Immunol., 131 :244-250 (1983), Loken et al, Blood, 70:1316-1324 (1987), Uckun et al, Blood, 71 :13- 29 (1988), Anderson et al, 1984. Blood, 63:1424-1433 (1984), Scheuermann, Leuk. Lymphoma, 18:385-397(1995)). The expression of CD 19 on plasma cells further suggests it may be expressed on differentiated B cell tumors such as multiple myeloma, plasmacytomas, Waldenstrom's tumors (Grossbard et al., Br. J. Haematol, 102:509- 15(1998); Treon et al, Semin. Oncol, 30:248-52(2003)).

Therefore, the CD 19 antigen is a target for immunotherapy in the treatment of non-Hodgkin's lymphoma (including each the subtypes described herein), chronic lymphocytic leukemia and/or acute lymphoblastic leukemia.

Certain CD19 therapies have been shown. T cells expressing an anti-CD19 chimeric antigen receptor (CAR) including both CD3- ζ and the 4-1BB costimulatory domain were administered to three patients with advanced CLL. Kalos et al., T cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia, Science Translational Medicine, vol. 3, no. 95 (10 August 2011), which is incorporated by reference in its entirety. Sadelain et al., The promise and potential pitfalls of chimeric antigen receptors, Current Opinion in Immunology, Elsevier, vol. 21, no.2, 2 April 2009, which is incorporated by reference in its entirety, also describes anti-CD19 chimeric antigen receptors (CARs).

The use of a CD19 antibody in non-specific B cell lymphomas is discussed in WO2007076950 (US2007154473), which are both incorporated by reference in their entireties.

The use of a CD19 antibody in CLL, NHL and ALL is described in Scheuermann et al., CD19 Antigen in Leukemia and Lymphoma Diagnosis and Immunotherapy, Leukemia and Lymphoma, Vol. 18, 385-397 (1995), which is incorporated by reference in its entirety.

Additional antibodies specific for CD19 are described in WO2005012493 (US7109304), WO2010053716 (US12/266,999) (Immunomedics); WO2007002223 (US US8097703) (Medarex); WO2008022152 (12/377,251) and WO2008150494 (Xencor), WO2008031056 (US11/852,106) (Medimmune); WO 2007076950 (US 11/648,505) (Merck Patent GmbH); WO 2009/052431 (US12/253,895) (Seattle Genetics); and WO2010095031 (12/710,442) (Glenmark Pharmaceuticals), WO2012010562 and WO2012010561 (International Drug Development), WO2011147834 (Roche Glycart), and WO 2012/156455 (Sanofi), which are all incorporated by reference in their entireties.

Combinations of antibodies specific for CD19 and other agents are described in WO2010151341 (US 13/377,514) (The Feinstein Institute); US5686072 (University of Texas), and WO2002022212 (PCT/US01/29026) (IDEC Pharmaceuticals), WO2013/024097 (14/126,928) (MorphoSys AG) and WO2013/024095 (14/127,217) (MorphoSys AG), which are all incorporated by reference in their entireties. Abstract 4765 of the AACR Annual Meeting 2014, April 5-9, 2014 in San Diego, CA, titled Drug synergies observed for antibody and toxin components of SAR3419 ADC contribute to overall conjugate efficacy and can be combination drug or tumor cell line dependent discloses the SAR3419 anti-CD19 antibody drug conjugate (ADC) with PI3K inhibitors in certain cell lines.

Certain Phosphoinositide 3-kinase inhibitors are commercially available. Idelalisib, also known as GS-1101 or CAL-101, is marketed by Gilead and has a trade name Zydely in the United States. Idelalisib is described in US Patent Nos: 6,800,620; 8,865,730; 8,980,901; RE44599; and RE44638, which are all incorporated by reference in their entireties.

It is clear that in spite of the recent progress in the discovery and development of anti-cancer agents, many forms of cancer involving CD19-expressing tumors still have a poor prognosis. Thus, there is a need for improved methods for treating such forms of cancer.

Summary

Neither alone nor in combination does the prior art suggest the synergistic effects of the combination of the exemplified antibody and Idelalisib in the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia.

In one aspect, the present disclosure relates to a synergistic combination of an antibody specific for CD19 and a phosphoinositide 3-kinase inhibitor. Such combinations are useful in the treatment of B cell malignancies, such as, non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia and/or acute lymphoblastic leukemia (ALL).

In vitro models are considered indicative of how a certain compound or combination of

compounds would behave in humans. Multiple cell lines were tested, for example, MEC-1 cells (DSMZ# ACC497) a chronic B-cell leukemia cell line. MEC-1 cells in this *in vitro* model are indicative of how the combination will work in the treatment of chronic lymphoid leukemia (CLL) in humans.

In addition, when compounds are combined *in vitro*, one expects that the combination has only additive effects. Surprisingly, the inventors found that the combination of a particular antibody specific for CD19 and Idelalisib mediated a synergistic level of specific cell killing *in vitro* in comparison to the antibody and Idelalisib alone.

Specifically, the inventors found that the combination of MOR00208 and Idelalisib mediated a synergistic level of specific cell killing *in vitro* in MEC-1 cells compared to the antibody and Idelalisib alone.

In addition, and also unexpectedly, the inventors found that the combination of a particular antibody specific for CD19 and Idelalisib had certain superior functional properties, in comparison to the antibody and Idelalisib alone.

In summary, the combination of the exemplified anti-CD19 antibody and Idelalisib behaved synergistically in models relevant to CLL. As CLL is a B cell related disorders and CD19 is highly expressed on B-cells, the exemplified combination would have the same mechanism of action and should also behave synergistically in the treatment of other B cell related disorders, e.g. NHL and ALL.

Therefore, the combination of the exemplified antibody specific for CD19 and Idelalisib should be effective in the treatment of humans in non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia. The expected efficacy of the combination of the antibody specific to CD19 exemplified and Idelalisib will be confirmed in clinical trials.

As the mechanism of action of Idelalisib and other phosphoinositide 3-kinase inhibitors are similar, as they work by inhibiting one or more of the phosphoinositide 3-kinase enzymes, which are part of the PI3K/AKT/mTOR pathway, an important signalling pathway for many cellular functions such as growth control, metabolism and translation initiation, it is believed that synergy should also be seen when treating humans having non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia with a combination of the exemplified anti-CD19 antibody and a phosphoinositide 3-kinase inhibitor other than Idelalisib.

As the exemplified anti-CD19 antibody and other anti-CD19 antibodies bind CD19, it is believed that synergy should also be seen when treating humans having non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia with a combination of any anti-CD19 antibody and a phosphoinositide 3-kinase inhibitor, e.g., Idelalisib .

An aspect of the present disclosure comprises a synergistic combination wherein the antibody specific for CD19 comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2

region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYFIT (SEQ ID NO: 6) and Idelalisib. In preferred aspects, the combination is used for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia.

Description of Drawings

Figure 1 shows the amino acid sequence of the variable domains of MOR00208.

Figure 2 shows the amino acid sequence of the Fc regions of MOR00208.

Figures 3-6 show ADCC dose response curves of the combination of MOR00208 and Idelalisib in MEC-1 cells from four independent experiments.

Figures 7-10 show the CI curves of the combination of MOR00208 and Idelalisib at differing doses from four independent experiments.

Detailed description of the invention

"Synergy", "synergism" or "synergistic" mean more than the expected additive effect of a combination. The "synergy", "synergism" or "synergistic" effect of a combination is determined herein by the methods of Chou et al., Clarke et al. and/or Webb et al. See Ting-Chao Chou, Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies, Pharmacol Rev 58:621-681 (2006), which is incorporated by reference in its entirety. See also Clarke et al., Issues in experimental design and endpoint analysis in the study of experimental cytotoxic agents *in vivo* in breast cancer and other models, Breast Cancer Research and Treatment 46:255-278 (1997), which is incorporated by reference in its entirety. See also Webb, J. L. (1963) Enzyme and Metabolic Inhibitors, Academic Press, New York, which is incorporated by reference in its entirety.

The term "antibody" means monoclonal antibodies, including any isotype, such as, IgG, IgM, IgA, IgD and IgE. An IgG antibody is comprised of two identical heavy chains and two identical light chains that are joined by disulfide bonds. Each heavy and light chain contains a constant region and a variable region. Each variable region contains three segments called "complementarity-determining regions" ("CDRs") or "hypervariable regions", which are primarily responsible for binding an epitope of an antigen. They are referred to as CDR1, CDR2, and CDR3, numbered sequentially from the N-terminus. The more highly conserved portions of the variable regions outside of the CDRs are called

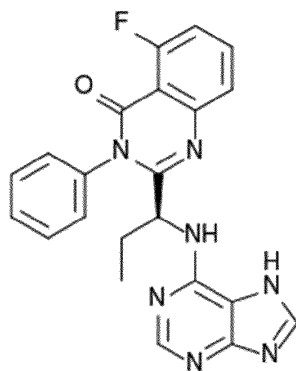
the "framework regions". An "antibody fragment" means an Fv, scFv, dsFv, Fab, Fab' F(ab')₂ fragment, or other fragment, which contains at least one variable heavy or variable light chain, each containing CDRs and framework regions.

A "phosphoinositide 3-kinase inhibitor" is a class of medical drug that functions by inhibiting one or more of the phosphoinositide 3-kinase enzymes, which are part of the PI3K/AKT/mTOR pathway, an important signalling pathway for many cellular functions such as growth control, metabolism and translation initiation.

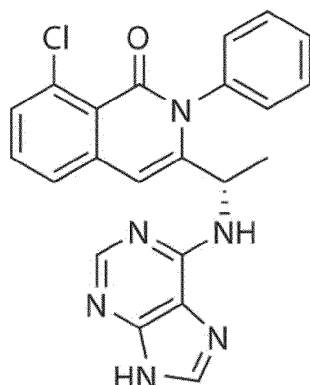
There are a number of different classes and isoforms of PI3Ks. Class 1 PI3Ks have a catalytic subunit known as p110, with four types (isoforms) - p110 alpha, p110 beta, p110 gamma and p110 delta. Current inhibitors being studied inhibit one or more isoforms of the class I PI3Ks.

Phosphoinositide 3-kinase inhibitors include at least Idelalisib, Duvelisib and Copanlisib.

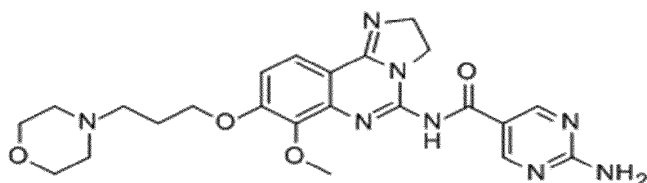
Idelalisib is marketed by Gilead Sciences, Inc. (trade name Zydelig, also named GS-1101 or CAL-101). Idelalisib is currently labelled for the treatment of relapsed chronic lymphocytic leukemia (CLL), in combination with rituximab, in patients for whom rituximab alone would be considered appropriate therapy due to other co-morbidities; relapsed follicular B-cell non-Hodgkin lymphoma (FL) in patients who have received at least two prior systemic therapies; relapsed small lymphocytic lymphoma (SLL) in patients who have received at least two prior systemic therapies. The substance acts as a phosphoinositide 3-kinase inhibitor; more specifically, it blocks P110 δ , the delta isoform of the enzyme phosphoinositide 3-kinase. The formula of Idelalisib is:



Duvelisib (IPI-145, INK1197) is a novel and selective PI3K δ/γ (delta and gamma) inhibitor. The formula of Duvelisib is:



Copanlisib (BAY 80-6946), developed by Bayer, is a selective Class I phosphoinositide 3-kinase inhibitor. The formula of Copanlisib is:



"VH" refers to the variable region of an immunoglobulin heavy chain of an antibody, or antibody fragment. "VL" refers to the variable region of the immunoglobulin light chain of an antibody, or antibody fragment.

The term "CD19" refers to the protein known as CD19, having the following synonyms: B4, B-lymphocyte antigen CD19, B-lymphocyte surface antigen B4, CVID3, Differentiation antigen CD19, MGC12802, and T-cell surface antigen Leu-12.

Human CD19 has the amino acid sequence of:

MPPPRLLFFLLFLTPMEVRPEEPLVVKVEEGDNAVLQCLKGTSDGPTQQLTWSRESPLKPFLKLSL
GLPGLGIHMRPLAIWLFIFNVSQQMGGFYLCQPGPPSEKAWQPGWTVNVEGSGELFRWNVSDLG
GLGCGLKNRSSEGPSKLMSPKLYVWAKDRPEIWEGEPPCLPPRDSLNLQSLSQDLTMAPGS

TLWLSCGVPPDSVSRGPLSWTHVHPKGPKSLLSLELKDDRPARDMWVMMETGLLLPRATAQDAGK
 YYCHRGNTMSFHLITARPVLWHWLLRTGGWKVSAVTLAYLIFCLCSLVGILHLQRALVLRKRK
 RMTDPTRRRFFKVTPPPGSGPQNQYGNVLSLPTPTSGLGRAQRWAAGLGGTAPSYGNPSSDVQA
 DGALGSRSPPGVGPEEEEEEGEYEEPDSEEDSEFYENDSNLGQDQLSQDGSQYENPEDEPLGPE
 DEDSFSNAESYENEDDELTQPVARTMDFLSPHGSAWDPSREATSLGSQSYEDMRGILYAAPQLR
 SIRGQPGPNHEEDADSYENMDNPDGPDPAWGGGGRMGWSTR. (SEQ ID NO: 7)

"MOR00208" is an anti-CD19 antibody. The amino acid sequence of the variable domains is provided in Figure 1. The amino acid sequence of the heavy and light chain Fc regions of MOR00208 are provided in Figure 2. "MOR00208," "XmAb 5574," and "MOR208" are used as synonyms to describe the antibody shown in Figures 1 and 2. The MOR00208 antibody is described in US patent application serial number 12/377,251, wherein the full light chain is SEQ ID NO: 106 and the full heavy chain is SEQ ID NO: 87, which is incorporated by reference in its entirety.

MOR00208 has been studied in human clinical trials in ALL, NHL, CLL, and Small Lymphocytic Lymphoma (SLL).

Additional antibodies specific for CD19 are described in US patent no. 7,109,304 (Immunomedics), which is incorporated by reference in its entirety; US application serial no. 11/917,750 (Medarex), which is incorporated by reference in its entirety; US application serial no. 11/852,106 (Medimmune), which is incorporated by reference in its entirety; US application serial no. 11/648,505 (Merck Patent GmbH), which is incorporated by reference in its entirety; US patent no. 7,968,687 (Seattle Genetics), which is incorporated by reference in its entirety; and US application serial no. 12/710,442 (Glenmark Pharmaceuticals), which is incorporated by reference in its entirety.

"Fc region" means the constant region of an antibody, which in humans may be of the IgG1, 2, 3, 4 subclass or others. The sequences of human Fc regions are available at IMGT, Human IGH C-REGIONS, http://www.imgt.org/IMGTrepertoire/Proteins/protein/human/IGH/IGHC/Hu_IGHCgenes.html (retrieved on 16 May 2011).

"RefmAb33" is an antibody whose amino acid sequence is as follows:

Heavy chain including the Fc region:

QVTLRESGPALVKPTQTLTLCTFSGFSLSTAGMSVGWIRQPPGKALEWLADIWWDDKKH
 YNPSLKDRLTISKDTSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWGQGTTVTVSSASTKG
 PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
 PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPDVFLFPPKPKDTLMIS
 RTPETCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSIVLTQVHQLDNLNGKE
 YKCKVSNKALPAAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN
 GPENNYKTTTPMLDSGSSFLYSKLTVDKSRWQQGNVVFSCVMHEALHNHYTQKSLSLSPGK
 (SEQ ID NO: 8)

Light chain including the Fc region:

DIQMTQSPSTLSASVGDRVTITCSASSRVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRF
SGSGSGTEFTLTISSLQPDDFATYYCFQGSQGYPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 9)

RefmAb33 is specific for RSV, and is used as isotype control, as it shares the same Fc region as MOR00208.

A "combination" means more than one item, e.g. a compound such as an antibody and Idelalisib.

The present disclosure also relates to combinations, pharmaceuticals, and pharmaceutical compositions containing the described combinations. The two components of the synergistic combination of the present invention, e.g. the antibody specific for CD19 and Idelalisib, may be administered together, simultaneously, separately or subsequently, either physically or in time.

Idelalisib is currently administered 150mg orally twice daily. MOR00208 is currently administered intravenously, and is currently dosed either once a week or once every two weeks. In an embodiment, Idelalisib, is administered prior to the administration of the antibody specific for CD19, e.g. MOR00208. In an embodiment, Idelalisib, is administered after the administration of the antibody specific for CD19, e.g. MOR00208.

Preferably, administration of both drugs allows for both drugs to be active in the patient at the same time. For example, if MOR00208 is dosed weekly and Idelalisib is dosed daily then the active substance of both drugs is desirably present in the patient at the same time even if both drugs are not always administered both on the same day.

"Simultaneously" or "administered together" means that the two components are administered at a time where both components (drugs) are active in the patient at the same time. It is implied by "synergism" that both drugs are active in the patient at the same time. It is not necessary for "simultaneously" or "administered together" to mean that the drugs are administered at the exact same time or always on the same day.

The two components may be formulated in different pharmaceutical compositions.

A pharmaceutical composition includes an active agent, eg. an antibody for therapeutic use in humans. A pharmaceutical composition may include acceptable carriers or excipients.

"Administered" or "administration" includes but is not limited to delivery by an injectable form, and includes, for example, intravenous, intramuscular, intradermal, topical, transdermally, intraperitoneally, intraorbitally, by implantation, or subcutaneous route or mucosal route, for example, as a nasal spray or aerosol for inhalation or as an ingestible solution, or orally, as a capsule or tablet.

A "therapeutically effective amount" of a compound or combination refers to an amount able to effect a measurable improvement, alleviate or partially arrest the clinical manifestations of a

given disease or disorder. The amount that is effective for a particular therapeutic purpose will depend on the severity of the disease or injury as well as on the weight and general state of the subject. It will be understood that determination of an appropriate dosage may be achieved, using routine experimentation, by constructing a matrix of values and testing different points in the matrix, all of which is within the ordinary skills of a trained physician or clinical scientist.

The "CDRs" herein are defined by either Chothia et al or Kabat et al. See Chothia C, Lesk AM. (1987) Canonical structures for the hypervariable regions of immunoglobulins. *J Mol Biol.*, 196(4):901-17, which is incorporated by reference in its entirety. See Kabat E.A, Wu T.T., Perry H.M., Gottesman K.S. and Foeller C. (1991). *Sequences of Proteins of Immunological Interest*. 5th edit., NIH Publication no. 91-3242, US Dept. of Health and Human Services, Washington, DC, which is incorporated by reference in its entirety.

"Cross competes" means the ability of an antibody or other binding agent to interfere with the binding of other antibodies or binding agents to CD19 in a standard competitive binding assay. The ability or extent to which an antibody or other binding agent is able to interfere with the binding of another antibody or binding molecule to CD19, and, therefore whether it can be said to cross-compete according to the invention, can be determined using standard competition binding assays. One suitable assay involves the use of the Biacore technology (e.g. by using the BIAcore 3000 instrument (Biacore, Uppsala, Sweden)), which can measure the extent of interactions using surface plasmon resonance technology. Another assay for measuring cross-competing uses an ELISA-based approach. A high throughput process for "epitope binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 2003/48731

The term "epitope" includes any protein determinant capable of specific binding to an antibody or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." The term "linear epitope" refers to an epitope with all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein (continuous). The term "conformational epitope" refers to an epitope in which discontinuous amino acids that come together in three dimensional conformation. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another.

"Binds the same epitope as" means the ability of an antibody or other binding agent to bind to CD19 and having the same epitope as the exemplified antibody. The epitopes of the exemplified antibody and other antibodies to CD19 can be determined using standard epitope

mapping techniques. Epitope mapping techniques, well known in the art, include Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66 (Glenn E. Morris, Ed., 1996) Humana Press, Totowa, New Jersey. For example, linear epitopes may be determined by e.g., concurrently synthesizing large numbers of peptides on solid supports, the peptides corresponding to portions of the protein molecule, and reacting the peptides with antibodies while the peptides are still attached to the supports. Such techniques are known in the art and described in, e.g., U.S. Patent No. 4,708,871 ; Geysen et al, (1984) Proc. Natl. Acad. Sci. USA 8:3998-4002; Geysen et al, (1985) Proc. Natl. Acad. Sci. USA 82:78-182; Geysen et al, (1986) Mol. Immunol. 23 :709-715. Similarly, conformational epitopes are readily identified by determining spatial conformation of amino acids such as by, e.g., hydrogen/deuterium exchange, x-ray crystallography and two-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, supra. Antigenic regions of proteins can also be identified using standard antigenicity and hydrophathy plots, such as those calculated using, e.g., the Omega version 1.0 software program available from the Oxford Molecular Group. This computer program employs the Hopp/Woods method, Hopp et al, (1981) Proc. Natl. Acad. Sci. USA 78:3824-3828; for determining antigenicity profiles, and the Kyte-Doolittle technique, Kyte et al, (1982) J.Mol. Biol. 157: 105-132; for hydrophathy plots.

Embodiments

An aspect of the present disclosure comprises a combination of an antibody specific for CD19 and a phosphoinositide 3-kinase inhibitor for use in the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia. In embodiments, the combination is synergistic.

Herein, the combination of the exemplified anti-CD19 antibody and Idelalisib behaved synergistically in *in vitro* models relevant to CLL. As CLL is a B cell related disorder and CD19 is highly expressed on B-cells, the exemplified combination should have the same mechanism of action and should also behave synergistically in the treatment of other B cell related disorders, e.g. NHL and ALL. Therefore, the combination of the exemplified antibody specific for CD19 and Idelalisib should be effective in the treatment of humans in non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia. The expected efficacy of the combination of the antibody specific to CD19 exemplified and Idelalisib will be confirmed in clinical trials.

MEC-1 cells (DSMZ# ACC497), a chronic B-cell leukemia cell line, were tested.

Additional cell lines are evaluated: Ramos cells (ATCC number CRL-1596), a human Burkitt's lymphoma cells. HG-3 (DSMZ#ACC765), and Cll (DSMZ#ACC773) are a chronic lymphocytic leukemia cell line. Su-DHL 6 (DSMZ#ACC572), and U2932 (DSMZ#ACC633) are a

Diffuse large B-cell lymphoma (DLBCL) cell line. JVM-2 (ATCC® CRL-3002) is a mantle cell lymphoma cell line. BALL-1 (DSMZ#ACC742) is an acute lymphoblastic leukemia cell line.

MEC-1 cells in this in vitro model are indicative of how the combination will work in the treatment of chronic lymphoid leukemia (CLL) in humans. Ramos cells in this in vitro model are indicative of how the combination will work in the treatment of non-Hodgkins lymphoma (NHL) in humans. HG-3 and Cll cells in this in vitro model are indicative of how the combination will work in the treatment of chronic lymphoid leukemia (CLL) in humans. Su-DHL 6 and U2932 cells in this in vitro model are indicative of how the combination will work in the treatment non-Hodgkin's lymphoma in humans. JVM-2 cells in this in vitro model are indicative of how the combination will work in the treatment non-Hodgkin's lymphoma in humans. BALL-1 cells in this in vitro model are indicative of how the combination will work in the treatment of acute lymphoblastic leukemia in humans.

The Chou index and Clarke et al. values indicate clear synergism of the combination of MOR00208 and Idelalisib in the specific killing of MEC-1 cells as compared to MOR00208 and Idelalisib alone.

In summary, the combination of the exemplified anti-CD19 antibody and Idelalisib behaved synergistically in models relevant to CLL. Therefore, the combination of the exemplified antibody specific for CD19 and Idelalisib should be effective in the treatment of humans in non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia.

As the mechanism of action of Idelalisib and other phosphoinositide 3-kinase inhibitors are similar, as they all work by inhibiting one or more of the phosphoinositide 3-kinase enzymes, which are part of the PI3K/AKT/mTOR pathway, an important signalling pathway for many cellular functions such as growth control, metabolism and translation initiation, it is believed that synergy should also be seen when treating humans having non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia with a combination of the exemplified anti-CD19 antibody and a phosphoinositide 3-kinase inhibitor other than Idelalisib.

As the exemplified anti-CD19 antibody and other anti-CD19 antibodies bind CD19, it is believed that synergy should also be seen when treating humans having non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia with a combination of any anti-CD19 antibody and a phosphoinositide 3-kinase inhibitor, where the anti-CD19 antibody is, for example, described in US patent application serial number 12/377,251 (Xencor), WO2005012493, WO2010053716 (Immunomedics); WO2007002223 (Medarex); WO2008022152 (Xencor); WO2008031056 (Medimmune); WO 2007/076950 (Merck Patent GmbH); WO 2009/052431 (Seattle Genetics); and WO2010095031 (Glenmark Pharmaceuticals), all of which are incorporated by reference in their entireties.

In embodiments, the antibody specific for CD19 comprises an antibody that cross-competes with the antibody comprising an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In embodiments, the antibody specific for CD19 comprises an antibody that binds to the same epitope as an antibody comprising an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In embodiments, the antibody specific for CD19 comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6).

In embodiments, the antibody specific for CD19 comprises a variable heavy chain of the sequence EVQLVESGGGLVKPGGSLKLSAASGYTFTSYVMHWVRQAPGKGLEWIGYINPY NDGTYNEKFQGRVTISDKSISTAYMELSSLRSEDAMYYCARGTYYYGTRVFDYWG QGTLTVSS (SEQ ID NO: 10) and a variable light chain of the sequence DIVMTQSPATLSLSPGERATLSCRSSKSLQNVNGNTYLYWFQQKPGQSPQLLIYR MSNLNSGVPDRFSGSGSGTEFTLTISLEPEDFAVYYCMQHLEYPITFGAGTKLEIK (SEQ ID NO: 11).

In certain embodiments said antibody comprises a heavy chain constant domain of the sequence
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPDVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQD
WLNQKEYKCKVSNKALPAAPEEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL
SLSPGK (SEQ ID NO: 12).

In embodiments, the antibody specific for CD19 comprises a light chain constant domain of the sequence

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. (SEQ ID NO: 13)

In embodiments, the phosphoinositide 3-kinase inhibitor is Idelalisib.

In embodiments, the components of the combination, the antibody specific for CD19 and Idelalisib, are administered separately. In an embodiment, Idelalisib is administered prior to administration of the antibody specific for CD19. In an embodiment, Idelalisib is administered after the administration of the antibody specific for CD19. In embodiments, the components of the combination, the antibody specific for CD19 and Idelalisib, are administered simultaneously or together.

In embodiments the combination is a pharmaceutical composition. In embodiments, the composition comprises an acceptable carrier. In embodiments, the combination is administered in an effective amount.

In another aspect the synergistic combination of an antibody specific for CD19 comprising an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6) and Idelalisib is able to mediate killing of MEC-1 cells by ADCC in the presence of isolated human PBMCs with an at least two-fold, three-fold, four-fold, or five-fold better efficacy than Idelalisib alone.

An aspect of the present disclosure comprises a synergistic combination of an antibody specific for CD19 comprising an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6) and Idelalisib for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia. In embodiments, the non-Hodgkin's lymphoma is selected from the group consisting of follicular lymphoma, small lymphocytic lymphoma, mucosa-associated lymphoid tissue, marginal zone, diffuse large B cell, Burkitt's, and mantle cell.

Another aspect comprises a method of treating non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia in an individual in need thereof, which method comprises administration of an antibody specific for CD19 and a phosphoinositide 3-kinase inhibitor. In embodiments of the method, the antibody specific for CD19 comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID

NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6). In embodiments of the method, the antibody comprises the exemplified antibody specific for CD19. In embodiments of the method the phosphoinositide 3-kinase inhibitor is Idelalisib.

Another aspect comprises the use of an antibody specific for CD19 and a phosphoinositide 3-kinase inhibitor in the manufacture of a medicament for treating non-Hodgkin's lymphoma, chronic lymphocytic leukemia and/or acute lymphoblastic leukemia in an individual in need thereof, which method comprises administration of the medicament comprising an antibody specific for CD19 and a phosphoinositide 3-kinase inhibitor. In embodiments of the method, the antibody specific for CD19 comprises an HCDR1 region of sequence SYVMH (SEQ ID NO: 1), an HCDR2 region of sequence NPYNDG (SEQ ID NO: 2), an HCDR3 region of sequence GTYYYGTRVFDY (SEQ ID NO: 3), an LCDR1 region of sequence RSSKSLQNVNGNTYLY (SEQ ID NO: 4), an LCDR2 region of sequence RMSNLNS (SEQ ID NO: 5), and an LCDR3 region of sequence MQHLEYPIT (SEQ ID NO: 6). In embodiments of the method, the antibody comprises the exemplified antibody specific for CD19. In embodiments of the method the phosphoinositide 3-kinase inhibitor is Idelalisib.

Examples

Example 1: Cytotoxicity of MEC-1 cells using MOR00208 and Idelalisib alone and in combination

Materials

Cell lines MEC-1 cells (DSMZ# ACC497) chronic B-cell leukemia cell line; JVM-2 (ATCC® CRL-3002) a mantle cell lymphoma cell line; Ramos cells (ATCC number CRL-1596), a human Burkitt's lymphoma cells; HG-3 (DSMZ#ACC765), and CII (DSMZ#ACC773) are a chronic lymphocytic leukemia cell line; Su-DHL 6 (DSMZ#ACC572), and U2932 (DSMZ#ACC633) are a Diffuse large B-cell lymphoma (DLBCL) cell line; and BALL-1 (DSMZ#ACC742) is an acute lymphoblastic leukemia cell line.

Culture conditions of cell lines used are according to supplier's information.

Cell Medium: Iscove's Modified Dulbecco's Medium (IMDM), Invitrogen, Cat No.: 31980-048; RPMI1640, Invitrogen, Cat No.: 31870-074; GlutaMAX, Invitrogen, CAT No.: 35050-38 LOT No.: 1654740; FCS: Sigma CAT No.: F7524 LOT No.: 111M3396.

NKs: RPMI1640, with GlutaMAX™, Invitrogen, Cat No.: 31870-074, 10% FCS; Biocoll: Biochrome AG CAT No.: L6115 LOT No.: 0034D; MACS NK cell isolation kit: Miltenyi Biotec CAT No.: 130-092-657 LOT No.: 5150327376; Idelalisib: Selleck Chem CAT No.: S2226; FCS: Sigma

CAT No.: F7524 LOT No.: 111M3396; and RefmAb33 (anti-RSV) with same Fc region as MOR00208.

Methods

The cytotoxicity of MOR00208 and Idelalisib alone and in combination were tested in the MEC-1 cell line..

Idelalisib is a phosphoinositide 3-kinase inhibitor; more specifically, it blocks P110 δ , the delta isoform of the enzyme phosphoinositide 3-kinase. Idelalisib alone has little to no cytotoxic effects against MEC-1 cells. MOR00208 targets CD19 and mediates target cell killing via ADCC.

The following were used as controls: a) MEC-1 cells + RefmAb33 + DMSO + NK cells, b) MEC-1 cells + DMSO + NK cells, c) MEC-1 cells + DMSO.

Target cell killing was measured using the following parameters: Idelalisib at concentrations of 0.3, 1, 3 and 10 μ M; MOR00208 at concentrations of 1.5, 0.015 and 0.0015 μ g/ml and the combination of MOR00208 and Idelalisib at the same concentrations.

In the Idelalisib group, MOR00208 alone group and in the MOR00208+Idelalisib combination group, target cells were pre-treated with Idelalisib for 7 days prior to the ADCC assay measurements. The target cells were counted and stained using 10 μ M CFSE end concentration. For DMSO treated target cells, an effector : target (E:T) ratio of 2:1 is chosen, corresponding to a cell density of 5x10⁵/ml. The proliferative effect on target cells caused by Idelalisib treatment was included by adjusting the E:T ratio in inhibitor treated cells. The NK cells are counted and adjusted to 1x10⁶/ml. The target cell killing assays were performed as follows: using 96 well plates, 100 μ l of target cell suspension was added per well, followed by 100 μ l cell suspension of NK cells to each well resulting in an E:T ratio of 2:1. The antibodies were diluted in a range of 10 – 0.00001 nM (corresponding to 1.5 – 0.0000015 μ g/ml) in medium. Cells were centrifuged and target : effector cell-pellets were re-suspended in 100 μ l antibody-containing medium or the according control solution. The assay was incubated for 4h in CO₂-incubator at 37°C. After 10 min incubation on ice, 50 μ l DAPI solution was added to each well (final concentration 1 μ g/ml) and incubated on ice for 10 min. The cell killing measurements were performed with FACS-Verse. Dead target cells were DAPI positive.

Data

In total, six experiments were performed in order to determine the mediation of ADCC on MEC-1 cells by the combination of MOR00208 and Idelalisib. In two out of the six experiments, the data was excluded from analysis because the RefmAb control and DMSO alone control showed 25% higher killing compared to the MEC-1 cells only control. The autoreactivity of the NK cells prevented a proper analysis in those two experiments.

The ADCC dose response curves for Experiments 1-4 are shown in Figures 3-6.

The percent (%) dead cells (raw data) for Experiments 1-4 are shown in Tables 1-16 below.

Experiment 1

Table 1: Idelalisib at 10 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,9	63,1	40,3
B: Idela alone 10μM	11,6	11,6	11,6
C: control (0,03%DMSO/Ref33)	7,4	7,4	7,4
AB: combination	88,0	86,4	68,2

Table 2 Idelalisib at 3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,9	63,1	40,3
B: Idela alone 3μM	11,3	11,3	11,3
C: control (0,03%DMSO/Ref33)	7,4	7,4	7,4
AB: combination	87,7	87,6	68,1

Table 3 Idelalisib at 1 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,9	63,1	40,3
B: Idela alone 1μM	10,8	10,8	10,8
C: control (0,03%DMSO/Ref33)	7,4	7,4	7,4
AB: combination	85,9	79,6	65,3

Table 4 Idelalisib at 0.3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,9	63,1	40,3
B: Idela alone 0.3 μ M	6,8	6,8	6,8
C: control (0,03%DMSO/Ref33)	7,4	7,4	7,4
AB: combination	77,5	78,5	57,6

Experiment 2

Table 5 Idelalisib at 10 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	85,6	78,9	52,9
B: Idela alone 10 μ M	14,7	14,7	14,7
C: control (0,03%DMSO/Ref33)	19,3	19,3	19,3
AB: combination	92,5	87,2	52,6

Table 6 Idelalisib at 3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	85,6	78,9	52,9
B: Idela alone 3 μ M	14,6	14,6	14,6
C: control (0,03%DMSO/Ref33)	19,3	19,3	19,3
AB: combination	90,9	83,9	51,8

Table 7 Idelalisib at 1 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	85,6	78,9	52,9
B: Idela alone 1 μ M	20,7	20,7	20,7
C: control (0,03%DMSO/Ref33)	19,3	19,3	19,3
AB: combination	94,2	86,5	63,4

Table 8 Idelalisib at 0.3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	85,6	78,9	52,9
B: Idela alone 0.3 μ M	20,8	20,8	20,8
C: control (0,03%DMSO/Ref33)	19,3	19,3	19,3
AB: combination	93,9	89,3	60,9

Experiment 3

Table 9 Idelalisib at 10 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,0	59,5	43,5
B: Idela alone 10 μ M	11,5	11,5	11,5
C: control (0,03%DMSO/Ref33)	19,1	19,1	19,1
AB: combination	81,3	73,0	48,9

Table 10 Idelalisib at 3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,0	59,5	43,5
B: Idela alone 3 μ M	14,0	14,0	14,0
C: control (0,03%DMSO/Ref33)	19,1	19,1	19,1
AB: combination	81,1	74,0	46,8

Table 11 Idelalisib at 1 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,0	59,5	43,5
B: Idela alone 1 μ M	18,7	18,7	18,7
C: control (0,03%DMSO/Ref33)	19,1	19,1	19,1
AB: combination	83,9	78,5	52,7

Table 12 Idelalisib at 0.3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	62,0	59,5	43,5
B: Idela alone 0.3 μ M	17,3	17,3	17,3
C: control (0,03%DMSO/Ref33)	19,1	19,1	19,1
AB: combination	80,4	73,7	50,8

Experiment 4

Table 13 Idelalisib at 10 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	74,6	73,3	56,3
B: Idela alone 10 μ M	12,1	12,1	12,1
C: control (0,03%DMSO/Ref33)	21,4	21,4	21,4
AB: combination	90,6	88,0	68,9

Table 14 Idelalisib at 3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	74,6	73,3	56,3
B: Idela alone 3 μ M	13,8	13,8	13,8
C: control (0,03%DMSO/Ref33)	21,4	21,4	21,4
AB: combination	91,9	88,5	66,2

Table 15 Idelalisib at 1 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	74,6	73,3	56,3
B: Idela alone 1 μ M	15,9	15,9	15,9
C: control (0,03%DMSO/Ref33)	21,4	21,4	21,4
AB: combination	91,7	89,9	67,4

Table 16 Idelalisib at 0.3 μ M

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
A: MOR00208 alone	74,6	73,3	56,3
B: Idela alone 0.3 μ M	15,5	15,5	15,5
C: control (0,03%DMSO/Ref33)	21,4	21,4	21,4
AB: combination	90,4	87,7	66,1

Calculation of synergism: Clarke et al.

Where one drug has low activity, as here, Idelalisib alone has low cytotoxicity activity against MEC-1 cells, synergy can be determined by statistical evidence that the combination is significantly different from the inhibitory drug alone. See Clarke et al., Issues in experimental design and endpoint analysis in the study of experimental cytotoxic agents *in vivo* in breast cancer and other models, Breast Cancer Research and Treatment 46:255-278 (1997), which is incorporated by reference in its entirety.

The % dead cells (raw data) from Tables 1-16 was analysed in the following way:

Antagonistic $(AB)/C < (A/C) \times (B/C)$

Additive $(AB)/C = (A/C) \times (B/C)$

Synergistic $(AB)/C > (A/C) \times (B/C)$

where A is the treatment with MOR00208 alone; B is the treatment with Idelalisib alone; C is response to the control DMSO + RefMab33; AB is the combination of treatments A and B.

Experiment 1

Table 17: Clarke analysis of Data shown in Table 1

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 μ g/ml	0.015 μ g/ml	0.0015 μ g/ml
(AB)/C	12,0	11,7	9,3
(A/C)x(B/C)	13,5	13,5	8,6

Table 18 Clarke analysis of Data shown in Table 2

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 $\mu\text{g/ml}$	0.015 $\mu\text{g/ml}$	0.0015 $\mu\text{g/ml}$
(AB)/C	11,9	11,9	9,3
(A/C)x(B/C)	13,1	13,1	8,4

Table 19 Clarke analysis of Data shown in Table 3

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 $\mu\text{g/ml}$	0.015 $\mu\text{g/ml}$	0.0015 $\mu\text{g/ml}$
(AB)/C	11,7	10,8	8,9
(A/C)x(B/C)	12,5	12,5	8,0

Table 20 Clarke analysis of Data shown in Table 4

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 $\mu\text{g/ml}$	0.015 $\mu\text{g/ml}$	0.0015 $\mu\text{g/ml}$
(AB)/C	10,5	10,7	7,8
(A/C)x(B/C)	7,9	7,9	5,0

Experiment 2

Table 21 Clarke analysis of Data shown in Table 5

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 $\mu\text{g/ml}$	0.015 $\mu\text{g/ml}$	0.0015 $\mu\text{g/ml}$
(AB)/C	4,8	4,5	2,7
(A/C)x(B/C)	3,4	3,1	2,1

Table 22 Clarke analysis of Data shown in Table 6

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 $\mu\text{g/ml}$	0.015 $\mu\text{g/ml}$	0.0015 $\mu\text{g/ml}$
(AB)/C	4,7	4,4	2,7
(A/C)x(B/C)	3,4	3,1	2,1

Table 23 Clarke analysis of Data shown in Table 7

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,9	4,5	3,3
(A/C)x(B/C)	4,8	4,4	3,0

Table 24 Clarke analysis of Data shown in Table 8

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,9	4,6	3,2
(A/C)x(B/C)	4,8	4,4	3,0

Experiment 3

Table 25 Clarke analysis of Data shown in Table 9

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,3	3,8	2,6
(A/C)x(B/C)	2,0	1,9	1,4

Table 26 Clarke analysis of Data shown in Table 10

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,2	3,9	2,5
(A/C)x(B/C)	2,4	2,3	1,7

Table 27 Clarke analysis of Data shown in Table 11

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,4	4,1	2,8
(A/C)x(B/C)	3,2	3,1	2,2

Table 28 Clarke analysis of Data shown in Table 12

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,2	3,9	2,7
(A/C)x(B/C)	2,9	2,8	2,1

Experiment 4

Table 29 Clarke analysis of Data shown in Table 13

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,2	4,1	3,2
(A/C)x(B/C)	2,0	1,9	1,5

Table 30 Clarke analysis of Data shown in Table 14

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,3	4,1	3,1
(A/C)x(B/C)	2,2	2,2	1,7

Table 31 Clarke analysis of Data shown in Table 15

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,3	4,2	3,1
(A/C)x(B/C)	2,6	2,5	2,0

Table 32 Clarke analysis of Data shown in Table 16

	MOR00208 Concentration		
	10 nM	0.1 nM	0.01 nM
	1.5 µg/ml	0.015 µg/ml	0.0015 µg/ml
(AB)/C	4,2	4,1	3,1
(A/C)x(B/C)	2,5	2,5	1,9

Results

Experiments 2-4 at each concentration showed clear synergy of the combination of MOR00208 + Idelalisib using the methods of Clarke et al. Experiment 1, however, at a few concentrations did not show synergism, because the Idelalisib group (see Tables 1-3) showed a small effect, which effect was slightly greater than the control (~4% greater than the control). This small (~ 4%) difference as compared to the control, is well within the range of the other controls, so can be attributed to experimental setup.

Calculation of synergism: Combination Index (CI)

In order to confirm the results of synergy as calculated using Clarke et al. above, the Combination Index (CI) method was applied to the % dead cells (raw data) of Tables 1-16. For CI calculations, we used 0.3, 1, 3 and 10 µM Idelalisib and three MOR208 concentrations (1.5, 0.015 and 0.0015 µg/ml).

Such calculations are described in Ting-Chao Chou, Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies, Pharmacol Rev 58:621–681 (2006), which is incorporated by reference in its entirety and Chou TC, Talalay P, Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22: 27-55 (1984), which is incorporated by reference in its entirety. The methods of Chou-Talalay are carried out using the CI-isobol method.

Median effect equation

The median-effect equation models the effect of an inhibitor (such as a drug) as $F_a/F_u = (D/D_{50})^m$, where D is the dose, F_a and F_u is the fraction of the system affected and unaffected by the dose D ($F_a + F_u = 1$); D_{50} is the dose producing the median effect (e.g. IC₅₀, ED₅₀, LD₅₀). The constant m determines the shape of the dose-effect curve.

We use GraphPad Prism to carry out a nonlinear regression calculation to estimate the parameters m and $D50$.

CI-isobol method

The CI-isobol method provides a quantitative assessment of synergism between drugs. A combination index (CI) is estimated from dose-effect data of single and combined drug treatments. A value of CI less than 1 indicates synergism; CI = 1 indicates additive effect; and CI > 1 indicates antagonism. Drug interaction (synergism or antagonism) is more pronounced the farther a CI value is from 1.

Formally, the combination index (CI) of a combined drug treatment is defined as

$$CI = D_1/D_{x1} + D_2/D_{x2}$$

Here D_1 and D_2 are the doses of drug 1 and drug 2 of the combination, respectively; and D_{x1} , and D_{x2} is the dose of a treatment with only drug 1 and drug 2 that would give the same effect as that of the combination. The doses D_{x1} and D_{x2} need to be estimated from the dose-effect data of single drug treatments. Essentially, a median effect equation is fitted to the data of each drug. From the median effect equation of a drug, we can estimate the dose (i.e. D) necessary to produce an effect (i.e. F_a , F_u). The further a point lies from the additive line, the bigger the different between 1 and its CI, thus the stronger the (synergistic or antagonistic) effect is.

Results

The curves generated for the Chou based synergy calculations are shown in Figures 7-10. The Chou index values indicate clear synergism in all Experiments 1-4 of the combination of MOR00208 and Idelalisib in the specific killing of MEC-1 cells as compared to MOR00208 and Idelalisib alone.

The combination of MOR00208 and Idelalisib behaved synergistically in the MEC-1 CLL cell line. Therefore, it is believed that the combination of MOR00208 and Idelalisib is synergistic in the treatment of CLL in humans.

In addition, it is also believed that the combination of MOR00208 and Idelalisib will behave synergistically in the treatment of non-Hodgkin's lymphoma (NHL), chronic lymphoid leukemia (CLL), and acute lymphoblastic leukemia (ALL) in humans.

It is to be understood that the description, specific examples and data, while indicating exemplary embodiments, are given by way of illustration and are not intended to limit the present invention. Various changes and modifications within the present invention will become apparent to the skilled artisan from the discussion, disclosure and data contained herein, and thus are considered part of the invention.